ANTIBODIES SPECIFIC TO CADHERIN-17

CROSS-REFERENCE TO RELATED APPLICATION

[001] This application claims under 35 USC 119(e) the benefit of US Application 61/170,980, filed April 20, 2009, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

[002] The present invention relates generally to the fields of immunology and molecular biology. More specifically, provided herein are antibodies and other therapeutic proteins directed against cell adhesion molecule Cadherin-17, nucleic acids encoding such antibodies and therapeutic proteins, methods for preparing inventive monoclonal antibodies and other therapeutic proteins, and methods for the treatment of diseases, such as cancers mediated by Cadherin-17 expression/activity and/or associated with abnormal expression/activity of ligands therefore.

BACKGROUND OF THE INVENTION

[003] Cadherins are calcium dependent cell adhesion molecules. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. The cadherin molecule Cadherin-17 is also known as liver-intestine cadherin or intestinal peptide-associated transporter HPT-1. Cadherin-17 may have a role in the morphological organization of liver and intestine. It is also involved in intestinal peptide transport. The Cadherin-17 structure is characterized as having an extracellular domain with 7 cadherin domains, a single hydrophobic transmembrane domain and a short C-terminal cytoplasmic tail. Only one human Cadherin-17 isoform is known, Genbank Accession No. NM_004063. Cadherin-17 has the accession number Q12864 in the SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI) which are available at www.expasy.com). The mouse Cadherin-17 orthologue (Q9R100) shows 76% identity to the human Cadherin-17.

[004] According to SWISS-PROT, Cadherin-17 is expressed in the gastrointestinal tract and pancreatic duct. It is not detected in kidney, lung, liver, brain, adrenal gland or skin. Cadherin-17 expression has been reported in gastric cancer (see, for example, Ito *et al.*, *Virchows Arch.* 2005 Oct;447(4):717-22; Su *et al.*, *Mod Pathol.* 2008 Nov;21(11):1379-86; Ko *et al.*, *Biochem Biophys Res Commun.* 2004 Jun 25;319(2):562-8; and Dong *et al.*, *Dig*

Dis Sci. 2007 Feb;52(2):536-42), pancreatic cancer and colorectal cancer (Su *et al.*, *Mod Pathol.* 2008 Nov;21(11):1379-86) and hepatocellular carcinoma (Wong *et al.*, *Biochem Biophys Res Commun.* 2003 Nov 21;311(3):618-24). International Patent Application WO2008/026008 discloses Cadherin-17 as a marker for colorectal cancer and as a biological target for therapeutic antibodies and other pharmaceutical agents.

SUMMARY OF THE INVENTION

[005] Described herein are antibodies directed against Cadherin-17, nucleic acids encoding such antibodies and therapeutic proteins, methods for preparing anti-Cadherin-17 monoclonal antibodies and other therapeutic proteins, and methods for the treatment of diseases, such as Cadherin-17 mediated disorders, e.g., human cancers, including colorectal cancer.

[005a] In a first aspect, the present invention refers to an isolated anti-Cadherin-17 antibody comprising:

a heavy chain variable region CDR 1 comprising SEQ ID NO: 4;

a heavy chain variable region CDR2 comprising SEQ ID NO: 8;

a heavy chain variable region CDR3 comprising SEQ ID NO: 17;

a light chain variable region CDR1 comprising SEQ ID NO: 24;

a light chain variable region CDR2 comprising SEQ ID NO: 30; and

a light chain variable region CDR3 comprising SEQ ID NO: 34;

optionally wherein any of the above SEQ ID NOs independently comprise one, two or three amino acid substiutions, additions or deletions.

[005b] In a second aspect, the present invention refers to an isolated anti-Cadherin-17 antibody comprising:

(a) the heavy chain variable region amino acid sequence set forth in SEQ ID NO:38 or an amino acid sequence at least 90% homologous thereto, and

(b) the light chain variable region amino acid sequence set forth in SEQ ID NO:49 or an amino acid sequence at least 90% homologous thereto.

[005c] In a third aspect, the present invention refers to the use of an anti-Cadherin-17 antibody of the present invention or an immunoconjugate of the present invention in the manufacture of a medicament for treating or preventing cancer associated with target cells expressing Cadherin-17.

[006] Thus, further described herein are isolated monoclonal antibodies, in particular murine, chimeric, humanized, and fully-human monoclonal antibodies, that bind to Cadherin-17 and that exhibit one or more desirable functional property. Such properties include, for example, high affinity specific binding to human Cadherin-17. Also provided are methods for treating a variety of Cadherin-17-mediated diseases using the antibodies, proteins, and compositions described herein.

[007] Described herein is an isolated monoclonal antibody, or an antigen-binding portion thereof, an antibody fragment, or an antibody mimetic which binds an epitope on human Cadherin-17 recognized by an antibody comprising a heavy chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisting of 38, 35, 36, 37, 39, 40, 41, 42, 43, 44, 45 and 46 and a light chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisting of 49, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57 and 58. In some examples the isolated antibody is a full-length antibody of an IgG1, IgG2, IgG3, or IgG4 isotype.

[008] In some examples, the antibody described herein is selected from the group consisting of: a whole antibody, an antibody fragment, a humanized antibody, a single chain antibody, an immunoconjugate, a defucosylated antibody, and a bispecific antibody. The antibody fragment may be selected from the group consisting of: a UniBody, a domain antibody, and a Nanobody. In some examples, the immunoconjugates described herein comprise a therapeutic agent. In another example, the therapeutic agent is a cytotoxin or a radioactive isotope.

[009] In some examples, the antibody described herein is selected from the group consisting of: an Affibody, a DARPin, an Anticalin, an Avimer, a Versabody, and a Duocalin.

[010] In alternative examples, compositions described herein comprise an isolated antibody or antigen-binding portion and a pharmaceutically acceptable carrier.

[011] In other examples, the antibody described herein is a composition comprising the isolated antibody or antigen-binding portion as described herein and a pharmaceutically acceptable carrier.

[012] In some examples, it is described an isolated nucleic acid molecule encoding the heavy or light chain of the isolated antibody or antigen-binding portion which binds an epitope on human Cadherin-17. Other examples comprise expression vectors comprising such nucleic acid molecules, and host cells comprising such expression vectors.

[013] In some examples, it is described a method for preparing an anti-Cadherin-17 antibody, said method comprising the steps of: obtaining a host cell that contains one or more nucleic acid molecules encoding the antibody described herein; growing the host cell in a host cell culture; providing host cell culture conditions wherein the one or more nucleic acid molecules are expressed; and recovering the antibody from the host cell or from the host cell culture.

[014] In other examples, described herein are methods for treating or preventing a disease associated with target cells expressing Cadherin-17, said method comprising the step of administering to a subject an anti-Cadherin-17 antibody, or antigen-binding portion thereof, in an amount effective to treat or prevent the disease. In some examples, the disease treated or prevented by the antibodies or antigen-binding portion thereof as described herein, is human cancer. In some examples, the disease treated or prevented by the antibodies described herein is colorectal cancer.

[015] In other examples, described herein an anti-Cadherin-17 antibody, or antigen-binding portion thereof, for use in treating or preventing a disease associated with target cells expressing Cadherin-17. In some examples, the disease treated or prevented by the antibodies or antigen-binding portion thereof as described herein, is human cancer. In some examples, the disease treated or prevented by the antibodies as described herein is colorectal cancer.

[016] In other examples, it is described the use of an anti-Cadherin-17 antibody, or antigen-binding portion thereof, for the manufacture of a medicament for use in

treating or preventing a disease associated with target cells expressing Cadherin-17. In some examples, the disease treated or prevented by the medicament described herein, is human cancer. In some examples, the disease treated or prevented by the medicament described herein is colorectal cancer.

[017] In other examples, described herein is an isolated monoclonal antibody or an antigen binding portion thereof, an antibody fragment, or an antibody mimetic which

binds an epitope on human Cadherin-17 recognized by an antibody comprising a heavy chain variable region and a light chain variable region selected from the group consisting of the heavy chain variable region amino acid sequence set forth in SEQ ID NO:35 and the light chain variable region amino acid sequence set forth in SEQ ID NO:47; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:36 and the light chain variable region amino acid sequence set forth in SEQ ID NO:48; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:37 and the light chain variable region amino acid sequence set forth in SEQ ID NO:48; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:38 and the light chain variable region amino acid sequence set forth in SEQ ID NO:49; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:39 and the light chain variable region amino acid sequence set forth in SEQ ID NO:50; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:40 and the light chain variable region amino acid sequence set forth in SEQ ID NO:51; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:40 and the light chain variable region amino acid sequence set forth in SEQ ID NO:54; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:40 and the light chain variable region amino acid sequence set forth in SEQ ID NO:55; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:41 and the light chain variable region amino acid sequence set forth in SEQ ID NO:52; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:42 and the light chain variable region amino acid sequence set forth in SEQ ID NO:53; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:43 and the light chain variable region amino acid sequence set forth in SEQ ID NO:56; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:44 and the light chain variable region amino acid sequence set forth in SEQ ID NO:55; the heavy chain variable region amino acid sequence set forth in SEQ ID NO:45 and the light chain variable region amino acid sequence set forth in SEQ ID NO:57; and the heavy chain variable region amino acid sequence set forth in SEQ ID NO:46 and the light chain variable region amino acid sequence set forth in SEQ ID NO:58. In further examples, the antibody is selected from the

group consisting of: a whole antibody, an antibody fragment, a humanized antibody, a single chain antibody, an immunoconjugate, a defucosylated antibody, and a bispecific antibody. In further examples, the antibody fragment is selected from the group consisting of: a UniBody, a domain antibody, and a Nanobody. In some examples, the antibody mimetic is selected from the group consisting of: an Affibody, a DARPin, an Anticalin, an Avimer, a Versabody, and a Duocalin. In further examples, the composition comprises the isolated antibody or antigen binding portion thereof and a pharmaceutically acceptable carrier.

[018] In some examples, described herein is an isolated nucleic acid molecule encoding the heavy or light chain of the isolated antibody or antigen binding portion thereof of antibody as described herein, and in further examples may include an expression vector comprising such nucleic acids, and host cells comprising such expression vectors.

[019] Another example is a hybridoma expressing the antibody or antigen binding portion thereof of any one of antibodies described herein.

[020] Other examples are directed to methods of making the antibodies as described herein, comprising the steps of:

immunizing an animal with a Cadherin-17 peptide;

recovering mRNA from the B cells of said animal;

converting said mRNA to cDNA;

expressing said cDNA in phages such that anti-Cadherin-17 antibodies encoded by said cDNA are presented on the surface of said phages;

selecting phages that present anti-Cadherin-17 antibodies;

recovering nucleic acid molecules from said selected phages that encode said anti-Cadherin-17 immunoglobulins;

expressing said recovered nucleic acid molecules in a host cell; and

recovering antibodies from said host cell that bind Cadherin-17.

[021] In some examples, the isolated monoclonal antibody, or an antigen binding portion thereof, binds an epitope on the Cadherin-17 polypeptide having an amino acid sequence of SEQ ID NOS: 136 or 137 recognized by an antibody comprising a heavy chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisting of 38, 35, 36, 37, 39, 40, 41, 42, 43, 44, 45, or 46 and a light chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisiting of 49, 47, 48, 50, 51, 52, 53, 54; 55, 56, 57, or 58.

[022] Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[023] Figure 1 shows the nucleotide sequence (SEQ ID NO:59) and amino acid sequence (SEQ ID NO:35) of the heavy chain variable region of the PTA001_A1 monoclonal antibody. The CDR1 (SEQ ID NO:1), CDR2 (SEQ ID NO:5) and CDR3 (SEQ ID NO:14) regions are delineated.

[024] Figure 2 shows the nucleotide sequence (SEQ ID NO:60) and amino acid sequence (SEQ ID NO:36) of the heavy chain variable region of the PTA001_A2 monoclonal antibody. The CDR1 (SEQ ID NO:2), CDR2 (SEQ ID NO:6) and CDR3 (SEQ ID NO:15) regions are delineated.

[025] Figure 3 shows the nucleotide sequence (SEQ ID NO:61) and amino acid sequence (SEQ ID NO:37) of the heavy chain variable region of the PTA001_A3 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:7) and CDR3 (SEQ ID NO:16) regions are delineated.

[026] Figure 4 shows the nucleotide sequence (SEQ ID NO:62) and amino acid sequence (SEQ ID NO:38) of the heavy chain variable region of the PTA001_A4 monoclonal antibody. The CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:8) and CDR3 (SEQ ID NO:17) regions are delineated.

[027] Figure 5 shows the nucleotide sequence (SEQ ID NO:63) and amino acid sequence (SEQ ID NO:39) of the heavy chain variable region of the PTA001_A5 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:7) and CDR3 (SEQ ID NO:18) regions are delineated.

[028] Figure 6 shows the nucleotide sequence (SEQ ID NO:64) and amino acid sequence (SEQ ID NO:40) of the heavy chain variable region of the PTA001_A6, PTA001_A9 and PTA001_A10 monoclonal antibodies. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:7) and CDR3 (SEQ ID NO:16) regions are delineated.

[029] Figure 7 shows the nucleotide sequence (SEQ ID NO:65) and amino acid sequence (SEQ ID NO:41) of the heavy chain variable region of the PTA001_A7 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:9) and CDR3 (SEQ ID NO:19) regions are delineated.

[030] Figure 8 shows the nucleotide sequence (SEQ ID NO:66) and amino acid sequence (SEQ ID NO:42) of the heavy chain variable region of the PTA001_A8 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:7) and CDR3 (SEQ ID NO:16) regions are delineated.

[031] Figure 9 shows the nucleotide sequence (SEQ ID NO:67) and amino acid sequence (SEQ ID NO:43) of the heavy chain variable region of the PTA001_A11 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:10) and CDR3 (SEQ ID NO:20) regions are delineated.

[032] Figure 10 shows the nucleotide sequence (SEQ ID NO:68) and amino acid sequence (SEQ ID NO:44) of the heavy chain variable region of the PTA001_A12 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:11) and CDR3 (SEQ ID NO:21) regions are delineated.

[033] Figure 11 shows the nucleotide sequence (SEQ ID NO:69) and amino acid sequence (SEQ ID NO:45) of the heavy chain variable region of the PTA001_A13 monoclonal antibody. The CDR1 (SEQ ID NO:3), CDR2 (SEQ ID NO:12) and CDR3 (SEQ ID NO:18) regions are delineated.

[034] Figure 12 shows the nucleotide sequence (SEQ ID NO:70) and amino acid sequence (SEQ ID NO:46) of the heavy chain variable region of the PTA001_A14 monoclonal antibody. The CDR1 (SEQ ID NO:2), CDR2 (SEQ ID NO:13) and CDR3 (SEQ ID NO:15) regions are delineated.

[035] Figure 13 shows the nucleotide sequence (SEQ ID NO:71) and amino acid sequence (SEQ ID NO:47) of the light chain variable region of the PTA001_A1 monoclonal antibody. The CDR1 (SEQ ID NO:22), CDR2 (SEQ ID NO:28) and CDR3 (SEQ ID NO:32) regions are delineated.

[036] Figure 14 shows the nucleotide sequence (SEQ ID NO:72) and amino acid sequence (SEQ ID NO:48) of the light chain variable region of the PTA001_A2 and PTA001_A3 monoclonal antibodies. The CDR1 (SEQ ID NO:23), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[037] Figure 15 shows the nucleotide sequence (SEQ ID NO:73) and amino acid sequence (SEQ ID NO:49) of the light chain variable region of the PTA001_A4 monoclonal antibody. The CDR1 (SEQ ID NO:24), CDR2 (SEQ ID NO:30) and CDR3 (SEQ ID NO:34) regions are delineated.

[038] Figure 16 shows the nucleotide sequence (SEQ ID NO:74) and amino acid sequence (SEQ ID NO:50) of the light chain variable region of the PTA001_A5 monoclonal

antibody. The CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:31) and CDR3 (SEQ ID NO:33) regions are delineated.

[039] Figure 17 shows the nucleotide sequence (SEQ ID NO:75) and amino acid sequence (SEQ ID NO:51) of the light chain variable region of the PTA001_A6 monoclonal antibody. The CDR1 (SEQ ID NO:23), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[040] Figure 18 shows the nucleotide sequence (SEQ ID NO:76) and amino acid sequence (SEQ ID NO:52) of the light chain variable region of the PTA001_A7 monoclonal antibody. The CDR1 (SEQ ID NO:26), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[041] Figure 19 shows the nucleotide sequence (SEQ ID NO:77) and amino acid sequence (SEQ ID NO:53) of the light chain variable region of the PTA001_A8 monoclonal antibody. The CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:31) and CDR3 (SEQ ID NO:33) regions are delineated.

[042] Figure 20 shows the nucleotide sequence (SEQ ID NO:78) and amino acid sequence (SEQ ID NO:54) of the light chain variable region of the PTA001_A9 monoclonal antibody. The CDR1 (SEQ ID NO:23), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[043] Figure 21 shows the nucleotide sequence (SEQ ID NOs:79 and 81) and amino acid sequence (SEQ ID NO:55) of the light chain variable region of the PTA001_A10 and PTA001_A12 monoclonal antibodies. The CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[044] Figure 22 shows the nucleotide sequence (SEQ ID NO:80) and amino acid sequence (SEQ ID NO:56) of the light chain variable region of the PTA001_A11 monoclonal antibody. The CDR1 (SEQ ID NO:27), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[045] Figure 23 shows the nucleotide sequence (SEQ ID NO:82) and amino acid sequence (SEQ ID NO:57) of the light chain variable region of the PTA001_A13 monoclonal antibody. The CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:31) and CDR3 (SEQ ID NO:33) regions are delineated.

[046] Figure 24 shows the nucleotide sequence (SEQ ID NO:83) and amino acid sequence (SEQ ID NO:58) of the light chain variable region of the PTA001_A14 monoclonal antibody. The CDR1 (SEQ ID NO:23), CDR2 (SEQ ID NO:29) and CDR3 (SEQ ID NO:33) regions are delineated.

[047] Figure 25 shows the alignment of the nucleotide sequences of the heavy chain CDR1 region of PTA001_A1 (SEQ ID NO:84) with nucleotides 240-269 of the mouse germline V_H 7-39 nucleotide sequence (SEQ ID NO:125) and the alignments of the nucleotide sequences of the heavy chain CDR1 regions of PTA001_A2 and PTA001_A14 (SEQ ID NO:85); PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12 and PTA001_A13 (SEQ ID NO:86); and PTA001_A4 (SEQ ID NO:87) with nucleotides 67-96 of the mouse germline V_H II gene H17 nucleotide sequence (SEQ ID NO:126).

[048] Figure 26 shows the alignments of the nucleotide sequences of the heavy chain CDR2 regions of PTA001_A2 (SEQ ID NO:89); PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A8, PTA001_A9 and PTA001_A10 (SEQ ID NO:90); PTA001_A4 (SEQ ID NO:91); PTA001_A7 (SEQ ID NO:92); PTA001_A11 (SEQ ID NO:93); PTA001_A12 (SEQ ID NO:94); PTA001_A13 (SEQ ID NO:95); and PTA001_A14 (SEQ ID NO:96) with nucleotides 1096-1146 of the mouse germline V_H II region VH105 nucleotide sequence (SEQ ID NO:127).

[049] Figure 27 shows the alignment of the nucleotide sequence of the light chain CDR1 region of PTA001_A1 (SEQ ID NO:105) with nucleotides 1738-1785 of the mouse germline V_K 1-110 nucleotide sequence (SEQ ID NO:128), the alignment of the nucleotide sequence of the light chain CDR1 region of PTA001_A4 (SEQ ID NO:107) with nucleotides 510-560 of the mouse germline V_K 8-30 nucleotide sequence (SEQ ID NO: 130) and the alignments of the nucleotide sequences of the light chain CDR1 regions of PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9 and PTA001_A14 (SEQ ID NO:106); PTA001_A5 and PTA001_A13 (SEQ ID NO:108); PTA001_A7 (SEQ ID NO:109); PTA001_A8 (SEQ ID NO:110); PTA001_A10 (SEQ ID NO:111); PTA001_A11 (SEQ ID NO:112); and PTA001_A12 (SEQ ID NO:113) with nucleotides 1807-1854 of the mouse germline V_K 24-140 nucleotide sequence (SEQ ID NO:133).

[050] Figure 28 shows the alignment of the nucleotide sequence of the light chain CDR2 region of PTA001_A4 (SEQ ID NO:116) with nucleotides 606-626 of the mouse germline V_K 8-30 nucleotide sequence (SEQ ID NO:131) and the alignments of the nucleotide sequences of the light chain CDR2 regions of PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9 and PTA001_A14 (SEQ ID NO:115); PTA001_A5 and PTA001_A13 (SEQ ID NO:117); PTA001_A7, PTA001_A10, PTA001_A11 and PTA001_A12 (SEQ ID NO:118); and PTA001_A8 (SEQ ID NO:119) with nucleotides 1900-1920 of the mouse germline V_K 24-140 nucleotide sequence (SEQ ID NO:134).

[051] Figure 29 shows the alignment of the nucleotide sequence of the light chain CDR3 region of PTA001_A1 (SEQ ID NO:120) with nucleotides 1948-1971 of the mouse germline V_K 1-110 nucleotide sequence (SEQ ID NO:129), the alignment of the nucleotide sequence of the light chain CDR3 region of PTA001_A4 (SEQ ID NO:122) with nucleotides 723-749 of the mouse germline V_K 8-30 nucleotide sequence (SEQ ID NO:132) and the alignments of the nucleotide sequences of the light chain CDR3 regions of PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12 and PTA001_A14 (SEQ ID NO:121); PTA001_A5, PTA001_A8 and PTA001_A13 (SEQ ID NO:123); and PTA001_A7 (SEQ ID NO:124) with nucleotides 2017-2043 of the mouse germline V_K 24-140 nucleotide sequence (SEQ ID NO:135).

[052] Figure 30 shows the Western Blotting analysis of Cadherin-17 using the PTA001_A4 monoclonal antibody.

[053] Figure 31 shows results of FACS analysis on PTA001_A4 in LoVo cells.

[054] Figure 32 shows results of FACS analysis on PTA001_A4 in LoVo and LS174T cells.

[055] Figure 33A shows surface binding of PTA001_A4/ secondary antibody FITC conjugate complex to LoVo cells after 60 minutes of incubation.

[056] Figure 33B shows internalization of PTA001_A4/ secondary antibody FITC conjugate complex after 120 minutes of incubation with LoVo cells.

[057] Figure 34A shows results of internalisation of PTA001_A4 by MabZAP assay in LoVo colon cancer cells.

[058] Figure 34B shows results of internalisation of PTA001_A4 by MabZAP assay in LoVo colon cancer cells.

[059] Figure 34C shows results of internalisation of PTA001_A4 by MabZAP assay in LS174T colon cancer cells.

[060] Figure 34D shows results of internalisation of PTA001_A4 by MabZAP assay in LS174T colon cancer cells.

[061] Figure 35A shows results of internalisation of PTA001_A4 by MabZAP assay in LoVo colon cancer cells.

[062] Figure 35B shows results of internalisation of PTA001_A4 by MabZAP assay in LS174T colon cancer cells.

DETAILED DESCRIPTION OF THE INVENTION

[063] The present invention relates to isolated antibodies, including, but not limited to monoclonal antibodies, for example, which bind specifically to Cadherin-17 with high

affinity. In certain embodiments, the antibodies of the invention comprise particular structural features such as CDR regions comprising particular amino acid sequences. The invention provides isolated antibodies, defucosylated antibodies, immunoconjugates, bispecific molecules, affibodies, domain antibodies, nanobodies, and unibodies, methods of making said molecules, and pharmaceutical compositions comprising said molecules and a pharmaceutical carrier. The invention also relates to methods of using the molecules, such as to detect Cadherin-17, as well as to treat diseases associated with expression of Cadherin-17, such as Cadherin-17 expressed on tumors, including those tumors of colorectal cancer.

[064] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[065] The terms "Cadherin-17", "Liver-intestine cadherin", "LI-cadherin", "Intestinal peptide-associated transported HPT-1" and "CDH17" are used interchangeably. Cadherin-17 has also been identified as OGTA001 in International Patent Application WO2008/026008, which is incorporated herein by reference in its entirety. Human antibodies of this disclosure may, in certain cases, cross-react with Cadherin-17 from species other than human. In certain embodiments, the antibodies may be completely specific for one or more human Cadherin-17 and may not exhibit species or other types of non-human cross-reactivity. The complete amino acid sequence of an exemplary human Cadherin-17 has Genbank accession number NM_004063.

[066] The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[067] A "signal transduction pathway" refers to the biochemical relationship between various of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the Cadherin-17 receptor.

[068] The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein which may comprise at least two heavy (H) chains and

two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L or V_K) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L / V_K regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L / V_K is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[069] The definition of "antibody" includes, but is not limited to, full length antibodies, antibody fragments, single chain antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[070] In one embodiment, the antibody is an antibody fragment. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody, (iv) the dAb fragment, which consists of a single variable domain, (v) isolated CDR regions, (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site, (viii) bispecific single chain Fv dimers, and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion. The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulfide bridges linking the VH and VL domains. Examples of antibody formats and architectures are described in Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136, and Carter 2006, Nature Reviews Immunology 6:343-357 and references cited therein, all expressly incorporated by reference.

[071] In one embodiment, an antibody disclosed herein may be a multispecific antibody, and notably a bispecific antibody, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens. Diabodies can be manufactured in a variety of ways known in the art, e.g., prepared chemically or from hybrid hybridomas. In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. In some cases, the scFv can be joined to the Fc region, and may include some or all of the hinge region. For a description of multispecific antibodies see Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136 and references cited therein, all expressly incorporated by reference.

[072] By "CDR" as used herein is meant a Complementarity Determining Region of an antibody variable domain. Systematic identification of residues included in the CDRs have been developed by Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda) and alternately by Chothia (Chothia & Lesk, 1987, J. Mol. Biol. 196: 901-917; Chothia et al., 1989, Nature 342: 877-883; Al-Lazikani et al., 1997, J. Mol. Biol. 273: 927-948). For the purposes of the present invention, CDRs are defined as a slightly smaller set of residues than the CDRs defined by Chothia. VL CDRs are herein defined to include residues at positions 27-32 (CDR1), 50-56 (CDR2), and 91-97 (CDR3), wherein the numbering is according to Chothia. Because the VL CDRs as defined by Chothia and Kabat are identical, the numbering of these VL CDR positions is also according to Kabat. VH CDRs are herein defined to include residues at positions 27-33 (CDR1), 52-56 (CDR2), and 95-102 (CDR3), wherein the numbering is according to Chothia. These VH CDR positions correspond to Kabat positions 27-35 (CDR1), 52-56 (CDR2), and 95-102 (CDR3).

[073] As will be appreciated by those in the art, the CDRs disclosed herein may also include variants. for example when backmutating the CDRs disclosed herein into different framework regions. Generally, the amino acid identity between individual variant CDRs are at least 80% to the sequences depicted herein, and more typically with preferably increasing identities of at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and almost 100%. In a similar manner, "percent (%) nucleic acid sequence identity" with respect to the nucleic acid sequence of the binding proteins identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the antigen binding protein. A specific method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

[074] Generally, the nucleic acid sequence identity between the nucleotide sequences encoding individual variant CDRs and the nucleotide sequences depicted herein are at least 80%, and more typically with preferably increasing identities of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and almost 100%.

[075] Thus, a "variant CDR" is one with the specified homology, similarity, or identity to the parent CDR of the invention, and shares biological function, including, but not limited to, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the specificity and/or activity of the parent CDR.

[076] While the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed antigen binding protein CDR variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done using assays of antigen binding protein activities as described herein.

[077] Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about one (1) to about twenty (20) amino acid residues, although considerably larger insertions may be tolerated. Deletions range from about one (1) to about twenty (20) amino acid residues, although in some cases deletions may be much larger.

[078] Substitutions, deletions, insertions or any combination thereof may be used to arrive at a final derivative or variant. Generally these changes are done on a few amino acids to minimize the alteration of the molecule, particularly the immunogenicity and specificity of the antigen binding protein. However, larger changes may be tolerated in certain circumstances.

[079] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein, or any other antibody embodiments as outlined herein.

[080] By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[081] By "framework" as used herein is meant the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable domain framework can

be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

[082] The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., Cadherin-17). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L / V_K, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fab' fragment, which is essentially an Fab with part of the hinge region (see, FUNDAMENTAL IMMUNOLOGY (Paul ed., 3.sup.rd ed. 1993); (iv) a Fd fragment consisting of the V_H and C_H1 domains; (v) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; (vi) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; (vii) an isolated complementarity determining region (CDR); and (viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Furthermore, although the two domains of the Fv fragment, V_L / V_K and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L / V_K and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[083] An "isolated antibody" as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds Cadherin-17 is substantially free of antibodies that specifically bind antigens other than Cadherin-17). An isolated antibody that specifically binds Cadherin-17 may, however, have cross-reactivity to other antigens, such as Cadherin-17 molecules from other species. Moreover, and/or alternatively an isolated antibody may be substantially free of other cellular material and/or chemicals in a form not normally found in nature.

[084] In some embodiments, the antibodies of the invention are recombinant proteins, isolated proteins or substantially pure proteins. An "isolated" protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, for example constituting at least about 5%, or at least about 50% by weight of the total protein in a given sample. It is understood that the isolated protein may constitute from 5 to 99.9% by weight of the total protein content depending on the circumstances. For example, the protein may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. In the case of recombinant proteins, the definition includes the production of an antibody in a wide variety of organisms and/or host cells that are known in the art in which it is not naturally produced.

[085] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[086] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgGI) that is encoded by the heavy chain constant region genes.

[087] The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

[088] The term "antibody derivatives" refers to any modified form of the antibody, e.g., a conjugate of the antibody and another agent or antibody. For example, antibodies of the present invention may be conjugated to a toxin, a label, etc. The antibodies of the present invention may be nonhuman, chimeric, humanized, or fully human. For a description of the concepts of chimeric and humanized antibodies see Clark et al., 2000 and references cited therein (Clark, 2000, *Immunol Today* 21:397-402). Chimeric antibodies comprise the variable region of a nonhuman antibody, for example VH and VL domains of mouse or rat origin, operably linked to the constant region of a human antibody (see for example U.S. Patent No. 4,816,567). In a preferred embodiment, the antibodies of the present invention are humanized. By "humanized" antibody as used herein is meant an antibody comprising a human framework region (FR) and one or more complementarity determining regions (CDR's) from a non-human (usually mouse or rat) antibody. The non-human antibody providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". Humanization relies principally on the grafting of donor

CDRs onto acceptor (human) VL and VH frameworks (US Patent No, 5,225,539). This strategy is referred to as "CDR grafting". "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5,530,101; US 5,585,089; US 5,693,761; US 5,693,762; US 6,180,370; US 5,859,205; US 5,821,337; US 6,054,297; US 6,407,213). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Methods for humanizing non-human antibodies are well known in the art, and can be essentially performed following the method of Winter and co-workers (Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*,1988, *Nature* 332:323-329; Verhoeyen *et al.*, 1988, *Science*, 239:1534-1536). Additional examples of humanized murine monoclonal antibodies are also known in the art, for example antibodies binding human protein C (O'Connor *et al.*, 1998, *Protein Eng* 11:321-8), interleukin 2 receptor (*Queen et al.*, 1989, *Proc Natl Acad Sci*, USA 86:10029-33), and human epidermal growth factor receptor 2 (Carter *et al.*, 1992, *Proc Natl Acad Sci USA 89:4285-9*). In an alternate embodiment, the antibodies of the present invention may be fully human, that is the sequences of the antibodies are completely or substantially human. A number of methods are known in the art for generating fully human antibodies, including the use of transgenic mice (Bruggemann *et al.*, 1997, *Curr Opin Biotechnol* 8:455-458) or human antibody libraries coupled with selection methods (Griffiths *et al.*, 1998, *Curr Opin Biotechnol* 9:102-108).

[089] The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

[090] The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[091] The term "specifically binds" (or "immunospecifically binds") is not intended to indicate that an antibody binds exclusively to its intended target. Rather, an antibody "specifically binds" if its affinity for its intended target is about 5-fold greater when compared to its affinity for a non-target molecule. Suitably there is no significant cross-reaction or cross-binding with undesired substances, especially naturally occurring proteins

or tissues of a healthy person or animal. The affinity of the antibody will, for example, be at least about 5 fold, such as 10 fold, such as 25-fold, especially 50-fold, and particularly 100-fold or more, greater for a target molecule than its affinity for a non-target molecule. In some embodiments, specific binding between an antibody or other binding agent and an antigen means a binding affinity of at least 10^6 M^{-1}. Antibodies may, for example, bind with affinities of at least about 10^7 M^{-1}, such as between about 10^8 M^{-1} to about 10^9 M^{-1}, about 10^9 M^{-1} to about 10^{10} M^{-1}, or about 10^{10} M^{-1} to about 10^{11} M^{-1}. Antibodies may, for example, bind with an EC_{50} of 50 nM or less, 10 nM or less, 1 nM or less, 100 pM or less, or more preferably 10 pM or less.

[092] The term "does not substantially bind" to a protein or cells, as used herein, means does not bind or does not bind with a high affinity to the protein or cells, i.e. binds to the protein or cells with a K_D of 1 x 10^{-6} M or more, more preferably 1 x 10^{-5} M or more, more preferably 1 x 10^{-4} M or more, more preferably 1 x 10^{-3} M or more, even more preferably 1 x 10^{-2} M or more.

[093] The term "EC_{50}" as used herein, is intended to refer to the potency of a compound by quantifying the concentration that leads to 50% maximal response/effect. EC_{50} may be determined by Scratchard or FACS.

[094] The term "K_{assoc}" or "K_a," as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D," as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e,. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

[095] As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 1 x 10^{-7} M or less, more preferably 5 x 10^{-8} M or less, even more preferably 1x10^{-8} M or less, even more preferably 5 x 10^{-9} M or less and even more preferably 1 x 10^{-9} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-6} M or less, more preferably 10^{-7} M or less, even more preferably 10^{-8} M or less.

[096] The term "epitope" or "antigenic determinant" refers to a site on an antigen to which an immunoglobulin or antibody specifically binds. Epitopes can be formed both from

contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include techniques in the art and those described herein, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance (see, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology,* Vol. 66, G. E. Morris, Ed. (1996)).

[097] Accordingly, also encompassed by the present invention are antibodies that bind to (i.e., recognize) the same epitope as the antibodies described herein (i.e., PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14). Antibodies that bind to the same epitope can be identified by their ability to cross-compete with (i.e., competitively inhibit binding of) a reference antibody to a target antigen in a statistically significant manner. Competitive inhibition can occur, for example, if the antibodies bind to identical or structurally similar epitopes (e.g., overlapping epitopes), or spatially proximal epitopes which, when bound, causes steric hindrance between the antibodies.

[098] Competitive inhibition can be determined using routine assays in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli *et al.*, *Methods in Enzymology* 9:242 (1983)); solid phase direct biotin-avidin EIA (see Kirkland *et al.*, *J. Immunol.* 137:3614 (1986)); solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Press (1988)); solid phase direct label RIA using I-125 label (see Morel *et al.*, *Mol. Immunol.* 25(1):7 (1988)); solid phase direct biotin-avidin EIA (Cheung *et al.*, *Virology* 176:546 (1990)); and direct labeled RIA. (Moldenhauer *et al.*, *Scand. J. Immunol.* 32:77 (1990)). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin

is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70% 70-75% or more.

[099] Other techniques include, for example, epitope mapping methods, such as x-ray analyses of crystals of antigen:antibody complexes which provides atomic resolution of the epitope. Other methods monitor the binding of the antibody to antigen fragments or mutated variations of the antigen where loss of binding due to a modification of an amino acid residue within the antigen sequence is often considered an indication of an epitope component. In addition, computational combinatorial methods for epitope mapping can also be used. These methods rely on the ability of the antibody of interest to affinity isolate specific short peptides from combinatorial phage display peptide libraries. The peptides are then regarded as leads for the definition of the epitope corresponding to the antibody used to screen the peptide library. For epitope mapping, computational algorithms have also been developed which have been shown to map conformational discontinuous epitopes.

[0100] As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

[0101] Various aspects of the invention are described in further detail in the following subsections.

Anti-Cadherin-17 Antibodies

[0102] The antibodies of the invention are characterized by particular functional features or properties of the antibodies. For example, the antibodies bind specifically to human Cadherin-17. Preferably, an antibody of the invention binds to Cadherin-17 with high affinity, for example with a K_D of 8×10^{-7} M or less, even more typically 1×10^{-8} M or less. The anti-Cadherin-17 antibodies of the invention preferably exhibit one or more of the following characteristics:

binds to human Cadherin-17 with a EC_{50} of 50 nM or less, 10 nM or less, 1 nM or less, 100 pM or less, or more preferably 10 pM or less;

binds to human cells expressing Cadherin-17.

[0103] In one embodiment, the antibodies preferably bind to an antigenic epitope present in Cadherin-17, which epitope is not present in other proteins. The antibodies typically bind Cadherin-17 but does not bind to other proteins, or binds to proteins with a low affinity, such as a K_D of 1×10^{-6} M or more, more preferably 1×10^{-5} M or more, more preferably 1×10^{-4} M or more, more preferably 1×10^{-3} M or more, even more preferably 1×10^{-2} M or more.

Preferably, the antibodies do not bind to related proteins, for example, the antibodies do not substantially bind to other cell adhesion molecules. In one embodiment, the antibody may be internalized into a cell expressing Cadherin-17. Standard assays to evaluate antibody internalization are known in the art, including, for example, a HumZap internalization assay.

[0104] Standard assays to evaluate the binding ability of the antibodies toward Cadherin-17 are known in the art, including for example, ELISAs, Western blots, RIAs, and flow cytometry analysis. Suitable assays are described in detail in the Examples. The binding kinetics (e.g., binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore® system analysis. To assess binding to Raji or Daudi B cell tumor cells, Raji (ATCC Deposit No. CCL-86) or Daudi (ATCC Deposit No. CCL-213) cells can be obtained from publicly available sources, such as the American Type Culture Collection, and used in standard assays, such as flow cytometric analysis.

Monoclonal Antibodies Of The Invention

[0105] Preferred antibodies of the invention are the monoclonal antibodies PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14, isolated and structurally characterized as described in Examples 1-6. The V_H amino acid sequences of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:35-46. The V_K amino acid sequences of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:47-58.

[0106] Given that each of these antibodies can bind to Cadherin-17, the V_H and V_K sequences can be "mixed and matched" to create other anti-Cadherin-17 binding molecules of the invention. Cadherin-17 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs). Preferably, when V_H and V_K chains are mixed and matched, a V_H sequence from a particular V_H/V_K pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_K sequence from a particular V_H/V_K pairing is replaced with a structurally similar V_K sequence.

[0107] Accordingly, in one aspect, the invention provides an antibody, comprising:
a heavy chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisting of 38, 35, 36, 37, 39, 40, 41, 42, 43, 44, 45 and 46 and a

light chain variable region comprising an amino acid sequence set forth in a SEQ ID NO: selected from the group consisting of 49, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57 and 58; wherein the antibody specifically binds Cadherin-17, preferably human Cadherin-17.

[0108] Preferred heavy and light chain combinations include:

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:47; or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:36; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:48, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:48, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:38; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:49, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:39; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:50, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:51, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:41; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:52, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:42; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:53, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:54, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:55, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:43; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:56, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:44; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:55, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:45; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:57, or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:46; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:58.

[0109] In another aspect, the invention provides antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s of PTA001_A1, PTA001_A2, PTA001_A3,

PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14, or combinations thereof. The amino acid sequences of the V_H CDR1s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs: 1-4. The amino acid sequences of the V_H CDR2s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs: 5-13. The amino acid sequences of the V_H CDR3s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:14-21. The amino acid sequences of the V_K CDR1s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:22-27. The amino acid sequences of the V_K CDR2s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:28-31. The amino acid sequences of the V_K CDR3s of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs:32-34. The CDR regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[0110] Given that each of these antibodies can bind to Cadherin-17 and that antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_K CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and matched, although each antibody generally contains a V_H CDR1, CDR2, and CDR3 and a V_K CDR1, CDR2, and CDR3) to create other anti-Cadherin-17 binding molecules of the invention. Accordingly, the invention specifically includes every possible combination of CDRs of the heavy and light chains.

[0111] Cadherin-17 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (e.g., ELISAs, Biacore® analysis). Preferably, when V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence is replaced with a structurally similar CDR sequence(s). Likewise, when V_K CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_K sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_K sequences can be created by substituting one or more V_H and/or V_L / V_K CDR region sequences with structurally similar sequences from the CDR sequences disclosed herein for monoclonal antibodies PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14.

[0112] Accordingly, in another aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-4;

a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:5-13;

a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14-21;

a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:22-27;

a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:28-31; and

a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:32-34;

with all possible combinations being possible, wherein the antibody specifically binds Cadherin-17, preferably human Cadherin-17

[0113] In a preferred embodiment, the antibody comprises:

a heavy chain variable region CDR1 comprising SEQ ID NO:1;

a heavy chain variable region CDR2 comprising SEQ ID NO:5;

a heavy chain variable region CDR3 comprising SEQ ID NO:14;

a light chain variable region CDR1 comprising SEQ ID NO:22;

a light chain variable region CDR2 comprising SEQ ID NO:28; and
a light chain variable region CDR3 comprising SEQ ID NO:32.

[0114] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:2;
a heavy chain variable region CDR2 comprising SEQ ID NO:6;
a heavy chain variable region CDR3 comprising SEQ ID NO:15;
a light chain variable region CDR1 comprising SEQ ID NO:23;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0115] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:7;
a heavy chain variable region CDR3 comprising SEQ ID NO:16;
a light chain variable region CDR1 comprising SEQ ID NO:23;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0116] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:4;
a heavy chain variable region CDR2 comprising SEQ ID NO:8;
a heavy chain variable region CDR3 comprising SEQ ID NO:17;
a light chain variable region CDR1 comprising SEQ ID NO:24;
a light chain variable region CDR2 comprising SEQ ID NO:30; and
a light chain variable region CDR3 comprising SEQ ID NO:34.

[0117] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:7;
a heavy chain variable region CDR3 comprising SEQ ID NO:18;
a light chain variable region CDR1 comprising SEQ ID NO:25;
a light chain variable region CDR2 comprising SEQ ID NO:31; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0118] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:9;
a heavy chain variable region CDR3 comprising SEQ ID NO:19;

a light chain variable region CDR1 comprising SEQ ID NO:26;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0119] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:7;
a heavy chain variable region CDR3 comprising SEQ ID NO:16;
a light chain variable region CDR1 comprising SEQ ID NO:25;
a light chain variable region CDR2 comprising SEQ ID NO:31; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0120] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:7;
a heavy chain variable region CDR3 comprising SEQ ID NO:16;
a light chain variable region CDR1 comprising SEQ ID NO:25;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0121] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:10;
a heavy chain variable region CDR3 comprising SEQ ID NO:20;
a light chain variable region CDR1 comprising SEQ ID NO:27;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0122] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:11;
a heavy chain variable region CDR3 comprising SEQ ID NO:21;
a light chain variable region CDR1 comprising SEQ ID NO:25;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0123] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:3;
a heavy chain variable region CDR2 comprising SEQ ID NO:12;

a heavy chain variable region CDR3 comprising SEQ ID NO:18;
a light chain variable region CDR1 comprising SEQ ID NO:25;
a light chain variable region CDR2 comprising SEQ ID NO:31; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0124] In another preferred embodiment, the antibody comprises:
a heavy chain variable region CDR1 comprising SEQ ID NO:2;
a heavy chain variable region CDR2 comprising SEQ ID NO:13;
a heavy chain variable region CDR3 comprising SEQ ID NO:15;
a light chain variable region CDR1 comprising SEQ ID NO:23;
a light chain variable region CDR2 comprising SEQ ID NO:29; and
a light chain variable region CDR3 comprising SEQ ID NO:33.

[0125] It is well known in the art that the CDR3 domain, independently from the CDR1 and/or CDR2 domain(s), alone can determine the binding specificity of an antibody for a cognate antigen and that multiple antibodies can predictably be generated having the same binding specificity based on a common CDR3 sequence. See, for example, Klimka *et al.*, *British J. of Cancer* 83(2):252-260 (2000) (describing the production of a humanized anti-CD30 antibody using only the heavy chain variable domain CDR3 of murine anti-CD30 antibody Ki-4); Beiboer *et al.*, *J. Mol. Biol.* 296:833-849 (2000) (describing recombinant epithelial glycoprotein-2 (EGP-2) antibodies using only the heavy chain CDR3 sequence of the parental murine MOC-31 anti-EGP-2 antibody); Rader *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95:8910-8915 (1998) (describing a panel of humanized anti-integrin $\alpha_v\beta_3$ antibodies using a heavy and light chain variable CDR3 domain of a murine anti-integrin $\alpha_v\beta_3$ antibody LM609 wherein each member antibody comprises a distinct sequence outside the CDR3 domain and capable of binding the same epitope as the parent murine antibody with affinities as high or higher than the parent murine antibody); Barbas *et al.*, *J. Am. Chem. Soc.* 116:2161-2162 (1994) (disclosing that the CDR3 domain provides the most significant contribution to antigen binding); Barbas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92:2529-2533 (1995) (describing the grafting of heavy chain CDR3 sequences of three Fabs (SI-1, SI-40, and SI-32) against human placental DNA onto the heavy chain of an anti-tetanus toxoid Fab thereby replacing the existing heavy chain CDR3 and demonstrating that the CDR3 domain alone conferred binding specificity); and Ditzel *et al.*, *J. Immunol.* 157:739-749 (1996) (describing grafting studies wherein transfer of only the heavy chain CDR3 of a parent polyspecific Fab LNA3 to a heavy chain of a monospecific IgG tetanus toxoid-binding Fab

p313 antibody was sufficient to retain binding specificity of the parent Fab). Each of these references is hereby incorporated by reference in its entirety.

[0126] Accordingly, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domains from an antibody derived from a human or non-human animal, wherein the monoclonal antibody is capable of specifically binding to Cadherin-17. Within certain aspects, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody, such as a mouse or rat antibody, wherein the monoclonal antibody is capable of specifically binding to Cadherin-17. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental non-human antibody.

[0127] Within other aspects, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domains from a human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the human antibody is capable of specifically binding to Cadherin-17. Within other aspects, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a first human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the first human antibody is capable of specifically binding to Cadherin-17 and wherein the CDR3 domain from the first human antibody replaces a CDR3 domain in a human antibody that is lacking binding specificity for Cadherin-17 to generate a second human antibody that is capable of specifically binding to Cadherin-17. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from the first human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental first human antibody.

Antibodies Having Particular Germline Sequences

[0128] In certain embodiments, an antibody of the invention comprises a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

[0129] For example, in a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain

variable region that is the product of or derived from a murineV_H 7-39 gene, a murine V_H II region VH105 gene or a murine V_H II gene H17, wherein the antibody specifically binds Cadherin-17. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a murine V_K 1-110 gene, a murine V_K 8-30 gene or a murine V_K 24-140 gene, wherein the antibody specifically binds Cadherin-17.

[0130] In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:
comprises a heavy chain variable region that is the product of or derived from a murine V_H 7-39 gene (which gene includes the nucleotide sequence set forth in SEQ ID NO: 125);
comprises a light chain variable region that is the product of or derived from a murine V_K 1-110 gene (which gene includes the nucleotide sequences set forth in SEQ ID NOs: 128 and 129); andspecifically binds to Cadherin-17, preferably human Cadherin-17. Examples of an antibody having V_H and V_K of V_H 7-39 and V_K 1-110, respectively, is PTA001_A1.

[0131] In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:
comprises a heavy chain variable region that is the product of or derived from a murine V_H II gene H17 or a murine V_H II region VH105 gene (which genes include the nucleotide sequences set forth in SEQ ID NO: 126 and 127 respectively);
comprises a light chain variable region that is the product of or derived from a murine V_K 8-30 gene (which gene includes the nucleotide sequences set forth in SEQ ID NOs: 130, 131 and 132); and
specifically binds to Cadherin-17, preferably human Cadherin-17.

[0132] Examples of an antibody having V_H of V_H II gene H17 or V_H II region VH105 and V_K of V_K 8-30 is PTA001_A4.

[0133] In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:
comprises a heavy chain variable region that is the product of or derived from a murine V_H II gene H17 or a murine V_H II region VH105 gene (which genes include the nucleotide sequences set forth in SEQ ID NO: 126 and 127 respectively);
comprises a light chain variable region that is the product of or derived from a murine V_K 24-140 gene (which gene includes the nucleotide sequences set forth in SEQ ID NOs: 133, 134 and 135); and
specifically binds to Cadherin-17, preferably human Cadherin-17. Examples of antibodies

having V_H of V_H II gene H17 or V_H II region VH105 and V_K of V_K 24-140 are PTA001_A2, PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14.

[0134] As used herein, an antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses murine germline immunoglobulin genes. Such systems include screening a murine immunoglobulin gene library displayed on phage with the antigen of interest. An antibody that is "the product of" or "derived from" a murine germline immunoglobulin sequence can be identified as such by comparing the nucleotide or amino acid sequence of the antibody to the nucleotide or amino acid sequences of murine germline immunoglobulins and selecting the murine germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the antibody. An antibody that is "the product of" or "derived from" a particular murine germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a murine germline immunoglobulin gene and contains amino acid residues that identify the antibody as being murine when compared to the germline immunoglobulin amino acid sequences of other species (e.g., human germline sequences). In certain cases, an antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, an antibody derived from a particular murine germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the murine germline immunoglobulin gene. In certain cases, the antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Homologous Antibodies

[0135] In yet another embodiment, an antibody of the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-Cadherin-17 antibodies of the invention.

For example, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

the heavy chain variable region comprises an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs:35-46;

the light chain variable region comprises an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs:47-58; and

the antibody binds to human Cadherin-17. Such antibodies may bind to human Cadherin-17 with an EC_{50} of 50 nM or less, 10 nM or less, 1 nM or less, 100 pM or less, or more preferably 10 pM or less.

[0136] The antibody may also bind to CHO cells transfected with human Cadherin-17.

[0137] In various embodiments, the antibody can be, for example, a human antibody, a humanized antibody or a chimeric antibody.

[0138] In other embodiments, the V_H and/or V_K amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_K regions having high (i.e., 80% or greater) identical to the V_H and V_K regions of the sequences set forth above, can be obtained by mutagenesis (e.g., site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs:59-83 followed by testing of the encoded altered antibody for retained function using the functional assays described herein.

[0139] The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[0140] The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0141] Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

Antibodies with Conservative Modifications

[0142] In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein (*e.g.*, PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-Cadherin-17 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:14-21, and conservative modifications thereof;

the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NOs:32-34, and conservative modifications thereof; and

the antibody binds to human Cadherin-17. Such antibodies may bind to human Cadherin-17 with an EC_{50} of 50 nM or less, 10 nM or less, 1 nM or less, 100 pM or less, or more preferably 10 pM or less.

[0143] The antibody may also bind to CHO cells transfected with human Cadherin-17.

[0144] In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:5-13, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:28-31, and conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:1-4, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:22-27, and conservative modifications thereof.

[0145] In various embodiments, the antibody can be, for example, human antibodies, humanized antibodies or chimeric antibodies.

[0146] As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function using the functional assays described herein.

[0147] The heavy chain CDR1 sequence of SEQ ID NO:1-4 may comprise one or more conservative sequence modification, such as one, two, three, four, five or more amino acid substitutions, additions or deletions; the light chain CDR1 sequence of SEQ ID NO:22-27 may comprise one or more conservative sequence modification, such as one, two, three, four,

five or more amino acid substitutions, additions or deletions; the heavy chain CDR2 sequence shown in SEQ ID NO:5-13 may comprise one or more conservative sequence modification, such as one, two, three, four, five or more amino acid substitutions, additions or deletions; the light chain CDR2 sequence shown in SEQ ID NO:28-31 may comprise one or more conservative sequence modification, such as one, two, three, four, five or more amino acid substitutions, additions or deletions; the heavy chain CDR3 sequence shown in SEQ ID NO:14-21 may comprise one or more conservative sequence modification, such as one, two, three, four, five or more amino acid substitutions, additions or deletions; and/or the light chain CDR3 sequence shown in SEQ ID NO:32-34 may comprise one or more conservative sequence modification, such as one, two, three, four, five or more amino acid substitutions, additions or deletions.

Antibodies that Bind to the Same Epitope as Anti-Cadherin-17 Antibodies of the Invention

[0148] In another embodiment, the invention provides antibodies that bind to the same epitope on human Cadherin-17 as any of the Cadherin-17 monoclonal antibodies of the invention (*i.e.*, antibodies that have the ability to cross-compete for binding to Cadherin-17 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody PTA001_A1 (having V_H and V_K sequences as shown in SEQ ID NOs:35 and 47, respectively), the monoclonal antibody PTA001_A2 (having V_H and V_K sequences as shown in SEQ ID NOs:36 and 48 respectively), the monoclonal antibody PTA001_A3 (having V_H and V_K sequences as shown in SEQ ID NOs:37 and 48 respectively), the monoclonal antibody PTA001_A4 (having V_H and V_K sequences as shown in SEQ ID NOs:38 and 49 respectively), the monoclonal antibody PTA001_A5 (having V_H and V_K sequences as shown in SEQ ID NOs:39 and 50 respectively), the monoclonal antibody PTA001_A6 (having V_H and V_K sequences as shown in SEQ ID NOs:40 and 51 respectively), the monoclonal antibody PTA001_A7 (having V_H and V_K sequences as shown in SEQ ID NOs:41 and 52 respectively), the monoclonal antibody PTA001_A8 (having V_H and V_K sequences as shown in SEQ ID NOs:42 and 53 respectively), the monoclonal antibody PTA001_A9 (having V_H and V_K sequences as shown in SEQ ID NOs:40 and 54 respectively), the monoclonal antibody PTA001_A10 (having V_H and V_K sequences as shown in SEQ ID NOs:40 and 55 respectively), the monoclonal antibody PTA001_A11 (having V_H and V_K sequences as shown in SEQ ID NOs:43 and 56 respectively), the monoclonal antibody PTA001_A12 (having V_H and V_K sequences as shown in SEQ ID NOs:44 and 55 respectively), the monoclonal antibody PTA001_A13 (having V_H and V_K sequences as shown in SEQ ID

NOs:45 and 57 respectively) or the monoclonal antibody PTA001_A14 (having V_H and V_K sequences as shown in SEQ ID NOs:46 and 58 respectively). Such cross-competing antibodies can be identified based on their ability to cross-compete with PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14 in standard Cadherin-17 binding assays. For example, BIAcore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14, to human Cadherin-17 demonstrates that the test antibody can compete with PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14 for binding to human Cadherin-17 and thus binds to the same epitope on human Cadherin-17 as PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14.

Engineered and Modified Antibodies

[0149] An antibody of the invention further can be prepared using an antibody having one or more of the V_H and/or V_L sequences disclosed herein which can be used as starting material to engineer a modified antibody, which modified antibody may have altered properties as compared to the starting antibody. An antibody can be engineered by modifying one or more amino acids within one or both variable regions (i.e., V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

[0150] In certain embodiments, CDR grafting can be used to engineer variable regions of antibodies. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring

antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; Queen, C. *et al.* (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*)

[0151] Accordingly, another embodiment of the invention pertains to an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-4, SEQ ID NOs:5-13, and SEQ ID NOs:14-21, respectively, and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:22-27, SEQ ID NOs:28-31, and SEQ ID NOs:32-34, respectively. Thus, such antibodies contain the V_H and V_K CDR sequences of monoclonal antibodies PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14 yet may contain different framework sequences from these antibodies.

[0152] Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for murine heavy and light chain variable region genes can be found in the IMGT (international ImMunoGeneTics) murine germline sequence database (available on the Internet at imgt.cines.fr/), as well as in Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; the contents of each of which are expressly incorporated herein by reference. As another example, the germline DNA sequences for murine heavy and light chain variable region genes can be found in the Genbank database.

[0153] Antibody protein sequences are compared against a compiled protein sequence database using one of the sequence similarity searching methods called the Gapped BLAST (Altschul *et al.* (1997) *Nucleic Acids Research* 25:3389-3402), which is well known to those skilled in the art. BLAST is a heuristic algorithm in that a statistically significant alignment between the antibody sequence and the database sequence is likely to contain high-scoring segment pairs (HSP) of aligned words. Segment pairs whose scores cannot be improved by extension or trimming is called a *hit*. Briefly, the nucleotide sequences in the database are translated and the region between and including FR1 through FR3 framework region is retained. The database sequences have an average length of 98 residues. Duplicate sequences

which are exact matches over the entire length of the protein are removed. A BLAST search for proteins using the program blastp with default, standard parameters except the low complexity filter, which is turned off, and the substitution matrix of BLOSUM62, filters for top 5 hits yielding sequence matches. The nucleotide sequences are translated in all six frames and the frame with no stop codons in the matching segment of the database sequence is considered the potential hit. This is in turn confirmed using the BLAST program tblastx, which translates the antibody sequence in all six frames and compares those translations to the nucleotide sequences in the database dynamically translated in all six frames.

[0154] The identities are exact amino acid matches between the antibody sequence and the protein database over the entire length of the sequence. The positives (identities + substitution match) are not identical but amino acid substitutions guided by the BLOSUM62 substitution matrix. If the antibody sequence matches two of the database sequences with same identity, the hit with most positives would be decided to be the matching sequence hit.

[0155] Preferred framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, e.g., similar to the V_H 7-39 framework sequence, the V_H II gene H17 framework sequence, the V_H II region VH105 framework sequence, the V_K 1-110 framework sequence, the V_K 8-30 framework sequence and/or the V_K 24-140 framework sequences used by preferred monoclonal antibodies of the invention. The V_H CDR1, CDR2, and CDR3 sequences, and the V_K CDR1, CDR2, and CDR3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see e.g., U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

[0156] Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_K CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (e.g., affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. In some embodiments, conservative modifications (as discussed above) are introduced. Alternatively, non-

conservative modifications can be made. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered, although as will be appreciated by those in the art, variants in other areas (framework regions for example) can be greater.

[0157] Accordingly, in another embodiment, the instant disclosure provides isolated anti-Cadherin-17 monoclonal antibodies, or antigen binding portions thereof, comprising a heavy chain variable region comprising: (a) a V_H CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-4, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:1-4; (b) a V_H CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:5-13, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:5-13; (c) a V_H CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14-21, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:14-21; (d) a V_K CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:22-27, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:22-27; (e) a V_K CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:28-31, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:28-31; and (f) a V_K CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:32-34, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:32-34.

[0158] Engineered antibodies of the invention include those in which modifications have been made to framework residues within V_H and/or V_K, e.g. to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

[0159] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 2003/0153043 by Carr *et al.*

[0160] In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

[0161] In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[0162] In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcal protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

[0163] In another embodiment, the antibody is modified to increase its biological half life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or C_L region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

[0164] In another embodiment, the antibody is produced as a UniBody as described in WO2007/059782 which is incorporated herein by reference in its entirety.

[0165] In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

[0166] In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent No. 6,194,551 by Idusogie *et al.*

[0167] In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.*

[0168] In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A. Further ADCC variants are described for example in WO2006/019447.

[0169] In yet another example, the Fc region is modified to increase the half-life of the antibody, generally by increasing binding to the FcRn receptor, as described for example in PCT/US2008/088053, US 7,371,826, US 7,670,600 and WO 97/34631.

[0170] In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*, and can be accomplished by removing the asparagine at position 297.

[0171] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. This is sometimes referred to in the art as a "engineered glycoform". Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can generally be accomplished in two ways; for example, in some embodiments, the antibody is expressed in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. Reference is made to the POTELLIGENT® technology. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 $FUT8^{-/-}$ cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 2004/0110704 by Yamane *et al.*, US Patent No. 7,517,670 and Yamane-Ohnuki *et al.* (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme. Hanai *et al.* also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also

resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A.L. *et al.* (1975) *Biochem.* 14:5516-23).

[0172] Alternatively, engineered glycoforms, particularly afucosylation, can be done using small molecule inhibitors of glycosylation pathway enzymes. See for example Rothman et al., Mol. Immunol. 26(12):113-1123 (1989); Elbein, FASEB J. 5:3055 (1991); PCT/US2009/042610 and US Patent No. 7,700,321.

[0173] Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

[0174] In additional embodiments, for example in the use of the antibodies of the invention for diagnostic or detection purposes, the antibodies may comprise a label. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic, electrical, thermal; and c) colored or luminescent dyes; although labels include enzymes and particles such as magnetic particles as well. Preferred labels include, but are not limited to, fluorescent

lanthanide complexes (including those of Europium and Terbium), and fluorescent labels including, but not limited to, quantum dots, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue, Texas Red, the Alexa dyes, the Cy dyes, and others described in the 6th Edition of the Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

Antibody Physical Properties

[0175] The antibodies of the present invention may be further characterized by the various physical properties of the anti-Cadherin-17 antibodies. Various assays may be used to detect and/or differentiate different classes of antibodies based on these physical properties.

[0176] In some embodiments, antibodies of the present invention may contain one or more glycosylation sites in either the light or heavy chain variable region. The presence of one or more glycosylation sites in the variable region may result in increased immunogenicity of the antibody or an alteration of the pK of the antibody due to altered antigen binding (Marshall *et al* (1972) *Annu Rev Biochem* 41:673-702; Gala FA and Morrison SL (2004) *J Immunol* 172:5489-94; Wallick *et al* (1988) *J Exp Med* 168:1099-109; Spiro RG (2002) *Glycobiology* 12:43R-56R; Parekh *et al* (1985) *Nature* 316:452-7; Mimura *et al.* (2000) *Mol Immunol* 37:697-706). Glycosylation has been known to occur at motifs containing an N-X-S/T sequence. Variable region glycosylation may be tested using a Glycoblot assay, which cleaves the antibody to produce a Fab, and then tests for glycosylation using an assay that measures periodate oxidation and Schiff base formation. Alternatively, variable region glycosylation may be tested using Dionex light chromatography (Dionex-LC), which cleaves saccharides from a Fab into monosaccharides and analyzes the individual saccharide content. In some instances, it is preferred to have an anti-Cadherin-17 antibody that does not contain variable region glycosylation. This can be achieved either by selecting antibodies that do not contain the glycosylation motif in the variable region or by mutating residues within the glycosylation motif using standard techniques well known in the art.

[0177] In a preferred embodiment, the antibodies of the present invention do not contain asparagine isomerism sites. A deamidation or isoaspartic acid effect may occur on N-G or D-G sequences, respectively. The deamidation or isoaspartic acid effect results in the creation of isoaspartic acid which decreases the stability of an antibody by creating a kinked structure off a side chain carboxy terminus rather than the main chain. The creation of isoaspartic acid can be measured using an iso-quant assay, which uses a reverse-phase HPLC to test for isoaspartic acid.

[0178] Each antibody will have a unique isoelectric point (pI), but generally antibodies will fall in the pH range of between 6 and 9.5. The pI for an IgG1 antibody typically falls within the pH range of 7-9.5 and the pI for an IgG4 antibody typically falls within the pH range of 6-8. Antibodies may have a pI that is outside this range. Although the effects are generally unknown, there is speculation that antibodies with a pI outside the normal range may have some unfolding and instability under *in vivo* conditions. The isoelectric point may be tested using a capillary isoelectric focusing assay, which creates a pH gradient and may utilize laser focusing for increased accuracy (Janini *et al* (2002) *Electrophoresis* 23:1605-11; Ma *et al.* (2001) *Chromatographia* 53:S75-89; Hunt *et al* (1998) *J Chromatogr A* 800:355-67). In some instances, it is preferred to have an anti-Cadherin-17 antibody that contains a pI value that falls in the normal range. This can be achieved either by selecting antibodies with a pI in the normal range, or by mutating charged surface residues using standard techniques well known in the art.

[0179] Each antibody will have a melting temperature that is indicative of thermal stability (Krishnamurthy R and Manning MC (2002) *Curr Pharm Biotechnol* 3:361-71). A higher thermal stability indicates greater overall antibody stability *in vivo*. The melting point of an antibody may be measured using techniques such as differential scanning calorimetry (Chen *et al* (2003) *Pharm Res* 20:1952-60; Ghirlando *et al* (1999) *Immunol Lett* 68:47-52). T_{M1} indicates the temperature of the initial unfolding of the antibody. T_{M2} indicates the temperature of complete unfolding of the antibody. Generally, it is preferred that the T_{M1} of an antibody of the present invention is greater than 60°C, preferably greater than 65°C, even more preferably greater than 70°C. Alternatively, the thermal stability of an antibody may be measure using circular dichroism (Murray *et al.* (2002) *J. Chromatogr Sci* 40:343-9).

[0180] In a preferred embodiment, antibodies are selected that do not rapidly degrade. Fragmentation of an anti-Cadherin-17 antibody may be measured using capillary electrophoresis (CE) and MALDI-MS, as is well understood in the art (Alexander AJ and Hughes DE (1995) *Anal Chem* 67:3626-32).

[0181] In another preferred embodiment, antibodies are selected that have minimal aggregation effects. Aggregation may lead to triggering of an unwanted immune response and/or altered or unfavorable pharmacokinetic properties. Generally, antibodies are acceptable with aggregation of 25% or less, preferably 20% or less, even more preferably 15% or less, even more preferably 10% or less and even more preferably 5% or less. Aggregation may be measured by several techniques well known in the art, including size-

exclusion column (SEC) high performance liquid chromatography (HPLC), and light scattering to identify monomers, dimers, trimers or multimers.

Methods of Engineering Antibodies

[0182] As discussed above, the anti-Cadherin-17 antibodies having V_H and V_K sequences disclosed herein can be used to create new anti-Cadherin-17 antibodies by modifying the V_H and/or V_K sequences, or the constant region(s) attached thereto. Thus, in another aspect of the invention, the structural features of an anti-Cadherin-17 antibody of the invention, e.g. PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14, are used to create structurally related anti-Cadherin-17 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to human Cadherin-17. For example, one or more CDR regions of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14, or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-Cadherin-17 antibodies of the invention, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is not necessary to actually prepare (i.e., express as a protein) an antibody having one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

[0183] Accordingly, in another embodiment, the invention provides a method for preparing an anti-Cadherin-17 antibody comprising:

providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs:1-4, a CDR2 sequence selected from the group consisting of SEQ ID NOs:5-13, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs:14-21; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs:22-27, a CDR2 sequence selected from the group consisting of SEQ ID NOs:28-31, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs:32-34;

altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

expressing the altered antibody sequence as a protein.

[0184] Standard molecular biology techniques can be used to prepare and express the altered antibody sequence.

[0185] Preferably, the antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-Cadherin-17 antibodies described herein, which functional properties include, but are not limited to:

binds to human Cadherin-17 with a K_D of $1x10^{-7}$ M or less;

binds to human CHO cells transfected with Cadherin-17.

[0186] The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples (e.g., flow cytometry, binding assays).

[0187] In certain embodiments of the methods of engineering antibodies of the invention, mutations can be introduced randomly or selectively along all or part of an anti-Cadherin-17 antibody coding sequence and the resulting modified anti-Cadherin-17 antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar *et al.* describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

Nucleic Acid Molecules Encoding Antibodies of the Invention

[0188] Another aspect of the invention pertains to nucleic acid molecules that encode the antibodies of the invention. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule.

[0189] Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas, cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), nucleic acids encoding the antibody can be recovered from the library.

[0190] Preferred nucleic acids molecules of the invention are those encoding the V_H and V_K sequences of the PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14 monoclonal antibodies. DNA sequences encoding the V_H sequences of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs: 59-70. DNA sequences encoding the V_K sequences of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 are shown in SEQ ID NOs: 71-83.

[0191] Other preferred nucleic acids of the invention are nucleic acids having at least 80% sequence identity, such as at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity, with one of the sequences shown in SEQ ID NOs: 59-83, which nucleic acids encode an antibody of the invention, or an antigen-binding portion thereof.

[0192] The percent identity between two nucleic acid sequences is the number of positions in the sequence in which the nucleotide is identical, taking into account the number of gaps and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, such as the algorithm of Meyers and Miller or the XBLAST program of Altschul described above.

[0193] Still further, preferred nucleic acids of the invention comprise one or more CDR-encoding portions of the nucleic acid sequences shown in SEQ ID NOs:59-83. In this embodiment, the nucleic acid may encode the heavy chain CDR1, CDR2 and/or CDR3 sequence of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14 or the light chain CDR1, CDR2 and/or CDR3 sequence of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5,

PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14.

[0194] Nucleic acids which have at least 80%, such as at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity, with such a CDR-encoding portion of SEQ ID NO:59-83 (V_H and V_K seqs) are also preferred nucleic acids of the invention. Such nucleic acids may differ from the corresponding portion of SEQ ID NO:59-83 in a non-CDR coding region and/or in a CDR-coding region. Where the difference is in a CDR-coding region, the nucleic acid CDR region encoded by the nucleic acid typically comprises one or more conservative sequence modifications as defined herein compared to the corresponding CDR sequence of PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 or PTA001_A14.

[0195] Once DNA fragments encoding V_H and V_K segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a V_K- or V_H-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0196] The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of murine heavy chain constant region genes are known in the art (see e.g., Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0197] The isolated DNA encoding the V_L/V_K region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences

of murine light chain constant region genes are known in the art (see e.g., Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

[0198] To create a scFv gene, the V_H- and V_L / V_K-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L / V_K sequences can be expressed as a contiguous single-chain protein, with the V_L / V_K and V_H regions joined by the flexible linker (see e.g., Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies

[0199] According to the invention Cadherin-17 or a fragment or derivative thereof may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means. One skilled in the art will recognize that many procedures are available for the production of antibodies, for example, as described in Antibodies, A Laboratory Manual, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988), Cold Spring Harbor, N.Y. One skilled in the art will also appreciate that binding fragments or Fab fragments which mimic antibodies can also be prepared from genetic information by various procedures (Antibody Engineering: A Practical Approach (Borrebaeck, C., ed.), 1995, Oxford University Press, Oxford; J. Immunol. 149, 3914-3920 (1992)).

[0200] In one embodiment of the invention, antibodies to a specific domain of Cadherin-17 are produced. In a specific embodiment, hydrophilic fragments of Cadherin-17 are used as immunogens for antibody production.

[0201] In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of Cadherin-17, one may assay generated hybridomas for a product which binds to a Cadherin-17 fragment containing such domain. For selection of an antibody that specifically binds a first Cadherin-17 homolog but which does not specifically bind to (or binds less avidly to) a second Cadherin-17 homolog, one can select on the basis of positive binding to the first Cadherin-17 homolog and a lack of binding to (or reduced binding to) the second Cadherin-17 homolog. Similarly, for selection of an antibody that specifically binds Cadherin-17 but which does not

specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as Cadherin-17), one can select on the basis of positive binding to Cadherin-17 and a lack of binding to (or reduced binding to) the different isoform (e.g. a different glycoform). Thus, the present invention provides an antibody (such as a monoclonal antibody) that binds with greater affinity (for example at least 2-fold, such as at least 5-fold, particularly at least 10-fold greater affinity) to Cadherin-17 than to a different isoform or isoforms (*e.g.* glycoforms) of Cadherin-17.

[0202] Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to Cadherin-17, a fragment of Cadherin-17, a Cadherin-17-related polypeptide, or a fragment of a Cadherin-17-related polypeptide. For example, one way is to purify polypeptides of interest or to synthesize the polypeptides of interest using, e.g. solid phase peptide synthesis methods well known in the art. See, e.g. *Guide to Protein Purification*, Murray P. Deutcher, ed., *Meth. Enzymol.* Vol 182 (1990); Solid Phase Peptide Synthesis, Greg B. Fields ed., *Meth. Enzymol.* Vol 289 (1997); Kiso *et al., Chem. Pharm. Bull.* (Tokyo) 38: 1192-99, 1990; Mostafavi *et al., Biomed. Pept. Proteins Nucleic Acids* 1: 255-60, 1995; Fujiwara *et al., Chem. Pharm. Bull.* (Tokyo) 44: 1326-31, 1996. The selected polypeptides may then be used to immunize by injection various host animals, including but not limited to rabbits, mice, rats, etc., to generate polyclonal or monoclonal antibodies. Various adjuvants (i.e. immunostimulants) may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. Additional adjuvants are also well known in the art.

[0203] For preparation of monoclonal antibodies (mAbs) directed toward Cadherin-17, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies

may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the monoclonal antibodies may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

[0204] The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known.

[0205] The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (e.g. human-mouse chimeras).

[0206] Chimeric or humanized antibodies of the present invention can be prepared based on the sequence of a non-human monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the non-human hybridoma of interest and engineered to contain non-murine (e.g., human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, murine variable regions can be linked to human constant regions using methods known in the art (see e.g., U.S. Patent No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, murine CDR regions can be inserted into a human framework using methods known in the art (see e.g., U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

[0207] Completely human antibodies can be produced using transgenic or transchromosomic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g. all or a portion of Cadherin-17. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. These transgenic and transchromosomic mice include mice of the HuMAb Mouse® (Medarex ®, Inc.) and KM Mouse® strains. The HuMAb Mouse® strain (Medarex ®, Inc.) is described in Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing

such antibodies, see, *e.g.* U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. The KM mouse® strain refers to a mouse that carries a human heavy chain transgene and a human light chain transchromosome and is described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

[0208] Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-Cadherin-17 antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Amgen, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6,150,584 and 6,162,963 to Kucherlapati *et al.*

[0209] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection". In this approach a selected non-human monoclonal antibody, e.g. a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) Bio/technology 12:899-903).

[0210] Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-Cadherin-17 antibodies. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894) and PCT application No. WO2002/092812 and can be used to raise anti-Cadherin-17 antibodies.

[0211] Human monoclonal antibodies of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767 to Wilson *et al.*

[0212] The antibodies of the present invention can be generated by the use of phage display technology to produce and screen libraries of polypeptides for binding to a selected target. See, e.g. Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87, 6378-82, 1990; Devlin et al., *Science* 249, 404-6, 1990, Scott and Smith, *Science* 249, 386-88, 1990; and Ladner et al., U.S. Patent No. 5,571,698. A basic concept of phage display methods is the establishment of a physical association between DNA encoding a polypeptide to be screened and the polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome which encodes the polypeptide.

The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means. See, e.g. U.S. Patent No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims. In particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g. human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.* using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0213] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g. as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0214] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology* 203:46-88 (1991); Shu *et al.*, *PNAS* 90:7995-7999 (1993); and Skerra *et al.*, *Science* 240:1038-1040 (1988).

[0215] The invention provides functionally active fragments, derivatives or analogs of the anti-Cadherin-17 immunoglobulin molecules. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotype antibodies (i.e., tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a particular embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

[0216] The present invention provides antibody fragments such as, but not limited to, $F(ab')_2$ fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. $F(ab')_2$ fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (e.g. as described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra et al., 1988, Science 242:1038-1041).

[0217] In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g. a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to

the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

[0218] The immunoglobulins of the invention include analogs and derivatives that are modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the immunoglobulins include those that have been further modified, e.g. by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

Immunization of Mice

[0219] Mice can be immunized with a purified or enriched preparation of Cadherin-17 antigen and/or recombinant Cadherin-17, or cells expressing Cadherin-17. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or recombinant preparation (100 μg) of Cadherin-17 antigen can be used to immunize the mice intraperitoneally.

[0220] Cumulative experience with various antigens has shown that the mice respond when immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below) to test for satisfactory titres. Mice can be boosted intravenously with antigen on 3 consecutive days with sacrifice and removal of the spleen taking place 5 days later. In one embodiment, A/J mouse strains (Jackson Laboratories, Bar Harbor, Me.) may be used.

Generation of Transfectomas Producing Monoclonal Antibodies

[0221] Antibodies of the invention can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (e.g., Morrison, S. (1985) *Science* 229:1202).

[0222] For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular

biology techniques (e.g., PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used.

[0223] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0224] The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_K segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0225] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the

transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. *et al.* (1988) *Mol. Cell. Biol.* 8:466-472).

[0226] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[0227] For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and

secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

[0228] Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus (Foecking et al., 1986, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2), dhfr-CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) *J. Mol. Biol.* 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462 (to Wilson), WO 89/01036 (to Bebbington) and EP 338,841 (to Bebbington).

[0229] A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g. *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g. *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g. baculovirus) containing the antibody coding sequences; 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 antibody coding sequences; or mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g. metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0230] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the

expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0231] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (e.g. an adenovirus expression system) may be utilized.

[0232] As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g. glycosylation) and processing (e.g. cleavage) of protein products may be important for the function of the protein.

[0233] For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (e.g. neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0234] The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will

increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

[0235] When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g. ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

[0236] Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Characterization of Antibody Binding to Antigen

[0237] The antibodies that are generated by these methods may then be selected by first screening for affinity and specificity with the purified polypeptide of interest and, if required, comparing the results to the affinity and specificity of the antibodies with polypeptides that are desired to be excluded from binding. The antibodies can be tested for binding to Cadherin-17 by, for example, standard ELISA. The screening procedure can involve immobilization of the purified polypeptides in separate wells of microtiter plates. The solution containing a potential antibody or groups of antibodies is then placed into the respective microtiter wells and incubated for about 30 min to 2 h. The microtiter wells are then washed and a labeled secondary antibody (for example, an anti-mouse antibody conjugated to alkaline phosphatase if the raised antibodies are mouse antibodies) is added to

the wells and incubated for about 30 min and then washed. Substrate is added to the wells and a color reaction will appear where antibody to the immobilized polypeptide(s) is present.

[0238] The antibodies so identified may then be further analyzed for affinity and specificity in the assay design selected. In the development of immunoassays for a target protein, the purified target protein acts as a standard with which to judge the sensitivity and specificity of the immunoassay using the antibodies that have been selected. Because the binding affinity of various antibodies may differ; certain antibody pairs (e.g. in sandwich assays) may interfere with one another sterically, etc., assay performance of an antibody may be a more important measure than absolute affinity and specificity of an antibody.

[0239] Those skilled in the art will recognize that many approaches can be taken in producing antibodies or binding fragments and screening and selecting for affinity and specificity for the various polypeptides, but these approaches do not change the scope of the invention.

[0240] To determine if the selected anti-Cadherin-17 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using Cadherin-17 coated-ELISA plates. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

[0241] To determine the isotype of purified antibodies, isotype ELISAs can be performed using reagents specific for antibodies of a particular isotype.

[0242] Anti-Cadherin-17 antibodies can be further tested for reactivity with Cadherin-17 antigen by Western blotting. Briefly, Cadherin-17 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested.

[0243] The binding specificity of an antibody of the invention may also be determined by monitoring binding of the antibody to cells expressing Cadherin-17, for example by flow cytometry. Typically, a cell line, such as a CHO cell line, may be transfected with an expression vector encoding Cadherin-17. The transfected protein may comprise a tag, such as a myc-tag, preferably at the N-terminus, for detection using an antibody to the tag. Binding of an antibody of the invention to Cadherin-17 may be determined by incubating the transfected cells with the antibody, and detecting bound antibody. Binding of an antibody to the tag on the transfected protein may be used as a positive control.

[0244] The specificity of an antibody of the invention for Cadherin-17 may be further studied by determining whether or not the antibody binds to other proteins, such as another member of the Cadherin family using the same methods by which binding to Cadherin-17 is determined.

Immunoconjugates

[0245] In another aspect, the present invention features an anti-Cadherin-17 antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (e.g., an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (e.g., kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0246] Other preferred examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg®; American Home Products).

[0247] Cytotoxins can be conjugated to antibodies of the invention using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (e.g., cathepsins B, C, D).

[0248] Examples of cytotoxins are described, for example, in U.S. Patent Nos. 6,989,452, 7,087,600, and 7,129,261, and in PCT Application Nos. PCT/US2002/17210, PCT/US2005/017804, PCT/US2006/37793, PCT/US2006/060050, PCT/US2006/060711, WO2006/110476, and in U.S. Patent Application No. 60/891,028, all of which are incorporated herein by reference in their entirety. For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. et al. (2003) Adv. Drug Deliv. Rev. 55:199-215; Trail, P.A. et al. (2003) Cancer Immunol. Immunother. 52:328-337; Payne, G. (2003) Cancer Cell 3:207-212; Allen, T.M. (2002) Nat. Rev. Cancer 2:750-763; Pastan, I. and Kreitman, R. J. (2002) Curr. Opin. Investig. Drugs 3:1089-1091; Senter, P.D. and Springer, C.J. (2001) Adv. Drug Deliv. Rev. 53:247-264.

[0249] Antibodies of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine131, indium111, yttrium90 and lutetium177. Method for preparing radioimmunoconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin® (IDEC Pharmaceuticals) and Bexxar® (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention.

[0250] The antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0251] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery," in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in

Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., Immunol. Rev., 62:119-58 (1982).

Bispecific Molecules

[0252] In another aspect, the present invention features bispecific molecules comprising an anti-Cadherin-17 antibody, or a fragment thereof, of the invention. An antibody of the invention, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g., another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody of the invention may in fact be derivatized or linked to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of the invention, an antibody of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

[0253] Accordingly, the present invention includes bispecific molecules comprising at least one first binding specificity for Cadherin-17 and a second binding specificity for a second target epitope. In a particular embodiment of the invention, the second target epitope is an Fc receptor, e.g., human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, the invention includes bispecific molecules capable of binding both to FcγR or FcαR expressing effector cells (e.g., monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing Cadherin-17. These bispecific molecules target Cadherin-17 expressing cells to effector cell and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of Cadherin-17 expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

[0254] In an embodiment of the invention in which the bispecific molecule is multispecific, the molecule can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-Cadherin-17 binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, e.g., a molecule

which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, e.g., an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the Fc receptor or target cell antigen. The "anti-enhancement factor portion" can bind an Fc receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell (e.g. via CD2, CD3, CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

[0255] In one embodiment, the bispecific molecules of the invention comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, e.g., an Fab, Fab', $F(ab')_2$, Fv, Fd, dAb or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in U.S. Patent No. 4,946,778 to Ladner *et al.*, the contents of which is expressly incorporated by reference.

[0256] In one embodiment, the binding specificity for an Fcγ receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), Fcγ RII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor is a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - 10^9 M^{-1}).

[0257] The production and characterization of certain preferred anti-Fcγ monoclonal antibodies are described in PCT Publication WO 88/00052 and in U.S. Patent No. 4,954,617 to Fanger *et al.*, the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22

(H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) *J. Immunol* 155 (10): 4996-5002 and PCT Publication WO 94/10332 to Tempest *et al.*. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

[0258] In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, e.g., an Fc-alpha receptor (Fcα RI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (Fcα RI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7$ M^{-1}) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) *Critical Reviews in Immunology* 16:423-440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.* (1992) *J. Immunol.* 148:1764).

[0259] FcαRI and FcγRI are preferred trigger receptors for use in the bispecific molecules of the invention because they are (1) expressed primarily on immune effector cells, e.g., monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (e.g., 5,000-100,000 per cell); (3) mediators of cytotoxic activities (e.g., ADCC, phagocytosis); and (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

[0260] Antibodies which can be employed in the bispecific molecules of the invention are murine, human, chimeric and humanized monoclonal antibodies.

[0261] The bispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, e.g., the anti-FcR and anti-Cadherin-17 binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see e.g., Karpovsky *et al.* (1984) *J. Exp. Med.*

160:1686; Liu, MA *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described in Paulus (1985) Behring Ins. Mitt. No. 78, 118-132; Brennan *et al.* (1985) *Science* 229:81-83, and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

[0262] When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

[0263] Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific molecule of the invention can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific molecules are described for example in U.S. Patent Numbers 5,260,203; 5,455,030; 4,881,175; 5,132,405; 5,091,513; 5,476,786; 5,013,653; 5,258,498; and 5,482,858, all of which are expressly incorporated herein by reference.

[0264] Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (e.g., growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using e.g., an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γcounter or a scintillation counter or by autoradiography.

Antibody Fragments and Antibody Mimetics

[0265] The instant invention is not limited to traditional antibodies and may be practiced through the use of antibody fragments and antibody mimetics. As detailed below, a wide variety of antibody fragment and antibody mimetic technologies have now been developed and are widely known in the art. While a number of these technologies, such as domain antibodies, Nanobodies, and UniBodies make use of fragments of, or other modifications to, traditional antibody structures, there are also alternative technologies, such as Affibodies, DARPins, Anticalins, Avimers, and Versabodies that employ binding structures that, while they mimic traditional antibody binding, are generated from and function via distinct mechanisms.

[0266] Domain Antibodies (dAbs) are the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy (V_H) or light (V_L) chains of human antibodies. Domain Antibodies have a molecular weight of approximately 13 kDa. Domantis has developed a series of large and highly functional libraries of fully human V_H and V_L dAbs (more than ten billion different sequences in each library), and uses these libraries to select dAbs that are specific to therapeutic targets. In contrast to many conventional antibodies, Domain Antibodies are well expressed in bacterial, yeast, and mammalian cell systems. Further details of domain antibodies and methods of production thereof may be obtained by reference to US Patent Nos 6,291,158; 6,582,915; 6,593,081; 6,172,197; 6,696,245; US Serial No. 2004/0110941; European patent application No. 1433846 and European Patents 0368684 & 0616640; WO05/035572, WO04/101790, WO04/081026, WO04/058821, WO04/003019 and WO03/002609, each of which is herein incorporated by reference in its entirety.

[0267] Nanobodies are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3). Importantly, the cloned and isolated VHH domain is a perfectly stable polypeptide harboring the full antigen-binding capacity of the original heavy-chain antibody. Nanobodies have a high homology with the VH domains of human antibodies and can be further humanized without any loss of activity. Importantly, Nanobodies have a low immunogenic potential, which has been confirmed in primate studies with Nanobody lead compounds.

[0268] Nanobodies combine the advantages of conventional antibodies with important features of small molecule drugs. Like conventional antibodies, Nanobodies show high target

specificity, high affinity for their target and low inherent toxicity. However, like small molecule drugs they can inhibit enzymes and readily access receptor clefts. Furthermore, Nanobodies are extremely stable, can be administered by means other than injection (see e.g. WO 04/041867, which is herein incorporated by reference in its entirety) and are easy to manufacture. Other advantages of Nanobodies include recognizing uncommon or hidden epitopes as a result of their small size, binding into cavities or active sites of protein targets with high affinity and selectivity due to their unique 3-dimensional, drug format flexibility, tailoring of half-life and ease and speed of drug discovery.

[0269] Nanobodies are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts e.g. E. coli (see e.g. US 6,765,087, which is herein incorporated by reference in its entirety), molds (for example Aspergillus or Trichoderma) and yeast (for example Saccharomyces, Kluyveromyces, Hansenula or Pichia) (see e.g. US 6,838,254, which is herein incorporated by reference in its entirety). The production process is scalable and multi-kilogram quantities of Nanobodies have been produced. Because Nanobodies exhibit a superior stability compared with conventional antibodies, they can be formulated as a long shelf-life, ready-to-use solution.

[0270] The Nanoclone method (see e.g. WO 06/079372, which is herein incorporated by reference in its entirety) is a proprietary method for generating Nanobodies against a desired target, based on automated high-throughout selection of B-cells and could be used in the context of the instant invention.

[0271] UniBodies are another antibody fragment technology; however this one is based upon the removal of the hinge region of IgG4 antibodies. The deletion of the hinge region results in a molecule that is essentially half the size of traditional IgG4 antibodies and has a univalent binding region rather than the bivalent binding region of IgG4 antibodies. It is also well known that IgG4 antibodies are inert and thus do not interact with the immune system, which may be advantageous for the treatment of diseases where an immune response is not desired, and this advantage is passed onto UniBodies. For example, UniBodies may function to inhibit or silence, but not kill, the cells to which they are bound. Additionally, UniBody binding to cancer cells do not stimulate them to proliferate. Furthermore, because UniBodies are about half the size of traditional IgG4 antibodies, they may show better distribution over larger solid tumors with potentially advantageous efficacy. UniBodies are cleared from the body at a similar rate to whole IgG4 antibodies and are able to bind with a similar affinity for their antigens as whole antibodies. Further details of UniBodies may be obtained by

reference to patent application WO2007/059782, which is herein incorporated by reference in its entirety.

[0272] Affibody molecules represent a new class of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG-binding domains of staphylococcal protein A. This three helix bundle domain has been used as a scaffold for the construction of combinatorial phagemid libraries, from which Affibody variants that target the desired molecules can be selected using phage display technology (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA, Binding proteins selected from combinatorial libraries of an α-helical bacterial receptor domain, Nat Biotechnol 1997;15:772-7. Ronmark J, Gronlund H, Uhlen M, Nygren PA, Human immunoglobulin A (IgA)-specific ligands from combinatorial engineering of protein A, Eur J Biochem 2002;269:2647-55.). The simple, robust structure of Affibody molecules in combination with their low molecular weight (6 kDa), make them suitable for a wide variety of applications, for instance, as detection reagents (Ronmark J, Hansson M, Nguyen T, et al, Construction and characterization of affibody-Fc chimeras produced in Escherichia coli, J Immunol Methods 2002;261:199-211) and to inhibit receptor interactions (Sandstorm K, Xu Z, Forsberg G, Nygren PA, Inhibition of the CD28-CD80 co-stimulation signal by a CD28-binding Affibody ligand developed by combinatorial protein engineering, Protein Eng 2003;16:691-7). Further details of Affibodies and methods of production thereof may be obtained by reference to US Patent No 5831012 which is herein incorporated by reference in its entirety.

[0273] Labelled Affibodies may also be useful in imaging applications for determining abundance of Isoforms.

[0274] DARPins (Designed Ankyrin Repeat Proteins) are one example of an antibody mimetic DRP (Designed Repeat Protein) technology that has been developed to exploit the binding abilities of non-antibody polypeptides. Repeat proteins such as ankyrin or leucine-rich repeat proteins, are ubiquitous binding molecules, which occur, unlike antibodies, intra- and extracellularly. Their unique modular architecture features repeating structural units (repeats), which stack together to form elongated repeat domains displaying variable and modular target-binding surfaces. Based on this modularity, combinatorial libraries of polypeptides with highly diversified binding specificities can be generated. This strategy includes the consensus design of self-compatible repeats displaying variable surface residues and their random assembly into repeat domains.

[0275] DARPins can be produced in bacterial expression systems at very high yields and they belong to the most stable proteins known. Highly specific, high-affinity DARPins to a

broad range of target proteins, including human receptors, cytokines, kinases, human proteases, viruses and membrane proteins, have been selected. DARPins having affinities in the single-digit nanomolar to picomolar range can be obtained.

[0276] DARPins have been used in a wide range of applications, including ELISA, sandwich ELISA, flow cytometric analysis (FACS), immunohistochemistry (IHC), chip applications, affinity purification or Western blotting. DARPins also proved to be highly active in the intracellular compartment for example as intracellular marker proteins fused to green fluorescent protein (GFP). DARPins were further used to inhibit viral entry with IC50 in the pM range. DARPins are not only ideal to block protein-protein interactions, but also to inhibit enzymes. Proteases, kinases and transporters have been successfully inhibited, most often an allosteric inhibition mode. Very fast and specific enrichments on the tumor and very favorable tumor to blood ratios make DARPins well suited for in vivo diagnostics or therapeutic approaches.

[0277] Additional information regarding DARPins and other DRP technologies can be found in US Patent Application Publication No. 2004/0132028, and International Patent Application Publication No. WO 02/20565, both of which are hereby incorporated by reference in their entirety.

[0278] Anticalins are an additional antibody mimetic technology, however in this case the binding specificity is derived from lipocalins, a family of low molecular weight proteins that are naturally and abundantly expressed in human tissues and body fluids. Lipocalins have evolved to perform a range of functions in vivo associated with the physiological transport and storage of chemically sensitive or insoluble compounds. Lipocalins have a robust intrinsic structure comprising a highly conserved ß-barrel which supports four loops at one terminus of the protein. These loops form the entrance to a binding pocket and conformational differences in this part of the molecule account for the variation in binding specificity between individual lipocalins.

[0279] While the overall structure of hypervariable loops supported by a conserved ß-sheet framework is reminiscent of immunoglobulins, lipocalins differ considerably from antibodies in terms of size, being composed of a single polypeptide chain of 160-180 amino acids which is marginally larger than a single immunoglobulin domain.

[0280] Lipocalins are cloned and their loops are subjected to engineering in order to create Anticalins. Libraries of structurally diverse Anticalins have been generated and Anticalin display allows the selection and screening of binding function, followed by the expression and production of soluble protein for further analysis in prokaryotic or eukaryotic systems.

Studies have successfully demonstrated that Anticalins can be developed that are specific for virtually any human target protein can be isolated and binding affinities in the nanomolar or higher range can be obtained.

[0281] Anticalins can also be formatted as dual targeting proteins, so-called Duocalins. A Duocalin binds two separate therapeutic targets in one easily produced monomeric protein using standard manufacturing processes while retaining target specificity and affinity regardless of the structural orientation of its two binding domains.

[0282] Modulation of multiple targets through a single molecule is particularly advantageous in diseases known to involve more than a single causative factor. Moreover, bi- or multivalent binding formats such as Duocalins have significant potential in targeting cell surface molecules in disease, mediating agonistic effects on signal transduction pathways or inducing enhanced internalization effects via binding and clustering of cell surface receptors. Furthermore, the high intrinsic stability of Duocalins is comparable to monomeric Anticalins, offering flexible formulation and delivery potential for Duocalins.

[0283] Additional information regarding Anticalins can be found in US Patent No. 7,250,297 and International Patent Application Publication No. WO 99/16873, both of which are hereby incorporated by reference in their entirety.

[0284] Another antibody mimetic technology useful in the context of the instant invention are Avimers. Avimers are evolved from a large family of human extracellular receptor domains by in vitro exon shuffling and phage display, generating multidomain proteins with binding and inhibitory properties. Linking multiple independent binding domains has been shown to create avidity and results in improved affinity and specificity compared with conventional single-epitope binding proteins. Other potential advantages include simple and efficient production of multitarget-specific molecules in Escherichia coli, improved thermostability and resistance to proteases. Avimers with sub-nanomolar affinities have been obtained against a variety of targets.

[0285] Additional information regarding Avimers can be found in US Patent Application Publication Nos. 2006/0286603, 2006/0234299, 2006/0223114, 2006/0177831, 2006/0008844, 2005/0221384, 2005/0164301, 2005/0089932, 2005/0053973, 2005/0048512, 2004/0175756, all of which are hereby incorporated by reference in their entirety.

[0286] Versabodies are another antibody mimetic technology that could be used in the context of the instant invention. Versabodies are small proteins of 3-5 kDa with >15% cysteines, which form a high disulfide density scaffold, replacing the hydrophobic core that typical proteins have. The replacement of a large number of hydrophobic amino acids,

comprising the hydrophobic core, with a small number of disulfides results in a protein that is smaller, more hydrophilic (less aggregation and non-specific binding), more resistant to proteases and heat, and has a lower density of T-cell epitopes, because the residues that contribute most to MHC presentation are hydrophobic. All four of these properties are well-known to affect immunogenicity, and together they are expected to cause a large decrease in immunogenicity.

[0287] The inspiration for Versabodies comes from the natural injectable biopharmaceuticals produced by leeches, snakes, spiders, scorpions, snails, and anemones, which are known to exhibit unexpectedly low immunogenicity. Starting with selected natural protein families, by design and by screening the size, hydrophobicity, proteolytic antigen processing, and epitope density are minimized to levels far below the average for natural injectable proteins.

[0288] Given the structure of Versabodies, these antibody mimetics offer a versatile format that includes multi-valency, multi-specificity, a diversity of half-life mechanisms, tissue targeting modules and the absence of the antibody Fc region. Furthermore, Versabodies are manufactured in E. coli at high yields, and because of their hydrophilicity and small size, Versabodies are highly soluble and can be formulated to high concentrations. Versabodies are exceptionally heat stable (they can be boiled) and offer extended shelf-life.

[0289] Additional information regarding Versabodies can be found in US Patent Application Publication No. 2007/0191272 which is hereby incorporated by reference in its entirety.

[0290] The detailed description of antibody fragment and antibody mimetic technologies provided above is not intended to be a comprehensive list of all technologies that could be used in the context of the instant specification. For example, and also not by way of limitation, a variety of additional technologies including alternative polypeptide-based technologies, such as fusions of complimentary determining regions as outlined in Qui *et al.*, Nature Biotechnology, 25(8) 921-929 (2007), which is hereby incorporated by reference in its entirety, as well as nucleic acid-based technologies, such as the RNA aptamer technologies described in US Patent Nos. 5,789,157, 5,864,026, 5,712,375, 5,763,566, 6,013,443, 6,376,474, 6,613,526, 6,114,120, 6,261,774, and 6,387,620, all of which are hereby incorporated by reference, could be used in the context of the instant invention.

Pharmaceutical Compositions

[0291] In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or

antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (e.g., two or more different) antibodies, or immunoconjugates or bispecific molecules of the invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

[0292] Pharmaceutical compositions of the invention also can be administered in combination therapy, i.e., combined with other agents. For example, the combination therapy can include an anti-Cadherin-17 antibody of the present invention combined with at least one other anti-tumor agent, or an anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

[0293] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., antibody, immunoconjugate, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[0294] The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0295] A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0296] Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0297] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0298] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0299] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol

(for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0300] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0301] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

[0302] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in

association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0303] For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-Cadherin-17 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

[0304] In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

[0305] Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or

complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0306] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0307] A "therapeutically effective dosage" of an anti-Cadherin-17 antibody of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of Cadherin-17^{+} tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit cell growth, such inhibition can be measured *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0308] A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The

phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[0309] Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0310] The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0311] Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

[0312] In certain embodiments, the monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of

the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods

[0313] The antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* diagnostic and therapeutic utilities involving the diagnosis and treatment of Cadherin-17 mediated disorders.

[0314] In some embodiments, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, e.g., *in vivo*, to treat, prevent and to diagnose a variety of disorders. As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles. Preferred subjects include human patients having disorders mediated by Cadherin-17 activity. The methods are particularly suitable for treating human patients having a disorder associated with aberrant Cadherin-17 expression. When antibodies to Cadherin-17 are administered together with another agent, the two can be administered in either order or simultaneously.

[0315] Given the specific binding of the antibodies of the invention for Cadherin-17, the antibodies of the invention can be used to specifically detect Cadherin-17 expression on the surface of cells and, moreover, can be used to purify Cadherin-17 via immunoaffinity purification.

[0316] Furthermore, given the expression of Cadherin-17 on tumor cells, the antibodies, antibody compositions and methods of the present invention can be used to treat a subject with a tumorigenic disorder, e.g., a disorder characterized by the presence of tumor cells expressing Cadherin-17 including, for example, colorectal cancer. Cadherin-17 has been demonstrated to be internalised on antibody binding as illustrated in Example 10 below, thus

enabling the antibodies of the invention to be used in any payload mechanism of action e.g. an ADC approach, radio immuno conjugate, or ADEPT approach.

[0317] In one embodiment, the antibodies (e.g., monoclonal antibodies, multispecific and bispecific molecules and compositions) of the invention can be used to detect levels of Cadherin-17, or levels of cells which contain Cadherin-17 on their membrane surface, which levels can then be linked to certain disease symptoms. Alternatively, the antibodies can be used to inhibit or block Cadherin-17 function which, in turn, can be linked to the prevention or amelioration of certain disease symptoms, thereby implicating Cadherin-17 as a mediator of the disease. This can be achieved by contacting a sample and a control sample with the anti-Cadherin-17 antibody under conditions that allow for the formation of a complex between the antibody and Cadherin-17. Any complexes formed between the antibody and Cadherin-17 are detected and compared in the sample and the control.

[0318] In another embodiment, the antibodies (e.g., monoclonal antibodies, multispecific and bispecific molecules and compositions) of the invention can be initially tested for binding activity associated with therapeutic or diagnostic use *in vitro*. For example, compositions of the invention can be tested using the flow cytometric assays described in the Examples below.

[0319] The antibodies (*e.g.*, monoclonal antibodies, multispecific and bispecific molecules, immunoconjugates and compositions) of the invention have additional utility in therapy and diagnosis of Cadherin-17 related diseases. For example, the monoclonal antibodies, the multispecific or bispecific molecules and the immunoconjugates can be used to elicit *in vivo* or *in vitro* one or more of the following biological activities: to inhibit the growth of and/or kill a cell expressing Cadherin-17; to mediate phagocytosis or ADCC of a cell expressing Cadherin-17 in the presence of human effector cells, or to block Cadherin-17 ligand binding to Cadherin-17.

[0320] In a particular embodiment, the antibodies (e.g., monoclonal antibodies, multispecific and bispecific molecules and compositions) are used *in vivo* to treat, prevent or diagnose a variety of Cadherin-17-related diseases. Examples of Cadherin-17-related diseases include, among others, human cancer tissues representing colorectal cancer.

[0321] Suitable routes of administering the antibody compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (e.g., intravenous or

subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

[0322] As previously described, anti-Cadherin-17 antibodies of the invention can be co-administered with one or other more therapeutic agents, e.g., a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, e.g., an anti-cancer therapy, e.g., radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/kg dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Other agents suitable for co-administration with the antibodies of the invention include other agents used for the treatment of cancers, e.g. pancreatic or colorectal cancer, such as Avastin®, 5FU and gemcitabine. Co-administration of the anti-Cadherin-17 antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

[0323] Target-specific effector cells, e.g., effector cells linked to compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules) of the invention can also be used as therapeutic agents. Effector cells for targeting can be human leukocytes such as macrophages, neutrophils or monocytes. Other cells include eosinophils, natural killer cells and other IgG- or IgA-receptor bearing cells. If desired, effector cells can be obtained from the subject to be treated. The target-specific effector cells can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^8-10^9 but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell, e.g., a tumor cell expressing Cadherin-17, and to affect cell killing by, e.g., phagocytosis. Routes of administration can also vary.

[0324] Therapy with target-specific effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy using the

compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules) of the invention and/or effector cells armed with these compositions can be used in conjunction with chemotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-Cadherin-17 antibodies linked to anti-Fc-gamma RI or anti-CD3 may be used in conjunction with IgG- or IgA-receptor specific binding agents.

[0325] Bispecific and multispecific molecules of the invention can also be used to modulate FcγR or FcγR levels on effector cells, such as by capping and elimination of receptors on the cell surface. Mixtures of anti-Fc receptors can also be used for this purpose.

[0326] The compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement, can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with a binding agent of the invention can be improved by binding of complement proteins. In another embodiment target cells coated with the compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules) of the invention can also be lysed by complement. In yet another embodiment, the compositions of the invention do not activate complement.

[0327] The compositions (e.g., monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention can also be administered together with complement. In certain embodiments, the instant disclosure provides compositions comprising antibodies, multispecific or bispecific molecules and serum or complement. These compositions can be advantageous when the complement is located in close proximity to the antibodies, multispecific or bispecific molecules. Alternatively, the antibodies, multispecific or bispecific molecules of the invention and the complement or serum can be administered separately.

[0328] Also within the scope of the present invention are kits comprising the antibody compositions of the invention (e.g., monoclonal antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain one more more additional reagents, such as an immunosuppressive reagent, a cytotoxic agent or a radiotoxic agent, or one or more additional antibodies of the invention (*e.g.*, an antibody

having a complementary activity which binds to an epitope in the Cadherin-17 antigen distinct from the first antibody).

[0329] Accordingly, patients treated with antibody compositions of the invention can be additionally administered (prior to, simultaneously with, or following administration of an antibody of the invention) with another therapeutic agent, such as a cytotoxic or radiotoxic agent, which enhances or augments the therapeutic effect of the antibodies.

[0330] In other embodiments, the subject can be additionally treated with an agent that modulates, e.g., enhances or inhibits, the expression or activity of Fcγ or Fcγ receptors by, for example, treating the subject with a cytokine. Preferred cytokines for administration during treatment with the multispecific molecule include of granulocyte colony-stimulating factor (G-CSF), granulocyte- macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN-γ), and tumor necrosis factor (TNF).

[0331] The compositions (e.g., antibodies, multispecific and bispecific molecules) of the invention can also be used to target cells expressing FcγR or Cadherin-17, for example for labeling such cells. For such use, the binding agent can be linked to a molecule that can be detected. Thus, the invention provides methods for localizing *ex vivo* or *in vitro* cells expressing Fc receptors, such as FcγR, or Cadherin-17. The detectable label can be, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.

[0332] In a particular embodiment, the invention provides methods for detecting the presence of Cadherin-17 antigen in a sample, or measuring the amount of Cadherin-17 antigen, comprising contacting the sample, and a control sample, with a monoclonal antibody, or an antigen binding portion thereof, which specifically binds to Cadherin-17, under conditions that allow for formation of a complex between the antibody or portion thereof and Cadherin-17. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of Cadherin-17 antigen in the sample.

[0333] In other embodiments, the invention provides methods for treating a Cadherin-17 mediated disorder in a subject, e.g., human cancers, including colorectal cancer.

[0334] In yet another embodiment, immunoconjugates of the invention can be used to target compounds (e.g., therapeutic agents, labels, cytotoxins, radiotoxins immunosuppressants, etc.) to cells which have Cadherin-17 cell surface receptors by linking such compounds to the antibody. For example, an anti-Cadherin-17 antibody can be conjugated to any of the toxin compounds described in US Patent Nos. 6,281,354 and 6,548,530, US patent publication Nos. 2003/0050331, 2003/0064984, 2003/0073852, and

2004/0087497, or published in WO 03/022806. Thus, the invention also provides methods for localizing *ex vivo* or *in vivo* cells expressing Cadherin-17 (e.g., with a detectable label, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor). Alternatively, the immunoconjugates can be used to kill cells which have Cadherin-17 cell surface receptors by targeting cytotoxins or radiotoxins to Cadherin-17.

[0335] The present invention is further illustrated by the following examples which should not be construed as further limiting.

[0336] All references cited in this specification, including without limitation all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, product fact sheets, and the like, one hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended to merely summarize the assertions made by their authors and no admission is made that any reference constitutes prior art and Applicants' reserve the right to challenge the accuracy and pertinence of the cited references.

[0337] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the dependant claims.

EXAMPLES

Example 1: Construction of a Phage-Display Library

[0338] A recombinant protein composed of domains 1-2 of the extracellular domain of Cadherin-17 (SEQ ID NO:136) was generated in bacteria by standard recombinant methods and used as antigen for immunization (see below). A recombinant protein composed of the full length extracellular domain of Cadherin-17 (SEQ ID NO:137) was also eurkaryotically synthesized by standard recombinant methods and used for screening.

Immunization and mRNA isolation

[0339] A phage display library for identification of Cadherin-17-binding molecules was constructed as follows. A/J mice (Jackson Laboratories, Bar Harbor, Me.) were immunized intraperitoneally with recombinant Cadherin-17 antigen (domains 1-2 of the extracellular domain), using 100 μg protein in Freund's complete adjuvant, on day 0, and with 100 μg antigen on day 28. Test bleeds of mice were obtained through puncture of the retro-orbital

sinus. If, by testing the titers, they were deemed high by ELISA using biotinylated Cadherin-17 antigen immobilized via neutravidin (Reacti-Bind(TM) NeutrAvidin(TM)-Coated Polystyrene Plates, Pierce, Rockford, Ill.), the mice were boosted with 100 μg of protein on day 70, 71 and 72, with subsequent sacrifice and splenectomy on day 77. If titers of antibody were not deemed satisfactory, mice were boosted with 100 μg antigen on day 56 and a test bleed taken on day 63. If satisfactory titers were obtained, the animals were boosted with 100 μg of antigen on day 98, 99, and 100 and the spleens harvested on day 105.

[0340] The spleens were harvested in a laminar flow hood and transferred to a petri dish, trimming off and discarding fat and connective tissue. The spleens were macerated quickly with the plunger from a sterile 5 cc syringe in the presence of 1.0 ml of solution D (25.0 g guanidine thiocyanate (Boehringer Mannheim, Indianapolis, Ind.), 29.3 ml sterile water, 1.76 ml 0.75 M sodium citrate pH 7.0, 2.64 ml 10% sarkosyl (Fisher Scientific, Pittsburgh, Pa.), 0.36 ml 2-mercaptoethanol (Fisher Scientific, Pittsburgh, Pa.)). This spleen suspension was pulled through an 18 gauge needle until all cells were lysed and the viscous solution was transferred to a microcentrifuge tube. The petri dish was washed with 100 μl of solution D to recover any remaining spleen. This suspension was then pulled through a 22 gauge needle an additional 5-10 times.

[0341] The sample was divided evenly between two microcentrifuge tubes and the following added, in order, with mixing by inversion after each addition: 50 μl 2 M sodium acetate pH 4.0, 0.5 ml water-saturated phenol (Fisher Scientific, Pittsburgh, Pa.), 100 μl chloroform/isoamyl alcohol 49:1 (Fisher Scientific, Pittsburgh, Pa.). The solution was vortexed for 10 seconds and incubated on ice for 15 min. Following centrifugation at 14 krpm for 20 min at 2-8 °C., the aqueous phase was transferred to a fresh tube. An equal volume of water saturated phenol:chloroform:isoamyl alcohol (50:49:1) was added, and the tube vortexed for ten seconds. After a 15 min incubation on ice, the sample was centrifuged for 20 min at 2-8 °C., and the aqueous phase transferred to a fresh tube and precipitated with an equal volume of isopropanol at -20 °C. for a minimum of 30 min. Following centrifugation at 14 krpm for 20 min at 4 °C., the supernatant was aspirated away, the tubes briefly spun and all traces of liquid removed from the RNA pellet.

[0342] The RNA pellets were each dissolved in 300 μl of solution D, combined, and precipitated with an equal volume of isopropanol at -20 °C. for a minimum of 30 min. The sample was centrifuged 14 krpm for 20 min at 4 °C., the supernatant aspirated as before, and the sample rinsed with 100 μl of ice-cold 70% ethanol. The sample was again centrifuged 14 krpm for 20 min at 4 °C., the 70% ethanol solution aspirated, and the RNA pellet dried in

vacuo. The pellet was resuspended in 100 µl of sterile diethyl pyrocarbonate-treated water. The concentration was determined by A260 using an absorbance of 1.0 for a concentration of 40 µg/ml. The RNAs were stored at -80 °C.

Preparation of Complementary DNA (cDNA)

[0343] The total RNA purified from mouse spleens as described above was used directly as template for cDNA preparation. RNA (50 µg) was diluted to 100 µL with sterile water, and 10 µL of 130 ng/µL oligo dT12 (synthesized on Applied Biosystems Model 392 DNA synthesizer) was added. The sample was heated for 10 min at 70 °C., then cooled on ice. Forty µL 5* first strand buffer was added (Gibco/BRL, Gaithersburg, Md.), along with 20 µL 0.1 M dithiothreitol (Gibco/BRL, Gaithersburg, Md.), 10 µL 20 mM deoxynucleoside triphosphates (dNTP's, Boehringer Mannheim, Indianapolis, Ind.), and 10 µL water on ice. The sample was then incubated at 37 °C. for 2 min. Ten µL reverse transcriptase (Superscript(TM) II, Gibco/BRL, Gaithersburg, Md.) was added and incubation was continued at 37 °C. for 1 hr. The cDNA products were used directly for polymerase chain reaction (PCR).

Amplification of Antibody Genes by PCR

[0344] To amplify substantially all of the H and L chain genes using PCR, primers were chosen that corresponded to substantially all published sequences. Because the nucleotide sequences of the amino termini of H and L contain considerable diversity, 33 oligonucleotides were synthesized to serve as 5' primers for the H chains, and 29 oligonucleotides were synthesized to serve as 5' primers for the kappa L chains as described in U.S. patent application Ser. No. 08/835,159, filed Apr. 4, 1997. The constant region nucleotide sequences for each chain required only one 3' primer for the H chains and one 3' primer for the kappa L chains.

[0345] A 50 µL reaction was performed for each primer pair with 50 µmol of 5' primer, 50 µmol of 3' primer, 0.25 µL Taq DNA Polymerase (5 units/µL, Boehringer Mannheim, Indianapolis, Ind.), 3 µL cDNA (prepared as described), 5 µL 2 mM dNTP's, 5 µL 10*Taq DNA polymerase buffer with MgCl2 (Boehringer Mannheim, Indianapolis, Ind.), and H2O to 50 µL. Amplification was done using a GeneAmp(R) 9600 thermal cycler (Perkin Elmer, Foster City, Calif.) with the following thermocycle program: 94 °C. for 1 min; 30 cycles of 94 °C. for 20 sec, 55 °C. for 30 sec, and 72 °C. for 30 sec; 72 °C. for 6 min; 4 °C.

[0346] The dsDNA products of the PCR process were then subjected to asymmetric PCR using only a 3' primer to generate substantially only the anti-sense strand of the target genes. A 100 µL reaction was done for each dsDNA product with 200 µmol of 3' primer, 2 µL of ds-

DNA product, 0.5 μL Taq DNA Polymerase, 10 μL 2 mM dNTP's, 10 μL 10*Taq DNA polymerase buffer with MgCl2 (Boehringer Mannheim, Indianapolis, Ind.), and H2O to 100 μL. The same PCR program as that described above was used to amplify the single-stranded (ss)-DNA.

Purification of Single-Stranded DNA by High Performance Liquid Chromatography and Kinasing Single-Stranded DNA

[0347] The H chain ss-PCR products and the L chain single-stranded PCR products were ethanol precipitated by adding 2.5 volumes ethanol and 0.2 volumes 7.5 M ammonium acetate and incubating at -20 °C. for at least 30 min. The DNA was pelleted by centrifuging in an Eppendorf centrifuge at 14 krpm for 10 min at 2-8 °C. The supernatant was carefully aspirated, and the tubes were briefly spun a 2nd time. The last drop of supernatant was removed with a pipette. The DNA was dried in vacuo for 10 min on medium heat. The H chain products were pooled in 210 μL water and the L chain products were pooled separately in 210 μL water. The single-stranded DNA was purified by high performance liquid chromatography (HPLC) using a Hewlett Packard 1090 HPLC and a Gen-Pak(TM) FAX anion exchange column (Millipore Corp., Milford, Mass.). The gradient used to purify the single-stranded DNA is shown in Table 1, and the oven temperature was 60 °C. Absorbance was monitored at 260 nm. The single-stranded DNA eluted from the HPLC was collected in 0.5 min fractions. Fractions containing single-stranded DNA were ethanol precipitated, pelleted and dried as described above. The dried DNA pellets were pooled in 200 μL sterile water.

Table 1 - HPLC gradient for purification of ss-DNA

Time (min)	%A	%B	%C	Flow (ml/min)
0	70	30	0	0.75
2	40	60	0	0.75
17	15	85	0	0.75
18	0	100	0	0.75
23	0	100	0	0.75
24	0	0	100	0.75
28	0	0	100	0.75
29	0	100	0	0.75
34	0	100	0	0.75
35	70	30	0	0.75

Buffer A is 25 mM Tris, 1 mM EDTA, pH 8.0

Buffer B is 25 mM Tris, 1 mM EDTA, 1 M NaCl, pH 8.0

Buffer C is 40 mm phosphoric acid

[0348] The single-stranded DNA was 5'-phosphorylated in preparation for mutagenesis. Twenty-four µL 10* kinase buffer (United States Biochemical, Cleveland, Ohio), 10.4 µL 10 mM adenosine-5'-triphosphate (Boehringer Mannheim, Indianapolis, Ind.), and 2 µL polynucleotide kinase (30 units/µL, United States Biochemical, Cleveland, Ohio) was added to each sample, and the tubes were incubated at 37 °C. for 1 hr. The reactions were stopped by incubating the tubes at 70 °C. for 10 min. The DNA was purified with one extraction of Tris equilibrated phenol (pH>8.0, United States Biochemical, Cleveland, Ohio):chloroform:isoamyl alcohol (50:49:1) and one extraction with chloroform:isoamyl alcohol (49:1). After the extractions, the DNA was ethanol precipitated and pelleted as described above. The DNA pellets were dried, then dissolved in 50 µL sterile water. The concentration was determined by measuring the absorbance of an aliquot of the DNA at 260 nm using 33 µg/ml for an absorbance of 1.0. Samples were stored at -20 °C.

Preparation of Uracil Templates Used in Generation of Spleen Antibody Phage Libraries

[0349] One ml of E. coli CJ236 (BioRAD, Hercules, Calif.) overnight culture was added to 50 ml 2*YT in a 250 ml baffled shake flask. The culture was grown at 37 °C. to OD600=0.6, inoculated with 10 µl of a 1/100 dilution of BS45 vector phage stock (described in U.S. patent application Ser. No. 08/835,159, filed Apr. 4, 1997) and growth continued for 6 hr. Approximately 40 ml of the culture was centrifuged at 12 krpm for 15 minutes at 4 °C. The supernatant (30 ml) was transferred to a fresh centrifuge tube and incubated at room temperature for 15 minutes after the addition of 15 µl of 10 mg/ml RNaseA (Boehringer Mannheim, Indianapolis, Ind.). The phage were precipitated by the addition of 7.5 ml of 20% polyethylene glycol 8000 (Fisher Scientific, Pittsburgh, Pa.)/3.5M ammonium acetate (Sigma Chemical Co., St. Louis, Mo.) and incubation on ice for 30 min. The sample was centrifuged at 12 krpm for 15 min at 2-8 °C. The supernatant was carefully discarded, and the tube briefly spun to remove all traces of supernatant. The pellet was resuspended in 400 µl of high salt buffer (300 mM NaCl, 100 mM Tris pH 8.0, 1 mM EDTA), and transferred to a 1.5 ml tube.

[0350] The phage stock was extracted repeatedly with an equal volume of equilibrated phenol:chloroform:isoamyl alcohol (50:49:1) until no trace of a white interface was visible, and then extracted with an equal volume of chloroform:isoamyl alcohol (49:1). The DNA was precipitated with 2.5 volumes of ethanol and 1/5 volume 7.5 M ammonium acetate and

incubated 30 min at -20 °C. The DNA was centrifuged at 14 krpm for 10 min at 4 °C., the pellet washed once with cold 70% ethanol, and dried in vacuo. The uracil template DNA was dissolved in 30 µl sterile water and the concentration determined by A260 using an absorbance of 1.0 for a concentration of 40 µg/ml. The template was diluted to 250 ng/µL with sterile water, aliquoted, and stored at -20 °C.

Mutagenesis of Uracil Template with ss-DNA and Electroporation into E. coli to Generate Antibody Phage Libraries

[0351] Antibody phage display libraries were generated by simultaneously introducing single-stranded heavy and light chain genes onto a phage display vector uracil template. A typical mutagenesis was performed on a 2 µg scale by mixing the following in a 0.2 ml PCR reaction tube: 8 µl of (250 ng/µL) uracil template, 8 µL of 10* annealing buffer (200 mM Tris pH 7.0, 20 mM MgCl2, 500 mM NaCl), 3.33 µl of kinased single-stranded heavy chain insert (100 ng/µL), 3.1 µl of kinased single-stranded light chain insert (100 ng/µL), and sterile water to 80 µl. DNA was annealed in a GeneAmp(R) 9600 thermal cycler using the following thermal profile: 20 sec at 94 °C., 85 °C. for 60 sec, 85 °C. to 55 °C. ramp over 30 min, hold at 55 °C. for 15 min. The DNA was transferred to ice after the program finished. The extension/ligation was carried out by adding 8 µl of 10* synthesis buffer (5 mM each dNTP, 10 mM ATP, 100 mM Tris pH 7.4, 50 mM MgCl2, 20 mM DTT), 8 µL T4 DNA ligase (1 U/µL, Bochringer Mannheim, Indianapolis, Ind.), 8 µL diluted T7 DNA polymerase (1 U/µL, New England BioLabs, Beverly, Mass.) and incubating at 37 °C. for 30 min. The reaction was stopped with 300 µL of mutagenesis stop buffer (10 mM Tris pH 8.0, 10 mM EDTA). The mutagenesis DNA was extracted once with equilibrated phenol (pH>8):chloroform:isoamyl alcohol (50:49:1), once with chloroform:isoamyl alcohol (49:1), and the DNA was ethanol precipitated at -20 °C. for at least 30 min. The DNA was pelleted and the supernatant carefully removed as described above. The sample was briefly spun again and all traces of ethanol removed with a pipetman. The pellet was dried in vacuo. The DNA was resuspended in 4 µL of sterile water.

[0352] One microliter of mutagenesis DNA (500 ng) was transferred into 40 µl electrocompetent E. coliDH12S (Gibco/BRL, Gaithersburg, Md.) using electroporation. The transformed cells were mixed with approximately 1.0 ml of overnight XL-1 cells which were diluted with 2*YT broth to 60% the original volume. This mixture was then transferred to a 15-ml sterile culture tube and 9 ml of top agar added for plating on a 150-mm LB agar plate. Plates were incubated for 4 hrs at 37° C. and then transferred to 20° C. overnight. First round antibody phage were made by eluting phage off these plates in 10 ml of 2*YT, spinning out

debris, and taking the supernatant. These samples are the antibody phage display libraries used for selecting antibodies against Cadherin-17. Efficiency of the electroporations was measured by plating 10 µl of a 10^{-4} dilution of suspended cells on LB agar plates, follow by overnight incubation of plates at 37 °C. The efficiency was calculated by multiplying the number of plaques on the 10^{-4} dilution plate by 106. Library electroporation efficiencies are typically greater than $1*10^{7}$ phage under these conditions.

Transformation of E. coli by Electroporation

[0353] Electrocompetent E. coli cells were thawed on ice. DNA was mixed with 40 L of these cells by gently pipetting the cells up and down 2-3 times, being careful not to introduce an air bubble. The cells were transferred to a Gene Pulser cuvette (0.2 cm gap, BioRAD, Hercules, Calif.) that had been cooled on ice, again being careful not to introduce an air bubble in the transfer. The cuvette was placed in the E. coli Pulser (BioRAD, Hercules, Calif.) and electroporated with the voltage set at 1.88 kV according to the manufacturer's recommendation. The transformed sample was immediately resuspended in 1 ml of 2*YT broth or 1 ml of a mixture of 400 µl 2*YT/600 µl overnight XL-1 cells and processed as procedures dictated.

Plating M13 Phage or Cells Transformed with Antibody Phage-Display Vector Mutagenesis Reaction

[0354] Phage samples were added to 200 µL of an overnight culture of E. coli XL1-Blue when plating on 100 mm LB agar plates or to 600 µL of overnight cells when plating on 150 mm plates in sterile 15 ml culture tubes. After adding LB top agar (3 ml for 100 mm plates or 9 ml for 150 mm plates, top agar stored at 55 °C. (see, Appendix A1, Sambrook et al., supra.), the mixture was evenly distributed on an LB agar plate that had been pre-warmed (37 °C.-55 °C.) to remove any excess moisture on the agar surface. The plates were cooled at room temperature until the top agar solidified. The plates were inverted and incubated at 37 °C. as indicated.

Preparation of Biotinylated Cadherin-17 and Biotinylated Antibodies

[0355] Concentrated recombinant Cadherin-17 antigen (full length extracellular domain) was extensively dialyzed into BBS (20 mM borate, 150 mM NaCl, 0.1% NaN3, pH 8.0). After dialysis, 1 mg of Cadherin-17 (1 mg/ml in BBS) was reacted with a 15 fold molar excess of biotin-XX-NHS ester (Molecular Probes, Eugene, Oreg., stock solution at 40 mM in DMSO). The reaction was incubated at room temperature for 90 min and then quenched with taurine (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 20 mM. The biotinylated reaction mixture was then dialyzed against BBS at 2-8 °C. After dialysis,

biotinylated Cadherin-17 was diluted in panning buffer (40 mM Tris, 150 mM NaCl, 20 mg/ml BSA, 0.1% Tween 20, pH 7.5), aliquoted, and stored at -80 °C. until needed.

[0356] Antibodies were reacted with 3-(N-maleimidylpropionyl)biocytin (Molecular Probes, Eugene, Oreg.) using a free cysteine located at the carboxy terminus of the heavy chain. Antibodies were reduced by adding DTT to a final concentration of 1 mM for 30 min at room temperature. Reduced antibody was passed through a Sephadex G50 desalting column equilibrated in 50 mM potassium phosphate, 10 mM boric acid, 150 mM NaCl, pH 7.0. 3-(N-maleimidylpropionyl)-biocytin was added to a final concentration of 1 mM and the reaction allowed to proceed at room temperature for 60 min. Samples were then dialyzed extensively against BBS and stored at 2-8 °C.

Preparation of Avidin Magnetic Latex

[0357] The magnetic latex (Estapor, 10% solids, Bangs Laboratories, Fishers, Ind.) was thoroughly resuspended and 2 ml aliquoted into a 15 ml conical tube. The magnetic latex was suspended in 12 ml distilled water and separated from the solution for 10 min using a magnet (PerSeptive Biosystems, Framingham, Mass.). While maintaining the separation of the magnetic latex with the magnet, the liquid was carefully removed using a 10 ml sterile pipette. This washing process was repeated an additional three times. After the final wash, the latex was resuspended in 2 ml of distilled water. In a separate 50 ml conical tube, 10 mg of avidin-HS (NeutrAvidin, Pierce, Rockford, Ill.) was dissolved in 18 ml of 40 mM Tris, 0.15 M sodium chloride, pH 7.5 (TBS). While vortexing, the 2 ml of washed magnetic latex was added to the diluted avidin-HS and the mixture mixed an additional 30 seconds. This mixture was incubated at 45 °C. for 2 hr, shaking every 30 minutes. The avidin magnetic latex was separated from the solution using a magnet and washed three times with 20 ml BBS as described above. After the final wash, the latex was resuspended in 10 ml BBS and stored at 4 °C.

[0358] Immediately prior to use, the avidin magnetic latex was equilibrated in panning buffer (40 mM Tris, 150 mM NaCl, 20 mg/ml BSA, 0.1% Tween 20, pH 7.5). The avidin magnetic latex needed for a panning experiment (200 µl/sample) was added to a sterile 15 ml centrifuge tube and brought to 10 ml with panning buffer. The tube was placed on the magnet for 10 min to separate the latex. The solution was carefully removed with a 10 ml sterile pipette as described above. The magnetic latex was resuspended in 10 ml of panning buffer to begin the second wash. The magnetic latex was washed a total of 3 times with panning buffer. After the final wash, the latex was resuspended in panning buffer to the starting volume.

Example 2: Selection of Recombinant Polyclonal Antibodies to Cadherin-17 Antigen

[0359] Binding reagents that specifically bind to Cadherin-17 were selected from the phage display libraries created from hyperimmunized mice as described in Example 1.

Panning

[0360] First round antibody phage were prepared as described in Example 1 using BS45 uracil template. Electroporations of mutagenesis DNA were performed yielding phage samples derived from different immunized mice. To create more diversity in the recombinant polyclonal library, each phage sample was panned separately.

[0361] Before the first round of functional panning with biotinylated Cadherin-17 antigen, antibody phage libraries were selected for phage displaying both heavy and light chains on their surface by panning with 7F11-magnetic latex (as described in Examples 21 and 22 of U.S. patent application Ser. No. 08/835,159, filed Apr. 4, 1997). Functional panning of these enriched libraries was performed in principle as described in Example 16 of U.S. patent application Ser. No. 08/835,159. Specifically, 10 µL of $1*10^{-6}$ M biotinylated Cadherin-17 antigen was added to the phage samples (approximately $1*10^{-8}$ M Cadherin-17 final concentration), and the mixture allowed to come to equilibrium overnight at 2-8 °C.

[0362] After reaching equilibrium, samples were panned with avidin magnetic latex to capture antibody phage bound to Cadherin-17. Equilibrated avidin magnetic latex (Example 1), 200 µL latex per sample, was incubated with the phage for 10 min at room temperature. After 10 min, approximately 9 ml of panning buffer was added to each phage sample, and the magnetic latex separated from the solution using a magnet. After a ten minute separation, unbound phage was carefully removed using a 10 ml sterile pipette. The magnetic latex was then resuspended in 10 ml of panning buffer to begin the second wash. The latex was washed a total of three times as described above. For each wash, the tubes were in contact with the magnet for 10 min to separate unbound phage from the magnetic latex. After the third wash, the magnetic latex was resuspended in 1 ml of panning buffer and transferred to a 1.5 mL tube. The entire volume of magnetic latex for each sample was then collected and resuspended in 200 µl 2*YT and plated on 150 mm LB plates as described in Example 1 to amplify bound phage. Plates were incubated at 37 °C. for 4 hr, then overnight at 20 °C.

[0363] The 150 mm plates used to amplify bound phage were used to generate the next round of antibody phage. After the overnight incubation, second round antibody phage were eluted from the 150 mm plates by pipetting 10 mL of 2*YT media onto the lawn and gently shaking the plate at room temperature for 20 min. The phage samples were then transferred to

15 ml disposable sterile centrifuge tubes with a plug seal cap, and the debris from the LB plate pelleted by centrifuging the tubes for 15 min at 3500 rpm. The supernatant containing the second round antibody phage was then transferred to a new tube.

[0364] A second round of functional panning was set up by diluting 100 µL of each phage stock into 900 µL of panning buffer in 15 ml disposable sterile centrifuge tubes. Biotinylated Cadherin-17 antigen was then added to each sample as described for the first round of panning, and the phage samples incubated for 1 hr at room temperature. The phage samples were then panned with avidin magnetic latex as described above. The progress of panning was monitored at this point by plating aliquots of each latex sample on 100 mm LB agar plates to determine the percentage of kappa positives. The majority of latex from each panning (99%) was plated on 150 mm LB agar plates to amplify the phage bound to the latex. The 100 mm LB agar plates were incubated at 37 °C. for 6-7 hr, after which the plates were transferred to room temperature and nitrocellulose filters (pore size 0.45 mm, BA85 Protran, Schleicher and Schuell, Keene, N.H.) were overlaid onto the plaques.

[0365] Plates with nitrocellulose filters were incubated overnight at room temperature and then developed with a goat anti-mouse kappa alkaline phosphatase conjugate to determine the percentage of kappa positives as described below. Phage samples with lower percentages (<70%) of kappa positives in the population were subjected to a round of panning with 7F11-magnetic latex before performing a third functional round of panning overnight at 2-8 °C. using biotinylated Cadherin-17 antigen at approximately $2*10^{-9}$ M. This round of panning was also monitored for kappa positives. Individual phage samples that had kappa positive percentages greater than 80% were pooled and subjected to a final round of panning overnight at 2-8 °C. at $5*10^{-9}$ M Cadherin-17. Antibody genes contained within the eluted phage from this fourth round of functional panning were subcloned into the expression vector, pBRncoH3.

[0366] The subcloning process was done generally as described in Example 18 of U.S. patent application Ser. No. 08/835,159. After subcloning, the expression vector was electroporated into DH10B cells and the mixture grown overnight in 2*YT containing 1% glycerol and 10 µg/ml tetracycline. After a second round of growth and selection in tetracycline, aliquots of cells were frozen at -80° C. as the source for Cadherin-17 polyclonal antibody production. Monoclonal antibodies were selected from these polyclonal mixtures by plating a sample of the mixture on LB agar plates containing 10 µg/ml tetracycline and screening for antibodies that recognized Cadherin-17.

Expression and Purification of Recombinant Antibodies Against Cadherin-17

[0367] A shake flask inoculum was generated overnight from a -70 °C. cell bank in an Innova 4330 incubator shaker (New Brunswick Scientific, Edison, N.J.) set at 37 °C., 300 rpm. The inoculum was used to seed a 20 L fermentor (Applikon, Foster City, Calif.) containing defined culture medium (Pack et al. (1993) Bio/Technology 11: 1271-1277) supplemented with 3 g/L L-leucine, 3 g/L L-isoleucine, 12 g/L casein digest (Difco, Detroit, Mich.), 12.5 g/L glycerol and 10 μg/ml tetracycline. The temperature, pH and dissolved oxygen in the fermentor were controlled at 26 °C., 6.0-6.8 and 25% saturation, respectively. Foam was controlled by addition of polypropylene glycol (Dow, Midland, Mich.). Glycerol was added to the fermentor in a fed-batch mode. Fab expression was induced by addition of L(+)-arabinose (Sigma, St. Louis, Mo.) to 2 g/L during the late logarithmic growth phase. Cell density was measured by optical density at 600 nm in an UV-1201 spectrophotometer (Shimadzu, Columbia, Md.). Following run termination and adjustment of pH to 6.0, the culture was passed twice through an M-210B-EH Microfluidizer (Microfluidics, Newton, Mass.) at 17,000 psi. The high pressure homogenization of the cells released the Fab into the culture supernatant.

[0368] The first step in purification was expanded bed immobilized metal affinity chromatography (EB-IMAC). Streamline(TM) chelating resin (Pharmacia, Piscataway, N.J.) was charged with 0.1 M NiCl2 and was then expanded and equilibrated in 50 mM acetate, 200 mM NaCl, 10 mM imidazole, 0.01% NaN3, pH 6.0 buffer flowing in the upward direction. A stock solution was used to bring the culture homogenate to 10 mM imidazole, following which it was diluted two-fold or higher in equilibration buffer to reduce the wet solids content to less than 5% by weight. It was then loaded onto the Streamline column flowing in the upward direction at a superficial velocity of 300 cm/hr. The cell debris passed through unhindered, but the Fab was captured by means of the high affinity interaction between nickel and the hexahistidine tag on the Fab heavy chain. After washing, the expanded bed was converted to a packed bed and the Fab was eluted with 20 mM borate, 150 mM NaCl, 200 mM imidazole, 0.01% NaN3, pH 8.0 buffer flowing in the downward direction.

[0369] The second step in the purification used ion-exchange chromatography (IEC). Q Sepharose FastFlow resin (Pharmacia, Piscataway, N.J.) was equilibrated in 20 mM borate, 37.5 mM NaCl, 0.01% NaN3, pH 8.0. The Fab elution pool from the EB-IMAC step was diluted four-fold in 20 mM borate, 0.01% NaN3, pH 8.0 and loaded onto the IEC column. After washing, the Fab was eluted with a 37.5-200 mM NaCl salt gradient. The elution fractions were evaluated for purity using an Xcell II(TM) SDS-PAGE system (Novex, San

Diego, Calif.) prior to pooling. Finally, the Fab pool was concentrated and diafiltered into 20 mM borate, 150 mM NaCl, 0.01% NaN3, pH 8.0 buffer for storage. This was achieved in a Sartocon Slice(TM) system fitted with a 10,000 MWCO cassette (Sartorius, Bohemia, N.Y.). The final purification yields were typically 50%. The concentration of the purified Fab was measured by UV absorbance at 280 nm, assuming an absorbance of 1.6 for a 1 mg/ml solution.

Example 3: Selection of Antibodies to Cadherin-17 Antigen From Tumor Membrane Preparations

[0370] Antibodies selected in Example 2 were further screened against tumor membrane preparations to isolate antibodies that preferentially bind to Cadherin-17 on cancer cells and not to normal intestinal epithelia.

[0371] Biotinylated plasma membrane preparations from paired colorectal cancer and normal adjacent tissue samples were used to pan phage samples with avidin magnetic latex to capture antibody phage bound to Cadherin-17 as described in Example 2. Antibodies were selected from these polyclonal mixtures by screening for antibodies that preferentially bind to Cadherin-17 on the colorectal cancer cells and not to the normal intestinal epithelia. These antibodies were then isolated as described in Example 4 and analyzed for binding to Cadherin-17.

Example 4: Selection of Monoclonal Antibodies to Cadherin-17 from the Recombinant Polyclonal Antibody Mixtures

[0372] Monoclonal antibodies against Cadherin-17 were isolated from clones containing the recombinant polyclonal mixtures (Example 3) by plating a diluted sample of the mixture on LB agar plates containing 10 μg/ml tetracycline. Individual colonies were then tested for the ability to produce antibody that recognized recombinant Cadherin-17 using surface plasmon resonance (BIACORE) (BIACORE, Uppsala, Sweden). Small scale production of these monoclonal antibodies was accomplished using a Ni-chelate batch-binding method (see below). Antibodies isolated from this method were diluted 1:3 in HBS-EP (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate 20 (v/v)), captured with a goat anti-mouse kappa antibody (Southern Biotechnology Associates, Inc, Birmingham, Ala.) coupled to a BIACORE CM5 sensor chip, and tested for the ability to bind recombinant Cadherin-17.

Minipreparation of Monoclonal Antibodies by Ni-Chelate Batch-Binding Method

[0373] Individual colonies were isolated from the recombinant polyclonal mixtures (Example 3) and used to inoculate 3 ml cultures of 2*YT medium containing 1% glycerol supplemented with 10 μg/ml tetracycline. These cultures were grown in an Innova 4330 incubator shaker (New Brunswick Scientific, Edison, N.J.) set at 37 °C., 300 rpm. The next morning 0.5 ml of each culture was used to inoculate shake flasks containing 50 ml of defined medium, (Pack et al. (1993) Bio/Technology 11: 1271-1277) supplemented with 3 g/L L-leucine, 3 g/L L-isoleucine, 12 g/L casein digest (Difco, Detroit, Mich.), 12.5 g/L glycerol and 10 μg/ml tetracycline. These cultures were shaken at 300 rpm, 37 °C. until an optical density of 4 was reached at 600 nm. Fab expression was then induced by adding L(+)-arabinose (Sigma, St. Louis, Mo.) to 2 g/L and shifting the temperature to 23 °C. with overnight shaking. The next day the following was added to the 50 ml cultures: 0.55 ml of 1 M imidazole, 5 ml B-PER (Pierce, Rockford, Ill.) and 2 ml Ni-chelating resin (Chelating Sepharose FastFlow(TM) resin Pharmacia, Piscataway, N.J.). The mixture was shaken at 300 rpm, 23 °C. for 1 hour after which time shaking was stopped and the resin allowed to settle to the bottom of the flasks for 15 minutes.

[0374] The supernatant was then poured off and the resin resuspended in 40 ml of BBS (20 mM borate, 150 mM NaCl, 0.1% NaN3, pH 8.0) containing 10 mM imidazole. This suspension was transferred to a 50 ml conical tube and the resin washed a total of 3 times with BBS containing 10 mM imidazole. Washing was accomplished by low speed centrifugation (1100 rpm for 1 minute), removal of supernatant and, resuspension of the resin in BBS containing 10 mM imidazole. After the supernatant of the final wash was poured off, 0.5 ml of 1 M imidazole was added to each tube, vortex briefly, and transferred to a sterile microcentrifuge tube. The samples were then centrifuged at 14 krpm for 1 minutes and the supernatant transferred to a new microcentrifuge tube. Antibodies contained in the supernatant were then analyzed for binding to Cadherin-17 using a BIACORE (BIACORE, Uppsala, Sweden).

Example 5: Specificity of Monoclonal Antibodies to Cadherin-17 Determined by Flow Cytometry Analysis

[0375] The specificity of antibodies against Cadherin-17 selected in Example 4 was tested by flow cytometry. To test the ability of the antibodies to bind to cell surface Cadherin-17 protein, the antibodies were incubated with Cadherin-17-expressing cells: LoVo and LS174T, human colorectal cancer lines. Cells were washed and resuspended in PBS. Four microliters of the suspensions were applied to wells of an eight well microscope slide and

allowed to air dry. The slides were lightly heated to fix the smears to the slide and covered with 0.1 mg/ml of antibody diluted in PBS containing 1% BSA. The smears were incubated with antibody for 1 h at 37 °C. in a moist chamber. After washing the slides three times by soaking in PBS for 5 min each, the smears were covered with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (H&L) F(ab')2 (Zymed Laboratories, Inc., South San Francisco, Calif.) diluted 1:80 in PBS, 1% BSA, 0.05% Evans Blue (Sigma). The slides were incubated for 1 h at 37 °C. in a moist chamber then washed as described above. After a final wash in deionized water, the slides were allowed to air dry in the dark. Coverslips were mounted using a 90% glycerol mounting medium containing 10 mg/ml p-phenylenediamine, pH 8.0.

[0376] The results of the flow cytometry analysis demonstrated that 14 monoclonal antibodies designated PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 bind effectively to cell-surface human Cadherin-17.

Example 6: Structural Characterization of Monoclonal Antibodies to Cadherin-17

[0377] The cDNA sequences encoding the heavy and light chain variable regions of the PTA001_A1, PTA001_A2, PTA001_A3, PTA001_A4, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13 and PTA001_A14 monoclonal antibodies were obtained using standard PCR techniques and were sequenced using standard DNA sequencing techniques.

[0378] The antibody sequences may be mutagenized to revert back to germline residues at one or more residues.

[0379] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A1 are shown in Figure 1 and in SEQ ID NO:59 and 35, respectively.

[0380] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A1 are shown in Figure 13 and in SEQ ID NO:71 and 47, respectively.

[0381] Comparison of the PTA001_A1 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A1 heavy chain utilizes a V_H segment from murine germline V_H 7-39. Further analysis of the PTA001_A1 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 1, and in SEQ ID NOs:1, 5 and 14, respectively. The alignment of the PTA001_A1 CDR1 V_H sequence to the germline V_H 7-39 sequence is shown in Figure 25.

[0382] Comparison of the PTA001_A1 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A1 light chain utilizes a V_K segment from murine germline V_K 1-110. Further analysis of the PTA001_A1 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 13 and in SEQ ID NOs:22, 28 and 32, respectively. The alignments of the PTA001_A1 CDR1 and CDR3 V_K sequences to the germline V_K 1-110 sequence are shown in Figures 27 and 29 respectively.

[0383] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A2 are shown in Figure 2 and in SEQ ID NO:60 and 36, respectively.

[0384] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A2 are shown in Figure 14 and in SEQ ID NO:72 and 48, respectively.

[0385] Comparison of the PTA001_A2 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A2 heavy chain utilizes a V_H segment from murine germline V_H region VH105 and V_H II gene H17. Further analysis of the PTA001_A2 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 2 and in SEQ ID NOs:2, 6 and 15, respectively. The alignment of the PTA001_A2 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A2 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0386] Comparison of the PTA001_A2 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A2 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A2 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 14, and in SEQ ID NOs:23, 29 and 33, respectively. The alignments of the PTA001_A2 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0387] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A3 are shown in Figure 3 and in SEQ ID NO:61 and 37, respectively.

[0388] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A3 are shown in Figure 14 and in SEQ ID NO:72 and 48, respectively.

[0389] Comparison of the PTA001_A3 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A3 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A3 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 3, and in SEQ ID NOs: 3, 7 and 16, respectively. The alignment of the PTA001_A3 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A3 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0390] Comparison of the PTA001_A3 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A3 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A3 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 14 and in SEQ ID NOs:23, 29, and 33, respectively. The alignments of the PTA001_A3 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0391] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A4 are shown in Figure 4 and in SEQ ID NO:62 and 38, respectively.

[0392] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A4 are shown in Figure 15 and in SEQ ID NO:73 and 49, respectively.

[0393] Comparison of the PTA001_A4 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A4 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A4 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 4 and in SEQ ID NOs: 4, 8 and 17, respectively. The alignment of the PTA001_A4 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A4 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0394] Comparison of the PTA001_A4 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A4 light chain utilizes a V_K segment from murine germline V_K 8-30. Further analysis of the PTA001_A4 V_K sequence using the Kabat system of CDR region determination led to the

delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 15 and in SEQ ID NOs:24, 30, and 34, respectively. The alignments of the PTA001_A4 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 8-30 sequence are shown in Figures 27, 28 and 29 respectively.

[0395] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A5 are shown in Figure 5 and in SEQ ID NO:63 and 39, respectively.

[0396] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A5 are shown in Figure 16 and in SEQ ID NO:74 and 50, respectively.

[0397] Comparison of the PTA001_A5 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A5 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A5 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 5, and in SEQ ID NOs: 3, 7 and 18, respectively. The alignment of the PTA001_A5 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A5 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0398] Comparison of the PTA001_A5 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A5 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A5 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 16, and in SEQ ID NOs:25, 31, and 33, respectively. The alignments of the PTA001_A5 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0399] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A6 are shown in Figure 6 and in SEQ ID NO:64 and 40, respectively.

[0400] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A6 are shown in Figure 17 and in SEQ ID NO:75 and 51, respectively.

[0401] Comparison of the PTA001_A6 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A6 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A6 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2

and CDR3 regions as shown in Figure 6 and in SEQ ID NOs: 3, 7 and 16, respectively. The alignment of the PTA001_A6 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A6 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0402] Comparison of the PTA001_A6 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A6 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A6 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 17 and in SEQ ID NOs:23, 29, and 33, respectively. The alignments of the PTA001_A6 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0403] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A7 are shown in Figure 7 and in SEQ ID NO:65 and 41, respectively.

[0404] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A7 are shown in Figure 18 and in SEQ ID NO:76 and 52, respectively.

[0405] Comparison of the PTA001_A7 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A7 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A7 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 7 and in SEQ ID NOs: 3, 9 and 19, respectively. The alignment of the PTA001_A7 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A7 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0406] Comparison of the PTA001_A7 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A7 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A7 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 18 and in SEQ ID NOs:26, 29, and 33, respectively. The alignments of the PTA001_A7 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0407] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A8 are shown in Figure 8 and in SEQ ID NO:66 and 42, respectively.

[0408] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A8 are shown in Figure 19 and in SEQ ID NO:77 and 53, respectively.

[0409] Comparison of the PTA001_A8 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A8 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A8 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 8 and in SEQ ID NOs: 3, 7 and 16, respectively. The alignment of the PTA001_A8 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A8 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0410] Comparison of the PTA001_A8 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A8 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A8 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 19 and in SEQ ID NOs:25, 31, and 33, respectively. The alignments of the PTA001_A8 CDR1, CDR2 and CDR3 V_k sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0411] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A9 are shown in Figure 6 and in SEQ ID NO:64 and 40, respectively.

[0412] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A9 are shown in Figure 20 and in SEQ ID NO:78 and 54, respectively.

[0413] Comparison of the PTA001_A9 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A9 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A9 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 6 and in SEQ ID NOs: 3, 7 and 16, respectively. The alignment of the PTA001_A9 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A9 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0414] Comparison of the PTA001_A9 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A9 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A9 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 20 and in SEQ ID NOs:23, 29, and 33, respectively. The alignments of the PTA001_A9 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0415] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A10 are shown in Figure 6 and in SEQ ID NO:64 and 40, respectively.

[0416] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A10 are shown in Figure 21 and in SEQ ID NO:79 and 55, respectively.

[0417] Comparison of the PTA001_A10 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A10 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A10 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 6 and in SEQ ID NOs: 3, 7 and 16, respectively. The alignment of the PTA001_A10 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A10 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0418] Comparison of the PTA001_A10 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A10 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A10 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 21 and in SEQ ID NOs:25, 29, and 33, respectively. The alignments of the PTA001_A10 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0419] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A11 are shown in Figure 9 and in SEQ ID NO:67 and 43, respectively.

[0420] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A11 are shown in Figure 22 and in SEQ ID NO:80 and 56, respectively.

[0421] Comparison of the PTA001_A11 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A11 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A11 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 9 and in SEQ ID NOs: 3, 10 and 20, respectively. The alignment of the PTA001_A11 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A11 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0422] Comparison of the PTA001_A11 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A11 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A11 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 22 and in SEQ ID NOs:27, 29, and 33, respectively. The alignments of the PTA001_A11 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0423] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A12 are shown in Figure 10 and in SEQ ID NO:68 and 44, respectively.

[0424] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A12 are shown in Figure 21 and in SEQ ID NO:81 and 55, respectively.

[0425] Comparison of the PTA001_A12 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A12 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A12 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 10 and in SEQ ID NOs: 3, 11 and 21, respectively. The alignment of the PTA001_A12 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A12 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0426] Comparison of the PTA001_A12 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A12 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A12 V_K sequence using the Kabat system of CDR region

determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 21 and in SEQ ID NOs:25, 29, and 33, respectively. The alignments of the PTA001_A12 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0427] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A13 are shown in Figure 11 and in SEQ ID NO:69 and 45, respectively.

[0428] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A13 are shown in Figure 23 and in SEQ ID NO:82 and 57, respectively.

[0429] Comparison of the PTA001_A13 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A13 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A13 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in Figure 11 and in SEQ ID NOs: 3, 12 and 18, respectively. The alignment of the PTA001_A13 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A13 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0430] Comparison of the PTA001_A13 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A13 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A13 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 23 and in SEQ ID NOs:25, 31, and 33, respectively. The alignments of the PTA001_A13 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

[0431] The nucleotide and amino acid sequences of the heavy chain variable region of PTA001_A14 are shown in Figure 12 and in SEQ ID NO:70 and 46, respectively.

[0432] The nucleotide and amino acid sequences of the light chain variable region of PTA001_A14 are shown in Figure 24 and in SEQ ID NO:83 and 58, respectively.

[0433] Comparison of the PTA001_A14 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the PTA001_A14 heavy chain utilizes a V_H segment from murine germline V_H II region VH105 and V_H II gene H17. Further analysis of the PTA001_A14 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2

and CDR3 regions as shown in Figure 12 and in SEQ ID NOs: 2, 13 and 15, respectively. The alignment of the PTA001_A14 CDR1 V_H sequence to the germline V_H II gene H17 sequence is shown in Figure 25 and the alignment of the PTA001_A14 CDR2 V_H sequence to the germline V_H II region VH105 is shown in Figure 26.

[0434] Comparison of the PTA001_A14 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the PTA001_A14 light chain utilizes a V_K segment from murine germline V_K 24-140. Further analysis of the PTA001_A14 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in Figure 24 and in SEQ ID NOs:23, 29, and 33, respectively. The alignments of the PTA001_A14 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 24-140 sequence are shown in Figures 27, 28 and 29 respectively.

Example 7: Immunohistochemistry on FFPE sections using anti-Cadherin-17 antibodies

[0435] Immunohistochemistry was performed on FFPE sections of colorectal tumor and normal adjacent tissue using the anti-Cadherin-17 antibodies PTA001_A3, PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9.

[0436] EX-De-Wax was from BioGenex, CA, USA. Tissue sections and arrays were from Biomax, MD, USA.

[0437] Slides were heated for 2 h at 60 ^{0}C in 50 ml Falcons in a water bath with no buffer. Each Falcon had one slide or two slides back-to back with long gel loading tip between them to prevent slides from sticking to each other. Slides were deparaffinised in EZ-DeWax for 5 min in black slide rack, then rinsed well with the same DeWax solution using 1 ml pipette, then washed with water from the wash bottle. Slides were placed in a coplin jar filled with water until the pressure cooker was ready; the water was changed a couple of times.

[0438] Water was exchanged for antigen retrieval solution = 1 x citrate buffer, pH 6 (DAKO). Antigen was retrieved by the pressure cooker method. The slides in the plastic coplin jar in antigen retrieval solution were placed into a pressure cooker which was then heated up to position 6 (the highest setting). 15-20 min into the incubation, the temperature was reduced to position 3 and left at that (when the temperature inside the pressure cooker was 117 ^{0}C) for another 20-25 minutes. Then the hob was switched off and the cooker was placed onto the cold hob and the pressure was released by carefully moving the handle into the position between "open" and "closed". The whole system was left to release the pressure and to cool down for another 20 minutes. The lid was opened and samples taken out to rest

on the bench. The slides were washed 1x5min with PBS-3T (0.5 L PBS + 3 drops of Tween-20) and placed in PBS.

[0439] After antigen retrieval, slides were mounted in the Shandon Coverplate system. Trapping of air bubbles between the slide and plastic coverplate was prevented by placing the coverplate into the coplin jar filled with PBS and gently sliding the slide with tissue sections into the coverplate. The slide was pulled out of the coplin jar while holding it tightly together with the coverplate. The assembled slide was placed into the rack, letting PBS trapped in the funnel and between the slide and coverplate to run through. Slides were washed with 2x2 ml (or 4x1 ml) PBS-3T, 1x2 ml PBS, waiting until all PBS had gone through the slide and virtually no PBS was left in the funnel.

[0440] Endogenous peroxide blockade was performed using 1-4 drops of peroxide solution per slide; the incubation time was 5 minutes. The slides were rinsed with water and then once with 2 ml PBS-3T and once with 2 ml PBS; it was important to wait until virtually no liquid was left in the funnel before adding a new portion of wash buffer.

[0441] The primary antibody was diluted with an Antibody diluent reagent (DAKO). Optimal dilution was determined to be 1:400. Up to 200 µl of diluted primary antibody was applied to each slide and incubated for 45 minutes at room temperature. Slides were washed with 2x2 ml (or 4x1 ml) PBS-3T and then 1x2 ml PBS.

[0442] The goat anti-mouse kappa HRP secondary (1 mg/ml, cat.1050-05, Southern Biotech) was applied 2x2 drops per slide and incubated for 35 min at room temperature. The slides were washed as above.

[0443] The DAB substrate was made up in dilution buffer; 2 ml containing 2 drops of substrate was enough for 10 slides. The DAB reagent was applied to the slides by applying a few drops at a time and left for 10 min. The slides were washed 1x2 ml (or 2x1 ml) with PBS-3T and 1x2 ml (or 2x1 ml) with PBS.

[0444] Hematoxylin (DAKO) was applied; 1 ml was enough for 10 slides and slides were incubated for 1 min at room temperature. The funnels of the Shandon Coverplate system were filled with 2 ml of water and let to run through. When slides were clear of the excess of hematoxylin, the system was disassembled, tissue sections and/or arrays were washed with water from the wash bottle and placed into black slide rack. Tissues were dehydrated by incubating in EZ-DeWax for 5 min and then in 95% ethanol for 2-5 min.

[0445] Slides were left to dry on the bench at room temperature and then mounted in mounting media and covered with coverslip.

[0446] Immunohistochemical analysis on antibodies PTA001_A3, PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9 revealed specific membrane staining of tumor cells in colorectal cancer and no appreciable staining of normal adjacent tissue in all cases. Antibody PTA001_A4, in particular, showed clear specific membrane staining of tumor cells.

Example 8: Immunohistochemistry on frozen sections using anti-Cadherin-17 antibodies

[0447] Immunohistochemistry was performed on frozen paired tumor and normal adjacent tissues using the anti-Cadherin-17 antibodies PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9.

[0448] Tissue sections were from BioChain Institute Inc., CA, USA.

[0449] Frozen sections were washed with PBS twice for 3 minutes each and were then placed in PBS.

[0450] Endogenous peroxide blockade was performed using Peroxidase Blocker (S2001, DAKO). 1-4 drops of peroxidase blocker was added to each slide and incubated for 5 minutes. The slides were rinsed three times with 3 ml PBS.

[0451] The primary antibody was diluted with an Antibody diluent reagent (DAKO). 150 µl of diluted primary antibody was applied to each slide and incubated for 45 minutes at room temperature. Slides were washed with twice for 3 minutes with PBS-3T (500 ml PBS + 3 drops of Tween-20) and then once for 3 minutes with PBS.

[0452] The goat anti-mouse kappa HRP secondary was applied at 1:1000 (1 mg/ml, cat.1050-05, Southern Biotech) and incubated for 35 min at room temperature. The slides were washed as above.

[0453] The DAB substrate was made up in dilution buffer; 2 ml containing 2 drops of substrate was enough for 10 slides. The DAB reagent was applied to the slides by applying a few drops at a time and incubated for 10 min. The slides were washed once for 3 minutes with PBS-3T and twice for 3 minutes with water.

[0454] Hematoxylin (DAKO) was applied; 1 ml was enough for 10 slides and slides were incubated for 1 min at room temperature.

[0455] Slides were left to dry on the bench at room temperature and then mounted in water-based mounting media from Vector and covered with coverslip.

[0456] Immunohistochemical analysis on antibodies PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9 on three colorectal cancer samples along with the paired normal adjacent tissue samples revealed strong specific membrane staining of tumor cells in

colorectal cancer and some weak staining of normal adjacent tissue. Antibody PTA001_A4, in particular, showed clear specific membrane staining of tumor cells.

Example 9: Western Blotting using anti-Cadherin-17 antibodies

[0457] Western blotting was performed using anti-Cadherin-17 antibodies PTA001_A3, PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9 to detect Cadherin-17 in a panel of genetically characterized colorectal cancer cell lines representing combinations of three critical mutation phenotypes (p53, APC and RER+/).

[0458] Snap frozen cell pellets were lysed in modified RIPA buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA) containing protease inhibitors (Roche) and cleared by spinning down at 18,000 g for 15 min at 4 C. The 4x LDS sample loading buffer (Invitrogen Inc.) was added to the cleared lysate to a final concentration of 1x; sample was heated for 10 min at 70 ^{0}C and kept at -80 ^{0}C afterwards. Before loading on a gel samples were re-heated.

[0459] Proteins from 10 μg (PC/JW) and 20 μg (other cell lines) of lysate per lane were separated by mini-gel electrophoresis on NuPAGE Novex precast mini-gel (Invitrogen, UK). Gels were blotted onto nitrocellulose membrane with iBlot Dry Blotting System (Invitrogen, UK).

[0460] Membrane was incubated with animal-free blocker (Vector) and probed with anti-Cadherin-17 antibody in animal-free blocker at 1:500 dilution, at 4 C, for 14-18 h, rotating. The secondary was anti-mouse DyLight 488 conjugate (Pierce).

[0461] A clear signal was detected at a size corresponding to the Cadherin-17 protein (92 kDa) in 11 of the 14 chosen colorectal cell lines for all 5 Cadherin-17 antibodies (PTA001_A3, PTA001_A4, PTA001_A6, PTA001_A8 and PTA001_A9). Figure 25 shows the Western blotting analysis of Cadherin-17 for antibody PTA001_A4. Cell lines were carefully selected for different genetic backgrounds relevant to the initiation and progression of CRC (RER+ = replication error positive tumor phenotype; RER- = replication error deficient tumor phenotype; p53 wild type or mutant and APC wild type or mutant genotype). Table 2 below shows the phenotype of the colorectal tumor derived cell lines.

Table 2 – Phenotype of colorectal tumor derived cell lines

Cell line	Characteristics
LS411	Colorectal carcinoma, Tumor stage: Dukes' type B; Tumorigenic in nude mice.
LoVo	Colorectal adenocarcinoma, Dukes' type C, grade IV derived from metastatic site; Tumorigenic in nude mice.
Vaco 5	Colorectal carcinoma.
DLD-1	Colorectal adenocarcinoma, Dukes' type C; Tumorigenic in nude mice.
LS 174T	Colorectal adenocarcinoma, Dukes' type B, Tumorigenic in nude mice.
HCT 116	Colorectal carcinoma, Tumorigenic in nude mice.
PC/JW	Adenocarcinoma. Tumorigenic in ethylic nude mice.
C99	Colorectal adenocarcinoma.
C84	Colorectal adenocarcinoma.
HT29	Colorectal adenocarcinoma, Tumorigenic in nude mice.
LS513	Colorectal carcinoma, Dukes' type C; Tumorigenic in nude mice.
NCI-H716	Colorectal adenocarcinoma; Tumorigenic in nude mice.
Caco2	Colorectal adenocarcinoma
Colo205	Colorectal adenocarcinoma

Example 10: Internalization of anti-Cadherin-17 antibodies

[0462] PTA001_A4 was shown to be internalized by LoVo cells upon binding to the cells using a Immunofluorescence microscopy assay. The Immunofluorescence microscopy assay showed internalization of the anti-Cadherin-17 monoclonal antibodies through binding of an anti-human IgG secondary antibody conjugated to Fluorescein isothiocyanate (GamK-FITC). First, PTA001_A4 were bound to the surface of the LoVo cells. Then, the secondary antibody conjugated to Fluorescein isothiocyanate were bound to the primary antibodies. Next, the PTA001_A4/secondary antibody FITC conjugate complex was internalized by the cells.

[0463] The Immunofluorescence microscopy assay was conducted as follows. LoVo cell were incubated at 37°C for 12 hours for cells to adhere to each other. PTA001_A4 and secondary antibody conjugated to Fluorescein isothiocyanate were serially diluted, washed with FACS buffer (PBS, 2% FBS) and then added to the culture media. The media was then washed again with FACS buffer (PBS, 2% FBS) and incubated at 37%, after which 200 ul 2% PFA was added. Coverslips were mounted using a 9 ul aqeous mountaing media and the cells were then visualized at regular time intervals using Leica fluorescent microscope. Figure 32A and Figure 32B shows surface binding of PTA001_A4/ secondary antibody FITC conjugate complex to LoVo cells after 60 minutes of incubation and internalization of PTA001_A4/ secondary antibody FITC conjugate complex after 120 minutes.

[0464] The monoclonal antibody, PTA001_A4, was shown to be internalized by LS147T and LoVo cells upon binding to the cells using a MabZap assay. The MabZAP assay showed internalization of the anti-CDH17 monoclonal antibodies through binding of an anti-human IgG secondary antibody conjugated to the toxin saporin. (Advanced Targeting System, San Diego, CA, IT-22-100). First, PTA001_A4 was bound to the surface of the LS147T and LoVo cells. Then, the MabZAP antibodies were bound to the primary antibodies. Next, the MabZAP complex was internalized by the cells. The entrance of Saporin into the cells resulted in protein synthesis inhibition and eventual cell death.

[0465] The MabZAP assay was conducted as follows. Each of the cells was seeded at a density of $5x10^3$ cells per well. The anti-CDH17 monoclonal antibodies or an isotype control human IgG were serially diluted then added to the cells. The MabZAP was then added at a concentration of 50 μg/ml and the plates allowed to incubate for 48 and 72 hours. Cell viability in the plates was detected by CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, G7571) and the plates were read at 490nM by a Luminomitor (Tuner BioSystems, Sunnyvale, CA). The data was analyzed by Prism (Graphpad). Cell death was proportional to the concentration of PTA001_A4 and monoclonal antibody. Figures 35A and 35B show that the anti-CDH17 monoclonal antibodies were efficiently internalized by LS174T and LoVo cells respectively as compared to the anti-human IgG isotype control antibody.

SEQUENCE LISTING

SEQ ID NO	SEQUENCE DESCRIPTION	SEQUENCE
1	VH CDR1 amino acid PTA001_A1	GFTFSNYGMS
2	VH CDR1 amino acid PTA001_A2, PTA001_A14	GYTFSDHAIH
3	VH CDR1 amino acid PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13	GYTFTDHAIH
4	VH CDR1 amino acid PTA001_A4	GYTLTDHTIH
5	VH CDR2 amino acid PTA001_A1	AINRDGGTTYYTDNVKG
6	VH CDR2 amino acid PTA001_A2	YIYPRHGTTNYNENFKG
7	VH CDR2 amino acid PTA001_A3, PTA001_A5,	YIYPEHGTIKYNEKFKG

	PTA001_A6, PTA001_A8, PTA001_A9, PTA001_A10	
8	VH CDR2 amino acid PTA001_A4	YIYPRDGITGYNEKFKG
9	VH CDR2 amino acid PTA001_A7	YIYPRDGFTKYNEKFKG
10	VH CDR2 amino acid PTA001_A11	YIYPEHGSITYNEKFKG
11	VH CDR2 amino acid PTA001_A12	YIYPRDDFAKVNEKFKG
12	VH CDR2 amino acid PTA001_A13	YIYPEHGTITYNEKFKG
13	VH CDR2 amino acid PTA001_A14	YIFPRDAFSLNNEKFKG
14	VH CDR3 amino acid PTA001_A1	FLLWDGWYFDV
15	VH CDR3 amino acid PTA001_A2, PTA001_A14	RNYFYVMDY
16	VH CDR3 amino acid PTA001_A3, PTA001_A6, PTA001_A8, PTA001_A9, PTA001_A10	TNYFYVMEY
17	VH CDR3 amino acid PTA001_A4	GYSYRNYAYYYDY
18	VH CDR3 amino acid PTA001_A5, PTA001_A13	RNYLYIMDY
19	VH CDR3 amino acid PTA001_A7	TNYFYTMDY
20	VH CDR3 amino acid PTA001_A11	RNYLYVMDY
21	VH CDR3 amino acid PTA001_A12	TNYLYIMDY
22	VK CDR1 amino acid PTA001_A1	RSSQSLLHSNGNTYLH
23	VK CDR1 amino acid PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9, PTA001_A14	TSSKSLLRSNGNTYLY
24	VK CDR1 amino acid PTA001_A4	KSSQSLLHSSNQKNYLA
25	VK CDR1 amino acid PTA001_A5, PTA001_A8, PTA001_A10, PTA001_A12, PTA001_A13	RSSKSLLRSNGNTYLY
26	VK CDR1 amino acid PTA001_A7	RSSKSLLRTNGNTYLH
27	VK CDR1 amino acid PTA001_A11	RSTKSLLRSNGNTYLY
28	VK CDR2 amino acid PTA001_A1	KVSNRFS
29	VK CDR2 amino acid PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A7, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A14	RMSNLAS
30	VK CDR2 amino acid PTA001_A4	WASTRES
31	VK CDR2 amino acid	RLSNLAS

	PTA001_A5, PTA001_A8, PTA001_A13	
32	VK CDR3 amino acid PTA001_A1	SQSTHVLT
33	VK CDR3 amino acid PTA001_A2, PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13, PTA001_A14	MQHLEYPFT
34	VK CDR3 amino acid PTA001_A4	QQYYSYPWT
35	VH amino acid PTA001_A1	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAEVQLLETGGGVVKPG GSLKLSCAASGFTFSNYGMSWVRQTPEKRLEWVAAINRDGGTTYYTDNVKG RFTISRDNAKNSLYLQMSSLRSEDTALYYCARQFLLWDGWYFDVWGAGTTV TVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSG VHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
36	VH amino acid PTA001_A2	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAELVKPG ASVKISCKVSGYTFSDHAIHWMSQRPGQGLKWIGYIYPRHGTTNYNENFKGK ATLTADTSSSTAYMQLNSLTSEDSAVYFCARMRNYFYVMDYWGQGTSVTVS SAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
37	VH amino acid PTA001_A3	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVLLQQSDAELVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGTIKYNEKFKGKA TLTADKSSSTAYMQLNSLTSEDSAVYFCSRLTNYFYVMEYWGQGTSVTVSSA KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
38	VH amino acid PTA001_A4	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAEVQLQQSVAELVKPG ASVKMSCKVSGYTLTDHTIHWMKQRPEQGLEWIGYIYPRDGITGYNEKFKGK ATLTADTSSSTAYMQLNSLTSEDSAVYFCARWGYSYRNYAYYYDYWGQGT TLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLS SGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPR DC
39	VH amino acid PTA001_A5	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDADLVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGTIKYNEKFKGKA TLTADKSSSTAYMQLNSLTSEDSAVYFCARLRNYLYIMDYWGQGTSVTVSSA KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
40	VH amino acid PTA001_A6, PTA001_A9, PTA001_A10	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAELVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGTIKYNEKFKGKA TLTADKSSSTAYMQLNSLTSEDSAVYFCSRLTNYFYVMEYWGQGTSVTVSSA KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
41	VH amino acid PTA001_A7	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAELVKPG ASVKISCKVSGYTFTDHAIHWMKQRPEQGLEWIGYIYPRDGFTKYNEKFKGK ATLTADTSSSTAYMQLNSLTSEDSTVYFCARMTNYFYTMDYWGQGTSVTVS SAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
42	VH amino acid PTA001_A8	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDADLVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGTIKYNEKFKGKA TLTADKSSSTAYMQLNSLTSEDSAVYFCSRLTNYFYVMEYWGQGTSVTVSSA KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
43	VH amino acid PTA001_A11	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAELVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGSITYNEKFKGKA TLTADKSSSTVYMHLNSLTSEDSAVYFCARLRNYLYVMDYWGQGTSVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
44	VH amino acid PTA001_A12	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSEAELVKPG ASVKLSCKASGYTFTDHAIHWMKQRPEQGLEWIGYIYPRDDFAKVNEKFKG KATLTADTSSSTAYMQLNSLTSEDSAVYFCARMTNYLYIMDYWGQGTSVTV SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGV HTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
45	VH amino acid PTA001_A13	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAELVKPG ASVKISCKASGYTFTDHAIHWVKQRPEQGLEWIGYIYPEHGTITYNEKFKGKA TLTADKSSSTVYMHLNSLTSEDSAVYFCARLRNYLYIMDYWGQGTSVTVSSA KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
46	VH amino acid PTA001_A14	LGKPWRYPRFVHGENKVKQSTIALALLPLLFTPVAKAQVQLQQSDAALVKPG ASVKISCKVSGYTFSDHAIHWMKQRPEQGLEWIGYIFPRDAFSLNNEKFKGK

		ATLSADTSSSTAYMELTSLTFEDSAVYFCARMRNYFYVMDYWGQGTSVTVS SAKTTPPSVYTLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
47	VK amino acid PTA001_A1	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADVVLT QTPLSLPVTLGDQASISCRSSQSLLHSNGNTYLHWYLLKPGQSPKLLIYKVSN RFSGVPDRFSGSGSGTDFTLKITRVEAEDLGVYFCSQSTHVLTFGAGTKLELK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
48	VK amino acid PTA001_A2, PTA001_A3	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCTSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
49	VK amino acid PTA001_A4	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMS QSPSSLAVSVGEKVTMSCKSSQSLLHSSNQKNYLAWYQQKPGQSPKVLIYWA STRESGVPDRFTGSGSGTDFTLTITSVKSEDLAVYYCQQYYSYPWTFGGGTRL EIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNG VLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNR NESYPYDVPDYAS
50	VK amino acid PTA001_A5	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCRSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRLSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
51	VK amino acid PTA001_A6	RILPDAFYRNSLLFLHTRFFGWSETIKYLLPTAAAGLLLLAAQPAMADIVMTQ AAPSVPVTPGESVSISCTSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNLA SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIKR ADAAPTVSILPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLN SWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNES YPYDVPDYAS
52	VK amino acid PTA001_A7	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCRSSKSLLRTNGNTYLHWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTVFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
53	VK amino acid PTA001_A8	RILPYAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCRSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRLSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
54	VK amino acid PTA001_A9	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCTSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSISPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
55	VK amino acid PTA001_A10, PTA001_A12	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCRSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
56	VK amino acid PTA001_A11	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVSVTPGESVSISCRSTKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
57	VK amino acid PTA001_A13	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCRSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRLSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK RADAAPTVSIFPQYSEQLTTGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE SYPYDVPDYAS
58	VK amino acid PTA001_A14	RILPDAFYRNSLLFLHTRFFGWSETMKYLLPTAAAGLLLLAAQPAMADIVMT QAAPSVPVTPGESVSISCTSSKSLLRSNGNTYLYWFLQRPGQSPQLLIYRMSNL ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTNLEIK RADAAPTVSIFTTSREQLTSGGASVVCFLNNFYPKDINVK
59	VH n.t. PTA001_A1	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC

		TGTGGCAAAAGCCGAAGTGCAGCTGTTGGAGACTGGGGGAGGCGTAGTG AAGCCCGGAGGGTCCCTTAAACTCTCCTGTGCAGCCTCTGGATTCACTTTC AGTAACTATGGCATGTCTTGGGTTCGCCAGACTCCGGAGAAGAGGCTGGA GTGGGTCGCAGCCATTAATCGTGATGGTGGTACCACCTACTATACAGACA ATGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACAGCCTG TACCTGCAAATGAGCAGTCTGAGGTCTGAGGACACAGCCTTGTATTACTG TGCAAGACAGTTCCTTCTCTGGGACGGCTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCT ATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGG GATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAAC TCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCT GACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCC AGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGT GGACAAGAAAATTGTGCCCAGGGATTGT
60	VH n.t. PTA001_A2	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGAGCTTCAGTGAAGATATCCTGCAAGGTTTCTGGCTACACCTTCA GTGACCATGCTATTCACTGGATGAGTCAGAGACCTGGACAGGGCCTGAAA TGGATTGGATATATTTATCCTAGACATGGGACTACTAACTACAATGAGAA CTTCAAGGGCAAGGCCACACTGACTGCAGACACATCCTCCAGCACAGCCT ACATGCAGCTCAACAGCCTGACATCTGAAGATTCTGCCGTCTATTTCTGTG CAAGAATGAGAAACTACTTCTATGTTATGGACTACTGGGGTCAAGGAACC TCAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTG GCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTG GTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATC CCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTA CACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGA CCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAG AAAATTGTGCCCAGGGATTGT
61	VH n.t. PTA001_A3	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCTGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGAACTATTAAGTATAATGAGAA GTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGCACTGCCT ATATGCAGCTCAACAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTT CAAGACTCACTAACTACTTCTATGTTATGGAGTATTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
62	VH n.t. PTA001_A4	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCGAGGTTCAGCTGCAGCAGTCTGTCGCTGAGTTGGTGA AACCTGGAGCTTCAGTGAAGATGTCATGCAAGGTTTCTGGCTACACCCTC ACTGACCATACTATTCACTGGATGAAGCAGAGGCCTGAACAGGGCCTGGA ATGGATTGGATATATTTACCCTAGAGATGGAATAACTGGGTACAATGAGA AGTTCAAGGGCAAGGCCACACTGACTGCAGACACTTCTTCCAGCACAGCC TACATGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTCTATTTCTGT GCCAGATGGGGCTATAGTTACAGGAATTACGCGTACTACTATGACTACTG GGGCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACGACACCCCCAT CTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGA CCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACC TGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTG CAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACC TGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCAC CAAGGTGGACAAGAAAATTGTGCCCAGGGATTGT
63	VH n.t. PTA001_A5	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGACTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAACAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGAACTATTAAGTATAATGAGAA GTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGCACTGCCT ATATGCAGCTCAACAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTG CAAGACTCAGGAACTATTTGTATATTATGGACTACTGGGGTCAAGGAACC TCAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTG GCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTG GTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATC CCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTA

		CACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGA CCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAG AAAATTGTGCCCAGGGATTGT
64	VH n.t. PTA001_A6, PTA001_A9, PTA001_A10	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGAACTATTAAGTATAATGAGAA GTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGCACTGCCT ATATGCAGCTCAACAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTT CAAGACTCACTAACTACTTCTATGTTATGGAGTATTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
65	VH n.t. PTA001_A7	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGAGCCTCAGTGAAGATATCCTGCAAGGTTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGATGAAACAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTAGAGATGGTTTTACTAAGTACAATGAGAAG TTCAAGGGCAAGGCCACACTGACTGCAGACACATCCTCCAGCACAGCCTA CATGCAGCTCAACAGCCTGACATCTGAGGATTCTACAGTCTATTTCTGTGC AAGAATGACTAACTACTTCTATACTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
66	VH n.t. PTA001_A8	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGACTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAACAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGAACTATTAAGTATAATGAGAA GTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGCACTGCCT ATATGCAGCTCAACAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTT CAAGACTCACTAACTACTTCTATGTTATGGAGTATTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
67	VH n.t. PTA001_A11	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGTAGTATTACGTATAATGAGAAG TTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGTACTGTCTA TATGCACCTCAATAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTGC AAGACTCAGGAACTACTTGTATGTTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
68	VH n.t. PTA001_A12	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGAGGCTGAGCTTGTGA AGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGATGAAACAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATCTACCCCAGAGATGATTTTGCTAAGGTGAATGAGAA GTTCAAGGGCAAGGCCACACTGACAGCAGACACATCCTCCAGCACAGCCT ACATGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTCTATTTCTGTG

		CAAGAATGACTAACTACCTCTATATTATGGACTACTGGGGTCAAGGAACC TCAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTG GCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTG GTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATC CCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTA CACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGA CCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAG AAAATTGTGCCCAGGGATTGT
69	VH n.t. PTA001_A13	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCA CTGACCATGCTATTCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTATCCTGAACATGGTACTATTACGTATAATGAGAAG TTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGTACTGTCTA TATGCACCTCAATAGCCTGACATCTGAGGATTCAGCAGTGTATTTCTGTGC AAGACTCAGGAACTATTTGTATATTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGG CCCCCGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTG GTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATC CCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTA CACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGA CCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAG AAAATTGTGCCCAGGGATTGT
70	VH n.t. PTA001_A14	TGACTGGGAAAACCCTGGCGTTACCCACGCTTTGTACATGGAGAAAATAA AGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGCTCTTATTTACCCC TGTGGCAAAAGCCCAGGTTCAGCTGCAACAGTCTGACGCCGCGTTGGTGA AACCTGGAGCTTCAGTGAAGATATCGTGCAAGGTTTCTGGCTACACCTTCA GTGACCATGCTATTCACTGGATGAAGCAGAGGCCTGAACAGGGCCTGGAA TGGATTGGATATATTTTTCCTAGAGATGCTTTTAGTTTGAACAATGAGAAG TTCAAGGGCAAGGCCACACTGAGTGCAGACACATCCTCCAGCACAGCCTA CATGGAGCTCACCAGCCTGACATTTGAGGATTCTGCAGTCTATTTCTGTGC AAGAATGAGAAACTACTTCTATGTTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATACACTGG CCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGG TCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGAC CGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGT
71	VK n.t. PTA001_A1	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TGTTGTGCTGACCCAGACTCCACTCTCCCTGCCTGTCACTCTTGGAGATCA AGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTTTACACAGTAATGGAAA CACCTATTTACATTGGTACCTGCTGAAGCCAGGCCAGTCTCCAAAGCTCCT GATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGG CAGTGGATCAGGGACAGATTTCACACTCAAGATCACCAGAGTGGAGGCTG AGGATCTGGGAGTTTATTTCTGCTCTCAAAGTACACATGTGCTCACGTTCG GTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTC GTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAA GATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATC AGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACC AAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAA GACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTTATCC ATATGATGTGCCAGATTATGCGAGCTAA
72	VK n.t. PTA001_A2, PTA001_A3	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCACGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAA CACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCGGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACG TTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
73	VK n.t. PTA001_A4	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT

		CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA CATCGTTATGTCTCAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAA GGTTACTATGAGCTGCAAGTCCAGCCAGAGCCTTTTACATAGTAGCAATC AAAAGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAA GTGCTGATTTACTGGGCATCCACTAGAGAATCTGGGGTCCCTGATCGCTTC ACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCACCAGTGTGAA GTCTGAAGACCTGGCAGTTTATTACTGTCAGCAATATTATAGCTATCCGTG GACGTTCGGTGGCGGCACCAGGCTGGAAATCAAACGGGCTGATGCTGCAC CAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTG CCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCA AGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGG ACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCAC GTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCA CTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAG TCTTATCCATATGATGTGCCAGATTATGCGAGCTAA
74	VK n.t. PTA001_A5	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCGCAGTAATGGCA ACACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCC TGATATATCGGCTGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCTGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACA TTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
75	VK n.t. PTA001_A6	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATAAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCACGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAA CACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCGGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACG TTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCCTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
76	VK n.t. PTA001_A7	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTTTCCATCTCCTGCAGGTCTTCTAAGAGTCTCCTGCGTACTAATGGCAA CACTTACTTGCATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGTTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCATTCACG TTCGGCTCGGGGACAAAGTTGGAAATAAAAAGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
77	VK n.t. PTA001_A8	TAAGATTAGCGGATCCTACCTTACGCTTTTTATCGCAACTCTCTACTGTTTC TCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCTA CGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGAT ATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAATCA GTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAAC ACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTG ATATATCGGCTGTCTAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTGGC AGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCTGA

		GGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACATT CGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAACTG TATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAG TCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGAT CAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGAC CAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACA AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTTAT CCATATGATGTGCCAGATTATGCGAGCTAA
78	VK n.t. PTA001_A9	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCACGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAA CACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCGGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACG TTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCTCCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
79	VK n.t. PTA001_A10	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCAGGTCCAGTAAGAGTCTCCTGCGTAGTAATGGCA ACACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCC TCATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCCTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACG TTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAA CTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCT CAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGT GGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACT GATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTT GACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTC ACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCT TATCCATATGATGTGCCAGATTATGCGAGCTAA
80	VK n.t. PTA001_A11	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTTCTGTTTC TCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCTA CGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGAT ATTGTGATGACCCAGGCTGCACCCTCTGTATCTGTCACTCCTGGAGAGTCA GTATCCATCTCCTGCAGGTCTACTAAGAGTCTCCTGCGTAGTAATGGCAAC ACTTACTTGTATTGGTTCCTCCAGAGGCCAGGCCAGTCTCCTCAGCTCCTG ATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTGG CAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCTG AGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACGT TCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
81	VK n.t. PTA001_A12	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACGTAGTAATGGCAA CACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCCTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACG TTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAA CTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCT CAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGT GGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACT GATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTT GACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTC

		ACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCT TATCCATATGATGTGCCAGATTATGCGAGCTAA
82	VK n.t. PTA001_A13	TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTT CTCCATACCCGTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCT ACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGA TATTGTGATGACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTC AGTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCGCAGTAATGGCA ACACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCC TGATATATCGGCTGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCT GAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACA TTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACAATACAGTGAGCAGTTAACAACTGGAGGTGCCTC AGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTG ATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCA CAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTCTT ATCCATATGATGTGCCAGATTATGCGAGCTAA
83	VK n.t. PTA001_A14	CGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCC GTTTTTTTGGATGGAGTGAAACGATGAAATACCTATTGCCTACGGCAGCC GCTGGATTGTTATTACTCGCTGCCCAACCAGCCATGGCCGATATTGTGATG ACCCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTCAGTATCCATC TCCTGCACGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAACACTTACTTG TATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGATATATCGG ATGTCCAACCTTGCCTCGGGAGTCCCAGACAGGTTCAGTGGCAGTGGGTC AGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAGGCTGAGGATGTGG GTGTTTATTACTGTATGCAACATCTAGAATATCCTTTCACGTTCGGCTCGG GGACAAATTTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATC TTCACAACATCCAGAGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTG CTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAG
84	VH CDR1 n.t. PTA001_A1	GGATTCACTTTCAGTAACTATGGCATGTCT
85	VH CDR1 n.t. PTA001_A2, PTA001_A14	GGCTACACCTTCAGTGACCATGCTATTCAC
86	VH CDR1 n.t. PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A7, PTA001_A8, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A13	GGCTACACCTTCACTGACCATGCTATTCAC
87	VH CDR1 n.t. PTA001_A4	GGCTACACCCTCACTGACCATACTATTCAC
88	VH CDR2 n.t. PTA001_A1	GCCATTAATCGTGATGGTGGTACCACCTACTATACAGACAATGTGAAGGG C
89	VH CDR2 n.t. PTA001_A2	TATATTTATCCTAGACATGGGACTACTAACTACAATGAGAACTTCAAGGG C
90	VH CDR2 n.t. PTA001_A3, PTA001_A5, PTA001_A6, PTA001_A8, PTA001_A9, PTA001_A10	TATATTTATCCTGAACATGGAACTATTAAGTATAATGAGAAGTTCAAGGG C
91	VH CDR2 n.t. PTA001_A4	TATATTTACCCTAGAGATGGAATAACTGGGTACAATGAGAAGTTCAAGGG C
92	VH CDR2 n.t. PTA001_A7	TATATTTATCCTAGAGATGGTTTTACTAAGTACAATGAGAAGTTCAAGGGC
93	VH CDR2 n.t. PTA001_A11	TATATTTATCCTGAACATGGTAGTATTACGTATAATGAGAAGTTCAAGGGC
94	VH CDR2 n.t. PTA001_A12	TATATCTACCCCAGAGATGATTTTGCTAAGGTGAATGAGAAGTTCAAGGG C
95	VH CDR2 n.t. PTA001_A13	TATATTTATCCTGAACATGGTACTATTACGTATAATGAGAAGTTCAAGGGC
96	VH CDR2 n.t. PTA001_A14	TATATTTTTCCTAGAGATGCTTTTAGTTTGAACAATGAGAAGTTCAAGGGC
97	VH CDR3 n.t. PTA001_A1	TTCCTTCTCTGGGACGGCTGGTACTTCGATGTC
98	VH CDR3 n.t. PTA001_A2, PTA001_A14	AGAAACTACTTCTATGTTATGGACTAC
99	VH CDR3 n.t. PTA001_A3, PTA001_A6, PTA001_A8, PTA001_A9, PTA001_A10	ACTAACTACTTCTATGTTATGGAGTAT
100	VH CDR3 n.t. PTA001_A4	GGCTATAGTTACAGGAATTACGCGTACTACTATGACTAC
101	VH CDR3 n.t. PTA001_A5, PTA001_A13	AGGAACTATTTGTATATTATGGACTAC

102	VH CDR3 n.t. PTA001_A7	ACTAACTACTTCTATACTATGGACTAC
103	VH CDR3 n.t. PTA001_A11	AGGAACTACTTGTATGTTATGGACTAC
104	VH CDR3 n.t. PTA001_A12	ACTAACTACCTCTATATTATGGACTAC
105	VK CDR1 n.t. PTA001_A1	AGATCTAGTCAGAGCCTTTTACACAGTAATGGAAACACCTATTTACAT
106	VK CDR1 n.t. PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9, PTA001_A14	ACGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAACACTTACTTGTAT
107	VK CDR1 n.t. PTA001_A4	AAGTCCAGCCAGAGCCTTTTACATAGTAGCAATCAAAAGAACTACTTGGC C
108	VK CDR1 n.t. PTA001_A5, PTA001_A13	AGGTCTAGTAAGAGTCTCCTGCGCAGTAATGGCAACACTTACTTGTAT
109	VK CDR1 n.t. PTA001_A7	AGGTCTTCTAAGAGTCTCCTGCGTACTAATGGCAACACTTACTTGCAT
110	VK CDR1 n.t. PTA001_A8	AGGTCTAGTAAGAGTCTCCTGCGTAGTAATGGCAACACTTACTTGTAT
111	VK CDR1 n.t. PTA001_A10	AGGTCCAGTAAGAGTCTCCTGCGTAGTAATGGCAACACTTACTTGTAT
112	VK CDR1 n.t. PTA001_A11	AGGTCTACTAAGAGTCTCCTGCGTAGTAATGGCAACACTTACTTGTAT
113	VK CDR1 n.t. PTA001_A12	AGGTCTAGTAAGAGTCTCCTACGTAGTAATGGCAACACTTACTTGTAT
114	VK CDR2 n.t. PTA001_A1	AAAGTTTCCAACCGATTTTCT
115	VK CDR2 n.t. PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9, PTA001_A14	CGGATGTCCAACCTTGCCTCG
116	VK CDR2 n.t. PTA001_A4	TGGGCATCCACTAGAGAATCT
117	VK CDR2 n.t. PTA001_A5, PTA001_A13	CGGCTGTCCAACCTTGCCTCA
118	VK CDR2 n.t. PTA001_A7, PTA001_A10, PTA001_A11, PTA001_A12	CGGATGTCCAACCTTGCCTCA
119	VK CDR2 n.t. PTA001_A8	CGGCTGTCTAACCTTGCCTCA
120	VK CDR3 n.t. PTA001_A1	TCTCAAAGTACACATGTGCTCACG
121	VK CDR3 n.t. PTA001_A2, PTA001_A3, PTA001_A6, PTA001_A9, PTA001_A10, PTA001_A11, PTA001_A12, PTA001_A14	ATGCAACATCTAGAATATCCTTTCACG
122	VK CDR3 n.t. PTA001_A4	CAGCAATATTATAGCTATCCGTGGACG
123	VK CDR3 n.t. PTA001_A5, PTA001_A8, PTA001_A13	ATGCAACATCTAGAATATCCTTTCACA
124	VK CDR3 n.t. PTA001_A7	ATGCAACATCTAGAATATCCATTCACG
125	VH7-39 (Genbank AJ851868.3) n.t. 240-269	GGATTCACTTTCAGTAGCTATGGCATGTCT
126	VHII gene H17 (GenBank X02466.1) n.t. 67-96	GGCTACACCTTCACTGACCATACTATTCAC
127	VHII region VII105 (Genbank J00507) n.t.1096-1146	TATATTTATCCTAGAGATGGTAGTACTAAGTACAATGAGAAGTTCAAGGG C
128	VK1-110 (GenBank AY591695.1) n.t. 1738-1785	AGATCTAGTCAGAGCCTTGTACACAGTAATGGAAACACCTATTTACAT
129	VK1-110 (GenBank AY591695.1) n.t. 1948-1971	TCTCAAAGTACACATGTTCCTCCC
130	VK8-30 (GenBank AJ235948.1) n.t. 510-560	AAGTCCAGTCAGAGCCTTTTATATAGTAGCAATCAAAAGAACTACTTGGC C
131	VK8-30 (GenBank AJ235948.1) n.t. 606-626	TGGGCATCCACTAGGGAATCT
132	VK8-30 (GenBank AJ235948.1) n.t. 723-749	CAGCAATATTATAGCTATCCTCCCACA
133	VK24-140 (GenBank AY591710.1) n.t. 1807-1854	AGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTAT
134	VK24-140 (GenBank AY591710.1) n.t. 1900-1920	CGGATGTCCAACCTTGCCTCA

135	VK24-140 (GenBank AY591710.1) n.t. 2017-2043	ATGCAACATCTAGAATATCCTTTCACA
136	Cadherin-17 ECD domains 1-2	QEGKFSGPLKPMTFSIYEGQEPSQIIFQFKANPPAVTFELTGETDNIFVIEREGLL YYNRALDRETRSTHNLQVAALDANGIIVEGPVPITIKVKDINDNRPTFLQSKYE GSVRQNSRPGKPFLYVNATDLDDPATPNGQLYYQIVIQLPMINNVMYFQINN KTGAISLTREGSQELNPAKNPSYNLVISVKDMGGQSENSFSDTTSVDIIVTENI WKAPKP
137	Cadherin-17 ECD	QEGKFSGPLKPMTFSIYEGQEPSQIIFQFKANPPAVTFELTGETDNIFVIEREGLL YYNRALDRETRSTHNLQVAALDANGIIVEGPVPITIKVKDINDNRPTFLQSKYE GSVRQNSRPGKPFLYVNATDLDDPATPNGQLYYQIVIQLPMINNVMYFQINN KTGAISLTREGSQELNPAKNPSYNLVISVKDMGGQSENSFSDTTSVDIIVTENI WKAPKPVEMVENSTDPHPIKITQVRWNDPGAQYSLVDKEKLPRPFSIDQEG DIYVTQPLDREEKDAYVFYAVAKDEYGKPLSYPLEIHVKVKDINDNPPTCPSP VTVFEVQENERLGNSIGTLTAHDRDEENTANSFLNYRIVEQTPKLPMDGLFLI QTYAGMLQLAKQSLKKQDTPQYNLTIEVSDKDFKTLCFVQINVIDINDQIPIFE KSDYGNLTLAEDTNIGSTILTIQATDADEPFTGSSKILYHIIKGDSEGRLGVDTD PHTNTGYVIIKKPLDFETAAVSNIVFKAENPEPLVFGVKYNASSFAKFTLIVTD VNEAPQFSQHVFQAKVSEDVAIGTKVGNVTAKDPEGLDISYSLRGDTRGWLK IDHVTGEIFSVAPLDREAGSPYRVQVVATEVGGSSLSSVSEFHLILMDVNDNPP RLAKDYTGLFFCHPLSAPGSLIFEATDDDQHLFRGPHFTFSLGSGSLQNDWEV SKINGTHARLSTRHTDFEEREYVVLIRINDGGRPPLEGIVSLPVTFCSCVEGSCF RPAGHQTGIPTVGM

WHAT IS CLAIMED:

1. An isolated anti-Cadherin-17 antibody comprising:
a heavy chain variable region CDR 1 comprising SEQ ID NO: 4;
a heavy chain variable region CDR2 comprising SEQ ID NO: 8;
a heavy chain variable region CDR3 comprising SEQ ID NO: 17;
a light chain variable region CDR1 comprising SEQ ID NO: 24;
a light chain variable region CDR2 comprising SEQ ID NO: 30; and
a light chain variable region CDR3 comprising SEQ ID NO: 34;
optionally wherein any of the above SEQ ID NOs independently comprise one, two or three amino acid substiutions, additions or deletions.

2. An isolated anti-Cadherin-17 antibody comprising:
(a) the heavy chain variable region amino acid sequence set forth in SEQ ID NO:38 or an amino acid sequence at least 90% homologous thereto, and
(b) the light chain variable region amino acid sequence set forth in SEQ ID NO:49 or an amino acid sequence at least 90% homologous thereto.

3. The antibody of claim 1 or 2, wherein said antibody is a full-length antibody of an IgG1, IgG2, IgG3, or IgG4 isotype.

4. The antibody of any one of the preceding claims, wherein said antibody is selected from the group consisting of: a whole antibody, a monoclonal antibody, an antibody fragment, a humanized antibody, a single chain antibody, a defucosylated antibody, an antibody mimetic and a bispecific antibody.

5. The antibody of claim 4, wherein said fragment is selected from the group consisting of: a UniBody, a domain antibody, and a Nanobody.

6. The antibody of claim 4, wherein the mimetic is selected from the group consisting of: an Affibody, a DARPin, an Anticalin, an Avimer, a Versabody, and a Duocalin.

7. An immunoconjugate comprising an antibody according to any one of the preceding claims conjugated to a therapeutic agent.

8. The immunoconjugate of claim 7 wherein the therapeutic agent is a cytotoxin or a radioactive isotope.

9. A composition comprising the isolated antibody or antigen-binding portion thereof of any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

10. An isolated nucleic acid molecule encoding the heavy or light chain of the antibody or antigen-binding portion thereof of any one of claims 1 to 8.

11. An expression vector comprising the nucleic acid molecule of claim 10.

12. A host cell comprising the expression vector of claim 11.

13. A method for preparing an anti-Cadherin-17 antibody, said method comprising the steps of: obtaining a host cell that contains one or more nucleic acid molecules encoding the antibody of any one of claims 1 to 8;

growing the host cell in a host cell culture;

providing host cell culture conditions wherein the one or more nucleic acid molecules are expressed; and

recovering the antibody from the host cell or from the host cell culture.

14. A method of making anti-Cadherin-17 antibodies, comprising the steps of:

recovering mRNA from the B cells of an animal which has been immunized with a Cadherin-17 peptide;

converting said mRNA to cDNA;

expressing said cDNA in phages such that anti-Cadherin-17 antibodies encoded by said cDNA are presented on the surface of said phages;

selecting phages that present anti-Cadherin-17 antibodies;

recovering nucleic acid molecules from said selected phages that encode said anti-Cadherin-17 immunoglobulins;

expressing said recovered nucleic acid molecules in a host cell; and

recovering antibodies as defined in claim 1 or claim 2 from said host cell that bind Cadherin-17.

15. Use of an anti-Cadherin-17 antibody of any one of claims 1 to 6 or an immunoconjugate of claim 7 or 8 in the manufacture of a medicament for treating or preventing cancer associated with target cells expressing Cadherin-17.

16. The use of claim 15, wherein said disease is a human cancer.

17. The use of claim 16, wherein said human cancer is colorectal cancer.