Anti-GCC Antibody Molecules and Use of Same to Test for Susceptibility to GCC-Targeted Therapy

Related Applications

The present application claims the benefit of and priority to U.S. Provisional Application Serial No. 61/639,376, filed April 27, 2012. The entire content of U.S. Provisional Application Serial No. 61/639,376 is incorporated herein by this reference.

Field of the Invention

The invention relates to antibody molecules which bind GCC, and methods for using the same to select patients for treatment with a GCC-targeted therapy, such as an immunoconjugate containing an anti-GCC antibody molecule, or fragment thereof, conjugated to an agent such as a therapeutic agent.

Background

Guanylyl cyclase C ("GCC") is a transmembrane cell surface receptor that functions in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation, see, e.g., Carrithers et al., Proc. Natl. Acad. Sci. USA 100:3018-3020 (2003). GCC is expressed at the mucosal cells lining the small intestine, large intestine and rectum (Carrithers et al., Dis Colon Rectum 39: 171-181 (1996)). GCC expression is maintained upon neoplastic transformation of intestinal epithelial cells, with expression in all primary and metastatic colorectal tumors (Carrithers et al., Dis Colon Rectum 39: 171-181 (1996); Buc et al. Eur J Cancer 41: 1618-1627 (2005); Carrithers et al., Gastroenterology 107: 1653-1661 (1994)).

Summary

The disclosure is based, at least in part, on the discovery of novel anti-GCC antibodies. Accordingly, in one aspect, the invention features an anti-GCC antibody molecule, as disclosed herein. The anti-GCC antibody molecules are useful as naked antibody molecules and as

components of immunoconjugates. Accordingly, in another aspect, the invention features immunoconjugates comprising an anti-GCC antibody molecule describd herein and a therapeutic agent or label. The invention also features methods of using the anti-GCC antibody molecules and immunoconjucates described herein, e.g., for detection of GCC and of cells or tissues that express GCC. Such methods are useful, *inter alia*, for diagnosis, prognosis, imaging, or staging of a GCC-mediated disease. Accordingly, in some aspects, the invention features methods of identifying a subject for treatment with a GCC- targeted therapy, e.g., an anti-GCC antibody therapy, e.g., an immunoconjugate comprising an anti-GCC antibody conjugated with a therapeutic agent. The invention also features an *in vitro* or *in vivo* method of determining of a subject having cancer is a potential candidate for a GCC-targeted therapy, e.g., a GCC-targeted therapy described herein.

Anti-GCC antibodies, e.g., the anti-GCC antibodies described herein, are also useful e.g., for modulating an activity or function of a GCC protein; and for treatment of a GCC-mediated disease, as described herein. In some aspects, the treatment includes acquiring knowledge and/or evaluating a sample or subject to determined GCC expression levels, and if the subject expresses GCC, then administering a GCC-targeted therapy, e.g., a GCC targeted therapy described herein. In other aspects, the method features generating a personalized treatment report, e.g., a GCC targeted treatment report, by obtaining a sample from a subject and determining GCC expression levels, e.g., by a detection method described herein, e.g., using an anti-GCC antibody described herein, and based upon the determination, selecting a targeted treatment report for the subject.

In another aspect, the invention also features isolated and/or recombinant nucleic acids encoding anti-GCC antibody molecule amino acid sequences, as well as vectors and host cells comprising such nucleic acids, and methods for producing anti-GCC antibody molecules. Also featured herein are reaction mixtures and kits comprising the anti-GCC antibodies, e.g., an immunocnjugate, described herein, as well as *in vitro* assays, e.g., comprising an anti-GCC antibody described herein, to detect GCC expression.

All publications, patent applications, patents and other references mentioned herein are incorporated by references in their entirety.

Other features, objects, and advantages of the invention(s) disclosed herein will be apparent from the description and drawings, and from the claims.

Brief Description of the Drawings

Figure 1 is a circular map of a protein expression vector used to generate a human GCC (hGCC) extracellular domain (ECD) mouse Fc (mFc) fusion protein (hGCC-ECD-mFc) of the invention.

Figures 2A-2D depict bar graphs summarizing the combined/aggregate (apical and cytoplasmic) H score distribution of GCC expression across tumor types from cancer patients screened for enrollment into the C26001 Study, a Phase I clinical trial of a GCC-targeted immunoconjugate. Figure 2A depicts the combined aggregate H score distribution across colorectal cancer patients screened; Figure 2B depicts the combined aggregate H score distribution across gastric cancer patients screened; Figure 2C depicts the combined aggregate H score distribution across pancreatic cancer patients screened; Figure 2D depicts the combined aggregate H score distribution across esophageal cancer patients screened.

Figures 3A-3C depict bar graphs summarizing the combined/aggregate (apical and cytoplasmic) H score distribution of GCC expression across various tumor specific microarrays that were screened. Figure 3A depicts the combined/aggregate H score distribution across samples on various colorectal tumor microarrays screened for GCC expression; Figure 3B depicts the combined/aggregate H score distribution across samples on a gastric tumor microarray screened for GCC expression; Figure 3C depicts the combined/aggregate H score distribution across samples on various pancreatic tumor microarrays screened for GCC expression.

Detailed Description

Guanylyl cyclase C (GCC) (also known as STAR, ST Receptor, GUC2C, and GUCY2C) is a transmembrane cell surface receptor that functions in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation (Carrithers et al., *Proc Natl Acad Sci* USA 100: 3018-3020 (2003); Mann et al., *Biochem Biophys Res Commun* 239: 463-466 (1997); Pitari et al., *Proc Natl Acad Sci* USA 100: 2695-2699 (2003)); GenBank Accession No. NM_004963, each of which is incorporated herein by reference in its entirety). This function is mediated through binding of guanylin (Wiegand et al. FEBS Lett. 311:150-154 (1992)). GCC also is a receptor for heat-stable enterotoxin (ST, e.g., having an amino acid sequence of NTFYCCELCCNPACAGCY, SEQ ID NO: 1) which is a peptide produced by E. coli, as well as other infectious organisms (Rao, M. C. *Ciba Found. Symp.* 112:74-93 (1985); Knoop F. C. and Owens, M. *J. Pharmacol. Toxicol. Methods* 28:67-72 (1992)). Binding of ST to GCC activates a signal cascade that results in enteric disease, e.g., diarrhea.

Nucleotide sequence for human GCC (GenBank Accession No. NM_004963; SEQ ID NO: 2):

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   1 gaccagagag aagcgtgggg aagagtgggc tgagggactc cactagaggc tgtccatctg
  61 gattccctgc ctccctagga gcccaacaga gcaaagcaag tgggcacaag gagtatggtt
 121 ctaacgtgat tggggtcatg aagacgttgc tgttggactt ggctttgtgg tcactgctct
 181 tccagcccgg gtggctgtcc tttagttccc aggtgagtca gaactgccac aatggcagct
 241 atgaaatcag cgtcctgatg atgggcaact cagcctttgc agagcccctg aaaaacttgg
 301 aagatgcggt gaatgagggg ctggaaatag tgagaggacg tctgcaaaat gctggcctaa
 361 atgtgactgt gaacgctact ttcatgtatt cggatggtct gattcataac tcaggcgact
 421 gccggagtag cacctgtgaa ggcctcgacc tactcaggaa aatttcaaat gcacaacgga
 481 tgggctgtgt cctcataggg ccctcatgta catactccac cttccagatg taccttgaca
 541 cagaattgag ctaccccatg atctcagctg gaagttttgg attgtcatgt gactataaag
 601 aaaccttaac caggctgatg tctccagcta gaaagttgat gtacttcttg gttaactttt
 661 ggaaaaccaa cgatctgccc ttcaaaactt attcctggag cacttcgtat gtttacaaga
 721 atggtacaga aactgaggac tgtttctggt accttaatgc tctggaggct agcgtttcct
 781 atttctccca cgaactcggc tttaaggtgg tgttaagaca agataaggag tttcaggata
 841 tcttaatgga ccacaacagg aaaagcaatg tgattattat gtgtggtggt ccagagttcc
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901 tctacaagct gaagggtgac cgagcagtgg ctgaagacat tgtcattatt ctagtggatc
961 ttttcaatga ccagtacttt gaggacaatg tcacagcccc tgactatatg aaaaatgtcc
1021 ttgttctgac gctgtctcct gggaattccc ttctaaatag ctctttctcc aggaatctat
1081 caccaacaaa acgagacttt gctcttgcct atttgaatgg aatcctgctc tttggacata
1141 tgctgaagat atttcttgaa aatggagaaa atattaccac ccccaaattt gctcatgctt
1201 tcaggaatct cacttttgaa gggtatgacg gtccagtgac cttggatgac tgggggggatg
1261 ttgacagtac catggtgctt ctgtatacct ctgtggacac caagaaatac aaggttcttt
1321 tgacctatga tacccacgta aataagacct atcctgtgga tatgagcccc acattcactt
1381 ggaagaactc taaacttcct aatgatatta caggccgggg ccctcagatc ctgatgattg
1441 cagtcttcac cctcactgga gctgtggtgc tgctcctgct cgtcgctctc ctgatgctca
1501 gaaaatatag aaaagattat gaacttcgtc agaaaaaatg gtcccacatt cctcctgaaa
1561 atatctttcc tctggagacc aatgagacca atcatgttag cctcaagatc gatgatgaca
1621 aaagacgaga tacaatccag agactacgac agtgcaaata cgacaaaaag cgagtgattc
1681 tcaaagatct caagcacaat gatggtaatt tcactgaaaa acagaagata gaattgaaca
1741 agttgcttca gattgactat tacaacctga ccaagttcta cggcacagtg aaacttgata
1801 ccatgatctt cggggtgata gaatactgtg agagaggatc cctccgggaa gttttaaatg
1861 acacaatttc ctaccctgat ggcacattca tggattggga gtttaagatc tctgtcttgt
1921 atgacattgc taagggaatg tcatatctgc actccagtaa gacagaagtc catggtcgtc
1981 tgaaatctac caactgcgta gtggacagta gaatggtggt gaagatcact gattttggct
2041 gcaattccat tttacctcca aaaaaggacc tgtggacagc tccagagcac ctccgccaag
2101 ccaacatctc tcagaaagga gatgtgtaca gctatgggat catcgcacag gagatcatcc
2161 tgcggaaaga aaccttctac actttgagct gtcgggaccg gaatgagaag attttcagag
2221 tggaaaattc caatggaatg aaaacccttcc gcccagattt attcttggaa acagcagagg
2281 aaaaagagct agaagtgtac ctacttgtaa aaaactgttg ggaggaagat ccagaaaaga
2341 gaccagattt caaaaaaatt gagactacac ttgccaagat atttggactt tttcatgacc
2401 aaaaaaatga aagctatatg gataccttga tccgacgtct acagctatat tctcgaaacc
2461 tggaacatct ggtagaggaa aggacacagc tgtacaaggc agagagggac agggctgaca
2521 gacttaactt tatgttgctt ccaaggctag tggtaaagtc tctgaaggag aaaggctttg
2581 tggagccgga actatatgag gaagttacaa tctacttcag tgacattgta ggtttcacta
2641 ctatctgcaa atacagcacc cccatggaag tggtggacat gcttaatgac atctataaga
2701 gttttgacca cattgttgat catcatgatg tctacaaggt ggaaaccatc ggtgatgcgt
2761 acatggtggc tagtggtttg cctaagagaa atggcaatcg gcatgcaata gacattgcca
2821 agatggcctt ggaaatcctc agcttcatgg ggacctttga gctggagcat cttcctggcc
2881 tcccaatatg gattcgcatt ggagttcact ctggtccctg tgctgctgga gttgtgggaa
2941 tcaagatgcc tcgttattgt ctatttggag atacggtcaa cacagcctct aggatggaat

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3001 ccactggcct ccctttgaga attcacgtga gtggctccac catagccatc ctgaagagaa
3061 ctgagtgcca gttcctttat gaagtgagag gagaaacata cttaaaggga agaggaaatg
3121 agactaccta ctggctgact gggatgaagg accagaaatt caacctgcca acccctccta
3181 ctgtggagaa tcaacagcgt ttgcaagcag aattttcaga catgattgcc aactctttac
3241 agaaaagaca ggcagcaggg ataagaagcc aaaaacccag acgggtagcc agctataaaa
3301 aaggcactct ggaatacttg cagctgaata ccacagacaa ggagagcacc tatttttaaa
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Amino acid sequence for human GCC (GenPept Accession No. NP_004954; SEQ ID NO: 3):

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   1 mktllldlal wsllfqpgwl sfssqvsqnc hngsyeisvl mmgnsafaep lknledavne
  61 gleivrgrlq naglnvtvna tfmysdglih nsgdcrsstc egldllrkis naqrmgcvli
 121 gpsctystfq myldtelsyp misagsfgls cdyketltrl msparklmyf lvnfwktndl
 181 pfktyswsts yvykngtete dcfwylnale asvsyfshel gfkvvlrqdk efqdilmdhn
 241 rksnviimcg gpeflyklkg dravaedivi ilvdlfndqy fednvtapdy mknvlvltls
 301 pgnsllnssf srnlsptkrd falaylngil lfghmlkifl engenittpk fahafrnltf
 361 egydgpvtld dwgdvdstmv llytsvdtkk ykvlltydth vnktypvdms ptftwknskl
 421 pnditgrgpq ilmiavftlt gavvllllva llmlrkyrkd yelrqkkwsh ippenifple
 481 tnetnhvslk idddkrrdti qrlrqckydk krvilkdlkh ndgnftekqk ielnkllqid
 541 yynltkfygt vkldtmifgv ieycergslr evlndtisyp dgtfmdwefk isvlydiakg
 601 msylhsskte vhgrlkstnc vvdsrmvvki tdfgcnsilp pkkdlwtape hlrqanisqk
 661 gdvysygiia qeiilrketf ytlscrdrne kifrvensng mkpfrpdlfl etaeekelev
 721 yllvkncwee dpekrpdfkk iettlakifg lfhdqknesy mdtlirrlql ysrnlehlve
 781 ertqlykaer dradrlnfml lprlvvkslk ekgfvepely eevtiyfsdi vgfttickys
 841 tpmevvdmln diyksfdhiv dhhdvykvet igdaymvasg lpkrngnrha idiakmalei
 901 lsfmgtfele hlpglpiwir igvhsgpcaa gvvgikmpry clfgdtvnta srmestglpl
 961 rihvsgstia ilkrtecqfl yevrgetylk grgnettywl tgmkdqkfnl ptpptvenqq
1021 rlqaefsdmi anslqkrqaa girsqkprrv asykkgtley lqlnttdkes tyf
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The GCC protein has some generally accepted domains each of which contributes a separable function to the GCC molecule. The portions of GCC include a signal sequence (for directing the protein to the cell surface) from amino acid residue 1 to about residue 23, or residue 1 to about residue 21 of SEQ ID NO: 3 (excised for maturation to yield functional mature protein

from about amino acid residues 22 or 24 to 1073 of SEQ ID NO: 3), an extracellular domain for ligand, e.g., guanylin or ST, binding from about amino acid residue 24 to about residue 420, or about residue 54 to about residue 384 of SEQ ID NO: 3, a transmembrane domain from about amino acid residue 431 to about residue 454, or about residue 436 to about residue 452 of SEQ ID NO: 3, a kinase homology domain, predicted to have tyrosine kinase activity from about amino acid residue 489 to about residue 749, or about residue 508 to about residue 745 of SEQ ID NO: 3 and a guanylyl cyclase catalytic domain from about residue 750 to about residue 1007, or about residue 816 to about residue 1002 of SEQ ID NO: 3.

In normal human tissues, GCC is expressed at the mucosal cells, e.g., at the apical brush border membranes, lining the small intestine, large intestine and rectum (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996)). GCC expression is maintained upon neoplastic transformation of intestinal epithelial cells, with expression in all primary and metastatic colorectal tumors (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. Eur J Cancer 41: 1618-1627 (2005); Carrithers et al., *Gastroenterology* 107: 1653-1661 (1994)). Neoplastic cells from the stomach, esophagus and the gastroesophageal junction also express GCC (see, e.g., U.S. Pat. No. 6,767,704; Debruyne et al. Gastroenterology 130:1191-1206 (2006)). The tissue-specific expression and association with cancer, e.g., of gastrointestinal origin, (e.g., colorectal cancer, stomach cancer, esophageal cancer or small intestine cancer), can be exploited for the use of GCC as a diagnostic marker for this disease (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. *Eur J Cancer* 41: 1618-1627 (2005)). Additionally, as demonstrated in the Examples herein, several different types of cancer of non-gastrointestinal origin have been shown to express GCC, including but not limited to pancreatic cancer, lung cancer, soft-tissue sarcomas (e.g., leiomyosarcoma and rhabdomyosarcoma) gastrointestinal or bronchopulmonary neuroendocrine tumors, and neuroectodermal tumors.

As a cell surface protein, GCC can also serve as a diagnostic or therapeutic target for receptor binding proteins such as antibodies or ligands. In normal intestinal tissue, GCC is expressed on the apical side of epithelial cell tight junctions that form an impermeable barrier between the luminal environment and vascular compartment (Almenoff et al., Mol Microbiol 8:

865-873); Guarino et al., Dig Dis Sci 32: 1017-1026 (1987)). As such, systemic intravenous administration of a GCC-binding protein therapeutic will have minimal effect on intestinal GCC receptors, while having access to neoplastic cells of the gastrointestinal system, including invasive or metastatic colon cancer cells, extraintestinal or metastatic colon tumors, esophageal tumors or stomach tumors, adenocarcinoma at the gastroesophageal junction. Additionally, GCC internalizes through receptor mediated endocytosis upon ligand binding (Buc et al. *Eur J Cancer* 41: 1618-1627 (2005); Urbanski et al., *Biochem Biophys Acta* 1245: 29-36 (1995)).

Polyclonal antibodies raised against the extracellular domain of GCC (Nandi et al. *Protein Expr. Purif.* 8:151-159 (1996)) were able to inhibit the ST peptide binding to human and rat GCC and inhibit ST-mediated cGMP production by human GCC.

GCC has been characterized as a protein involved in cancers, including colon cancers. See also, Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. Eur J Cancer 41: 1618-1627 (2005); Carrithers et al., *Gastroenterology* 107: 1653-1661 (1994); Urbanski et al., *Biochem Biophys Acta* 1245: 29-36 (1995). Antibody molecules directed to GCC can thus be used alone in unconjugated form to inhibit the GCC-expressing cancerous cells. Additionally, antibody molecules directed to GCC can be used in naked or labeled form, to detect GCC-expressing cancerous cells. Anti-GCC antibody molecules of the invention can bind human GCC. In some embodiments, an anti-GCC antibody molecule of the invention can inhibit the binding of a ligand, e.g., guanylin or heat-stable enterotoxin to GCC. In other embodiments, an anti-GCC antibody molecule of the invention does not inhibit the binding of a ligand, e.g., guanylin or heat-stable enterotoxin to GCC.

Monoclonal antibodies specific for GCC include GCC:B10 (Nandi et al., *J. Cell. Biochem.* 66:500-511 (1997)), GCC:4D7 (Vijayachandra et al. *Biochemistry* 39:16075-16083 (2000)) and GCC:C8 (Bakre et al. *Eur. J. Biochem.* 267:179-187 (2000)). GCC:B10 has a kappa light chain and an IgG2a isotype and cross-reacts to rat, pig and monkey GCC. GCC:B10 binds to the first 63 amino acids of the intracellular domain of GCC, specifically to residues 470-480 of SEQ ID NO: 3 (Nandi et al. *Protein Sci.* 7:2175-2183 (1998)). GCC:4D7 binds to the kinase

homology domain, within residues 491-568 of GCC (Bhandari et al. *Biochemistry* 40:9196-9206 (2001)). GCC:C8 binds to the protein kinase-like domain in the cytoplasmic portion of GCC.

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those known in the art. GenBank or GenPept accession numbers and useful nucleic acid and peptide sequences can be found at the website maintained by the National Center for Biotechnological Information, Bethesda Md. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation and transfection (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to methods known in the art, e.g., as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000)) or see generally, Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, described herein are known in the art. Furthermore, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

As used herein, the term "antibody molecule" refers to an antibody, antibody peptide(s) or immunoglobulin, or an antigen binding fragment of any of the foregoing, e.g., of an antibody. Antibody molecules include single chain antibody molecules, e.g., 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), and single domain antibody molecules, see, e.g., WO9404678. Although not within the

term "antibody molecules," the invention also includes "antibody analog(s)," other non-antibody molecule protein-based scaffolds, e.g., fusion proteins and/or immunoconjugates that use CDRs to provide specific antigen binding.

An "anti-GCC antibody molecule" refers to an antibody molecule (i.e., an antibody, antigen-binding fragment of an antibody or antibody analog) which interacts with or recognizes, e.g., binds (e.g., binds specifically) to GCC, e.g., human GCC. Exemplary anti-GCC antibody molecules are such as those summarized in Tables 1 and 2.

As used herein, the term "antibody," "antibody peptide(s)" or "immunoglobulin" refers to single chain, two-chain, and multi-chain proteins and glycoproteins. The term antibody includes polyclonal, monoclonal, chimeric, CDR-grafted and human or humanized antibodies, all of which are discussed in more detail elsewhere herein. Also included within the term are camelid antibodies, see, e.g., US2005/0037421, and nanobodies, e.g., IgNARs (shark antibodies), see, e.g., WO03/014161. The term "antibody" also includes synthetic and genetically engineered variants.

As used herein, the term "antibody fragment" or "antigen binding fragment" of an antibody refers, e.g., to Fab, Fab', $F(ab')_2$, and Fv fragments, single chain antibodies, functional heavy chain antibodies (nanobodies), as well as any portion of an antibody having specificity toward at least one desired epitope, that competes with the intact antibody for specific binding (e.g., a fragment having sufficient CDR sequences and having sufficient framework sequences so as to bind specifically to an epitope). E.g., an antigen binding fragment can compete for binding to an epitope which binds the antibody from which the fragment was derived. Derived, as used in this and similar contexts, does not imply any particular method or process of derivation, but can refer merely to sequence similarity. Antigen binding fragments can be produced by recombinant techniques, or by enzymatic or chemical cleavage of an intact antibody. The term, antigen binding fragment, when used with a single chain, e.g., a heavy chain, of an antibody having a light and heavy chain means that the fragment of the chain is sufficient such that when paired

with a complete variable region of the other chain, e.g., the light chain, it will allow binding of at least 25, 50, 75, 85 or 90% of that seen with the whole heavy and light variable region.

The term, "antigen binding constellation of CDRs" or "a number of CDRs sufficient to allow binding" (and similar language), as used herein, refers to sufficient CDRs of a chain, e.g., the heavy chain, such that when placed in a framework and paired with a complete variable region of the other chain, or with a portion of the other chain's variable region of similar length and having the same number of CDRs, e.g., the light chain, will allow binding, e.g., of at least 25, 50, 75, 85 or 90% of that seen with the whole heavy and light variable region.

As used herein, the term "humanized antibody" refers to an antibody that is derived from a non-human antibody e.g., rabbit, rodent (e.g., murine), sheep or goat, that retains or substantially retains the antigen-binding properties of the parent antibody but is less immunogenic in humans. Humanized as used herein is intended to include deimmunized antibodies. Typically, humanized antibodies include non-human CDRs and human or human derived framework and constant regions.

The term "modified" antibody, as used herein, refers to antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial antibody library, antibodies isolated from a non-human animal (e.g., a rabbit, mouse, rat, sheep or goat) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such modified antibodies include humanized, CDR grafted (e.g., an antibody having CDRs from a first antibody and a framework region from a different source, e.g., a second antibody or a consensus framework), chimeric, *in vitro* generated (e.g., by phage display) antibodies, and may optionally include variable or constant regions derived from human germline immunoglobulin sequences or human immunoglobulin genes or antibodies which have been prepared, expressed, created or isolated by any means that involves splicing of human immunoglobulin gene sequences to alternative

immunoglobulin sequences. In embodiments a modified antibody molecule includes an antibody molecule having a sequence change from a reference antibody.

The term "monospecific antibody" refers to an antibody or antibody preparation that displays a single binding specificity and affinity for a particular epitope. This term includes a "monoclonal antibody" or "monoclonal antibody composition."

The term "bispecific antibody" or "bifunctional antibody" refers to an antibody that displays dual binding specificity for two epitopes, where each binding site differs and recognizes a different epitope.

The terms "non-conjugated antibody" and "naked antibody" are used interchangeably to refer to an antibody molecule that is not conjugated to a non-antibody moiety, e.g., an agent or a label.

Each of the terms "immunoconjugate", “antibody-drug conjugate” and "antibody conjugate", are used interchangeably and refer to an antibody that is conjugated to a non-antibody moiety, e.g., an agent or a label. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The term "therapeutic agent" refers to an agent that has biological activity. Exemplary therapeutic agents are chemotherapeutic agents.

The term "anti-cancer agent" or "chemotherapeutic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia Inhibition of metastasis or angiogenesis is frequently a property of anti-cancer or chemotherapeutic agents. A chemotherapeutic agent may be a cytotoxic or cytostatic agent. The term "cytostatic agent" refers to an agent which inhibits or suppresses cell growth and/or multiplication of cells.

"Cytotoxic agents" refer to compounds which cause cell death primarily by interfering directly with the cell's functioning, including, but not limited to, alkylating agents, tumor

necrosis factor inhibitors, intercalators, microtubule inhibitors, kinase inhibitors, proteasome inhibitors and topoisomerase inhibitors. A "toxic payload" as used herein refers to a sufficient amount of cytotoxic agent which, when delivered to a cell results in cell death. Delivery of a toxic payload may be accomplished by administration of a sufficient amount of immunoconjugate comprising an antibody or antigen binding fragment of the invention and a cytotoxic agent. Delivery of a toxic payload may also be accomplished by administration of a sufficient amount of an immunoconjugate comprising a cytotoxic agent, wherein the immunoconjugate comprises a secondary antibody or antigen binding fragment thereof which recognizes and binds an antibody or antigen binding fragment of the invention.

As used herein the phrase, a sequence "derived from" or "specific for a designated sequence" refers to a sequence that comprises a contiguous sequence of approximately at least 6 nucleotides or at least 2 amino acids, at least about 9 nucleotides or at least 3 amino acids, at least about 10-12 nucleotides or 4 amino acids, or at least about 15-21 nucleotides or 5-7 amino acids corresponding, i.e., identical or complementary to, e.g., a contiguous region of the designated sequence. In certain embodiments, the sequence comprises all of a designated nucleotide or amino acid sequence. The sequence may be complementary (in the case of a polynucleotide sequence) or identical to a sequence region that is unique to a particular sequence as determined by techniques known in the art. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, regions encoding CDRs, regions encoding framework sequences, regions encoding constant domain regions, regions encoding variable domain regions, as well as non-translated and/or non-transcribed regions. The derived sequence will not necessarily be derived physically from the sequence of interest under study, but may be generated in any manner, including, but not limited to, chemical synthesis, replication, reverse transcription or transcription, that is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified or combined in ways known in the art to be consistent with the intended use. For example, a

sequence may comprise two or more contiguous sequences which each comprise part of a designated sequence, and are interrupted with a region which is not identical to the designated sequence but is intended to represent a sequence derived from the designated sequence. With regard to antibody molecules, "derived therefrom" includes an antibody molecule which is functionally or structurally related to a comparison antibody, e.g., "derived therefrom" includes an antibody molecule having similar or substantially the same sequence or structure, e.g., having the same or similar CDRs, framework or variable regions. "Derived therefrom" for an antibody also includes residues, e.g., one or more, e.g., 2, 3, 4, 5, 6 or more residues, which may or may not be contiguous, but are defined or identified according to a numbering scheme or homology to general antibody structure or three-dimensional proximity, i.e., within a CDR or a framework region, of a comparison sequence. The term "derived therefrom" is not limited to physically derived therefrom but includes generation by any manner, e.g., by use of sequence information from a comparison antibody to design another antibody.

As used herein, the phrase "encoded by" refers to a nucleic acid sequence that codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, at least 8 to 10 amino acids, or at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence.

As used herein, the term “colorectal cancer”, also commonly known as colon cancer or bowel cancer, refers to cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix.

The terms “stomach cancer” and “gastric cancer” are used herein interchangeably.

Calculationsof "homology" between two sequences can be performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is at least 30%, 40%, or 50%, at least 60%, or at least 70%, 80%, 90%, 95%, 100% of the length of the reference sequence. The amino acid residues or

nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, 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 homology between two sequences can be accomplished using a mathematical algorithm. The percent homology between two amino acid sequences can be determined using any method known in the art. For example, 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, 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. The percent homology between two nucleotide sequences can also be determined using the GAP program in the GCG software package (Accelerys, Inc. San Diego, Calif.), using an NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. An exemplary set of parameters for determination of homology are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45° C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50° C. (the temperature of the washes can be increased to 55° C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at

about 45° C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60° C; 3) high stringency hybridization conditions in 6X SSC at about 45° C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65° C; and 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65° C, followed by one or more washes at 0.2X SSC, 1% SDS at 65° C. Very high stringency conditions (4) are often the preferred conditions and the ones that should be used unless otherwise specified.

It is understood that the antibodies and antigen binding fragment thereof of the invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect desired biological properties, such as binding activity, can be determined as described in Bowie, J U et al. *Science* 247:1306-1310 (1990) or Padlan et al. *FASEB J.* 9:133-139 (1995). A "conservative amino acid substitution" is one 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., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. In an antibody, an essential amino acid residue can be a specificity determining residue (SDR).

As used herein, the term "isolated" refers to material that is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same

polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide or polypeptide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, e.g., a mixture, solution or suspension or comprising an isolated cell or a cultured cell which comprises the polynucleotide or polypeptide, and still be isolated in that the vector or composition is not part of its natural environment.

As used herein, the term "replicon" refers to any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

As used herein, the term "operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequence.

As used herein, the term "vector" refers to a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

As used herein, the term "control sequence" refers to a polynucleotide sequence that is necessary to effect the expression of a coding sequence to which it is ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include a promoter, a ribosomal binding site and terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

As used herein, the term "purified product" refers to a preparation of the product which has been isolated from the cellular constituents with which the product is normally associated and/or from other types of cells that may be present in the sample of interest.

As used herein, the term "epitope" refers to a protein determinate capable of binding specifically to an antibody. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Some epitopes are linear epitopes while others are conformational epitopes. A linear epitope is an epitope wherein a contiguous amino acid primary sequence comprises the epitope recognized. A linear epitope typically includes at least 3, and more usually, at least 5, for example, about 8 to about 10 contiguous amino acids. A conformational epitope can result from at least two situations, such as: a) a linear sequence which is only exposed to antibody binding in certain protein conformations, e.g., dependent on ligand binding, or dependent on modification (e.g., phosphorylation) by signaling molecules; or b) a combination of structural features from more than one part of the protein, or in multisubunit proteins, from more than one subunit, wherein the features are in sufficiently close proximity in 3-dimensional space to participate in binding.

As used herein, "isotype" refers to the antibody class (e.g., IgM, IgA, IgE or IgG) that is encoded by heavy chain constant region genes.

As used herein, the terms "detectable agent," "label" or "labeled" are used to refer to incorporation of a detectable marker on a polypeptide or glycoprotein. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., indium (^{111}In), iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), bismuth (^{212}Bi or ^{213}Bi), sulfur (^{35}S), carbon (^{14}C), tritium (^{3}H), rhodium (^{188}Rh), technetium (^{99} mTc), praseodymium, or phosphorous (^{32}P) or a positron-emitting radionuclide, e.g., carbon-11 (^{11}C), potassium-40 (^{40}K), nitrogen-13 (^{13}N), oxygen-15 (^{15}O) $_{9}$, fluorine-18 (^{18}F), gallium-68 (^{68}Ga), and iodine-121 (^{121}I)), fluorescent labels (e.g., FITC, rhodamine, lanthanide

phosphors), enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups (which can be detected by a marked avidin, e.g., a molecule containing a streptavidin moiety and a fluorescent marker or an enzymatic activity that can be detected by optical or calorimetric methods), and predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

As used herein, "specific binding," "bind(s) specifically" or "binding specificity" means, for an anti-GCC antibody molecule, that the antibody molecule binds to GCC, e.g., human GCC protein, with greater affinity than it does to a non-GCC protein, e.g., BSA. Typically an anti-GCC molecule will have a K_d for the non-GCC protein, e.g., BSA, which is greater than 2, greater than 10, greater than 100, greater than 1,000 times, greater than 10^4, greater than 10^5, or greater than 10^6 times its K_d for GCC, e.g., human GCC protein. In determination of K_d, the K K_d for GCC and the non-GCC protein, e.g., BSA, should be done under the same conditions.

The term “affinity” or “binding affinity” refers to the apparent association constant or K_a. The K_a is the reciprocal of the dissociation constant (K_d). An antibody may, for example, have a binding affinity of at least 10^5, 10^6, 10^7,10^8, 10^9, 10^{10} and 10^{11} M^{-1} for a particular target molecule. Higher affinity binding of an antibody to a first target relative to a second target can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first target than the K_A (or numerical value K_D) for binding the second target. In such cases, the antibody has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, or 10^5 fold.

Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface 19act cc resonance, or spectroscopy

(e.g., using a fluorescence assay). For example, relative affinity of an anti-GCC antibody molecule can be measured from ELISA measurements against GCC protein (e.g., the immunogen used to raise anti-GCC antibody molecules), by FACS measurements with GCC expressing cells.

Exemplary conditions for evaluating binding affinity are in TRIS-buffer (50mM TRIS, 150mM NaCl, 5mM $CaCl_2$ at pH7.5). These techniques can be used to measure the concentration of bound and free binding protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[Bound] = N \cdot [Free]/((1/K_A) + [Free]).$$

It is not always necessary to make an exact determination of K_A, though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A, and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an *in vitro* or *in vivo* assay. Affinity of anti-GCC antibody molecules can also be measured using a technology such as real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S, and Urbaniczky, C., 1991, Anal. Chem. 63:2338-2345 and Szabo et al., 1995, Curr. Opin. Struct. Biol. 5:699-705). As used herein, "BIA" or "surface plasmon resonance" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIACORE™). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

The measurement of affinity of anti-GCC antibody molecules using a BIACORE™ T100 system (GE Healthcare, Piscataway, N.J.) is described in Example 1 of U.S. Patent Application Publication No. US2011/0110936, the contents of which are incorporated by reference in its

entirety. Briefly, an anti-GCC antibody (Prep A) is diluted to an appropriate concentration (e.g., 20 μg/mL) 10 mM sodium acetate, pH 4.0 and a referenc/control antibody (Prep B) is diluted to an appropriate concentration (e.g., 10 μg/mL) in 10 mM sodium acetate, pH 4.0. Each antibody is covalently immobilized to several CM4 BIACORE chips using standard amine coupling. For each CM4 chip prepared, Prep A antibody is immobilized over two flow cells at around 75-100 RU while Prep B antibody is immobilized to one flow cell at around 70-80 RU. The remaining fourth flow cell of each CM4 chip is used as the reference flow cell. The concentration of GCC protein can be determined using the methods detailed by Pace et al. in Protein Science, 4:2411 (1995), and Pace and Grimsley in Current Protocols in Protein Science 3.1.1-3.1.9 (2003). For each prepared CM4 chip, GCC protein is injected for 2 minutes at a concentration range of 202 nM -1.6 nM (2.times. serial dilution) followed by a 7 minute dissociation. Samples are randomly injected in triplicate with several buffer inject cycles interspersed for double referencing. To obtain more significant off-rate decay data, three additional 101 nM GCC protein injections and three additional buffer injections are performed with a 2 minute injection and a 4 hour dissociation time. A flow rate of 100 μL/min is used for all experiments and all surfaces are regenerated with a 20 second pulse of 10 mM Glycine-HCl (pH 2.0). All samples are prepared in the running buffer (e.g., Hepes-buffered saline, 0.005% polysorbate 20, pH 7.4 (HBS-P)) with 100 μg/mL of BSA added. All sensorgram (plot of surface plasmon resonance vs time) data can be processed with Scrubber 2.0 software (BioLogic Software, Campbell, Australia) and globally fit to a 1:1 interaction model including a term for the mass transport constant k_m using CLAMP^{TM} software (Myszka and Morton Trends Biochem. Sci. 23:149-150 (1998)).

As used herein, the term "treat" or "treatment" is defined as the administration of an anti-GCC antibody molecule to a subject, e.g., a patient, or administration, e.g., by application, to an isolated tissue or cell from a subject which is returned to the subject. The anti-GCC antibody molecule can be administered alone or in combination with a second agent. The treatment can be to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder, e.g., a cancer. While not wishing to be bound by theory, treating is believed to cause the inhibition, ablation, or killing of

a cell *in vitro* or *in vivo*, or otherwise reducing capacity of a cell, e.g., an aberrant cell, to mediate a disorder, e.g., a disorder as described herein (e.g., a cancer).

As used herein, the term "subject" is intended to include mammals, primates, humans and non-human animals. For example, a subject can be a patient (e.g., a human patient or a veterinary patient), having a cancer in which at least some of the cells express GCC, such as a cancer of gastrointestinal origin (e.g., colorectal cancer, stomach cancer, small intestine cancer, or esophageal cancer), pancreatic cancer, lung cancer (e.g., squamous cell carcinoma, adenosquamous carcinoma, adenocarcinoma), soft-tissue sarcoma (e.g., leiosacroma or rhabdomyosarcoma), neuroendocrine tumors (e.g., gastrointestinal or bronchopulmonary), or neuroectodermal tumors; a symptom of such GCC expressing cancers; or a predisposition toward such GCC-expressing cancers. As another example, the subject can be a patient having a gastrointestinal disorder in which at least some of the cells of the gastrointestinal system express GCC, such in as inflammatory bowel syndrome, Crohn's disease and constipation. As yet another example, the subject can be a patient having a neurological disorder in which at least some neurons within the patients central nervous system express GCC, such as Parkinson's Disease. The term "non-human animals" of the invention includes all non-human vertebrates, e.g., non-human mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, mouse, rat, rabbit or goat etc., unless otherwise noted. In an embodiment, "subject" excludes one or more or all of a mouse, rat, rabbit or goat.

As used herein, an amount of an anti-GCC antibody molecule "effective" or "sufficient" to treat a disorder, or a "therapeutically effective amount" or "therapeutically sufficient amount" refers to an amount of the antibody molecule which is effective, upon single or multiple dose administration to a subject, in treating a cell, e.g., cancer cell (e.g., a GCC-expressing tumor cell), or in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment. As used herein, "inhibiting the growth" of the tumor or cancer refers to slowing, interrupting, arresting or stopping its growth and/or metastases and does not necessarily indicate a total elimination of the tumor growth.

As used herein, "GCC," also known as "STAR", "GUC2C", "GUCY2C" or "ST receptor" protein refers to mammalian GCC, preferably human GCC protein. Human GCC refers to the protein shown in SEQ ID NO: 3 and naturally occurring allelic protein variants thereof. The allele in SEQ ID NO: 3 can be encoded by the nucleic acid sequence of GCC shown in SEQ ID NO: 2. Other variants are known in the art. See, e.g., accession number Ensp0000261170, Ensembl Database, European Bioinformatics Institute and Wellcome Trust Sanger Institute, which has a leucine at residue 281; SEQ ID NO: 14 of published US patent application number 20060035852; or GenBank accession number AAB 19934. Typically, a naturally occurring allelic variant has an amino acid sequence at least 95%, 97% or 99% identical to the GCC sequence of SEQ ID NO: 3. The transcript encodes a protein product of 1073 amino acids, and is described in GenBank accession no.: NM_004963. GCC protein is characterized as a transmembrane cell surface receptor protein, and is believed to play a critical role in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation.

Anti-GCC Antibodies

Described herein are anti-GCC antibody molecules useful, *inter alia* to detect GCC expression. The anti-GCC antibody molecules, e.g., useful for GCC detection, can include non-human anti-GCC antibody molecules (e.g., non-human and non-murine antibody molecules) that specifically bind to GCC, e.g., with a binding affinity at least 10^3, 10^4, 10^5, 10^6, 10^7,10^8, 10^9, 10^{10} and 10^{11} M^{-1} for GCC. The anti-GCC antibody molecule can be a non-human, non-murine and non-rat antibody molecule, e.g., a rabbit anti-GCC antibody molecule, e.g., as described herein.

In certain aspects, the invention relates to anti-GCC antibody molecules that include features such as those summarized in Tables 1 and 2. In other aspects, the invention relates to anti-GCC antibody molecules that include features such as those summarized in Tables 3, 4, 5 and/or 6.

In an embodiment, the anti-GCC antibody molecule is a rabbit hybridoma antibody and is one of antibody MIL-44-148-2 or MIL-44-67-4. In an embodiment, the anti-GCC antibody molecule is derived from antibody MIL-44-148-2 or MIL-44-67-4.

In an embodiment an anti-GCC antibody molecule will have an affinity for GCC, e.g., as measured by direct binding or competition binding assays. In an embodiment the anti-GCC antibody molecule has a K_d of less than $1x10^{-6}$ M, less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, less than $1x10^{-9}$ M, less than $1x10^{-10}$ M, less than $1x\ 10^{-11}$ M, less than $1x10^{-12}$ M, or less than $1x10^{-13}$ M. In an embodiment the antibody molecule is an IgG, or antigen-binding fragment thereof, and has a K_d of less than $1x10^{-6}$ M, less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, or less than $1x10^{-9}$ M. In an embodiment, an anti-GCC antibody molecule, e.g., a MIL-44-148-2 antibody or antibody derived therefrom has a K_d of about 80 to about 200 pM, preferably about 100 to about 150 pM or about 120 pM. In an embodiment, an anti-GCC antibody molecule, e.g., a MIL-44-148-2 antibody or antibody derived therefrom has a k_a of about 0.9 to about $1.25x10^5$ M^{-1} s^{-1}, preferably about $1.1x10^5$ M^{-1} s^{-1}. In an embodiment the antibody molecule is an ScFv and has a K_d of less than $1x10^{-6}$ M, less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, less than $1x10^{-9}$ M, less than $1x10^{-10}$ M, $1x\ 10^{-11}$ M, less than $1x10^{-12}$ M, or less than $1x10^{-13}$ M.

In embodiments, the antibody molecules are not immunoconjugates, i.e., are "naked" and in embodiments cause a cellular reaction upon binding to GCC. In related embodiments, the cellular reaction is performed by the GCC-expressing cell to which the antibody binds. Such a cellular reaction can be signal transduction mediated by GCC, e.g., if the antibody molecule is an agonist of GCC (see, e.g., US Patent Application publication no. US20040258687). In other embodiments, the cellular reaction is performed by a second cell, e.g., an immune effector cell (e.g., a natural killer cell) which recognizes the antibody molecule bound to GCC on the first cell. In some embodiments, surveillance molecules, e.g., complement molecules, contact the GCC-bound antibody molecule prior to the cellular reaction. The cellular reactions in these embodiments can cause death of the GCC-expressing cell.

In further embodiments, antibody molecules which are immunoconjugates can both cause a cellular reaction upon binding to GCC and internalize to deliver an agent to the GCC-expressing cell to which it binds.

In some embodiments, an anti-GCC antibody molecule of the invention can block ligand binding to GCC.

In an embodiment, the antibody molecule is not GCC:B10, GCC:4D7 or GCC:C8. In another embodiment, an anti-GCC antibody molecule does not bind an intracellular domain of GCC, about amino acid residue 455 to 1073 of SEQ ID NO: 3. For example, in this embodiment, an anti-GCC antibody molecule does not bind the kinase homology domain or the guanylyl cyclase domain of GCC.

The naturally occurring mammalian antibody structural unit is typified by a tetramer. Each tetramer is composed of two pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains can be classified as kappa and lambda light chains. Heavy chains can be classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). The variable regions of each light/heavy chain pair form the antibody binding site. Preferred isotypes for the anti-GCC antibody molecules are IgG immunoglobulins, which can be classified into four subclasses, IgG1, IgG2, IgG3 and IgG4, having different gamma heavy chains. Most therapeutic antibodies are human, chimeric, or humanized antibodies of the IgG1 isotype. In a particular embodiment, the anti-GCC antibody molecule is a rabbit IgG antibody.

The variable regions of each heavy and light chain pair form the antigen binding site. Thus, an intact IgG antibody has two binding sites which are the same. However, bifunctional or bispecific antibodies are artificial hybrid constructs which have two different heavy/light chain pairs, resulting in two different binding sites.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989). As used herein, CDRs are referred to for each of the heavy (HCDR1, HCDR2, HCDR3) and light (LCDR1, LCDR2, LCDR3) chains.

An anti-GCC antibody molecule can comprise all, or an antigen binding subset of the CDRs, of one or both, the heavy and light chain, of one of the above-referenced rabbit antibodies. Amino acid sequences of rabbit hybridoma antibodies, including variable regions and CDRs, can be found in Table 3 and Table 5.

Thus, in an embodiment the antibody molecule includes one or both of:

(a) one, two, three, or an antigen binding number of, light chain CDRs (LCDR1, LCDR2 and/or LCDR3) of one of the above-referenced rabbit hybridoma antibodies. In embodiments the CDR(s) may comprise an amino acid sequence of one or more or all of LCDR1-3 as follows: LCDR1, or modified LCDR1 wherein one to seven amino acids are conservatively substituted) LCDR2, or modified LCDR2 wherein one or two amino acids are conservatively substituted); or LCDR3, or modified LCDR3 wherein one or two amino acids are conservatively substituted; and

(b) one, two, three, or an antigen binding number of, heavy chain CDRs (HCDR1, HCDR2 and/or HCDR3) of one of the above-referenced rabbit hybridoma antibodies. In embodiments the CDR(s) may comprise an amino acid sequence of one or more or all of HCDR1-3 as follows: HCDR1, or modified HCDR1 wherein one or two amino acids are conservatively substituted; HCDR2, or modified HCDR2 wherein one to four amino acids are

conservatively substituted; or HCDR3, or modified HCDR3 wherein one or two amino acids are conservatively substituted.

Useful immunogens for production of anti-GCC antibody molecules include GCC e.g., human GCC-expressing cells (e.g., a tumor cell line, e.g., T84 cells, or fresh or frozen colon tumor cells, recombinant cells expressing GCC); membrane fractions of GCC-expressing cells (e.g., a colon tumor cell line, e.g., T84 cells), or fresh or frozen colonic tumor cells; recombinant cells expressing GCC; isolated or purified GCC, e.g., human GCC protein (e.g., biochemically isolated GCC, e.g., isolated from gastrointestinal tumor cells or recombinant cells expressing GCC or a variant thereof), or a portion thereof (e.g., the extracellular domain of GCC, the kinase homology domain of GCC or the guanylyl cyclase catalytic domain of GCC or peptide corresponding to a portion thereof, e.g., comprising at least about 8, 10, 12, 14, 16, 20, 24, 28 or 32 amino acid residues of SEQ ID NO: 3); or an immunogen comprising SEQ ID NO: 46 or comprising a mature portion thereof without the signal sequence (i.e., without amino acid residues 1 to about 21 or 23 of SEQ ID NO: 46), e.g., the mature hGCC(ECD)-mIgG2a FcR r-mutII (also referred to herein as "pLKTOK108") protein, SEQ ID NO: 48.

Immunogens can be fused to heterologous sequences to aid in biochemical manipulation, purification, immunization or antibody titer measurement. Such immunogens can comprise a portion of GCC, e.g., the extracellular domain, and a portion comprising a non-GCC polypeptide. Many options exist for constructing a fusion protein for ease of purification or immobilization onto a solid support, e.g., an affinity column or a microtiter plate or other suitable assay substrate/chip. For example, a fusion moiety can add a domain, e.g., glutathione-S-transferase/kinase (GST), which can bind glutathione; an Fc region of an immunoglobulin, which can bind to protein A or protein G; amino acid residues, e.g., two, three, four, five, preferably six histidine residues which can bind nickel or cobalt on an affinity column; an epitope tag, e.g., a portion of c-myc oncogene (myc-tag), a FLAG tag (U.S. Pat. No. 4,703,004), a hemagglutinin (HA) tag, a T7 gene 10 tag, a V5 tag, an HSV tag, or a VSV-G tag which can bind a tag-specific antibody; or a cofactor, e.g., biotin, which can bind streptavidin.

Immunogens which comprise the Fc portion of an immunoglobulin can hold the GCC, either in solution or attached to a cell, in a configuration which allows structural access to GCC epitopes by the host immune surveillance components for efficient antibody generation. Because immunoglobulin heavy chains comprising the Fc regions associate into dimers through interchain disulfide bonds, immunogens resulting from fusion with Fc regions are dimers. Valency of fusion proteins can reflect the type of immunoglobulin contributing an Fc region. For example, fusions with IgG proteins can be dimers, IgA fusions can make tetrameric immunogens, and IgM fusions can make decameric immunogens, the latter two is facilitated with co-transfection of the J chain. An exemplary immunoglobulin for an Fc fusion protein is IgG1. The portion used typically has the IgG1 hinge, CH2 and CH3 domains encoded by a single exon. Because this exon also has a portion of the CH1 region, which has a cysteine oriented to disulfide bond with a cysteine from the light chain, a useful modification is to mutate the CH1 cysteine, e.g., to a serine, to ensure there is no unpaired cysteine in the fusion protein. Such a mutation also increases flexibility of the hinge.

An Fc portion derived from a non-host species, e.g., human Ig Fc region, for fusing to an immunogen for immunization in a host species, e.g., mouse, rat, rabbit, goat, acts as an adjuvant. This adjuvant function can trigger specific antibodies against both Fc and GCC epitopes. Fc-reactive antibodies can be identified and discarded during screening. The Fc portion can have a wild type sequence or a sequence which is mutated to modify effector function. For example, a mutated constant region (variant) can be incorporated into a fusion protein to minimize binding to Fc receptors and/or ability to fix complement (see e.g. Winter et al, GB 2,209,757 B; Morrison et al., WO 89/07142; Morgan et al., WO 94/29351). In a preferred example, lysine 235 and glycine 237, numbered according to Fc region standards, are mutated, e.g., to alanine. An immunogen/fusion protein with Fc-mutated IgG can have reduced interaction with Fc receptors in the host. A preferred soluble immunogen fusion protein (after maturation to cleave the signal peptide and secretion) is hGCC(ECD)-mIgG2a FcR r-mutII (pLKTOK108), which consists of amino acid residues 24 to 430 of SEQ ID NO: 3 fused to mutated mouse IgG2a immunoglobulin Fc (collectively SEQ ID NO:48).

To prepare a cell-expressed immunogen, the immunoglobulin portion can be structured to mimic an immunoglobulin portion of the B cell receptor. For example, the immunoglobulin Fc region can be further fused to a polypeptide comprising a transmembrane region from an immune receptor, such as Fcγ receptors, Fcα receptors, Fcα/μ receptor or Fcε receptors. Proper orientation of such an Fc receptor cell-bound immunogen with adequate exposure on the cell surface may be improved if the cell expressing the immunogen fusion protein further comprises additional components of the antigen receptor complex, e.g., B cell IgM receptor or IgD receptor. Suitable components of the complex include immunoglobulin (Ig) sheath proteins, such as MB-1 and B29 (CD79A and CD79B; Hombach et al. Eur. J. Immunol. 20:2795-2799 (1990) for IgM receptor), which form a heterodimer. The Ig sheath proteins can be provided endogenously by the transfected cell, e.g., if transfecting a B cell lymphoma cell line; or by co-transfection of the immunogen with sheath proteins, e.g., in a separate vector or in the same vector.

Useful epitopes, e.g., reference epitopes, from the GCC molecule, to which the anti-GCC antibody molecules, e.g., rabbit monoclonal antibodies, or humanized versions thereof, as described herein, can bind, can be found on the extracellular portion of GCC. Such GCC epitopes can bind antibody molecules on the surface of cells, e.g., on the cell exterior.

For example, an epitope for an anti-GCC antibody molecule can reside within, or include a residue(s) from, residues 1-50 of SEQ ID NO: 3, or a fragment thereof that binds an anti-GCC antibody molecule of the invention, e.g., a MIL-44-148-2-binding fragment thereof. Such fragments can comprise residues 1-25, 5-30, 10-35, 15-40, 20-45, 25-50, 5-45, 10-40, 15-35, 20-30 or 33-50 of SEQ ID NO: 3. In some embodiments, an epitope for an anti-GCC antibody molecule, e.g., a MIL-44-148-2 antibody, is a conformational epitope further comprising one or more additional amino acid residues in the GCC amino acid sequence beyond residue 50, i.e., selected from about residue 50 to 1073 of SEQ ID NO: 3.

Antibodies raised against such epitopes or the extracellular domain, e.g., epitopes that reside within, or include a residue(s) from amino acid residues 24 to 420 of SEQ ID NO: 3, or a

reference portion thereof, e.g., residues 24 to 75, 75 to 150, 150 to 225, 225 to 300, 300 to 375 or 375 to 420 of GCC, or antibody molecules derived therefrom, can be useful as therapeutic or diagnostic antibodies, as described herein.

In an embodiment, the anti-GCC antibody molecule has one or more of the following properties:

a) it competes for binding, e.g., binding to cell surface GCC or purified GCC, with one of the above-referenced anti-GCC antibody molecules summarized in Tables 1 and 2 e.g., rabbit hybridoma antibodies (e.g., MIL-44-148-2);

b) it binds to the same, or substantially the same, epitope on GCC as one of the above-referenced anti-GCC antibody molecules summarized in Tables 1 and 2, e.g., e.g., rabbit hybridoma antibodies (e.g., MIL-44-148-2). In an embodiment, the antibody binds the same epitope, as determined by one or more of a peptide array assay or by binding to truncation mutants, chimeras or point mutants expressed on the cell surface or membrane preparations, e.g., as those assays are described herein;

c) it binds to an epitope which has at least 1, 2, 3, 4, 5, 8, 10, 15 or 20 contiguous amino acid residues in common with the epitope of one of the above-referenced anti-GCC antibody molecules summarized in Tables 1 and 2, e.g., rabbit hybridoma antibodies (e.g., MIL-44-148-2);

d) it binds a region of human GCC that is bound by an anti-GCC antibody of the invention, wherein the region e.g., an extracellular or cytoplasmic region, is 10-15, 10-20, 20-30, or 20-40 residues in length, and binding is determined, e.g., by binding to truncation mutants; In an embodiment the anti-GCC antibody molecule binds the extracellular region of human GCC. In an embodiment an anti-GCC antibody molecule can bind the human GCC portion of the extracellular domain defined by amino acid residues 24 to 420 of SEQ ID NO: 3. In an embodiment an anti-GCC antibody molecule can bind the guanylate cyclase signature site at amino acid residues 931 to 954 of SEQ ID NO: 3; or

e) it binds to a reference epitope described herein.

In an embodiment the anti-GCC antibody molecule binds the GCC sequence ILVDLFNDQYFEDNVTAPDYMKNVLVLTLS (SEQ ID NO: 8).

In an embodiment the anti-GCC antibody molecule binds the GCC sequence FAHAFRNLTFEGYDGPVTLDDWGDV (SEQ ID NO: 9).

In an embodiment the antibody molecule binds a conformational epitope. In other embodiments an antibody molecule binds a linear epitope.

The anti-GCC antibody molecules can be polyclonal antibodies, monoclonal antibodies, monospecific antibodies, chimeric antibodies (See U.S. Pat. No. 6,020,153) or humanized antibodies or antibody fragments or derivatives thereof. Synthetic and genetically engineered variants (See U.S. Pat. No. 6,331,415) of any of the foregoing are also contemplated by the present invention. Monoclonal antibodies can be produced by a variety of techniques, including conventional murine monoclonal antibody methodology (e.g., the standard somatic cell hybridization technique of Kohler and Milstein, Nature 256: 495 (1975); see generally, Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y), and the rabbit monoclonal antibody technology and services provided by Epitomics (Burlingame, CA) which produces custom rabbit monoclonal antibodies (RabMAbs®) using rabbit-rabbit hybridomas generated by fusing isolated B-cells from an immunized rabbit with Epitomics' proprietary fusion partner cell line, as described in U.S. Patents 7,402,409, 7,429,487, 7,462,697, 7,575,896, 7,732,168, and 8,062,867, each of which are incorporated by reference herein in their entireties.

Immunization with protein, e.g., GCC or a soluble portion, or fusion protein comprising a portion of GCC (e.g., hGCC(ECD)-mIgG2a FcRbr-mutII (pLKTOK108), or cells or membrane fractions therefrom, e.g., cells expressing surface-exposed GCC or a portion thereof (e.g., the pLKTOK4 product), can be performed with the immunogen prepared for injection in a manner to induce a response, e.g., with adjuvant, e.g., complete Freund's adjuvant. Other suitable adjuvants

include, Titermax Gold® adjuvant (CYTRX Corporation, Los Angeles, Calif.) and alum. Small peptide immunogens can be linked to a larger molecule, such as keyhole limpet hemocyanin. Mice or rabbits can be injected in a number of manners, e.g., subcutaneous, intravenous or intramuscular at a number of sites, e.g., in the peritoneum (i.p.), base of the tail, or foot pad, or a combination of sites, e.g., iP and base of tail (BIP). Booster injections can include the same or a different immunogen and can additionally include adjuvant, e.g., incomplete Freund's adjuvant. Immunization with DNA, e.g., DNA encoding GCC or a portion thereof or fusion protein comprising GCC or a portion thereof (e.g., encoding hGCC(ECD)-mIgG2a FcRbr-mutII) can be injected using gene gun technology. For example, DNA is loaded onto microscopic gold particles and injected into mice or rabbits at frequent intervals over a brief period.

Generally, where a monoclonal antibody is desired, a hybridoma is produced by fusing a suitable cell from an immortal cell line (e.g., a myeloma cell line such as SP2/0, P3X63Ag8.653 or a heteromyeloma) with antibody-producing cells. Antibody-producing cells can be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans, human-antibody transgenic animals or other suitable animals (e.g., rabbits) immunized with the antigen of interest. Cells that produce antibodies of human origin (e.g., a human antibody) can be produced using suitable methods, for example, fusion of a human antibody-producing cell and a heteromyeloma or trioma, or immortalization of an activated human B cell via infection with Epstein Barr virus. (See, e.g., U.S. Pat. No. 6,197,582 (Trakht); Niedbala et al., Hybridoma, 17:299-304 (1998); Zanella et al., J Immunol Methods, 156:205-215 (1992); Gustafsson et al., Hum Antibodies Hybridomas, 2:26-32 (1991).) The fused or immortalized antibody-producing cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired specificity can be identified using a suitable assay (e.g., ELISA (e.g., with immunogen, e.g., hGCC(ECD)-mIgG2a FcRbr-mutII, immobilized on the microtiter well) or by FACS on a cell expressing GCC or a portion thereof, e.g., a cell expressing the pLKTOK4 product). For example, if the GCC-immunogen comprises a fusion moiety that is an affinity reagent, this moiety can allow the fusion protein comprising GCC or a portion thereof to be bound to a matrix, e.g., protein G-coated, streptavidin-coated,

glutathione-derivatized or antibody-coated microtitre plates or assay chips, which are then combined with the immune serum or conditioned medium from a hybridoma or antibody-expressing recombinant cell, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the microtitre plate wells or chip cells are washed to remove any unbound components and binding by anti-GCC antibody is measured.

In embodiments, for therapeutic applications, the antibodies of the present invention are humanized antibodies. The advantage of humanized antibodies is that they potentially decrease or eliminate the immunogenicity of the antibody in a host recipient, thereby permitting an increase in the bioavailability and a reduction in the possibility of adverse immune reaction, thus potentially enabling multiple antibody administrations.

Modified antibodies include humanized, chimeric or CDR-grafted antibodies. Human anti-mouse antibody (HAMA) responses have led to development of chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a non-human variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. The presence of such non-human (e.g., murine, rat, rabbit, sheep or goat) derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of non-human derived antibodies, humanized antibodies where sequences are introduced to an antibody sequence to make it closer to human antibody sequence, or fully human antibodies generated by the introduction of human antibody function into a non-human species, such as a mouse, rat, rabbit, sheep or goat, have been developed so that the non-human species would produce antibodies having fully human sequences. Human antibodies avoid certain of the problems associated with antibodies that possess rabbit, rodent, sheep or goat variable and/or constant regions.

Humanization and Display Technologies and Modifications to Antibodies

Humanized antibody molecules can minimize the immunogenic and allergic responses intrinsic to non-human or non-human-derivatized mAbs and thus to increase the efficacy and safety of the administered antibodies. The use of humanized antibody molecules can provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated antibody administrations.

The production of humanized antibodies with reduced immunogenicity can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that antibodies from non-human species, such as mice, rats, rabbits, sheep, goats, etc., can be humanized or primatized using techniques known in the art. See e.g., Winter and Harris Immunol Today 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (see WO 92/02190 and U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al. *Proc Natl Acad Sci* USA. 84:3439 (1987) and J. Immunol. 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA: The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202).

Alternatively, phage display technology (see, e.g., McCafferty et al, *Nature*, 348:552-553 (1990)) can be used to produce human antibodies or antibodies from other species, as well as antibody fragments *in vitro*, from immunoglobulin variable (V) domain genes, e.g., from repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage

genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson and Chiswell, *Current Opinion in Structural Biology*, 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., *J. Mol. Biol.*, 222:581-597 (1991), or Griffith et al, *EMBO J.*, 12:725-734 (1993). See, also, U.S. Pat. Nos. 5,565,332 and 5,573,905. Display libraries can contain antibodies or antigen-binding fragments of antibodies that contain artificial amino acid sequences. For example, the library can contain Fab fragments which contain artificial CDRs (e.g., random amino acid sequences) and human framework regions. (See, for example, U.S. Pat. No. 6,300,064 (Knappik, et al.).)

The sequences of human constant region genes may be found in Kabat et al. (1991) Sequences of Proteins of Immunological Interest, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Isotypes can be IgG1, IgG2, IgG3 or IgG4. In particular embodiments, antibody molecules of the invention are IgG1 and IgG2. Either of the human light chain constant regions, kappa or lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

In some embodiments, an anti-GCC antibody molecule of the invention can draw antibody-dependent cellular cytotoxicity (ADCC) to a cell expressing GCC, e.g., a tumor cell. Antibodies with the IgG1 and IgG3 isotypes are useful for eliciting effector function in an antibody-dependent cytotoxic capacity, due to their ability to bind the Fc receptor. Antibodies with the IgG2 and IgG4 isotypes are useful to minimize an ADCC response because of their low

ability to bind the Fc receptor. In related embodiments substitutions in the Fc region or changes in the glycosylation composition of an antibody, e.g., by growth in a modified eukaryotic cell line, can be made to enhance the ability of Fc receptors to recognize, bind, and/or mediate cytotoxicity of cells to which anti-GCC antibodies bind (see, e.g., U.S. Pat. Nos. 7,317,091, 5,624,821 and publications including WO 00/42072, Shields, et al. *J. Biol. Chem.* 276:6591-6604 (2001), Lazar et al. *Proc. Natl. Acad. Sci.* U.S.A. 103:4005-4010 (2006), Satoh et al. *Expert Opin Biol. Ther.* 6:1161-1173 (2006)). In certain embodiments, the antibody or antigen-binding fragment (e.g., antibody of human origin, human antibody) can include amino acid substitutions or replacements that alter or tailor function (e.g., effector function). For example, a constant region of human origin (e.g., γ1 constant region, γ2 constant region) can be designed to reduce complement activation and/or Fc receptor binding. (See, for example, U.S. Pat. Nos. 5,648,260 (Winter et al.), 5,624,821 (Winter et al.) and 5,834,597 (Tso et al.), the entire teachings of which are incorporated herein by reference.) Preferably, the amino acid sequence of a constant region of human origin that contains such amino acid substitutions or replacements is at least about 95% identical over the full length to the amino acid sequence of the unaltered constant region of human origin, more preferably at least about 99% identical over the full length to the amino acid sequence of the unaltered constant region of human origin.

In still another embodiment, effector functions can also be altered by modulating the glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. For example, antibodies with enhanced ADCC activities with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in U.S. Patent Application Publication No. 2003/0157108 (Presta). See also U.S. Patent Application Publication No. 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Glycofi has also developed yeast cell lines capable of producing specific glycoforms of antibodies.

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. Such altered glycosylation patterns

have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody 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 are engineered to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang 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. 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).

Humanized antibodies can also be made using a CDR-grafted approach. Techniques of generation of such humanized antibodies are known in the art. Generally, humanized antibodies are produced by obtaining nucleic acid sequences that encode the variable heavy and variable light sequences of an antibody that binds to GCC, identifying the complementary determining region or "CDR" in the variable heavy and variable light sequences and grafting the CDR nucleic acid sequences on to human framework nucleic acid sequences. (See, for example, U.S. Pat. Nos. 4,816,567 and 5,225,539). The location of the CDRs and framework residues can be determined (see, 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 Chothia, C. et al. J. Mol. Biol. 196:901-917 (1987)). Anti-GCC antibody molecules described herein have the CDR amino acid sequences and nucleic acid sequences encoding CDRs listed in Tables 5 and 6. In some embodiments sequences from Tables 5 and 6 can be incorporated into molecules which recognize GCC for use in the therapeutic or diagnostic methods described herein. The human

framework that is selected is one that is suitable for *in vivo* administration, meaning that it does not exhibit immunogenicity. For example, such a determination can be made by prior experience with *in vivo* usage of such antibodies and studies of amino acid similarities. A suitable framework region can be selected from an antibody of human origin having at least about 65% amino acid sequence identity, and preferably at least about 70%, 80%, 90% or 95% amino acid sequence identity over the length of the framework region within the amino acid sequence of the equivalent portion (e.g., framework region) of the donor antibody, e.g., an anti-GCC antibody molecule (e.g., 3G1). Amino acid sequence identity can be determined using a suitable amino acid sequence alignment algorithm, such as CLUSTAL W, using the default parameters. (Thompson J. D. et al., Nucleic Acids Res. 22:4673-4680 (1994).)

Once the CDRs and FRs of the cloned antibody that are to be humanized are identified, the amino acid sequences encoding the CDRs are identified and the corresponding nucleic acid sequences grafted on to selected human FRs. This can be done using known primers and linkers, the selection of which are known in the art. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen. After the CDRs are grafted onto selected human FRs, the resulting "humanized" variable heavy and variable light sequences are expressed to produce a humanized Fv or humanized antibody that binds to GCC. Preferably, the CDR-grafted (e.g., humanized) antibody binds a GCC protein with an affinity similar to, substantially the same as, or better than that of the donor antibody. Typically, the humanized variable heavy and light sequences are expressed as a fusion protein with human constant domain sequences so an intact antibody that binds to GCC is obtained. However, a humanized Fv antibody can be produced that does not contain the constant sequences.

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, humanized antibodies can have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a selected, small number of acceptor framework residues of the humanized

immunoglobulin chain can be replaced by the corresponding donor amino acids. Locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., U.S. Pat. No. 5,585,089 or 5,859,205). The acceptor framework can be a mature human antibody framework sequence or a consensus sequence. As used herein, the term "consensus sequence" refers to the sequence found most frequently, or devised from the most common residues at each position in a sequence in a region among related family members. A number of human antibody consensus sequences are available, including consensus sequences for the different subgroups of human variable regions (see, Kabat, E. A., et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991)). The Kabat database and its applications are freely available on line, e.g. via IgBLAST at the National Center for Biotechnology Information, Bethesda, Md. (also see, Johnson, G. and Wu, T. T., Nucleic Acids Research 29:205-206 (2001)).

Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on Dec. 23, 1992.

The anti-GCC antibody molecule includes other humanized antibodies which may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in PCT Publication Nos. WO 98/52976 and WO 00/34317, the contents of which are incorporated herein by reference. Briefly, the rabbit, or other non-human species, heavy and light chain variable regions of an anti-GCC antibody can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes. For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the rabbit VH and VL sequences, as described in PCT Publication Nos. WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible, conservative substitutions are made, often but

not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in Tomlinson, I. A. et al., *J. Mol. Biol.* 227:776-798 (1992); Cook, G. P. et al., Immunol. Today Vol. 16 (5): 237-242 (1995); Chothia, D. et al., *J. Mol. Bio.* 227:799-817 (1992). The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I. A. et al. MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunized VH and VL of an anti-GCC antibody are constructed by mutagenesis of the rabbit VH and VL genes, the mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or K (kappa) constant regions.

In other embodiments, reduction of an immunogenic response by a CDR-grafted antibody can be achieved by changes, e.g., deletions, substitutions, of amino acid residues in CDRs (Kashmiri et al. Methods 36:25-34 (2005), U.S. Pat. No. 6,818,749, Tan et al. *J. Immunol.* 169:1119-1125 (2006)). For example, residues at positions involved in contact with the antigen preferably would not be changed. Typically, such residues, the specificity determining residues (SDRs), are in positions which display high levels of variability among antibodies. Consensus sequences derived, e.g., by the Clustal method (Higgins D. G. et al., Meth. Enzymol. 266:383-402 (1996)), from anti-GCC antibody molecules, e.g., from antibodies described herein, aid in identifying SDRs. In the anti-GCC antibody molecules described herein, the SDRs are the following, at least the first residue or in some embodiments, the first four residues of heavy chain CDR1; at least the N-terminal portion, e.g., the first seven, ten or 13 residues of heavy chain CDR2; nearly all of heavy chain CDR3; the C-terminal portion, e.g., after residue six, eight, or nine of light chain CDR1; about the first, middle and/or last residue of light chain CDR2; and most of light chain CDR3, or at least after residue two or three. Accordingly, to maintain binding to GCC protein after humanization or modification of an anti-GCC antibody molecule, such SDR residues in CDRs of the anti-GCC antibody molecules are less amenable to changes, e.g., from rabbit residues to human consensus residues than are residues in other residues of the CDRs or the framework regions. Conversely, it can be beneficial to change residues in non-human, e.g., rabbit CDRs to residues identified as consensus in human CDRs, e.g., CDRs of anti-GCC

antibody molecules described in US Published Patent Application No. 20110110936, the contents of which are incorporated by reference herein in its entirety.

Anti-GCC antibodies that are not intact antibodies are also useful in this invention. Such antibodies may be derived from any of the antibodies described above. Useful antibody molecules of this type include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature 341:544-546 (1989)), which consists of a VH domain; (vii) a single domain functional heavy chain antibody, which consists of a VHH domain (known as a nanobody) see e.g., Cortez-Retamozo, et al., *Cancer Res.* 64: 2853-2857 (2004), and references cited therein; and (vii) an isolated CDR, e.g., one or more isolated CDRs together with sufficient framework to provide an antigen binding fragment. Furthermore, although the two domains of the Fv fragment, VL and VH, 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 VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. *Science* 242:423-426 (1988); and Huston et al. *Proc. Natl. Acad. Sci.* USA 85:5879-5883 (1988). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" 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. Antibody fragments, such as Fv, $F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage.

In embodiments, some or all of the CDRs sequences, of one or both the heavy and light chain, can be used in another antibody molecule, e.g., in a CDR-grafted, humanized, or chimeric antibody molecule.

Embodiments include an antibody molecule that comprises sufficient CDRs, e.g., all six CDRs from one of the rabbit hybridoma antibodies described herein to allow binding to cell surface GCC.

In an embodiment the CDRs, e.g., all of the HCDRs, or all of the LCDRs, or all six, are embedded in human or human derived framework region(s). Examples of human framework regions include human germline framework sequences, human germline sequences that have been affinity matured (either *in vivo* or *in vitro*), or synthetic human sequences, e.g., consensus sequences. In an embodiment the heavy chain framework is an IgG1 or IgG2 framework. In an embodiment the light chain framework is a kappa framework.

In an embodiment the anti-GCC antibody molecule, e.g., a CDR-grafted or humanized antibody molecule, comprises sufficient CDRs, e.g., all six CDRs from one of the antibodies described herein, e.g., sequences listed in Table 5, to allow binding to GCC. (Exemplary nucleic acid sequences which can encode the CDR amino acid sequences listed in Table 5, are provided, in Table 6 herein). In particular embodiments, an anti-GCC antibody molecule can comprise CDRs from MIL-44-148-2 or MIL-44-67-4.

Antibody fragments for *in vivo* therapeutic or diagnostic use can benefit from modifications which improve their serum half lives. Suitable organic moieties intended to increase the *in vivo* serum half-life of the antibody can include one, two or more linear or branched moiety selected from a hydrophilic polymeric group (e.g., a linear or a branched polymer (e.g., a polyalkane glycol such as polyethylene glycol, monomethoxy-polyethylene glycol and the like), a carbohydrate (e.g., a dextran, a cellulose, a polysaccharide and the like), a polymer of a hydrophilic amino acid (e.g., polylysine, polyaspartate and the like), a polyalkane oxide and polyvinyl pyrrolidone), a fatty acid group (e.g., a mono-carboxylic acid or a di-carboxylic acid), a fatty acid ester group, a lipid group (e.g., diacylglycerol group, sphingolipid group (e.g., ceramidyl)) or a phospholipid group (e.g., phosphatidyl ethanolamine group). Preferably, the organic moiety is bound to a predetermined site where the organic moiety does not impair the function (e.g., decrease the antigen binding affinity) of the resulting

immunoconjugate compared to the non-conjugated antibody moiety. The organic moiety can have a molecular weight of about 500 Da to about 50,000 Da, preferably about 2000, 5000, 10,000 or 20,000 Da. Examples and methods for modifying polypeptides, e.g., antibodies, with organic moieties can be found, for example, in U.S. Pat. Nos. 4,179,337 and 5,612,460, PCT Publication Nos. WO 95/06058 and WO 00/26256, and U.S. Patent Application Publication No. 20030026805.

An anti-GCC antibody molecule can comprise all, or an antigen binding fragment of the variable region, of one or both, the heavy and light chain, of one of the above-referenced rabbit hybridoma antibodies.

In an embodiment the light chain amino acid sequence of (a) can differ from one of the reference amino acid sequence(s) referred to in (a)(i-ii) by as many as 1, 2, 3, 4, 5, 10, or 15 residues. In embodiments the differences are conservative substitutions. In embodiments, the differences are in the framework regions. In an embodiment the heavy chain amino acid sequence of (b) can differ from one of the reference amino acid sequence(s) referred to in (b)(i-ii) by as many as 1, 2, 3, 4, 5, 10, or 15 residues. In embodiments the differences are conservative substitutions. In embodiments the differences are in the framework regions.

In an embodiment the anti-GCC antibody molecule comprises one or both of:

(a) a light chain amino acid sequence of all, or an antigen binding fragment of, either, (i) a light chain variable region amino acid sequence from Table 3, e.g., SEQ ID NO:13, or (ii) a light chain variable region amino acid encoded by a nucleotide sequence from Table 4, e.g., SEQ ID NO:12; and

(b) a heavy chain amino acid sequence of all, or an antigen binding fragment of, either (i) a heavy chain variable region amino acid sequence from Table 3, e.g., SEQ ID NO:11, or (ii) a heavy chain amino acid sequence encoded by a nucleotide sequence from Table 4, e.g., SEQ ID NO:10.

In an embodiment the anti-GCC antibody molecule comprises one or both of:

a) a light chain variable region, or an antigen binding fragment thereof, having at least 85, 90, 95, 97 or 99% homology with the light chain variable region of an anti-GCC antibody molecule of the invention; and

(b) a heavy chain variable region, or an antigen binding fragment thereof, having at least 85, 90, 95, 97 or 99% homology with the heavy chain variable region of an anti-GCC antibody molecule of the invention.

Amino acid sequences of the variable regions of the anti-GCC antibodies of the invention can be found in Table 3.

In one approach, consensus sequences encoding the heavy and light chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Suitable expression vectors can contain a number of components, for example, an origin of replication, a selectable marker gene, one or more expression control elements, such as a transcription control element (e.g., promoter, enhancer, terminator) and/or one or more translation signals, a signal sequence or leader sequence, and the like. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter. Examples of suitable vectors that can be used include those that are suitable for mammalian hosts and based on viral replication systems, such as simian virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus 2, bovine papilloma virus

(BPV), papovavirus BK mutant (BKV), or mouse and human cytomegalovirus (CMV), and moloney murine leukemia virus (MMLV), native Ig promoters, etc. A variety of suitable vectors are known in the art, including vectors which are maintained in single copy or multiple copies, or which become integrated into the host cell chromosome, e.g., via LTRs, or via artificial chromosomes engineered with multiple integration sites (Lindenbaum et al. Nucleic Acids Res. 32:e172 (2004), Kennard et al. Biotechnol. Bioeng. Online May 20, 2009). Additional examples of suitable vectors are listed in a later section.

Thus, the invention provides an expression vector comprising a nucleic acid encoding an antibody, antigen-binding fragment of an antibody (e.g., a humanized, chimeric antibody or antigen-binding fragment of any of the foregoing), antibody chain (e.g., heavy chain, light chain) or antigen-binding portion of an antibody chain that binds a GCC protein.

Expression in eukaryotic host cells is useful because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to known methods (Kim and Baldwin, "Specific Intermediates in the Folding Reactions of Small Proteins and the Mechanism of Protein Folding", Ann. Rev. Biochem. 51, pp. 459-89 (1982)). It is possible that the host cells will produce portions of intact antibodies, such as light chain dimers or heavy chain dimers, which also are antibody homologs according to the present invention.

Further, as described elsewhere herein, antibodies or antibodies from human or non-human species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are known in the art. Winter and Harris Immunol Today 14:43-46 (1993) and Wright et al. Crit. Reviews in Immunol. 12125-168 (1992), Hanes and Plucthau PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith Gene 73:305-318 (1988) (phage display), Scott TIBS 17:241-245 (1992), Cwirla et al. Proc Natl Acad

Sci USA 87:6378-6382 (1990), Russel et al. Nucl. Acids Research 21:1081-1085 (1993), Hoganboom et al. Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty TIBTECH 10:80-84 (1992), and U.S. Pat. No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

It will be appreciated that antibodies that are generated need not initially possess a particular desired isotype but, rather, the antibody as generated can possess any isotype. For example, the antibody produced by the MIL-44-148-2 rabbit hybridoma has an IgG isotype. The isotype of the antibody can be switched thereafter, e.g., to IgG2, or IgG3 to elicit an ADCC response when the antibody binds GCC on a cell, using conventional techniques that are known in the art. Such techniques include the use of direct recombinant techniques (see e.g., U.S. Pat. No. 4,816,397), cell-cell fusion techniques (see e.g., U.S. Pat No 5,916,771), among others. In the cell-cell fusion technique, a myeloma or other cell line is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line is prepared that possesses the light chain. Such cells can, thereafter, be fused and a cell line expressing an intact antibody can be isolated.

In certain embodiments, the GCC antibody molecule is a rabbit anti-GCC IgG1 antibody. Since such antibodies possess desired binding to the GCC molecule, any one of such antibodies can be readily isotype-switched to generate another isotype while still possessing the same variable region (which defines the antibody's specificity and affinity, to a certain extent). Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain additional "functional" attributes that are desired through isotype switching.

In an embodiment the variable region or antigen binding fragment thereof can be coupled to a constant region (or fragment thereof) other than the constant region it was generated with, e.g., a constant region (or fragment thereof) from another antibody or to a synthetic constant region (or fragment thereof). In embodiments the constant region is an IgG1 or IgG2 constant

region (or fragment thereof). Sequence changes can be made in the variable or constant regions to modify effector activity of the antibody molecule.

Design and Generation of Other Therapeutics

The antibodies that are produced and characterized herein with respect to GCC provide for the design of other therapeutic modalities including other antibodies, other antagonists, or chemical moieties other than antibodies is facilitated. Such modalities include, without limitation, antibodies having similar binding activity or functionality, advanced antibody therapeutics, such as bispecific antibodies, immunoconjugates, and radiolabeled therapeutics, generation of peptide therapeutics, particularly intrabodies, and small molecules. Furthermore, as discussed above, the effector function of the antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA1, IgA2, IgE, or IgM for various therapeutic uses.

In connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies, one with a specificity to GCC and another to a second molecule that are conjugated together, (ii) a single antibody that has one chain specific to GCC and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to GCC and the other molecule. Such bispecific antibodies can be generated using techniques that are known. For example, bispecific antibodies may be produced by crosslinking two or more antibodies (of the same type or of different types). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill. See also, e.g., Fanger et al. *Immunomethods* 4:72-81 (1994) and Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12125-168 (1992) and in connection with (iii) see e.g., Traunecker et al. *Int. J. Cancer* (Suppl.) 7:51-52 (1992). Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J. Immunol.* 148:1547-1553 (1992).

In addition, "Kappabodies" (Ill. et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" *Protein Eng* 10:949-57 (1997)), "Minibodies" (Martin et al. *EMBO J.* 13:5303-9 (1994), U.S. Pat. No. 5,837,821), "Diabodies" (Holliger et al. *Proc Natl Acad Sci* USA 90:6444-6448 (1993)), or "Janusins" (Traunecker et al. *EMBO J.* 10:3655-3659 (1991) and Traunecker et al. *Int J Cancer Suppl* 7:51-52 (1992)) may also be prepared.

Nucleic Acids and Polypeptides

In another embodiment, the present invention relates to polynucleotide and polypeptide sequences that encode for or represent the antibody molecules described herein. Such polynucleotides encode for both the variable and constant regions of each of the heavy and light chains, although other combinations are also contemplated by the present invention in accordance with the compositions described herein. The present invention also contemplates oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides.

The polynucleotides can be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence that encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

In embodiments provided, polynucleotides encode at least one heavy chain variable region and at least one light chain variable region of the present invention, e.g., as summarized in Table 4.

The present invention also includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions, and any polypeptide modification

resulting from the variant polynucleotide sequence. A polynucleotide of the present invention may also have a coding sequence that is a variant of the coding sequence provided herein. For example, a variant polynucleotide can have at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 97% identity with a polynucleotide listed in Table 4. In embodiments, the variant polynucleotide encodes for an anti-GCC antibody molecule.

The present invention further relates to polypeptides that represent the antibodies of the present invention as well as fragments, analogs and derivatives of such polypeptides. The polypeptides of the present invention may be recombinant polypeptides, naturally produced polypeptides or synthetic polypeptides. The fragment, derivative or analogs of the polypeptides of the present invention may be one in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence that is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present invention. In various aspects, the polypeptides of the invention may be partially purified, or purified product.

A polypeptide of the present invention can have an amino acid sequence that is identical to that of the antibodies described herein, e.g., summarized in Tables 2 or 3, or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or threonine with serine; replacement of lysine with arginine or histidine. In contrast, variations may include nonconservative changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions or both.

Guidance in determining which and how many amino acid residues may be substituted, inserted, or deleted without changing biological or immunological activity may be found using computer programs known in the art, for example DNASTAR software (DNASTAR, Inc., Madison, Wis.).

In another aspect, the invention features, isolated and/or recombinant nucleic acids encoding anti-GCC antibody molecules. In embodiments, the nucleic acids encode one or more of an antibody molecule, a heavy chain, a light chain, a light chain variable region, a heavy chain variable region, portions of the heavy chains and light chains of the antibody molecules described herein (e.g., a light chain variable region fragment which when paired with a full length heavy chain variable region is antigen binding, or a heavy chain variable region fragment which when paired with a full length light chain variable region is antigen binding), and CDRs. Embodiments include such nucleic acids disposed in vectors, e.g., expression vectors. Still further, the invention encompasses antibody molecules produced by host cells, e.g., expressing the antibody molecules encoded by such plasmids

In an embodiment, is provided a vector, e.g., an expression vector, comprising one or both of:

sequences encoding a light chain variable region, e.g., a light chain variable region described in Table 3, e.g., a sequence listed in Table 4, an antigen binding fragment thereof, or one, two or three CDRs from a light chain (and optionally a framework region), described herein, e.g., CDRs described in Table 5,e.g., a CDR encoding sequence in Table 6; and

sequences encoding a heavy chain variable region, e.g., a heavy chain variable region described in Table 3, e.g., a sequence listed in Table 4, an antigen binding fragment thereof, or one, two or three CDRs from a heavy chain (and optionally a framework region), described herein, e.g., CDRs described in Table 5, e.g., a CDR encoding sequence in Table 6.

In embodiments provided, polynucleotides encode at least one heavy chain variable region or at least one light chain variable region of the antibodies of the present invention. In

embodiments provided, polypeptides can encode at least one heavy chain variable region and one light chain variable region of the antibodies of the present invention.

In an embodiment the anti-GCC antibody molecule comprises one or both of:

(a) a light chain variable region, or an antigen binding fragment thereof, encoded by a nucleic acid that hybridizes under selected stringency conditions with, (i) the complement of an anti-GCC antibody molecule-encoding-nucleic acid sequence described herein, e.g., in Table 4, or (ii) any nucleic acid sequence that encodes a light chain of an anti-GCC antibody molecule of the invention, e.g., one of the above-referenced rabbit antibodies summarized in Tables 1 and 2; and

(b) a heavy chain variable region, or an antigen binding fragment thereof, encoded by a nucleic acid that hybridizes under selected stringency conditions with, (i) the complement of an anti-GCC antibody molecule-encoding-nucleic acid sequence described herein, e.g., in Table 4, or (ii) any nucleic acid sequence that encodes a heavy chain of an anti-GCC antibody molecule of the invention, e.g., one of the above-referenced rabbit antibodies summarized in Tables 1 and 2.

In an embodiment selected stringency conditions are high stringency or very high stringency conditions, e.g., as those conditions are described herein.

The present invention also provides vectors that include the polynucleotides of the present invention, host cells which are genetically engineered with vectors of the present invention and the production of the antibodies of the present invention by recombinant techniques.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction endonuclease sites by procedures known in the art. The polynucleotide sequence in the expression vector is operatively linked to an appropriate expression control sequence (i.e. promoter) to direct mRNA synthesis. Examples of such promoters include, but are not limited to, the Rous sarcoma virus

LTR or the early or late SV40 promoter, the *E. coli* lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic (e.g., tac, T3, T7 promoters for *E. coli*) or eukaryotic (e.g., cytomegalovirus promoter, adenovirus late promoter, EF-1a promoter) cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. For example, the vector can contain enhancers, which are transcription-stimulating DNA sequences of viral origin, such as those derived form simian virus such as SV40, polyoma virus, cytomegalovirus, bovine papilloma virus or Moloney sarcoma virus, or genomic, origin. The vector preferably also contains an origin of replication. The vector can be constructed to contain an exogenous origin of replication or, such an origin of replication can be derived from SV40 or another viral source, or by the host cell chromosomal replication mechanism.

In addition, the vectors optionally contain a marker gene for selection of transfected host cells such as dihydrofolate reductase marker genes to permit selection with methotrexate in a variety of hosts, or antibiotics, such as .beta.-lactamase gene (ampicillin resistance), Tet gene (for tetracycline resistance) used in prokaryotic cells or neomycin, GA418 (geneticin, a neomycin-derivative) gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes, or genes which complement a genetic lesion of the host cells such as the absence of thymidine kinase, hypoxanthine phosphoribosyl transferase, dihydrofolate reductase, etc. Genes encoding the gene product of auxotrophic markers of the host (e.g., LEU2, URA3, HIS3) are often used as selectable markers in yeast.

In order to obtain the antibodies of the present invention, one or more polynucleotide sequences that encode for the light and heavy chain variable regions and light and heavy chain constant regions of the antibodies of the present invention should be incorporated into a vector. Polynucleotide sequences encoding the light and heavy chains of the antibodies of the present invention can be incorporated into one or multiple vectors and then incorporated into the host cells.

Suitable expression vectors for expression in mammalian cells include, for example, pCDM8, pcDNA1.1/amp, pcDNA3.1, pRc/RSV, pEF-1 (Invitrogen Life Technologies, Carlsbad, Calif.), pCMV-SCRIPT, pFB, pSG5, pXT1 (Stratagene, La Jolla, Calif.), pCDEF3 (Goldman, L. A., et al., *Biotechniques*, 21:1013-1015 (1996)), pSVSPORT (GIBCO division of Invitrogen Life Technologies, Carlsbad, Calif.), pEF-Bos (Mizushima, S., et al., *Nucleic Acids Res.*, 18:5322 (1990)), Bicistronic GPEX® Retrovector (Gala Biotech, Middleton, Wis.) and the like. Expression vectors which are suitable for use in various expression hosts, such as prokaryotic cells (*E. coli*), insect cells (*Drosophila* Schnieder S2 cells, Sf9) and yeast (*P. methanolica, P. pastoris, S. cerevisiae*) are also available. Exemplary vectors are pLKTOK58 (wild type IgG1 Fc sequence) and pLKTOK59 (mutated IgG1 Fc sequence) (see U.S. Patent Application publication no. 20060147445).

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for a suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, for introducing heterologous polynucleotides into mammalian cells, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) into liposomes and direct microinjection of the DNA molecule. The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

In another aspect, the invention features, a host cell comprising a nucleic acid described herein. In embodiments the cell expresses an antibody molecule, or component thereof,

described herein. Still further embodiment provides a method of producing an antibody molecule, e.g., an anti-GCC antibody molecule described herein, e.g. a rabbit antibody molecule, or a humanized version thereof, comprising maintaining the host cell under conditions appropriate for expression, whereby immunoglobulin chain(s) are expressed and an antibody molecule is produced. An additional embodiment provides a host cell comprising any of the foregoing expression vectors encoding heavy and light chain antibody sequences. The host cell can be a eukaryotic cell, e.g., a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, e.g., *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (e.g., NS0), Chinese hamster ovary cells (CHO), COS cells. In a particular embodiment, the cultured host cell is a CHO cell comprising nucleic acid sequences encoding a MIL-44-148-2 antibody molecule. In another embodiment, the host cell is Hybridoma MIL-44-148-2 (PTA-8132). Additionally cells include oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cell. For example, nucleic acids encoding an antibody molecule described herein can be expressed in a transgenic nonhuman animal.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive GCC binding properties.

A still further embodiment provides a method of producing an anti-GCC antibody molecule, e.g., a rabbit antibody molecule or a humanized version thereof, comprising maintaining the host cell comprising nucleic acids described herein, e.g., one or more nucleic

acid sequence listed in Table 4 or 6, under conditions appropriate for expression of an immunoglobulin, whereby immunoglobulin chains, are expressed and an antibody molecule, e.g., a rabbit antibody molecule, or a humanized version thereof, that binds GCC, or a fragment or variant thereof, is produced. For example, methods of expression of antibody molecules include the use of host cells wherein a first recombinant nucleic acid molecule encoding an antibody molecule, e.g., a rabbit antibody light chain or a humanized version thereof, and a second recombinant nucleic acid molecule encoding an antibody molecule, e.g., a rabbit antibody heavy chain or a humanized version thereof, are comprised in a single expression vector. In other embodiments, they are in separate vectors. The method can further comprise the step of isolating or recovering the antibody, antigen-binding fragment of an antibody, antibody chain or antigen-binding fragment of an antibody chain, if desired.

For example, a nucleic acid molecule (i.e., one or more nucleic acid molecules) encoding the heavy and light chains of a rabbit (or humanized) antibody that binds a GCC protein, or an expression construct (i.e., one or more constructs) comprising such nucleic acid molecule(s), can be introduced into a suitable host cell to create a recombinant host cell using any method appropriate to the host cell selected (e.g., transformation, transfection, electroporation, infection), such that the nucleic acid molecule(s) are operably linked to one or more expression control elements (e.g., in a vector, in a construct created by processes in the cell, integrated into the host cell genome). The resulting recombinant host cell can be maintained under conditions suitable for expression (e.g., in the presence of an inducer, in a suitable non-human animal, in suitable culture media supplemented with appropriate salts, growth factors, antibiotics, nutritional supplements, etc.), whereby the encoded polypeptide(s) are produced. If desired, the encoded protein can be isolated or recovered (e.g., from the animal, the host cell, medium, milk). This process encompasses expression in a host cell of a transgenic non-human animal (see, e.g., WO 92/03918, GenPharm International) or plant.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase and DHFR gene expression systems are common approaches for enhancing

expression under certain conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning, Microdrop technology, or any other methods known in the art. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In an exemplary system for recombinant expression of a modified antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

Antibodies of the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Pat. Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

The antibodies, antigen-binding fragments, antibody chains and antigen-binding portions thereof described herein also can be produced in a suitable *in vitro* expression system, by chemical synthesis or by any other suitable method.

Fusion Proteins and Immunoconjugates

The anti-GCC antibodies described herein can be functionally linked by any suitable method (e.g., chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more non-antibody molecular entities.

Fusion proteins can be produced in which an anti-GCC antibody molecule as described herein and a non-antibody moiety are components of a single continuous polypeptide chain. The non-antibody moiety can be located N-terminally, C-terminally, or internally, with respect to the antibody moiety. For example, some embodiments can be produced by the insertion of a nucleic acid encoding immunoglobulin sequences into a suitable expression vector, such as a pET vector (e.g., pET-15b, Novagen), a phage vector (e.g., pCNATAB 5 E, Pharmacia), or other vector, e.g., pRIT2T Protein A fusion vector, Pharmacia). The resulting construct can be expressed to produce antibody chains that comprise a non-antibody moiety (e.g., Histidine tag, E tag, or Protein A IgG binding domain). Fusion proteins can be isolated or recovered using any suitable technique, such as chromatography using a suitable affinity matrix (see, e.g., *Current Protocols in Molecular Biology* (Ausubel, F. M et al., eds., Vol. 2, Suppl. 26, pp. 16.4.1-16.7.8 (1991)).

The invention provides anti-GCC antibody molecules which are directed to and, in embodiments, are internalized into cells. They are capable of delivering therapeutic agents or detectable agents to or into cells expressing GCC, but not to or into cells where the target is not expressed. Thus, the invention also provides anti-GCC immunoconjugates comprising an anti-GCC antibody molecule as described herein, which is conjugated to a therapeutic agent or a detectable agent. In embodiments, the affinity for GCC of an anti-GCC immunoconjugate is at least 10, 25, 50, 75, 80, 90, or 95% of that for the unconjugated antibody. This can be determined using cell surface GCC or isolated GCC. In an embodiment the anti-GCC antibody molecule,

e.g., an immunoconjugate, has an LD50, as determined by an assay described herein, of less than 1,000, 500, 250, 100, or 50 pM.

The anti-GCC antibody molecule can be modified to act as an immunoconjugate utilizing techniques that are known in the art. See e.g., *Vitetta Immunol Today* 14:252 (1993). See also U.S. Pat. No. 5,194,594. The preparation of radiolabeled antibodies can also be readily prepared utilizing techniques that are known in the art. See e.g., Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). See also U.S. Pat. Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (U.S. Re. Pat. No. 35,500), 5,648,471, and 5,697,902.

In some embodiments, the antibody molecule and non-antibody moiety are connected by means of a linker In such embodiments, the immunoconjugate is represented by formula (I):

$$Ab-(X-Z)_m$$

wherein,

Ab is an anti-GCC antibody molecule described herein;

X is a moiety which connects Ab and Z, e.g., the residue of a linker described herein after covalent linkage to one or both of Ab and Z;

Z is a therapeutic agent or label; and

m ranges from about 1 to about 15.

The variable m represents the number of --X--Z moieties per antibody molecule in an immunoconjugate of formula (I). In various embodiments, m ranges from 1 to 15, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, m ranges from 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, m is 1, 2, 3, 4, 5 or 6. In compositions comprising a plurality of immunoconjugates of formula (I), m is the average

number of --X--Z moieties per Ab, also referred to as the average drug loading. Average drug loading may range from 1 to about 15-X--Z moieties per Ab. In some embodiments, when m represents the average drug loading, m is about 1, about 2, about 3, about 4, about 5, about 6, about 7, or about 8. In exemplary embodiments, m is from about 2 to about 8. In one embodiment, m is about 8. In another embodiment, m is about 4. In another embodiment, m is about 2.

The average number of --X--Z moieties per Ab may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of immunoconjugates in terms of m may also be determined. In some instances, separation, purification, and characterization of homogeneous immunoconjugates where m is a certain value, as distinguished from immunoconjugates with other drug loadings, may be achieved by means such as reverse phase HPLC or electrophoresis.

The immunoconjugates of formula (I) may exist as mixtures, wherein each component of the mixture has a different m value. For example, an immunoconjugate of formula (I) may exist as a mixture of two separate immunoconjugate components: one immunoconjugate component wherein m is 7, and the other immunoconjugate component wherein m is 8.

In one embodiment, the immunoconjugate of formula (I) exists as a mixture of three separate immunoconjugates wherein m for the three separate immunoconjugates is 1, 2, and 3, respectively; 3, 4, and 5, respectively; 5, 6, and 7, respectively; 7, 8, and 9, respectively; 9, 10, and 11, respectively; 11, 12, and 13, respectively; or 13, 14, and 15, respectively.

A variety of suitable linkers (e.g., heterobifunctional reagents for connecting an antibody molecule to a therapeutic agent or label) and methods for preparing immunoconjugates are known in the art. (See, for example, Chari et al., Cancer Research 52:127-131 (1992).) The linker can be cleavable, e.g., under physiological conditions.,e.g., under intracellular conditions, such that cleavage of the linker releases the drug (i.e., therapeutic agent or label) in the intracellular environment. In other embodiments, the linker is not cleavable, and the drug is released, for example, by antibody degradation.

The linker can be bonded to a chemically reactive group on the antibody moiety, e.g., to a free amino, imino, hydroxyl, thiol or carboxyl group (e.g., to the N- or C-terminus, to the epsilon amino group of one or more lysine residues, the free carboxylic acid group of one or more glutamic acid or aspartic acid residues, or to the sulfhydryl group of one or more cysteinyl residues). The site to which the linker is bound can be a natural residue in the amino acid sequence of the antibody moiety or it can be introduced into the antibody moiety, e.g., by DNA recombinant technology (e.g., by introducing a cysteine or protease cleavage site in the amino acid sequence) or by protein biochemistry (e.g., reduction, pH adjustment or proteolysis).

One of the most commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody molecule. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule. Also available for attachment of drug (i.e., therapeutic agent or label) to antibody molecules is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the antibody molecule. Attachment occurs via formation of a Schiff base with amino groups of the antibody molecule. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to antibody molecule. Other techniques are known to the skilled artisan and within the scope of the present invention.

In certain embodiments, an intermediate, which is the precursor of the linker (X), is reacted with the drug (Z) under appropriate conditions. In certain embodiments, reactive groups are used on the drug and/or the intermediate. The product of the reaction between the drug (i.e., therapeutic agent or label) and the intermediate, or the derivatized drug, is subsequently reacted with the antibody molecule under appropriate conditions.

The immunoconjugate can be purified from reactants by employing methodologies well known to those of skill in the art, e.g., column chromatography (e.g., affinity chromatography, ion exchange chromatography, gel filtration, hydrophobic interaction chromatography), dialysis,

diafiltration or precipitation. The immunoconjugate can be evaluated by employing methodologies well known to those skilled in the art, e.g., SDS-PAGE, mass spectroscopy, or capillary electrophoresis.

In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (e.g., within a lysosome or endosome or caveolea). The linker can be, e.g., a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug (i.e., therapeutic agent or label) inside target cells (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Most typical are peptidyl linkers that are cleavable by enzymes that are present in GCC-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker (SEQ ID NO:319)). Other examples of such linkers are described, e.g., in U.S. Pat. No. 6,214,345, incorporated herein by reference in its entirety and for all purposes. In a specific embodiment, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker). One advantage of using intracellular proteolytic release of the drug (i.e., therapeutic agent or label) is that the drug is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are

unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929).

In yet other embodiments, the linker is cleavable under reducing conditions (e.g., a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Pat. No. 4,880,935.)

In yet other specific embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg Med. Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

In yet other embodiments, the linker unit is not cleavable and the drug (i.e., therapeutic agent or label) is released by antibody degradation. (See for example U.S. Publication No. 20050238649 incorporated by reference herein in its entirety and for all purposes).

Typically, the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, typically no more than about 15%, more typically no more than about 10%, and even more typically no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of immunoconjugate, are cleaved when the immunoconjugate presents in an extracellular environment (e.g., in plasma). Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the immunoconjugate for a predetermined time period (e.g., 2, 4, 8, 16, or 24 hours) and then quantifying the amount of free drug present in the plasma.

In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent or label (Z). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the Z moiety and the anti-GCC antibody molecule.

A variety of exemplary linkers that can be used with the present compositions and methods are described in WO 2004-010957, U.S. Publication No. 20060074008, U.S. Publication No. 20050238649, and U.S. Publication No. 20060024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

Examples of linkers capable of being used to couple an antibody molecule to a therapeutic agent or label include, for example, maleimidocaproyl (mc); maleimidocaproyl-p-aminobenzylcarbamate; maleimidocaproyl-peptide-aminobenzylcarbamate linkers, e.g., maleimidocaproyl-L-phenylalanine-L-lysine-p-aminobenzylcarbamate and maleimidocaproyl-L-valine-L-citrulline-p-aminobenzylcarbamate (vc); N-succinimidyl 3-(2-pyridyldithio)proprionate (also known as N-succinimidyl 4-(2-pyridyldithio)pentanoate or SPP); 4-succinimidyl-oxycarbonyl-2-methyl-2-(2-pyridyldithio)-toluene (SMPT); N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP); N-succinimidyl 4-(2-pyridyldithio)butyrate (SPDB); 2-iminothiolane; S-acetylsuccinic anhydride; disulfide benzyl carbamate; carbonate; hydrazone linkers; N-(α-Maleimidoacetoxy) succinimide ester; N-[4-(p-Azidosalicylamido) butyl]-3'-(2'-pyridyldithio)propionamide (AMAS); N-[.beta.-Maleimidopropyloxy]succinimide ester (BMPS); [N-ε-Maleimidocaproyloxy]succinimide ester (EMCS); N-[γ-Maleimidobutyryloxy]succinimide ester (GMBS); Succinimidyl-4-[N-Maleimidomethyl]cyclohexane-1-carboxy-[6-amidocaproate] (LC-SMCC); Succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate (LC-SPDP); m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS); N-Succinimidyl[4-iodoacetyl]aminobenzoate (SIAB); Succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC); N-Succinimidyl 3-[2-pyridyldithio]-propionamido (SPDP); [N- ε-Maleimidocaproyloxy]sulfosuccinimide ester (Sulfo-EMCS); N-[γ-Maleimidobutyryloxy]sulfosuccinimide ester (Sulfo-GMBS); 4-Sulfosuccinimidyl-6-methyl-α-

(2-pyridyldithio)toluamido]hexanoate) (Sulfo-LC-SMPT); Sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido)hexanoate (Sulfo-LC-SPDP); m-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester (Sulfo-MBS); N-Sulfosuccinimidyl[4-iodoacetyl]aminobenzoate (Sulfo-SIAB); Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC); Sulfosuccinimidyl 4-[p-maleimidophenyl]butyrate (Sulfo-SMPB); ethylene glycol-bis(succinic acid N-hydroxysuccinimide ester) (EGS); disuccinimidyl tartrate (DST); 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA); diethylenetriamine-pentaacetic acid (DTPA); and thiourea linkers.

In some embodiments, the therapeutic agent is a cytostatic or cytotoxic agent. Examples include, without limitation, antimetabolites (e.g., azathioprine, 6-mercaptopurine, 6-thioguanine, fludarabine, pentostatin, cladribine, 5-fluorouracil (5FU), floxuridine (FUDR), cytosine arabinoside (cytarabine), methotrexate, trimethoprim, pyrimethamine, pemetrexed); alkylating agents (e.g., cyclophosphamide, mechlorethamine, uramustine, melphalan, chlorambucil, thiotepa/chlorambucil, ifosfamide, carmustine, lomustine, streptozocin, busulfan, dibromomannitol, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin, triplatin tetranitrate, procarbazine, altretamine, dacarbazine, mitozolomide, temozolomide); anthracyclines (e.g., daunorubicin, doxorubicin, epirubicin, idarubicin, valrubicin); antibiotics (e.g., dactinomycin, bleomycin, mithramycin, anthramycin, streptozotocin, gramicidin D, mitomycins (e.g., mitomycin C), duocarmycins (e.g., CC-1065), calicheamicins); antimitotic agents (including, e.g., maytansinoids, auristatins, dolastatins, cryptophycins, vinca alkaloids (e.g., vincristine, vinblastine, vindesine, vinorelbine), taxanes (e.g., paclitaxel, docetaxel, or a novel taxane (see, e.g., International Patent Publication No. WO 01/38318, published May 31, 2001)), and colchicines; topoisomerase inhibitors (e.g., irinotecan, topotecan, amsacrine, etoposide, teniposide, mitoxantrone); and proteasome inhibitors (e.g., peptidyl boronic acids).

In some embodiments, the therapeutic agent is a maytansinoid. Maytansinoid compounds and methods for their conjugation to antibodies are described, for example, in Chari et al., *Cancer Res.,* 52: 127-131 (1992); Widdison et al., *J. Med. Chem.* 49: 4392-4408 (2006); and U.S. Pat. Nos. 5,208,020 and 6,333,410. Examples of maytansinoids include maytansine

analogues having a modified aromatic ring (e.g., C-19-dechloro, C-20-demethoxy, C-20-acyloxy) and those having modifications at other positions (e.g., C-9-CH, C-14-alkoxymethyl, C-14-hydroxymethyl or acyloxymethyl, C-15-hydroxy/acyloxy, C-15-methoxy, C-18-N-demethyl, 4,5-deoxy). In certain embodiments, the maytansinoid is N.sup.2'-deacetyl-N.sup.2'-(4-mercapto-1-oxopentyl)maytansine (DM3), N.sup.2'-deacetyl-N.sup.2'-(3-mercapto-1-oxopropyl)-maytansine (DM1), or N.sup.2'-deacetyl-N.sup.2'-(4-mercapto-4-methyl-1-oxopentyl)maytansine (DM4).

Maytansinoid compounds that comprise a sulfhydryl group can be coupled to antibodies using a heterobifunctional linker that is connected to the maytansinoid compound by way of a thioether or disulfide linkage. In some such embodiments, the linker is coupled to an amino group on the antibody (e.g., a terminal amino group or the epsilon amino group of a lysine residue. In some embodiments, the heterobifunctional linker that is used to couple a maytansinoid compounds to an antibody is N-succinimidyl 3-(2-pyridyldithio)proprionate (also known as N-succinimidyl 4-(2-pyridyldithio)pentanoate, or SPP), 4-succinimidyl-oxycarbonyl-2-methyl-2-(2-pyridyldithio)-toluene (SMPT), N-succinimidyl 4[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC), N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP); N-succinimidyl 4-(2-pyridyldithio)butyrate (SPDB), 2-iminothiolane, or S-acetylsuccinic anhydride.

In some other embodiments the therapeutic agent is a dolastatin. In some embodiments, the therapeutic agent is an auristatin, such as auristatin E (also known in the art as a derivative of dolastatin-10) or a derivative thereof. Auristatin compounds and methods for their conjugation to antibodies are described, for example, in Doronina et al., *Nature Biotech.,* 21: 778-784 (2003); Hamblett et al, *Clin. Cancer Res.,* 10: 7063-7070 (2004); Carter and Senter, *Cancer J.,* 14 154-169 (2008); U.S. Pat. Nos. 7,498,298, 7,091,186, 6,884,869; 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414; U.S. Patent Publication Nos. 20090010945, 20060074008, 20080300192, 20050009751, 20050238649, and 20030083236; and International Patent Publication Nos. WO

04/010957 and WO 02/088172, each of which is incorporated by reference herein in its entirety and for all purposes.

The auristatin can be, for example, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatins include auristatin phenylalanine phenylenediamine (AFP), monomethyl auristatin E (MMAE), and monomethyl auristatin F (MMAF).

In some embodiments, the therapeutic agent is a radionuclide. Examples of radionuclides useful as toxins in radiation therapy include: ^{47}Sc, ^{67}Cu, ^{90}Y, ^{109}Pd, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{199}Au, ^{211}At, ^{212}Pb and ^{212}B. Other radionuclides which have been used by those having ordinary skill in the art include: ^{32}P and ^{33}P, ^{71}Ge, ^{77}As, ^{103}Pb, ^{105}Rh, ^{111}Ag, ^{119}Sb, ^{121}Sn, ^{131}Cs, ^{143}Pr, ^{161}Tb, ^{177}Lu, ^{191}Os, ^{193}MPt, ^{197}Hg, all beta negative and/or auger emitters. Some preferred radionuclides include: ^{90}Y, ^{131}I, ^{211}At and $^{212}Pb/^{212}$ Bi.

One having ordinary skill in the art may conjugate an anti-GCC antibody molecule to a radionuclide using well-known techniques. For example, Magerstadt, M. (1991) Antibody Conjugates And Malignant Disease, CRC Press, Boca Raton, Fla.,; and Barchel, S. W. and Rhodes, B. H., (1983) Radioimaging and Radiotherapy, Elsevier, NY, N.Y., each of which is incorporated herein by reference, teach the conjugation of various therapeutic and diagnostic radionuclides to amino acids of antibodies. Such reactions may be applied to conjugate radionuclides to anti-GCC antibody molecules of the invention with an appropriate chelating agent and/or linker.

Anti-GCC Antibody Sequences

Rabbit monoclonal anti-GCC antibodies were generated by several methods, as is discussed in more detail in the Examples. Briefly, rabbit monoclonal antibodies MIL-44-148-2 and MIL-44-67-4 were generated by traditional immunization technology in rabbits. True rabbit-rabbit hybridomas were generated at Epitomics (Burlingame, CA) by fusing isolated B-cells

from an immunized rabbit with Epitomics' proprietary fusion partner cell line (see U.S. Patents 7,402,409; 7,429,487; 7,462,697; 7,575,896; 7,732,168; and 8,062,867). Specificity of the antibodies against GCC was tested by ELISA and flow cytometry (FCM).

Table 1 below summarizes the rabbit monoclonal anti-GCC antibodies of the invention generated using the hGCC(ECD)/mIgG2a FcR-mutII immunogen.

The sequences of the light and heavy chain variable regions were determined Table 2 below is a summary of the SEQ ID NOs for the variable regions of several antibodies. The amino acid and nucleic acid sequences for the variable regions of each of the heavy and light chains for rabbit anti-GCC antibodies are shown in Tables 3 and 4, respectively.

The amino acid and nucleic acid sequences for each of the CDRs of the heavy and light chains for anti-GCC antibodies are shown in Tables 5 and 6, respectively.

Sequencing of the CDRs allowed determination of the abundance of residues that might serve as toxin conjugation sites. For example, an unpaired free cysteine in the antigen binding region could be a site for auristatin conjugation and a lysine could be a site for maytansine conjugation. Toxin conjugation to an amino acid of the CDR would raise the concern of altering the binding affinity of the antibody to GCC. Thus, in embodiments the CDRs lack an amino acid which can be conjugated to a therapeutic agent.

Table 1: Summary of SEQ ID NOs for heavy and light chains of anti-GCC rabbit mAbs

mAb	IgG Chain	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
MIL-44-148-2 H2	Heavy	4	42
MIL-44-148-2 L5	Light	5	43
MIL-44-67-4 H2	Heavy	6	44
MIL-44-67-4 L4	Light	7	45

MIL-44-148-2 H2 Nucleic Acid (SEQ ID NO: 4)

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCAGTGAAGGAGTCCGG
GGGAGGCCTCTTCAAGCCAACGGATACCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAGTAGTCATAGAA
TGAACTGGGTCCGCCAGACTCCAGGGAAGGGGCTGGAATGGATCGCAATCATTACTCATAATAGTATCACATACTAC
GCGAGCTGGGCGAAAAGCCGATCCACCATCACCAGAAACACCAGCGAGAACACGGTGACTCTGAAAATGACCAGTCT
GACAGCCGCGGACACGGCCACTTATTTCTGTGCCAGAGAGGATAGTATGGGGTATTATTTTGACTTGTGGGGCCCAG
GCACCCTGGTCACCATCTCCTCA

GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

MIL-44-148-2 H2 Amino Acid (SEQ ID NO: 42)

METGLRWLLLVAVLKGVQCQSVKESGGGLFKPTDTLTLTCTVSGFSLSSHRMNWVRQTPGKGLEWIAIITHNSITYY
ASWAKSRSTITRNTSENTVTLKMTSLTAADTATYFCAREDSMGYYFDLWGPGTLVTISSGQPKAPSVFPLAPCCGDT
PSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTV
APSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQF
NSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFY
PSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

MIL-44-148-2 L5 Nucleic Acid (SEQ ID NO: 5)

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAGATGTGCCTATGATAT
GACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTA
GTAACTGGTTAGCCTGGTATCAGCAGAAACCAGGGCAGTCTCCCAAGCCCCTGATCTACAGGGCATCCACTCTGGCA
TCTGGGGTCTCATCGCGGTTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGTGGCGTGGAGTGTGC

CGATGCTGCCACTTACTACTGTCAGCAGACTTATACTAATAATCATCTTGATAATGGTTTCGGCGGAGGGACCGAGG
TGGTGGTCAAA
GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTCACCATCGT
GTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCG
AGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGACACTGACCAGCACACAG
TACAACAGCCACAAAGAGTACACCTGCAGGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGA
CTGTTAG

MIL-44-148-2 L5 Amino Acid (SEQ ID NO: 43)

MDTRAPTQLLGLLLLWLPGARCAYDMTQTPASVEVAVGGTVTIKCQASQSISNWLAWYQQKPGQSPKPLIYRASTLA
SGVSSRFRGSGSGTQFTLTISGVECADAATYYCQQTYTNNHLDNGFGGGTEVVVKGDPVAPTVLIFPPAADQVATGT
VTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCRVTQGTTSVVQSF
NRGDC

MIL-44-67-4 H2 Nucleic Acid (SEQ ID NO: 6)

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAGTCGGTGGAGGAGTCCGG
GGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGCCTCTGGATCCGACATCAGTAACTATGCAA
TATCCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATTCATCGGATATATTAGTTATGGTAAAAGTATATACTAC
GCGAGCTGGGCGAAAGGCCGGTTCGCCATCTCCAAAACCTCGTCGACCACGGTGGATCTGGAAATCACCAGTCCGAC
AACCGAGGACACGGCCACCTATTTTTGTGCCAGAGAGGATAGTGCTACTTATAGTCCTAACTTGTGGGGCCCAGGCA
CCCTGGTCACCGTCTCCTCA
GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGG
CTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA
CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCC
CACGTGCCCACCCCCTGAACTCCTGGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTAC
ATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAG
CACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGG
CCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGG
GAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTG
GGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCT
ACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG
CACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

MIL-44-67-4 H2 Amino Acid (SEQ ID NO: 44)

METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTASGSDISNYAISWVRQAPGKGLEFIGYISYGKSIYY
ASWAKGRFAISKTSSTTVDLEITSPTTEDTATYFCAREDSATYSPNLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTP
SSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVA
PSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFN
STIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYP
SDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

MIL-44-67-4 L4 Nucleic acid (SEQ ID NO: 7)

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAGATGTGCCTATGATAT
GACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGTATTA
ACACCTACTTAGCCTGGTATCAGCAGAAACCAGGGCAGCGTCCCAAGCTCCTGATCTACAGGGCATCCACTCTGGCA
TCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGCGGCGTGGAGTGTGC
CGATGCTGCCACTTACTACTGTCAACAGGGTTATAGTTATAATAATCTTGATCGTGCTTTCGGCGGAGGGACCGAGG
TGGTGGTCACA

GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTCACCATCGT
GTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCG
AGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGACACTGACCAGCACACAG
TACAACAGCCACAAAGAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGA
CTGTTAG

MIL-44-67-4 L4 Amino acid (SEQ ID NO: 45)

MDTRAPTQLLGLLLLWLPGARCAYDMTQTPASVEVAVGGTVTIKCQASQSINTYLAWYQQKPGQRPKLLIYRASTLA
SGVSSRFKGSGSGTEFTLTISGVECADAATYYCQQGYSYNNLDRAFGGGTEVVVTGDPVAPTVLIFPPAADQVATGT
VTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSF
NRGDC

Table 2: Summary of SEQ ID NOs for variable regions of anti-GCC rabbit mAbs

mAb	IgG Chain	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
MIL-44-148-2 H2	Heavy	10	11
MIL-44-148-2 L5	Light	12	13
MIL-44-67-4 H2	Heavy	14	15
MIL-44-67-4 L4	Light	16	17

Table 3: Amino Acid Sequences of mAb variable regions of anti-GCC rabbit mAbs

mAb	IgG Chain	SEQ ID NO:	Amino Acid Sequence
MIL-44-148-2	Heavy	11	QSVKESGGGLFKPTDTLTLTCTVSGFSLSSHRMNWVRQTPGKGLEWIA IITHNSITYYASWAKSRSTITRNTSENTVTLKMTSLTAADTATYFCAR EDSMGYYFDLWGPGTLVTISS
MIL-44-148-2	Light	13	AYDMTQTPASVEVAVGGTVTIKCQASQSISNWLAWYQQ KPGQSPKPLIYRASTLASGVSSRFRGSGSGTQFTLTISGVECADAATYYC QQTYTNNHLDNGFGGGTEVVVK
MIL-44-67-4	Heavy	15	QSVEESGGRLVTPGTPLTLTCTASGSDISNYAISWVRQAPG KGLEFIGYISYGKSIYYASWAKGRFAISKTSSTTVDLEITSPTTEDTATYFCAR EDSATYSPNLWGPGTLVTVSS
MIL-44-67-4	Light	17	AYDMTQTPASVEVAVGGTVTIKCQASQSINTYLAWYQQ KPGQRPKLLIYRASTLASGVSSRFKGSGSGTEFTLTISGVECADAATYYC QQGYSYNNLDRAFGGGTEVVVT

Table 4: Nucleic Acid Sequences of mAb variable regions of anti-GCC rabbit mAbs

mAb	IgG Chain	SEQ ID NO:	Nucleic Acid Sequence
MIL-44-148-2	Heavy	10	CAGTCAGTGAAGGAGTCCGGGGGAGGCCTCTTCAAGCCAACGGATACCCTGACACT CACCTGCACCGTCTCTGGATTCTCCCTCAGTAGTCATAGAATGAACTGGGTCCGCC AGACTCCAGGGAAGGGGCTGGAATGGATCGCAATCATTACTCATAATAGTATCACA TACTACGCGAGCTGGGCGAAAAGCCGATCCACCATCACCAGAAACACCAGCGAGAA CACGGTGACTCTGAAAATGACCAGTCTGACAGCCGCGGACACGGCCACTTATTTCT GTGCCAGAGAGGATAGTATGGGGTATTATTTTGACTTGTGGGGCCCAGGCACCCTG GTCACCATCTCCTCA
MIL-44-148-2	Light	12	GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGT CACCATCAAGTGCCAGGCCAGTCAGAGCATTAGTAACTGGTTAGCCTGGTATCAGC AGAAACCAGGGCAGTCTCCCAAGCCCCTGATCTACAGGGCATCCACTCTGGCATCT GGGGTCTCATCGCGGTTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCACCAT CAGTGGCGTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAGCAGACTTATACTA ATAATCATCTTGATAATGGTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA
MIL-44-67-4	Heavy	14	CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACT CACCTGCACAGCCTCTGGATCCGACATCAGTAACTATGCAATATCCTGGGTCCGCC AGGCTCCAGGGAAGGGGCTGGAATTCATCGGATATATTAGTTATGGTAAAAGTATA TACTACGCGAGCTGGGCGAAAGGCCGGTTCGCCATCTCCAAAACCTCGTCGACCAC GGTGGATCTGGAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTTTTGTG CCAGAGAGGATAGTGCTACTTATAGTCCTAACTTGTGGGGCCCAGGCACCCTGGTC ACCGTCTCCTCA
MIL-44-67-4	Light	16	GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGT CACCATCAAGTGCCAGGCCAGTCAGAGTATTAACACCTACTTAGCCTGGTATCAGC AGAAACCAGGGCAGCGTCCCAAGCTCCTGATCTACAGGGCATCCACTCTGGCATCT GGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTTCACTCTCACCAT CAGCGGCGTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAACAGGGTTATAGTT ATAATAATCTTGATCGTGCTTTCGGCGGAGGGACCGAGGTGGTGGTCACA

Table 5: Amino Acid Sequences of CDRs of anti-GCC rabbit mAbs

mAb	IgG	SEQ ID NO:	Amino Acid Sequence
MIL-44-148-2-H2	VH CDR1	21	SHRMN
MIL-44-148-2-H2	VH CDR2	22	IITHNSITYYASWAKS
MIL-44-148-2-H2	VH CDR3	23	EDSMGYYFDL
MIL-44-148-2-L5	VK CDR1	27	QASQSISNWLA
MIL-44-148-2-L5	VK CDR2	28	RASTLAS

mAb	IgG	SEQ ID NO:	Amino Acid Sequence
MIL-44-148-2-L5	VK CDR3	29	QQTYTNNHLDNG
MIL-44-67-4 H2	VH CDR1	33	NYAIS
MIL-44-67-4 H2	VH CDR2	34	YISYGKSIYYASWAKG
MIL-44-67-4 H2	VH CDR3	35	EDSATYSPNL
MIL-44-67-4 L4	VK CDR1	39	QASQSINTYLA
MIL-44-67-4 L4	VK CDR2	40	RASTLAS
MIL-44-67-4 L4	VK CDR3	41	QQGYSYNNLDRA

Table 6: Nucleic Acid Sequences of CDRs of anti-GCC rabbit mAbs

mAb	IgG	SEQ ID NO:	Nucleic Acid Sequence
MIL-44-148-2-H2	VH CDR1	18	AGTCATAGAATGAAC
MIL-44-148-2-H2	VH CDR2	19	ATCATTACTCATAATAGTATCACATACTACGCGAGCTGGGCGAAAAGC
MIL-44-148-2-H2	VH CDR3	20	GAGGATAGTATGGGGTATTATTTTGACTTG
MIL-44-148-2-L5	VK CDR1	24	CAGGCCAGTCAGAGCATTAGTAACTGGTTAGCC
MIL-44-148-2-L5	VK CDR2	25	AGGGCATCCACTCTGGCATCT
MIL-44-148-2-L5	VK CDR3	26	CAGCAGACTTATACTAATAATCATCTTGATAATGGT
MIL-44-67-4 H2	VH CDR1	30	AACTATGCAATATCC
MIL-44-67-4 H2	VH CDR2	31	TATATTAGTTATGGTAAAAGTATATACTACGCGAGCTGGGCGAAAGGC

mAb	IgG	SEQ ID NO:	Nucleic Acid Sequence
MIL-44-67-4 H2	VH CDR3	32	AGTCCTAACTTG
MIL-44-67-4 L4	VK CDR1	36	CAGGCCAGTCAGAGTATTAACACCTACTTAGCC
MIL-44-67-4 L4	VK CDR2	37	AGGGCATCCACTCTGGCATCT
MIL-44-67-4 L4	VK CDR3	38	CAACAGGGTTATAGTTATAATAATCTTGATCGTGCT

Therapeutic Uses

The rabbit monoclonal anti-GCC antibody molecules described herein have *in vitro* and *in vivo* utilities. For example, these antibody molecules can be administered to cells in culture, e.g. *in vitro* or *ex vivo*, or administered in a subject, e.g., *in vivo*, to treat and/or prevent, a variety of disorders. In certain emobidments of herapeutic applications of the invention, the rabbit monoclonal anti-GCC antibody molecules of the invention are humanized, using one or more techniques described above herein.

The antibody molecules, immunoconjugates, and fusion proteins described herein can be used to modulate an activity or function of a GCC protein, such as ligand binding (e.g., binding of ST or guanylin), GCC-mediated signal transduction, maintenance of intestinal fluid, electrolyte homeostasis, intracellular calcium release (calcium flux), cell differentiation, cell proliferation, or cell activation.

In one aspect, the invention features a method of killing, inhibiting or modulating the growth of, or interfering with the metabolism of, a GCC-expressing cell. In one embodiment, the invention provides a method of inhibiting GCC-mediated cell signaling or a method of killing a cell. The method may be used with any cell or tissue which expresses GCC, such as a cancerous cell. Examples of cancerous cells which express GCC include, but are not limited to, a cell from

a cancer of gastrointestinal origin (e.g., colorectal cancer, stomach cancer, small intestine cancer, or esophageal cancer), pancreatic cancer, lung cancer (e.g., squamous cell carcinoma, adenosquamous carcinoma, adenocarcinoma), soft-tissue sarcomas such as leiosacroma or rhabdomyosarcoma, gastrointestinal or bronchopulmonary neuroendocrine tumors, or neuroectodermal tumors, or any metastatic lesions thereof. Nonlimiting examples of GCC-expressing cells include T84 human colonic adenocarcinoma cells, fresh or frozen colonic tumor cells, and cells comprising a recombinant nucleic acid encoding GCC or a portion thereof.

Methods of the invention include the steps of contacting the cell with an anti-GCC antibody molecule or immunoconjugate thereof, as described herein, in an effective amount, i.e., amount sufficient to inhibit GCC-mediated cell signaling or an amount sufficient to kill the cell. The method can be used on cells in culture, e.g. *in vitro*, *in vivo*, *ex vivo*, or *in situ*. For example, cells that express GCC (e.g., cells collected by biopsy of a tumor or metastatic lesion; cells from an established cancer cell line; or recombinant cells), can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the anti-GCC antibody molecule or immunoconjugate to the culture medium. In methods of killing a cell, the method comprises using a naked anti-GCC antibody molecule, or an immunoconjugate comprising an anti-GCC antibody molecule and a cytotoxic agent. The method will result in killing of cells expressing GCC, including in particular tumor cells expressing GCC (e.g., colonic tumor cells).

The rabbit monoclonal antibodies of the invention, or humanized versions thereof, can be tested for cellular internalization after binding to GCC using immunofluorescence microscopy techniquies well known to those skilled in the art. Such antibodies that are confirmed to internalize would be useful when linked to a cytotoxic moiety for therapeutic purposes, or to a moiety for cell imaging. Antibodies which do not internalize can still be used for diagnostic purposes or for therapeutic methods using naked antibody designed to elicit an antibody-dependent cell-mediated cytotoxic response, or perhaps for liposome delivery methods.

Anti-GCC antibody molecules of the present invention bind to extracellular domains of GCC or portions thereof in cells expressing the antigen. As a result, when practicing the methods

of the present invention to kill, suppress, or detect cancerous cells, the antibodies or antigen binding fragments, bind to all such cells, not only to cells which are fixed or cells whose intracellular antigenic domains are otherwise exposed to the extracellular environment. Consequently, binding of the antibodies or antigen binding fragments, is concentrated in areas where there are cells expressing GCC, irrespective of whether these cells are fixed or unfixed, viable or necrotic. Additionally or alternatively, the anti-GCC antibody molecules, bind to and are internalized with GCC upon binding cells expressing the antigen.

The method also can be performed on cells present in a subject, as part of an *in vivo* protocol. In one embodiment, the subject is a human subject. Alternatively, the subject can be a mammal expressing a GCC antigen with which an anti-GCC antibody molecule disclosed herein cross-reacts. An anti-GCC antibody molecule or immunoconjugate thereof can be administered to a human subject for therapeutic purposes. An anti-GCC antibody molecule or immunoconjugate also can be administered to a non-human mammal expressing the GCC-like antigen with which the antibody cross-reacts (e.g., a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration). For *in vivo* embodiments, the contacting step is effected in a subject and includes administering an anti-GCC antibody molecule or immunoconjugate thereof to the subject under conditions effective to permit both binding of the antibody molecule to the extracellular domain of GCC expressed on the cell, and the treating of the cell.

In one embodiment, the invention provides a method of treating cancer by administering an anti-GCC antibody molecule or an immunoconjugate comprising an anti-GCC antibody molecule and a cytotoxic agent to a patient in need of such treatment. The method can be used for the treatment of any cancerous disorder which includes at least some cells that express the GCC antigen. As used herein, the term "cancer" is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The terms "cancer" and

"tumor" may be used interchangeably (e.g., when used in the context of treatment methods, "treatment of a cancer" and "treatment of a tumor" have the same meaning).

In embodiments, the treatment is sufficient to reduce or inhibit the growth of the subject's tumor, reduce the number or size of metastatic lesions, reduce tumor load, reduce primary tumor load, reduce invasiveness, prolong survival time, or maintain or improve the quality of life.

Examples of cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting colon. Adenocarcinomas include malignancies such as non-small cell carcinoma of the lung. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention.

In some embodiments, the GCC-expressing cancer to be treated is a primary or metastatic cancer of gastrointestinal origin, such as colorectal cancer, stomach cancer, small intestine cancer, or esophageal cancer. In some embodiments, the GCC_expressing cancer to be treated is primary or metastatic pancreatic cancer. In some embodiments, the GCC_expressing cancer to be treated is primary or metastatic lung cancer, such as squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma. In some embodiments, the GCC-expressing cancer to be treated is a sarcoma, such as leiomyosarcoma or rhabdomyosarcoma. In some embodiments, the GCC-expressing cancer to be treated is a primary or metastasized neuroectodermal tumor, such as aphaechromotcytoma or a paraganglioma. In some embodiments, the GCC-expressing cancer is a primary or a metastatized bronchopulmonary or a gastrointestinal neuroendocrine tumor.

The method can be useful in treating a relevant disorder at any stage or subclassification. For example, method can be used to treat early or late stage colon cancer, or colon cancer of any of stages 0, I, IIA, IIB, IIIA, IIIB, IIIC, and IV.

In some embodiments, the method for treating GCC-expressing cancer (e.g., colorectal cancer, stomach cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung

cancer, leiomyosarcoma, rhabdomyosarcoma, neuroendocrine tumor, neuroectodermal tumor etc.) comprises administering to a patient in need of such treatment a naked anti-GCC antibody molecule described herein. In other embodiments, the method comprises administering an immunoconjugate comprising an anti-GCC antibody molecule described herein and a cytotoxic agent such as a maytansanoid or an auristatin, or derivatives thereof. Methods of administering antibody molecules and immunoconjugates are described above. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular compound used.

In some embodiments, the anti-GCC antibody molecule or immunoconjugate is administered in treatment cycles. A "treatment cycle" consists of a treatment period, during which the anti-GCC antibody molecule or immunoconjugate is administered as described above, followed by a rest period, during which no anti-GCC antibody molecule or immunoconjugate is administered. The treatment cycle can be repeated as necessary to achieve the desired effect.

The anti-GCC antibodies described herein (e.g., naked anti-GCC antibody molecules or immunoconjugates comprising an anti-GCC antibody molecule and a therapeutic agent) may be used in combination with other therapies. For example, the combination therapy can include a composition of the present invention co-formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more anti-cancer agents, e.g., cytotoxic or cytostatic agents, hormone treatment, vaccines, and/or other immunotherapies. In other embodiments, the anti-GCC antibodies are administered in combination with other therapeutic treatment modalities, including surgery, radiation, cryosurgery, and/or thermotherapy. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

Administered "in combination," as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with

the disorder and before the disorder has been cured or eliminated. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

In some embodiments, the anti-GCC antibody molecule or immunoconjugate thereof is used in combination with a chemotherapeutic agent. Non-limiting examples of DNA damaging chemotherapeutic agents include topoisomerase I inhibitors (e.g., irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (e.g., etoposide, teniposide, and daunorubicin); alkylating agents (e.g., melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, mitomycin C, and cyclophosphamide); DNA intercalators (e.g., cisplatin, oxaliplatin, and carboplatin); DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (e.g., 5-fluorouracil, capecitibine, gemcitabine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea).

Chemotherapeutic agents that disrupt cell replication include: paclitaxel, docetaxel, and related analogs; vincristine, vinblastin, and related analogs; thalidomide, lenalidomide, and related analogs (e.g., CC-5013 and CC-4047); protein tyrosine kinase inhibitors (e.g., imatinib mesylate and gefitinib); proteasome inhibitors (e.g., bortezomib); NF-κB inhibitors, including

inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

The selection of therapeutic agent(s) or treatment modality to be combined with an anti-GCC antibody molecule or immunoconjugate of the invention will depend on the disorder to be treated. The additional agent(s) or treatment modality may include, for example, standard approved therapies for the indication being treated. For example, when the anti-GCC antibody molecule or immunoconjugate thereof is used to treat colon cancer, it may be used in combination with, e.g., surgery; radiation therapy; 5-fluorouricil (5-FU), capecitibine, leucovorin, irinotecan, oxaliplatin, bevacizumab, cetuximab, panitumum, or combinations thereof (e.g., oxaliplatin/capecitibine (XELOX), 5-fluorouricil/leucovorin/-oxaliplatin (FOLFOX), 5-fluorouricil/leucovorin/irinotecan (FOLFIRI), FOLFOX plus bevacizumab, or FOLFIRI plus bevacizumab).

In another aspect, the invention features the use of an anti-GCC antibody molecule or immunoconjugate as described herein in the manufacture of a medicament. In an embodiment, the medicament is for treating cancer, e.g., a gastrointestinal cancer. In some embodiments, the medicament comprises an anti-GCC antibody molecule having features summarized in Tables 1-6. In some embodiments, the medicament comprises a MIL-44-148-2 or a MIL-44-67-4 antibody molecule, or humanized versions thereof.

Antibody Labeling and Detection

Anti-GCC antibody molecules used in methods described herein, e.g., in the *in vitro* and *in vivo* detection, e.g., diagnostic, staging, or imaging methods, can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various biologically active enzymes, ligands, prosthetic groups, fluorescent materials, luminescent materials, chemiluminescent materials, bioluminescent materials, chromophoric materials, electron dense materials, paramagnetic (e.g.,

nuclear magnetic resonance active) materials, and radioactive materials. In some embodiments, the anti-GCC antibody molecule is coupled to a radioactive ion, e.g., indium (^{111}In), iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), bismuth (^{212}Bi or ^{213}Bi), sulfur (^{35}S), carbon (^{14}C), tritium (^{3}H), rhodium (^{188}Rh), technetium (^{99}mTc), praseodymium, or phosphorous (^{32}P); or a positron-emitting radionuclide, e.g., carbon-11 (^{11}C) potassium-40 (^{40}K), nitrogen-13 (^{13}N), oxygen-15 (^{15}O), fluorine-18 (^{18}F), gallium (^{68}Ga), and iodine-121 (^{121}I). Additional radioactive agents that can be conjugated to the antibodies of the invention for use in in *in vitro* or *in vivo* diagnostic/detection methods are described below.

Exemplary labels include fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, and 2,3-dihydrophthalazinediones. Other exemplary labels include horseradish peroxidase (HRP), alkaline phosphatase, galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose 6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Fluorophore and chromophore labeled antibody molecules can be prepared from standard moieties known in the art. Since antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluorescent compounds and chromophores are described by Stryer Science, 162:526 (1968) and Brand, L. et al. *Annual Review of Biochemistry*, 41:843-868 (1972). The antibodies can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Pat. Nos. 3,940,475, 4,289,747, and 4,376,110.

One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins derived from 3,6-dihydroxy-9-

henylxanthhydrol and resamines and rhodamines derived from 3,6-diamino-9-phenylxanthydrol and lissanime rhodamine B. The rhodamine and fluorescein derivatives of 9-o-carboxyphenylxanthhydrol have a 9-o-carboxyphenyl group. Fluorescein compounds having reactive coupling groups such as amino and isothiocyanate groups such as fluorescein isothiocyanate and fluorescamine are readily available. Another group of fluorescent compounds are the naphthylamines, having an amino group in the α or β position.

Labeled antibody molecules can be used, for example, diagnostically and/or experimentally in a number of contexts, including (i) to isolate a predetermined antigen by standard techniques, such as affinity chromatography or immunoprecipitation; (ii) to detect a predetermined antigen (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein; (iii) to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen.

In Vitro Diagnostics

The anti-GCC antibodies and immunoconjugates described herein can be used to detect the presence or absence of GCC, e.g., to detect the presence or absence of GCC in an *ex vivo* biological sample obtained from a subject (i.e., in vitro detection), or to detect the presence or distribution or absence of GCC in a subject (i.e., *in vivo* detection). Such detection methods are useful to detect or diagnose a variety of disorders, or to guide therapeutic decisions. The term "detecting" as used herein encompasses quantitative or qualitative detection. Detecting GCC or GCC protein, as used herein, means detecting intact GCC protein or detecting a portion of the GCC protein that comprises the epitope to which the anti-GCC antibody molecule binds.

Accordingly, in another aspect, the invention features, a method of detecting GCC expression in a biological sample such as a cell or tissue, e.g., a tumor cell, or a tumor having one or more cells that express GCC. The method comprises: contacting a biological sample, with an anti-GCC antibody molecule described herein (e.g., MIL-44-148-2 or MIL-44-67-4), under conditions which allow formation of a complex between the anti-GCC antibody molecule and GCC protein; and detecting formation of a complex between the anti-GCC antibody molecule

and GCC protein, to thereby detect the presence of GCC protein, e.g., to detect a GCC expressing cell or tumor.

In an embodiment, the anti-GCC antibody molecule is an immunoconjugate comprising a detectable label. The detectable label can be a radioactive agent. Alternatively, the detectable label is a non-radioactive agent (e.g. a fluorophore or a chromophore as described above).

In certain embodiments, the biological sample include normal and/or cancerous cells or tissues that express GCC. Examples of normal cells or tissues that express GCC include but are not limited cells or tissue or gastrointestinal origin, particularly normal colorectal cells or tissue. Examples of cancerous cells or tissues that express GCC include but are not limited to: cancer of gastrointestinal origin, such as colorectal cancer, stomach cancer, small intestine cancer and esophageal cancer; pancreatic cancer; lung cancer such as squamous cell carcinoma, adenosquamous carcinoma and adenocarcinoma; soft-tissue sarcomas such as leiomyosarcoma and rhabdomyosarcoma; gastrointential and bronchopulmonary neuroendocrine tumors; and neuroectodermal tumors. In particular embodiments, the normal and/or cancerous cells tissues may express GCC at higher levels relative to other tissues, for example other tissue such as B cells and/or B cell associated tissues.

Methods of detection described herein, whether *in vitro* or *in vivo*, can be used to evaluate a disorder in a subject. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor, e.g., colorectal cancer, stomach cancer or pancreatic cancer.

In one aspect, the invention provides, a method for detecting the presence or absence of GCC protein in a biological sample *in vitro* (e.g., e.g., in a cell or tissue biopsy obtainedfrom a subject) . The method comprises: (i) contacting a biological sample obtained from a subject with an anti-GCC antibody molecule or immunoconjugate thereofand (ii) detecting formation of a complex between the anti-GCC antibody molecule and GCC protein. Complex formation is indicative of the presence or level of GCC protein in the biological sample, whereas no complex formation is indicative of the absence of GCC protein in the biological sample.

Exemplary biological samples for methods described herein comprise a cell, cells, tissue or body fluid, such as an inflammatory exudate, blood, serum, bowel fluid, stool sample. In particular embodiments, the biological sample comprises a cancerous cell(s) or tissue. For example, the sample can be a tumor biopsy, e.g., biopsy of a colorectal tumor, a gastric tumor, an esophageal tumor, a small intestine tumor, a lung tumor, a soft-tissue sarcoma, a neuroendocrine tumor, a neuroectodermal tumor, or from a tissue sample from any metastatic site thereof. In other embodiments, the biological sample can be blood or another fluid, where the fluid comprises a cancer cell. A biological sample can be obtained using any of a number of methods in the art. Further, a biological sample can be treated with a fixative such as formaldehyde and embedded in paraffin and sectioned for use. Alternatively, fresh or frozen tissue can be employed. In other embodiments, fine-needle aspirates may be used.

In certain embodiments, a test cell or tissue is obtained from an individual suspected of having a disorder associated with GCC expression. In certain embodiments, a test cell or tissue is obtained from an individual suspected of having a disorder associated with GCC expression in a location other than the apical surface of intestinal epithelial cells (e.g., cytoplasmic GCC expression in intestinal epithelial cells), or a disorder associated with GCC expression in non-intestinal cells or tissue, such as pancreatic, lung, soft-tissue, or tissue of neuroendocrine or neuroectodermal origin. In certain embodiments, a test cell or tissue is obtained from an individual suspected of having a disorder associated with increased expression of GCC.

In an embodiment the level of GCC, in a sample from the subject, or in the subject, is compared with a reference level, e.g., the level of GCC in a control material, e.g., a normal cell of the same tissue origin as the subject's cell or a cell having GCC at levels comparable to such a normal cell. The method can comprise, e.g., responsive to the detected level of GCC, providing a diagnosis, a prognosis, an evaluation of the efficacy of treatment, or the staging of a disorder. A higher level of GCC in the sample or subject, as compared to the control material, indicates the presence of a disorder associated with increased expression of GCC. A higher level of GCC in the sample or subject, as compared to the control material, can also indicate, the relative lack of efficacy of a treatment, a relatively poorer prognosis, or a later stage of disease. The level of

GCC can also be used to evaluate or select future treatment, e.g., the need for more or less aggressive treatment, or the need to switch from one treatment regimen to another. In some embodiments, the methods further comprise selecting a GCC-targetd therapy, e.g., a GCC-targeted therapy described herein, based, at least in part, on the determined GCC levels, and optionally administering the selected GCC-targeted therapy to the subject.

Complex formation between the anti-GCC antibody molecule and GCC can be detected by measuring or visualizing either the antibody (or antibody fragment) bound to the GCC antigen or unbound antibody molecule. One having ordinary skill in the art can readily appreciate the multitude of ways to to detect binding of anti-GCC antibodies to GCC. Such methods include, but are not limited to, antigen-binding assays that are known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" Immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

In a particular embodiment, GCC is detected or measured by immunohistochemistry using an anti-GCC antibody of the invention. Immunohistochemistry techniques may be used to identify and essentially stain cells that express GCC. Such "staining" allows for analysis of metastatic migration. Anti-GCC antibodies such as those described herein are contacted with fixed cells and the GCC present in the cells reacts with the antibodies. The antibodies are detectably labeled or detected using labeled second antibody or protein A to stain the cells. In one particular embodiment, the MIL-44-148-2 antibody is used in an IHC assay to detect or measure GCC expression in a biological sample.

Other conventional detection assays can be used, e.g., western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC) or radioimmunoassay (RIA).

Alternative to labeling the anti-GCC antibody molecule, the presence of GCC can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable

substance and an unlabeled anti-GCC antibody molecule. In this assay, the biological sample, the labeled standards and the GCC binding agent are combined and the amount of labeled standard bound to the unlabeled antibody is determined. The amount of GCC in the sample is inversely proportional to the amount of labeled standard bound to the GCC binding agent.

It is also possible to directly detect GCC to anti-GCC antibody molecule complex formation without further manipulation or labeling of either component (GCC or antibody molecule), for example by utilizing the technique of fluorescence energy transfer (FET, see, for example, Lakowicz et al., U.S. Pat. No. 5,631,169; Stavrianopoulos, et al., U.S. Pat. No. 4,868,103). A fluorophore label on the first, "donor" molecule is selected such that, upon excitation with incident light of appropriate wavelength, its emitted fluorescent energy will be absorbed by a fluorescent label on a second "acceptor" molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the "donor" protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the "acceptor" molecule label may be differentiated from that of the "donor". Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, spatial relationships between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the `"acceptor" molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another example, determination of the ability of an antibody molecule to recognize GCC can be accomplished without labeling either assay component (GCC or antibody molecule) by utilizing a technology such as real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S, and Urbaniczky, C., 1991, Anal. Chem. 63:2338-2345 and Szabo et al., 1995, Curr. Opin. Struct. Biol. 5:699-705). As used herein, "BIA" or "surface plasmon resonance" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIACORE^{TM}). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical

phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In some aspects, the disclosure features a reaction mixture that includes an antibody molecule described herein (e.g., an immunoconjugate that includes an antibody molecule described herein and, e.g., a label) and a biological sample, e.g., a biological sample described herein. In other embodiments, the reaction mixture can include an antibody molecule described herein (e.g., an immunoconjugate that includes an antibody molecule described herein and, e.g., a label) and GCC obtained from a biological sample, e.g., a biological sample described herein.

In certain embodiments, a method, such as those described above, comprises detecting binding of an anti-GCC antibody to GCC expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing GCC on its surface. In certain embodiments, the method comprises contacting a cell with an anti-GCC antibody under conditions permissive for binding of the anti-GCC antibody to GCC, and detecting whether a complex is formed between the anti-GCC antibody and GCC on the cell surface. An exemplary assay for detecting binding of an anti-GCC antibody to GCC expressed on the surface of a cell is a "FACS" assay.

In Vivo Diagnostics

In still another embodiment, the invention provides a method for detecting the presence or absence of GCC-expressing cells or tissues *in vivo*. The method includes (i) administering to a subject (e.g., a patient having a cancer) an anti-GCC antibody molecule of the invention (i.e., MIL-44-148-2 or MIL-44-67-4), or antigen binding fragment thereof, preferably an antibody or antigen binding fragment thereof conjugated to a detectable label or marker; (ii) exposing the subject to a means for detecting said detectable label or marker to the GCC-expressing tissues or cells. Such *in vivo* methods can be used for evaluation, diagnosis, staging and/or prognosis of a patient suffering from a disorder such as cancer. The method comprises: (i) administering to a subject, an anti-GCC antibody molecule or immunoconjugate thereof; and (ii) detecting formation of a complex between the anti-GCC antibody molecule and GCC protein. Complex

formation is indicative of the presence or level of GCC in the subject whereas no complex formation is indicative of the absence of GCC in the subject.

Such individuals may be diagnosed as suffering from metastasized GCC-expressing cancer and the metastasized GCC-expressing cancer cells may be detected by administering to the individual, preferably by intravenous administration, a pharmaceutical composition that comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an anti-GCC antibody molecule and an active moiety wherein the active moiety is a radioactive agent, and detecting the presence of a localized accumulation or aggregation of radioactivity, indicating the presence of cells expressing GCC. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an anti-GCC antibody molecule and an active moiety wherein the active moiety is a radioactive agent and the anti-GCC antibody molecule is the MIL-44-148-2 antibody described herein, or fragments or derivatives thereof.

In one particular embodiment, radionuclides may be conjugated to an anti-GCC antibody molecule of the invention for use as an imaging agent in *in vivo* imaging procedures. Imaging agents are useful diagnostic procedures as well as the procedures used to identify the location of metastasized cells. For example, individuals may be diagnosed as suffering from metastasized colorectal cancer and the metastasized colorectal cancer cells may be detected by administering to the individual, preferably by intravenous administration, a pharmaceutical composition that comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an anti-GCC antibody molecule of the invention and an active moiety wherein the active moiety is a radionuclide and detecting the presence of a localized accumulation or aggregation of radioactivity, indicating the presence of cells that express GCC.

Imaging can be performed by many procedures well-known to those having ordinary skill in the art and the appropriate imaging agent useful in such procedures may be conjugated to an anti-GCC antibody molecule of the invention by well-known means. Examples of labels useful for diagnostic imaging in accordance with the present invention are radiolabels such as ^{32}P, ^{3}H,

^{14}C, ^{188}Rh, ^{43}K, ^{52}Fe, ^{57}Co, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{99}Tc, ^{111}In, $^{113}M\ In$, ^{123}I, ^{125}I, ^{127}Cs, ^{129}Cs, ^{131}I, ^{132}I, ^{197}Hg, ^{203}Pb and ^{206}Bi, and ^{213}Bi; fluorescent labels such as fluorescein and rhodamine; nuclear magnetic resonance active labels; positron emitting isotopes of oxygen, nitrogen, iron, carbon, or gallium (e.g., ^{68}Ga, ^{18}F) detectable by a single photon emission computed tomography ("SPECT") detector or positron emission tomography ("PET") scanner; chemiluminescers such as luciferin; and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes, such as a transrectal probe, can also be employed. Imaging can also be performed, for example, by radioscintigraphy, nuclear magnetic resonance imaging (MRI) or computed tomography (CT scan). Imaging by CT scan may employ a heavy metal such as iron chelates. MRI scanning may employ chelates of gadolinium or manganese.

The antibody can be labeled with such reagents using techniques known in the art. For example, Magerstadt, M. (1991) Antibody Conjugates And Malignant Disease, CRC Press, Boca Raton, Fla.; and Barchel, S. W. and Rhodes, B. H., (1983) Radioimaging and Radiotherapy, Elsevier, NY, N.Y., each of which is incorporated herein by reference, teach the conjugation of various therapeutic and diagnostic radionuclides to amino acids of antibodies. Such reactions may be applied to conjugate radionuclides to anti-GCC antibody molecules of the invention with an appropriate chelating agent and/or linker. See also Wensel and Meares (1983) Radioimmunoimaging and Radioimmunotherapy, Elsevier, N.Y., for techniques relating to the radiolabeling of antibodies. See also, D. Colcher et al. Meth. Enzymol. 121: 802-816 (1986).

In the case of a radiolabeled antibody, the antibody is administered to the patient, is localized to the tumor bearing the antigen with which the antibody reacts, and is detected or "imaged" *in vivo* using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography or computed tomography. See e.g., A. R. Bradwell et al., "Developments in Antibody Imaging", Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp 65-85 (Academic Press 1985). Alternatively, a positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven

National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N, ^{68}Ga).

In other embodiments, the invention provides methods for determining the dose, e.g., radiation dose, that different tissues are exposed to when a subject, e.g., a human subject, is administered an anti-GCC antibody molecule that is conjugated to a radioactive isotope. The method includes: (i) administering an anti-GCC antibody molecule as described herein, e.g., a anti-GCC antibody molecule, that is labeled with a radioactive isotope to a subject; (ii) measuring the amount of radioactive isotope located in different tissues, e.g., tumor, or blood, at various time points until some or all of the radioactive isotope has been eliminated from the body of the subject; and (iii) calculating the total dose of radiation received by each tissue analyzed. The measurements can be taken at scheduled time points, e.g., day 1, 2, 3, 5, 7, and 12, following administration (at day 0) of the radioactively labeled anti-GCC antibody molecule to the subject. The concentration of radioisotope present in a given tissue, integrated over time, and multiplied by the specific activity of the radioisotope can be used to calculate the dose that a given tissue receives. Pharmacological information generated using anti-GCC antibody molecules labeled with one radioactive isotope, e.g., a gamma-emitter, e.g., ^{111}In can be used to calculate the expected dose that the same tissue would receive from a different radioactive isotope which cannot be easily measured, e.g., a beta-emitter, e.g., ^{90}Y.

Companion Diagnostic for GCC-Targeted Therapy

The *in vitro* and *in vivo* diagnostic methods described herein are useful to inform whether a patient suffering from a profliferative disease such as cancer, or a gastrointestinal disorder such as inflammatory bowel syndrome, Crohn's Disease or constipation, or should be treated or not with a GCC-targeted therapy, based on the presence or absence, respectively, of GCC expression on the surface of or within the patient's cells or tissue. A patient having one more cells that express GCC on the cell surface or within the cell is a candidate for treatment with a GCC-targeted therapy.

In certain aspects, the invention provides a method of determining sensitivity of a patient suspected of suffering from a GCC-expressing disease or disorder to a GCC-targeted therapy, comprising the steps of: (i) contacting a biological sample obtained from a subject with an anti-GCC antibody molecule of the invention; (ii) detecting formation of a complex between the anti-GCC antibody molecule and GCC protein; wherein complex formation is indicative of the presence or level of GCC protein in the biological sample, whereas no complex formation is indicative of the absence of GCC protein in the biological sample, thereby determining the sensitivity of the patient to a GCC-targeted therapy. In a particular embodiment, complex formation between the anti-GCC antibody molecule and GCC protein in the biological sample is detected via immunohistochemistry using an antibody molecule described herein, e.g., the MIL-44-1482 antibody described herein.

Exemplary biological samples can comprise a cell, cells, tissue or body fluid, such as an inflammatory exudate, blood, serum, bowel fluid, stool sample. In particular embodiments, the biological sample comprises a cancerous cell(s) or tissue. For example, the sample can be a tumor biopsy, e.g., biopsy of a colorectal tumor, a gastric tumor, an esophageal tumor, a small intestine tumor, a lung tumor, a soft-tissue sarcoma, a neuroendocrine tumor, a neuroectodermal tumor, or from a tissue sample from any metastatic site thereof. In other embodiments, the biological sample can be blood or another fluid, where the fluid comprises a cancer cell. A biological sample can be obtained using any of a number of methods in the art. Further, a biological sample can be treated with a fixative such as formaldehyde and embedded in paraffin and sectioned for use. Alternatively, fresh or frozen tissue can be employed. In other embodiments, fine-needle aspirates may be used.

Exemplary diseases/disorders that may be evaluated (e.g., diagnosed) and treated using the companion diagnostic methods described herein include , but not limited to proliferative disorders including but not limited to colorectal cancer, stomach cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung cancer (e.g., squamous cell carcinoma, adenosquamous carcinoma, adenocarcinoma), soft tissue sarcoma such as leiomyosarcoma and rhabdomyosarcoma, gastrointestinal and bronchopulmonary neuroendocrine tumors, and

neuroectodermal tumors, gastrointestinal disorders such as inflammatory bowel syndrome, Crohn's Disease, and constipation, and neurological disorders such as Parkinson's Disease.

The methods of the invention guide physician's decisions in determining whether to treat a patient with a GCC-targeted therapy. The methods provided herein also allow for the generation of a personalized treatment report, e.g., a personalized cancer treatment report, e.g., with a GCC-targeted therapy described herein.

A GCC-targeted therapy is a therapeutic agent that treats or prevents a GCC-expressing disease or a GCC-mediated disease, e.g., a GCC-expressing disease or GCC-mediated disease described herein. In certain aspects of the invention, the GCC-targeted therapy is a GCC-ligand such as an anti-GCC antibody molecule or a peptide ligand (e.g., an ST peptide) conjugated to an agent, such as a therapeutic agent. Exemplary GCC-ligands conjugated to therapeutic agents (i.e., immunoconjugates) are described, e.g., U.S. Published Patent Application No. 20110110936, the contents of which are incorporated by reference herein in its entirety. In a particular embodiment, the GCC-targeted therapeutic agent is anti-GCC human IgG1 monoclonal antibody conjugated to a cytotoxic agent, wherein the mAb includes a light chain variable region (VL) having the three light chain complementarity determining regions (CDR1, CDR2, and CDR3) and a heavy chain variable region (VH) having the three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) listed in Tables 7 (amino acid sequences) and 8 (corresponding nucleic acid sequences) below, and a heavy chain variable region and light chain variable region listed in Tables 9 (amino acid sequences) and 10 (corresponding nucleic acid sequence) below.

Table 7: Amino acid sequence of VL CDRs and VH CDRs

VH CDR1	SEQ ID NO: 67	GYYWS
VH CDR2	SEQ ID NO: 68	EINHRGNTNDNPSLKS

VH CDR3	SEQ ID NO: 69	ERGYTYGNFDH
VL CDR1	SEQ ID NO: 70	RASQSVSRNLA
VL CDR2	SEQ ID NO: 71	GASTRAT
VL CDR3	SEQ ID NO: 72	QQYKTWPRT

Table 8: Nucleic acid sequence of VL CDRs and VH CDRs

VH CDR1	SEQ ID NO: 73	GGTTACTACTGGAGC
VH CDR2	SEQ ID NO: 74	GAAATCAATCATCGTGGAAACACCAACGAC AACCCGTCCCTCAAG
VH CDR3	SEQ ID NO: 75	GAACGTGGATACACCTATGGTAACTTTGACC AC
VL CDR1	SEQ ID NO: 76	AGGGCCAGTCAGAGTGTTAGCAGAAACTTA GCC
VL CDR2	SEQ ID NO: 77	GGTGCATCCACCAGGGCCACT
VL CDR3	SEQ ID NO: 78	CAGCAGTATAAAACCTGGCCTCGGACG

Table 9: Amino acid sequence of mAb variable region

Heavy chain	SEQ ID NO: 79	QVQLQQWGAGLLKPSETLSLTCAVFGGSFSGYYWSWI RQPPGKGLEWIGEINHRGNTNDNPSLKSRVTISVDTSK NQFALKLSSVTAADTAVYYCARERGYTYGNFDHWGQ GTLVTVSS
Light chain	SEQ ID NO: 80	EIVMTQSPATLSVSPGERATLSCRASQSVSRNLAWYQ QKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTIG SLQSEDFAVYYCQQYKTWPRTFGQGTNVEIK

Table 10: Nucleic acid sequence of mAb variable region

Heavy chain	SEQ ID NO: 81	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGT TGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGCT GTCTTTGGTGGGTCCTTCAGTGGTTACTACTGGAG CTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAG TGGATTGGGGAAATCAATCATCGTGGAAACACCA ACGACAACCCGTCCCTCAAGAGTCGAGTCACCAT ATCAGTAGACACGTCCAAGAACCAGTTCGCCCTG AAGCTGAGTTCTGTGACCGCCGCGGACACGGCTG TTTATTACTGTGCGAGAGAACGTGGATACACCTAT GGTAACTTTGACCACTGGGGCCAGGGAACCCTGG TCACCGTCTCCTCA
Light chain	SEQ ID NO: 82	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGT CTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGC AGGGCCAGTCAGAGTGTTAGCAGAAACTTAGCCT GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCT CCTCATCTATGGTGCATCCACCAGGGCCACTGGA ATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGA CAGAGTTCACTCTCACCATCGGCAGCCTGCAGTCT GAAGATTTTGCAGTTTATTACTGTCAGCAGTATAA AACCTGGCCTCGGACGTTCGGCCAAGGGACCAAC GTGGAAATCAAA

The amino acid and corresponding nucleic acid sequences for the full hIgG1 heavy chain and hKappa light chain sequences containing the VL CDRs and VH CDRs shown in Tables 7 and 8, and variable heavy and light chain regions shown in Tables 9 and 10 are listed below:

The hIgG1 heavy chain nucleotide sequence is:

GAATTCCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACA
GGTGTCCACTCCCAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTC
GGAGACCCTGTCCCTCACCTGCGCTGTCTTTGGTGGGTCTTTCAGTGGTTACTACTGG
AGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCA
TCGTGGAAACACCAACGACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAG
ACACGTCCAAGAACCAGTTCGCCCTGAAGCTGAGTTCTGTGACCGCCGCGGACACG
GCTGTTTATTACTGTGCGAGAGAACGTGGATACACCTATGGTAACTTTGACCACTGG
GGCCAGGGAACCCTGGTCACCGTCAGCTCAGCCTCCACCAAGGGCCCATCGGTCTTC
CCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGAC
CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAG
CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGT
GAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCA
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG
AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGA
GAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC
CCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG

TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATAATAGGGATAACAGGGTAATACTAGAG (SEQ ID NO: 83)

The hIgG1 heavy chain protein sequence is;

MGWSCIILFLVATATGVHSQVQLQQWGAGLLKPSETLSLTCAVFGGSFSGYYWSWIRQ
PPGKGLEWIGEINHRGNTNDNPSLKSRVTISVDTSKNQFALKLSSVTAADTAVYYCARE
RGYTYGNFDHWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO: 84)

The Kappa light chain nucleotide sequence is:

GCGGCCGCCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTA
CAGGTGTCCACTCCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTC
CAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGAAACTTA
GCCTGGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCC
ACCAGGGCCACTGGAATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTT
CACTCTCACCATCGGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCA
GTATAAAACCTGGCCTCGGACGTTCGGCCAAGGGACCAACGTGGAAATCAAACGTA
CGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
GAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
AGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACCCTGAGCAAAGC
AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCT
CGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGTCTAGA (SEQ ID NO: 85)

The hKappa light chain protein sequence is:

MGWSCIILFLVATATGVHSEIVMTQSPATLSVSPGERATLSCRASQSVSRNLAWYQQKP GQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTIGSLQSEDFAVYYCQQYKTWPRTFGQ GTNVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 86)

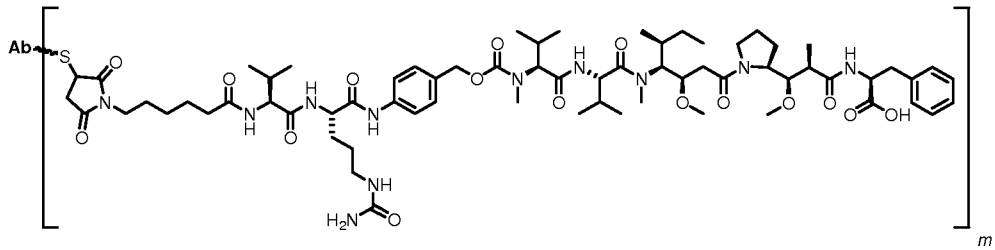
In one particular aspect of the invention, the GCC-targeted therapy is an immunoconjugate having an anti-GCC antibody molecule conjugated to an auristatin molecule. In one embodiment the immunoconjugate comprises an anti-GCC antibody molecule that includes three heavy chain (VH) CDRs according to SEQ ID NOs: 67, 68 and 69, and three light chain (VL) CDR regions according to SEQ ID NOs: 70, 71 and 72, conjugated to an auristatin molecule. In another embodiment, the immunoconjugate comprises an anti-GCC antibody molecule that includes a heavy chain variable region according to SEQ ID NO: 79, and a light chain variable region according to SEQ ID NO: 80, conjugated to an auristatin molecule. In still another embodiment, the immunoconjugate comprises an anti-GCC antibody molecule that includes the heavy and light chain variable regions according to SEQ ID NOs 79 and 80, respectively, conjugated to an auristatin molecule.

In some embodiments, the auristatin molecule is linked to a cysteine moiety on the anti-GCC antibody molecule by way of a linker containing a maleimide moiety, e.g., a maleimidocaproyl moiety.

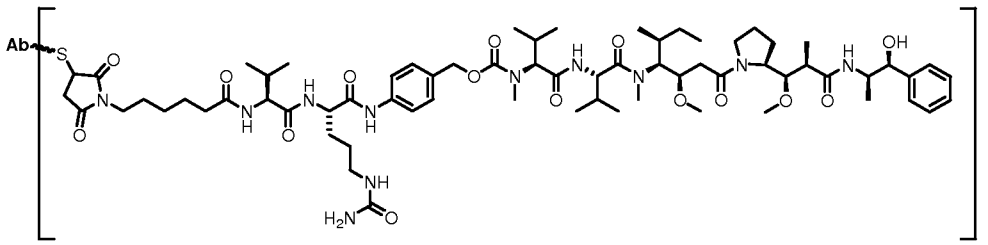
In some embodiments, the auristatin molecule is coupled to an anti-GCC antibody molecule using a heterobifunctional linker that is connected to a hydroxyl group on the auristatin molecule. In some such embodiments, the linker comprises a hydrazone. In some embodiments, the linker is a hydrazone compound formed by reaction of maleimidocaproylhydrazide and a ketocarboxylic acid, e.g., 5-benzoylvaleric acid. In particular embodiments, the linker is (Z)-6-(2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)hydrazono)-6-phenylhexanoic acid.

In some other embodiments the auristatin molecule is coupled to the anti-GCC antibody molecule using a heterobifunctional linker that is connected to a monomethyl amino group on the auristatin molecule. In some embodiments, the linker comprises a cleavable moiety, e.g., a peptide moiety, and a self-immolative *p*-aminobenzylcarbamate spacer. Exemplary linkers include maleimidocaproyl (mc), maleimidocaproyl-L-phenylalanine-L-lysine-*p*-aminobenzyl-carbamate, and maleimidocaproyl-L-valine-L-citrulline-*p*-aminobenzylcarbamate (vc).

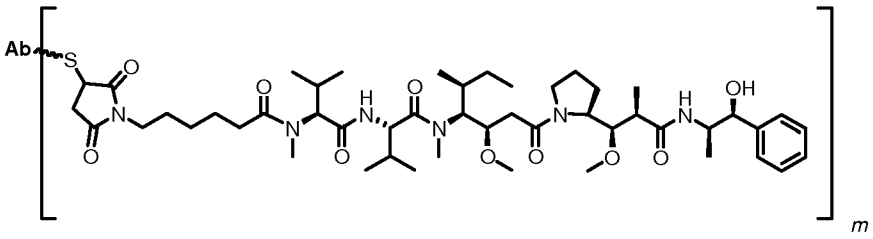
In certain embodiments, the GCC-targeted therapy is an immunoconjugate characterized by the formula Ab-(vc-MMAF)$_m$ (formula (***I-4***)); Ab-(vc-MMAE)$_m$ (formula (***I-5***)); Ab-(mc-MMAE)$_m$ (formula (***I-6***)); or Ab-(mc-MMAF)$_m$, (formula (***I-7***)), wherein Ab is an anti-GCC antibody molecule that includes features such as the features described in any one of Tables 7, 8, 9 or 10, S is a sulfur atom of the antibody, and *m* ranges from about 1 to about 15. In certain embodiments, *m* is an integer from 1 to about 5.



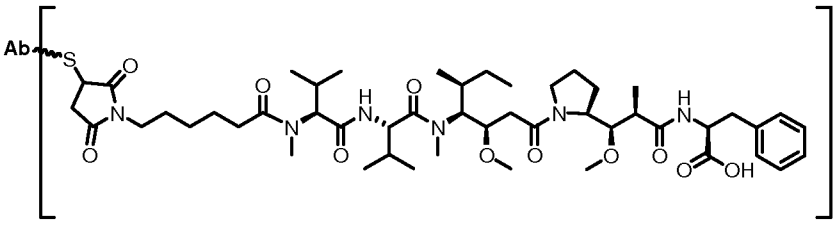
(***I-4***)



(***I-5***)



(***I-6***)



(*I-7*)

In some embodiments, the variable *m* in formula (***I-4***), (***I-5***), (***I-6***), or (***I-7***) ranges from about 2 to about 10, from about 6 to about 8, from about 4 to about 6, from about 3 to about 5, or from about 1 to about 3 .

In certain particular embodiments, the targeted GCC therapy is an immunoconjugate of formula (***I-4***), (***I-5***), (***I-6***), or (***I-7***), wherein Ab is a an antibody molecule that includes three heavy chain (VH) CDRs according to SEQ ID NOs: 67, 68 and 69, and three light chain (VL) CDR regions according to SEQ ID NOs: 70, 71 and 72, and *m* is about 3 to about 5 (e.g., about 4). In certain aspects, the Ab that includes the heavy chain CDRs according to SEQ ID NOs: 67, 68 and 69, and the three light chain CDRs according to SEQ ID NOs: 70, 71 and 72, respectively, is a human monoclonal antibody, preferably human IgG1 antibody.

In other particular embodiments, the GCC-targeted therapy is an immunoconjugate of formula (***I-4***), (***I-5***), (***I-6***), or (***I-7***), wherein Ab is a monoclonal antibody molecule that includes a heavy chain variable region according to SEQ ID NO: 79, and a light chain variable region according to SEQ ID NO: 80, and *m* is about 3 to about 5 (e.g., about 4). In certain aspects, the Ab that includes the heavy and light chain variable regions according to SEQ ID NOs 79 and 80, respectively, is a human monoclonal antibody, preferably human IgG1 antibody.

In still other certain embodiments, the GCC-targeted therapy is an immunoconjugate of formula (***I-4***), (***I-5***), (***I-6***), or (***I-7***), wherein Ab is a human IgG monoclonal antibody molecule that includes a heavy chain IgG1 sequence according to SEQ ID NO: 84, and a Kappa light chain sequence according to SEQ ID NO: 86, and *m* is about 3 to about 5 (e.g., about 4).

In yet another embodiment, the GCC-targeted therapy is a GCC-ligand conjugate capable of crossing the blood-brain barrier. For example, in certain aspects the invention relates to a GCC-ligand

conjugated to a neuroprotective agent such as L-dopa, which is capable of crossing the blood-brain barrier. Examples of such GCC-ligand conjugates are described in published PCT application WO2013/016662, the contents of which are incorporated by reference herein in its entirety. In embodiments of the invention, patients suffering from or suspected of having a neurological disorder (e.g., Parkinson's Disease) whose neurons express GCC would be considered good candidates for treatment with a GCC-ligand conjugated to a neuroprotective agent.

In some aspects of the invention, the GCC-targeted therapy is a GCC antagonist. In one emodiment, the GCC-targeted therapy is a peptide antagonist (e.g., an ST peptide) or a a small molecule inhibitor of GCC.

In some aspects, the GCC-targeted therapy is a GCC agonist. In one embodiment, the GCC agonist is an ST peptide. In a particular embodiment, the GCC agonist is an ST peptide comprising the amino acid sequence: H–Cys^1–Cys^2–Glu^3–Tyr^4–Cys^5–Cys^6–Asn^7–Pro^8–Ala^9–Cys^{10}–Thr^{11}–Gly^{12}–Cys^{13}–Tyr^{14}–OH (SEQ ID NO: 87), wherein there are three disulfide bonds: Between Cys^1 and Cys^6, between Cys^2 and Cys^{10}, and between Cys^5 and Cys^{13}. In a particular embodiment the GCC agonist is a peptide agonist that binds GCC such as Linaclotide (Ironwood Pharmaceuticals).

In certain embodiments of the invention, patients whose tumor cells express GCC on their surfaces would be considered good candidates for treatment with toxin-conjugated anti-GCC antibody molecules, such as an immunoconjugate as described herein, or the toxin-conjugated antibodies as described in U.S. Published Patent Application No. 20110110936, the contents of which are incorporated by reference herein in its entirety. Without intending to be bound by any theory, patients whose tumor cells express low amounts of GCC on their surfaces may not be as good candidates for this or might be candidates for combining the GCC-targeted therapy with an additional treatment method, or be candidates for naked antibody therapy. In another example, the dose of theGCC-targeted therapy could be adjusted to reflect the number of GCC molecules expressed on the surfaces of tumor cells. For example, patients with high numbers of GCC molecules on their tumor cell surfaces might be treated with lower doses of a GCC-targeted therapy than patients with low numbers of GCC molecules expressed on the tumor

cell surface. Detecting the presence of GCC-expressing tumor cells *in vivo* can allow identification of tissues into the primary GCC-expressing tumor has metastasized. Knowledge of which tissues have metastases can lead to targeted application of tumor therapy.

As discussed above, the antibody molecules described herein permit assessment of the presence of a GCC protein in normal versus neoplastic tissues, through which the presence or severity of disease, disease progress and/or the efficacy of therapy can be assessed. For example, therapy can be monitored and efficacy assessed. In one example, a GCC protein can be detected and/or measured in a first sample obtained from a subject having a proliferative disease and therapy can be initiated. Later, a second sample can be obtained from the subject and GCC protein in the sample can be detected and/or measured. A decrease in the quantity of GCC protein detected or measured in the second sample can be indicative of therapeutic efficacy.

Without intending to be bound by any theory, vascularization may be required for a GCC-targeted therapeutic to access a GCC expressing tumor, particularly in instances where the GCC-targeted therapeutic is administered intravenously. Thus, in certain embodiments of of the methods of the invention, it may be useful to evaluate or characterize tumor vasculature in addition to or in conjunction with the detection of GCC protein. For example, a tissue sample can be stained with an agent that identifies a vascular endothelial cell, such as an anti-CD-31 antibody or an anti-von Willebrand Factor antibody molecule, and an anti-GCC antibody of the invention to simultaneously or contemporaneously characterize GCC expression and tissue vascularization. In certain aspects of the invention, such simultaneously or contemporaneous characterization of GCC expression and vasculature is useful as a patient selection tool for a targeted -targetedtherapeutic.

In another aspect, cell surface expression of GCC may be required for a GCC-targeted therapeutic to affect the killing of a GCC expressing tumor cell. For example, some tumor cells may be expressing GCC, but not on the cell surface. If a therapeutic depends on cell surface GCC expression, such a therapeutic may not be able to kill a cell where GCC is primarily intracellular. Thus, in some embodiments of the invention, the diagnostic or prognostic assay

can further include analysis and/or quantification of cellular location of GCC, e.g., a method which can distinguish and/or quantify cell surface expression from intracellular expression. Without intending to be boundy by any theory, a patient whose tumor primarly has intracellular GCC expression may not be a good candidate for an anti-GCC antibody molecule which binds to the extracellular domain of GCC. Alternatively, such an analytical result may prompt initial treatment with an agent which induces cell surface expression of GCC (see, e.g., PCT publication No. WO04/071436). Following, or in conjunction with GCC cell surface induction, an anti-GCC antibody molecule which has access to or binds only to extracellularly expressed GCC can be administered.

Kits

Also within the scope of the invention are kits comprising an anti-GCC antibody molecule or immunoconjugate as described herein. Further included are kits comprising liposome compositions comprising an anti-GCC antibody molecule or immunoconjugate. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject. Instructions for use can include instructions for diagnostic applications of the anti-GCC antibody molecule or immunoconjugate to detect GCC, *in vitro*, e.g., in a sample, e.g., a biopsy or cells from a patient having a cancer, or *in vivo*. The instructions can include guidance for therapeutic application including suggested dosages and/or modes of administration, e.g., in a patient with a cancer (e.g., a cancer of gastrointestinal origin, such as, for example, colon cancer, stomach cancer, esophageal cancer). Other instructions can include instructions on coupling of the antibody to a chelator, a label or a therapeutic agent, or for purification of a conjugated antibody, e.g., from unreacted conjugation components. As discussed above, the kit can include a label, e.g., any of the labels described herein. As discussed above, the kit can include a therapeutic agent, e.g., a therapeutic agent described herein. In some applications the antibody will be reacted with other components, e.g., a chelator or a label or

therapeutic agent, e.g., a radioisotope, e.g., yttrium or lutetium. In such cases the kit can include one or more of a reaction vessel to carry out the reaction or a separation device, e.g., a chromatographic column, for use in separating the finished product from starting materials or reaction intermediates.

The kit can further contain at least one additional reagent, such as a diagnostic or therapeutic agent, e.g., a diagnostic or therapeutic agent as described herein, and/or one or more additional anti-GCC antibody molecules or immunoconjugates, formulated as appropriate, in one or more separate pharmaceutical preparations.

The kit can further contain a radioprotectant. The radiolytic nature of isotopes, e.g., ^{90}Yttrium (^{90}Y) is known. In order to overcome this radiolysis, radioprotectants may be included, e.g., in the reaction buffer, as long as such radioprotectants are benign, meaning that they do not inhibit or otherwise adversely affect the labeling reaction, e.g., of an isotope, such as of ^{90}Y, to the antibody. The formulation buffer of the present invention may include a radioprotectant such as human serum albumin (HSA) or ascorbate, which minimize radiolysis due to yttrium or other strong radionuclides. Other radioprotectants are known in the art and can also be used in the formulation buffer of the present invention, i.e., free radical scavengers (phenol, sulfites, glutathione, cysteine, gentisic acid, nicotinic acid, ascorbyl palmitate, HOP(:O)H_2I glycerol, sodium formaldehyde sulfoxylate, Na_2S_2O, $Na_2S_2O_3$, and SO_2, etc.).

A provided kit is one useful for radiolabeling a chelator-conjugated protein or peptide with a therapeutic radioisotope for administration to a patient. The kit includes (i) a vial containing chelator-conjugated antibody, (ii) a vial containing formulation buffer for stabilizing and administering the radiolabeled antibody to a patient, and (iii) instructions for performing the radiolabeling procedure. The kit provides for exposing a chelator-conjugated antibody to the radioisotope or a salt thereof for a sufficient amount of time under amiable conditions, e.g., as recommended in the instructions. A radiolabeled antibody having sufficient purity, specific activity and binding specificity is produced. The radiolabeled antibody may be diluted to an appropriate concentration, e.g., in formulation buffer, and administered directly to the patient

with or without further purification. The chelator-conjugated antibody may be supplied in lyophilized form.

The following examples are illustrative but are not meant to be limiting of the present invention.

EXAMPLES

Example 1: Generation of a human GCC extracellular domain-mouse Fc (hGCC-ECD-mFc) fusion protein

The generation of a secreted human (h) guanylyl cyclase (GCC) (hGCC) extracellular domain (ECD)/mouse immunoglobulin (Ig)G2a heavy chain constant (Fc) (with receptor binding region mutation (FcRbr-mutII) fusion protein (i.e., hGCC(ECD)-mIgG2a RcRbr-mutII fusion protein, also referred to herein as pLKTOK108 and MIL-44) for immunization and screening was performed as follows. GCC antigen was prepared by subcloning a portion of the GCC gene encoding a sequenced comprising the following GCC sequence (signal sequence and extracellular domain) into the pLKTOK4 expression vector:

MKTLLLDLALWSLLFQPGWLSFSSQVSQNCHNGSYEISVLMMGNSAFAEP
LKNLEDAVNEGLEIVRGRLQNAGLNVTVNATFMYSDGLIHNSGDCRSSTC
EGLDLLRKISNAQRMGCVLIGPSCTYSTFQMYLDTELSYPMISAGSFGLS
CDYKETLTRLMSPARKLMYFLVNFWKTNDLPFKTYSWSTSYVYKNGTETE
DCFWYLNALEASVSYFSHELGFKVVLRQDKEFQDILMDHNRKSNVIIMCG
GPEFLYKLKGDRAVAEDIVIILVDLFNDQYFEDNVTAPDYMKNVLVLTLS
PGNSLLNSSFSRNLSPTKRDFALAYLNGILLFGHMLKIFLENGENITTPK
FAHAFRNLTFEGYDGPVTLDDWGDVDSTMVLLYTSVDTKKYKVLLTYDTH
VNKTYPVDMSPTFTWKNSKL (SEQ ID NO: 46)

The amino acid sequence GLy-Arg-Gly-Pro-Gln (SEQ ID NO: 66), at positions 427 to 430, was selected to terminate the extracellular GCC fragment. In GCC, this sequence is immediately followed by a Pro that aligns well with a Pro at the position homologous to the Pro that is historically used to initiate human IgG1 Fc fusion proteins.

The mouse IgG2a Fc region of pLKTOK108 was designed to start with the amino acid sequence that functionally is the end of the CH1 domain [Pro-Arg-Valine (Val)-Pro-Isoleucine (Ile)-Threonine (Thr)-Glu-Asparagine (Asn)] (SEQ ID NO: 58). Two regions were mutated in the mouse IgG2a constant region. In addition to the leucine (Leu)-Leu-Gly-Gly (SEQ ID NO:

59) to Leu-alanine (Ala)-Gly-Ala (SEQ ID NO: 60) mutations (positions 234 to 237 Lysine [Lys]-Lys-Gly-Gly (SEQ ID NO: 61) to Lys-Ala-Gly-Ala (SEQ ID NO: 62), the second Fc receptor region at positions 318 to 322 was also mutated as follows: glutamic acid (Glu)-Phenylalanine (Phe)-Lys-Cysteine (Cys)-Lys (SEQ ID NO: 63) to Ala-Phe-Lys-Cys-Lys (SEQ ID NO: 64) and then to Phe-Lys-Cys-Lys (SEQ ID NO: 65).

Once the complete fusion protein sequence was designed, flanking restriction enzyme sequences for BamHI and XbaI, as well as the Kozak sequence (CTCACC) and a terminal stop codon were added to complete the fusion protein cDNA. The nucleotide and amino acid sequences of the fusion protein pLKTOK108 (hGCC/mIgG2a FcRmutII) is provided below (the BamHI and XbaI restriction sites are shown in lower case letters in SEQ ID NO: 47):

Human GCC-ECD/mouse IgG2a Fc nucleotide sequence (SEQ ID NO: 47)

cgcggatccctcaccATGAAGACGTTGCTGTTGGACTTGGCTTTGTGGTCACTGCTCTTCCAG
CCCGGGTGGCTGTCCTTTAGTTCCCAGGTGAGTCAGAACTGCCACAATGGCAGCTAT
GAAATCAGCGTCCTGATGATGGGCAACTCAGCCTTTGCAGAGCCCCTGAAAAACTTG
GAAGATGCGGTGAATGAGGGGCTGGAAATAGTGAGAGGACGTCTGCAAAATGCTGG
CCTAAATGTGACTGTGAACGCTACTTTCATGTATTCGGATGGTCTGATTCATAACTCA
GGCGACTGCCGGAGTAGCACCTGTGAAGGCCTCGACCTACTCAGGAAAATTTCAAA
TGCACAACGGATGGGCTGTGTCCTCATAGGGCCCTCATGTACATACTCCACCTTCCA
GATGTACCTTGACACAGAATTGAGCTACCCCATGATCTCAGCTGGAAGTTTTGGATT
GTCATGTGACTATAAAGAAACCTTAACCAGGCTGATGTCTCCAGCTAGAAAGTTGAT
GTACTTCTTGGTTAACTTTTGGAAAACCAACGATCTGCCCTTCAAAACTTATTCCTGG
AGCACTTCGTATGTTTACAAGAATGGTACAGAAACTGAGGACTGTTTCTGGTACCTT
AATGCTCTGGAGGCTAGCGTTTCCTATTTCTCCCACGAACTCGGCTTTAAGGTGGTGT
TAAGACAAGATAAGGAGTTTCAGGATATCTTAATGGACCACAACAGGAAAAGCAAT
GTGATTATTATGTGTGGTGGTCCAGAGTTCCTCTACAAGCTGAAGGGTGACCGAGCA
GTGGCTGAAGACATTGTCATTATTCTAGTGGATCTTTTCAATGACCAGTACTTGGAG
GACAATGTCACAGCCCCTGACTATATGAAAAATGTCCTTGTTCTGACGCTGTCTCCT
GGGAATTCCCTTCTAAATAGCTCTTTCTCCAGGAATCTATCACCAACAAAACGAGAC

TTTGCTCTTGCCTATTTGAATGGAATCCTGCTCTTTGGACATATGCTGAAGATATTTC
TTGAAAATGGAGAAAATATTACCACCCCCAAATTTGCTCATGCTTTCAGGAATCTCA
CTTTTGAAGGGTATGACGGTCCAGTGACCTTGGATGACTGGGGGGATGTTGACAGTA
CCATGGTGCTTCTGTATACCTCTGTGGACACCAAGAAATACAAGGTTCTTTTGACCT
ATGATACCCACGTAAATAAGACCTATCCTGTGGATATGAGCCCCACATTCACTTGGA
AGAACTCTAAACTTCCTAATGATATTACAGGCCGGGGCCCTCAGCCCAGAGTGCCCA
TAACACAGAACCCCTGTCCTCCACTCAAAGAGTGTCCCCCATGCGCAGCTCCAGACC
TCGCAGGTGCACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGA
TCTCCCTGAGCCCCATGGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAG
ACGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAA
ACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAG
CACCAGGACTGGATGAGTGGCAAGGCATTCAAATGCAAGGTCAACAACAGAGCCCT
CCCATCCCCCATCGAGAAAACCATCTCAAAACCCAGAGGGCCAGTAAGAGCTCCAC
AGGTATATGTCTTGCCTCCACCAGCAGAAGAGATGACTAAGAAAGAGTTCAGTCTG
ACCTGCATGATCACAGGCTTCTTACCTGCCGAAATTGCTGTGGACTGGACCAGCAAT
GGGCGTACAGAGCAAAACTACAAGAACACCGCAACAGTCCTGGACTCTGATGGTTC
TTACTTCATGTACAGCAAGCTCAGAGTACAAAAGAGCACTTGGGAAAGAGGAAGTC
TTTTCGCCTGCTCAGTGGTCCACGAGGGTCTGCACAATCACCTTACGACTAAGACCA
TCTCCCGGTCTCTGGGTAAATAAtctagagca

Human GCC-ECD/mouse IgG2a Fc amino acid sequence (SEQ ID NO: 48):

MKTLLLDLALWSLLFQPGWLSFSSQVSQNCHNGSYEISVLMMGNSAFAEPLKNLEDAV
NEGLEIVRGRLQNAGLNVTVNATFMYSDGLIHNSGDCRSSTCEGLDLLRKISNAQRMGC
VLIGPSCTYSTFQMYLDTELSYPMISAGSFGLSCDYKETLTRLMSPARKLMYFLVNFWK
TNDLPFKTYSWSTSYVYKNGTETEDCFWYLNALEASVSYFSHELGFKVVLRQDKEFQDI
LMDHNRKSNVIIMCGGPEFLYKLKGDRAVAEDIVIILVDLFNDQYLEDNVTAPDYMKN
VLVLTLSPGNSLLNSSFSRNLSPTKRDFALAYLNGILLFGHMLKIFLENGENITTPKFAHA
FRNLTFEGYDGPVTLDDWGDVDSTMVLLYTSVDTKKYKVLLTYDTHVNKTYPVDMSP
TFTWKNSKLPNDITGRGPQPRVPITQNPCPPLKECPPCAAPDLAGAPSVFIFPPKIKDVLMI

SLSPMVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQ
DWMSGKAFKCKVNNRALPSPIEKTISKPRGPVRAPQVYVLPPPAEEMTKKEFSLTCMIT
GFLPAEIAVDWTSNGRTEQNYKNTATVLDSDGSYFMYSKLRVQKSTWERGSLFACSVV
HEGLHNHLTTKTISRSLGK

As stated above, the recombinant protein pLKTOK108 combines the extracellular region of human GCC fused to a mouse IgG2a Fc region in which the two mutated Fc receptor binding regions (FcRs) were mutated to prevent Fc receptor binding (mIgG2a FcRmutII). The recombinant DNA insert for pLKTOK108 was created by a three-step PCR process as follows:

The first step created the adapted extracellular human GCC and the adapted mouse IgG2a FcRmutII DNA fragments containing 35 nucleotides of overlapping sequences. These PCR reactions used the templates and primers described in Table 11 and Table 12 with the protocol described in Table 13 to create the two fragments. These DNA fragments were isolated from a 1% agarose gel using a Qiagen Gel Purification kit (Valencia, CA). The human GCC template was provided by a protein expression vector containing the sequence for human GCC (Clontech Laboratories, Inc., Mountain View, CA, USA. The template for the Fc domain was obtained from an expression construct for human 1228 fused to mouse IgG2aFc with two mutated Fc receptor binding regions (FcRmutII), referred to as pLKTOK84, that itself were created using the vector pLKTOK61 (described in U.S. Patent 7,053,202, the contents of which are incorporated by reference herein in its entirety) as a template.

Table 11: Templates Used in First Step PCR Assembly Reactions to Create Recombinant DNA for pLKTOK108

Number	Product	Template	Primer 1	Primer 2	Size
1A	Extracellular GCC	Human GCC-Vect	pGCCFC5	pGCCFCMuA	1300 bp
1B	Mouse IgG2a-FcRmutII	pLKTOK84	pGCCFCMuB	pMICOS-4	700 bp

Table 12: Primers Used in All PCR Reactions to Create pLKTOK108

Primer Name	Sequence	SEQ ID NO:
pGCCFC5	5'-CGCGGATCCCTCACCATGAAGACGTTGCTGTTGGACTTGGC-3'	49
pGCCFCMuA	5'- TGGGCACTCTGGGCTGAGGGCCCCGGCCTGTAATATCATTAG -3'	50
pGCCFCMuB	5'-CAGGCCGGGGCCCTCAGCCCAGAGTGCCCATAACACAGAACCCCTGTCC -3'	51
pMICOS-4	5'-TGCTCTAGATTATTTACCCAGAGACCGGGAGATGGTCTTA	52
pSMUCH2	5'-ACCTGTGGAGCTCTTACTGG-3'	53
EF5S	5'-CATTTCAGGTGTCGTGAGGA-3'	54
SP6	5´-ATTTAGGTGACACTATAG-3´	55
M13f	5'-GTTTTCCCAGTCACGAC-3'	56
M13r	5'-AACAGCTATGACCATG-3'	57

Table 13: Reaction Protocol Used in First Step PCR Assembly Reactions to Create Recombinant DNA for pLKTOK108

Reaction Mixture	Machine settings
1 uL DNA (1:100 miniprep of template)	94°C- 2 minutes
0.2 uL 200mM Primer 1	
0.2 uL 200mM Primer 2	30 cycles
10 uL 10x PCR buffer	94°C- 1 minutes
3 uL 50mM $MgCl_2$	55°C- 30 seconds
2 uL 10mM dNTP mix	72°C- 2 minutes
83.5 uL H_2O	
0.5 uL Taq polymerase	72°C- 10 minutes

The second PCR reaction combined the templates in the concentrations listed in **Error! Reference source not found.**. The reaction protocol listed in **Error! Reference source not found.** created a single recombinant fusion protein gene. The product of this reaction was used directly as the template in the third PCR reaction.

Table 14: Templates Used in the Second Step PCR Assembly Reactions to Create Recombinant DNA for pLKTOK108

Number	Template 1	Template 2	Concentration
2A	Extracellular GCC	Mouse IgG2a-FcRmutII	10 uM (2.5 ul each)
2B	Extracellular GCC	Mouse IgG2a-FcRmutII	30 uM (7.5 ul each)

Table 15: Reaction Protocol Used in the Second Step PCR Assembly Reactions to Create Recombinant DNA for pLKTOK108

Reaction Mixture	Machine Settings
2.5 or 7.5 uL each DNA	8 cycles
10 uL 10x PCR buffer	94°C- 1 minute
3 uL $MgCl_2$	30 sec ramp
2 uL dNTP	72°C- 2 minutes
79.5 or 69.5 uL H_2O	30 sec ramp
0.5 uL Taq	

The third PCR reaction used the templates and primers described in **Error! Reference source not found.** and **Error! Reference source not found.** with the protocol described in **Error! Reference source not found.** below to create the complete fragments. These DNA fragments were isolated from a 0.7% agarose gel using a Qiagen Gel Purification kit (Appendix F), and a thiamine adenosine (TA) overhang TOPO® TA Cloning Kit (Appendix F). Unique clones were isolated and DNA purified using Qiagen's DNA miniprep kit (Appendix F). The DNA was sequenced with the primers M13f, M13r and pSMUCH2 to identify those with the desired sequence. The intermediate TOPO clone TOK108-15 contained the desired recombinant DNA sequence.

Table 16: Templates Used in the Third Step PCR Assembly Reactions to Create Recombinant DNA for pLKTOK108

Number	Product	Template	Primer 1	Primer 2	Size
3A	TOK108 Insert	Reaction 2A (5 ul)	pGCCFC5	pMICOS-4	2028 bp
3B	TOK108 Insert	Reaction 2B (5 ul)	pGCCFC5	pMICOS-4	2028 bp

Table 17: Reaction Protocol Used in the Third Step PCR Assembly Reaction to Create Recombinant DNA for pLKTOK108

Reaction Mixture	Machine settings
5 uL PCR Reaction 2A or 2B	94°C- 2 minutes
0.2 uL 200mM Primer 1	
0.2 uL 200mM Primer 2	30 cycles
10 uL 10x PCR buffer	94°C- 1 minute
3 uL 50mM MgCl2	55°C- 30 seconds
2 uL 10mM dNTP mix	72°C- 2 minutes
79.5 uL H2O	
0.5 uL Taq polymerase	72°C- 10 minutes

To create the expression vector pLKTOK4, pcDNA3.1™ was used as a backbone vector. It contains the neomycin (NEO) gene for resistance to G-418 (Geneticin®) to allow for easy selection under research conditions. The SpeI restriction site was eliminated from pcDNA™3.1 by site-directed mutagenesis. The EF-1α promoter from plasmid pcDEF3 (originally pEF-BOS[4]) was inserted into pcDNA™3.1, thus eliminating the CMV promoter. A circular map for the pLKTOK4 expression vector is depicted in Figure 1.

Cloning was performed on the final PCR products using a TOPO® TA Cloning kit. After digestion with BamHI and XbaI restriction enzymes, the desired fragment from the TOPO clone was ligated to the expression vector pLKTOK4 that was also digested with BamHI and XbaI. The ligation reaction was used to transform K12 chemically competent *E.coli* cells and then selected on Luria broth (LB)/ampicillin agar plates. Plasmids from individual *E. Coli* clones were isolated using QIAGEN's DNA miniprep kit and sequenced with the primers SP6 (SEQ ID NO: 55), EF5S (SEQ ID NO: 54) and pSMUCH2 (SEQ ID NO: 53).

A clone determined to contain the desired recombinant DNA by DNA sequencing and used to make a large quantity of pure plasmid DNA using a QIAGEN Maxiprep kit. This maxiprep DNA was used for transfection into dihydrofolate reductase-deficient Chinese hamster ovary (CHO-DG44) cells.

A serum-free, suspension adapted CHO-DG44 cell line, called S1-CHO-DG44, was used for developing pLKTOK108 production cell lines. Briefly, transfections were done using a Nucleofector® device from Amaxa Biosystems and Nucleofection® kit V using either non-linearized, circular DNA or linearized plasmid DNA treated with Pvu I restriction enzyme. Transfected cells were maintained in IS-CHO-V-GS growth media for 48 hours before exchanging into G-418 selection media. The live, transfected cells were fed with fresh G-418 selection media and maintained in culture until confluence (~10 to 14 days). The pLKTOK108 productivity of each transfection pool was assessed using a mouse IgG2a ELISA assay and the cells expanded for making frozen cell banks. The transfection pool with the highest productivity by mouse IgG2a ELISA was identified for limited dilution cloning where cells were plated into 5 × 96-well tissue culture plates in G-418 Selection Medium (approximately 1 cell in every other well). The 96-well plates were incubated in a 37°C incubator with 5% CO_2 for 2 weeks without feeding. Fifty µL of supernatant from each well that had a single colony was transferred directly into a 96-well assay plate to perform the mouse IgG2a ELISA assay. Twenty three clones with high productivity were identified and expanded sequentially through 24-well cell culture plates and then 6-well cell culture plates. The antibody titer of the supernatant from these clones was measured at 3 different dilutions in the mouse IgG2a ELISA assay.

The best 6 clones based on the mouse IgG2a titer were expanded in G-418 Selection Medium for making frozen cell banks and were adapted to serum-free, suspension Sigma #21 medium. The cell density and viability were determined using the Cedex Automated Cell Culture analyzer, and the protein concentration in the supernatant was measured using the mouse IgG2a ELISA assay. Once the cells reached logarithmic growth phase, they were harvested and frozen at -80°C overnight and then transferred to a liquid nitrogen cryochamber for storage.

To produce the fusion protein, cells were thawed and plated, and subsequently serially expanded into larger T-flasks and then into shaker flasks at starting densities of 3.0×10^5 cells/mL and incubated in a humidified incubator set at 37°C, with 5% CO_2 in an orbital shaker set at 105 rpm. The final cultures were fed with 10% volume of the Sigma #21 Special Feed Medium on Days 4 and 7, and 5% volume on Day 10. Sigma #21 Feed Medium consists of Sigma #21

Medium supplemented with 40 g/L glucose, 10 g/L L-glutamine, 10 g/L yeast extract, and 10 g/L soy peptone. The shake culture was harvested by centrifugation. The supernatant containing secreted pLKTOK108 protein was filtered through a 0.2-μm low protein binding polyethersulfone (PES) membrane filter unit, with the crude pLKTOK108-containing filtrate ready for purification or stored at -80°C for future purification.

Initial purification involved circulating filtered supernatants containing pLKTOK108 over Protein A Sepharose column at approximately 4°C. The resin was then washed with PBS pH7.4, and the protein eluted with 0.1M glycine in PBS at pH 3.0 and neutralized with 1M sodium phosphate at pH 6.5. The neutralized eluate was concentrated using a Vivaspin concentrator with a molecular weight cut-off (MWCO) of 30kDa and loaded onto a Superdex 200 size-exclusion chromatography (SEC) column (Appendix G) that was pre-equilibrated with PBS pH 7.4 buffer in order to separate out aggregates of this protein. Purified pLKTOK108 protein elutes as a single peak, with purity confirmed on SDS-PAGE and Coomassie staining. Fractions containing the hGCC(ECD)/mIgG2a Fc homodimers were pooled. After the concentration of the pooled material was determined by UV absorbance at 280 nm on a NanoDrop™ ND1000 spectrophotometer, the purified pLKTOK108 protein was aliquoted and stored at – 80°C.

Example 2: Generation of rabbit mAbs by protein immunization

Rabbit monoclonal antibodies against the hGCC(ECD)-mIgG2a RcRbr-mutII fusion protein (pLKTOK108) were generated using the RabMAb® service provided by Epitomics (Burlingame, CA). For the purposes of MAb generation, the hGCC(ECD)-mIgG2a RcRbr-mutII fusion protein (pLKTOK108) fusion protein is referred to herein as MIL-44.

Three rabbits (ML1009, ML1010 and ML1011) were immunized with MIL-44 using conventional immunization techniques. The serum titer against MIL-44 and a non-GCC counterscreen antigen (hMadCAM-mFc) was evaluated using test bleeds. Booster immunizations were given subsequent to the initial immunizations. The rabbit with the highest serum titer,

rabbit ML1010, was chosen as a candidate for splenectomy and monoclonal fusion using Epitomics' proprietary fusion partner cell line and methods.

On two separate days (Day 1 and Day 2), two hundred million lymphocyte cells were fused with 100 million fusion partner cells and plated on 20X 96-well plates, respectively. The plates were kept in tissue culture incubators under standard conditions. Cell growth was examined 2-3 weeks after fusion and fusion efficiency computed using the number of wells with growth divided by the total number of wells examined. The fusion efficiency for the fusion on Day 1 was measured at 72% fusion efficiency, whereas the fusion efficiency on Day 2 was 79%. A minimum of two plates were examined for each fusion as follows:

All 40 plates were screened using standard ELISA methods with plates coated with 50 ng of MIL-44/well. A bleed of ML1010 at 1:10K dilution was used as a positive control. 151 clones having an O.D. greater than 0.5 were considered putatively positive and were further expanded into a 24-well plate.

A subsequent confirmatory screen was performed by ELISA using plates coated with 50 ng of MIL-44 or 50 ng of hMadCAM-mFc/well. 143 clones were confirmed positive against MIL-44 and among them 72 were identified as MIL-44 specific, i.e., they were negative against hMadCAM-mFc protein.

Following the multiclone supernatant evaluation, several of the MIL-44 specific multiclones were sub-cloned: including multiclone #148 and #67. Subcloning was done using limited cell dilution method. Several subclone supernatatants were screened by ELISA. The hybridoma cells for subclones #148-2 and #67-4 were selected for freezing/banking and for further screening and analysis as a GCC detection reagent in an immunohistochemistry (IHC) assay, as described in Example 3.

The MIL-44-148-2 and MIL-44-67-4 antibodies were also cloned into pcDNA3.1+ neo (Invitrogen) for production by transient transfection in mammalian cells and for sequencing. The nucleic acid and amino acid sequences for the heavy and light chains for MIL-44-148-2 and

MIL-44-67-4 antibodies are provided below. The signal sequence in each IgG chain is shown italicized; the variable region in each IgG chain is shown in bold font; the CDR's are shown underlined.

MIL-44-148-2 H2 Nucleic Acid Sequence (SEQ ID NO: 4)

ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGT

CAGTCAGTGAAGGAGTCCGGGGGAGGCCTCTTCAAGCCAACGGATACCCTGACACTCACCTGCA

CCGTCTCTGGATTCTCCCTCAGTAGTCATAGAATGAACTGGGTCCGCCAGACTCCAGGGAAGGG

GCTGGAATGGATCGCAATCATTACTCATAATAGTATCACATACTACGCGAGCTGGGCGAAAAGC

CGATCCACCATCACCAGAAACACCAGCGAGAACACGGTGACTCTGAAAATGACCAGTCTGACAG

CCGCGGACACGGCCACTTATTTCTGTGCCAGAGAGGATAGTATGGGGTATTATTTTGACTTGTG

GGGCCCAGGCACCCTGGTCACCATCTCCTCA

GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCA

CGGTGACCCTGGGCTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTC

GGGCACCCTCACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCG

CTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCAG

CCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACC

CCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATG

ATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGC

AGTTCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCA

GTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGC

AAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCA

AAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAG

CAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAG

TGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACG

GCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCC

GTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

MIL-44-148-2 H2 Amino Acid Sequence (SEQ ID NO: 42)

*METGLRWLLLVAVLKGVQC***QSVKESGGGLFKPTDTLTLTCTVSGFSLSSHRMNWVRQTPGKGLE WIAIITHNSITYYASWAKSRSTITRNTSENTVTLKMTSLTAADTATYFCAREDSMGYYFDLWGP GTLVTISS**

GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYS LSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLM ISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAHQDWLRG KEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRS PGK

MIL-44-148-2 L5 Nucleic Acid Sequence (SEQ ID NO: 5)

*ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAGAT GT***GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGTCACCAT CAAGTGCCAGGCCAGTCAGAGCATTAGTAACTGGTTAGCCTGGTATCAGCAGAAACCAGGGCAG TCTCCCAAGCCCCTGATCTACAGGGCATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAGAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGTGGCGTGGAGTGTGCCGATGCTGCCAC TTACTACTGTCAGCAGACTTATACTAATAATCATCTTGATAATGGTTTCGGCGGAGGGACCGAG GTGGTGGTCAAA**

GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAA CAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGA TGGCACCACCCAAACAACTGGCATCGAGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACC TACAACCTCAGCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCT GCAGGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGACTGTTAG

MIL-44-148-2 L5 Amino Acid Sequence (SEQ ID NO: 43)

*MDTRAPTQLLGLLLLWLPGARC***AYDMTQTPASVEVAVGGTVTIKCQASQSISNWLAWYQQKPGQ**
SPKPLIYRASTLASGVSSRFRGSGSGTQFTLTISGVECADAATYYCQQTYTNNHLDNGFGGGTE
VVVK

GDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCT
YNLSSTLTLTSTQYNSHKEYTCRVTQGTTSVVQSFNRGDC

MIL-44-67-4 H2 Nucleic Acid Sequence (SEQ ID NO: 6)

*ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGT***CAGTCGG**
TGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGCCTC
TGGATCCGACATCAGTAACTATGCAATATCCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAA
TTCATCGGATATATTAGTTATGGTAAAAGTATATACTACGCGAGCTGGGCGAAAGGCCGGTTCG
CCATCTCCAAAACCTCGTCGACCACGGTGGATCTGGAAATCACCAGTCCGACAACCGAGGACAC
GGCCACCTATTTTTGTGCCAGAGAGGATAGTGCTACTTATAGTCCTAACTTGTGGGGCCCAGGC
ACCCTGGTCACCGTCTCCTCA

GGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGCTCCA
CGGTGACCCTGGGCTGCCTGGTCAAAGGGTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTC
GGGCACCCTCACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCG
CTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCAG
CCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACC
CCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATG
ATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGC
AGTTCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCA
GTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGC
AAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCA
AAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAG
CAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAG
TGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACG

GCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTT
CACCTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCT
CCGGGTAAATGA

MIL-44-67-4 H2 Amino Acid Sequence (SEQ ID NO: 44)

*METGLRWLLLVAVLKGVQC***QSVEESGGRLVTPGTPLTLTCTASGSDISNYAISWVRQAPGKGLE**
FIGYISYGKSIYYASWAKGRFAISKTSSTTVDLEITSPTTEDTATYFCAREDSATYSPNLWGPG
TLVTVSS

GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYS
LSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLM
ISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAHQDWLRG
KEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE
WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRS
PGK

MIL-44-67-4 L4 Nucleic Acid Sequence (SEQ ID NO: 7)

ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAGAT
*GT***GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACAGTCACCAT**
CAAGTGCCAGGCCAGTCAGAGTATTAACACCTACTTAGCCTGGTATCAGCAGAAACCAGGGCAG
CGTCCCAAGCTCCTGATCTACAGGGCATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAG
GCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGCGGCGTGGAGTGTGCCGATGCTGCCAC
TTACTACTGTCAACAGGGTTATAGTTATAATAATCTTGATCGTGCTTTCGGCGGAGGGACCGAG
GTGGTGGTCACA

GGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAA
CAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTGGA
TGGCACCACCCAAACAACTGGCATCGAGAACAGTAAAACACCGCAGAATTCTGCAGATTGTACC
TACAACCTCAGCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCT
GCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAGGGGTGACTGTTAG

MIL-44-67-4 L4 Amino Acid Sequence (SEQ ID NO: 45)

*MDTRAPTQLLGLLLLWLPGARC***AYDMTQTPASVEVAVGGTVTIKCQASQSINTYLAWYQQKPGQ RPKLLIYRASTLASGVSSRFKGSGSGTEFTLTISGVECADAATYYCQQGYSYNNLDRAFGGGTE VVVT**

GDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCT YNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFNRGDC

Example 3: Immunohistochemistry using anti-GCC antibodies

Detection of GCC expression in human tumor xenograft models of mCRC

An IHC assay using the MIL-44-148-2 antibody was developed to evaluate GCC expression in HEK293-GCC xenograft tumors and several primary human tumor xenografts (PHTX) derived from metastatic colorectal cancer (mCRC) patient samples in female SCID mice.

GCC protein levels in Formalin-Fixed, Paraffin-Embedded (FFPE) tissues were assessed on 5 μm thick sections and incubated with MIL-44-148-2 antibody (3.5 μg/mL) for 1 hour on the Ventana Medical Systems (Tucson, AZ) Discovery XT® automated stainer. Antibodies were biotinylated with a rabbit anti-goat secondary antibody (Vector Laboratories) and developed with the 3,3'-diaminobexidine (DAB) substrate map system (Ventana Medical Systems). Slides were counterstained with hematoxylin and imaged using the Aperio whole slide scanning system.

GCC levels differed significantly among these tumors with H-scores (scoring system described below) ranging from 4+ in HEK293-GCC tumor xenografts, and from 1+, 1-2+, 2+, 2-3+, and 4+ in various PHTX tumor xenografts. In general, in tumors with moderate/well differentiated tumor cells that maintained a polarized epithelial structure, GCC was concentrated on the luminal side of the tumor tissue.

Detection of GCC expression in human colon samples and tumor microarrays

The MIL-44-148-2 and MIL-44-67-4 antibodies described herein were also screened as a GCC detection reagent in an IHC protocol described above using the above-reference primary human tumor xenografts (PHTX), HT29 and HEK293 GCC transfected cell pellets, in addition to malignant and benign human colon samples (FFPE and tumor microarrays (TMAs)).

HT-29 and HEK293 GCC transfected cell pellets stained as expected. The PHTXs demonstrated a wide range of staining intensities with the MIL-44-148-2 and MIL-44-67-4 clones. Both MIL-44-148-2 and MIL-44-67-4 stained positive in well or moderately differentiated colon carcinoma *in situ* or metastasis. Poorly differentiated tumors stained less intensely. Normal colon tissue demonstrated positive apical staining using antibodies produced by both the MIL-44-148-2 and MIL-44-67-4 subclones.

Antibodies from both the MIL-44-148-2 and MIL-44-67-4 subclones provided readily apparent, intense and specific staining in the GCC transfected HEK293 cells and HT29 cells without any non-specific staining in the HEK293 and HT29 parental cell lines. Both clones also stained positive in well or moderately differentiated colon carcinoma in situ or metastasis. Less staining was seen for both subclones in poorly differentiated carcinomas. Normal colon demonstrated positive apical staining for both clones, as expected. MIL-44-148-2 demonstrated an overall higher sensitivity and specificity than MIL-44-67-4 in IHC. While MIL-44-67-4 demonstrated a better dynamic range than MIL-44-148-2 in cell pellets, MIL-44-148-2 demonstrated a superior dynamic range over that of MIL-44-67-4 in TMAs. Overall, the MIL-44-148-2 subclone showed superiority over the MIL-67-4, demonstrating higher sensitivity and specificity in IHC, and a full dynamic range in TMA's, and an intense staining (+2 IHC score) in normal colon at a low concentration.

Based on the results of the initial IHC experiments described above, the MIL-44-148-2 subclone was selected for the development and validation of an automated protocol equivalent to

the IHC protocol described above using a Tek-Mate automated stainer. The automated IHC assay is a useful tool for screening cancer patients for GCC expressing tumors as a clinical trial enrollment criteria for a GCC-targeted cancer therapeutic, and generally as a screening tool for selecting patients (e.g., cancer patients) who should receive a GCC-targeted therapy.

The IHC protocol shown in Table 18 was developed for detection of GCC in FFPE human cells and tissues, and approximately 53 colorectal tumors and 20 normal colon tissues, as well as 2 colon cancer TMAs (purchased from US Biomax) were screened for GCC expression. These tumors covered a range of tumor grades as well as colon cancer metastatic tissues.

Four-micron sections were prepared from the various tissue samples. Tissue sections were dewaxed through 4, 5-minute changes of xylene followed by a graded alcohol series to distilled water. Steam heat induced epitope recovery (SHIER) was used with SHIER2 solution for 20 minutes in the capillary gap in the upper chamber of a Black and Decker Steamer.

Table 18A: IHC Procedure

TechMate Steps	UltraVision Detection (UV)
1.	UltraVision Block- 15 minutes
2.	Primary Antibody Incubation – Overnight
3.	Primary Antibody Enhancer- 25 minutes
4.	Hydrogen peroxide block- 3 x 2.5 minutes each
5.	Polymer Detection- 25 minutes
6.	DAB Chromogen- 3 x 5.0 minutes each
7.	Hematoxylin Counter Stain - 1 minute

Table 18B: Antibody Reactivity Spec Sheet

Antibody	GCC
Supplier	MLNM Takeda in-house antibody
Catalog No.	N/A
Source/Isotype	RbIgG
Supplier Lot #	Not determined
QualTek Lot #	R3512
Clone	148-2
Concentration	0.475µg/ml
Suggested Dilution	1.0µg/ml
Incubation Time	Overnight
Pretreatments	SHIER2, no enzyme
TechMate Protocol	MIP
Detection system	UltraVision Detection System
Sub-Cellular Localization	Cytoplasmic and/or apical

One skilled in the art would recognize that the primary antibody enhancer can be an anti-rabbit secondary antibody raised in a species other than rabbit (e.g., human, rat, goat, mouse,

etc.) having the same isotype as the MIL-44-148-2 or MIL-44-67 rabbit mAbs (rabbit IgG) or a similar reagent that is suitable to amplify the MIL-44 signal.

The above protocol used an overnight antibody incubation of MIL-44-148-2 at 1.0µg/ml with a non-biotin based peroxidase detection (Ultravision kit from Thermo/Lab Vision) and DAB as chromogen. This procedure was completely automated using the TechMate 500 or TechMate 1000 (Roche Diagnostics). After staining, slides were dehydrated through an alcohol series to absolute ethanol followed by xylene rinses. Slides were permanently coverslipped with glass coverslips and CytoSeal. Slides were examined under a microscope to assess staining. Positive staining is indicated by the presence of a brown (DAB-HRP) reaction product. Hematoxylin counterstain provides a blue nuclear stain to assess cell and tissue morphology.

Upon evaluating the GCC staining, it was determined that an H-score approach would be the best approach for quantifying GCC expression. The H-score approach provides optimal data resolution for determining variation in intensity and tumor percentage of staining within and among tumor types. It also provides a good tool for determining thresholds for positive staining. In this method, the percentage of cells (0-100) within a tumor with staining intensities ranging from 0-3+ are provided. With the instant method, scores with intensities of 0, 0.5, 1, 2 and 3 were provided. Depending on the marker, 0.5 staining can be scored as positive or negative, and reflects light but perceptible staining for the marker. To obtain an H-score, the percentage of tumor cells are multiplied by each intensity and added together:

H score = (% tumor*1) + (% tumor*2) + (% tumor*3). For example, if a tumor is 20% negative (0), 30% +1, 10% +2, 40% +3, this would give an H score of 170.

The maximum H-score is 300 (100% * +3), per sub-cellular localization (i.e., apical or cytoplasmic), if 100% of tumor cells label with 3+ intensity. Initially, as a control, the total H-score alone was not be used to compare samples, but evaluated in addition to a review of the break-down of the percentage of cells at each intensity. For example, a score of 90 could represent 90% of tumor cells staining with 1+ intensity or 30% of cells with 3+ intensity. These samples have the same H-score but very different GCC expression. The percentage of cells to be

scored at each intensity can vary, but are normally scored in increments of 10%; however, a small percentage of scoring of a single component can be estimated at 1% and 5% as well in order to demonstrate that some level of staining is present. For GCC, apical staining may be considered for evaluating at low level increments, such as 1 and 5%.

Two different sub-cellular localizations were scored for GCC using the H-score approach. These include cytoplasmic staining and apical associated staining. The cytoplasmic staining pattern was generally observed as diffuse throughout the cytoplasm of tumor cells. However, in some cases there were variations of the cytoplasmic staining, which included intense globular staining or punctate, coarse granular staining. Intense globular staining was scored as 3+ cytoplasmic staining. The punctate staining was associated with apical staining and was not given a separate score for this type of cytoplasmic staining (n=4 samples for punctate staining). GCC apical staining was observed when lumen were present. Other GCC staining patterns observed included membrane-like, non-lumen staining (one case) and extra-cellular staining present in tumor lumen. In normal colon tissues, staining was generally apical along with diffuse cytoplasmic staining.

Since H scores were obtained for both cytoplasmic and apical GCC expression, and since it is not known whether one type of localization is more critical over another for efficacy of a GCC-targeted therapy, all data was captured and an some instances, an aggregate H score was generated by using the sum of both apical and cytoplasmic GCC expression. In such instances, the maximum H score became 600 for the aggregate score (300 apical + 300 cytoplasmic).

Overall, staining in a normal colon samples illustrated that GCC is anatomically privileged, being expressed on the apical surface. GCC was expressed on more than 95% of tumor samples and, in contrast to normal tissue, demonstrated diffuse cytoplasmic staining in some cases. Strong focal GCC staining in human CRC liver metastasis samples was also seen.

Tables 19A shows cytoplasmic and apical H-score staining results for normal and tumor tissues that were screened. Data shown in Table 19A is broken-out according to sample origin (in-house (denoted as MLNM), TMAs (denoted as BIOMAX), and CRO (denoted as QualTek))

and tumor grade. Summary data of positivity is provided when using thresholds of 0.5 and 1.0+ staining intensity. A total of 173 tumor samples were scored. When using a 0.5+ cut-off for positive staining intensity for either cytoplasmic or apical staining, 95% of tumors are considered positive. When using a 1.0+ cut-off, 92% of samples are considered positive.

The source of tissues shows variation in the percentage of positive tumors cells as well as the H-scores. For 1+ staining positivity threshold, the range is from 84% (CRO tumor MTB samples) to 100% (in-house samples or CRO single tissue samples – note the smaller number of samples in these groups). The in-house tumor tissues showed a very high apical H-score of 253 (n=9 samples). There were also differences in the scoring results of the 2 TMAs. US Biomax TMA C0992 stained stronger than C0701. Without intending to be bound by any theory, the difference in the TMAs may be due to a difference with fixation with the source of tissues or one block could have been cut more recently than the other.

The stability of the antigen in a cut section and the freshness of the cut samples were considered. Samples tested the instant study included samples that were cut and stored and samples that were cut fresh, indicating a need to further research the stability of the tissue samples over time.

Some differences were observed in GCC positivity and tumor grade (see Table 19B), with greater positive staining associated with well differentiated tumors vs. poorly differentiated tumors (six tumors from US Biomax did not include a grade). Grade 1 tumors (n=20) showed 100% positivity; Grade 2 tumors (n=95) labeled with 98% of positive cases; and grade 3 tumors (n=44) labeled at a positivity rate of 88%. Poorly differentiated tumors generally lack lumen, which may account for some of this decrease in staining due to a lack of apical staining. This percent positivity was based on a 0.5+ staining intensity threshold. Seven of 7 distant mets were positive (from in-house and CRO tissues). Metastatic tumors from the US Biomax TMA were listed as mets to lymph nodes.

Overall, GCC stains a very high percentage of colon tumors and normal colon tissues regardless of the source of the tissue or the tumor grade.

Table 19A: Summary of colon cancer staining by sample source

Colon CA Samples	Total Colon CA Samples	No. Sample Positive				Mean H-Score	
		0.5+ & Greater		1.0+ & Greater		0-300	
		No.	%	No.	%	Cyto	Apical
MLNM Samples	9	9	100%	9	100%	83	253
QualTek Single Samples	4	4	100%	4	100%	69	98
QualTek Colon CA MTBS	43	39	91%	36	84%	66	118
BIOMAX C0992 Array	65	63	97%	63	97%	144	164
BIOMAX C0701 Array	52	49	94%	48	92%	98	118
Total	**173**	**164**	**95%**	**160**	**92%**	**102**	**138**

Table 19B: Summary of colon cancer staining by tumor grade

Colon Samples	No. Positive	Total Samples	%
Normal	57	58	98%
Grade 1	20	20	100%
Grade 2	95	97	98%
Grade 3	44	50	88%
Total	**216**	**225**	**96%**

Intra-assay precision of the GCC IHC assay was evaluated within one run utilizing 5 replicates each from three cell pellets and 14 different colon carcinoma tissues. Cell pellets were prepared on separate slides. Colon tumor samples were included in two different multi-tumor blocks. These tissues were scored for GCC IHC reactivity.

Precision staining of cell pellets: Near identical staining was observed in all of the 5 intra-run replicates of the 3 cell pellets.

Precision staining of colon tumor samples: Very similar to near identical staining was observed among the 5 intra-run replicates of the 14 colon tumor samples. Samples were scored by a certified pathologist using the H-score approach as described previously above. The standard deviation demonstrated that in all cases the variance was minimal, thus demonstrating

good precision of staining within the same run. Overall, there was very consistent intra-run GCC IHC staining of the cell pellet and colon carcinoma samples tested.

Between-run assay variability and variability due to different operators was evaluated in 5 separate GCC IHC staining runs. Four runs were performed on different days by one operator and a second operator performed the fifth run. Staining included testing of the same tissues in the precision testing described above.

Reproducibility staining of cell pellets: Near identical staining as observed in all of the 5 inter-run replicates of the 3 cell pellets.

Reproducibility staining of colon tumor samples: Very similar to near identical staining was observed among the 5 inter-run replicates of the 14 colon tumor samples. Samples were scored by a certified pathologist using the H-score approach as described previously. The standard deviation demonstrated that in all cases the variance as minimal, thus demonstrating good reproducibility of staining from day to day and with a different operator. Overall, there was very consistent inter-run and inter-operator GCC IHC staining of the cell pellet and colon carcinoma samples tested.

Specificity of the GCC IHC assay was evaluated by testing a panel of normal human tissues. These normal human tissues included 30 different tissue types: adrenal, bladder, bone marrow, breast, cerebral cortex, cervix, fallopian tube, heart, kidney, liver, lung, lymph node, nerve, ovary, pancreas, parotid (salivary gland), pituitary, placenta, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testis, thymus, thyroid, tonsil, ureter, and uterus. For each tissue type, at least 3 unique specimens were stained and evaluated for GCC immunoreactivity.

Overall, the GCC IHC assay using the MIL-44-148-2 antibody was shown by the IHC assay described herein to be very specific for colon tumor samples compared to normal tissue staining, particularly for apical staining. Apical staining was only detected in 2 of the stomach samples; however, this staining was also observed in the negative control. Cytoplasmic staining, generally light, was observed in several tissue types, including ovarian follicle (1 of 3 samples),

skin (follicle and dermis, 2 of 3 samples), stomach parietal cells (2 of 3 samples), prostate glandular epithelium (light in 3 of 3 cases), pituitary (2 of 3 cases), uterus epithelium (3 of 3 cases), fallopian tube epithelium (2 of 3 cases), placenta trophoblast (light in 2 of 3 cases) and lung (endothelium in 3 of 3 cases and bronchiole epithelium in 1 of 3 cases). The strongest cytoplasmic staining (2+) was present in one case of fallopian tube and one case of pituitary. In both cases there was lighter staining in the same compartments in the negative control. Plasma cells were positive in a number of tissues, including spleen, tonsil and lymph node. Histiocytes were positive in spleen, lung and lymph node. Stromal staining was present in testis (2 of 3 cases), uterus (3 of 3 cases) and ovary (1 of 3 cases). Extracellular staining of blood vessels was widely observed and appears to be non-specific binding of serum.

The GCC assay, using the rabbit monoclonal antibody, MIL-44-148-2, on the TechMate staining platform shows consistent inter and intra-run staining of tumors and control cell pellets. The GCC assay appears to be highly sensitive in colon carcinoma as it stains the vast majority of colon tumor samples tested. The GCC assay also appears to be much more specific for colon tumors compared to normal tissues. GCC expression observed in many colon tumors is far stronger than any staining observed in a 30 tissue normal panel with at least 3 replicates of each tissue type. No specific apical GCC staining was detected in any of the normal tissues, whereas apical staining is common in the majority of GCC samples. Only cytoplasmic staining is observed in some normal tissue types and this staining is generally light. The MIL-44-148-2 antibody appears to be a reproducible, sensitive and relatively specific IHC marker for staining formalin-fixed, paraffin-embedded (FFPE) colon tumors.

Example 4: Additional Immunohistochemistry in Non-Colorectal PHTX Models and Tumor MicroArrays and Colorectal and Non-Colorectal Human Clinical Samples

The automated IHC assay described in Example 3 was used to evaluate GCC expression (i.e., apical, cytoplasmic and/or aggregate GCC expression) in a variety of non-colorectal samples from different sources, including primary human tumor xenografts (PHTX) derived

from gastric cancer and pancreatic cancer patient samples in female SCID mice, and various tumor microarrays (TMAs) purchased from US BioMax, Pantomics and other commercial sources) specific for pancreatic cancer, gastric cancer, esophageal cancer, lung cancer and leiomyosarcomas/rhabdomyosarcomas. GCC expression was also assessed via the automated IHC assay described in Example 3 in a variety human clinical samples, including human gastric, pancreatic and esophageal tumor samples obtained from the tissue database of a specialty CRO (QualTek) engaged to run the automated IHC assay, and colorectal cancer, gastric cancer, pancreatic cancer, esophageal cancer, and small intestine cancer derived from cancer patients tested for GCC expression prior to enrollment in an open-label, multicenter, dose escalation, first-in-human study of a GCC-targeted antibody-drug conjugate, designated as MLN0264, in adult patients with advanced gastrointestinal malignancies expressing Guanylyl Cyclase C (Study C26001, ClinicalTrials.gov identifier NCT01577758).

The results of the IHC staining in various normal and cancerous colorectal tissue samples and non-colorectal tissues (gastric, pancreatic, small intestine, esophageal, lung, rhabdomyosarcoma, leiomyosarcoma) from various sources (PHTX, human clinical samples and tumormicroarrays) are shown in Tables 20-32 below. Tables 20-32 include the IHC scores (0, 0.5+, 1+, 2+ and 3+) and the corresponding H scores calculated based on the IHC scores for both Apical ("A") and cytoplasmic ("C" or "Cyto") GCC staining in the various tissues tested. In addition, Table 32 shows a comparison of GCC IHC expression data in human clinical samples of primary and metastatic cancer, in each case obtained from the same patient. As shown in the first lefthand column, the "A" samples (i.e., 1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A 10A, etc.), refer to primary tumor samples, whereas the "B" samples (i.e., 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, etc.) refer to metastatic tumor samples obtained from the same patient from which the corresponding primary tumor sample (the corresponding"A" sample) was obtained. In otherwords, the designation "1A" and "1B" refer to primary and metastatic tumors, respectively, obtained from the same patient; the designation "2A" and "2B" refer to primary and metastatic tumors, respectively, obtained from another patient, and so forth. A majority of the tumor samples shown in Table 32 were obtained from patients having primary and metastatic colorectal

cancer, except where indicated otherwise as reflected in the column labeled "Comments", in which case the particular type of non-colorectal cancer is specified. As can be seen in the "Comments" column in Table 32, some samples of neuroendocrine tumors, renal cell carcinomas, gastric tumors, gastric GIST, pancreatic tumors, and uterine leiomyosarcoma were also for GCC expression by the IHC assay.

Table 20: IHC Assay in PHTX Model of Human Gastric Cancer

Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score
		0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cyto	Apical
GAF-023	Gastric CA Xenograft			100							100	50	300
GAF-025	Gastric CA Xenograft		100			90		5		5		115	0
GAF-055	Gastric CA Xenograft					70		30			100	130	300
GAF-074	Gastric CA Xenograft	100	90						10			0	20
GAF-075	Gastric CA Xenograft	90	100	10								5	0
GAF-087	Gastric CA Xenograft	100	100									0	0
GAF-114	Gastric CA Xenograft	100	100									0	0
GAF-152	Gastric CA Xenograft	100	100									0	0
GAF-318	Gastric CA Xenograft					60		40	20		80	140	280
GAF-019	Gastric CA Xenograft					60		20		20	100	160	300
GAF-151	Gastric CA Xenograft	50	50	50					40		10	25	110
GAM-006	Gastric CA Xenograft		100	50		40		10				85	0
GAM-016	Gastric CA Xenograft	100	100									0	0
GAM-022	Gastric CA Xenograft		100	100								50	0
GAM-031	Gastric CA Xenograft	90	100	10								5	0
GAM-033	Gastric CA Xenograft	70	30	20		10			40		30	20	170
GAM-037	Gastric CA Xenograft	100	100									0	0
GAM-042	Gastric CA Xenograft	100	100									0	0
GAM-044	Gastric CA Xenograft					50		50	50		50	150	250
GAM-046	Gastric CA Xenograft	50	100	50								25	0
GAM-060	Gastric CA Xenograft					20		30		50	100	230	300

Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score
		0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cyto	Apical
GAM-080	Gastric CA Xenograft	80	100	20								10	0
GAM-093	Gastric CA Xenograft	10	20	10		20	10	30	40	30	30	175	180
GAM-095	Gastric CA Xenograft	80	100	10		10						15	0
GAM-098	Gastric CA Xenograft		10	70		30			40		50	65	230
GAM-110	Gastric CA Xenograft	50	100	50								25	0
GAM-119	Gastric CA Xenograft		100					40		60		260	0
GAM-138	Gastric CA Xenograft	100	100									0	0
GAM-139	Gastric CA Xenograft	90	90	10			10					5	10

Table 21: PHTX Model of Human Pancreatic Cancer

Sample #	Tumor Type	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
117	Gastric		40	100	20		20		20	POS	100	60	100	120
118	Pancreatic		100	50		40		10		POS	100	0	160	0
119	Pancreatic		50		50	60		40		POS	100	50	240	50
120	Gastric	30	70	70	30					POS	70	30	70	30
121	Pancreatic	20	20	80	70		10			POS	80	80	80	90
122	Pancreatic				50	50	50	50		POS	100	100	250	150
123	Gastric		100			50		50		POS	100	0	250	0
124	Pancreatic	30	80	50	10	20	10			POS	70	20	90	30

Sample #	Tumor Type	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
125	Pancreatic				20	50	50	50	30	**POS**	100	100	250	210
126	Pancreatic		50	80	20	20	20		10	**POS**	100	50	120	90
127	Pancreatic	50		50			20		80	**POS**	50	100	50	280
128	Pancreatic			20		40		40	100	**POS**	100	100	220	300
129	Pancreatic	50	100	50						**POS**	50	0	50	0
130	Pancreatic		80	80	20	20				**POS**	100	20	120	20
131	Pancreatic					50		50	100	**POS**	100	100	250	300
132	Pancreatic		30	20	30	40	30	40	10	**POS**	100	70	220	120
133	Pancreatic	100	100							**NEG**	0	0	0	0
134	Pancreatic			40	40	40	40	20	20	**POS**	100	100	180	180
135	Pancreatic		70	70	20	30	10			**POS**	100	30	130	40
136	Pancreatic		30		30	100	30		10	**POS**	100	70	200	120
137	Pancreatic					70	50	30	50	**POS**	100	100	230	250
138	Pancreatic		0	100						**POS**	100	0	100	0
139	Pancreatic			50	40	50	30		30	**POS**	100	100	150	190
140	Pancreatic		50	100	50					**POS**	100	50	100	50
141	Pancreatic	30	40	40	30	30	30			**POS**	70	60	100	90
142	Pancreatic		100	30		70				**POS**	100	0	170	0
143	Pancreatic		30		30	50	20	50	20	**POS**	100	70	250	130
144	Pancreatic		20	50	20	50	20		40	**POS**	100	80	150	180
145	Pancreatic			40		30		30	100	**POS**	100	100	190	300

Table 22A: GCC IHC Staining in US Biomax P1921 Pancreatic Tumor MicroArray (TMA)

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A1	48	F	Duct adenocarcinoma (chronic inflammation of pancreas tissue)	-	100	100							NEG	0	0	0	0
A2	41	F	Duct adenocarcinoma	1													
A3	57	M	Duct adenocarcinoma	1	100							100	POS	0	100	0	300
A4	42	F	Duct adenocarcinoma	1	100	100							NEG	0	0	0	0
A5	47	F	Duct adenocarcinoma	1													
A6	54	F	Duct adenocarcinoma	1													
A7	40	F	Duct adenocarcinoma	2			30		70			100	POS	100	100	170	300
A8	54	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
A9	48	F	Duct adenocarcinoma	1	100	100							NEG	0	0	0	0
A10	41	F	Duct adenocarcinoma (sparse)	1	100	100							NEG	0	0	0	0
A11	57	M	Duct adenocarcinoma	1	100					50		50	POS	0	100	0	250

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A12	42	F	Duct adenocarcinoma	1	100	100							NEG	0	0	0	0
A13	47	F	Duct adenocarcinoma (sparse)	1													
A14	54	F	Duct adenocarcinoma	1	50	10	50	40		40		10	POS	50	90	50	150
A15	40	F	Duct adenocarcinoma	1			30		70			100	POS	100	100	170	300
A16	54	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
B1	51	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
B2	54	M	Duct adenocarcinoma	2	80	80	20	20					POS	20	20	20	20
B3	60	M	Duct adenocarcinoma	1	90	90	10	10					POS	10	10	10	10
B4	47	M	Duct adenocarcinoma	1	100	100							NEG	0	0	0	0
B5	39	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
B6	54	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
B7	62	F	Duct adenocarcinoma	1	100	80		10		10			POS	0	20	0	30
B8	64	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
B9	51	F	Duct	2	100	90		10					POS	0	10	0	10

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			adenocarcinoma														
B10	54	M	Duct adenocarcinoma	2	50	70	50	30					**POS**	50	30	50	30
B11	60	M	Duct adenocarcinoma	1	50	80	50	20					**POS**	50	20	50	20
B12	47	M	Duct adenocarcinoma	1	100	100							**NEG**	0	0	0	0
B13	39	M	Duct adenocarcinoma	2	50	100	50						**POS**	50	0	50	0
B14	54	M	Duct adenocarcinoma	2	100	100							**NEG**	0	0	0	0
B15	62	F	Duct adenocarcinoma	1	100	80		20					**POS**	0	20	0	20
B16	64	F	Duct adenocarcinoma (sparse)	-	100	100							**NEG**	0	0	0	0
C1	67	F	Duct adenocarcinoma	2	100	90		10					**POS**	0	10	0	10
C2	65	M	Duct adenocarcinoma	2	50	80	50	20					**POS**	50	20	50	20
C3	57	M	Duct adenocarcinoma	2	100	50		50					**POS**	0	50	0	50
C4	48	M	Duct adenocarcinoma	2		50	100	50					**POS**	100	50	100	50
C5	76	M	Duct adenocarcinoma	2		80	100	20					**POS**	100	20	100	20

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
C6	43	F	Duct adenocarcinoma (fibrofatty tissue)	-	100	100							NEG	0	0	0	0
C7	57	M	Duct adenocarcinoma	2	100	70				30			POS	0	30	0	60
C8	49	M	Duct adenocarcinoma	1	100	80		20					POS	0	20	0	20
C9	67	F	Duct adenocarcinoma	2	100	80		20					POS	0	20	0	20
C10	65	M	Duct adenocarcinoma	2	80	50	20	50					POS	20	50	20	50
C11	57	M	Duct adenocarcinoma	2	50	80	50	20					POS	50	20	50	20
C12	48	M	Duct adenocarcinoma	2	50	80	50	20					POS	50	20	50	20
C13	76	M	Duct adenocarcinoma	2	50	40	50	50		10			POS	50	60	50	70
C14	43	F	Duct adenocarcinoma (chronic inflammation of fibrofatty tissue)	-	90	90	10	10					POS	10	10	10	10
C15	57	M	Duct adenocarcinoma	2													
C16	49	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D1	52	M	Duct adenocarcinoma	2		20	80	20		20	20	40	POS	100	80	140	180

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
D2	72	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D3	53	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D4	55	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D5	51	M	Duct adenocarcinoma	2	100	90		10					POS	0	10	0	10
D6	57	M	Duct adenocarcinoma (fibrofatty tissue)	-		100	100						POS	100	0	100	0
D7	49	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D8	64	M	Duct adenocarcinoma	2		100	100						POS	100	0	100	0
D9	52	M	Duct adenocarcinoma	2		10	100	30		30		30	POS	100	90	100	180
D10	72	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D11	53	M	Duct adenocarcinoma	2	100	90		10					POS	0	10	0	10
D12	55	M	Duct adenocarcinoma	2	100	90		10					POS	0	10	0	10
D13	51	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D14	57	M	Duct adenocarcinoma (chronic	-	100	100							NEG	0	0	0	0

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			inflammation of fibrofatty tissue)														
D15	49	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
D16	64	M	Duct adenocarcinoma	2		90	100	10					POS	100	10	100	10
E1	57	M	Duct adenocarcinoma	2	50	50	50	50					POS	50	50	50	50
E2	72	M	Duct adenocarcinoma (sparse)	-	50	90	50	10					POS	50	10	50	10
E3	42	M	Duct adenocarcinoma	2	50	40	50	30		30			POS	50	60	50	90
E4	55	M	Duct adenocarcinoma	2	50	100	50						POS	50	0	50	0
E5	47	M	Duct adenocarcinoma (pancreas duct tissue)	-			100	100					POS	100	100	100	100
E6	44	M	Duct adenocarcinoma	2													
E7	59	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
E8	34	M	Duct adenocarcinoma	1		50	100	30		20			POS	100	50	100	70
E9	57	M	Duct adenocarcinoma	2	50	80	50	20					POS	50	20	50	20

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
E10	72	M	Duct adenocarcinoma	2	50	70	50	30					POS	50	30	50	30
E11	42	M	Duct adenocarcinoma	2													
E12	55	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
E13	47	M	Duct adenocarcinoma	2	50	50	50	50					POS	50	50	50	50
E14	44	M	Duct adenocarcinoma	2													
E15	59	M	Duct adenocarcinoma (sparse)	2	100	100							NEG	0	0	0	0
E16	34	M	Duct adenocarcinoma	1													
F1	61	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
F2	39	F	Duct adenocarcinoma	2	90	100	10						POS	10	0	10	0
F3	44	M	Duct adenocarcinoma	2		90	100	10					POS	100	10	100	10
F4	59	M	Duct adenocarcinoma	1	80		20	70		20		10	POS	20	100	20	140
F5	67	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
F6	72	F	Duct adenocarcinoma	2	50	90	50	10					POS	50	10	50	10
F7	41	F	Duct	2	50	100	50						POS	50	0	50	0

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			adenocarcinoma														
F8	51	M	Duct adenocarcinoma	2	80		20	70		30			**POS**	20	100	20	130
F9	61	M	Duct adenocarcinoma	2	50	80	50	20					**POS**	50	20	50	20
F10	39	F	Duct adenocarcinoma	2	100	100							**NEG**	0	0	0	0
F11	44	M	Duct adenocarcinoma	2		70	80		20	30			**POS**	100	30	120	60
F12	59	M	Duct adenocarcinoma	2			80	30	20	30		40	**POS**	100	100	120	210
F13	67	F	Duct adenocarcinoma	2	100	100							**NEG**	0	0	0	0
F14	72	F	Duct adenocarcinoma	2	50	90	50	10					**POS**	50	10	50	10
F15	41	F	Duct adenocarcinoma	2	50	100	50						**POS**	50	0	50	0
F16	51	M	Duct adenocarcinoma	2	50	50	50	40		10			**POS**	50	50	50	60
G1	41	M	Duct adenocarcinoma	2	100	100							**NEG**	0	0	0	0
G2	58	F	Duct adenocarcinoma	2	100	100							**NEG**	0	0	0	0
G3	60	M	Duct adenocarcinoma	2	50	50	50	50					**POS**	50	50	50	50
G4	41	M	Duct adenocarcinoma	2	50	90	50	10					**POS**	50	10	50	10

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G5	68	F	Duct adenocarcinoma	2	50	100	50						POS	50	0	50	0
G6	52	M	Duct adenocarcinoma (sparse) with necrosis	-	50	100	50						POS	50	0	50	0
G7	51	F	Duct adenocarcinoma	2		90	100	10					POS	100	10	100	10
G8	76	F	Duct adenocarcinoma	2		20	80	40	20	40			POS	100	80	120	120
G9	41	M	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
G10	58	F	Duct adenocarcinoma	2	50	100	50						POS	50	0	50	0
G11	60	M	Duct adenocarcinoma	2	50	50	50	50					POS	50	50	50	50
G12	41	M	Duct adenocarcinoma	2		50	100	30		20			POS	100	50	100	70
G13	68	F	Duct adenocarcinoma	2		80	100	20					POS	100	20	100	20
G14	52	M	Duct adenocarcinoma (sparse) with necrosis	-	50	100	50						POS	50	0	50	0
G15	51	F	Duct adenocarcinoma	2	50	50	50	50					POS	50	50	50	50
G16	76	F	Duct adenocarcinoma	2													

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H1	62	F	Adenocarcinoma	3	100	100							NEG	0	0	0	0
H2	51	M	Adenocarcinoma	3	50	100	50						POS	50	0	50	0
H3	60	F	Duct adenocarcinoma	2		100	100						POS	100	0	100	0
H4	76	M	Duct adenocarcinoma	2	60	100	30		10				POS	40	0	50	0
H5	78	M	Duct adenocarcinoma	2	50	80	50	20					POS	50	20	50	20
H6	53	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
H7	48	F	Duct adenocarcinoma	2	50	100	50						POS	50	0	50	0
H8	55	M	Duct adenocarcinoma	3	50	80	50	20					POS	50	20	50	20
H9	62	F	Adenocarcinoma	3	100	100							NEG	0	0	0	0
H10	51	M	Adenocarcinoma	3	50	100	50						POS	50	0	50	0
H11	60	F	Duct adenocarcinoma	2		100	100						POS	100	0	100	0
H12	76	M	Duct adenocarcinoma (fibrous tissue and blood vessel)	-	100	100							NEG	0	0	0	0
H13	78	M	Duct adenocarcinoma	2	50	100	50						POS	50	0	50	0
H14	53	F	Duct adenocarcinoma	2		90	100	10					POS	100	10	100	10

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H15	48	F	Duct adenocarcinoma	2	100	100							NEG	0	0	0	0
H16	55	M	Duct adenocarcinoma	2		100	90		10				POS	100	0	110	0
I1	59	M	Duct adenocarcinoma	2--3	50	90	50	10					POS	50	10	50	10
I2	62	M	Duct adenocarcinoma	3	100	100							NEG	0	0	0	0
I3	67	M	Adenocarcinoma (sparse)	-		100	50		50				POS	100	0	150	0
I4	66	F	Duct adenocarcinoma	3	50	100	50						POS	50	0	50	0
I5	49	M	Adenosquamous carcinoma (fibrous tissue and blood vessel)	-													
I6	50	M	Squamous cell carcinoma	3	50	100	50						POS	50	0	50	0
I7	73	M	Undifferentiated carcinoma	-	100	100							NEG	0	0	0	0
I8	65	M	Undifferentiated carcinoma	-	50	100	50						POS	50	0	50	0
I9	59	M	Duct adenocarcinoma	2	50	80	50	20					POS	50	20	50	20
I10	62	M	Duct adenocarcinoma (sparse)	2	100	100							NEG	0	0	0	0
I11	67	M	Adenocarcinoma	3		100	50		50				POS	100	0	150	0

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
I12	66	F	Duct adenocarcinoma	3	50	100	50						POS	50	0	50	0
I13	49	M	Adenosquamous carcinoma	-		100	100						POS	100	0	100	0
I14	50	M	Squamous cell carcinoma	3		80	100	20					POS	100	20	100	20
I15	73	M	Undifferentiated carcinoma	-		100	100						POS	100	0	100	0
I16	65	M	Undifferentiated carcinoma	-													
J1	56	F	Undifferentiated carcinoma	-	100	100							NEG	0	0	0	0
J2	52	F	Carcinoid	-	100	100							NEG	0	0	0	0
J3	51	M	Atypical carcinoid	-	100	100							NEG	0	0	0	0
J4	42	M	Neuroendocrine carcinoma	-	100	100							NEG	0	0	0	0
J5	52	F	Adenocarcinoma	1	50	90	50	10					POS	50	10	50	10
J6	45	F	Adenocarcinoma	2	100	90		10					POS	0	10	0	10
J7	49	M	Adenocarcinoma	2													
J8	65	M	Adenocarcinoma	2													
J9	56	F	Undifferentiated carcinoma	-	90	100	10						POS	10	0	10	0
J10	52	F	Carcinoid	-	100	100							NEG	0	0	0	0
J11	51	M	Atypical carcinoid	-	100	100							NEG	0	0	0	0
J12	42	M	Neuroendocrine	-	100	100							NEG	0	0	0	0

USBiomax PA1921 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			carcinoma														
J13	52	F	Adenocarcinoma (chronic inflammation of pancreas tissue)	-		100	100						**POS**	100	0	100	0
J14	45	F	Adenocarcinoma	2	50	80	50			20			**POS**	50	20	50	40
J15	49	M	Adenocarcinoma	2													
J16	65	M	Adenocarcinoma	2													
K1	52	M	Adenocarcinoma	2		80	100	20					**POS**	100	20	100	20
K2	56	F	Adenocarcinoma	2		90	100	10					**POS**	100	10	100	10
K3	76	F	Adenocarcinoma	2	100	100							**NEG**	0	0	0	0
K4	45	M	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
K5	41	M	Adenocarcinoma	3			50		50	50		50	**POS**	100	100	150	250
K6	62	M	Adenocarcinoma	3													
K7	50	M	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
K8	60	M	Adenocarcinoma	3													
K9	52	M	Adenocarcinoma	2	100	90		10					**POS**	0	10	0	10
K10	56	F	Adenocarcinoma	2	50	80	50	20					**POS**	50	20	50	20
K11	76	F	Adenocarcinoma (sparse) of liver	3	100	100							**NEG**	0	0	0	0
K12	45	M	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
K13	41	M	Adenocarcinoma	3													

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
K14	62	M	Adenocarcinoma	3	100	100							NEG	0	0	0	0
K15	50	M	Adenocarcinoma	3	100	100							NEG	0	0	0	0
K16	60	M	Adenocarcinoma	3	100	100							NEG	0	0	0	0
L1	56	M	Duct adenocarcinoma	3													
L2	49	F	Adenosquamous carcinoma	-	100	100							NEG	0	0	0	0
L3	25	M	Adenocarcinoma	2	100	90		10					POS	0	10	0	10
L4	38	M	Adenocarcinoma	2	50	90	50	10					POS	50	10	50	10
L5	42	M	Duct adenocarcinoma	2													
L6	59	M	Adenocarcinoma (pancreas tissue)	-		100			50		50		POS	100	0	250	0
L7	60	F	Duct adenocarcinoma	3	100	100							NEG	0	0	0	0
L8	13	F	Solid pseudopapillary carcinoma	-	100	100							NEG	0	0	0	0
L9	56	M	Duct adenocarcinoma	3	100	100							NEG	0	0	0	0
L10	49	F	Adenosquamous carcinoma	-	90	100	10						POS	10	0	10	0
L11	25	M	Adenocarcinoma (sparse)	2	100	100							NEG	0	0	0	0
L12	38	M	Adenocarcinoma	2	100	90		10					POS	0	10	0	10

USBiomax PA1921 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
L13	42	M	Duct adenocarcinoma	2	100	40		40		20			POS	0	60	0	80
L14	59	M	Adenocarcinoma	3		100			50		50		POS	100	0	250	0
L15	60	F	Duct adenocarcinoma	3	100	100							NEG	0	0	0	0
L16	13	F	Solid pseudopapillary carcinoma	-	100	100							NEG	0	0	0	0

Table 22B: Summary GCC Staining in US Biomax P1921 Pancreatic Tumor MicroArray (TMA)

AVG % POS Cyto	AVG % POS Apical	AVG H Cyto	AVG H Apical	Total Pos	Total N	Pct Pos
33	16	38	25	106	171	62%

Table 23A: GCC IHC Staining in US Biomax PA1002 Pancreatic Tumor MicroArray (TMA)

USBiomax PA1002 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A1	65	M	Adenocarcinoma	2													
A2	52	M	Adenocarcinoma	1													
A3	45	F	Adenocarcinoma	2		80	100			10		10	POS	100	20	100	50
A4	44	M	Adenocarcinoma	2				100		100			POS	0	200	0	300
A5	47	M	Adenocarcinoma (sparse)	1		90	100			10			POS	100	10	100	20
B1	55	M	Adenocarcinoma	2	100	100							NEG	0	0	0	0
B2	57	M	Adenocarcinoma (sparse) with necrosis	2	100	100							NEG	0	0	0	0
B3	34	M	Adenocarcinoma	1	80	80			20	20			POS	20	20	40	40
B4	56	F	Adenocarcinoma	1	100	80				20			POS	0	20	0	40
B5	42	M	Adenocarcinoma	1	100	100							NEG	0	0	0	0
C1	39	F	Adenocarcinoma	2	100	100							NEG	0	0	0	0
C2	44	M	Adenocarcinoma	2		100	100						POS	100	0	100	0
C3	59	M	Adenocarcinoma	2	70	80	20	10					POS	20	10	20	10
C4	65	M	Adenocarcinoma (fibrofatty tissue)	-													
C5	67	F	Adenocarcinoma (chronic inflammation of	-	80	100	20						POS	20	0	20	0

USBiomax PA1002 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			pancreas tissue)														
D1	53	M	Adenocarcinoma	1	100	100							NEG	0	0	0	0
D2	52	M	Adenocarcinoma	2	80	60	20	20		20			POS	20	40	20	60
D3	72	F	Adenocarcinoma	1	50	90	50	10					POS	50	10	50	10
D4	58	F	Adenocarcinoma	2	30	100	70						POS	70	0	70	0
D5	41	M	Adenocarcinoma	2	100	100							NEG	0	0	0	0
E1	51	M	Adenocarcinoma	2	90	80	10	20					POS	10	20	10	20
E2	41	M	Adenocarcinoma (sparse)	-													
E3	68	F	Adenocarcinoma	2	90	100	10						POS	10	0	10	0
E4	41	F	Adenocarcinoma	2		100	100						POS	100	0	100	0
E5	72	F	Adenocarcinoma	2	100	100							NEG	0	0	0	0
F1	76	F	Adenocarcinoma with necrosis	2	100	100							NEG	0	0	0	0
F2	52	M	Adenocarcinoma	2	30	100	70						POS	70	0	70	0
F3	60	F	Adenocarcinoma	2	70	100	30						POS	30	0	30	0
F4	76	M	Adenocarcinoma (sparse)	2	80	90	20	10					POS	20	10	20	10
F5	78	M	Adenocarcinoma	2	100	100							NEG	0	0	0	0
G1	41	M	Adenocarcinoma	2	70	50	30			20		30	POS	30	50	30	130
G2	62	F	Adenocarcinoma	3	100	100							NEG	0	0	0	0
G3	51	M	Adenocarcinoma (sparse)	-		100	100						POS	100	0	100	0

USBiomax PA1002 Pancreatic TMA																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G4	50	M	Adenocarcinoma	3		100	100						POS	100	0	100	0
G5	60	M	Adenocarcinoma	2													
H1	53	F	Adenocarcinoma	2	100	100							NEG	0	0	0	0
H2	59	M	Adenocarcinoma	2		100	70		30				POS	100	0	130	0
H3	56	M	Adenocarcinoma	3		100	50		50				POS	100	0	150	0
H4	60	F	Adenocarcinoma	3													
H5	66	F	Adenocarcinoma (fibrous tissue and blood vessel)	-													
I1	40	M	Normal pancreas tissue	-	100	100							NEG	0	0	0	0
I2	47	M	Normal pancreas tissue	-										0	0	0	0
I3	25	M	Normal pancreas tissue	-		70	100			30			POS	100	30	100	60
I4	35	F	Normal pancreas tissue	-										0	0	0	0
I5	30	M	Normal pancreas tissue	-		70	100	30					POS	100	30	100	30
J1	50	M	Normal pancreas tissue	-		100	100						NEG	100	0	100	0
J2	30	M	Normal pancreas tissue	-		100	100						NEG	100	0	100	0
J3	40	M	Normal pancreas tissue	-										0	0	0	0
J4	35	M	Normal pancreas	-										0	0	0	0

USBiomax PA1002 Pancreatic TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			tissue														
J5	21	F	Normal pancreas tissue	-										0	0	0	0

Table 23B: Summary of GCC Staining in US Biomax PA1002 Pancreatic Tumor MicroArray (TMA)

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
35	12	38	21	22	33	67%

Table 24A: GCC IHC Staining in Pantomics PAC481 Pancreatic Tumor MicroArray-Part 1

Pantomics PAC481 Pancreatic TMA-Part 1																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A01	46	M	Normal	-										0	0	0	0
A02	48	M	Norma	-										0	0	0	0
A03	58	F	Normal	-	100	100							NEG	0	0	0	0
A04	28	F	Islet cell tumor	-	100	100							NEG	0	0	0	0
A05	44	F	Adenocarcinoma	I	50	100	50						POS	50	0	50	0
A06	64	F	Adenocarcinoma	I	100	100							NEG	0	0	0	0
A07	39	F	Adenocarcinoma	I			50		50			100	POS	100	100	150	300
A08	49	F	Adenocarcinoma	I	90	100			10				POS	10	0	20	0
C01	56	F	Adenocarcinoma	I													
C02	49	F	Adenocarcinoma	II	100	100							NEG	0	0	0	0
C03	52	F	Adenocarcinoma	II	100	100							NEG	0	0	0	0
C04	42	F	Adenocarcinoma	II	90	100	10						POS	10	0	10	0
C05	54	M	Adenocarcinoma	II	100	100							NEG	0	0	0	0
C06	59	M	Adenocarcinoma	II	100	100	30						POS	30	0	30	0
C07	34	F	Adenocarcinoma	II		100	100	20					POS	100	20	100	20
C08	69	M	Adenocarcinoma	II		100	100						POS	100	0	100	0
E01	40	F	Adenocarcinoma	II	100	100							NEG	0	0		
E02	79	F	Adenocarcinoma	III	100	100							NEG	0	0	0	0
E03	76	F	Adenocarcinoma	III	100	100							NEG	0	0	0	0

Pantomics PAC481 Pancreatic TMA-Part 1																
					Apical & Cytoplasmic Staining (Percent)								POS/	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
E04	52	M	Adenocarcinoma	III	80	100	20						POS	20	0	20	0
E05	51	F	Adenocarcinoma	III	50	100	50						POS	50	0	50	0
E06	42	F	Adenocarcinoma	III													
E07	40	M	Adenocarcinoma	III	50	100	50						POS	50	0	50	0
E08	64	M	Metastatic Carcinoma	II													

Table 24B: Summary of GCC Staining in Pantomics PAC481 Pancreatic TMA-Part 1

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
29	7	34	19	10	18	56%

Table 24C: GCC IHC Staining in Pantomics PAC481 Pancreatic Tumor MicroArray-Part 2

Pantomics PAC 481 Pancreatic TMA-Part 2																	
					Apical & Cytoplasmic Staining (Percent)								POS/ NEG	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
B01	46	M	Normal	-													
B02	48	M	Norma	-													
B03	58	F	Norma	-													
B04	28	F	Islet cell tumor	-	100	100							NEG	0	0	0	0
B05	44	F	Adenocarcinoma	I	100	100							NEG	0	0	0	0
B06	64	F	Adenocarcinoma	I	70	70	30	30					POS	30	30	30	30
B07	39	F	Adenocarcinoma	I			100					100	POS	100	100	100	300
B08	49	F	Adenocarcinoma	I	90	100	10						POS	10	0	10	0
D01	56	F	Adenocarcinoma	I	100	100							NEG	0	0	0	0
D02	49	F	Adenocarcinoma	II	100	100							NEG	0	0	0	0
D03	52	F	Adenocarcinoma	II	100	100							NEG	0	0	0	0
D04	42	F	Adenocarcinoma	II	90	100	10						POS	10	0	10	0
D05	54	M	Adenocarcinoma	II	100	100							NEG	0	0	0	0
D06	59	M	Adenocarcinoma	II	80	100	20						POS	20	0	20	0
D07	34	F	Adenocarcinoma	II		100	100						POS	100	0	100	0
D08	69	M	Adenocarcinoma	II	100	100							NEG	0	0	0	0
F01	40	F	Adenocarcinoma	II	100	100							NEG	0	0	0	0

Pantomics PAC481 Pancreatic TMA-Part 2																	
					Apical & Cytoplasmic Staining (Percent)								POS/ NEG	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
F02	79	F	Adenocarcinoma	III	100	100							NEG	0	0	0	0
F03	76	F	Adenocarcinoma	III	100	100							NEG	0	0	0	0
F04	52	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
F05	51	F	Adenocarcinoma	III	60	100	40						POS	40	0	40	0
F06	42	F	Adenocarcinoma	III	50	100	50						POS	50	0	50	0
F07	40	M	Adenocarcinoma	III	50	100	50						POS	50	0	50	0
F08	64	M	Metastatic Carcinoma	II	100	70			30				POS	30	0	60	0

Table 24D: Summary of GCC Staining in Pantomics PAC481 Pancreatic TMA-Part 2

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
21	7	21	17	10	21	48%

Table 25: GCC IHC Staining in US Biomax STC1501 Gastric Tumor MicroArray-Part 1

USBiomax STC1501 Gastric TMA – Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A1	35	M	Normal	-													
A2	83	M	Normal	-		100	100						POS	100	0	100	0
A3	53	M	Chronic gastritis	-		70	100					30	POS	100	30	100	90
A4	54	M	Chronic gastritis	-			50		20	50	30	50	POS	100	100	180	250
A5	59	M	Gastric stromal tumor	-	100	100							NEG	0	0	0	0
A6	68	F	Signet-ring cell adenocarcinoma	-		100			100				POS	100	0	200	0
A7	42	M	Adenocarcinoma	III		100	100						POS	100	0	100	0
A8	44	F	Adenocarcinoma	II~III		20	50		50	30		50	POS	100	80	150	210
A9	54	F	Adenocarcinoma	I		50			100	20		30	POS	100	50	200	130
A10	51	M	Adenocarcinoma	II~III		100	100						POS	100	0	100	0
A11	61	M	Adenocarcinoma	II~III					100	50		50	POS	100	100	200	250
A12	50	M	Adenocarcinoma	II		70			100			30	POS	100	30	200	90
A13	53	F	Adenocarcinoma	III		100	70		30				POS	100	0	130	0
A14	55	M	Adenocarcinoma	III		100			70		30		POS	100	0	230	0
A15	73	F	Adenocarcinoma	II													
C1	47	F	Adenocarcinoma	II~III	100	100							NEG	0	0	0	0
C2	70	M	Adenocarcinoma	II~III	50	50	50					50	POS	50	50	50	150
C3	36	M	Adenocarcinoma	I~II	100	50						50	POS	0	50	0	150

US Biomax STC1501 Gastric TMA – Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
C4	65	M	Adenocarcinoma	III	60	70			20		20	30	POS	40	30	100	90
C5	62	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
C6	77	M	Adenocarcinoma	II~III	50	100	50						POS	50	0	50	0
C7	45	F	Adenocarcinoma	I~II	100	30						70	POS	0	70	0	210
C8	67	M	Adenocarcinoma	II~III	70	90	30			10			POS	30	10	30	20
C9	62	M	Adenocarcinoma	II~III	100	100							NEG	0	0	0	0
C10	55	F	Adenocarcinoma	II~III	50	100	50						POS	50	0	50	0
C11	75	M	Adenocarcinoma	III		100	100						POS	100	0	100	0
C12	73	M	Undifferentiated carcinoma	-	100	100							NEG	0	0	0	0
C13	41	M	Adenocarcinoma	II~III	50	80	50	10		10			POS	50	20	50	30
C14	58	M	Adenocarcinoma	II~III	70	80	30	20					POS	30	20	30	20
C15	52	F	Adenocarcinoma	I~II		40			100	30		30	POS	100	60	200	150
E1	63	M	Gastric stromal tumor	-	60	100	20		20				POS	40	0	60	0
E2	72	M	Adenocarcinoma	II		100	20		30		50		POS	100	0	230	0
E3	57	M	Signet-ring cell adenocarcinoma	-	100	100							NEG	0	0	0	0
E4	60	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
E5	66	F	Adenocarcinoma	III													
E6	62	M	Adenocarcinoma	I~II			10		60		30	100	POS	100	100	220	300
E7	48	F	Adenocarcinoma	III	50	100	20		30				POS	50	0	80	0

US Biomax STC1501 Gastric TMA – Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
E8	60	M	Adenocarcinoma	II~III		100	20		30		50		POS	100	0	230	0
E9	56	M	Adenocarcinoma	III		100	50				50		POS	100	0	200	0
E10	54	M	Adenocarcinoma	III		100	100						POS	100	0	100	0
E11	64	F	Adenocarcinoma	III		100	30				70		POS	100	0	240	0
E12	59	M	Adenocarcinoma	III	80	100			20				POS	20	0	40	0
E13	75	M	Undifferentiated carcinoma	-	30	100					70		POS	70	0	210	0
E14	65	M	Signet-ring cell adenocarcinoma	-		100					100		POS	100	0	300	0
E15	35	F	Adenocarcinoma	III		100			70		30		POS	100	0	230	0
G1	71	M	Adenocarcinoma	III	90	100	10						POS	10	0	10	0
G2	38	M	Signet-ring cell adenocarcinoma	-	50	100			50				POS	50	0	100	0
G3	68	M	Gastric stromal tumor	-	100	100							NEG	0	0	0	0
G4	56	F	Adenocarcinoma	I~II			50		30		20	100	POS	100	100	170	300
G5	45	M	Adenocarcinoma	II		100	100						POS	100	0	100	0
G6	45	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
G7	74	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
G8	24	F	Adenocarcinoma	II	30	50	50		20	50			POS	70	50	90	100
G9	51	M	Lymphoma?	-		100	100						POS	100	0	100	0
G10	53	M	Signet-ring cell adenocarcinoma	-	50	100	50						POS	50	0	50	0

US Biomax STC1501 Gastric TMA – Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G11	58	F	Lymphoma	-													
G12	58	F	Lymphoma	-													
G13	81	M	Adenocarcinoma	I~II	20	70	50	30	30				POS	80	30	110	30
G14	56	M	Signet-ring cell adenocarcinoma	-													
G15	35	M	Adenocarcinoma	I~II			100			100			POS	100	100	100	200
I1	61	M	Adenocarcinoma	II	100	100							NEG	0	0	0	0
I2	42	M	Undifferentiated carcinoma	-	100	100							NEG	0	0	0	0
I3	78	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
I4	65	F	Adenocarcinoma	III	40	80	20		40			20	POS	60	20	100	60
I5	68	M	Adenocarcinoma	II~III	100	100							NEG	0	0	0	0
I6	60	M	Adenocarcinoma	II~III													
I7	53	M	Adenocarcinoma	II	100	50						50	POS	0	50	0	150
I8	76	F	Mucinous adenocarcinoma	II		100			20		80		POS	100	0	280	0
I9	50	M	Adenocarcinoma	II													
I10	74	M	Adenocarcinoma	III	100	100							NEG	0	0	0	0
I11	75	F	Signet-ring cell adenocarcinoma	-		100					100		POS	100	0	300	0
I12	68	F	Adenocarcinoma	II~III		70	100					30	POS	100	30	100	90
I13	56	F	Adenocarcinoma	II~III													

US Biomax STC1501 Gastric TMA – Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
I14	53	F	Lymphoma	-													
I15	80	M	Adenocarcinoma	II		100			100				POS	100	0	200	0

Table 25B: Summary of GCC Staining in US Biomax STC1501 Gastric TMA-Part 1

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
57	17	100	45	47	61	77%

Table 25C: GCC IHC Staining in US Biomax STC1501 Gastric TumorMicroArray-Part 2

					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
B1	M	35	Normal	-	100	100								0	0	0	0
B2	M	83	Normal	-		90	100					10		100	10	100	30
B3	M	53	Chronic gastritis	-		100	100							100	0	100	0
B4	M	54	Chronic gastritis	-					50		50	100		100	100	250	300
B5	M	59	Gastric stromal tumor	-	100	100								0	0	0	0
B6	F	68	Signet-ring cell adenocarcinoma	-	50	100			50					50	0	100	0
B7	M	42	Adenocarcinoma	III		100	50		50					100	0	150	0
B8	F	44	Adenocarcinoma	II~III		100			50		50			100	0	250	0
B9	F	54	Adenocarcinoma	I					100	30		70		100	100	200	270
B10	M	51	Adenocarcinoma	II~III		90	100			10				100	10	100	20
B11	M	61	Adenocarcinoma	II~III					100			100		100	100	200	300
B12	M	50	Adenocarcinoma	II													
B13	F	53	Adenocarcinoma	III		100	90				10			100	0	120	0
B14	M	55	Adenocarcinoma	III		100	40		30		30			100	0	190	0
B15	F	73	Adenocarcinoma	II		100	100							100	0	100	0
D1	F	47	Adenocarcinoma	II~III	100	100								0	0	0	0
D2	M	70	Adenocarcinoma	II~III		50	100					50		100	50	100	150
D3	M	36	Adenocarcinoma	I~II	100	50						50		0	50	0	150
D4	M	65	Adenocarcinoma	III		**100**	**20**		**30**		**50**			100	0	230	0
D5	M	62	Adenocarcinoma	III	90	90	10	10						10	10	10	10

					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
D6	M	77	Adenocarcinoma	II~III	40	100	30		30					60	0	90	0
D7	F	45	Adenocarcinoma	I~II	100	50						50		0	50	0	150
D8	M	67	Adenocarcinoma	II~III	80	90	10		10	10				20	10	30	20
D9	M	62	Adenocarcinoma	II~III	100	100								0	0	0	0
D10	F	55	Adenocarcinoma	II~III	100	100								0	0	0	0
D11	M	75	Adenocarcinoma	III	50	100			50					50	0	100	0
D12	M	73	Undifferentiated carcinoma	-	100	100								0	0	0	0
D13	M	41	Adenocarcinoma	II~III					100			100		100	100	200	300
D14	M	58	Adenocarcinoma	II~III	100	90				10				0	10	0	20
D15	F	52	Adenocarcinoma	I~II		80			100			20		100	20	200	60
F1	M	63	Gastric stromal tumor	-	80	100	20							20	0	20	0
F2	M	72	Adenocarcinoma	II		100	20		30		50			100	0	230	0
F3	M	57	Signet-ring cell adenocarcinoma	-	100	100								0	0	0	0
F4	M	60	Adenocarcinoma	III													
F5	F	66	Adenocarcinoma	III		100	30		50		20			100	0	190	0
F6	M	62	Adenocarcinoma	I~II					100			100		100	100	200	300
F7	F	48	Adenocarcinoma	III	50	100	50							50	0	50	0
F8	M	60	Adenocarcinoma	II~III	40	50			50	10	10	50		60	60	130	170
F9	M	56	Adenocarcinoma	III		100			100					100	0	200	0
F10	M	54	Adenocarcinoma	III	80	100	20							20	0	20	0
F11	F	64	Adenocarcinoma	III		100	50		20		30			100	0	180	0
F12	M	59	Adenocarcinoma	III	80	100			20					20	0	40	0

					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
F13	M	75	Undifferentiated carcinoma	-	100	100								0	0	0	0
F14	M	65	Signet-ring cell adenocarcinoma	-		100					100			100	0	300	0
F15	F	35	Adenocarcinoma	III		100	30		50		20			100	0	190	0
H1	M	71	Adenocarcinoma	III	90	100	10							10	0	10	0
H2	M	38	Signet-ring cell adenocarcinoma	-													
H3	M	68	Gastric stromal tumor	-	100	100								0	0	0	0
H4	F	56	Adenocarcinoma	I~II					100			100		100	100	200	300
H5	M	45	Adenocarcinoma	II		100	100							100	0	100	0
H6	M	45	Adenocarcinoma	III		100	100							100	0	100	0
H7	M	74	Adenocarcinoma	III	100	100								0	0	0	0
H8	F	24	Adenocarcinoma	II	80	80			20			20		20	20	40	60
H9	M	51	Lymphoma?	-													
H10	M	53	Signet-ring cell adenocarcinoma	-	70	100	30							30	0	30	0
H11	F	58	Lymphoma	-													
H12	F	58	Lymphoma	-													
H13	M	81	Adenocarcinoma	I~II		90	100	10						100	10	100	10
H14	M	56	Signet-ring cell adenocarcinoma	-													
H15	M	35	Adenocarcinoma	I~II		70	80		20			30		100	30	120	90
J1	M	61	Adenocarcinoma	II	80	100	20							20	0	20	0
J2	M	42	Undifferentiated carcinoma	-													

					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
J3	M	78	Adenocarcinoma	III	100	100								0	0	0	0
J4	F	65	Adenocarcinoma	III		50	50		50			50		100	50	150	150
J5	M	68	Adenocarcinoma	II~III	100	80				20				0	20	0	40
J6	M	60	Adenocarcinoma	II~III		100			50		50			100	0	250	0
J7	M	53	Adenocarcinoma	II	50	80	50			10		10		50	20	50	50
J8	F	76	Mucinous adenocarcinoma	II													
J9	M	50	Adenocarcinoma	II		40			100	30		30		100	60	200	150
J10	M	74	Adenocarcinoma	III	100	100								0	0	0	0
J11	F	75	Signet-ring cell adenocarcinoma	-		100					100			100	0	300	0
J12	F	68	Adenocarcinoma	II~III	90	70	10			30				10	30	10	60
J13	F	56	Adenocarcinoma	II~III													
J14	F	53	Lymphoma	-													
J15	M	80	Adenocarcinoma	II		100			100					100	0	200	0

Table 25D: Summary of GCC Staining in US Biomax STC1501 Gastric TMA-Part 2

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
58	17	102	48	49	59	83%

Table 26A: GCC IHC Staining in Pantomics ESC1021 Esophageal Tumor Microarray

Pantomics ESC1021 Esophageal TMA																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A01	56	M	Normal		100	100							NEG	0	0	0	0
A02	53	M	Normal		100	100							NEG	0	0	0	0
A03	53	M	Normal		100	100							NEG	0	0	0	0
A04	62	M	Esophagitis		100	100							NEG	0	0	0	0
A05	66	M	Esophagitis		100	100							NEG	0	0	0	0
A06	58	M	Squamous cell carcinoma	I	30	100			20		50		POS	70	0	190	0
A07	36	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
A08	72	F	Squamous cell carcinoma	I	50	100			50				POS	50	0	100	0
A09	62	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
A10	56	M	Squamous cell carcinoma	I	70	100			30				POS	30	0	60	0
A11	68	F	Squamous cell carcinoma	I	100	100							NEG	0	0	0	0
A12	62	M	Squamous cell carcinoma	I	80	100	20						POS	20	0	20	0
A13	56	M	Squamous cell carcinoma	I	70	100	30						POS	30	0	30	0
B01	60	M	Squamous cell carcinoma	I	100	100							NEG	0	0	0	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
B02	50	M	Squamous cell carcinoma	I	90	100					10		POS	10	0	30	0
B03	44	M	Squamous cell carcinoma	I	70	100			30				POS	30	0	60	0
B04	60	M	Squamous cell carcinoma	I	100	100							NEG	0	0	0	0
B05	64	F	Squamous cell carcinoma	I	70	100	30						POS	30	0	30	0
B06	55	M	Squamous cell carcinoma	I	70	100	30						POS	30	0	30	0
B07	56	M	Squamous cell carcinoma	I	60	100			20		20		POS	40	0	100	0
B08	48	M	Squamous cell carcinoma	I	80	100			20				POS	20	0	40	0
B09	51	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
B10	56	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
B11	71	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
B12	55	M	Squamous cell carcinoma	I~II	80	100			10		10		POS	20	0	50	0
B13	58	M	Squamous cell carcinoma	I~II	70	100	30						POS	30	0	30	0
C01	26	F	Squamous cell carcinoma	I~II	30	100			70				POS	70	0	140	0
C02	56	F	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
C03	65	M	Squamous cell carcinoma	I~II	30	100			70				POS	70	0	140	0
C04	43	M	Squamous cell carcinoma	I~II	30	100	60		10				POS	70	0	80	0
C05	62	M	Squamous cell carcinoma	I~II	90	100	10						POS	10	0	10	0
C06	55	M	Squamous cell carcinoma	I~II	50	100	50						POS	50	0	50	0
C07	59	M	Squamous cell carcinoma	I~II	70	100			30				POS	30	0	60	0
C08	42	M	Squamous cell carcinoma	I~II	90	100	10						POS	10	0	10	0
C09	61	M	Squamous cell carcinoma	I~II	20	100	80						POS	80	0	80	0
C10	57	M	Squamous cell carcinoma	I~II	80	100	20						POS	20	0	20	0
C11	62	M	Squamous cell carcinoma	I~II		100	100						POS	100	0	100	0
C12	62	M	Squamous cell carcinoma	I~II	80	100			10		10		POS	20	0	50	0
C13	55	M	Squamous cell carcinoma	I~II	50	100	50						POS	50	0	50	0
D01	70	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
D02	68	M	Squamous cell carcinoma	I~II	90	100	10						POS	10	0	10	0
D03	55	M	Squamous cell carcinoma	I~II	90	100			10				POS	10	0	20	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
D04	65	F	Squamous cell carcinoma	I~II	80	100	20						POS	20	0	20	0
D05	60	M	Squamous cell carcinoma	I~II		100	100						POS	100	0	100	0
D06	66	M	Squamous cell carcinoma	I~II	80	100	20						POS	20	0	20	0
D07	56	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
D08	68	F	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
D09	55	M	Squamous cell carcinoma	I~II	50	100	50						POS	50	0	50	0
D10	60	M	Squamous cell carcinoma	I~II	80	100	20						POS	20	0	20	0
D11	61	F	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
D12	56	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
D13	71	F	Squamous cell carcinoma	I~II		100			100				POS	100	0	200	0
E01	57	M	Squamous cell carcinoma	I~II	90	100	10						POS	10	0	10	0
E02	64	M	Squamous cell carcinoma	I~II	50	100	20		30				POS	50	0	80	0
E03	72	M	Squamous cell carcinoma	I~II	90	100	10						POS	10	0	10	0
E04	74	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
E05	51	M	Squamous cell carcinoma	I~II	80	100			20				POS	20	0	40	0
E06	61	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
E07	62	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
E08	63	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
E09	71	M	Squamous cell carcinoma	I~II	100	100							NEG	0	0	0	0
E10	51	M	Squamous cell carcinoma	II													
E11	54	M	Squamous cell carcinoma	II	70	100	30						POS	30	0	30	0
E12	77	M	Adenocarcinoma	II	80	70	20					30	POS	20	30	20	90
E13	64	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
F01	45	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
F02	52	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
F03	59	M	Squamous cell carcinoma	II		100	50						POS	50	0	50	0
F04	68	M	Squamous cell carcinoma	II		100	100						POS	100	0	100	0
F05	53	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
F06	62	M	Squamous cell carcinoma	II		100			50		50		POS	100	0	250	0
F07	67	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
F08	54	M	Squamous cell carcinoma	II	70	100	30						POS	30	0	30	0
F09	67	M	Squamous cell carcinoma	II	100	100							NEG	0	0	0	0
F10	56	F	Squamous cell carcinoma	II	80	100	20						POS	20	0	20	0
F11	56	M	Adenocarcinoma	II	20				80			100	POS	80	100	160	300
F12	57	F	Squamous cell carcinoma	II~III	80	100			20				POS	20	0	40	0
F13	53	M	Squamous cell carcinoma	II~III	70	100			30				POS	30	0	60	0
G01	57	M	Squamous cell carcinoma	II~III	100	100							NEG	0	0	0	0
G02	47	M	Squamous cell carcinoma	II~III	100	100							NEG	0	0	0	0
G03	66	M	Squamous cell carcinoma	II~III	100	100							NEG	0	0	0	0
G04	47	F	Squamous cell carcinoma	II~III	70	100	30						POS	30	0	30	0
G05	53	M	Squamous cell carcinoma	II~III		100	100						POS	100	0	100	0
G06	57	M	Squamous cell carcinoma	II~III	90	100	10						POS	10	0	10	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G07	59	M	Squamous cell carcinoma	II~III	100	100							NEG	0	0	0	0
G08	42	M	Squamous cell carcinoma	II~III		100	100						POS	100	0	100	0
G09	49	M	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
G10	48	M	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
G11	67	M	Squamous cell carcinoma	III		100	100						POS	100	0	100	0
G12	58	M	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
H01	58	M	Adenocarcinoma ?	III		100	100						POS	100	0	100	0
H02	56	M	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
H03	61	F	Squamous cell carcinoma	III	90	100	10						POS	10	0	10	0
H04	72	F	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
H05	60	M	Squamous cell carcinoma	III		100	100						POS	100	0	100	0
H06	49	M	Squamous cell carcinoma	III		100	50		50				POS	100	0	150	0
H07	56	M	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
H08	53	M	Squamous cell carcinoma	III		100	100						POS	100	0	100	0

Pantomics ESC1021 Esophageal TMA																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H09	66	F	Squamous cell carcinoma	III	100	100							NEG	0	0	0	0
H10	65	M	Carcinoid		100	100							NEG	0	0	0	0
H11	55	M	Adenosquamous carcinoma		100	100							NEG	0	0	0	0
H12	60	M	Undifferentiated carcinoma		100	100							NEG	0	0	0	0

Table 26B: Summary of GCC Staining in Pantomics ESC1021 Esophageal Tumor MicroArray

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
28	1	39	4	57	96	59%

Table 27A: GCC IHC Staining in US BioMax ES8010 Esophageal Tumor MicroArray-Part 1

USBioMax ES8010 Esophageal TMA-Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A1	69	M	Squamous cell carcinoma	1	50	100	50						POS	50	0	50	0
A3	56	M	Squamous cell carcinoma	1	50	100					50		POS	50	0	150	0
A5	62	M	Squamous cell carcinoma	2	80	100			20				POS	20	0	40	0
A7	58	M	Squamous cell carcinoma	1		100			70		30		POS	100	0	230	0
A9	46	M	Squamous cell carcinoma	1	50	100			50				POS	50	0	100	0
B1	43	M	Squamous cell carcinoma	1	40	100	30				30		POS	60	0	120	0
B3	61	F	Squamous cell carcinoma	1	100	100							NEG	0	0	0	0
B5	62	M	Squamous cell carcinoma	2	90	100					10		POS	10	0	30	0
B7	50	M	Squamous cell carcinoma	1	40	100	30		30				POS	60	0	90	0
B9	68	M	Squamous cell carcinoma	2	70	100	30						POS	30	0	30	0
C1	65	M	Squamous cell carcinoma	1	100	100							NEG	0	0	0	0
C3	50	M	Squamous cell carcinoma	2	90	100	10						POS	10	0	10	0
C5	60	M	Squamous cell	2	70	100			30				POS	30	0	60	0

USBioMax ES8010 Esophageal TMA-Part 1																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			carcinoma														
C7	49	F	Squamous cell carcinoma	2	80	100			20				POS	20	0	40	0
C9	59	F	Squamous cell carcinoma	2	80	100	20						POS	20	0	20	0
D1	43	M	Squamous cell carcinoma	2	80	100			20				POS	20	0	40	0
D3	62	M	Squamous cell carcinoma	2	60	100	40						POS	40	0	40	0
D5	62	M	Squamous cell carcinoma	3	90	100			10				POS	10	0	20	0
D7	60	F	Squamous cell carcinoma	2		100	70		30				POS	100	0	130	0
D9	54	F	Squamous cell carcinoma	2	80	100	20						POS	20	0	20	0
E1	48	M	Squamous cell carcinoma	2	80	100	20						POS	20	0	20	0
E3	57	M	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
E5	58	M	Squamous cell carcinoma	2	80	100	20						POS	20	0	20	0
E7	53	F	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
E9	56	F	Squamous cell carcinoma	2	30	100	70						POS	70	0	70	0
F1	76	M	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0

USBioMax ES8010 Esophageal TMA-Part 1																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
F3	60	M	Squamous cell carcinoma (sparse esophagus tissue)	-	20	100	80						POS	80	0	80	0
F5	53	M	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
F7	49	F	Carcinoma in situ	-		100	100						POS	100	0	100	0
F9	62	F	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
G1	48	F	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
G3	57	M	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
G5	45	M	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
G7	55	F	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
G9	53	F	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
H1	68	M	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
H3	64	M	Squamous cell carcinoma	2	90	100			10				POS	10	0	20	0
H5	55	M	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0
H7	63	F	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0

USBioMax ES8010 Esophageal TMA-Part 1																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H9	54	F	Squamous cell carcinoma	3	100	100							NEG	0	0	0	0

Table 27B: Summary of GCC Staining in US BioMax ES8010 Esophageal TMA-Part 1

% POS	% POS	H	H	Total	Total	Pct
Cyto	Apical	Cyto	Apical	Pos	N	Pos
25	0	38	0	24	40	60%

Table 27C: GCC IHC Staining in US BioMax ES8010 Esophageal Tumor MicroArray-Part 2

USBioMax ES8010 Esophageal TMA (80 Cores)																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A2	69	M	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-	100	100							NEG	0	0	0	0
A4	56	M	Cancer adjacent normal esophageal tissue	-	50	100	50						POS	50	0	50	0
A6	62	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
A8	58	M	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-													
A10	46	M	Cancer adjacent normal esophageal tissue	-	20	100	80						POS	80	0	80	0
B2	43	M	Cancer adjacent normal esophageal tissue	-	70	100	30						POS	30	0	30	0
B4	61	F	Cancer adjacent normal esophageal tissue	-	100	100							NEG	0	0	0	0
B6	62	M	Cancer adjacent normal esophageal tissue	-	70	100					30		POS	30	0	90	0
B8	50	M	Cancer adjacent normal esophageal tissue	-	20	100	80						POS	80	0	80	0
B10	68	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
C2	65	M	Cancer adjacent normal esophageal tissue	-		100	70		30				POS	100	0	130	0
C4	50	M	Cancer adjacent normal	-		100	100						POS	100	0	100	0

USBioMax ES8010 Esophageal TMA (80 Cores)																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			esophageal tissue														
C6	60	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
C8	49	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
C10	59	F	Cancer adjacent normal esophageal tissue	-	30	100	70						POS	70	0	70	0
D2	43	M	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-	50	100	50						POS	50	0	50	0
D4	62	M	Cancer adjacent normal esophageal tissue (sparse mucosa)	-		100	100						POS	100	0	100	0
D6	62	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
D8	60	F	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-	100	100							NEG	0	0	0	0
D10	54	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
E2	48	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
E4	57	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
E6	58	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
E8	53	F	Cancer adjacent normal esophageal tissue (chronic	-		100	100						POS	100	0	100	0

USBioMax ES8010 Esophageal TMA (80 Cores)																
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
			inflammation of mucosa)														
E10	56	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
F2	76	M	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-	70	100	30						POS	30	0	30	0
F4	60	M	Cancer adjacent normal esophageal tissue	-	20	100	80						POS	80	0	80	0
F6	53	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
F8	49	F	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-		100	100						POS	100	0	100	0
F10	62	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
G2	48	F	Cancer adjacent normal esophageal tissue (smooth muscle and mucous gland tissue)	-	20	100	80						POS	80	0	80	0
G4	57	M	Cancer adjacent normal esophageal tissue (fibrous tissue, blood vessel and smooth muscle tissue)	-	20	100	80						POS	80	0	80	0
G6	45	M	Cancer adjacent normal esophageal tissue (smooth muscle tissue)	-													
G8	55	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0

USBioMax ES8010 Esophageal TMA (80 Cores)																	
					Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G10	53	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
H2	68	M	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-	70	100	30						POS	30	0	30	0
H4	64	M	Cancer adjacent normal esophageal tissue	-	100	100							NEG	0	0	0	0
H6	55	M	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0
H8	63	F	Cancer adjacent normal esophageal tissue (chronic inflammation of mucosa)	-		100	100						POS	100	0	100	0
H10	54	F	Cancer adjacent normal esophageal tissue	-		100	100						POS	100	0	100	0

Table 27D: Summary of GCC Staining in US BioMax ES8010 Esophageal TMA -Part 2

% POS Cyto	% POS Apical	H Cyto	H Apical	Total Pos	Total N	Pct Pos
76	0	78	0	34	38	89%

Table 28A: GCC IHC Staining in US BioMax LC20813 Lung Tumor MicroArray

USBiomax LC20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A1	72	M	Lung	Squamous cell carcinoma	1	100	100							NEG	0	0	0	0
A2	63	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A3	55	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A4	50	F	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A5	61	F	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A6	63	M	Lung	Squamous cell carcinoma (sparse)	-	100	100							NEG	0	0	0	0
A7	73	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A8	53	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A9	69	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A10	66	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A11	61	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A12	64	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
A13	48	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A14	59	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A15	51	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
A16	70	F	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B1	63	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B2	55	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B3	76	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B4	68	M	Lung	Squamous cell carcinoma	2	50	100	50						POS	50	0	50	0
B5	45	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B6	54	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B7	50	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B8	57	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B9	43	F	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B10	46	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
B11	72	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B12	62	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B13	64	M	Lung	Squamous cell carcinoma with necrosis	2	100	100							NEG	0	0	0	0
B14	61	M	Lung	Squamous cell carcinoma with necrosis	3	90	100	10						POS	10	0	10	0
B15	54	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
B16	61	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C1	35	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C2	53	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C3	76	M	Lung	Squamous cell carcinoma (sparse) with necrosis	2	100	100							NEG	0	0	0	0
C4	53	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C5	66	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C6	76	M	Lung	Squamous cell carcinoma (lung	-	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				tissue)														
C7	70	M	Lung	Squamous cell carcinoma (tumoral necrosis)	-													
C8	64	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
C9	68	M	Lung	Squamous cell carcinoma (bronchus, fibrous tissue and blood vessel)	-	100	100							NEG	0	0	0	0
C10	25	M	Lung	Squamous cell carcinoma	2													
C11	66	M	Lung	Squamous cell carcinoma	2	90	100	10						POS	10	0	10	0
C12	66	M	Lung	Squamous cell carcinoma	2	90	100	10						POS	10	0	10	0
C13	53	M	Lung	Squamous cell carcinoma	2		100	100						POS	100	0	100	0
C14	39	M	Lung	Squamous cell carcinoma	2	60	100	40						POS	40	0	40	0
C15	60	M	Lung	Squamous cell carcinoma	2	70	100	30						POS	30	0	30	0
C16	59	M	Lung	Squamous cell carcinoma (cartilage,	-													

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				chronic inflammation of fibrous tissue and blood vessel)														
D1	62	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D2	54	M	Lung	Squamous cell carcinoma	-	100	100							**NEG**	0	0	0	0
D3	67	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D4	54	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D5	49	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D6	46	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D7	56	M	Lung	Squamous cell carcinoma	3	90	100			10				**POS**	10	0	20	0
D8	55	F	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D9	45	M	Lung	Squamous cell carcinoma	2	80	100	20						**POS**	20	0	20	0
D10	47	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D11	72	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D12	66	M	Lung	Squamous cell	2	50	100	50						**POS**	50	0	50	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				carcinoma														
D13	62	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D14	48	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D15	57	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
D16	70	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E1	55	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E2	64	M	Lung	Squamous cell carcinoma	2	50	100	50						**POS**	50	0	50	0
E3	67	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E4	75	M	Lung	Squamous cell carcinoma	-	100	100							**NEG**	0	0	0	0
E5	56	F	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E6	77	M	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E7	51	F	Lung	Squamous cell carcinoma	2	100	100							**NEG**	0	0	0	0
E8	64	M	Lung	Squamous cell carcinoma (lung tissue)	-													
E9	65	M	Lung	Squamous cell	2	100	100							**NEG**	0	0	0	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				carcinoma with necrosis														
E10	63	M	Lung	Squamous cell carcinoma	2	100	100							NEG	0	0	0	0
E11	52	M	Lung	Squamous cell carcinoma	2		100	100						POS	100	0	100	0
E12	55	F	Lung	Squamous cell carcinoma	2		100	100						POS	100	0	100	0
E13	71	M	Lung	Squamous cell carcinoma	2		100	50		30		20		POS	100	0	170	0
E14	52	M	Lung	Squamous cell carcinoma	2		100	100						POS	100	0	100	0
E15	63	F	Lung	Adenosquamous carcinoma	-	50	100	50						POS	50	0	50	0
E16	67	F	Lung	Adenosquamous carcinoma	-		100	50		40		10		POS	100	0	160	0
F1	46	F	Lung	Adenosquamous carcinoma	-	50	100	50						POS	50	0	50	0
F2	61	M	Lung	Adenosquamous carcinoma	-	100	100							NEG	0	0	0	0
F3	54	M	Lung	Adenosquamous carcinoma	-	100	100							NEG	0	0	0	0
F4	55	F	Lung	Adenosquamous carcinoma	-	50	100	50						POS	50	0	50	0
F5	68	M	Lung	Adenosquamous carcinoma	-		100	50		50				POS	100	0	150	0
F6	54	F	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
F7	61	M	Lung	Adenocarcinoma	2			80		20	50		50	POS	100	100	120	250
F8	60	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
F9	62	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
F10	38	M	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
F11	42	F	Lung	Adenocarcinoma	2	50	80	50	20					POS	50	20	50	20
F12	56	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
F13	59	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
F14	33	M	Lung	Adenosquamous carcinoma	-	80	100	20						POS	20	0	20	0
F15	68	F	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
F16	49	F	Lung	Adenocarcinoma with necrosis	2	100	100							NEG	0	0	0	0
G1	56	F	Lung	Adenocarcinoma (chronic inflammation of fibrous tissue and blood vessel)	-													
G2	60	M	Lung	Papillary adenocarcinoma	2	80	100	10		10				POS	20	0	30	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
G3	39	M	Lung	Papillary adenocarcinoma	2	80	100	20						POS	20	0	20	0
G4	58	M	Lung	Papillary adenocarcinoma	2													
G5	56	F	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0
G6	55	M	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0
G7	62	M	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0
G8	64	F	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0
G9	72	M	Lung	Adenocarcinoma	3	50	100	50						POS	50	0	50	0
G10	53	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
G11	65	M	Lung	Papillary adenocarcinoma	2	90	100			10				POS	10	0	20	0
G12	52	F	Lung	Papillary adenocarcinoma	2		100	100						POS	100	0	100	0
G13	47	F	Lung	Papillary adenocarcinoma	2	70	100	20		10				POS	30	0	40	0
G14	71	F	Lung	Papillary adenocarcinoma	2	100	100							NEG	0	0	0	0
G15	49	M	Lung	Adenocarcinoma	2		100	100						POS	100	0	100	0
G16	58	M	Lung	Adenocarcinoma	2		100	100						POS	100	0	100	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H1	62	M	Lung	Adenocarcinoma	2	100	90		10					POS	0	10	0	10
H2	54	F	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
H3	38	M	Lung	Adenocarcinoma with necrosis	2	100	100							NEG	0	0	0	0
H4	68	M	Lung	Adenocarcinoma	2	50	100	50						POS	50	0	50	0
H5	64	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
H6	41	F	Lung	Adenocarcinoma	-	90	100	10						POS	10	0	10	0
H7	40	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
H8	58	F	Lung	Adenocarcinoma with necrosis	3	100	100							NEG	0	0	0	0
H9	64	M	Lung	Adenocarcinoma with necrosis	3	100	100							NEG	0	0	0	0
H10	56	F	Lung	Papillary adenocarcinoma	2		90	100	10					POS	100	10	100	10
H11	57	F	Lung	Papillary adenocarcinoma	2	80	100	20						POS	20	0	20	0
H12	62	F	Lung	Adenocarcinoma with necrosis	2		100	80		20				POS	100	0	120	0
H13	60	M	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0
H14	64	F	Lung	Adenocarcinoma	2	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
H15	82	M	Lung	Adenocarcinoma	3	100	90		10					**POS**	0	10	0	10
H16	60	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I1	50	F	Lung	Adenocarcinoma	3	50	100	50						**POS**	50	0	50	0
I2	46	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I3	69	M	Lung	Adenocarcinoma (sparse)	3													
I4	51	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I5	70	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I6	67	M	Lung	Adenocarcinoma	3		10	60		20	30	20	60	**POS**	100	90	160	240
I7	36	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I8	49	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I9	60	M	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I10	39	F	Lung	Adenocarcinoma	3	100	100							**NEG**	0	0	0	0
I11	65	M	Lung	Adenocarcinoma	3		100	100						**POS**	100	0	100	0
I12	69	F	Lung	Adenocarcinoma	3		100	70		30				**POS**	100	0	130	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
I13	75	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
I14	75	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
I15	61	F	Lung	Adenocarcinoma	3		100	100						POS	100	0	100	0
I16	44	M	Lung	Adenocarcinoma	3	100	90		10					POS	0	10	0	10
J1	59	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J2	68	F	Lung	Adenocarcinoma	3	80	100	20						POS	20	0	20	0
J3	65	M	Lung	Adenocarcinoma with necrosis	3	100	100							NEG	0	0	0	0
J4	65	M	Lung	Adenosquamous carcinoma	-	70	100	30						POS	30	0	30	0
J5	39	M	Lung	Adenocarcinoma	3	80	100	20						POS	20	0	20	0
J6	74	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J7	50	M	Lung	Adenocarcinoma	-	100	100							NEG	0	0	0	0
J8	36	F	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J9	46	M	Lung	Adenocarcinoma	3	90	100	10						POS	10	0	10	0
J10	69	M	Lung	Adenocarcinoma	3	50	100	50						POS	50	0	50	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
J11	30	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J12	65	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J13	52	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J14	66	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J15	47	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
J16	52	F	Lung	Adenocarcinoma	3	90	100	10						POS	10	0	10	0
K1	58	F	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
K2	46	F	Lung	Adenocarcinoma	3	50	100	50						POS	50	0	50	0
K3	69	M	Lung	Adenocarcinoma	3	100	100							NEG	0	0	0	0
K4	58	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K5	51	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K6	63	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K7	63	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K8	60	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
K9	63	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K10	66	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K11	71	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K12	60	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K13	31	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K14	61	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K15	52	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
K16	69	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L1	43	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L2	56	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L3	62	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L4	42	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L5	59	F	Lung	Small cell carcinoma (fibrous tissue and blood	-	100	100							NEG	0	0	0	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				vessel)														
L6	55	F	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L7	28	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L8	39	M	Lung	Small cell carcinoma	-	100	100							NEG	0	0	0	0
L9	61	M	Lung	Large cell carcinoma	-	100	100							NEG	0	0	0	0
L10	72	F	Lung	Large cell carcinoma	-	90	100	10						POS	10	0	10	0
L11	46	M	Lung	Large cell carcinoma	-	70	100	30						POS	30	0	30	0
L12	64	F	Lung	Large cell carcinoma	-	100	100							NEG	0	0	0	0
L13	55	F	Lung	Bronchioloalveolar carcinoma	-	100	100							NEG	0	0	0	0
L14	52	M	Lung	Bronchioloalveolar carcinoma	-	90	100	10						POS	10	0	10	0
L15	55	M	Lung	Bronchioloalveolar carcinoma	-	100	100							NEG	0	0	0	0
L16	64	F	Lung	Bronchioloalveolar carcinoma	-	100	100							NEG	0	0	0	0
M1	59	M	Lung	Mucinous bronchioloalveolar carcinoma	-	100	100							NEG	0	0	0	0
M2	50	F	Lung	Mucinous	-													

USBiomax LC 20813 Lung TMA																	
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				bronchioloalveolar carcinoma (sparse)														
M3	27	M	Lung	Mucinous bronchioloalveolar carcinoma	-	100	100							**NEG**	0	0	0	0
M4	48	M	Lung	Mucinous bronchioloalveolar carcinoma	-	100	100							**NEG**	0	0	0	0
M5	56	M	Lung	Mucoepidermoid carcinoma	-	90	100	10						**POS**	10	0	10	0
M6	51	M	Lung	Mucoepidermoid carcinoma	-													
M7	48	M	Lung	Mucoepidermoid carcinoma	-	100	100							**NEG**	0	0	0	0
M8	58	M	Lung	Mucoepidermoid carcinoma	-	90	100	10						**POS**	10	0	10	0
M9	49	M	Lung	Atypical carcinoid	-	100	100							**NEG**	0	0	0	0
M10	47	M	Lung	Atypical carcinoid	-	100	100							**NEG**	0	0	0	0
M11	67	M	Lung	Atypical carcinoid	-	100	100							**NEG**	0	0	0	0
M12	65	M	Lung	Large cell neuroendocrine carcinoma	-	100	100							**NEG**	0	0	0	0
M13	43	M	Lung	Mixed large cell neuroendocrine	-	100	100							**NEG**	0	0	0	0

USBiomax LC 20813 Lung TMA																		
						Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Array Position	Age	Sex	Organ	Pathology	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
				carcinoma														
M14	58	M	Lung	Giant cell carcinoma	-	100	100							NEG	0	0	0	0
M15	36	F	Lung	Basal cell carcinoma	-	100	100							NEG	0	0	0	0
M16	66	M	Lung	Pleomorphic carcinoma	-	100	100							NEG	0	0	0	0

Table 28B: Summary of GCC Staining in US BioMax LC20813 Lung TMA

AVG % POS Cyto	AVG % POS Apical	AVG H Cyto	AVG H Apical	Total Pos	Total N	Pct Pos
14	1	15	3	56	199	28%
11	0	12	0	15	74	20%
23	3	26	7	36	82	44%
0	0	0	0	0	21	0%
3	0	3	0	5	22	23%

Table 29A: GCC IHC Staining in Leiomyosarcoma/Rhabdomyosarcoma Tumor MicroArray

						Apical & Cytoplasmic Staining (Percent)									%	%	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	POS /NEG	POS Cyto	POS Apical	Cyto	Apical
A1	M	85	Fibrous tissue	High malignant leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
A2	M	85	Fibrous tissue	High malignant leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
A3	M	26	Fibrous tissue	Moderate malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A4	M	26	Fibrous tissue	Moderate malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A5	F	56	Fibrous tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
A6	F	56	Fibrous tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
A7	F	74	Fibrous tissue	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A8	F	74	Fibrous tissue	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A9	M	34	Fibrous tissue	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
B1	M	34	Fibrous tissue	Moderate malignant leiomyosarcoma	IIA		100	100						POS	100	0	100	0
B2	F	20	Smooth muscle	Moderate malignant leiomyosarcoma	IIIA	100	100							NEG	0	0	0	0
B3	F	20	Smooth muscle	Moderate malignant leiomyosarcoma	IIIA	100	100							NEG	0	0	0	0
B4	F	49	Fatty tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
B5	F	49	Fatty tissue	Moderate malignant leiomyosarcoma	IIB	NET	100							n/a				
B6	F	34	Fatty tissue	Moderate malignant epithelioid leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
B7	F	34	Fatty tissue	Moderate malignant epithelioid leiomyosarcoma	IIB		100	100						POS	100	0	100	0
B8	M	38	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
B9	M	38	Smooth muscle	Low malignant leiomyosarcoma	IA	80	100	20						POS	20	0	20	0
C1	F	44	Ligament	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
C2	F	44	Ligament	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
C3	F	69	Smooth muscle	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
C4	F	69	Smooth muscle	Moderate malignant leiomyosarcoma	IIB		100	100						POS	100	0	100	0
C5	F	88	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
C6	F	88	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
C7	F	54	Smooth muscle	Malignant pleomorphic leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
C8	F	54	Smooth muscle	Malignant pleomorphic	IIIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
				leiomyosarcoma														
C9	F	58	Smooth muscle	Low malignant leiomyosarcoma	IIB		100	100						POS	100	0	100	0
D1	F	58	Smooth muscle	Low malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
D2	F	57	Smooth muscle	Low malignant leiomyosarcoma	IB	90	100	10						POS	10	0	10	0
D3	F	57	Smooth muscle	Low malignant leiomyosarcoma	IB	50	100	50						POS	50	0	50	0
D4	M	78	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	NET	100							n/a				
D5	M	78	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	NET	100							n/a				
D6	F	52	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
D7	F	52	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	50	100	50						POS	50	0	50	0
D8	M	61	Smooth muscle	Moderate malignant leiomyosarcoma	IIA		100	100						POS	100	0	100	0
D9	M	61	Smooth muscle	Moderate malignant leiomyosarcoma (fibrous tissue, blood vessel and smooth muscle)	IIA		100	100						POS	100	0	100	0
E1	F	33	Fibrous tissue	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
E2	F	33	Fibrous tissue	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
E3	M	21	Retroperitoneum	Embryonic rhabdomyosarcoma	IIIA		100	100						POS	100	0	100	0
E4	M	21	Retroperitoneum	Embryonic rhabdomyosarcoma	IIIA		100	100						POS	100	0	100	0
E5	F	51	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
E6	F	51	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
E7	M	16	Testis	Spindle cell rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
E8	M	16	Testis	Spindle cell rhabdomyosarcoma	IIA		100	100						POS	100	0	100	0
E9	F	49	Uterus	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F1	F	49	Uterus	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F2	M	30	Striated muscle	Rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
F3	M	30	Striated muscle	Rhabdomyosarcoma	IIB	70	100	30						POS	30	0	30	0
F4	M	18	Tongue	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
F5	M	18	Tongue	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
F6	M	91	Striated muscle	Pleomorphic rhabdomyosarcoma	IIIB		100	100						POS	100	0	100	0
F7	M	91	Striated	Pleomorphic	IIIB		100	10						POS	100	0	100	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
			muscle	rhabdomyosarcoma				0										
F8	F	74	Bladder	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F9	F	74	Bladder	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G1	F	67	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G2	F	67	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G3	F	40	Abdominal cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G4	F	40	Abdominal cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G5	F	23	Pelvic cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G6	F	23	Pelvic cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G7	M	50	Striated muscle	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
G8	M	50	Striated muscle	Pleomorphic rhabdomyosarcoma	IIA		100	100						POS	100	0	100	0
G9	M	10	Striated muscle	Alveolus rhabdomyosarcoma	IIB		100	70		30				POS	100	0	130	0
H1	M	10	Striated muscle	Alveolus rhabdomyosarcoma	IIB	50	100	50						POS	50	0	50	0
H2	F	32	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	70	100	30						POS	30	0	30	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
H3	F	32	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							**NEG**	0	0	0	0
H4	M	48	Soft tissue	Alveolus rhabdomyosarcoma	IIB	80	100	20						**POS**	20	0	20	0
H5	M	48	Soft tissue	Alveolus rhabdomyosarcoma	IIB	100	100							**NEG**	0	0	0	0
H6	M	40	Soft tissue	Spindle cell rhabdomyosarcoma	IA	80	100	20						**POS**	20	0	20	0
H7	M	40	Soft tissue	Spindle cell rhabdomyosarcoma	IA	80	100	20						**POS**	20	0	20	0
H8	M	49	Testis	Embryonic rhabdomyosarcoma	IIB		100	100						**POS**	100	0	100	0
H9	M	49	Testis	Embryonic rhabdomyosarcoma	IIB		100	100						**POS**	100	0	100	0
I1	F	48	Uterus	Smooth muscle	-	100	100							**NEG**	0	0	0	0
I2	F	8	Heart	Cardiac muscle	-	100	100							**NEG**	0	0	0	0
I3	F	14	Heart	Cardiac muscle	-	100	100							**NEG**	0	0	0	0
n/a			Bladder	Normal Bladder	n/a	100	100							**NEG**	0	0	0	0
n/a			1007 (GCC293); Xenograft	Human Embryonic Kidney Xenograft - GCC Transfected	n/a		100					100		**POS**	100	0	300	0
n/a			Kidney Cell Line - 293 HEK-	Human Embryonic Kidney Cell Line - GCC Transfected	n/a		100					100		**POS**	100	0	300	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
			GCC#2															
n/a			Kidney Cell Line - 293 [HEK-293]	Human Embryonic Kidney Cell Line	n/a	100	100							**NEG**	0	0	0	0
n/a			Tissue 1	Colon Cancer MTB	n/a	100					10		90	**POS**	0	100	0	290
n/a			Tissue 2	Colon Cancer MTB	n/a	NET								**n/a**				
n/a			Tissue 3	Colon Cancer MTB	n/a	80	60	20	10		20		10	**POS**	20	40	20	80
n/a			Tissue 4	Colon Cancer MTB	n/a	90	90	10	10					**POS**	10	10	10	10
n/a			Tissue 5	Colon Cancer MTB	n/a	NET								**n/a**				
n/a			Tissue 6	Colon Cancer MTB	n/a	NET								**n/a**				
n/a			Tissue 7	Colon Cancer MTB	n/a	40		40		20	50		50	**POS**	60	100	80	250
n/a			Tissue 8	Colon Cancer MTB	n/a	80		20	20		30		50	**POS**	20	100	20	230

Table 29B: Negative Control Ab Scoring Results in Leiomyosarcoma/Rhabdomyosarcoma Tumor MicroArray

						Apical & Cytoplasmic Staining (Percent)									%	%	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	POS /NEG	POS Cyto	POS Apical	Cyto	Apical
A1	M	85	Fibrous tissue	High malignant leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
A2	M	85	Fibrous tissue	High malignant leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
A3	M	26	Fibrous tissue	Moderate malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A4	M	26	Fibrous tissue	Moderate malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A5	F	56	Fibrous tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
A6	F	56	Fibrous tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
A7	F	74	Fibrous tissue	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A8	F	74	Fibrous tissue	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
A9	M	34	Fibrous tissue	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
B1	M	34	Fibrous tissue	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
B2	F	20	Smooth muscle	Moderate malignant leiomyosarcoma	IIIA	100	100							NEG	0	0	0	0
B3	F	20	Smooth muscle	Moderate malignant leiomyosarcoma	IIIA	100	100							NEG	0	0	0	0
B4	F	49	Fatty tissue	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
B5	F	49	Fatty tissue	Moderate malignant leiomyosarcoma	IIB	100	100							n/a				
B6	F	34	Fatty tissue	Moderate malignant epithelioid leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
B7	F	34	Fatty tissue	Moderate malignant epithelioid leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
B8	M	38	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
B9	M	38	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
C1	F	44	Ligament	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
C2	F	44	Ligament	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
C3	F	69	Smooth muscle	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
C4	F	69	Smooth muscle	Moderate malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
C5	F	88	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
C6	F	88	Smooth muscle	Low malignant leiomyosarcoma	IA	100	100							NEG	0	0	0	0
C7	F	54	Smooth muscle	Malignant pleomorphic leiomyosarcoma	IIIB	100	100							NEG	0	0	0	0
C8	F	54	Smooth muscle	Malignant pleomorphic	IIIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
				leiomyosarcoma														
C9	F	58	Smooth muscle	Low malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
D1	F	58	Smooth muscle	Low malignant leiomyosarcoma	IIB	100	100							NEG	0	0	0	0
D2	F	57	Smooth muscle	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
D3	F	57	Smooth muscle	Low malignant leiomyosarcoma	IB	100	100							NEG	0	0	0	0
D4	M	78	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							n/a				
D5	M	78	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							n/a				
D6	F	52	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
D7	F	52	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
D8	M	61	Smooth muscle	Moderate malignant leiomyosarcoma	IIA	100	100							NEG	0	0	0	0
D9	M	61	Smooth muscle	Moderate malignant leiomyosarcoma (fibrous tissue, blood vessel and smooth muscle)	IIA	100	100							NEG	0	0	0	0
E1	F	33	Fibrous tissue	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
E2	F	33	Fibrous tissue	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
E3	M	21	Retroperitoneum	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
E4	M	21	Retroperitoneum	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
E5	F	51	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
E6	F	51	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
E7	M	16	Testis	Spindle cell rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
E8	M	16	Testis	Spindle cell rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
E9	F	49	Uterus	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F1	F	49	Uterus	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F2	M	30	Striated muscle	Rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
F3	M	30	Striated muscle	Rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
F4	M	18	Tongue	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
F5	M	18	Tongue	Embryonic rhabdomyosarcoma	IIIA	100	100							NEG	0	0	0	0
F6	M	91	Striated muscle	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F7	M	91	Striated	Pleomorphic	IIIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
			muscle	rhabdomyosarcoma														
F8	F	74	Bladder	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
F9	F	74	Bladder	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G1	F	67	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G2	F	67	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G3	F	40	Abdominal cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G4	F	40	Abdominal cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G5	F	23	Pelvic cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G6	F	23	Pelvic cavity	Embryonic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
G7	M	50	Striated muscle	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
G8	M	50	Striated muscle	Pleomorphic rhabdomyosarcoma	IIA	100	100							NEG	0	0	0	0
G9	M	10	Striated muscle	Alveolus rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
H1	M	10	Striated muscle	Alveolus rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
H2	F	32	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
H3	F	32	Uterine cervix	Pleomorphic rhabdomyosarcoma	IIIB	100	100							NEG	0	0	0	0
H4	M	48	Soft tissue	Alveolus rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
H5	M	48	Soft tissue	Alveolus rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
H6	M	40	Soft tissue	Spindle cell rhabdomyosarcoma	IA	100	100							NEG	0	0	0	0
H7	M	40	Soft tissue	Spindle cell rhabdomyosarcoma	IA	100	100							NEG	0	0	0	0
H8	M	49	Testis	Embryonic rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
H9	M	49	Testis	Embryonic rhabdomyosarcoma	IIB	100	100							NEG	0	0	0	0
I1	F	48	Uterus	Smooth muscle	-	100	100							NEG	0	0	0	0
I2	F	8	Heart	Cardiac muscle	-	100	100							NEG	0	0	0	0
I3	F	14	Heart	Cardiac muscle	-	100	100							NEG	0	0	0	0
n/a			Bladder	Normal Bladder	n/a	100	100							NEG	0	0	0	0
n/a			1007 (GCC293); Xenograft	Human Embryonic Kidney Xenograft - GCC Transfected	n/a	100	100							NEG	0	0	0	0
n/a			Kidney Cell Line -	Human Embryonic Kidney Cell Line - GCC Transfected	n/a	100	100							NEG	0	0	0	0

						Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS Cyto	% POS Apical	H Cyto	H Apical
Core Position	sex	age	Organ/ Tissue	Pathology Diagnosis/Tissue Description	Type	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A					
			293 HEK-GCC#2															
n/a			Kidney Cell Line - 293 [HEK-293]	Human Embryonic Kidney Cell Line	n/a	100	100							NEG	0	0	0	0
n/a			Tissue 1	Colon Cancer MTB	n/a	100	100							NEG	0	0	0	0
n/a			Tissue 2	Colon Cancer MTB	n/a	NET								n/a				
n/a			Tissue 3	Colon Cancer MTB	n/a	100	100							NEG	0	0	0	0
n/a			Tissue 4	Colon Cancer MTB	n/a	100	100							NEG	0	0	0	0
n/a			Tissue 5	Colon Cancer MTB	n/a	NET								n/a				
n/a			Tissue 6	Colon Cancer MTB	n/a	NET								n/a				
n/a			Tissue 7	Colon Cancer MTB	n/a	100	100							NEG	0	0	0	0
n/a			Tissue 8	Colon Cancer MTB	n/a	100	100							NEG	0	0	0	0

Table 30A: GCC IHC Staining in Non-Colorectal Cancer Human Clinical Samples from a CRO (QualTek) Tissue Database

QualTek Tissue Database Samples															
	QML Slide		Apical & Cytoplasmic Staining (Percent)								POS /	% POS	% POS	H	H
Sample	No.	Grade	0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	NEG	Cyto	Apical	Cyto	Apical
Pancreas Ca	MIL#7	2	80	100	20						POS	20	0	20	0
Pancreas Ca	MIL#7	3	100	100							NEG	0	0	0	0
Pancreas Ca	MIL#7	2	60	80	30		10	10		10	POS	40	20	50	50
Gastric Adeno	MIL#8	3	60	80	20	0	20	10	10	10	POS	50	20	90	50
Gastric Adeno	MIL#9	3	100	100							NEG	0	0	0	0
Gastric Adeno	MIIL#10	2-3	80	100	20						POS	20	0	20	0
Esophageal Sq Ca	MIL#33	2	90	100	10						POS	10	0	10	0
Esophageal Sq Ca	MIL#12	2	30		50		20				POS	70	0	90	0
Esophageal Sq Ca	MIL#13	2	100	100							NEG	0	0	0	0

Table 30B: GCC IHC Staining in Human Clinical Samples of Normal Colon and Colorectal Cancer (Tumor Grade 1, 2, 2&3 or 3) from a CRO (QualTek) Tissue Database

QualTek Sample No.	MLNM Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score	Tissue
			0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cytoplasm	Apical	Grade
Q34	PRGNX000525	Colon							100	30		70	200	270	Normal
Q35	PRGNX000526	Colon			50		50					100	75	300	2
Q36	PRGNX000527	Colon			50		50			50		50	75	250	Normal
Q37	PRGNX000528	Colon			50		20		30			100	105	300	2
Q38	PRGNX000529	Colon			50		50			20		80	75	280	Normal
Q39	PRGNX000530	Colon					50			50	50	50	200	250	3
Q40	PRGNX000531	Colon			50		50			50		50	75	250	Normal
Q41	PRGNX000532	Colon		30	100			10		30		30	50	160	2
Q42	PRGNX000533	Colon					50		50	20		80	150	280	Normal
Q43	PRGNX000534	Colon					50		50	30		70	150	270	2
Q44	PRGNX000535	Colon		80	100			20					50	20	Normal
Q45	PRGNX000536	Colon					50		50			100	150	300	2
Q46	PRGNX000537	Colon							100	30		70	200	270	Normal
Q47	PRGNX000538	Colon			80		20					100	60	300	2
Q48	PRGNX000539	Colon			50		50			50		50	75	250	Normal
Q49	PRGNX000540	Colon							100	80		20	200	220	2
Q50	PRGNX000541	Colon			50		50	50		50			75	150	Normal
Q51	PRGNX000542	Colon							100			100	200	300	2
Q52	PRGNX000543	Colon					70		30			100	130	300	Normal
Q53	PRGNX000544	Colon					100					100	100	300	2

QualTek Sample No.	MLNM Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score	Tissue
			0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cytoplasm	Apical	Grade
Q54	PRGNX000545	Colon			50		50					100	75	300	Normal
Q55	PRGNX000546	Colon		20	90		10			30		50	55	210	2
Q56	PRGNX000547	Colon			100	50		50					50	75	Normal
Q57	PRGNX000548	Colon			40		30		30			100	110	300	2
Q58	PRGNX000549	Colon			100			30		40		30	50	200	Normal
Q59	PRGNX000550	Colon			30		50		20			100	105	300	2
Q60	PRGNX000551	Colon					80		10	50	10	50	130	250	Normal
Q61	PRGNX000552	Colon	80	80			10		10			20	30	60	2 & 3
Q62	PRGNX000553	Colon			50		50			50		50	75	250	Normal
Q63	PRGNX000554	Colon					20		80			100	180	300	2
Q64	PRGNX000555	Colon					100			50		50	100	250	Normal
Q65	PRGNX000556	Colon					90		10			100	110	300	2
Q66	PRGNX000557	Colon			30		50		20	50		50	105	250	Normal
Q67	*PRGNX000558*	*Colon*													
Q68	PRGNX000559	Colon			50		50					100	75	300	Normal
Q69	PRGNX000560	Colon	50		50			20		30		50	25	230	2
Q70	PRGNX000561	Colon			50		50	20		30		50	75	230	Normal
Q71	*PRGNX000562*	*Colon*													
Q72	PRGNX000563	Colon		40	70		30	20		20		20	65	120	Normal
Q73	PRGNX000564	Colon			100							100	50	300	2
Q74	PRGNX000565	Colon			50		50					100	75	300	Normal
Q75	PRGNX000566	Colon			100			10		20		70	50	260	2
Q76	PRGNX000567	Colon			50		50			50		50	75	250	Normal
Q77	PRGNX000568	Colon	20				70		10			100	90	300	2

QualTek Sample No.	MLNM Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score	Tissue
			0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cytoplasm	Apical	Grade
Q78	PRGNX000569	Colon		30	100			20		30		20	50	140	Normal
Q79	PRGNX000570	Colon			100							100	50	300	2
Q80	PRGNX000571	Colon					50		50			100	150	300	Normal
Q81	PRGNX000572	Colon			80		20					100	60	300	2
Q82	PRGNX000573	Colon			50		50					100	75	300	Normal
Q83	PRGNX000574	Colon			50		50			20		80	75	280	2
Q84	PRGNX000575	Colon					100			20		80	100	280	Normal
Q85	PRGNX000576	Colon			50		20		30	20		80	105	280	2
Q86	PRGNX000577	Colon			50		50			50		50	75	250	Normal
Q87	PRGNX000578	Colon			20		60		20			100	110	300	2
Q88	PRGNX000579	Colon					50		50			100	150	300	Normal
Q89	PRGNX000580	Colon					50		50			100	150	300	3
Q90	PRGNX000581	Colon			50		50			50		50	75	250	Normal
Q91	PRGNX000582	Colon			30		50		20	20		80	105	280	2
Q92	PRGNX000583	Colon			50		50			50		50	75	250	Normal
Q93	PRGNX000584	Colon		20	100			30				50	50	180	2
Q94	PRGNX000585	Colon			50		50			20		80	75	280	Normal
Q95	*PRGNX000586*	*Colon*													
Q96	PRGNX000587	Colon			50		50			50		50	75	250	Normal
Q97	PRGNX000588	Colon					20		80			100	180	300	2
Q98	PRGNX000589	Colon			50		50					100	75	300	Normal
Q99	PRGNX000590	Colon			50		30		20	50		50	95	250	2
Q100	PRGNX000591	Colon			50		50			50		50	75	250	Normal
Q101	PRGNX000592	Colon		20	100					50		30	50	190	2

QualTek Sample No.	MLNM Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score	Tissue
			0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cytoplasm	Apical	Grade
Q102	PRGNX000593	Colon			50		50					100	75	300	Normal
Q103	PRGNX000594	Colon	100	100									0	0	3
Q104	PRGNX000595	Colon			50		50			90		10	75	210	Normal
Q105	PRGNX000596	Colon			60		40	20		30		50	70	230	2
Q106	PRGNX000597	Colon							100			100	200	300	Normal
Q107	PRGNX000598	Colon	10	10	10		30		30	40	20	50	155	230	2 & 3
Q108	PRGNX000599	Colon							100			100	200	300	Normal
Q109	PRGNX000600	Colon	80	100			20						20	0	2
Q110	PRGNX000601	Colon			50		50					100	75	300	Normal
Q111	PRGNX000602	Colon	10		20		60		10			100	90	300	2
Q112	PRGNX000603	Colon			50		50			50		50	75	250	Normal
Q113	PRGNX000604	Colon			50		50			20		80	75	280	2
Q114	PRGNX000605	Colon			50		50			50		50	75	250	Normal
Q115	PRGNX000606	Colon			50				20		30	100	155	300	2
Q116	PRGNX000607	Colon					100			50		50	100	250	Normal
Q117	PRGNX000608	Colon		100	70		30						65	0	3
Q118	PRGNX000609	Colon					100			50		50	100	250	Normal
Q119	PRGNX000610	Colon			30		50		20			100	105	300	2
Q120	PRGNX000611	Colon					100			50		50	100	250	Normal
Q121	PRGNX000612	Colon			30		60		10	20		80	95	280	2
Q122	PRGNX000613	Colon					100					100	100	300	Normal
Q123	PRGNX000614	Colon			30		50		20			100	105	300	2
Q124	PRGNX000615	Colon		60			100	20				20	100	80	Normal
Q125	PRGNX000616	Colon			100	20		20		30		30	50	180	2

QualTek Sample No.	MLNM Sample No.	Sample Type	GCC Apical & Cytoplasmic Staining (Percent)										H-Score	H-Score	Tissue
			0C	0A	0.5+ C	0.5+ A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A	Cytoplasm	Apical	Grade
Q126	PRGNX000617	Colon					100			30		70	100	270	Normal
Q127	PRGNX000618	Colon					80		20			100	120	300	2
Q128	PRGNX000619	Colon			50		50	40		40		20	75	180	Normal
Q129	PRGNX000620	Colon	100									100	0	300	2
Q130	PRGNX000621	Colon					80		20			100	120	300	Normal
Q131	PRGNX000622	Colon			20		30		50			100	140	300	2
Q132	PRGNX000623	Colon					70		30			100	130	300	Normal
Q133	PRGNX000624	Colon					70		30			100	130	300	2

Table 31: Clinical Samples from C260001 Phase I Dose Escalation Study of GCC Targeted Therapeutic MLN0264

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0 A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
58001-102**	CRC	M	03-SEP-2008	5 unstained slides	5/22/2012	100	0	0	0	0	0	0	100	**POS**	0	100	0	300
58001-103**	CRC	F	17-FEB-2010	5 unstained slides	5/22/2012	100	20	0	30	0	30	0	20	**POS**	0	80	0	150
58001-101**	Pancreatic	F	15-MAY-2012	1 paraffin block	5/22/2012	0	0	0	0	0	0	100	100	**POS**	100	100	300	300
58001-104	CRC	M	12-APR-2011	5 unstained slides	6/6/2012	10	0	50	0	20	0	20	100	**POS**	90	100	150	300
58001-105	Pancreatic	M	23-AUG-2006	20 unstained slides	7/3/2012	90	100	10	0	0	0	0	0	**POS**	10	0	10	0

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0A	1+C	1+A	2+C	2+A	3+C	3+A		Cyto	Apical	Cyto	Apical
58002-103	Esophageal	M	17-AUG-2010	5 unstained slides	7/3/2012	90	100	0	0	10	0	0	0	POS	10	0	20	0
58002-101	Pancreatic	M	20-JAN-2011	5 unstained slides	7/3/2012	90	100	10	0	0	0	0	0	POS	10	0	10	0
58002-102	CRC	M	09-AUG-2007	1 FFPE block	7/3/2012	100	100	0	0	0	0	0	0	NEG	0	0	0	0
58002-106	CRC	F	22-JUN-2011	5 unstained slides	7/11/2012	100	100	0	0	0	0	0	0	NEG	0	0	0	0
58001-107	CRC	F	28-NOV-2008	5 unstained slides	7/24/2012	80	20	20	0	0	20	0	60	POS	20	80	20	220
58001-107	CRC	F	28-NOV-2008	5 unstained slides	7/24/2012	90	10	10	10	0	30	0	50	POS	10	90	10	220
58001-107	CRC	F	28-NOV-2008	5 unstained slides	7/24/2012	40	0	50	0	10	20	0	80	POS	60	100	70	280
58002-111	CRC	F	23-OCT-2009	5 unstained slides	7/25/2012	0	0	100	0	0	0	0	100	POS	100	100	100	300
58002-112	CRC	F	01-DEC-2011	5 unstained slides	7/25/2012	0	0	80	0	20	20	0	80	POS	100	100	120	280
58002-107	CRC	F	12-FEB-2007	5 unstained slides	7/26/2012	80	50	10	0	10	50	0	0	POS	20	50	30	100
58002-108	Gastric	M	27-JUL-2012	5 unstained slides	7/31/2012	20	100	20	0	50	0	10	0	POS	80	0	150	0
58002-113	CRC	M	09-JUL-2008	5 unstained slides	7/31/2012	50	20	30	10	20	20	0	50	POS	50	80	70	200
58001-108	Esophageal	M	12-MAY-2010	20 unstained slides	8/8/2012	100	100							NEG	0	0	0	0
58002-114	CRC	F	12-SEP-2005	5 unstained slides	8/10/2012	100			10		80		10	POS	0	100	0	200
58002-105	CRC	M	19-APR-2010	1 FFPE block	8/16/2012	10		70		20	20		80	POS	90	100	110	280

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0A	1+C	1+A	2+C	2+A	3+C	3+A		Cyto	Apical	Cyto	Apical
58001-109	Esophageal	M	20-SEP-2011	6 unstained slides	8/21/2012	100	100							NEG	0	0	0	0
58002-115	CRC	M	24-AUG-2010	5 unstained slides	8/21/2012	100			10		20		70	POS	0	100	0	260
58002-109	Pancreatic	M	08-AUG-2012	5 unstained slides	8/22/2012	20	10	40	30	20	30	20	30	POS	80	90	140	180
58002-117	Pancreatic	M	28-FEB-2011	5 unstained slides	8/22/2012	40	10	40	50	20	30		10	POS	60	90	80	140
58002-118	CRC	M	22-NOV-2010	1 FFPE block	9/7/2012		10		10	70	10	30	70	POS	100	90	230	240
58001-110	Small Intestine	F	31-AUG-2012	1 FFPE block	9/11/2012		80			30		70	20	POS	100	20	270	60
58002-120	CRC	F	12-MAY-2010	1 FFPE block	9/12/2012		70	30		40		30	30	POS	100	30	200	90
58002-121	Gastric	F	03-MAY-2012	1 FFPE block	9/12/2012	40	100	10		40		10		POS	60	0	120	0
58002-123	Pancreatic	F	20-MAY-2011	5 unstained slides	9/13/2012	100	80						20	POS	0	20	0	60
51001-101	CRC	M	03-AUG-2010	5 unstained slides	9/17/2012					100			100	POS	100	100	200	300
58002-119	CRC	M	09-NOV-2005	5 unstained slides + 2 H&E's	9/19/2012	40	20	40	20	20	20		40	POS	60	80	80	180
58002-122	Esophageal	M	10-MAY-2010	1 FFPE block and 2 H&E's	9/19/2012	20	100	20		30		30		POS	80	0	170	0
51001-102	CRC	M	23-NOV-2010	5 unstained slides	10/10/2012					50		50	100	POS	100	100	250	300
51001-103	Pancreatic	F	05-AUG-2009	5 unstained slides	10/15/2012	50	80	50			10		10	POS	50	20	50	50

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0A	1+C	1+A	2+C	2+A	3+C	3+A		Cyto	Apical	Cyto	Apical
51001-104	CRC	F	24-MAR-2010	5 unstained slides	10/22/2012	70		30			10		90	POS	30	100	30	290
58002-116	CRC	M	19-OCT-2010	5 unstained slides	10/24/2012					50		50	100	POS	100	100	250	300
51001-105	Pancreatic	M	29-DEC-2010	5 unstained slides	10/24/2012	90	90	10	10					POS	10	10	10	10
51001-106	CRC	F	17-SEP-2010	5 unstained slides	10/25/2012	80		20			20		80	POS	20	100	20	280
58001-111	CRC	F	05-FEB-2005	10 unstained slides	10/31/2012	90		10	10		10		80	POS	10	100	10	270
58003-105	CRC	F	09-JUN-2010	5 unstained slides	11/6/2012	70						30	100	POS	30	100	90	300
51001-107	CRC	M	31-AUG-2009	5 unstained slides	11/7/2012	80		20					100	POS	20	100	20	300
58002-127	Gastric	F	03-AUG-2011	5 unstained slides	11/8/2012	100	100							NEG	0	0	0	0
58003-101	CRC	F	13-SEP-2010	1 paraffin block	11/8/2012		10	70	10	30	10		70	POS	100	90	130	240
58003-107	Pancreatic	F	29-FEB-2012	5 unstained slides	11/8/2012	100	100							NEG	0	0	0	0
58003-106	CRC	F	25-JUN-2009	5 unstained slides	11/9/2012	100			10		10		80	POS	0	100	0	270
58003-108	CRC	F	28-JUL-2011	5 unstained slides	11/9/2012	100					20		80	POS	0	100	0	280
51001-108	CRC	M	UK-UK-UK	5 unstained slides	11/9/2012			30	0	30	30	40	70	POS	100	100	210	270
51001-109	CRC	M	09-OCT-2007	5 unstained slides	11/12/2012	70		30			30		70	POS	30	100	30	270
58003-103	Esophageal	M	09-MAR-2011	5 unstained slides	11/13/2012	100	100							NEG	0	0	0	0

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0A	1+C	1+A	2+C	2+A	3+C	3+A		Cyto	Apical	Cyto	Apical
58001-112	CRC	M	11-JUN-2012	20 unstained slides	11/13/2012	30		30		30		10	100	**POS**	70	100	120	300
58003-110	CRC	F	30-MAY-2012	5 unstained slides	11/14/2012		100			30		70		**POS**	100	0	270	0
58003-102	CRC	M	17-MAR-2010	1 paraffin block	11/16/2012	20		30	10	40	20	10	70	**POS**	80	100	140	260
58002-126	Pancreatic	F	04-AUG-2011	5 unstained slides	11/16/2012			30		70				**POS**	100		170	
58003-112	Esophageal	M	21-OCT-2011	5 unstained slides	11/29/2012	40	100	50		10				**POS**	60	0	70	0
58001-114	Pancreatic	M	30-NOV-2011	5 unstained slides	11/29/2012	100	100							**NEG**	0	0	0	0
58003-104	Pancreatic	M	16-MAR-2012	5 unstained slides	12/3/2012	20	100	40		20		20		**POS**	80	0	140	0
51001-110	CRC	F	10-FEB-2009	5 unstained slides	12/3/2012		20	70	10	30	20		50	**POS**	100	80	130	200
51001-111	CRC	M	16-SEP-2009	5 unstained slides	12/7/2012		30	100	10		10		50	**POS**	100	70	100	180
58003-113	Gastric	F	07-FEB-2011	5 unstained slides	12/13/2012	100	100							**NEG**	0	0	0	0
58001-113	Esophageal	F	06-JUN-2012	5 unstained slides	12/21/2012			100						**POS**	100		100	
58002-128	Pancreatic	M	09-NOV-2011	1 paraffin block	12/21/2012													
58002-130	Pancreatic	M	10-DEC-2008	1 paraffin block	12/21/2012			50		50			100	**POS**	100	100	150	300
58002-130	Pancreatic	M	10-DEC-2008	1 paraffin block	12/21/2012			50		50	25		75	**POS**	100	100	150	275
58002-130	Pancreatic	M	10-DEC-2008	1 paraffin block	12/21/2012			50		50			100	**POS**	100	100	150	300

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0A	1+C	1+A	2+C	2+A	3+C	3+A		Cyto	Apical	Cyto	Apical
58002-130	Pancreatic	M	13-APR-2011	1 paraffin block	12/21/2012			25		75	25		75	**POS**	100	100	175	275
58003-114	CRC	M	29-MAR-2010	5 unstained slides	12/26/2012	25		50	25	25	25		50	**POS**	75	100	100	225
58003-117	CRC	F	20-MAY-2011	5 unstained slides	12/28/2012		100	70		30				**POS**	100	0	130	0
58001-116	Pancreatic	M	26-JAN-2006	5 unstained slides	1/4/2013 & 1/18/2013 **	100	100							**NEG**	0	0	0	0
58001-115	Esophageal	M	14-DEC-2010	5 unstained slides	1/9/2013	50		40		10			100	**POS**	50	100	60	300
58003-119	Esophageal	M	27-DEC-2011	9 unstained slides	1/14/2013	70	10	30			10		80	**POS**	30	90	30	260
58002-132	Esophageal	M	23-NOV-2010	5 unstained slides & 1 FFPE block	1/16/2013		50	40		30	20	30	30	**POS**	100	50	190	130
51001-112	Gastric	M	26-JAN-2009	5 unstained slides	1/16/2013	70	70	10		20			30	**POS**	30	30	50	90
58003-115	CRC	M	08-SEP-2009	5 unstained slides	1/17/2013	20		80					100	**POS**	80	100	80	300
58003-120	Esophageal	M	15-NOV-2011	5 unstained slides	1/25/2013	10	10	60	10	30	10		70	**POS**	90	90	120	240
51001-113	CRC	M	20-JUL-2009	5 unstained slides	1/28/2013	50		50					100	**POS**	50	100	50	300
51001-114	Pancreatic	F	08-NOV-2007	5 unstained slides	1/28/2013	80	70	20	20		10			**POS**	20	30	20	40
58001-117	Gastric	M	15-SEP-2012	5 unstained slides	1/29/2013		80	90		10	10		10	**POS**	100	20	110	50
58003-	Gastric	M	28-MAY-	5 unstained	2/4/2013	100			10		40		50	**POS**	0	100	0	240

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0 A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
118			2010	slides														
58003-123	CRC	M	01-NOV-2011	5 unstained slides	2/6/2013			30		70			100	POS	100	100	170	300
58003-121	Pancreatic	M	07-SEP-2012	5 unstained slides	2/6/2013	100	95				5			POS	0	5	0	10
58003-125	Pancreatic	M	04-SEP-2012	5 unstained slides	2/11/2013		100	100						POS	100	0	100	0
58003-127	Intestinal	M	11-APR-2011	5 unstained slides	2/11/2013		20	100					80	POS	100	80	100	240
58002-135		F	19-DEC-2012	5 unstained slides	2/14/2013	100	20		30		50			POS	0	80	0	130
58001-119	Gastric	M	29-OCT-2012	5 unstained slides	2/15/2013	100	95				5			POS	0	5	0	10
58001-120	CRC	F	22-MAY-2012	16 unstained slides	2/15/2013	100	20				40		40	POS	0	80	0	200
58003-126	CRC	M	29-DEC-2010	5 unstained slides	2/18/2013	70		30					100	POS	30	100	30	300
58003-129	Pancreatic	M	23-MAR-2012	5 unstained slides	2/18/2013		90	100	10					POS	100	10	100	10
58003-131	CRC	M	10-MAY-2012	5 unstained slides	2/18/2013	100	100							NEG	0	0	0	0
58002-133	Pancreatic	M	03-MAR-2011	5 unstained slides	2/20/2013													
58002-133	Pancreatic	M	03-MAR-2011	5 unstained slides	2/20/2013													
58003-122	CRC	M	24-MAR-2009	5 unstained slides	2/25/2013			90		10			100	POS	100	100	110	300
58003-128	Pancreatic	M	21-DEC-2011	5 unstained slides	2/25/2013	100	100							NEG	0	0	0	0
58001-	CRC	F	22-JUN-	20 unstained	2/25/2013	100	90		10					POS	0	10	0	10

Subject #	Cancer Type	Sex	Collection Date	Sample Type/Quantity	Rcv'd Date	Apical & Cytoplasmic Staining (Percent)								POS /NEG	% POS	% POS	H	H
						0C	0 A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A		Cyto	Apical	Cyto	Apical
121			2010	slides														
58001-115	Esophageal	F	25-FEB-2011	5 unstained slides	2/25/2013	100	55		30		10		5	**POS**	0	45	0	65
58003-124	Gastric	M	07-JUN-2012	5 unstained slides	2/28/2013		100	20		20		60		**POS**	100	0	240	0
58002-133	Pancreatic	M	03-MAR-2011	10 unstained slides	2/28/2013													
58002-129	Esophageal	M	20-MAR-2012	5 unstained slides	2/28/2013			70		30			100	**POS**	100	100	130	300
58002-129	Esophageal	M	08-MAR-2012*	5 unstained slides	2/28/2013		70	80		20			30	**POS**	100	30	120	90
58002-137		F	03-OCT-2011	1 paraffin block	3/1/2013	20	100	40		30		10		**POS**	80	0	130	0

Table 32: GCC IHC Expression in Primary and Metastatic Colorectal Tumor Samples

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
1A	1			20		80			100		POS	100	100	180	300
1B	2			80		20			100		POS	100	100	120	300
2A	3		50	100	20		20		10		POS	100	50	100	90
2B	4		100	60		40					POS	100	0	140	0
3A	5			20		80			100		POS	100	100	180	300
3B	6			80		20			100		POS	100	100	120	300
4A	7	50		50	50		20		30		POS	50	100	50	180
4B	8		50	100	20		30				POS	100	50	100	80
5A	9			80		20	20		80		POS	100	100	120	280
5B	10	50	40	30	20	20	20		20		POS	50	60	70	120
6A	11			80	10	20	30		60		POS	100	100	120	250
6B	12			50	10	50	20		70		POS	100	100	150	260
7A	13	30		50		20	20		80		POS	70	100	90	280
7B	14	30		50		20	20		80		POS	70	100	90	280
8A	15					100			100		POS	100	100	200	300
8B	16			50		50			100		POS	100	100	150	300
9A	17		100			50		50		High grade - staining in vacuoles	POS	100	0	250	0

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
9B	18		60	100	20		20				POS	100	40	100	60
10A	19			80		20			100		POS	100	100	120	300
10B	20			50		50			100		POS	100	100	150	300
11A	21	50	10	50	30		30		30		POS	50	90	50	180
11B	22	50	60	50	20		10		10		POS	50	40	50	70
12A	23			80		20	50		50		POS	100	100	120	250
12B	24				20	10	30	90	50	**High grade - staining in vacuoles mainly - a few lumens present**	POS	100	100	290	230
13A	25	50	20	50	10		20		50		POS	50	80	50	200
13B	26		80	100	10		10				POS	100	20	100	30
14A	27			80		20			100	**Signet Ring cell component not scored**	POS	100	100	120	300
14B	28			50		50			100		POS	100	100	150	300
15A	29	50	50	40	10	10	30		10		POS	50	50	60	100
15B	31	50	100	50							POS	50	0	50	0
16A	32	60	20	30	20	10	30		30	**Mucinous**	POS	40	80	50	170
16B	33			70		30			100		POS	100	100	130	300
17A	34			90		10			100		POS	100	100	110	300

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
17B	35	50	20	50	20		20		40		POS	50	80	50	180
18A	36			70	20	30	30		50		POS	100	100	130	230
18B	37	10	50	80	10	10	20		20		POS	90	50	100	110
19A	38			80		20			100		POS	100	100	120	300
19B	39	70	70	30			20		10		POS	30	30	30	70
20A	40	80	100	20						Neuroendocrine	POS	20	0	20	0
20B	41	50	90	20		30	10				POS	50	10	80	20
21A	42	50		50					100		POS	50	100	50	300
21B	43	NET								No Evidence of Tumor					
22A	44	60	10	30	10	10	20		60		POS	40	90	50	230
22B	45		10	100			10		80		POS	100	90	100	260
23A	46	50	10	50					90		POS	50	90	50	270
23B	47			70		30			100		POS	100	100	130	300
24A	48		10	80		20	40		50		POS	100	90	120	230
24B	49	80	40	10		10	30		30	Only small amt of tumor	POS	20	60	30	150
25A	50	50	30	50	20		20		30		POS	50	70	50	150
25B	51			80		20			100		POS	100	100	120	300
26A	52			80		20	50		50		POS	100	100	120	250

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
26B	53	Net								**No Evidence of Tumor**					
27A	54	40	70	20	10	20	10	20	10		**POS**	60	30	120	60
27B	55			60		20	30	20	70		**POS**	100	100	160	270
28A	56			50		50			100		**POS**	100	100	150	300
28B	57			30		50		20	100		**POS**	100	100	190	300
29A	58			50		50			100		**POS**	100	100	150	300
29B	59	50		40	20	10	30		50		**POS**	50	100	60	230
30A	61	10		70		20	20		80		**POS**	90	100	110	280
30B	62			50		50	50		50		**POS**	100	100	150	250
31A	63					50		50	100		**POS**	100	100	250	300
31B	64			50		50	50		50		**POS**	100	100	150	250
32A	65			80		20			100		**POS**	100	100	120	300
32B	66	50	50	50					50		**POS**	50	50	50	150
33A	67			80		20	50		50		**POS**	100	100	120	250
33B	68			20		80			100		**POS**	100	100	180	300
34A	69			100			30		70		**POS**	100	100	100	270
34B	70			100	20		40		40		**POS**	100	100	100	220
35A	71			70		20		10	100		**POS**	100	100	140	300
35B	72			70		20		10	100		**POS**	100	100	140	300

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
36A	73			50	40	40	50	10	10		**POS**	100	100	160	170
36B	74			30	40	40	30	30	30		**POS**	100	100	200	190
37A	75		20	100	10		20		50		**POS**	100	80	100	200
37B	76	50	90	50					10		**POS**	50	10	50	30
38A	77	30		70			50		50		**POS**	70	100	70	250
38B	78	80		20					100		**POS**	20	100	20	300
39A	79			100			50		50		**POS**	100	100	100	250
39B	80			100					100		**POS**	100	100	100	300
40A	81			40		60	20		80		**POS**	100	100	160	280
40B	82	NET								**No Evidence of Tumor**					
41A	83	20	60	80	20		10		10		**POS**	80	40	80	70
41B	84	50	40	50	20		20		20		**POS**	50	60	50	120
42A	85		10	80	10	20	40		40		**POS**	100	90	120	210
42B	86		10	80	10	20	30		50		**POS**	100	90	120	220
43A	87		10	30	30	50	30	20	30		**POS**	100	90	190	180
43B	88			50		40	30	10	70		**POS**	100	100	160	270
44A	89			80		20			100		**POS**	100	100	120	300
44B	91	60	100	30		10					**POS**	40	0	50	0
45A	92			30		50		20	100		**POS**	100	100	190	300

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
45B	93			70		20		10	100	**Golgi-like staining in cytoplasm**	**POS**	100	100	140	300
46A	94	90	90	10	10						**POS**	10	10	10	10
46B	95	20	80	80			10		10		**POS**	80	20	80	50
47A	96	50	100	50						**Adenoma - no tumor**	**POS**	50	0	50	0
47B	97		40	80	20	20	20		20		**POS**	100	60	120	120
48A	98	30		70			20		80		**POS**	70	100	70	280
48B	99	90		10			20		80		**POS**	10	100	10	280
49A	100	30		40		30	30		70		**POS**	70	100	100	270
49B	101	30	70	50		20			30		**POS**	70	30	90	90
50A	102		10	90	30	10			60		**POS**	100	90	110	210
50B	103	100	70		10				20		**POS**	0	30	0	70
51A	104	50	80	50			20				**POS**	50	20	50	40
51B	105	NET								**No Evidence of Tumor**					
52A	106	50	90	50			10				**POS**	50	10	50	20
52B	107	90	90	10	10						**POS**	10	10	10	10
53A	108		40	80	20	20	20		20		**POS**	100	60	120	120
53B	109	50	80	50	20						**POS**	50	20	50	20

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
54A	110		50	70	20	30	20		10		POS	100	50	130	90
54B	111		20	80		20	20		60		POS	100	80	120	220
55A	112	100	100								NEG	0	0	0	0
55B	113	100	100								NEG	0	0	0	0
56A	114	50	70	50	30						POS	50	30	50	30
56B	115	100	100								NEG	0	0	0	0
57A	116	50	70	50	10		10		10		POS	50	30	50	60
57B	117		80	50		50	10		10	Small amount of tumor	POS	100	20	150	50
58A	118	10		80	20	10	40		40		POS	90	100	100	220
58B	119		40	100	20		20		20	Small amount of tumor	POS	100	60	100	120
59A	121			70		30	30		70		POS	100	100	130	270
59B	122			30		70			100		POS	100	100	170	300
60A	123	50	70	50	10		10		10		POS	50	30	50	60
60B	124	10	30	70	20	20	20		30		POS	90	70	110	150
61A	125	50		30		20	50		50		POS	50	100	70	250
61B	126	100			20		30		50		POS	0	100	0	230
62A	127		80	100			10		10		POS	100	20	100	50
62B	128		90	80		20	10				POS	100	10	120	20

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
63A	129	10	50	80		10	20		30		POS	90	50	100	130
63B	130		100	90		10					POS	100	0	110	0
64A	131		90	80		20	10				POS	100	10	120	20
64B	132		60	70	10	30	20		10		POS	100	40	130	80
65A	133	50	90	50			10				POS	50	10	50	20
65B	134	70	100	30						Tumor high Grade	POS	30	0	30	0
66A	135		30	80		20	50		20		POS	100	70	120	160
66B	136		100	100						Small amount of tumor	POS	100	0	100	0
67A	137	10	20	80		10			80		POS	90	80	100	240
67B	138	30	20	70			40		40		POS	70	80	70	200
68A	139	20	90			20		60	10	Vacuolar staining	POS	80	10	220	30
68B	140		100	70				30			POS	100	0	160	0
69A	141		40	20		80	30		30		POS	100	60	180	150
69B	142		20	20	20	50	30	30	30		POS	100	80	210	170
70A	143			50		50			100		POS	100	100	150	300
70B	144		10	40	30	40	30	20	30		POS	100	90	180	180
71A	145									Only a few tumor cells- cannot score					

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
71B	71-2	60	50	20		20	20		30		POS	40	50	60	130
72A	72-1		50	50		30	20	20	30	Higher grade - apicically negative	POS	100	50	170	130
72B	72-2	100	100								NEG	0	0	0	0
73A	73-1	20	80	80					20		POS	80	20	80	60
73B	73-2	40	50	40	10	10	20	10	20		POS	60	50	90	110
74A	74-1							100	100	Vacuolar staining	POS	100	100	300	300
74B	74-2		20	50	10	50	30		40		POS	100	80	150	190
75A	75-1			70		30	30		70		POS	100	100	130	270
75B	75-2	10	50	90	10		20		20		POS	90	50	90	110
76A	76-1		10	60	10	40	20		60		POS	100	90	140	230
76B	76-2			50	10	50	30		60		POS	100	100	150	250
77A	77-1		20	90	20	10	20		40		POS	100	80	110	180
77B	77-2	10	20	90	10		20		50		POS	90	80	90	200
78A	78-1			100					100		POS	100	100	100	300
78B	78-2			100					100		POS	100	100	100	300
79A	79-1			80		20			100		POS	100	100	120	300
79B	79-2			50		50			100		POS	100	100	150	300
80A	80-1	90		10					100		POS	10	100	10	300

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
80B	80-2			20		50		30	100		POS	100	100	210	300
81A	81-1			80		20	30		70		POS	100	100	120	270
81B	81-2		10	90	10	10	20		60		POS	100	90	110	230
82A	82-1			50		50			100		POS	100	100	150	300
82B	82-2	20		80					100		POS	80	100	80	300
83A	83-1	30		70	50		20		30		POS	70	100	70	180
83B	83-2			80		20	20		80	Golgi-like staining in cytoplasm	POS	100	100	120	280
84A	84-1	80		20					100		POS	20	100	20	300
84B	84-2	100	60		20		10		10		POS	0	40	0	70
85A	85-1			100					100		POS	100	100	100	300
85B	85-2	100							100		POS	0	100	0	300
86A	86-1	10	40	80		10			60		POS	90	60	100	180
86B	86-2			50		50			100		POS	100	100	150	300
87A	87-1	10	10	80	20	10	20		50		POS	90	90	100	210
87B	87-2	10	10	50	30	30	30	10	30		POS	90	90	140	180
88A	88-1		30	80	20	20	20		30		POS	100	70	120	150
88B	88-2		100	50		50					POS	100	0	150	0
89A	89-1	100	100							Renal Cell	NEG	0	0	0	0

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
89B	89-2	100	100								NEG	0	0	0	0
90A	90-1	50	100	20				30		Pancreas - Neuroendocrine	POS	50	0	110	0
90B	90-2		100			20		80		Vacuolar staining	POS	100	0	280	0
91A	91-1	100	100							Peritoneal - Liposarcoma	NEG	0	0	0	0
91B	91-2	100	100								NEG	0	0	0	0
92A	92-1	100	100							Gastric GIST	NEG	0	0	0	0
92B	92-2	100	100								NEG	0	0	0	0
93A	93-1	50		30		20			100	Golgi-like staining in cytoplasm	POS	50	100	70	300
93B	93-2	50		30		20			100		POS	50	100	70	300
94A	94-1	50		50					100		POS	50	100	50	300
94B	94-2			80		20			100		POS	100	100	120	300
95A	95-1			40		50		10	100		POS	100	100	170	300
95B	95-2	50		50					100		POS	50	100	50	300
96A	96-1					100			100		POS	100	100	200	300
96B	96-2	100	40		20		20		20		POS	0	60	0	120
97A	97-1	100	100							Pancreatic	NEG	0	0	0	0

Sample #	Slide #	Apical & Cytoplasmic Staining (Percent)								Comments	POS /NEG	% POS	% POS	H	H
		0C	0A	1+ C	1+ A	2+ C	2+ A	3+ C	3+ A			Cyto	Apical	Cyto	Apical
97B	97-2	100	100								NEG	0	0	0	0
98A	98-1		100					100		Uterus - Leiomyosarcoma	POS	100	0	300	0
98B	98-2		100					100			POS	100	0	300	0
99A	99-1	10	100					90		Pancreas - Neuroendocrine	POS	90	0	270	0
99B	99-2	100	100								NEG	0	0	0	0
100A	100-1			100			50		50	Gastric	POS	100	100	100	250
100B	100-2		20	60		40	40		40		POS	100	80	140	200

Summary of GCC IHC Staining Results

The GCC IHC staining results shown in Examples 3 and 4 demonstrate that GCC is expressed in a variety of gastrointestinal malignancies, including colorectal, stomach, small intestine and esophageal tumors, as well as non-gastrointestinal tumors including pancreatic tumors and lung adenocarcinomas, lung squamous cell carcinomas, leiomyosarcomas and rhabdomyosarcomas. A summary of the various tumor microarray analyses is shown in Table 33 below.

Table 33: TMA analysis shows GCC is expressed in a variety of maligancies

		% GCC Positive Staining*		
Tumor Type	**N Tested**	**Apical/Membranous**	**Cytoplasmic**	**% positive with either**
Colorectal	298	76	90	95
Gastric	154	33.1	63	79
Pancreatic	221	15.4	43.4	63
Esophageal	138	1.4	30	30
Lung Adenocarcinoma	81	2.4	25.6	44
Lung Squamous	74	0	10.8	10.8
Leiomyosarcoma	18	0	44.4	44.4
Rhabdomyosarcoma	18	0	55.6	55.6

*(% positive is defined by H score >10)

Human clinical samples of gastrointestinal and GI-related malignancies also tested positive for varying levels of GCC expression, as summarized in Table 34 below.

Table 34: Percent of Tumors Screend in C26001 positive

	N	% any positive	% greater 400 (on combined/aggregate apical and cytoplasmic H score)
Colorectal	46	93.5	26.1
Gastric	9	77.8	0
Esophageal	14	78.6	7.1
Pancreatic	22	81.8	22.7
Small Intestine	2	100	0
Total	93	86.0	

(Positive is defined as an H score > = to 10 in either apical or cytoplasmic)

The results summarized in Table 34 are similar to the results observed throughout the TMA screenings summarized in Table 33.

The combined/aggregate H Score distribution of GCC expression across tumor types from patient enrollment screening for the the C26001 trial is depicted in Figures 2A-2D.

The combined/aggregate H score distribute of GCC expression from the various colorectal, gastric, and pancreatic tumor microarrays screened is depicted in Figures 3A-3C.

While this invention has been shown and described with references to provided embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed is:

1. An anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOs: 21, 22 and 23, respectively, or SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOs: 27, 28 and 29, respectively, or SEQ ID NOS: 39, 40 and 41, respectively.

2. An anti-GCC antibody molecule the competes for binding or binds to the same epitope as an anti-GCC antibody comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOs: 21, 22 and 23, respectively, and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOs: 27, 28 and 29, respectively.

3. An anti-GCC antibody molecule the competes for binding or binds to the same epitope as an anti-GCC antibody comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences SEQ ID NOS: 39, 40 and 41, respectively.

4. The anti-GCC antibody molecule of any of the preceding claims, wherein said anti-GCC antibody molecule is a monoclonal antibody.

5. The anti-GCC antibody molecule of any of the preceeding claims, wherein said anti-GCC antibody molecule is a rabbit or rabbit-derived antibody.

6. The anti-GCC antibody molecule of claim 5, wherein said antibody is a rabbit monoclonal antibody.

7. The anti-GCC antibody molecule of claim 1, further comprising a heavy chain variable region comprising an amino acid sequence according to SEQ ID NO: 11 or SEQ ID NO: 15, and a light chain variable region comprising an amino acid sequence according to SEQ ID NO: 13 or SEQ ID NO: 17.

8. The anti-GCC antibody molecule of claim 1, wherein the heavy chain CDR1, CDR2 and CDR3 comprise the amino acid sequences according to SEQ ID NOs: 21, 22 and 23, respectively, and the light chain CDRs comprise the amino acid sequences according to SEQ ID NOs: 27, 28 and 29, respectively.

9. The anti-GCC antibody molecule of claim 7, wherein the heavy chain variable region comprises an amino acid sequence according to SEQ ID NO: 11 and the light chain variable region comprises an amino acid sequence according to SEQ ID NO: 13.

10. The anti-GCC antibody molecule of any of the preceding claims, wherein said anti-GCC antibody molecule is conjugated to a detectable label.

11. The anti-GCC antibody molecule of claim 10, wherein said detectable label is selected from the group consisting of horseradish peroxidase (HRP), alkaline phosphatase, galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and

glucose 6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, and stable free radicals.

12. The anti-GCC antibody molecule of claim 10, wherein said detectable label is a fluorophores selected from fluorescein or a derivatives thereof, rhodamine or a derivative thereof, dansyl, umbelliferone, a luceriferase, luciferin, and 2,3-dihydrophthalazinediones.

13. The anti-GCC antibody molecule of claim 10, wherein said detectable label is a radioactive agent selected from the group consisting of ^{32}P, ^{3}H, ^{14}C, ^{188}Rh, ^{43}K, ^{52}Fe, ^{57}Co, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{77}Br, $^{81}Rb/\ ^{81M}Kr$, ^{87M}Sr, ^{99}Tc, ^{111}In, $^{113}M\ In$, ^{123}I, ^{125}I, ^{127}Cs, ^{129}Cs, ^{131}I, ^{132}I, ^{197}Hg, ^{203}Pb and ^{206}Bi, and ^{213}Bi.

14. Isolated nucleic acid sequences that encode an antibody molecule of any of claims 1, 7, 8 and 9.

15. A cell comprising the isolated nucleic acid sequences of claim 14.

16. A method of producing an antibody molecule of claim 1, 7, 8 or 9, comprising culturing the cell of claim 15 under conditions that allow production of an antibody molecule, thereby producing the antibody molecule of claim 1, 7, 8 or 9.

17. A vector comprising one or both of the light chain and heavy chain of an antibody molecule of claim 1, 7, 8 or 9.

18. A method of detecting a GCC molecule in a biological sample comprising contacting the biological sample with an antibody molecule of any of claims 1-13 and determining if said antibody molecule binds to said GCC molecule.

19. The method of claim 18, wherein said method detection method comprises an immunohistochemistry assay.

20. The method of claim 18 or claim 19, wherein said biological sample is a tumor biopsy derived from a patient suspected of having a GCC expressing cancer.

21. The method of any of claims 18-20, further comprising the step of quantifying GCC expression in said biological sample.

22. The method of claim 21, wherein said quantification of GCC expression comprises apical GCC expression in said biological sample, cytoplasmic GCC expression in said biological sample, or both.

23. The method of claim 21 or 22, wherein said quantification step comprises an H-score approach.

24. The method of claim 20, wherein said GCC expressing cancer is selected from the group consisting of: colorectal cancer, gastric cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung cancer, a soft tissue sarcoma, a neuroectodermal tumor and a neuroendocrine tumor.

25. The method of claim 24, wherein said lung cancer is squamous cell carcinoma or adenocarcinoma.

25. The method of claim 24, wherein said soft tissue sarcoma is leiomyosarcoma or rhabdomyosarcoma.

26. The method of claim 24, wherein said neuroendocrine tumor is a gastrointestinal or a bronchopulmonary neuroendocrine tumor.

27. A kit comprising the anti-GCC antibody molecule of any of claims 1-13 and instructions for use.

28. The kit of claim 27, further comprising a GCC-targeted therapeutic agent.

29. The kit of claim 28, wherein the GCC-targeted therapeutic agent comprises an anti-GCC antibody molecule.

30. The kit of claim 29, wherein said therapeutic anti-GCC antibody molecule is a monoclonal antibody.

31. The kit of claim 29 or 30, wherein said therapeutic anti-GCC antibody molecule is a human IgG1 antibody.

32. The kit of claim 29, 30 or 31, wherein said therapeutic anti-GCC antibody molecule comprises three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70, 71 and 72, respectively.

33. The kit of claim 32, wherein said therapeutic anti-GCC antibody molecule comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 79, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 80.

34. The kit of claim 29-33, wherein said therapeutic anti-GCC antibody molecule is conjugated to a cytotoxic agent.

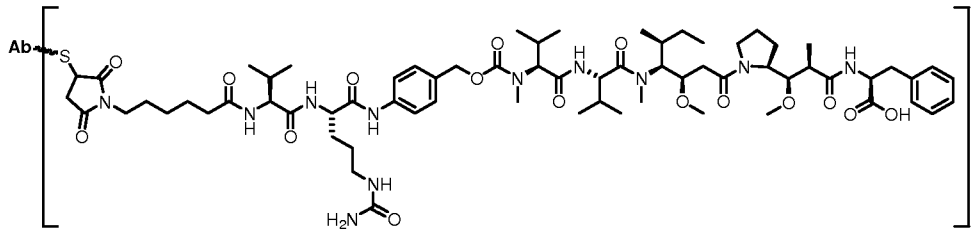
35. The kit of claim 34, wherein said cytotoxic agent is an auristatin molecule.

36. The kit of claim 35, wherein said auristatin molecule is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

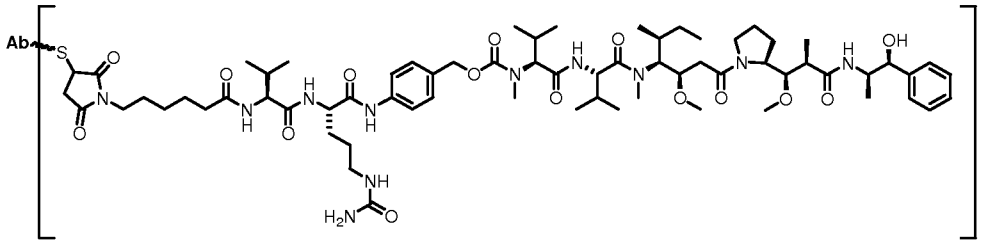
37. The kit of claim 34-36, wherein said cytotoxic agent is conjugated to said antibody via a linker containing a maleimide moiety.

38. The kit of claim 37, wherein said maleimide linker is selected from the group consisting of maleimidocaproyl (mc), maleimidocaproyl-L-phenylalanine-L-lysine-*p*-aminobenzyl-carbamate, and maleimidocaproyl-L-valine-L-citrulline-*p*-aminobenzylcarbamate (vc).

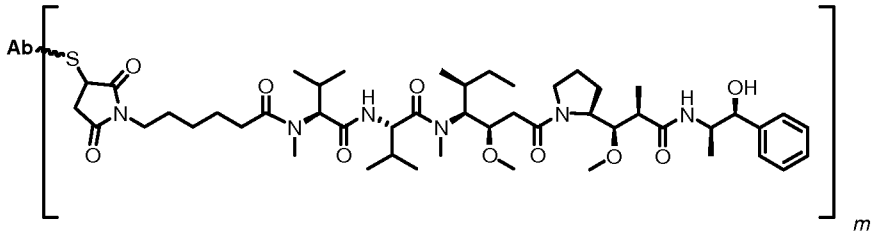
39. The kit of claim 28, wherein said GCC-targeted therapeutic agent is an immunoconjugate characterized by any one of formulas (***I-4***), (***I-5)*** or (***I-6***):



(***I-4***),



(***I-5***),



(*I-6*),

wherein Ab is an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70, 71 and 72, respectively, and wherein m is an integer from 1 to 8.

40. The kit of claim 39, wherein m is from 3 to 5.

41. The kit of claim 39, wherein m is about 4.

42. A method of treating a patient having a disease characterized by one or more GCC-expressing cells, comprising:

a. detecting GCC protein expression in a biological sample obtained from the patient, e.g., by a method of any of claims 18-26; and

b. administering a GCC-targeted therapeutic agent, e.g., a GCC-targeted therapeutic agent described herein, to the patient if the biological sample expresses GCC.

43. The method of claim 42, wherein said detection step comprises the steps of:

i) contacting the biological sample with an anti-GCC antibody molecule described herein, e.g., an anti-GCC antibody molecule of any of claims 1-13, e.g., an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 21, 22 and 23, or SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 27, 28 and 29, or SEQ ID NOS: 39, 40 and 41, respectively; and

ii) detecting formation of a complex between the anti-GCC antibody molecule and GCC protein.

44. The method of claim 43, wherein the detection step is performed via immunohistochemistry.

45. The method of claim 43, wherein the GCC-targeted therapeutic agent comprises an anti-GCC antibody molecule.

46. The method of claim 45, wherein said therapeutic anti-GCC antibody molecule is a monoclonal antibody.

47. The method of claim 45 or 46, wherein said therapeutic anti-GCC antibody molecule is a human IgG1 antibody.

48. The method of claim 45, wherein the anti-GCC antibody molecule comprises three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70,71 and 72, respectively.

49. The method of claim 48, wherein the anti-GCC antibody molecule further comprises a heavy chain variable region comprising an amino acid sequence according to SEQ ID NO: 79, and a light chain variable region comprising an amino acid sequence according to SEQ ID NO: 80.

50. The method of claim 45 wherein the anti-GCC antibody molecule is conjugated to a cytotoxic agent.

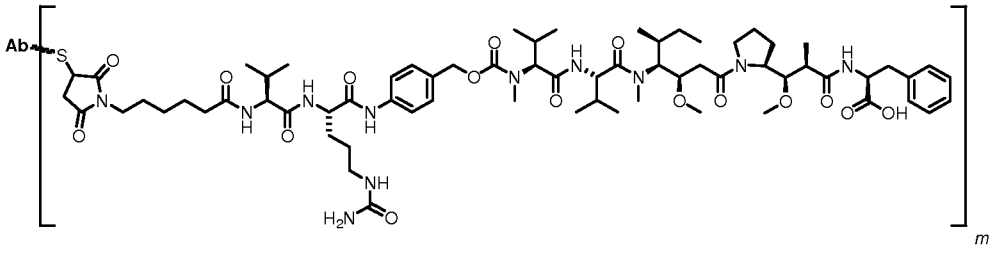
51. The method of claim 50, wherein said cytotoxic agent is an auristatin molecule.

52. The method of claim 51, wherein said auristatin molecule is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

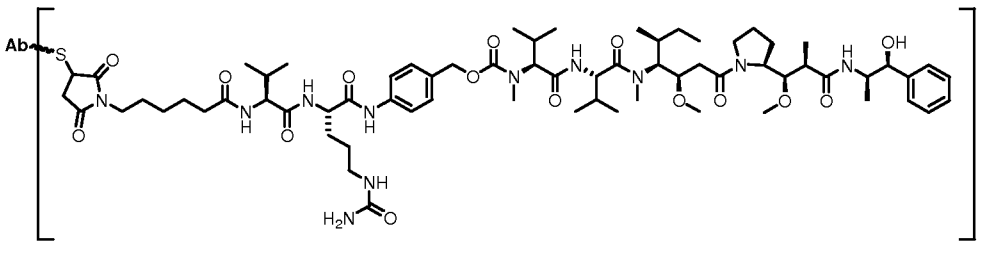
53. The method of claim 50-53, wherein said cytotoxic agent is conjugated via a linker containing a maleimide moiety.

54. The method of claim 53, wherein said maleimide linker is selected from the group consisting of maleimidocaproyl (mc), maleimidocaproyl-L-phenylalanine-L-lysine-*p*-amino-benzylcarbamate, and maleimidocaproyl-L-valine-L-citrulline-*p*-aminobenzylcarbamate (vc).

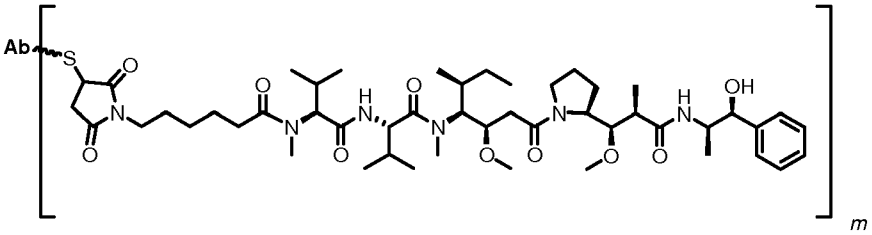
55. The method of claim 42, wherein said GCC-targeted therapy is an immunoconjugate characterized by any one of formulas (***I-4***), (***I-5)*** or (***I-6***):



(***I-4***),



(***I-5***),



(***I-6***),

wherein Ab is an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70, 71 and 72, respectively, and wherein m is an integer from 1 to 8.

56. The method of claim 55, wherein m is from about 3 to about 5.

57. The method of claim 56, wherein m is about 4.

58. The method of any of claims 42-57, wherein the disease characterized by one or more GCC-expressing cells is selected from the group consisting of: cancer, inflammatory bowel syndrome, Crohn's disease or Parkinson's disease.

59. The method of claim 58, wherein the cancer is selected from the group consisting of colorectal cancer, gastric cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung cancer, a soft tissue sarcoma, a neuroectodermal tumor and a neuroendocrine tumor.

60. The method of claim 59, wherein said lung cancer is squamous cell carcinoma or adenocarcinoma.

61. The method of claim 59, wherein said soft tissue sarcoma is leiomyosarcoma or rhabdomyosarcoma.

62. The method of claim 59, wherein said neuroendocrine tumor is a gastrointestinal or a bronchopulmonary neuroendocrine tumor.

63. The method of claim 42-62, wherein the biological sample is a cell or a tissue biopsy.

64. The method of claim 63, wherein the cell or tissue biopsy is a tumor biopsy.

65. A method of determining sensitivity of cancer cells to a GCC-targeted therapeutic agent and/or evaluating whether a subject is a potential candidate for a GCC-targeted therapy, the method comprising the steps of:

i) providing a sample from cancer cells from a patient that has cancer;

ii) contacting the sample with an anti-GCC antibody molecule described herein, e.g., an anti-GCC antibody of any of claims 1-13, e.g., an anti-GCC antibody comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 21, 22 and 23, or SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 27, 28 and 29, or SEQ ID NOS: 39, 40 and 41, respectively; and

iiii) detecting formation of a complex between the anti-GCC antibody molecule and GCC protein, thereby determining the sensitivity of the cancer to the GCC-targeted therapeutic agent, e.g., a GC-targeted therapeutic agent dscribed herein, and/or determining if a subject is a

candidate for treatment with a GCC-targeted therapy, e.g., a GCC-targeted therapy described herein.

66. The method of claim 65, wherein the detection step is performed via immunohistochemistry.

67. The method of claim 65 or 66, wherein the cancer is selected from the group consisting of colorectal cancer, gastric cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung cancer, a soft tissue sarcoma, a neuroectodermal tumor and a neuroendocrine tumor.

68. The method of claim 67, wherein said lung cancer is squamous cell carcinoma or adenocarcinoma.

69. The method of claim 67, wherein said soft tissue sarcoma is leiomyosarcoma or rhabdomyosarcoma.

70. The method of claim 67, wherein said neuroendocrine tumor is a gastrointestinal or a bronchopulmonary neuroendocrine tumor.

71. The method of any of claims 65-70, further comprising the step of administering the GCC-targeted therapeutic agent, e.g., a GCC-targeted therapeutic agent described herein, to the patient.

72. The method of claim 71, wherein the GCC-targeted therapeutic agent comprises an anti-GCC antibody molecule.

73. The method of claim 72, wherein said therapeutic anti-GCC antibody molecule is a monoclonal antibody.

74. The method of claim 72 or 73, wherein said therapeutic anti-GCC antibody molecule is a human IgG1 antibody.

75. The method of any of claims 72, 73 or 74, wherein said therapeutic anti-GCC antibody molecule comprises three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70,71 and 72, respectively.

76. The method of claim 75, wherein said therapeutic anti-GCC antibody molecule further comprises a heavy chain variable region comprising an amino acid sequence according to SEQ ID NO: 79, and a light chain variable region comprising an amino acid sequence according to SEQ ID NO: 80.

77. The method of any of claims 72-76 wherein the anti-GCC antibody molecule is conjugated to a cytotoxic agent.

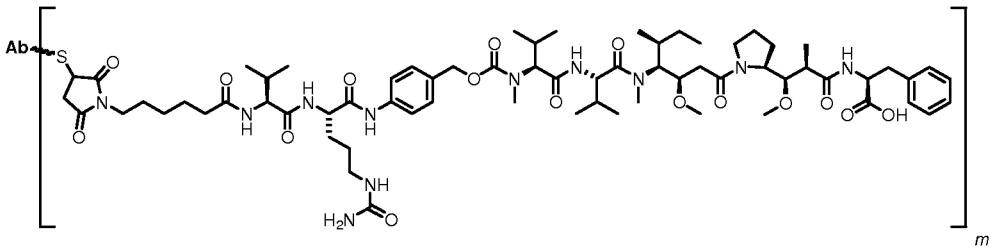
78. The method of claim 77, wherein said cytotoxic agent is an auristatin molecule.

79. The method of claim 78, wherein said auristatin molecule is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

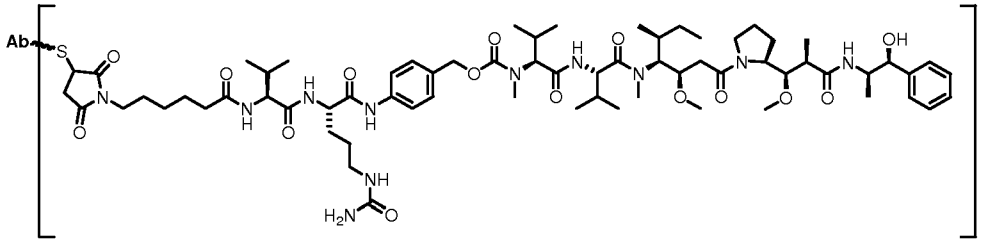
80. The method of any of claims 77, 78 or 79, wherein said cytotoxic agent is conjugated via a linker containing a maleimide moiety.

81. The method of claim 80, wherein said maleimide linker is selected from the group consisting of maleimidocaproyl (mc), maleimidocaproyl-L-phenylalanine-L-lysine-*p*-amino-benzylcarbamate, and maleimidocaproyl-L-valine-L-citrulline-*p*-aminobenzylcarbamate (vc).

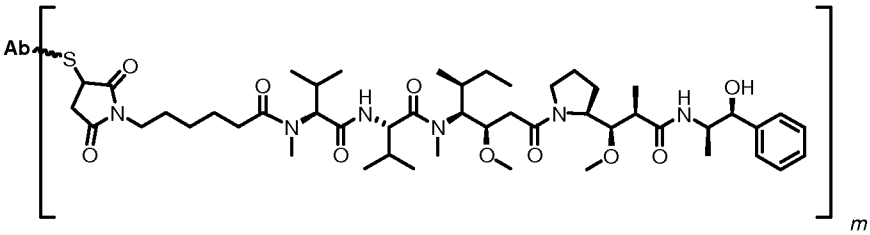
82. The method of claim 65-71, wherein said GCC-targeted therapeutic agent is an immunoconjugate characterized by any one of formulas (***I-4***), (***I-5)*** or (***I-6***):



(***I-4***),



(*I-5*),



(*I-6*),

wherein Ab is an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 67, 68 and 69, respectively, and three light chain complementarity determining regions (CDR1, CDR2 and CDR3) comprising amino acid sequences according to SEQ ID NOs: 70, 71 and 72, respectively, and wherein m is an integer from 1 to 8.

83. The method of claim 82, wherein m is from about 3 to about 5.

84. The method of claim 83, wherein m is about 4.

85. A reaction mixture comprising a biological sample and an anti-GCC antibody molecule described herein, e.g., an anti-GCC antibody of any of claims 1-13, e.g., an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 21, 22 and 23, or SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 27, 28 and 29, or SEQ ID NOS: 39, 40 and 41, respectively.

86. The reaction mixture of claim 85, wherein the biological sample comprises one or more cells.

87. The reaction mixture of claim 85, wherein the biological sample comprises a tissue sample.

88. The reaction mixture of claim 87, wherein the tissue sample is a paraffin-embedded tissue sample.

89. The reaction mixture of claim 85, wherein the biological sample is a primary or metastatic tumor biopsy sample.

90. The reaction mixture of claim 85, wherein the biological sample is mounted on a slide.

91. The reaction mixture of claim 89, wherein the tumor is a colorectal tumor, a gastric tumor, a small intestine tumor, an esophageal tumor, a pancreatic tumor, a lung tumor, a soft-tissue sarcoma, a neuroectodermal tumor or a neuroendocrine tumor.

92. The reaction mixture of claim 91, wherein the lung tumor is a squamous cell carcinoma or an adenocarcinoma.

93. The reaction mixture of claim 91, wherein the soft-tissue sarcoma is a leiomyosarcoma or a rhabdomyosarcoma.

94. The reaction mixture of claim 91, wherein the neuroendocrine tumor is a gastrointestinal or a bronchopulmonary neuroendocrine tumor.

95. The reaction mixture of claim 85, wherein the biological sample is suspected of containing GCC protein.

96. The reaction mixture of any of claims 85-95, further comprising a reagent suitable for detecting formation of a complex between the anti-GCC antibody and GCC protein.

97. A method for generating a personalized cancer treatment report, said method comprising the steps of:

contacting a biological sample comprising one or more cancer cells obtained from a cancer patient suspected of having a GCC-expressing cancer with an anti-GCC antibody

molecule described herein, e.g., an anti-GCC antibody molecule of any of claims 1-13, e.g., an anti-GCC antibody molecule comprising three heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 21, 22 and 23, or SEQ ID NOs: 33, 34 and 35, respectively; and three light chain complementarity determining regions (CDR1, CDR2, and CDR3) comprising amino acid sequences according to SEQ ID NOs: 27, 28 and 29, or SEQ ID NOS: 39, 40 and 41, respectively;

detecting formation of a complex between the anti-GCC molecule and GCC protein in the biological sample, e.g., by a method of any of claims 18-26;

quantifying GCC expression in the biological sample;

comparing the GCC expression level against a database comprising GCC-targeted therapy, e.g., a GCC-targeted therapy described herein; and

selecting a GCC-targeted therapy and, optionally, a dosing regimen based on the GCC expression level.

98. The method of claim 97, wherein the GCC-expressing cancer is colorectal cancer, gastric cancer, small intestine cancer, esophageal cancer, pancreatic cancer, lung cancer, soft-tissue sarcoma, a neuroectodermal tumor or a neuroendocrine tumor.

99. The method of claim 98, wherein the lung tumor is a squamous cell carcinoma or an adenocarcinoma.

100. The method of claim 98, wherein the soft-tissue sarcoma is a leiomyosarcoma or a rhabdomyosarcoma.

101. The reaction mixture of claim 98, wherein the neuroendocrine tumor is a gastrointestinal or a bronchopulmonary neuroendocrine tumor.

102. The method of any of claims 97-101, wherein said detection step is performed via immunohistochemistry.

103. The method of any of claims 97-102, wherein said quantification of GCC expression comprises apical GCC expression in said biological sample, cytoplasmic GCC expression in said biological sample, or both.

104. The method of claim 97-103, wherein said quantification step comprises an H-score approach.