US 20090269345A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2009/0269345 A1**

(43) **Pub. Date: Oct. 29, 2009**

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(54) **CLN248 ANTIBODY COMPOSITIONS AND METHODS OF USE**

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(21) Appl. No.: **12/158,729**

(22) PCT Filed: **Dec. 22, 2006**

(86) PCT No.: **PCT/US06/62535**

§ 371 (c)(1),
(2), (4) Date: **Mar. 4, 2009**

Related U.S. Application Data

(60) Provisional application No. 60/753,993, filed on Dec. 23, 2005.

Publication Classification

(51) **Int. Cl.**

A61K 39/395	(2006.01)
C07K 16/00	(2006.01)
C12N 9/96	(2006.01)
C12N 5/16	(2006.01)
C12N 5/02	(2006.01)
A61K 39/00	(2006.01)
G01N 33/53	(2006.01)
G01N 33/566	(2006.01)

(52) **U.S. Cl.** **424/138.1**; 530/387.1; 530/387.3; 530/388.1; 530/387.9; 530/391.1; 530/391.7; 435/188; 435/326; 424/130.1; 435/375; 424/178.1; 435/7.1; 436/501

(57) **ABSTRACT**

Isolated anti-Cln248 antibodies that bind to Cln248 and cells that produce the anti-Cln248 antibodies are provided. Also provided are compositions of an anti-Cln248 antibody and a carrier. In addition, isolated nucleic acids encoding an anti-Cln248 antibody, as well as an expression vector for the isolated nucleic acids are provided. Methods for identifying anti-Cln248 antibodies, methods for producing the anti-Cln248 antibodies, as well as methods for their use in killing a Cln248-expressing cancer cells and alleviating or treating a Cln248-expressing cancer in a mammal are also provided.

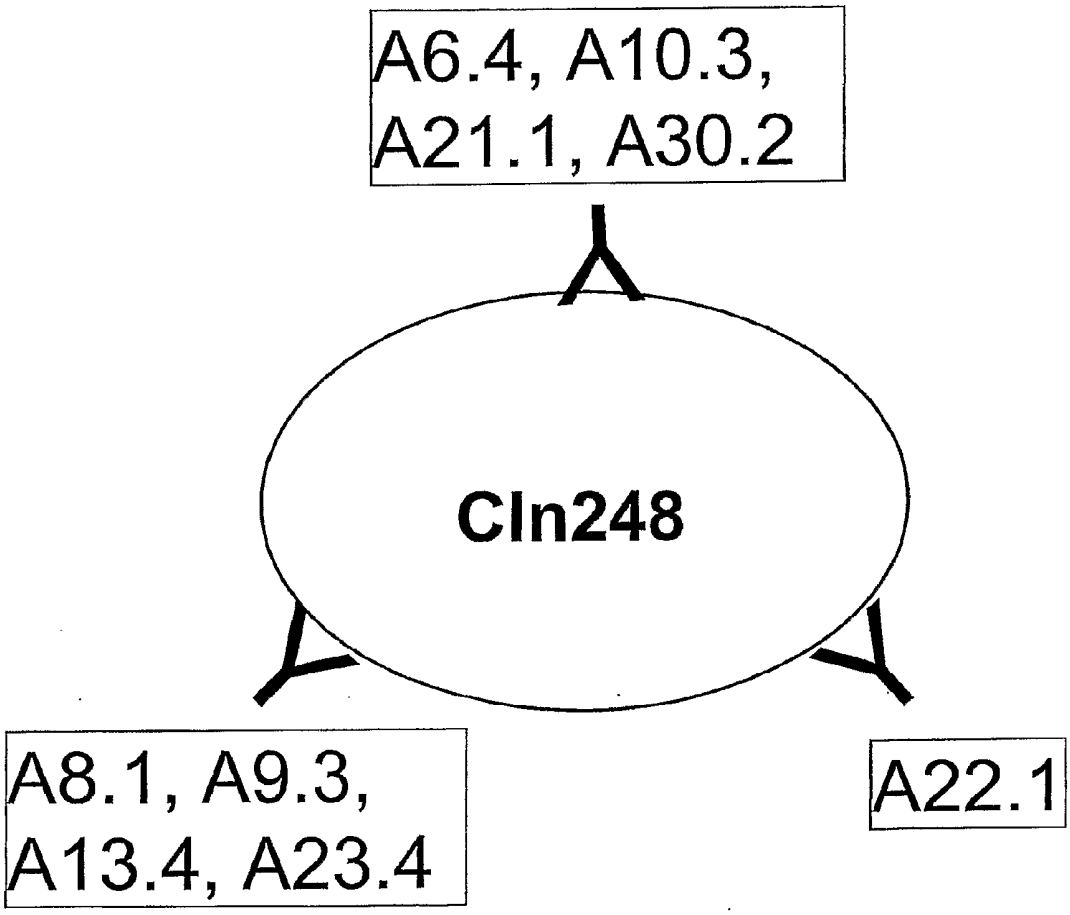

Figure 1

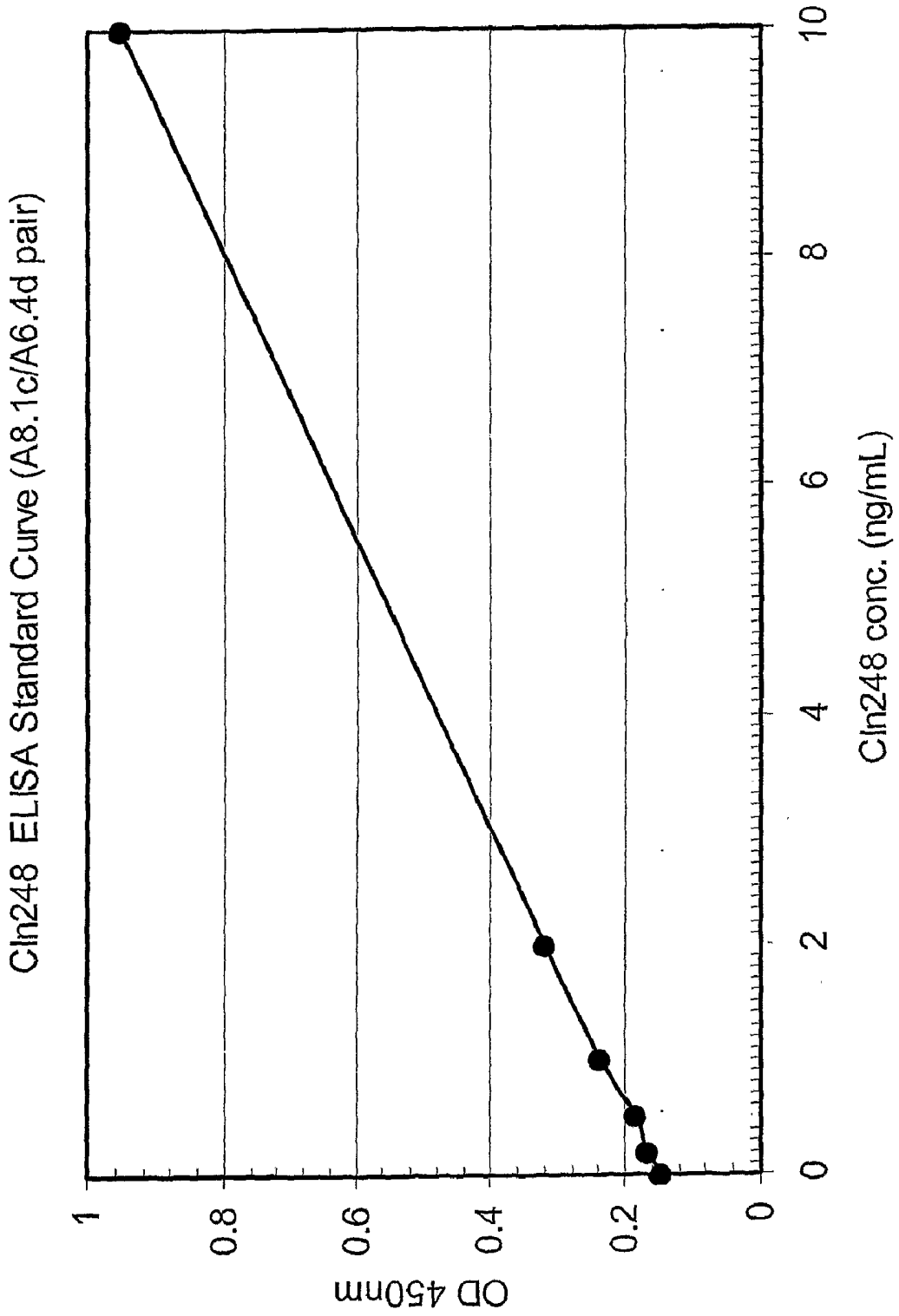
Cln248 ELISA Standard Curve (A8.1c/A6.4d pair)
OD 450nm
1
0.8
0.6
0.4
0.2
0
Cln248 conc. (ng/mL)
0
2
4
6
8
10

Figure 2

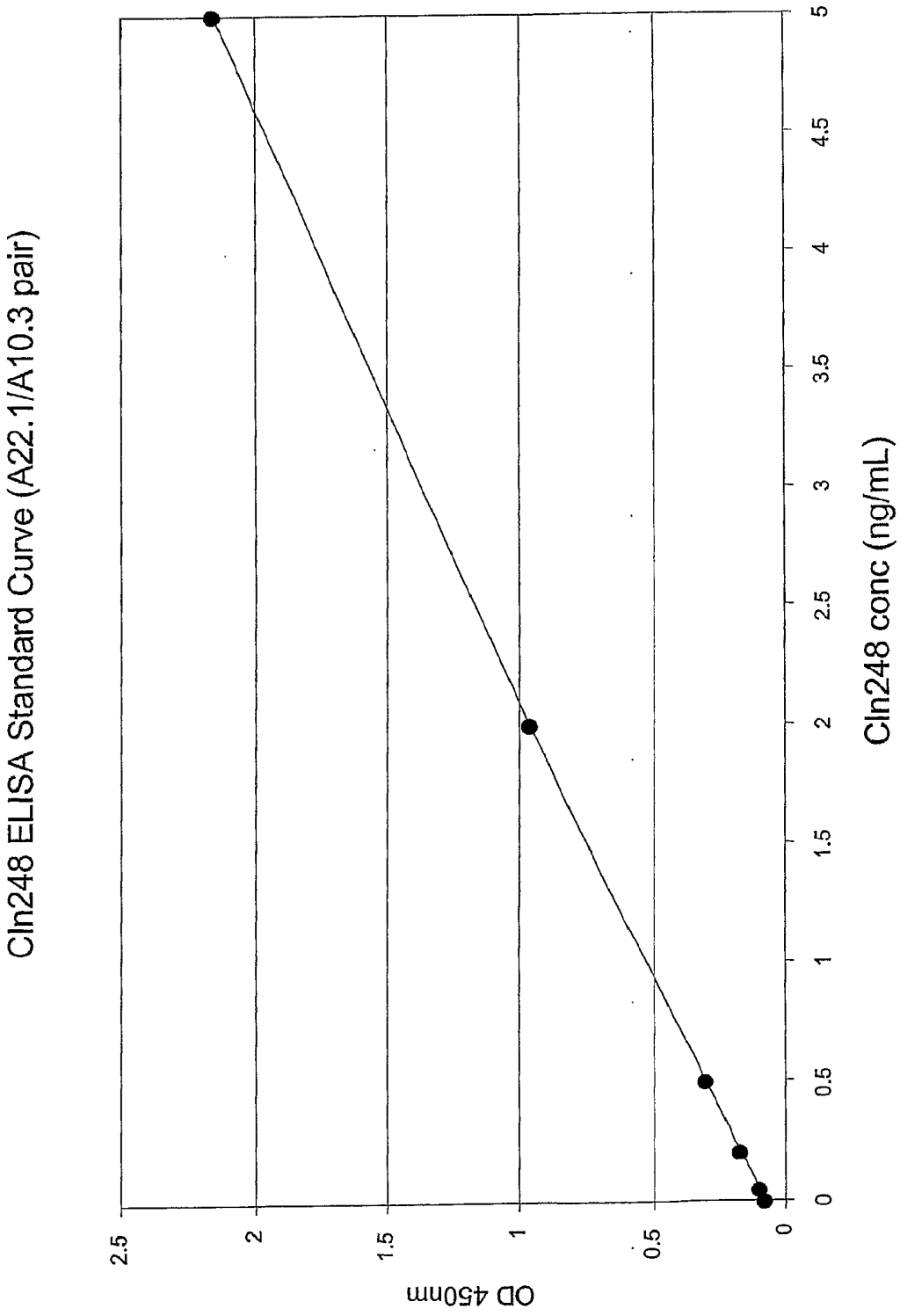
Cln248 ELISA Standard Curve (A22.1/A10.3 pair)
OD 450nm
Cln248 conc (ng/mL)
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

Figure 3

CLN248 ANTIBODY COMPOSITIONS AND METHODS OF USE

[0001] This patent application claims the benefit of priority from U.S. Provisional Application Ser. No. 60/753,993, filed Dec. 23, 2005, the teachings of which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to anti-Cln248 antibody compositions and methods of killing Cln248-expressing colon, ovarian, lung or prostate cancer cells.

BACKGROUND OF THE INVENTION

Colon Cancer

[0003] Colorectal cancer is the second most common cause of cancer death in the United States and the third most prevalent cancer in both men and women. M. L. Davila & A. D. Davila, *Screening for Colon and Rectal Cancer*, in *Colon and Rectal Cancer* 47 (Peter S. Edelstein ed., 2000). Colorectal cancer is categorized as a digestive system cancer by the American Cancer Society (ACS) which also includes cancers of the esophagus, stomach, small intestine, anus, anal canal, anorectum, liver & intrahepatic bile duct, gallbladder & other biliary, pancreas, and other digestive organs. The ACS estimates that there will be about 253,500 new cases of digestive system cancers in 2005 in the United States alone. Digestive system cancers will cause an estimated 136,060 deaths combined in the United States in 2005. Specifically, The ACS estimates that there will be about 104,950 new cases of colon cancer, 40,340 new cases of rectal cancer and 5,420 new cases of small intestine cancer in the 2005 in the United States alone. Colon, rectal and small intestine cancers will cause an estimated 57,360 deaths combined in the United States in 2005. ACS Website: cancer with the extension org of the world wide web. Nearly all cases of colorectal cancer arise from adenomatous polyps, some of which mature into large polyps, undergo abnormal growth and development, and ultimately progress into cancer. Davila at 55-56. This progression would appear to take at least 10 years in most patients, rendering it a readily treatable form of cancer if diagnosed early, when the cancer is localized. Davila at 56; Walter J. Burdette, *Cancer: Etiology, Diagnosis, and Treatment* 125 (1998).

[0004] Although our understanding of the etiology of colon cancer is undergoing continual refinement, extensive research in this area points to a combination of factors, including age, hereditary and nonhereditary conditions, and environmental/dietary factors. Age is a key risk factor in the development of colorectal cancer, Davila at 48, with men and women over 40 years of age become increasingly susceptible to that cancer, Burdette at 126. Incidence rates increase considerably in each subsequent decade of life. Davila at 48. A number of hereditary and nonhereditary conditions have also been linked to a heightened risk of developing colorectal cancer, including familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (Lynch syndrome or HNPCC), a personal and/or family history of colorectal cancer or adenomatous polyps, inflammatory bowel disease, diabetes mellitus, and obesity. Id. at 47; Henry T. Lynch & Jane F. Lynch, *Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndromes)*, in *Colon and Rectal Cancer* 67-68 (Peter S. Edelstein ed., 2000).

[0005] Environmental/dietary factors associated with an increased risk of colorectal cancer include a high fat diet, intake of high dietary red meat, and sedentary lifestyle. Davila at 47; Reddy, B. S., *Prev. Med.* 16(4): 460-7 (1987). Conversely, environmental/dietary factors associated with a reduced risk of colorectal cancer include a diet high in fiber, folic acid, calcium, and hormone-replacement therapy in post-menopausal women. Davila at 50-55. The effect of antioxidants in reducing the risk of colon cancer is unclear. Davila at 53.

[0006] Because colon cancer is highly treatable when detected at an early, localized stage, screening should be a part of routine care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer. One major advantage of colorectal cancer screening over its counterparts in other types of cancer is its ability to not only detect precancerous lesions, but to remove them as well. Davila at 56. The key colorectal cancer screening tests in use today are fecal occult blood test, sigmoidoscopy, colonoscopy, double-contrast barium enema, and the carcinoembryonic antigen (CEA) test. Burdette at 125; Davila at 56.

[0007] The fecal occult blood test (FOBT) screens for colorectal cancer by detecting the amount of blood in the stool, the premise being that neoplastic tissue, particularly malignant tissue, bleeds more than typical mucosa, with the amount of bleeding increasing with polyp size and cancer stage. Davila at 56-57. While effective at detecting early stage tumors, FOBT is unable to detect adenomatous polyps (premalignant lesions), and, depending on the contents of the fecal sample, is subject to rendering false positives. Davila at 56-59. Sigmoidoscopy and colonoscopy, by contrast, allow direct visualization of the bowel, and enable one to detect, biopsy, and remove adenomatous polyps. Davila at 59-60, 61. Despite the advantages of these procedures, there are accompanying downsides: sigmoidoscopy, by definition, is limited to the sigmoid colon and below, colonoscopy is a relatively expensive procedure, and both share the risk of possible bowel perforation and hemorrhaging. Davila at 59-60. Double-contrast barium enema (DCBE) enables detection of lesions better than FOBT, and almost as well a colonoscopy, but it may be limited in evaluating the winding rectosigmoid region. Davila at 60. The CEA blood test, which involves screening the blood for carcinoembryonic antigen, shares the downside of FOBT, in that it is of limited utility in detecting colorectal cancer at an early stage. Burdette at 125.

[0008] Once colon cancer has been diagnosed, treatment decisions are typically made in reference to the stage of cancer progression. A number of techniques are employed to stage the cancer (some of which are also used to screen for colon cancer), including pathologic examination of resected colon, sigmoidoscopy, colonoscopy, and various imaging techniques. *AJCC Cancer Staging Handbook* 84 (Irvin D. Fleming et al. eds., 5th ed. 1998); Montgomery, R. C. and Ridge, J. A., *Semin. Surg. Oncol.* 15(3): 143-150 (1998). Moreover, chest films, liver functionality tests, and liver scans are employed to determine the extent of metastasis. Fleming at 84. While computerized tomography and magnetic resonance imaging are useful in staging colorectal cancer in its later stages, both have unacceptably low staging accuracy for identifying early stages of the disease, due to the difficulty that both methods have in (1) revealing the depth of bowel

wall tumor infiltration and (2) diagnosing malignant adenopathy. Thoeni, R. F., *Radiol. Clin. N. Am.* 35(2): 457-85 (1997). Rather, techniques such as transrectal ultrasound (TRUS) are preferred in this context, although this technique is inaccurate with respect to detecting small lymph nodes that may contain metastases. David Blumberg & Frank G. Opelka, *Neoadjuvant and Adjuvant Therapy for Adenocarcinoin a of the Rectum*, in *Colon and Rectal Cancer* 316 (Peter S. Edelstein ed., 2000).

[0009] Several classification systems have been devised to stage the extent of colorectal cancer, including the Dukes' system and the more detailed International Union against Cancer-American Joint Committee on Cancer TNM staging system, which is considered by many in the field to be a more useful staging system. Burdette at 126-27. The TNM system, which is used for either clinical or pathological staging, is divided into four stages, each of which evaluates the extent of cancer growth with respect to primary tumor (T), regional lymph nodes (N), and distant metastasis (M). Fleming at 84-85. The system focuses on the extent of tumor invasion into the intestinal wall, invasion of adjacent structures, the number of regional lymph nodes that have been affected, and whether distant metastasis has occurred. Fleming at 81.

[0010] Stage 0 is characterized by in situ carcinoma (Tis), in which the cancer cells are located inside the glandular basement membrane (intraepithelial) or lamina propria (intramucosal). In this stage, the cancer has not spread to the regional lymph nodes (N0), and there is no distant metastasis (M0). In stage I, there is still no spread of the cancer to the regional lymph nodes and no distant metastasis, but the tumor has invaded the submucosa (T1) or has progressed further to invade the muscularis propria (T2). Stage II also involves no spread of the cancer to the regional lymph nodes and no distant metastasis, but the tumor has invaded the subserosa, or the nonperitonealized pericolic or perirectal tissues (T3), or has progressed to invade other organs or structures, and/or has perforated the visceral peritoneum (T4). Stage III is characterized by any of the T substages, no distant metastasis, and either metastasis in 1 to 3 regional lymph nodes (N1) or metastasis in four or more regional lymph nodes (N2). Lastly, stage IV involves any of the T or N substages, as well as distant metastasis. Fleming at 84-85; Burdette at 127.

[0011] Currently, pathological staging of colon cancer is preferable over clinical staging as pathological staging provides a more accurate prognosis. Pathological staging typically involves examination of the resected colon section, along with surgical examination of the abdominal cavity. Fleming at 84. Clinical staging would be a preferred method of staging were it at least as accurate as pathological staging, as it does not depend on the invasive procedures of its counterpart.

[0012] Turning to the treatment of colorectal cancer, surgical resection results in a cure for roughly 50% of patients. Irradiation is used both preoperatively and postoperatively in treating colorectal cancer. Chemotherapeutic agents, particularly 5-fluorouracil, are also powerful weapons in treating colorectal cancer. Other agents include irinotecan and floxuridine, cisplatin, levamisole, methotrexate, interferon-α, and leucovorin. Burdette at 125, 132-33. Nonetheless, thirty to forty percent of patients will develop a recurrence of colon cancer following surgical resection, which in many patients is the ultimate cause of death. Wayne De Vos, *Follow-up After Treatment of Colon Cancer, Colon and Rectal Cancer* 225 (Peter S. Edelstein ed., 2000). Accordingly, colon cancer patients must be closely monitored to determine response to therapy and to detect persistent or recurrent disease and metastasis.

[0013] The next few paragraphs describe the some of molecular bases of colon cancer. In the case of FAP, the tumor suppressor gene APC (adenomatous polyposis coli), chromosomally located at 5q21, has been either inactivated or deleted by mutation. Alberts et al., *Molecular Biology of the Cell* 1288 (3d ed. 1994). The APC protein plays a role in a number of functions, including cell adhesion, apoptosis, and repression of the c-myc oncogene. N. R. Hall & R. D. Madoff, *Genetics and the Polyp-Cancer Sequence, Colon and Rectal Cancer* 8 (Peter S. Edelstein, ed., 2000). Of those patients with colorectal cancer who have normal APC genes, over 65% have such mutations in the cancer cells but not in other tissues. Alberts et al., supra at 1288. In the case of HPNCC, patients manifest abnormalities in the tumor suppressor gene HNPCC, but only about 15% of tumors contain the mutated gene. Id. A host of other genes have also been implicated in colorectal cancer, including the K-ras, N-ras, H-ras and c-myc oncogencs, and the tumor suppressor genes DCC (deleted in colon carcinoma) and p^{53}. Hall & Madoff, supra at 8-9; Alberts et al., supra at 1288.

[0014] Abnormalities in Wg/Wnt signal transduction pathway are also associated with the development of colorectal carcinoma. Taipale, J. and Beachy, P. A. *Nature* 411: 349-354 (2001). Wnt1 is a secreted protein gene originally identified within mouse mammary cancers by its insertion into the mouse mammary tumor virus (MMTV) gene. The protein is homologous to the wingless (Wg) gene product of *Drosophila*, in which it functions as an important factor for the determination of dorsal-ventral segmentation and regulates the formation of fly imaginal discs. Wg/Wnt pathway controls cell proliferation, death and differentiation. Taipal (2001). There are at least 13 members in the Wnt family. These proteins have been found expressed mainly in the central nervous system (CNS) of vertebrates as well as other tissues such as mammary and intestine. The Wnt proteins are the ligands for a family of seven transmembrane domain receptors related to the Frizzled gene product in *Drosophila*. Binding Wnt to Frizzled stimulates the activity of the downstream target, Disheveled, which in turn inactivates the glycogen synthetase kinase 3 (GSK3β). Taipal (2001). Usually active GSK3β will form a complex with the adenomatous polyposis coli (APC) protein and phosphorylate another complex member, β-catenin. Once phosphorylated, β-catenin is directed to degradation through the ubiquitin pathway. When GSK3β or APC activity is down regulated, β-catenin is accumulated in the cytoplasm and binds to the T-cell factor or lymphocyte excitation factor (Tcf/Lef) family of transcriptional factors. Binding of β-catenin to Tcf releases the transcriptional repression and induces gene transcription. Among the genes regulated by β-catenin are a transcriptional repressor Engrailed, a transforming growth factor-β (TGF-β) family member Decapentaplegic, and the cytokine Hedgehog in *Drosophila*. β-Catenin also involves in regulating cell adhesion by binding to α-catenin and E-cadherin. On the other hand, binding of β-catenin to these proteins controls the cytoplasmic β-catenin level and its complexing with TCF. Taipal (2001). Growth factor stimulation and activation of c-src or v-src also regulate β-catenin level by phosphorylation of α-catenin and its related protein, $p120^{cas}$. When phosphorylated, these proteins decrease their binding to E-cadherin and β-catenin resulting in the accumulation of cytoplasmic

β-catenin. Reynolds, A. B. et al. *Mol. Cell. Biol.* 14: 8333-8342 (1994). In colon cancer, c-src enzymatic activity has been shown increased to the level of v-src. Alternation of components in the Wg/Wnt pathway promotes colorectal carcinoma development. The best known modifications are to the APC gene. Nicola S et al. *Hum. Mol. Genet.* 10:721-733 (2001). This germline mutation causes the appearance of hundreds to thousands of adenomatous polyps in the large bowel. It is the gene defect that accounts for the autosomally dominantly inherited FAP and related syndromes. The molecular alternations that occur in this pathway largely involve deletions of alleles of tumor-suppressor genes, such as APC, p53 and Deleted in Colorectal Cancer (DCC), combined with mutational activation of proto-oncogenes, especially c-Ki-ras. Aoki, T. et al. *Human Mutat.* 3: 342-346 (1994). All of these lead to genomic instability in colorectal cancers.

[0015] Another source of genomic instability in colorectal cancer is the defect of DNA mismatch repair (MMR) genes. Human homologues of the bacterial mutHLS complex (hMSH2, hMLH1, hPMS1, hPMS2 and hMSH6), which is involved in the DNA mismatch repair in bacteria, have been shown to cause the HNPCC (about 70-90% HNPCC) when mutated. Modrich, P. and Lahue, R. *Ann Rev. Biochem.* 65: 101-133 (1996); and Peltomaki, P. *Hum. Mol. Genet.* 10: 735-740 (2001). The inactivation of these proteins leads to the accumulation of mutations and causes genetic instability that represents errors in the accurate replication of the repetitive mono-, di-, tri- and tetra-nucleotide repcats, which are scattered throughout the genome (microsatellite regions). Jass, J. R. et al. *J. Gastroenterol Hepatol* 17: 17-26 (2002). Like in the classic FAP, mutational activation of c-Ki-ras is also required for the promotion of MSI in the alternative HNPCC. Mutations in other proteins such as the tumor suppressor protein phosphatase PTEN (Zhou, X. P. ct al. *Hum. Mol. Genet.* 11: 445-450 (2002)), BAX (Buttler, L. M. *Aus. N. Z. J. Surg.* 69: 88-94 (1999)), Caspase-5 (Planck, M. *Cancer Genet Cytogenet.* 134: 46-54 (2002)), TGFβ-RII (Fallik, D. et al. *Gastroenterol Clin Biol.* 24: 917-22 (2000)) and IGFII-R (Giovannucci E. *J. Nutr.* 131: 3109S-20S (2001)) have also been found in some colorectal tumors possibly as the cause of MMR defect.

[0016] Some tyrosine kinases have been shown up-regulated in colorectal tumor tissues or cell lines like HT29. Skoudy, A. et al. *Biochem J* 317 (Pt 1): 279-84 (1996). Focal adhesion kinase (FAK) and its up-stream kinase c-src and c-yes in colonic epithelia cells may play an important role in the promotion of colorectal cancers through the extracellular matrix (ECM) and integrin-mediated signaling pathways. Jessup, J. M. et al., *The molecular biology of colorectal carcinoma*, in: *The Molecular Basis of Human Cancer,* 251-268 (Coleman W. B. and Tsongalis G. J. Eds. 2002). The formation of c-src/FAK complexes may coordinately deregulate VEGF expression and apoptosis inhibition. Recent evidences suggest that a specific signal-transduction pathway for cell survival that implicates integrin engagement leads to FAK activation and thus activates PI-3 kinase and akt. In turn, akt phosphorylates BAD and blocks apoptosis in epithelial cells. The activation of c-src in colon cancer may induce VEGF expression through the hypoxia pathway. Other genes that may be implicated in colorectal cancer include Cox enzymes (Ota, S. et al. *Aliment Pharmacol. Ther.* 16 (Suppl 2): 102-106 (2002)), estrogen (al-Azzawi, F. and Wahab, M. *Climacteric* 5: 3-14 (2002)), peroxisome proliferator-activated receptor-γ (PPAR-γ) (Gelman, L. et al. *Cell Mol. Life. Sci.* 55: 932-943 (1999)), IGF-I (Giovannucci (2001)), thymine DNA glycosylase (TDG) (Hardeland, U. et al. *Prog. Nucleic Acid Res. Mol. Biol.* 68: 235-253 (2001)) and EGF (Mendelsohn, J. *Endocrine-Related Cancer* 8: 3-9 (2001)).

[0017] Gene deletion and mutation are not the only causes for development of colorectal cancers. Epigenetic silencing by DNA methylation also accounts for the lost of function of colorectal cancer suppressor genes. A strong association between MSI and CpG island methylation has been well characterized in sporadic colorectal cancers with high MSI but not in those of hereditary origin. In one experiment, DNA methylation of MLH1, CDKN2A, MGMT, TBBS1, RARD, APC, and p14ARF genes has been shown in 80%, 55%, 23%, 23%, 58%, 35%, and 50% of 40 sporadic colorectal cancers with high MSI respectively. Yamamoto, H. et al. *Genes Chromosomes Cancer* 33: 322-325 (2002); and Kim, K. M. et al. *Oncogene.* 12; 21(35): 5441-9 (2002). Carcinogen metabolism enzymes such as GST, NAT, CYP and MTHFR are also associated with an increased or decreased colorectal cancer risk. Pistorius, S. et al. *Kongressbd Dtsch Ges Chir Kongr* 118: 820-824 (2001); and Potter, J. D. *J. Natl. Cancer Inst.* 91: 916-932 (1999).

[0018] From the foregoing, it is clear that procedures used for detecting, diagnosing, monitoring, staging, prognosticating, and preventing the recurrence of colorectal cancer are of critical importance to the outcome of the patient. Moreover, current procedures, while helpful in each of these analyses, are limited by their specificity, sensitivity, invasiveness, and/or their cost. As such, highly specific and sensitive procedures that would operate by way of detecting novel markers in cells, tissues, or bodily fluids, with minimal invasiveness and at a reasonable cost, would be highly desirable.

[0019] Accordingly, there is a great need for more sensitive and accurate methods for predicting whether a person is likely to develop colorectal cancer, for diagnosing colorectal cancer, for monitoring the progression of the disease, for staging the colorectal cancer, for determining whether the colorectal cancer has metastasized, and for imaging the colorectal cancer. Following accurate diagnosis, there is also a need for less invasive and more effective treatment of colorectal cancer.

Ovarian Cancer

[0020] Cancer of the ovaries is the fourth-most common cause of cancer death in women in the United States, with more than 23,000 new cases and roughly 14,000 deaths predicted for the year 2001. Shridhar, V. et al., *Cancer Res.* 61(15): 5895-904 (2001); Memarzadeh, S. & Berek, J. S., *J. Reprod. Med.* 46(7): 621-29 (2001). The American Cancer Society (ACS) estimates that there will be about 25,580 new cases of ovarian cancer in 2004 and ovarian cancer will cause about 16,090 deaths in the United States. ACS Website: cancer with the extension org of the world wide web. More women die annually from ovarian cancer than from all other gynecologic malignancies combined. The incidence of ovarian cancer in the US is estimated to 14.2 pcr 100,000 women per year and 9 women per 100,000 die every year from ovarian cancer. In 2004, approximately 70-75% of new diagnoses will be stage III and IV carcinoma with a predicted 5-year survival of ~15%. Jemal et al., Annual Report to the Nation on the Status of Cancer, 1975-2001, with a Special Feature Regarding Survival. Cancer 2004; 101: 3-27. The incidence of ovarian cancer is of serious concern worldwide, with an estimated 191,000 new cases predicted annually. Run-

nebaum, I. B. & Stickcler, E., *J. Cancer Res. Clin. Oncol.* 127(2): 73-79 (2001). Unfortunately, women with ovarian cancer are typically asymptomatic until the disease has metastasized. Because effective screening for ovarian cancer is not available, roughly 70% of women diagnosed have an advanced stage of the cancer with a five-year survival rate of ~25-30%. Memarzadeh, S. & Berek, J. S., supra; Nunns, D. et al., *Obstet. Gynecol. Surv.* 55(12): 746-51. Conversely, women diagnosed with early stage ovarian cancer enjoy considerably higher survival rates. Werness, 13. A. & Eltabbakh, G. H., *Int'l. J. Gynecol. Pathol* 20(1): 48-63 (2001). Although our understanding of the etiology of ovarian cancer is incomplete, the results of extensive research in this area point to a combination of age, genetics, reproductive, and dietary/environmental factors. Age is a key risk factor in the development of ovarian cancer: while the risk for developing ovarian cancer before the age of 30 is slim, the incidence of ovarian cancer rises linearly between ages 30 to 50, increasing at a slower rate thereafter, with the highest incidence being among septagenarian women. Jeanne M. Schilder et al., *Hereditary Ovarian Cancer: Clinical Syndromes and Management*, in *Ovarian Cancer* 182 (Stephen C. Rubin & Gregory P. Sutton eds., 2d ed. 2001).

[0021] With respect to genetic factors, a family history of ovarian cancer is the most significant risk factor in the development of the disease, with that risk depending on the number of affected family members, the degree of their relationship to the woman, and which particular first degree relatives are affected by the disease. Id. Mutations in several genes have been associated with ovarian cancer, including BRCA1 and BRCA2, both of which play a key role in the development of breast cancer, as well as hMSH2 and hMLH1, both of which are associated with hereditary non-polyposis colon cancer. Katherine Y. Look, *Epidemiology, Etiology, and Screening of Ovarian Cancer*, in *Ovarian Cancer* 169, 171-73 (Stephen C. Rubin & Gregory P. Sutton eds., 2d ed. 2001). BRCA1, located on chromosome 17, and BRCA2, located on chromosome 13, are tumor suppressor genes implicated in DNA repair; mutations in these genes are linked to roughly 10% of ovarian cancers. Id. at 171-72; Schilder et al., supra at 185-86. hMSSH2 and HMLH1 are associated with DNA mismatch repair, and are located on chromosomes 2 and 3, respectively; it has been reported that roughly 3% of hereditary ovarian carcinomas are due to mutations in these genes. Look, supra at 173; Schilder et al., supra at 184, 188-89.

[0022] Reproductive factors have also been associated with an increased or reduced risk of ovarian cancer. Late menopause, nulliparity, and early age at menarche have all been linked with an elevated risk of ovarian cancer. Schilder et al., supra at 182. One theory hypothesizes that these factors increase the number of ovulatory cycles over the course of a woman's life, leading to "incessant ovulation," which is thought to be the primary cause of mutations to the ovarian epithelium. Id.; Laura J. Havrilesky & Andrew Berchuck, *Molecular Alterations in Sporadic Ovarian Cancer*, in *Ovarian Cancer* 25 (Stephen C. Rubin & Gregory P. Sutton eds., 2d ed. 2001). The mutations may be explained by the fact that ovulation results in the destruction and repair of that epithelium, necessitating increased cell division, thereby increasing the possibility that an undetected mutation will occur. Id. Support for this theory may be found in the fact pregnancy, lactation, and the use of oral contraceptives, all of which suppress ovulation, confer a protective effect with respect to developing ovarian cancer. Id.

[0023] Among dietary/environmental factors, there would appear to be an association between high intake of animal fat or red meat and ovarian cancer, while the antioxidant Vitamin A, which prevents free radical formation and also assists in maintaining normal cellular differentiation, may offer a protective effect. Look, supra at 169. Reports have also associated asbestos and hydrous magnesium trisilicate (talc), the latter of which may be present in diaphragms and sanitary napkins. Id. at 169-70.

[0024] Current screening procedures for ovarian cancer, while of some utility, are quite limited in their diagnostic ability, a problem that is particularly acute at early stages of cancer progression when the disease is typically asymptomatic yet is most readily treated. Walter J. Burdette, *Cancer: Etiology, Diagnosis, and Treatment* 166 (1998); Memarzadeh & Berek, supra; Runnebaum & Stickeler, supra; Werness & Eltabbakh, supra. 0Commonly used screening tests include biannual rectovaginal pelvic examination, radioimmunoassay to detect the CA-125 serum tumor marker, and transvaginal ultrasonography. Burdette, supra at 166. Currently, CA-125 is the only clinically approved serum marker for use in ovarian cancer. CA-125 is found elevated in the majority of serous cancers, but is elevated in only half of those women with early stage disease. The major clinical application of CA125 is in monitoring treatment success or detection of recurrence in women undergoing treatment for ovarian cancer. Markinan M. *The Oncologist;* 2: 6-9 (1997). The use of CA125 as a screening marker is limited because it is frequently elevated in women with benign diseases such as endometriosis. Hence, there is a critical need for novel serum markers that are more sensitive and specific for the detection of ovarian cancer when used alone, or in combination with CA125. Bast R C. Et al., *Early Detection of Ovarian Cancer: Promise and Reality* in *Ovarian Cancer. Cancer Research and Treatment* Vol 107 (Stack M S, Fishman, D A, eds., 2001).

[0025] Pelvic examination has failed to yield adequate numbers of early diagnoses, and the other methods are not sufficiently accurate. Id. One study reported that only 15% of patients who suffered from ovarian cancer were diagnosed with the disease at the time of their pelvic examination. Look, supra at 174. Moreover, the CA-125 test is prone to giving false positives in pre-menopausal women and has been reported to be of low predictive value in post-menopausal women. Id. at 174-75. Although transvaginal ultrasonography is now the preferred procedure for screening for ovarian cancer, it is unable to distinguish reliably between benign and malignant tumors, and also cannot locate primary peritoneal malignancies or ovarian cancer if the ovary size is normal. Schilder et al., supra at 194-95. While genetic testing for mutations of the BRCA1, BRCA2, hMSH2, and hMLH1 genes is now available, these tests may be too costly for some patients and may also yield false negative or indeterminate results. Schilder et al., supra at 191-94.

[0026] Additionally, current efforts focus on the identification of panels of biomarkers that can be used in combination. Bast R C Jr., J Clin Oncol 2003; 21: 200-205. Currently, other markers being evaluated as potential ovarian serum markers which may serve as members of a multi-marker panel to improve detection of ovarian cancer are HE4; mesothelin; kallikrein 5, 8, 10 and 11; and prostasin. Urban et al. Ovarian cancer screening Hematol Oncol Clin North Am. 2003 August; 17(4):989-1005; Hellstrom et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma, Cancer Res.

2003 Jul. 1; 63(13):3695-700; Ordonez, Application of mesothelin immunostaining in tumor diagnosis, Am J Surg Pathol. 2003 November; 27(11):1418-28; Diamandis E P et al., Cancer Research 2002; 62: 295-300; Yousef G M et al., Cancer Research 2003; 63: 3958-3965; Kishi T et al., Cancer Research 2003; 63: 2771-2774; Luo L Y et al., Cancer Research 2003; 63: 807-811; Mok S C et al., J Natl Cancer Inst 2001; 93 (19): 1437-1439.

[0027] The staging of ovarian cancer, which is accomplished through surgical exploration, is crucial in determining the course of treatment and management of the disease. *AJCC Cancer Staging Handbook* 187 (Irvin D. Fleming et al. eds., 5th ed. 1998); Burdette, supra at 170; Memarzadeh & Berek, supra; Shridhar et al., supra. Staging is performed by reference to the classification system developed by the International Federation of Gynecology and Obstetrics. David H. Moore, *Primary Surgical Management of Early Epithelial Ovarian Carcinoma*, in *Ovarian Cancer* 203 (Stephen C. Rubin & Gregory P. Sutton eds., 2d ed. 2001); Fleming et al. eds., supra at 188. Stage I ovarian cancer is characterized by tumor growth that is limited to the ovaries and is comprised of three substages. Id. In substage IA, tumor growth is limited to one ovary, there is no tumor on the external surface of the ovary, the ovarian capsule is intact, and no malignant cells are present in ascites or peritoneal washings. Id. Substage IB is identical to A1, except that tumor growth is limited to both ovaries. Id. Substage IC refers to the presence of tumor growth limited to one or both ovaries, and also includes one or more of the following characteristics: capsule rupture, tumor growth on the surface of one or both ovaries, and malignant cells present in ascites or peritoneal washings. Id.

[0028] Stage II ovarian cancer refers to tumor growth involving one or both ovaries, along with pelvic extension. Id. Substage IIA involves extension and/or implants on the uterus and/or fallopian tubes, with no malignant cells in the ascites or peritoneal washings, while substage IIB involves extension into other pelvic organs and tissues, again with no malignant cells in the ascites or peritoneal washings. Id. Substage IIC involves pelvic extension as in IIA or IIB, but with malignant cells in the ascites or peritoneal washings. Id.

[0029] Stage III ovarian cancer involves tumor growth in one or both ovaries, with peritoneal metastasis beyond the pelvis confirmed by microscope and/or metastasis in the regional lymph nodes. Id. Substage IIIA is characterized by microscopic peritoneal metastasis outside the pelvis, with substage IIIB involving macroscopic peritoneal metastasis outside the pelvis 2 cm or less in greatest dimension. Id. Substage IIIC is identical to IIIB, except that the metastasis is greater than 2 cm in greatest dimension and may include regional lymph node metastasis. Id. Lastly, Stage IV refers to the presence distant metastasis, excluding peritoneal metastasis. Id.

[0030] While surgical staging is currently the benchmark for assessing the management and treatment of ovarian cancer, it suffers from considerable drawbacks, including the invasiveness of the procedure, the potential for complications, as well as the potential for inaccuracy. Moore, supra at 206-208, 213. In view of these limitations, attention has turned to developing alternative staging methodologies through understanding differential gene expression in various stages of ovarian cancer and by obtaining various biomarkers to help better assess the progression of the disease. Vartiainen, J. et al., *Int'l J. Cancer,* 95(5): 313-16 (2001); Shridhar et al. supra; Baekelandt, M. et al., *J. Clin. Oncol.* 18(22): 3775-81.

[0031] The treatment of ovarian cancer typically involves a multiprong attack, with surgical intervention serving as the foundation of treatment. Dennis S. Chi & William J. Hoskins, *Primary Surgical Management of Advanced Epithelial Ovarian Cancer*, in *Ovarian Cancer* 241 (Stephen C. Rubin & Gregory P. Sutton eds., 2d ed. 2001). For example, in the case of epithelial ovarian cancer, which accounts for ~90% of cases of ovarian cancer, treatment typically consists of: (1) cytoreductive surgery, including total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and lymphadenectomy, followed by (2) adjuvant chemotherapy with paclitaxel and either cisplatin or carboplatin. Eltabbakh, G. H. & Awtrey, C. S., *Expert Op. Pharmacother.* 2(10): 109-24. Despite a clinical response rate of 80% to the adjuvant therapy, most patients experience tumor recurrence within three years of treatment. Id. Certain patients may undergo a second cytoreductive surgery and/or second-line chemotherapy. Memarzadeh & Berek, supra.

[0032] From the foregoing, it is clear that procedures used for detecting, diagnosing, monitoring, staging, prognosticating, and preventing the recurrence of ovarian cancer are of critical importance to the outcome of the patient. Moreover, current procedures, while helpful in each of these analyses, are limited by their specificity, sensitivity, invasiveness, and/or their cost. As such, highly specific and sensitive procedures that would operate by way of detecting novel markers in cells, tissues, or bodily fluids, with minimal invasiveness and at a reasonable cost, would be highly desirable.

[0033] Accordingly, there is a great need for more sensitive and accurate methods for predicting whether a person is likely to develop ovarian cancer, for diagnosing ovarian cancer, for monitoring the progression of the disease, for staging the ovarian cancer, for determining whether the ovarian cancer has metastasized, and for imaging the ovarian cancer. There is also a need for better treatment of ovarian cancer.

Prostate Cancer

[0034] Prostate cancer is the most prevalent cancer in men and is the second leading cause of death from cancer among males in the United States. *AJCC Cancer Staging Handbook* 203 (Irvin D. Fleming et al. eds., 5th ed. 1998); Walter J. Burdette, *Cancer: Etiology Diagnosis, and Treatment* 147 (1998). In 1999, it was estimated that 37,000 men in the United States would die as result of prostate cancer. Elizabeth A. Platz et al., & Edward Giovannucci, *Epidemiology of and Risk Factors for Prostate Cancer*, in *Management of Prostate Cancer* 21 (Eric A Klein, ed. 2000). More recently, the American Cancer Society estimated there will be 232,090 new cases of prostate cancer and 30,350 deaths in 2005. Additionally, the rate of prostate cancer deaths in the United States for 1997-2001 was 31.5 per 100,000 men, second only to lung and bronchus cancer. American Cancer Society website: cancer with the extension.org of the world wide web. Cancer of the prostate typically occurs in older males, with a median age of 74 years for clinical diagnosis. Burdette, supra at 147. A man's risk of being diagnosed with invasive prostate cancer in his lifetime is one in six. Platz et al., supra at 21.

[0035] Although our understanding of the etiology of prostate cancer is incomplete, the results of extensive research in this area point to a combination of age, genetic and environmental/dietary factors. Platz et al., supra at 19; Burdette, supra at 147; Steven K. Clinton, *Diet and Nutrition in Prostate Cancer Prevention and Therapy*, in *Prostate Cancer: a Multidisciplinary Guide* 246-269 (Philip W. Kantoff et al.

eds. 1997). Broadly speaking, genetic risk factors predisposing one to prostate cancer include race and a family history of the disease. Platz et al., supra at 19, 28-29, 32-34. Aside from these generalities, a deeper understanding of the genetic basis of prostate cancer has remained elusive. Considerable research has been directed to studying the link between prostate cancer, androgens, and androgen regulation, as androgens play a crucial role in prostate growth and differentiation. Meena Augustus et al., *Molecular Genetics and Markers of Progression*, in *Management of Prostate Cancer* 59 (Eric A Klein ed. 2000). While a number of studies have concluded that prostate tumor development is linked to elevated levels of circulating androgen (e.g., testosterone and dihydrotestosterone), the genetic determinants of these levels remain unknown. Platz et al., supra at 29-30.

[0036] Several studies have explored a possible link between prostate cancer and the androgen receptor (AR) gene, the gene product of which mediates the molecular and cellular effects of testosterone and dihydrotestosterone in tissues responsive to androgens. Id. at 30. Differences in the number of certain trinucleotide repeats in exon 1, the region involved in transactivational control, have been of particular interest. Augustus et al., supra at 60. For example, these studies have revealed that as the number of CAG repeats decreases the transactivation ability of the gene product increases, as does the risk of prostate cancer. Platz et al., supra at 30-31. Other research has focused on the α-reductase Type 2 gene, the gene which codes for the enzyme that converts testosterone into dihydrotestosterone. Id. at 30. Dihydrotestosterone has greater affinity for the AR than testosterone, resulting in increased transactivation of genes responsive to androgens. Id. While studies have reported differences among the races in the length of a TA dinucleotide repeat in the 3' untranslated region, no link has been established between the length of that repeat and prostate cancer. Id. Interestingly, while ras gene mutations are implicated in numerous other cancers, such mutations appear not to play a significant role in prostate cancer, at least among Caucasian males. Augustus, supra at 52.

[0037] Environmental/dietary risk factors which may increase the risk of prostate cancer include intake of saturated fat and calcium. Platz et al., supra at 19, 25-26. Conversely, intake of selenium, vitamin E and tomato products (which contain the carotenoid lycopene) apparently decrease that risk. Id. at 19, 26-28 The impact of physical activity, cigarette smoking, and alcohol consumption on prostate cancer is unclear. Platz et al., supra at 23-25.

[0038] Periodic screening for prostate cancer is most effectively performed by digital rectal examination (DRE) of the prostate, in conjunction with determination of the serum level of prostate-specific antigen (PSA). Burdette, supra at 148. While the merits of such screening are the subject of considerable debate, Jerome P. Richie & Irving D. Kaplan, *Screening for Prostate Cancer: The Horns of a Dilemma*, in *Prostate Cancer: A Multidisciplinary Guide* 1-10 (Philip W. Kantoff et al. eds. 1997), the American Cancer Society and American Urological Association recommend that both of these tests be performed annually on men 50 years or older with a life expectancy of at least 10 years, and younger men at high risk for prostate cancer. Tan M. Thompson & John Foley, *Screening for Prostate Cancer*, in *Management of Prostate Cancer* 71 (Eric A Klein ed. 2000). If necessary, these screening methods may be followed by additional tests, including biopsy, ultrasonic imaging, computerized tomography, and magnetic resonance imaging. Christopher A. Haas & Martin I. Resnick, *Trends in Diagnosis, Biopsy, and Imaging*, in *Management of Prostate Cancer* 89-98 (Eric A Klein ed. 2000); Burdette, supra at 148.

[0039] Once the diagnosis of prostate cancer has been made, treatment decisions for the individual are typically linked to the stage of prostate cancer present in that individual, as well as his age and overall health. Burdette, supra at 151. One preferred classification system for staging prostate cancer was developed by the American Urological Association (AUA). Id. at 148. The AUA classification system divides prostate tumors into four broad stages, A to D, which are in turn accompanied by a number of smaller substages. Burdette, supra at 152-153; Anthony V. D'Amico et al., *The Staging of Prostate Cancer*, in *Prostate Cancer: A Multidisciplinary Guide* 41 (Philip W. Kantoff et al. eds. 1997).

[0040] Stage A prostate cancer refers to the presence of microscopic cancer within the prostate gland. D'Amico, supra at 41. This stage is comprised of two substages: A1, which involves less than four well-differentiated cancer foci within the prostate, and A2, which involves greater than three well-differentiated cancer foci or alternatively, moderately to poorly differentiated foci within the prostate. Burdette, supra at 152; D'Amico, supra at 41. Treatment for stage A1 preferentially involves following PSA levels and periodic DRE. Burdette, supra at 151. Should PSA levels rise, preferred treatments include radical prostatectomy in patients 70 years of age and younger, external beam radiotherapy for patients between 70 and 80 years of age, and hormone therapy for those over 80 years of age. Id.

[0041] Stage B prostate cancer is characterized by the presence of a palpable lump within the prostate. Burdette, supra at 152-53; D'Amico, supra at 41. This stage is comprised of three substages: B1, in which the lump is less than 2 cm and is contained in one lobe of the prostate; B2, in which the lump is greater than 2 cm yet is still contained within one lobe; and B3, in which the lump has spread to both lobes. Burdette, supra, at 152-53. For stages B1 and B2, the treatment again involves radical prostatectomy in patients 70 years of age and younger, external beam radiotherapy for patients between 70 and 80 years of age, and hormone therapy for those over 80 years of age. Id. at 151. In stage B3, radical prostatectomy is employed if the cancer is well-differentiated and PSA levels are below 15 ng/mL; otherwise, external beam radiation is the chosen treatment option. Id.

[0042] Stage C prostate cancer involves a substantial cancer mass accompanied by extraprostatic extension. Burdette, supra at 153; D'Amico, supra at 41. Like stage A prostate cancer, Stage C is comprised of two substages: substage C1, in which the tumor is relatively minimal, with minor pro static extension, and substage C2, in which the tumor is large and bulky, with major prostatic extension. Id. The treatment of choice for both substages is external beam radiation. Burdette, supra at 151.

[0043] The fourth and final stage of prostate cancer, Stage D, describes the extent to which the cancer has metastasized. Burdette, supra at 153; D'Amico, supra at 41. This stage is comprised of four substages: (1) D0, in which acid phosphatase levels are persistently high, (2) D1, in which only the pelvic lymph nodes have been invaded, (3) D2, in which the lymph nodes above the aortic bifurcation have been invaded, with or without distant metastasis, and (4) D3, in which the metastasis progresses despite intense hormonal therapy. Id.

Treatment at this stage may involve hormonal therapy, chemotherapy, and removal of one or both testes. Burdette, supra at 151.

[0044] Despite the need for accurate staging of prostate cancer, current staging methodology is limited. The wide variety of biological behavior displayed by neoplasms of the prostate has resulted in considerable difficulty in predicting and assessing the course of prostate cancer. Augustus et al., supra at 47. Indeed, despite the fact that most prostate cancer patients have carcinomas that are of intermediate grade and stage, prognosis for these types of carcinomas is highly variable. Andrew A Renshaw & Christopher L. Corless, *Prognostic Features in the Pathology of Prostate Cancer*, in *Prostate Cancer: A Multidisciplinary Guide* 26 (Philip W. Kantoff et al. eds. 1997). Techniques such as transrectal ultrasound, abdominal and pelvic computerized tomography, and MR1 have not been particularly useful in predicting local tumor extension. D'Amico, supra at 53 (editors' comment). While the use of serum PSA in combination with the Gleason score is currently the most effective method of staging prostate cancer, id., PSA is of limited predictive value, Augustus et al., supra at 47; Renshaw et al., supra at 26, and the Gleason score is prone to variability and error, King, C. R. & Long, J. P., *Int'l. J. Cancer* 90(6): 326-30 (2000). As such, the current focus of prostate cancer research has been to obtain biomarkers to help better assess the progression of the disease. Augustus et al., supra at 47; Renshaw et al., supra at 26; Pettaway, C. A., *Tech. Urol.* 4(1): 35-42 (1998).

[0045] Accordingly, there is a great need for more sensitive and accurate methods for predicting whether a person is likely to develop prostate cancer, for diagnosing prostate cancer, for monitoring the progression of the disease, for staging the prostate cancer, for determining whether the prostate cancer has metastasized and for imaging the prostate cancer. There is also a need for better treatment of prostate cancer.

Angiogenesis in Cancer

[0046] Growth and metastasis of solid tumors are also dependent on angiogenesis. Folkman, J., 1986, *Cancer Research,* 46, 467-473; Folkman, J., 1989, *Journal of the National Cancer Institute,* 82, 4-6. It has been shown, for example, that tumors which enlarge to greater than 2 mm must obtain their own blood supply and do so by inducing the growth of new capillary blood vessels. Once these new blood vessels become embedded in the tumor, they provide a means for tumor cells to enter the circulation and metastasize to distant sites such as liver, lung or bone. Weidner, N., et al., 1991, *The New England Journal of Medicine,* 324(1), 1-8.

[0047] Angiogenesis, defined as the growth or sprouting of new blood vessels from existing vessels, is a complex process that primarily occurs during embryonic development. The process is distinct from vasculogenesis, in that the new endothelial cells lining the vessel arise from proliferation of existing cells, rather than differentiating from stem cells. The process is invasive and dependent upon proteolysis of the extracellular matrix (ECM), migration of new endothelial cells, and synthesis of new matrix components. Angiogenesis occurs during embryogenic development of the circulatory system; however, in adult humans, angiogenesis only occurs as a response to a pathological condition (except during the reproductive cycle in women).

[0048] Under normal physiological conditions in adults, angiogenesis takes place only in very restricted situations such as hair growth and wounding healing. Auerbach, W. and Auerbach, R., 1994, *Pharmacol Ther.* 63(3):265-3 11; Ribatti et al., 1991, *Haematologica* 76(4):3 11-20; Risau, 1997, *Nature* 386(6626):67 1-4. Angiogenesis progresses by a stimulus which results in the formation of a migrating column of endothelial cells. Proteolytic activity is focused at the advancing tip of this "vascular sprout", which breaks down the ECM sufficiently to permit the column of cells to infiltrate and migrate. Behind the advancing front, the endothelial cells differentiate and begin to adhere to each other, thus forming a new basement membrane. The cells then cease proliferation and finally define a lumen for the new arteriole or capillary.

[0049] Unregulated angiogenesis has gradually been recognized to be responsible for a wide range of disorders, including, but not limited to, cancer, cardiovascular disease, rheumatoid arthritis, psoriasis and diabetic retinopathy. Folklman, 1995, *Nat Med* 1(1):27-31; Isner, 1999, Circulation 99(13): 1653-5; Koch, 1998, *Arthritis Rheum* 41(6):951-62; Walsh, 1999, *Rheumatology* (Oxford) 38(2):103-12; Ware and Simons, 1997, *Nat Med* 3(2): 158-64.

[0050] Of particular interest is the observation that angiogenesis is required by solid tumors for their growth and metastases. Folkman, 1986 supra; Folkman 1990, *J Natl. Cancer Inst.,* 82(1) 4-6; Folkman, 1992, *Semin Cancer Biol* 3(2):65-71; Zetter, 1998, *Annu Rev Med* 49:407-24. A tumor usually begins as a single aberrant cell which can proliferate only to a size of a few cubic millimeters due to the distance from available capillary beds, and it can stay 'dormant' without further growth and dissemination for a long period of time. Some tumor cells then switch to the angiogenic phenotype to activate endothelial cells, which proliferate and mature into new capillary blood vessels. These newly formed blood vessels not only allow for continued growth of the primary tumor, but also for the dissemination and recolonization of metastatic tumor cells. The precise mechanisms that control the angiogenic switch is not well understood, but it is believed that neovascularization of tumor mass results from the net balance of a multitude of angiogenesis stimulators and inhibitors Folkman, 1995, supra.

[0051] One of the most potent angiogenesis inhibitors is endostatin identified by O'Reilly and Folkman. O'Reilly et al., 1997, *Cell* 88(2):277-85; O'Reilly et al., 1994, *Cell* 79(2):3 15-28. Its discovery was based on the phenomenon that certain primary tumors can inhibit the growth of distant metastases. O'Reilly and Folkman hypothesized that a primary tumor initiates angiogenesis by generating angiogenic stimulators in excess of inhibitors. However, angiogenic inhibitors, by virtue of their longer half life in the circulation, reach the site of a secondary tumor in excess of the stimulators. The net result is the growth of primary tumor and inhibition of secondary tumor. Endostatin is one of a growing list of such angiogenesis inhibitors produced by primary tumors. It is a proteolytic fragment of a larger protein: endostatin is a 20 kDa fragment of collagen XVIII (amino acid H1132-K1315 in murine collagen XVIII). Endostatin has been shown to specifically inhibit endothelial cell proliferation in vitro and block angiogenesis in vivo. More importantly, administration of endostatin to tumor-bearing mice leads to significant tumor regression, and no toxicity or drug resistance has been observed even after multiple treatment cycles. Boehm et al., 1997, Nature 390(6658):404-407. The fact that endostatin targets genetically stable endothelial cells and inhibits a variety of solid tumors makes it a very attractive candidate for anticancer therapy. Fidler and Ellis, 1994, Cell 79(2):185-8; Gastl et al., 1997, Oncology 54(3):177-84;

Hinsbergh et al., 1999, Ann Oncol 10 Suppl 4:60-3. In addition, angiogenesis inhibitors have been shown to be more effective when combined with radiation and chemotherapeutic agents. Klement, 2000, J. Clin Invest, 105(8) R15-24. Browder, 2000, Cancer Res. 6-(7) 1878-86, Arap et al., 1998, Science 279(5349):377-80; Mauceri et al., 1998, Nature 394 (6690):287-91.

[0052] As discussed above, each of the methods for diagnosing and staging colon, ovarian, lung or prostate cancer is limited by the technology employed. Accordingly, there is need for sensitive molecular and cellular markers for the detection of colon, ovarian, lung or prostate cancer. There is a need for molecular markers for the accurate staging, including clinical and pathological staging, of colon, ovarian, lung or prostate cancers to optimize treatment methods. In addition, there is a need for sensitive molecular and cellular markers to monitor the progress of cancer treatments, including markers that can detect recurrence of colon, ovarian, lung or prostate cancers following remission.

[0053] The present invention provides alternative methods of treating colon, ovarian, lung or prostate cancer that overcome the limitations of conventional therapeutic methods as well as offer additional advantages that will be apparent from the detailed description below.

SUMMARY OF THE INVENTION

[0054] This invention is directed to an isolated Cln248 antibody that binds to Cln248 on a mammalian cell. The invention is further directed to an isolated Cln248 antibody that internalizes upon binding to Cln248 on a mammalian cell. The antibody may be a monoclonal antibody. Alternatively, the antibody is an antibody fragment or a chimeric or a humanized antibody. The monoclonal antibody may be produced by a hybridoma selected from the group of hybridomas deposited under American Type Culture Collection selected from the group comprising PTA-7172 and PTA-7175.

[0055] The antibody may compete for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma selected from the group of hybridomas deposited under the American Type Culture Collection comprising PTA-7172 and PTA-7175.

[0056] The invention is also directed to conjugated antibodies. They may be conjugated to a growth inhibitory agent or a cytotoxic agent. The cytotoxic agent may be selected from the group consisting of toxins, antibiotics, radioactive isotopes and nucleolytic enzymes and toxins. Examples of toxins include, but are not limited to, maytansin, maytansinoids, saporin, gelonin, ricin or calicheamicin.

[0057] The mammalian cell may be a cancer coll. Preferably, the anti-Cln248 monoclonal antibody that inhibits the growth of Cln248-expressing cancer cells.

[0058] The antibody may be produced in bacteria. Alternatively, the antibody may be a humanized form of an anti-C n248 antibody produced by a hybridoma selected from the group of hybridomas deposited with the ATCC comprising PTA-7172 and PTA-7175.

[0059] Preferably, the cancer is selected from the group consisting of ovarian, colon, prostate, and lung cancer. The invention is also directed to a method of producing the antibodies comprising culturing an appropriate cell and recovering the antibody from the cell culture.

[0060] The invention is also directed to compositions comprising the antibodies and a carrier. The antibody may be conjugated to a cytotoxic agent. The cytotoxic agent may be a radioactive isotope or other chemotherapeutic agent.

[0061] The invention is also directed to a method of killing an Cln248-expressing cancer cell, comprising contacting the cancer cell with the antibodies of this invention, thereby killing the cancer cell. The cancer cell may be selected from the group consisting of ovarian, colon, prostate, and lung cancer cell.

[0062] The ovarian, colon, prostate or lung may be metastatic cancer. The breast cancer may be HER-2 negative breast cancer. The invention is also directed to a method of alleviating an Cln248-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the antibodies to the mammal.

[0063] In addition, the invention is directed to an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody as described herein. The article of manufacture may also comprise an additional component, e.g., a package insert indicating that the composition can be used to treat colon, ovarian, lung or prostate cancer.

BRIEF DESCRIPTION OF THE FIGURES

[0064] FIG. **1** shows the Cln248 epitope map for anti-Cln248 antibodies.

[0065] FIG. **2** shows the Cln248 A8.1/A6.4 ELISA Standard Curve.

[0066] FIG. **3** shows the Cln248 A22.1/A10.3 ELISA Standard Curve.

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

[0067] Human "Cln248" as used herein, refers to a protein of 300 amino acids that is secreted by cells, whose nucleotide and amino acid sequence sequences are as disclosed in e.g., WO 98/30694-A2, WO 98/30694-A2 and US 2003-0198640-A1 as Tumor necrosis factor Receptor 6 (TR6); EP861850-A1 as human tumor necrosis related receptor (TR4); WO 99/04001-A1 as Human tumor necrosis factor receptor ZTNFR-5; WO 99/07738-A2 as Orphan receptor (HUMAN NTR-1); WO 99/14330-A1 as Decoy Receptor 3 (DcR3); and WO 99/26977-A1 as Mammalian tumor necrosis factor receptor OPG-2; WO 2000/46247-A1 as M68 TNF receptor related protein; the disclosures of which are hereby expressly incorporated by reference. Amino acids 1-300, or 30-300 (without the secretory signal peptide at amino acids 1-29) of Cln248 are secreted from cells. Cln248 as used herein includes full length protein (amino acids 1-300), mature protein (amino acids 30-300), functional metabolic degradation fragments (amino acids 1-218), allelic variants and conservative substitution mutants of the protein which have Cln248 biological activity.

[0068] Cln248 is related to tumor necrosis factor (TNF) family and is identified in the RefSeq database as accessions NM_003823 and NP_003814 (accessible at ncbi with the extension.nln.nih.gov of the world wide web) and titled "*Homo sapiens* tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), transcript valiant M68E, mRNA". Other synonyms for Cln248 include: M68, TR6, DcR3, and DJ583P15.1.1. The refseq database includes the following summary of Cln248:

[0069] This gene belongs to the tumor necrosis factor receptor superfamily. The encoded protein is postulated

to play a regulatory role in suppressing FasL- and LIGHT-mediated cell death. It acts as a decoy receptor that competes with death receptors for ligand binding. Overexpression of this gene has been noted in gastrointestinal tract tumors, and it is located in a gene-rich cluster on chromosome 20, with other potentially tumor-related genes. Two transcript variants encoding the same isoform, but differing in the 5' UTR, have been observed for this gene.

[0070] Transcript Variant: This variant (M68E) lacks the 5' noncoding exons present in variant M68C, hence contains a shorter 5' UTR. Both variants encode the same isoform.

Many publications have described the identification, characterization, association with carcinomas, and clinical development of Cln248 as a molecular target for cancer therapy and cancer vaccination including the following which are hereby incorporated by reference in their entirety.

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J Immunol. 2005 Oct 15; 175(8): 5135-45.
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Teaching resource. Beta-catenin signaling and axis specification.
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[0071] As described in the publications above, Cln248 is secreted protein with an approximate relative molecular mass of 35,000, is differentially expressed cancer versus normal tissues and plays a role in the regulation of a number of processes including apoptosis, tumorigenesis, chemotaxis, cell differentiation, immune cell interactions, growth inhibition signaling, macrophage differentiation, monocyte adhesion, angiogenesis, osteoclast formation and inflammation. Cln248 binds FasL, LIGHT and TL1A and acts as a decoy receptor to regulate FasL-, LIGHT- and TL1A-mediated apoptosis. It has also been disclosed that DcR3 endows tumor cells with survival advantages by blocking Fas-mediated apoptosis and inhibits T cell activation by interfering with two-way T cell costimulation between LIGHT and HveA.

[0072] Taken together, the differential expression in cancer, demonstrated receptor-ligand interactions and role in regulation of cellular processes, make Cln248 a promising target for diagnosis and immunotherapy of various tumor types. Anti-Cln248 antibodies are useful in diagnostic or therapeutic applications alone or in combination with antibodies against other TNF family members.

[0073] Antibodies of the instant invention, described herein, specifically bind Cln248 and have demonstrated characteristics which make them ideal therapeutic candidates for modulating DcR3 functions including T cell (and other immune cell) regulation such as activation, proliferation and tumor infiltration, FasL-, LIGHT- and TL1A-mediated apoptosis, chemotaxis. Anti-Cln248 antibodies Furthermore, the antibodies of the instant invention are useful as therapeutic agents for those suffering from colon, ovarian, lung, and prostate cancers. The antibodies have therapeutic effect by killing Cln248 expressing cancer cells, inhibiting growth of Cln248 expressing tumors, shrinking Cln248 expressing tumors, extending survival time of individuals with Cln248 expressing tumors, reducing metastases of Cln248 expressing tumors, inducing immune response against Cln248 expressing tumors, reducing inhibition of immune response against Cln248 expressing tumors or reducing angiogenesis or vascularization of Cln248 expressing tumors. Anti-Cln248 antibodies bind DcR3 and block binding of DcR3 to FasL, LIGHT or TL1A thereby reducing DcR3 inhibition of FasL-, LIGHT- and TL1A-mediated apoptosis. In general Anti-Cln248 antibodies bind DcR3 reducing DcR3 regulation of DcR3 functions described above. The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

[0074] An "isolated antibody" is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. Preferably, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0075] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody

consists of 5 of the basic heterotetramer unit along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain). In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the α and γ chains and four CH domains for [L and F isotypes. Each 6 L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end.

[0076] The VL is aligned with the VH and the CL is aligned with the first constant domain of the heavy chain (CHI).

[0077] Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Teff and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6.

[0078] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated α, δ, ϵ, γ, and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1; and IgA2.

[0079] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and define specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 1-10-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a P-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the P-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0080] The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. around about residues 24-34 (LI), 5056 (L2) and 89-97 (L3) in the VL, and around about 1-35 (H1), 50-65 (H2) and 95-102 (113) in the VH; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (LI), 50-52 (L2) and 91-96 (U) in the VL, and 26-32 (HI), 53-55 (1-12) and 96-101 (H3) in the VH; Chothia and Lesk J. Mol. Biol. 196: 901-917 (1987)).

[0081] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler et al., Nature, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Pat. No. 4,816, 567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

[0082] The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey, Ape etc), and human constant region sequences.

[0083] An "intact" antibody is one which comprises an antigen-binding site as well as a CL and at least heavy chain constant domains, CHI, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

[0084] An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments

include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CHI). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')2 fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the CHI domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of 8 Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0085] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

[0086] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0087] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, infra.

[0088] The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, i.e., fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "cross-over" sFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404, 097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993). Furthermore, effects of linker sequence alterations in engineering bispecific tandem diabodies are described in Le Gall et al., Protein Eng Des Sel. 17(4):357-66 (2004).

[0089] A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (e.g., antibody) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of a naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

[0090] The term "amino acid sequence variant" refers to a polypeptide that has amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants of Cln248 will possess at least about 70% homology with the native sequence Cln248, preferably, at least about 80%, more preferably at least about 85%, even more preferably at least about 90% homology, and most preferably at least 95%. The amino acid sequence variants can possess substitutions, deletions, insertions and/or alterations due to allelic variation or Single Nucleotide Polymorphisms (SNPs) within the native nucleic acid sequence encoding the amino acid sequence.

[0091] Several definitions of SNPs exist. See, e.g., Brooks, 235 *Gene* 177-86 (1999). As used herein, the term "single nucleotide polymorphism" or "SNP" includes all single base variants, thus including nucleotide insertions and deletions in addition to single nucleotide substitutions and any resulting amino acid variants due to codon alteration. There are two types of nucleotide substitutions. A transition is the replacement of one purine by another purine or one pyrimidine by another pyrimidine. A transversion is the replacement of a purine for a pyrimidine, or vice versa.

[0092] Numerous methods exist for detecting SNPs within a nucleotide sequence. A review of many of these methods can be found in Landegren et al., 8 *Genome Res.* 769-76 (1998). For example, a SNP in a genomic sample can be detected by preparing a Reduced Complexity Genome (RCG) from the genomic sample, then analyzing the RCG for the presence or absence of a SNP. See, e.g., WO 00/18960. Multiple SNPs in a population of target polynucleotides in parallel can be detected using, for example, the methods of WO 00/50869. Other SNP detection methods include the methods of U.S. Pat. Nos. 6,297,018 and 6,322,980. Furthermore, SNPs can be detected by restriction fragment length polymorphism (RFLP) analysis. See, e.g., U.S. Pat. Nos. 5,324,631; 5,645,995. RFLP analysis of SNPs, however, is limited to cases where the SNP either creates or destroys a restriction enzyme cleavage site. SNPs can also be detected by direct sequencing of the nucleotide sequence of interest. In addition, numerous assays based on hybridization have also been developed to detect SNPs and mismatch distinction by polymerases and ligases. Several web sites provide information about SNPs including Ensembl (ensembl with the extension org of the world wide web), Sanger Institute (sanger with the extension .ac. uk/genetics/exon/ of the world wide web), National Center for Biotechnology Information (NCBI) (ncbi with the extension nln.nih. gov/SNP/ of the world wide web), The SNP Consortium Ltd. (snp with the extension .cshl.org of the world wide web). The chromosomal locations for the compositions disclosed herein are provided below. In addition, one of ordinary skill in the art could perform a search against the genome or any of the databases cited above using BLAST to find the chromosomal location or locations of

SNPs. Another a preferred method to find the genomic coordinates and associated SNPs would be to use the BLAT tool (genome.ucsc.edu, Kent et al. 2001, The Human Genome Browser at UCSC, Genome Research 996-1006 or Kent 2002 BLAT, The BLAST-Like Alignment Tool Genome Research, 1-9). All web sites above were accessed Dec. 3, 2003.

[0093] Preferred amino acid sequence variants of Cln248 are described in the table below. The nucleic acid and amino acid sequences of Cln248 are disclosed in WO 98/30694-A2 which is incorporated by reference in its entirety. The polynucleotides encoding the amino acids of the present invention were analyzed and single nucleotide polymorphism (SNP) attributes were identified. Specifically identified were SNPs occurring the coding region of the nucleotide, the Alleles of the SNP, the nucleotide ambiguity code for the SNP, the position in the codon of the SNP if within the Open Reading Frame (1, 2, 3 or UTR for untranslated regions), and the SNP type (synonymous or non-synonymous to the protein translation). In addition to the attributes above, the SNP rs# ID for the NCBI SNP database (dbSNP) which is accessible at ncbi with the extension .nlm.nih.gov/SNP/ of the world wide web is referenced for each SNP. Additional single nucleotide polymorphism (SNP) information can be accessed at the databases listed above.

[0094] The table below includes the polynucleotide target, dbSNP rs# ID, Nucleic acid residue affected by the SNP (Polynucleotide) in NM_003823, SNP alleles, Nucleotide ambiguity code, Condon Position of the SNP if within the ORF (1, 2, 3 or UTR if not within ORF), and the SNP type (synonymous "syn" or non-synonymous "non-syn"), Amino acid residue affected by the SNP (AA Residue) in NP_003814, and the Alternate amino acid residue.

	dbSNP rs# ID	Nucleic Acid Residue	Alleles	Ambiguity Code	Codon Pos	SNP type	Amino Acid Residue	Alternate Amino Acid
Cln248	2257440	247	T/C	Y	3	Syn	49	C/C
Cln248	2738787	355	G/A	R	3	Syn	85	L/L
Cln248	17851469	355	G/A	R	3	Syn	85	L/L
Cln248	2258056	433	C/T	Y	3	Syn	111	R/R
Cln248	909341	586	T/C	Y	3	Syn	162	S/S
Cln248	1291205	673	G/C	S	3	Syn	191	T/T

[0095] Variants of Cln248 as described above and antibodies which bind to these variants are part of the invention described herein.

[0096] The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-IgE antibody is one which can bind to an IgE immunoglobulin in such a manner so as to prevent or substantially reduce the ability of such molecule from having the ability to bind to the high affinity receptor, FcεRI.

[0097] "Homology" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. Sequence similarity may be measured by any common sequence analysis algorithm, such as GAP or BESTFIT or other variation Smith-Waterman alignment. See, T. F. Smith and M. S. Waterman, J. Mol. Biol. 147:195-197 (1981) and W. R. Pearson, Genomics 11:635-650 (1991).

[0098] "Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332: 323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

[0099] As used herein, an anti-Cln248 antibody that "internalizes" is one that is taken up by (i.e., enters) the cell upon binding to Cln248 on a mammalian cell (i.e. cell surface Cln248). The internalizing antibody will of course include antibody fragments, human or humanized antibody and antibody conjugate. For therapeutic applications, internalization in vivo is contemplated. The number of antibody molecules internalized will be sufficient or adequate to kill an Cln248-expressing cell, especially an Cln248-expressing cancer cell. Depending on the potency of the antibody or antibody conjugate, in some instances, the uptake of a single antibody molecule into the cell is sufficient to kill the target cell to which the antibody binds. For example, certain toxins are highly potent in killing such that internalization of one molecule of the toxin conjugated to the antibody is sufficient to kill the tumor cell.

[0100] Whether an anti-Cln248 antibody internalizes upon binding Cln248 on a mammalian cell can be determined by various assays including those described in the experimental examples below. For example, to test internalization in vivo, the test antibody is labeled and introduced into an animal known to have Cln248 expressed on the surface of certain cells. The antibody can be radiolabeled or labeled with fluo-

rescent or gold particles, for instance. Animals suitable for this assay include a mammal such as a NCR nude mouse that contains a human Cln248-expressing tumor transplant or xenograft, or a mouse into which cells transfected with human Cln248 have been introduced, or a transgenic mouse expressing the human Cln248 transgene. Appropriate controls include animals that did not receive the test antibody or that received an unrelated antibody, and animals that received an antibody to another antigen on the cells of interest, which antibody is known to be internalized upon binding to the antigen. The antibody can be administered to the animal, e.g., by intravenous injection. At suitable time intervals, tissue sections of the animal can be prepared using known methods or as described in the experimental examples below, and analyzed by light microscopy or electron microscopy, for internalization as well as the location of the internalized antibody in the cell. For internalization in vitro, the cells can be incubated in tissue culture dishes in the presence or absence of the relevant antibodies added to the culture media and processed for microscopic analysis at desired time points. The presence of an internalized, labeled antibody in the cells can be directly visualized by microscopy or by autoradiography if radiolabeled antibody is used. Alternatively, in a quantitative biochemical assay, a population of cells comprising Cln248-expressing cells are contacted in vitro or in vivo with a radiolabeled test antibody and the cells (if contacted in vivo, cells are then isolated after a suitable amount of time) are treated with a protease or subjected to an acid wash to remove uninternalized antibody on the cell surface. The cells are ground up and the amount of protease resistant, radioactive counts per minute (cpm) associated with each batch of cells is measured by passing the homogenate through a scintillation counter. Based on the known specific activity of the radiolabeled antibody, the number of antibody molecules internalized per cell can be deduced from the scintillation counts of the ground-up cells. Cells are "contacted" with antibody in vitro preferably in solution form such as by adding the cells to the cell culture media in the culture dish or flask and mixing the antibody well with the media to ensure uniform exposure of the cells to the antibody. Instead of adding to the culture media, the cells can be contacted with the test antibody in an isotonic solution such as PBS in a test tube for the desired time period. In vivo, the cells are contacted with antibody by any suitable method of administering the test antibody such as the methods of administration described below when administered to a patient.

[0101] The faster the rate of internalization of the antibody upon binding to the Cln248-expressing cell in vivo, the faster the desired killing or growth inhibitory effect on the target Cln248-expressing cell can be achieved, e.g., by a cytotoxic immunoconjugate. Preferably, the kinetics of internalization of the anti-Cln248 antibodies are such that they favor rapid killing of the Cln248-expressing target cell. Therefore, it is desirable that the anti-Cln248 antibody exhibit a rapid rate of internalization preferably, within 24 hours from administration of the antibody in vivo, more preferably within about 12 hours, even more preferably within about 30 minutes to 1 hour, and most preferably, within about 30 minutes. The present invention provides antibodies that internalize as fast as about 15 minutes from the time of introducing the anti-Cln248 antibody in vivo. The antibody will preferably be internalized into the cell within a few hours upon binding to Cln248 on the cell surface, preferably within 1 hour, even more preferably within 15-30 minutes.

[0102] To determine if a test antibody can compete for binding to the same epitope as the epitope bound by the anti-Cln248 antibodies of the present invention including the antibodies produced by the hybridomas deposited with the ATCC, a cross-blocking assay e.g., a competitive ELISA assay can be performed. In an exemplary competitive ELISA assay, Cln248-coated wells of a microtiter plate, or Cln248-coated sepharose beads, axe pre-incubated with or without candidate competing antibody and then a biotin-labeled anti-Cln248 antibody of the invention is added. The amount of labeled anti-Cln248 antibody bound to the Cln248 antigen in the wells or on the beads is measured using avidin-peroxidase conjugate and appropriate substrate.

[0103] Alternatively, the anti-Cln248 antibody can be labeled, e.g., with a radioactive or fluorescent label or some other detectable and measurable label. The amount of labeled anti-Cln248 antibody that binds to the antigen will have an inverse correlation to the ability of the candidate competing antibody (test antibody) to compete for binding to the same epitope on the antigen, i.e., the greater the affinity of the test antibody for the same epitope, the less labeled anti-Cln248 antibody will be bound to the antigen-coated wells. A candidate competing antibody is considered an antibody that binds substantially to the same epitope or that competes for binding to the same epitope as an anti-Cln248 antibody of the invention if the candidate competing antibody can block binding of the anti-Cln248 antibody by at least 20%, preferably by at least 20-50%, even more preferably, by at least 50% as compared to a control performed in parallel in the absence of the candidate competing antibody (but may be in the presence of a known noncompeting antibody). It will be understood that variations of this assay can be performed to arrive at the same quantitative value.

[0104] An antibody having a "biological characteristic" of a designated antibody, such as any of the monoclonal antibodies Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15, Cln248.A16, Cln248.A17, Cln248.A18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46, is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen, Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15, Cln248.A16, Cln248.A17, Cln248.A18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46 will bind the same epitope as that bound by Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15,

Cln248.A16, Cln248.A17, Cln248.A18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46 (e.g. which competes for binding or blocks binding of monoclonal antibody Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15, Cln248.A16, Cln248.A17, Cln248.A18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46), be able to target an Cln248-expressing tumor in vivo and may internalize upon binding to Cln248 on a mammalian cell in vivo. Likewise, an antibody with the biological characteristic of the Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15, Cln248.A16, Cln248.A17, Cln248.A 18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46 antibody will have the same epitope binding, targeting, internalizing, tumor growth inhibitory and cytotoxic properties of the antibody.

[0105] The term "antagonist" antibody is used in the broadest sense, and includes an antibody that partially or fully blocks, inhibits, or neutralizes a biological activity of a native Cln248 protein disclosed herein. Methods for identifying antagonists of an Cln248 polypeptide may comprise contacting an Cln248 polypeptide or a cell expressing Cln248 on the cell surface, with a candidate antagonist antibody and measuring a detectable change in one or more biological activities normally associated with the Cln248 polypeptide.

[0106] The term "agonistic" antibody is used in the broadest sense, and includes an antibody the partially or fully promotes, activates, or increases biological activity of Cln248. Additionally, an agonistic antibody may mimic an Cln248 binding partner (e.g. receptor or ligand) wherein binding of the Cln248 antibody has substantially the same effect on biologic activity of Cln248 as binding of the binding partner. Methods for identifying agonists of an Cln248 polypeptide may comprise contacting an Cln248 polypeptide or a cell expressing Cln248 on the cell surface, with a candidate agonistic antibody and measuring a detectable change in one or more biological activities normally associated with the Cln248 polypeptide.

[0107] An "antibody that inhibits the growth of tumor cells expressing Cln248" or a "growth inhibitory" antibody is one which binds to and results in measurable growth inhibition of cancer cells expressing or overexpressing Cln248. Preferred growth inhibitory anti-Cln248 antibodies inhibit growth of Cln248-expressing tumor cells (e.g., ovarian, colon, prostate or lung cancer cells) by greater than 20%, preferably from about 20% to about 50%, and even more preferably, by greater than 50% (e.g. from about 50% to about 100%) as compared to the appropriate control, the control typically being tumor cells not treated with the antibody being tested. Growth inhibition can be measured at an antibody concentration of about 0.1 to 30 pg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. Growth inhibition of tumor cells in vivo can be determined in various ways such as is described in the Experimental Examples section below. The antibody is growth inhibitory in vivo if administration of the anti-Cln248 antibody at about 1 pg/kg to about 100 mg/kg body weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

[0108] An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses Cln248. Preferably the cell is a tumor cell, e.g. an ovarian, colon, prostate, or lung cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cells in an annexin binding assay.

[0109] Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); complement dependent cytotoxicity (CDC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0110] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activ-

ity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

[0111] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRI receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daeron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126.330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer, of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)).

[0112] "Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source, e.g. from blood.

[0113] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996) may be performed.

[0114] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma; multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases.

[0115] A "Cln248-expressing cell" is a cell which expresses endogenous or transfected Cln248 on the cell surface. A "Cln248-expressing cancer" is a cancer comprising cells that have Cln248 protein present on the cell surface. A "Cln248-expressing cancer" produces sufficient levels of Cln48 on the surface of cells thereof, such that an anti-Cln248 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which "overexpresses" Cln248 is one which has significantly higher levels of Cln248 at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. Cln248 overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the Cln248 protein present on the surface of a cell (e.g. via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of Cln248-encoding nucleic acid or mRNA in the cell, e.g. via fluorescent in situ hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study Cln248 overexpression by measuring shed antigen in a biological fluid such as serum, e.g., using antibody-based assays (see also, e.g., U.S. Pat. No. 4,933,294 issued Jun. 12, 1990; WO91/05264 published Apr. 18, 1991; U.S. Pat. No. 5,401,638 issued Mar. 28, 1995; and Sias et al. J. Immunol. Methods 132: 73-80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. An Cln248-expressing cancer includes colon, ovarian, lung or prostate cancer.

[0116] A "mammal" for purposes of treating a cancer or alleviating the symptoms of cancer, refers to any mammal, including-humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

[0117] "Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for an Cln248-expressing cancer if, after receiving a therapeutic amount of an anti-Cln248 antibody according to the methods of the present invention, the patient shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (i.e., slow to some extent and preferably stop) of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition (i.e., slow to some extent and preferably stop) of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief to some extent, one or more of the symptoms associated with the specific cancer; reduced morbidity and mor-

tality, and improvement in quality of life issues. To the extent the anti-Cln248 antibody may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. Reduction of these signs or symptoms may also be felt by the patient.

[0118] The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

[0119] The term "therapeutically effective amount" refers to an amount of an antibody or a drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

[0120] "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time.

[0121] "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0122] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[0123] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed.

[0124] Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0125] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, e.g., gelonin, ricin, saporin, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0126] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an Cln248-expressing cancer cell, either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of Cln248-expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce GI arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogencs, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0127] "Label" as used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0128] The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-Cln248 antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

[0129] A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

[0130] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0131] An "isolated nucleic acid molecule" is a nucleic acid molecule, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid molecule which has been

removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure nucleic acid molecule includes isolated forms of the nucleic acid molecule.

[0132] "Vector" includes shuttle and expression vectors and includes, e.g., a plasmid, cosmid, or phagemid. Typically, a plasmid construct will also include an origin of replication (e.g., the ColE1 origin of replication) and a selectable marker (e.g., ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in prokaryotic, e.g., bacterial, or eukaryotic cells. Suitable vectors are disclosed below.

[0133] The cell that produces an anti-Cln248 antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

[0134] RNA interference refers to the process of sequence-specific post transcriptional gene silencing in animals mediated by short interfering RNAs (siRNA) (Fire et al., 1998, Nature, 391, 806). The corresponding process in plants is commonly referred to as post transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post transcriptional gene silencing is thought to be an evolutionarily conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire et al., 1999, Trends Genet., 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double stranded RNAs (dsRNA) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

[0135] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNA) (Berstein et al., 2001, Nature, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21-23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21 and 22 nucleotide small temporal RNAs (stRNA) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, Science, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir et al., 2001, Genes Dev., 15, 188).

[0136] Short interfering RNA mediated RNAi has been studied in a variety of systems. Fire et al., 1998, Nature, 391, 806, wore the first to observe RNAi in *C. Elegans*. Wianny and Goetz, 1999, Nature Cell Biol., 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond et al., 2000, Nature, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir et al., 2001, Nature, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir et al., 2001, EMBO J., 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two nucleotide 3'-overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir et al., 2001, EMBO J., 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen et al., 2001, Cell, 107, 309).

[0137] Studies have shown that replacing the 3'-overhanging segments of a 21-mer siRNA duplex having 2 nucleotide 3' overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to 4 nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir et al., 2001, EMBO J., 20, 6877). In addition, Elbashir et al., supra, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li et al., International PCT Publication No. WO 00/44914, and Beach et al., International PCT Publication No. WO 01/68836 both suggest that siRNA "may include modifications to either the phosphate-sugar back bone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom", however neither application teaches to what extent these modifications are tolerated in siRNA molecules nor provide any examples of such modified siRNA. Kreutzer and Limmer, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double stranded-RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer and Limmer similarly fail to show to what extent these modifications are tolerated in siRNA molecules nor do they provide any examples of such modified siRNA.

[0138] Parrish et al., 2000, Molecular Cell, 6, 1977-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate

nucleotide analogs with T7 and T3 RNA polymerase and observed that "RNAs with two (phosphorothioate) modified bases also had substantial decreases in effectiveness as RNAi triggers (data not shown); (phosphorothioate) modification of more than two residues greatly destabilized the RNAs in vitro and we were not able to assay interference activities." Id. at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and observed that substituting deoxynucleotides for ribonucleotides "produced a substantial decrease in interference activity", especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. Id. In addition, the authors tested certain base modifications, including substituting 4-thiouracil, 5-bromouracil, 5-iodouracil, 3-(aminoallyl)uracil for uracil, and inosine for guanosine in sense and antisense strands of the siRNA, and found that whereas 4-thiouracil and 5-bromouracil were all well tolerated, inosine "produced a substantial decrease in interference activity" when incorporated in either strand. Incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in substantial decrease in RNAi activity as well.

[0139] Beach et al., International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously derived dsRNA. Tuschl et al., International PCT Publication No. WO 01/75164, describes a *Drosophila* in vitro RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, Chem. Biochem., 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due "to the danger of activating interferon response". Li et al., International PCT Publication No. WO 00/44914, describes the use of specific dsRNAs for use in attenuating the expression of certain target genes. Zernicka-Goetz et al., International PCT Publication No. WO 01/36646, describes certain methods for inhibiting the expression of particular genes in mammalian cells using certain dsRNA molecules. Fire et al., International PCT Publication No. WO 99/32619, describes particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck et al., International PCT Publication No. WO 00/01846, describes certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello et al., International PCT Publication No. WO 01/29058, describes the identification of specific genes involved in dsRNA mediated RNAi. Deschamps Depaillette et al., International PCT Publication No. WO 99/07409, describes specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Driscoll et al., International PCT Publication No. WO 01/49844, describes specific DNA constructs for use in facilitating gene silencing in targeted organisms. Parrish et al., 2000, Molecular Cell, 6, 1977-1087, describes specific chemically modified siRNA constructs targeting the unc-22 gene of *C. elegans*. Tuschl et al., International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs.

Compositions and Methods of the Invention

[0140] The invention provides anti-Cln248 antibodies. Preferably, the anti-Cln248 antibodies internalize upon binding to cell surface Cln248 on a mammalian cell. The anti-Cln248 antibodies may also destroy or lead to the destruction of tumor cells bearing Cln248.

[0141] It was not apparent that Cln248 was internalization-competent. In addition the ability of an antibody to internalize depends on several factors including the affinity, avidity, and isotype of the antibody, and the epitope that it binds. We have demonstrated herein that the cell surface Cln248 is internalization competent upon binding by the anti-Cln248 antibodies of the invention. Additionally, it was demonstrated that the anti-Cln248 antibodies of the present invention can specifically target Cln248-expressing tumor cells. These tumor targeting, internalization and growth inhibitory properties of the anti-Cln248 antibodies make these antibodies very suitable for therapeutic uses, e.g., in the treatment of various cancers including colon, ovarian, lung or prostate cancer. Internalization of the anti-Cln248 antibody is preferred, e.g., if the antibody or antibody conjugate has an intracellular site of action and if the cytotoxic agent conjugated to the antibody does not readily cross the plasma membrane (e.g., the toxin calicheamicin). Internalization is not necessary if the antibodies or the agent conjugated to the antibodies do not have intracellular sites of action, e.g., if the antibody can kill the tumor cell by ADCC or some other mechanism.

[0142] The anti-Cln248 antibodies of the invention also have various non-therapeutic applications. The anti-Cln248 antibodies of the present invention can be useful for diagnosis and staging of Cln248-expressing cancers (e.g., in radioimaging). They may be used alone or in combination with other ovarian cancer markers, including, but not limited to, CA125, HE4 and mesothelin. The antibodies are also useful for purification or immunoprecipitation of Cln248 from cells, for detection and quantitation of Cln248 in vitro, e.g. in an ELISA or a Western blot, to kill and eliminate Cln248-expressing cells from a population of mixed cells as a step in the purification of other cells. The internalizing anti-Cln248 antibodies of the invention can be in the different forms encompassed by the definition of "antibody" herein. Thus, the antibodies include full length or intact antibody, antibody fragments, native sequence antibody or amino acid variants, humanized, chimeric or fusion antibodies, immunoconjugates, and functional fragments thereof. In fusion antibodies, an antibody sequence is fused to a heterologous polypeptide sequence. The antibodies can be modified in the Fc region to provide desired effector functions. As discussed in more detail in the sections below, with the appropriate Fc regions, the naked antibody bound on the cell surface can induce cytotoxicity, e.g., via antibody-dependent cellular cytotoxicity (ADCC) or by recruiting complement in complement dependent cytotoxicity, or some other mechanism. Alternatively, where it is desirable to eliminate or reduce effector function, so as to minimize side effects or therapeutic complications, certain other Fc regions may be used.

[0143] The antibody may compete for binding, or binds substantially to, the same epitope bound by the antibodies of the invention. Antibodies having the biological characteristics of the present anti-Cln248 antibodies of the invention are also contemplated, e.g., an anti-Cln248 antibody which has the biological characteristics of a monoclonal antibody produced by the hybridomas deposited with the ATCC on 14 Oct. 2005 and 18 Oct. 2005 comprising PTA-7172 and PTA-7175, specifically including the in vivo tumor targeting, internalization and any cell proliferation inhibition or cytotoxic characteristics. Specifically provided are anti-Cln248 antibodies that bind to an epitope present in amino acids 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, 140-150, 150-160,

160-170, 170-180, 180-190, 190-200, 200-210, 210-220, 220-230, 230-240, 240-250, 250-260, 260-270, 270-280, 280-290, 290-300 or 1-15, 10-25, 15-25, 21-35, 31-45, 41-55, 51-65, 61-75, 71-85, 81-95, 91-105, 101-115, 111-125, 121-135, 131-145, 141-155, 151-165, 161-175, 171-185, 181-195, 191-205, 201-215, 211-225, 221-235, 231-245, 241-255, 251-265, 261-275, 271-285, 281-295, 291-300 of human Cln248.

[0144] Methods of producing the above antibodies are described in detail below.

[0145] The present anti-Cln248 antibodies are useful for treating a Cln248-expressing cancer or alleviating one or more symptoms of the cancer in a mammal. Such a cancer includes colon, ovarian, lung or prostate cancer, cancer of the urinary tract, lung cancer, breast cancer, colon cancer, pancreatic cancer, and ovarian cancer, more specifically, prostate adenocarcinoma, renal cell carcinomas, colorectal adenocarcinomas, lung adenocarcinomas, lung squamous cell carcinomas, and pleural mesothelioma. The cancers encompass metastatic cancers of any of the preceding, e.g., colon, ovarian, lung or prostate cancer metastases. The antibody is able to bind to at least a portion of the cancer cells that express Cln248 in the mammal and preferably is one that does not induce or that minimizes HAMA response. Preferably, the antibody is effective to destroy or kill Cln248-expressing tumor cells or inhibit the growth of such tumor cells, in vitro or in vivo, upon binding to Cln248 on the cell. Such an antibody includes a naked anti-Cln248 antibody (not conjugated to any agent). Naked anti-Cln248 antibodies having tumor growth inhibition properties in vivo include the antibodies described in the Experimental Examples below. Naked antibodies that have cytotoxic or cell growth inhibition properties can be further conjugated with a cytotoxic agent to render them even more potent in tumor cell destruction. Cytotoxic properties can be conferred to an anti-Cln248 antibody by, e.g., conjugating the antibody with a cytotoxic agent, to form an immunoconjugate as described below. The cytotoxic agent or a growth inhibitory agent is preferably a small molecule. Toxins such as maytansin, maytansinoids, saporin, gelonin, ricin or calicheamicin and analogs or derivatives thereof, are preferable.

[0146] The invention provides a composition comprising an anti-Cln248 antibody of the invention, and a carrier. For the purposes of treating cancer, compositions can be administered to the patient in need of such treatment, wherein the composition can comprise one or more anti-Cln248 antibodies present as an immunoconjugate or as the naked antibody. Further, the compositions can comprise these antibodies in combination with other therapeutic agents such as cytotoxic or growth inhibitory agents, including chemotherapeutic agents. The invention also provides formulations comprising an anti-Cln248 antibody of the invention, and a carrier. The formulation may be a therapeutic formulation comprising a pharmaceutically acceptable carrier.

[0147] Another aspect of the invention is isolated nucleic acids encoding the internalizing anti-Cln248 antibodies. Nucleic acids encoding both the H and L chains and especially the hypervariable region residues, chains which encode the native sequence antibody as well as variants, modifications and humanized versions of the antibody, are encompassed.

[0148] The invention also provides methods useful for treating an Cln248-expressing cancer or alleviating one or more symptoms of the cancer in a mammal, comprising administering a therapeutically effective amount of an internalizing anti-Cln248 antibody to the mammal. The antibody therapeutic compositions can be administered short term (acute) or chronic, or intermittent as directed by physician. Also provided are methods of inhibiting the growth of, and killing an Cln248 expressing cell. Finally, the invention also provides kits and articles of manufacture comprising at least one antibody of this invention, preferably at least one internalizing anti-Cln248 antibody of this invention. Kits containing anti-Cln248 antibodies find use in detecting Cln248 expression, or in therapeutic or diagnostic assays, e.g., for Cln248 cell killing assays or for purification and/or immunoprecipitation of Cln248 from cells. For example, for isolation and purification of Cln248, the kit can contain an anti-Cln248 antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantitation of Cln248 in vitro, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

Production of Anti-Cln248 Antibodies

[0149] The following describes exemplary techniques for the production of the antibodies useful in the present invention. Some of these techniques are described further in Example 1. The Cln248 antigen to be used for production of antibodies may be, e.g., the full length polypeptide or a portion thereof, including a soluble form of Cln248 lacking the membrane spanning sequence, or synthetic peptides to selected portions of the protein.

[0150] Alternatively, cells expressing Cln248 at their cell surface (e.g. CHO or NIH-3T3 cells transformed to overexpress Cln248; ovarian, pancreatic, lung, breast or other Cln248-expressing tumor cell line), or membranes prepared from such cells can be used to generate antibodies. The nucleotide and amino acid sequences of human and murine Cln248 are available as provided above. Cln248 can be produced recombinantly in and isolated from, prokaryotic cells, e.g., bacterial cells, or eukaryotic cells using standard recombinant DNA methodology. Cln248 can be expressed as a tagged (e.g., epitope tag) or other fusion protein to facilitate its isolation as well as its identification in various assays.

[0151] Antibodies or binding proteins that bind to various tags and fusion sequences are available as elaborated below. Other forms of Cln248 useful for generating antibodies will be apparent to those skilled in the art.

[0152] Tags

[0153] Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 (Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990)). The FLAG-peptide (Hopp et al., BioTechnology, 6:1204-1210 (1988)) is recognized by an anti-FLAG M2 monoclonal antibody (Eastman Kodak Co., New Haven, Conn.). Purification of a protein containing the FLAG peptide can be performed by immunoaffinity chromatography using an affinity matrix comprising the anti-FLAG M2 monoclonal antibody covalently attached to agarose (Eastman Kodak Co., New Haven, Conn.). Other tag

polypeptides include the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an α-tubulin epitope peptide (Skinner et al., J. Biol. Chenz., 266:15163-15166 (1991)); and the T7 gene protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)).

[0154] Polyclonal Antibodies

[0155] Polyclonal antibodies are preferably raised in animals, preferably non-human animals, by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups. Conjugates also can be made in recombinant cell culture as protein fusions.

[0156] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 5-100 pg of the protein or conjugate (for rabbits or nice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with ⅕ to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0157] Monoclonal Antibodies

[0158] Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567). In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. After immunization, lymphocytes are isolated and then fused with a "fusion partner", e.g., a mycloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies. Principles and Practice, pp 103 (Academic Press, 1986)).

[0159] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, fusion partner, e.g., the parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0160] Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-II mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); and Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0161] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[0162] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980). Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp 103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal e.g., by i.p. injection of the cells into mice.

[0163] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

[0164] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed or transfected into prokaryotic or eukaryotic host cells such as, e.g., *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells, that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., Curr. Opinion in Immunol., 5:256-262 (1993) and Phickthun, Immunol. Revs., 130:151-188 (1992).

[0165] Further, the monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990). Clackson et al., Nature, 352: 624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., Bio/Technology, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., Nuc. Acids.

Res., 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0166] The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain (CH and CL) sequences for the homologous murine sequences (U.S. Pat. No. 4,816,567; and Morrison, et al., Proc. Natl. Acad. Sci. USA, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The nonimmunoglobulin polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[0167] Humanzized Antibodies

[0168] Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is nonhuman. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321: 522-525 (1986); Reichmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In, practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0169] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993)).

[0170] It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art.

[0171] Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0172] Various forms of a humanized anti-Cln248 antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

[0173] Human Antibodies

[0174] As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggemann et al., Year in Immuno., 7:33 (1993); U.S. Pat. Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; and Alternatively, phage display technology (McCafferty et al., Nature 348: 552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene 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, reviewed in, e.g., Johnson, Kevin S. and Chiswell, David J., 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. As discussed above, human antibodies may also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275).

[0175] Antibody Fragments

[0176] In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab)_2$ fragments (Carter et al., Bio/Technology 10: 163-167 (1992)). According to another approach, F(ab)2 fragments can be isolated directly from recombinant host cell culture. Fab and F(ab)2 fragment with increased in vivo half-life comprising a salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. The antibody of choice may also be a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during in vivo use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

[0177] Bispecific Antibodies

[0178] Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the Cln248 protein. Other such antibodies may combine an Cln248 binding site with a binding site for another protein. Alternatively, an anti-Cln248. Arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a Tcell receptor molecule (e.g. C133), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRI (CD32) and FcγRIII (CD16), so as to focus and localize cellular defense mechanisms to the Cln248-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Cln248. These antibodies possess an Cln248-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab)2 bispecific antibodies). WO 96/16673 describes a bispecific anti-ErbB2/anti-FcγRII antibody and U.S. Pat. No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcγRI antibody. A bispecific anti-ErbB2/Fcα antibody is shown in WO98/02463. U.S. Pat. No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

[0179] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature,* 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J,* 10:3655-3659 (1991).

[0180] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (CHI) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

[0181] Preferably, the bispecific antibodies in this approach are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

[0182] According to another approach described in U.S. Pat. No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0183] Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

[0184] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')2 fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0185] Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')2 molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the BrbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0186] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers.

[0187] The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a VH connected to a VL by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

[0188] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. J. Immunol. 147: 60 (1991).

[0189] Multivalent Antibodies

[0190] A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain (s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1(X1n-VD2-(X2)$_n$-Fc, wherein VDI is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, XI and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CHI-flexible linker-VH-CHI-Fc region chain; or VH-CHI-VH-CHI-Fc region chain. The multivalent antibody herein preferably farther comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

[0191] Other Amino Acid Sequence Modifications

[0192] Amino acid sequence modification(s) of the anti-Cln248 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-Cln248 antibody are prepared by introducing appropriate nucleotide changes into the anti-Cln248 antibody nucleic acid, or by peptide synthesis.

[0193] Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the anti-Cln248 antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the anti-Cln248 antibody, such as changing the number or position of glycosylation sites.

[0194] A useful method for identification of certain residues or regions of the anti-Cln248 antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in Science, 244:1081-1085 (1989). Here, a residue or group of target residues within the anti-Cln248 antibody are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with Cln248 antigen.

[0195] Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substi-

tution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at a target codon or region and the expressed anti-Cln248 antibody variants are screened for the desired activity.

[0196] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-Cln248 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-Cln248 antibody molecule include the fusion to the N- or C-terminus of the anti-Cln248 antibody to an enzyme (e.g. for ADEPT) or a fusion to a polypeptide which increases the serum half-life of the antibody.

[0197] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-Cln248 antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table I under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in the table below, or as further described below in reference to amino acid classes, may be introduced and the products screened for a desired characteristic.

Amino Acid Substitutions

Original	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	Val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe;	leu
Leu (L)	norleucine; ile; val; met; ala;	ile
Lys (K)	arg; gin; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	Phe
Val (V)	ile; leu; met; phe; ala;	leu

[0198] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr; (3) acidic: asp, glu; (4) basic: asn, gin, his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic: trp, tyr, phe.

[0199] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Any cysteine residue not involved in maintaining the proper conformation of the anti-Cln248 antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0200] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human Cln248. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0201] Another type of amino acid variant of the antibody alters the original 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. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or

substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0202] Nucleic acid molecules encoding amino acid sequence variants of the anti-Cln248 antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared nucleic acid molecule encoding a variant or a non-variant version of the anti-Cln248 antibody.

[0203] It may be desirable to modify the antibody of the invention with respect to effector function, e.g. so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. Anti-Cancer Drug Design 3:219-230 (1989).

[0204] To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of the antibody.

Screening for Antibodies with the Desired Properties

[0205] Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

[0206] The growth inhibitory effects of an anti-Cln248 antibody of the invention may be assessed by methods known in the art, e.g., using cells which express Cln248 either endogenously or following transfection with the Cln248 gene. For example, the tumor cell lines and Cln248-transfected cells provided in Example 1 below may be treated with an anti-Cln248 monoclonal antibody of the invention at various concentrations for a few days (e.g., 2-7) days and stained with crystal violet or MTT or analyzed by some other calorimetric assay. Another method of measuring proliferation would be by comparing 3H-thymidine uptake by the cells treated in the presence or absence an anti-Cln248 antibody of the invention. After antibody treatment, the cells are harvested and the amount of radioactivity incorporated into the DNA quantitated in a scintillation counter. Appropriated positive controls include treatment of a selected cell line with a growth inhibitory antibody known to inhibit growth of that cell line. Growth inhibition of tumor cells in vivo can be determined in various ways such as is described in the Experimental Examples section below. Preferably, the tumor cell is one that over-expresses Cln248. Preferably, the anti-Cln248 antibody will inhibit cell proliferation of an Cln248-expressing tumor cell in vitro or in vivo by about 25-100% compared to the untreated tumor cell, more preferably, by about 30-100%, and even more preferably by about 50-100% or 70-100%, at an antibody concentration of about 0.5 to 30 μg/ml. Growth inhibition can be measured at an antibody concentration of about 0.5 to 30 μg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. The antibody is growth inhibitory in vivo if administration of the anti-Cln248 antibody at about 1 μg/kg to about 100 mg/kg body weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

[0207] To select for antibodies which induce cell death, loss of membrane integrity as indicated by, e.g., propidium iodide (PI), trypan blue or 7AAD uptake may be assessed relative to a control. A PI uptake assay can be performed in the absence of complement and immune effector cells. Cln248-expressing tumor cells are incubated with medium alone or medium containing of the appropriate monoclonal antibody at e.g., about 10 μg/ml. The cells are incubated for a 3 day time period. Following each treatment, cells are washed and aliquoted into 35 mm strainer-capped 12×75 tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing antibodies.

[0208] To screen for antibodies which bind to an epitope on Cln248 bound by an antibody of interest, e.g., the Cln248 antibodies of this invention, a routine cross-blocking assay such as that describe in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if a test antibody binds the same site or epitope as an anti-Cln248 antibody of the invention. Alternatively, or additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. The mutant antibody is initially tested for binding with polyclonal antibody to ensure proper folding. In a different method, peptides corresponding to different regions of Cln248 can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

[0209] For example, a method to screen for antibodies that bind to an epitope which is bound by an antibody this invention may comprise combining an Cln248-containing sample with a test antibody and an antibody of this invention to form a mixture, the level of Cln248 antibody bound to Cln248 in the mixture is then determined and compared to the level of Cln248 antibody bound in the mixture to a control mixture, wherein the level of Cln248 antibody binding to Cln248 in the mixture as compared to the control is indicative of the test antibody's binding to an epitope that is bound by the anti-Cln248 antibody of this invention. The level of Cln248 antibody bound to Cln248 is determined by ELISA. The control may be a positive or negative control or both. For example, the

control may be a mixture of Cln248, Cln248 antibody of this invention and an antibody known to bind the epitope bound by the Cln248 antibody of this invention. The anti-Cln248 antibody labeled with a label such as those disclosed herein. The Cln248 may be bound to a solid support, e.g., a tissue culture plate or to beads, e.g., sepharose beads.

Immunoconjugates

[0210] The invention also pertains to therapy with immunoconjugates comprising an antibody conjugated to an anticancer agent such as a cytotoxic agent or a growth inhibitory agent.

[0211] Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

[0212] Maytansine and Maytansinoids

[0213] Preferably, an anti-Cln248 antibody (full length or fragments) of the invention is conjugated to one or more maytansinoid molecules.

[0214] Maytansinoids are mitotic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the cast African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, the disclosures of which are hereby expressly incorporated by reference.

[0215] Maytansinoid-Antibody Conjugates

[0216] In an attempt to improve their therapeutic index, maytansine and maytansinoids have been conjugated to antibodies specifically binding to tumor cell antigens. Immunoconjugates containing maytansinoids and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DMI linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay. Chari et al. Cancer Research 52:127-131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3×10 5 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansonoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

[0217] Anti-Cln248 antibody-Maytansinoid Conjugates (Immunoconjugates)

[0218] Anti-Cln248 antibody-maytansinoid conjugates are prepared by chemically linking an anti-Cln248 antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Pat. No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytantsinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

[0219] There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Pat. No. 5,208,020 or EP Patent 0 425 235 B1, and Chari et al. Cancer Research 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred. Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl (2-pyridyldithio) propionate (SPDP), succinimidyl-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as his (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl (2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 [1978]) and N-succinimidyl (2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

[0220] The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. Preferably, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

Calicheamicin

[0221] Another immunoconjugate of interest comprises an anti-Cln248 antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at subpicomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710,

5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ_1^I, (Hinman et al. Cancer Research 53: 3336 (1993), Lode et al. Cancer Research 5 8: 2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

Other Cytotoxic Agents

[0222] Other antitumor agents that can be conjugated to the anti-Cln248 antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Pat. Nos. 5,053,394, 5,770,710, as well as esperamicins (U.S. Pat. No. 5,877,296). Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, 15 nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993. The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (e.g. a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0223] For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated anti-Cln248 antibodies. Examples include At^{211}, I^{131}, I^{25}, In^{111}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, and radioactive isotopes of Lu. When the conjugate is used for diagnosis, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99M} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0224] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as Tc^{99M}, I^{123}, In^{111}, Re^{186}, Re^{188}, can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate iodine "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0225] Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl (2-pyridyldithio) propionate (SPDP), succinimidyl (N-maleinidomethyl)cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediatnine), diisocyanates (such as tolyene 2,6diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. Science 238: 1098 (1987). Carbon labeled 1-isothiocyanatobenzyl methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al. Cancer Research 52: 127-131 (1992); U.S. Pat. No. 5,208,020) may be used.

[0226] Alternatively, a fusion protein comprising the anti-Cln248 antibody and cytotoxic agent may be made, e.g. by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

[0227] In addition, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

[0228] The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see W081/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Pat. No. 4,975,278.

[0229] The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thernolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as O-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with P-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, Nature 328: 457-458 (1987)).

Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population. The enzymes of this invention can be covalently bound to the anti-Cln248 antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above.

[0230] Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., Nature, 312: 604-608 (1984).

Other Antibody Modifications

[0231] Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

[0232] The anti-Cln248 antibodies disclosed herein may also be formulated as immunoliposomes. A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82:3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published Oct. 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al. J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst. 81(19)1484 (1989).

Vectors, Host Cells, and Recombinant Methods

[0233] The invention also provides isolated nucleic acid molecule encoding the humanized anti-Cln248 antibody, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody. For recombinant production of the antibody, the nucleic acid molecule encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or inserted into a vector in operable linkage with a promoter for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to nucleic acid molecules encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

[0234] Signal Sequence Component

[0235] The anti-Cln248 antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native anti-Cln248 antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, e.g., the yeast invertase leader, oc factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such precursor region is ligated in reading frame to DNA encoding the anti-Cln248 antibody.

[0236] Origin of Replication

[0237] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Selection Gene Component

[0238] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0239] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the anti-Cln248 antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -11, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc. For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

[0240] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding anti-Cln248 antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

[0241] A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., Nature, 282:39 (1979)). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4 Jones, Genetics, 85:12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

[0242] In addition, vectors derived from the 1.6 pm circular plasmid pKDI can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, Bio/Technology, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al., Bio/Technology, 9:968-975 (1991).

[0243] Promoter Component

[0244] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the anti-Cln248 antibody nucleic acid. Promoters suitable for use with prokaryotic hosts include the phoA promoter, P-lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgamo (S.D.) sequence operably linked to the DNA encoding the anti-Cln248 antibody.

[0245] Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors. Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde phosphate dehydrogenase, hexokinase, pyravate decarboxylase, phosphofructokinase, glucose phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0246] Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

[0247] Anti-Cln248 antibody transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0248] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982) on expression of human P-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

[0249] Enhancer Element Component

[0250] Transcription of a DNA encoding the anti-Cln248 antibody of this invention by higher eukaryotes is often increased by inserting all enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the anti-Cln248 antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

[0251] Transcription Termination Component

[0252] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain

nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding anti-Cln248 antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO 94/11026 and the expression vector disclosed therein.

[0253] Selection and Transformation of Host Cells

[0254] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella*, e.g., *Salmonella typhimurium, Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 Apr. 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

[0255] Full length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half life in circulation. Production in *E. coli* is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Pat. No. 5,648,237 (Carter et. al.), U.S. Pat. No. 5,789,199 (Joly et al.), and U.S. Pat. No. 5,840,523 (Simmons et al.) which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion, these patents incorporated herein by reference. After expression, the antibody is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells.

[0256] In addition to prokaryotes, eukaryotic microbes such as filamentous fingi or yeast are suitable cloning or expression hosts for anti-Cln248 antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe; Kluyveromyces* hosts such as, e.g., *K. lactis, K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (BP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa; Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger.*

[0257] Suitable host cells for the expression of glycosylated anti-Cln248 antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

[0258] Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, *Arabidopsis* and tobacco can also be utilized as hosts. Cloning and expression vectors useful in the production of proteins in plant cell culture are known to those of skill in the art. See e.g. Hiatt et al., Nature (1989) 342: 76-78, Owen et al. (1992) Bio/Technology 10: 790-794, Artsaenko et al. (1995) The Plant J 8: 745-750, and Fecker et al. (1996) Plant Mol Biol 32: 979-986.

[0259] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/−DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRLI 587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, 1413 8065); mouse mammary tumor (MMT 060562, ATCC CCL5 1); TR1 cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0260] Host cells are transformed with the above-described expression or cloning vectors for anti-Cln248 antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0261] Culturing Host Cells

[0262] The host cells used to produce the anti-Cln248 antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's FIO (Sigma), Minimal Essential Medium (MEM)(Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM)(Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. No. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that

would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0263] Purification of Anti-Cln248 Antibody

[0264] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0265] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, get electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SEDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0266] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

Pharmaceutical Formulations

[0267] Pharmaceutical formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as acetate, Tris, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbonzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol, and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyllolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; tonicifiers such as trehalose and sodium chloride; sugars such as sucrose, mannitol, trehalose or sorbitol; surfactant such as polysorbate; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). The antibody preferably comprises the antibody at a concentration of between 5-200 mg/ml, preferably between 10-100 mg/ml.

[0268] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to the anti-Cln248 antibody which internalizes, it may be desirable to include in the one formulation, an additional antibody, e.g. a second anti-Cln248 antibody which binds a different epitope on Cln248, or an antibody to some other target such as a growth factor that affects the growth of the particular cancer. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0269] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0270] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−) hydroxybutyric acid.

[0271] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Methods and Treatment Using Anti-Cln248 Antibodies

[0272] According to the present invention, the anti-Cln248 antibody that internalizes upon binding Cln248 on a cell surface is used to treat a subject in need thereof having a cancer characterized by Cln248-expressing cancer cells, in particular, colon, ovarian, lung or prostate cancer, and associated metastases.

[0273] The cancer will generally comprise Cln248-expressing cells, such that the anti-Cln248 antibody is able to bind thereto. While the cancer may be characterized by overexpression of the Cln248 molecule, the present application further provides a method for treating cancer which is not considered to be an Cln248-overexpressing cancer.

[0274] This invention also relates to methods for detecting cells or tissues which overexpress Cln248 and to diagnostic kits useful in detecting cells or tissues expressing Cln248 or in detecting Cln248 in bodily fluids from a patient. Bodily fluids include blood, serum, plasma, urine, ascites, peritoneal wash, saliva, sputum, seminal fluids, mucous membrane secretions, and other bodily excretions such as stool. The methods may comprise combining a cell-containing test sample with an antibody of this invention, assaying the test sample for antibody binding to cells in the test sample and comparing the level of antibody binding in the test sample to the level of antibody binding in a control sample of cells. A suitable control is, e.g., a sample of normal cells of the same type as the test sample or a cell sample known to be free of Cln248 overexpressing cells. A level of Cln248 binding higher than that of such a control sample would be indicative of the test sample containing cells that overexpress Cln248. Alternatively the control may be a sample of cells known to contain cells that overexpress Cln248. In such a case, a level of Cln248 antibody binding in the test sample that is similar to, or in excess of, that of the control sample would be indicative of the test sample containing cells that overexpress Cln248.

[0275] Cln248 overexpression may be detected with a various diagnostic assays. For example, over expression of Cln248 may be assayed by immunohistochemistry (IUC). Parrafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded an Cln248 protein staining intensity criteria as follows.

[0276] Score 0 no staining is observed or membrane staining is observed in less than 10% of tumor cells.

[0277] Score 1+ a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

[0278] Score 2+ a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

[0279] Score 3+ a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

[0280] Those tumors with 0 or 1+ scores for Cln248 expression may be characterized as not overexpressing Cln248, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing Cln248.

[0281] Alternatively, or additionally, FISH assays such as the INFORM™ (sold by Ventana, Ariz.) or PATHVISION™ (VySiS, Illinois) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of Cln248 overexpression in the tumor. Cln248 overexpression or amplification may be evaluated using an in vivo diagnostic assay, e.g. by administering a molecule (such as an antibody of this invention) which binds Cln248 and which is labeled with a detectable label (e.g. a radioactive isotope or a fluorescent label) and externally scanning the patient for localization of the label.

[0282] A sample suspected of containing cells expressing or overexpressing Cln248 is combined with the antibodies of this invention under conditions suitable for the specific binding of the antibodies to Cln248. Binding and/or internalizing the Cln248 antibodies of this invention is indicative of the cells expressing Cln248. The level of binding may be determined and compared to a suitable control, wherein an elevated level of bound Cln248 as compared to the control is indicative of Cln248 overexpression. The sample suspected of containing cells overexpressing Cln248 may be a cancer cell sample, particularly a sample of ovarian, colon, prostate or lung cancer. A serum sample from a subject may also be assayed for levels of Cln248 by combining a serum sample from a subject with an Cln248 antibody of this invention, determining the level of Cln248 bound to the antibody and comparing the level to a control, wherein an elevated level of Cln248 in the serum of the patient as compared to a control is indicative of overexpression of Cln248 by cells in the patient. The subject may have a cancer such as ovarian, colon, prostate or lung cancer.

[0283] Currently, depending on the stage of the cancer, colon, ovarian, lung or prostate cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, androgen deprivation (e.g., hormonal therapy), and chemotherapy. Anti-Cln248 antibody therapy may be especially desirable in elderly patients who do not tolerate the toxicity and side effects of chemotherapy well, in metastatic disease where radiation therapy has limited usefulness, and for the management of prostatic carcinoma that is resistant to androgen deprivation treatment. The tumor targeting and internalizing anti-Cln248 antibodies of the invention are useful to alleviate Cln248-expressing cancers, e.g., colon, ovarian, lung or prostate cancers upon initial diagnosis of the disease or during relapse. For therapeutic applications, the anti-Cln248 antibody can be used alone, or in combination therapy with, e.g., hormones, antiangiogens, or radiolabelled compounds, or with surgery, cryotherapy, and/or radiotherapy, notably for colon, ovarian, lung or prostate cancers, also particularly where shed cells cannot be reached. Anti-Cln248 antibody treatment can be administered in conjunction with other forms of conventional therapy, either consecutively with, pre- or post-conventional therapy, Chemotherapeutic drugs such as Taxotere® (docotaxel), Taxol® (paclitaxel), estramustine and mitoxantrone are used in treating metastatic and hormone refractory colon, ovarian, lung or prostate cancer, in particular, in good risk patients. In the present method of the invention for treating or alleviating cancer, in particular, androgen independent and/or metastatic colon, ovarian, lung or prostate cancer, the cancer patient can be administered anti-Cln248 antibody in conjunction with treatment with the one or more of the preceding chemotherapeutic agents. In particular, combination therapy with paclitaxel and modified derivatives (see, e.g., EP0600517) is contemplated. The anti-Cln248 antibody will be administered with a therapeutically effective dose of the chemotherapeutic agent. The anti-Cln248 antibody may also be administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent, e.g., paclitaxel. The Physicians' Desk Reference

(PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill in the art and can be determined by the physician.

[0284] Particularly, an immunoconjugate comprising the anti-Cln248 antibody conjugated with a cytotoxic agent may be administered to the patient. Preferably, the immunoconjugate bound to the Cln248 protein is internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. Preferably, the cytotoxic agent targets or interferes with the nucleic acid in the cancer cell. Examples of such cytotoxic agents are described above and include maytansin, maytansinoids, saporin, gelonin, ricin, calicheamicin, ribonucleases and DNA endonucleases.

[0285] The anti-Cln248 antibodies or immunoconjugates are administered to a human patient, in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The antibodies or immunoconjugates may be injected directly into the tumor mass. Intravenous or subcutaneous administration of the antibody is preferred. Other therapeutic regimens may be combined with the administration of the anti-Cln248 antibody.

[0286] The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preferably such combined therapy results in a synergistic therapeutic effect.

[0287] It may also be desirable to combine administration of the anti-Cln248 antibody or antibodies, with administration of an antibody directed against another tumor antigen associated with the particular cancer. As such, this invention is also directed to an antibody "cocktail" comprising one or more antibodies of this invention and at least one other antibody which binds another tumor antigen associated with the Cln248-expressing tumor cells. The cocktail may also comprise antibodies that are directed to other epitopes of Cln248. Preferably the other antibodies do not interfere with the binding and or internalization of the antibodies of this invention.

[0288] The antibody therapeutic treatment method of the present invention may involve the combined administration of an anti-Cln248 antibody (or antibodies) and one or more chemotherapeutic agents or growth inhibitory agents, including co-administration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include, e.g., estramustine phosphate, prednimustine, cisplatin, 5-fluorouracil, melphalan, cyclophosphamide, hydroxyurea and hydroxyureataxanes (such as paclitaxel and doxetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

[0289] The antibody may be combined with an anti-hormonal compound; e.g., an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (see, EP 616 812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is androgen independent cancer, the patient may previously have been subjected to anti-androgen therapy and, after the cancer becomes androgen independent, the anti-Cln248 antibody (and optionally other agents as described herein) may be administered to the patient.

[0290] Sometimes, it may be beneficial to also co-administer a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy, before, simultaneously with, or post antibody therapy. Suitable dosages for any of the above co-administered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-Cln248 antibody.

[0291] For the prevention or treatment of disease, the dosage and mode of administration will be chosen by the physician according to known criteria. The appropriate dosage of antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Preferably, the antibody is administered by intravenous infusion or by subcutaneous injections. Depending on the type and severity of the disease, about 1 pg/kg to about 50 mg/kg body weight (e.g. about 0.1-15 mg/kg/dose) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A dosing regimen can comprise administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the anti-Cln248 antibody. However, other dosage regimens may be useful. A typical daily dosage might range from about 1 pg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The progress of this therapy can be readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art.

[0292] Aside from administration of the antibody protein to the patient, the present application contemplates administration of the antibody by gene therapy. Such administration of a nucleic acid molecule encoding the antibody is encompassed by the expression "administering a therapeutically effective amount of an antibody". See, for example, WO 96/07321 published Mar. 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

[0293] There are two major approaches to introducing the nucleic acid molecule (optionally contained in a vector) into the patient's cells; in vivo and ex vivo. For in vivo delivery the nucleic acid molecule is injected directly into the patient, usually at the site where the antibody is required. For ex vivo treatment, the patient's cells are removed, the nucleic acid molecule is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for

example, encapsulated within porous membranes which are implanted into the patient (see, e.g. U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acid molecules into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for ex vivo delivery of the gene is a retroviral vector.

[0294] The currently preferred in vivo nucleic acid molecule transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). For review of the currently known gene marking and gene therapy protocols see Anderson et at., Science 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

Articles of Manufacture and Kits

[0295] The invention also relates to an article of manufacture containing materials useful for the detection for Cln248 overexpressing cells and/or the treatment of Cln248 expressing cancer, in particular colon, ovarian, lung or prostate cancer. The article of manufacture comprises a container and a composition contained therein comprising an antibody of this invention. The composition may further comprise a carrier. The article of manufacture may also comprise a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for detecting Cln248 expressing cells and/or treating a cancer condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-Cln248 antibody of the invention. The label or package insert indicates that the composition is used for detecting Cln248 expressing cells and/or for treating colon, ovarian, lung or prostate cancer, in a patient in need thereof. The label or package insert may further comprise instructions for administering the antibody composition to a cancer patient. Additionally, the article of manufacture may further comprise a second container comprising a substance which detects the antibody of this invention, e.g., a second antibody which binds to the antibodies of this invention. The substance may be labeled with a detectable label such as those disclosed herein. The second container may contain e.g., a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0296] Kits are also provided that are useful for various purposes, e.g., for Cln248 cell killing assays, for purification or immunoprecipitation of Cln248 from cells or for detecting the presence of Cln248 in a serum sample or detecting the presence of Cln248-expressing cells in a cell sample. For isolation and purification of Cln248, the kit can contain an anti-Cln248 antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of Cln248 in vitro, e.g. in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a composition contained therein comprising an antibody of this invention. The kit may further comprise a label or package insert on or associated with the container. The kits may comprise additional components, e.g., diluents and buffers, substances which bind to the antibodies of this invention, e.g., a second antibody which may comprise a label such as those disclosed herein, e.g., a radiolabel, fluorescent label, or enzyme, or the kit may also comprise control antibodies. The additional components may be within separate containers within the kit. The label or package insert may provide a description of the composition as well as instructions for the intended in vitro or diagnostic use.

[0297] The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1

Production and Isolation of Monoclonal Antibody Producing Hybridomas

[0298] The following MAb/hybridomas of the present invention are described below:

[0299] Cln248.A1, Cln248.A2, Cln248.A3, Cln248.A4, Cln248.A5, Cln248.A6, Cln248.A7, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A11, Cln248.A12, Cln248.A13, Cln248.A14, Cln248.A15, Cln248.A16, Cln248.A17, Cln248.A18, Cln248.A19, Cln248.A20, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A28, Cln248.A29, Cln248.A30, Cln248.A31, Cln248.A32, Cln248.A33, Cln248.A34, Cln248.A35, Cln248.A36, Cln248.A37, Cln248.A38, Cln248.A39, Cln248.A40, Cln248.A41, Cln248.A42, Cln248.A43, Cln248.A44, Cln248.A45 and Cln248.A46.

[0300] If the MAb producing hybridoma has been cloned, it will get the nomenclature "X#.1," e.g., the first clone of Cln248.A12 will be referred to as A12.1, the second clone of A12 will be referred to as A12.2, etc. Sub-clones are designated by a subsequent ".#", e.g. the first sub-clone of Cln248. A12.1 is referred to as A12.1.1, the second sub-clone of A12.1 is A12.1.2, etc. Further generations of sub-clones are annotated in the same format. For the purposes of this invention, a reference to an anti-Cln248 antibody producing hybridoma, e.g. Cln248.A12 or A12, will include all clones and sub-clones of the antibody, e.g., A12.1, A12.2, A12.1.1, etc. Furthermore, the nomenclature Cln248.A12.l, for example, may reference the antibody producing hybridoma, or the antibody itself.

Immunogens and Antigens (Recombinant Proteins, His Tags)

[0301] For the Cln248 Constructs described below, nucleic acid molecules encoding regions of Cln248 were inserted into various expression vectors to produce recombinant proteins. These nucleic acid sequences were isolated using the primers included in the descriptions below of each construct.

[0302] For purposes of illustration, the predicted amino acid sequence encoded by each construct is also included. However, the constructs may include naturally occurring

variants (e.g. allelic variants, SNPs) within the Cln248 region as isolated by the primers. These variant sequences, and antibodies which bind to them are considered part of the invention as described herein.

Cln248 Construct 1 Sequence and Protein Production

[0303] A nucleic acid molecule encoding the full length of Cln248 (Met1-His300), was inserted into a pCMV5His2 vector at the PmeI/NheI site. The nucleic acid molecule was isolated using the following primers:

(SEQ ID NO: 1)
5'primer: CTTTGTTTAAACATGAGGGCGCTGGAGGGGCCA

(SEQ ID NO: 2)
3'primer: CGGCTAGCGTGCACAGGGAGGAAGCGCTC

[0304] The vector comprises a sequence encoding 2 transitional amino acids and a 10 His tag in-frame at the 3' side of the insertion site. The resulting vector with the inserted Cln248 nucleic acid fragment encodes a recombinant Cln248 fusion protein with the 10 His-tag fused to the C-terminus of the protein. This recombinant plasmid is herein referred to as "Cln248 Construct 1". A representative amino acid sequence encoded by Cln248 Construct 1 is presented in SEQ ID NO:3.

Cln248 Construct 2 Sequence and Protein Production

[0305] A nucleic acid molecule encoding the mature form of Cln248 (no secretion signal), Val30 to His300, was inserted into a modified pCMV5His2 vector at the NsiI/NheI sites. The nucleic acid molecule was isolated using the following primers:

(SEQ ID NO: 4)
5'primer: CCAATGCATGTGGCAGAAACACCCACCTAC

(SEQ ID NO: 2)
3'primer: CGGCTAGCGTGCACAGGGAGGAAGCGCTC

[0306] The modified vector comprises a nucleotide sequence encoding a 23 amino acid secretion signal sequence from human stanniocalcin 1 (STC1) plus 4 transitional amino acids in frame on the 5' side of the insertion site, and a sequence encoding 2 transitional amino acids and a 10 His tag in-frame at the 3' side of the insertion site. The resulting vector with the inserted Cln248 nucleic acid fragment encodes a recombinant Cln248 fusion protein with the STC1 secretion signal fused to the N-terminus and the 10 His-tag fused to the C-terminus of the Cln248 protein fragment (Val30-His300). This recombinant plasmid encoding the Cln248 His-tagged protein is herein referred to as "Cln248 Construct 2". A representative amino acid sequence encoded by Cln248 Construct 2 is presented in SEQ ID NO:5.

Cln248 Construct 1 Amino Acid Sequence (SEQ ID NO:3)

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    1          11         21         31         41         51
    |          |          |          |          |          |
1   MRALEGPGLS LLCLVLALPA LLPVPAVRGV AETPTYPWRD AETGERLVCA QCPPGTFVQR
61  PCRRDSPTTC GPCPPRHYTQ FWNYLERCRY CNVLCGEREE EARACHATHN RACRCRTGFF
121 AHAGFCLEHA SCPPGAGVIA PGTPSQNTQC QPCPPGTFSA SSSSSEQCQP HRNCTALGLA
181 LNVPGSSSHD TLCTSCTGFP LSTRVPGAEE CERAVIDFVA FQDISIKRLQ RLLQALEAPE
241 GWGPTPRAGR AALQLKLRRR LTELLGAQDG ALLVRLLQAL RVARMPGLER SVRERFLPVH
301 ASHHHHHHHH HH
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Cln248 Construct 2 Amino Acid Sequence (SEQ ID NO:5)

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    1          11         21         31         41         51
    |          |          |          |          |          |
1   MLQNSAVLLV LVISASATHE AEQSRMHVAE TPTYPWRDAE TGERLVCAQC PPGTFVQRPC
61  RRDSPTTCGP CPPRHYTQFW NYLERCRYCN VLCGEREEEA RACHATHNRA CRCRTGFFAH
121 AGFCLEHASC PPGAGVIAPG TPSQNTQCQP CPPGTFSASS SSSEQCQPHR NCTALGLALN
181 VPGSSSHDTL CTSCTGFPLS TRVPGAEECE RAVIDFVAFQ DISIKRLQRL LQALEAPEGW
241 GPTPRAGRAA LQLKLRRRLT ELLGAQDGAL LVRLLQALRV ARMPGLERSV RERFLPVHAS
301 HHHHHHHHHH
```

[0307] The recombinant plasmids, Cln248 Construct 1 and Cln248 Construct 2, were used to independently transfect HEK293F cells in suspension culture (1-10 liter serum free medium) in spinner flasks. Culture medium was harvested at 48 hours post-transfection. Medium was concentrated 10-100 fold, and diafiltrated with 20 mM Tris/HCl, 500 mM NaCl, 10% glycerol, pH 7.8. Concentrated medium containing protein encoded by either Cln248 Construct 1 or Cln248 Construct 2 was passed through a 5-mL nickel metal chelating column (His-Select-Ni, Sigma Inc.), which had been previously equilibrated with 50 mM sodium phosphate, 1000 mM NaCl, 10% glycerol, pH 7.8. The column was then washed with 6 column volume (CV) of 50 mM sodium phosphate, 1000 mM NaCl, 20 mM imidazole, 10% glycerol, pH 7.8. Protein encoded by Cln248 Construct 1 and 2 was eluted from the column using 6 CV of 50 mM sodium phosphate, 500 mM NaCl, 10% glycerol, pH 7.7 containing 500 mM imidazole. Samples from collected fractions were subjected to SDS-PAGE and Western blot analysis for assessing the purity of the protein. Purified fractions were pooled and dialyzed against PBS, pH 7.4.

Lng108 Construct 1 Sequence and Protein Production

[0308] A nucleic acid molecule encoding the full length of Lng108 (Met1-Ala247), was inserted into a pCMV5His2 vector at the PmeI/NheI site. The nucleic acid molecule was isolated using the following primers:

(SEQ ID NO: 6)
5'primer: CTTTGTTTAAACATGCTCAAAACTCAGCAGTG

(SEQ ID NO: 7)
3'primer: CGGCTAGCTGCACTCTCATGGGATGTGCGTTTGA

[0309] The vector comprises a sequence encoding 2 transitional amino acids and a 10 His tag in-frame at the 3' end of the insertion site. The resulting vector with the inserted Lng108 nucleic acid fragment encodes a recombinant Lng108 fusion protein with the 10 His-tag fused to the C-terminus of the protein. This recombinant plasmid is herein referred to as "Lng108 Construct 1". A representative amino acid sequence encoded by Lng108 Construct 1 is presented in SEQ ID NO:8.

Lng108 Construct 1 Amino Acid Sequence (SEQ ID NO:8)

```
    1          11          21          31          41          51
    |          |           |           |           |           |
1   MLQNSAVLLV LVISASATHE AEQNDSVSPR KSRVAAQNSA EVVRCLNSAL QVGCGAFACL
61  ENSTCDTDGM YDICKSFLYS AAKFDTQGKA FVKESLKCIA NGVTSKVFLA IRRCSTFQRM
121 IAEVQEECYS KLNVCSIAKR NPEAITEVVQ LPNHFSNRYY NRLVRSLLEC DEDTVSTIRD
181 SLMEKIGPNM ASLFHILQTD HCAQTHPRAD FNRRRTNEPQ KLKVLLRNLR GEEDSPSHIK
241 RTSHESAASH HHHHHHHHH
```

[0310] The recombinant plasmid Lng108 Construct 1 was used to transfect HEK 293F cells in suspension culture (1-10 liter serum free medium) in a bioreactor. Culture medium was harvested at 48 hours post-transfection. Medium was concentrated 10-100 fold, and diafiltrated with 20 mM Tris/HCl, 500 mM NaCl, 5% glycerol, pH 7.8. Concentrated medium containing protein encoded by Lng108 Construct 1 was passed through a 10-mL nickel metal chelating column (His-Select-Ni, Sigma Inc.), which had been previously equilibrated with 50 mM sodium phosphate, 500 mM NaCl, 5% glycerol, pH 8.0. The column was then washed with 7 column volume (CV) of 50 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, 10% glycerol, pH 8.0. Protein encoded by Lng108 Construct 1 was eluted from the column using 9 CV of 50 mM sodium phosphate, 500 mM NaCl, 10% glycerol, pH 7.6 containing 50 mM imidazole and 10 CV of 50 mM sodium phosphate, 500 mM NaCl, 10% glycerol, pH 7.6 containing 100 mM imidazole. Samples from collected fractions were subjected to SDS-PAGE and Western blot analysis for assessing the purity of the protein. Purified fractions were pooled and concentrated.

Immunization

[0311] Eight BALB/c mice were immunized intradermally in both rear footpads with Cln248 Construct 1. All injections were 25 uL per foot. The first injection of 10 ug of antigen per mouse was in Dulbecco's phosphate buffered saline (DPBS) mixed in equal volume to volume ratio with Titermax gold adjuvant (Sigma, Saint Louis, Miss.). Subsequently, mice were immunized twice weekly for 5 weeks. For the 2^{nd} through 10^{th} injection, mice were immunized with 10 ug of antigen in 20 uL of DPBS plus 5 uL of Adju-phos adjuvant (Accurate Chemical & Scientific Corp., Westbury, N.Y.) per mouse. The final immunization consisted of 10 ug antigen diluted in DPBS alone.

Hybridoma Fusion

[0312] Four days after the final immunization, mice were sacrificed and draining lymph node (popliteal) tissue was collected by sterile dissection. Lymph node cells were dispersed using a Tenbroeck tissue grinder (Wheaton #347426, VWR, Brisbane, Calif.) followed by pressing through a sterile sieve (VWR) into DMEM and removing T-cells via anti-CD90 (Thy1.2) coated magnetic beads (Miltenyi Biotech, Bergisch-Gladbach, Germany).

[0313] These primary B-cell enriched lymph node cells were then immortalized by electro-cell fusion (BTX, San Diego, Calif.) with the continuous myeloma cell line P3x63Ag8.653. See Kearney, J. F. et al., J. Immunology 123: 1548-1550, 1979. The mycloma and B-cells were pooled at a 1:1 ratio for the fusion. These fusion cultures were distributed at 2 million cells per plate into wells of 96 well culture plates (Costar #3585, VWR). Successfully fused cells were selected by culturing in selection medium (DMEM/15% FBS) containing 2.85 μM Azaserine, 50 μM Hypoxanthine (HA) (Sigma) or 50 μM Hypoxanthine, 0.2 μM Aminopterin, 8 μM Thymidine (HAT) (Sigma) supplemented with recombinant human IL-6 (Sigma) at 0.5 ng/mL. Cultures were transitioned

into medium (DMEM/10% FBS) without selection or IL-6 supplements for continued expansion and antibody production.

[0314] Supernatants from wells were screened by enzyme linked solid phase immunoassay (ELISA) and the dissociation constant from Cln248 protein of antibodies was determined. Monoclonal cultures, consisting of the genetically uniform progeny from single cells, were established after the screening procedure, by sorting of single viable cells into wells of two 96 well plates, using flow cytometry (Coulter Elite; Beckman-Coulter, Miami, Fla.). The resulting murine B-cell hybridoma cultures were expanded using standard tissue culture techniques. Selected hybridomas were cryopreserved in fetal bovine serum (FBS) with 10% DMSO and stored in Liquid Nitrogen at −196° C. to assure maintenance of viable clone cultures.

Direct ELISA Screening & Selection of Hybridomas Producing Cln248 Specific Antibodies

[0315] Hybridoma cell lines were selected for production of Cln248 specific antibody by direct ELISA. Wells were coated with either protein encoded by Cln248 Construct 1 or Lng108 Construct 1 (negative control) as antigen. For plate coating, one ug/mL protein in PBS (100 uL/well) was incubated overnight in 96 well polystyrene EIA plates (Costar #9018, VWR) at 4° C. The plate wells were washed twice with Tris buffered saline with 0.05% Tween20, pH 7.4 (TBST). Nonspecific binding capacity was blocked by filling the wells (300 ul/well) with TBST/0.5% bovine serum albumin (TBST/BSA) and incubating for >30 minutes at room temperature (RT). The wells were emptied and filled with 50 uL/well TBST/BSA to prevent them from drying out during the sample collection process. Hybridoma culture medium sample (50 uL) was added to the wells coated with Cln248 Construct 1 protein or Lng108 Construct 1 protein and incubated for 1 hour at RT. The wells were washed 3 times with TBST. One hundred uL of alkaline phosphatase conjugated goat anti-mouse IgG (Fc) with minimal cross-reactivity to human Fc (P/N115-055-071, Jackson Immunoresearch), diluted 1:5000 in TBST/BSA, was added to each well and incubated for >1 hour at RT. The wells were washed 3 times with TBST. One hundred uL of alkaline phosphatase substrate para-nitrophenylphosphate (pNPP) (Sigma) at 1 mg/mL in 1 M Diethanolamine buffer pH 8.9 (Pierce) was added to each well and incubated for 20 min at RT. The enzymatic reaction was quantified by measuring the solution's absorbance at 405 nm wavelength on a Spectramax Plus384 plate reader from Molecular Devices (Sunnyvale, Calif.).

[0316] Supernatants from hybridomas that produced an absorbance value of greater than 1.0 in wells coated with Cln248 Construct 1 protein and less than 0.15 in wells coated with Lng108 Construct 1 protein demonstrated specific binding to Cln248. Hybridomas producing antibodies with the highest signal-to-noise ratios were selected for expansion.

Kinetics Analysis of Cln248 Antibody Hybridoma Supernatants

[0317] Dissociation constants (kd) were calculated from surface plasmon resonance measurements using a BIACORE 3000 instrument (BiaCore, Piscataway, N.J.). A RAM-Fc surface was used to capture each antibody supernatant, followed by an injection of the protein encoded by Cln248 Construct 1 over the captured antibody.

[0318] Flow cell 1 of a CM5 sensor chip (BiaCore) was used as a blank surface for reference subtractions, and was activated and then inactivated with ethanolamine per standard BiaCore protocols. Flow cell 2 was used to immobilize RAM Fc using an injection time of 12 minutes and a flow of 5 ul/min. The RAM-FC (BiaCore) was diluted to 35 ug/mL in 10mM acetate as suggested. Standard amine coupling (BiaCore) was used to immobilize 10349 RU. Hybridoma supernatants were diluted 1:2 in HBS-EP running buffer (BiaCore) and passed over flow cells 1 and 2. Antibodies were captured at 5 ul/min flow rate, 3 minute injection, and Cln248 Construct 1 protein was injected at 5 ug/mL for 2 minutes. The dissociation time was 3 minutes. The regeneration of the chip surface, or removal of captured hybridoma supernatants binding to the antigen between cycles, was performed by injecting 10mM glycine pH 1.75 for 30 seconds at 100 uL/minute.

[0319] The above procedure was performed by using the BiaCore's surface preparation and binding wizard included in the BiaCore control software. The off-ranking results presented in Table 1 below were automatically fitted using the separate ka/kd function included in the BiaCore analysis software, assuming a 1:1 Langmuir binding model.

[0320] It has been previously disclosed that typical association rates for protein-protein complexes, specifically including antibody-protein complexes, are on the order of 105 to 10^6 M^{-1} s^{-1}. See Seewald et al. (Molecular and Cellular Biology, November 2003, p. 8124-8136, Vol. 23, No. 22). Based on this, the binding affinity for the Cln248 antibodies were determined.

[0321] The table below includes the Cln248 antibody producing hybridoma (Hyb), the response unit of antigen binding (Unit), the dissociation constant (kd), and the calculated binding affinity (KD) range.

TABLE 1

Antibody kinetics analysis of Cln248-specific hybridoma supernatants

Hyb	Unit	kd	KD
A1	119.5	9.60E−04	9.60E−09-9.60E−10
A5	543.9	3.73E−04	3.73E−09-3.73E−10
A6	504.2	1.36E−05	1.36E−10-1.36E−11
A8	414.9	4.13E−05	4.13E−10-4.13E−11
A9	435.2	8.59E−05	8.59E−10-8.59E−11
A10	603.0	1.27E−04	1.27E−09-1.27E−10
A11	112.6	1.37E−03	1.37E−08-1.37E−09
A12	148.2	6.38E−04	6.38E−09-6.38E−10
A13	481.7	1.87E−04	1.87E−09-1.87E−10
A14	354.5	1.23E−03	1.23E−08-1.23E−09
A15	503.5	5.55E−04	5.55E−09-5.55E−10
A16	442.9	5.58E−04	5.58E−09-5.58E−10
A18	110.2	4.49E−04	4.49E−09-4.49E−10
A19	116.5	2.22E−04	2.22E−09-2.22E−10
A21	393.8	3.04E−04	3.04E−09-3.04E−10
A22	354.6	3.86E−05	3.86E−10-3.86E−11
A23	215.8	2.13E−04	2.13E−09-2.13E−10
A24	123.2	4.79E−04	4.79E−09-4.79E−10
A25	521.7	3.53E−04	3.53E−09-3.53E−10
A26	423.5	3.92E−04	3.92E−09-3.92E−10
A27	528.5	3.55E−05	3.55E−10-3.55E−11
A28	107.9	1.11E−04	1.11E−09-1.11E−10
A29	426.5	3.50E−04	3.50E−09-3.50E−10
A30	432.5	3.14E−04	3.14E−09-3.14E−10
A31	90.7	1.99E−04	1.99E−09-1.99E−10
A32	102.4	7.97E−04	7.97E−09-7.97E−10
A33	113.8	5.10E−04	5.10E−09-5.10E−10
A36	160.4	4.97E−04	4.97E−09-4.97E−10

TABLE 1-continued

Antibody kinetics analysis of Cln248-specific hybridoma supernatants

Hyb	Unit	kd	KD
A37	292.0	1.29E−04	1.29E−09-1.29E−10
A38	125.9	3.18E−04	3.18E−09-3.18E−10
A39	210.2	1.38E−04	1.38E−09-1.39E−10
A40	125.2	8.30E−04	8.30E−09-8.30E−10
A41	202.8	2.19E−04	2.19E−09-2.19E−10
A42	114.3	1.18E−03	1.18E−08-1.18E−09
A44	258.8	1.28E−03	1.28E−08-1.28E−09
A45	359.8	7.54E−05	7.54E−10-7.54E−11
A46	76.4	5.43E−04	5.43E−09-5.43E−10

The dissociation constants of anti-Cln248 antibodies were evaluated to select antibodies for use in ELISAs. Anti-Cln248 antibodies with nanoMolar affinity (at least 10^{-9}) are useful as therapeutic agents. Specifically, Cln248.A1, Cln248.A6, Cln248.A8, Cln248.A9, Cln248.A12, Cln248.A15, Cln248.A16, Cln248.A18, Cln248.A22, Cln248.A24, Cln248.A25, Cln248.A26, Cln248.A27, Cln248.A29, Cln248.A32, Cln248.A33, Cln248.A36, Cln248.A40, Cln248.A45 and Cln248.A45 are useful as therapeutic agents alone or in combination for the treatment of cancer.

Cloning of Hybridomas Producing Cln248 Specific MAb

[0322] Based on data from ELISA and Biacore binding experiments above, the following hybridomas were selected for single cell cloning into 96 well culture plates by cell sorting (Coulter Elite): Cln248.A6, Cln248.A8, Cln248.A9, Cln248.A10, Cln248.A12, Cln248.A13, Cln248.A15, Cln248.A16, Cln248.A19, Cln248.A21, Cln248.A22, Cln248.A23, Cln248.A27, Cln248.A28, Cln248.A30, Cln248.A32, Cln248.A39, Cln248.A40, Cln248.A41, and Cln248.A45. After 2 weeks of culture, serial dilutions of supernatants from selected clones from each parent hybridoma were tested by direct ELISA on wells coated with Cln248 Construct 1 protein and Lng108 Construct 1 protein (negative control) as described above. Absorbance at 405 nm is reported for antibodies in wells coated with Cln248 Construct 1 protein in tables 2A and 2B below.

TABLE 2A

Direct ELISA with Anti-Cln248 Ab Hybridoma Supernatants

	Serial Dilution of Supernatant							
Clone	undiluted	1:3	1:9	1:27	1:81	1:243	1:729	1:2187
Cln248.A6.4	1.519	1.766	1.835	1.693	1.597	1.183	0.774	0.451
Cln248.A8.1	1.231	1.366	1.402	1.358	1.292	1.048	0.651	0.365
Cln248.A9.3	1.097	1.327	1.229	1.127	0.920	0.677	0.377	0.211
Cln248.A12.2	0.830	0.885	0.688	0.433	0.216	0.131	0.102	0.094
Cln248.A15.1	1.513	1.565	1.638	1.579	1.291	0.823	0.471	0.257
Cln248.A16.1	1.545	1.615	1.537	1.247	0.749	0.387	0.206	0.129

TABLE 2B

Direct ELISA with Anti-Cln248 Ab Hybridoma Supernatants

	Serial Dilution of Supernatant							
Clone	undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cln248.A22.1	0.115	0.197	0.190	0.168	0.154	0.124	0.108	0.089
Cln248.A22.2	0.348	0.612	0.486	0.305	0.187	0.127	0.103	0.096
Cln248.A27.1	1.269	1.403	1.369	1.377	1.287	0.889	0.516	0.279
Cln248.A32.2	0.418	0.371	0.287	0.218	0.148	0.119	0.095	0.091
Cln248.A40.1	1.233	1.229	1.242	1.112	0.874	0.567	0.310	0.188
Cln248.A10.3	1.162	1.174	1.150	1.134	1.095	1.148	1.138	1.113
Cln248.A13.4	1.265	1.230	1.223	1.163	1.167	1.251	1.171	1.219
Cln248.A19.4	0.620	0.062	0.602	0.547	0.536	0.463	0.445	0.345
Cln248.A21.1	1.331	1.432	1.437	1.391	1.392	1.402	1.338	1.316
Cln248.A23.4	0.834	0.863	0.825	0.813	0.812	0.820	0.825	0.890
Cln248.A30.2	1.256	1.275	1.271	1.291	1.313	1.132	0.963	0.817
Cln248.A41.3	1.304	1.205	1.175	1.138	1.119	1.143	1.166	1.171

Cln248 MAb Isotypes

[0323] The isotypes of anti-Cln248 MAbs were determined using commercially available mouse monoclonal antibody isotyping immunoassay test kits (IsoStrip, Roche Diagnostic Corp., Indianapolis, Ind.). Results of the isotyping are listed in Table 3.

TABLE 3

Cln248 MAb Isotypes

Clone	Isotype
Cln248.A10.3	IgG1 kappa
Cln248.A22.1	IgG1 kappa

Cln248 A-series MAb Checkerboard ELISA

[0324] High binding polystyrene plates (Corning Life Sciences) were coated overnight at 4° C. with 1 μg/well of a first anti-Cln248 MAb. The coating solution was aspirated off and free binding sites were blocked with 300 μl/well Superblock-TBS (Pierce Biotechnology, Illinois) on a shaker for 1 hour at room temperature (RT). After washing 4 times with washing buffer (TBS+0.1% Tween2), 75 μl of Assay Buffer (TBS, 1% BSA, 1% Mouse Serum, 1% Calf Serum, and 0.1% proclin) was added to each well and then 25 μl of antigen was added for 60 minutes incubation on shaker. For each sandwich ELISA, standards of specified concentrations of recombinant Cln248 Construct 2 protein were run in parallel with test samples. Standards and test samples were diluted in TBS with 1% BSA. For detection, 100 μl of a second biotinylated MAb (0.5 μg/ml) was added to each well and incubated for 1 hour at room temperature (RT), while shaking. After washing, 100 μL of Streptavidin-BP conjugate (Jackson Lab) at 1:40,000 dilution in TBS, was added to each well. Plates were then incubated with shaking at RT for 30 min. After washing the plate, 100 uL/well of TMB plus substrate (DAKO) was added to each well and the plate was incubated at RT, covered and on the shaker for 30 minutes. The reaction was stopped using 100 μl/well 1N HCl, and the plates were read at 450 nm using a Spectramax 190 plate reader (Molecular Devices).

[0325] Cln248-A Series MAb ELISA Pairing Results

[0326] The results of the checkerboard ELISA on anti-Cln248 MAbs are shown in the Table 4 below. Each antibody was tested as both a coating and detecting antibody, in all possible combination. All pairs were tested in duplicate with 10 ng/ml of recombinant Cln248 Construct 2 protein in buffer or with buffer alone as a blank (negative control).

[0327] The results in table 4 below are shown as specific signal/noise ratio. Capturing MAbs are listed on the Y-axis with detecting MAbs on the X-axis.

TABLE 4

Pairing of Cln248 A-series MAb by Sandwich ELISA

		detecting 2° MAb								
CLN248		A6.4	A8.1	A9.3	A10.3	A13.4	A15.1	A16.1	A19.4	A21.1
coating 1° MAb	A6.4	1	5	2	1	1	1	1	2	1
	A8.1	23	2	3	40	7	5	2	3	26
	A9.3	28	3	1	43	23	10	4	4	29
	A10.3	1	4	3	1	14	1	1	2	1
	A13.4	23	3	3	31	3	4	2	2	24
	A15.1	1	4	3	1	14	1	1	2	1
	A16.1	1	6	6	1	10	1	1	1	1
	A19.4	9	2	3	14	3	2	2	1	13
	A21.1	1	4	4	1	12	1	1	2	1
	A22.2	19	23	4	25	19	3	2	3	19
	A23.4	21	1	2	44	5	5	3	2	26
	A30.2	1	3	3	1	7	1	1	2	1
	A32.3	5	3	3	11	3	2	2	2	7
	A40.1	8	2	2	14	4	2	1	2	9
	A41.3	1	1	1	1	1	1	1	1	7

		detecting 2° MAb					
CLN248		A22.2	A23.4	A30.2	A32.3	A40.1	A41.3
coating 1° MAb	A6.4	1	6	4	1	1	3
	A8.1	16	3	22	2	1	3
	A9.3	3	3	30	4	1	6
	A10.3	1	4	4	1	1	3
	A13.4	2	2	18	2	1	3
	A15.1	1	4	3	1	1	2
	A16.1	2	6	3	1	1	3
	A19.4	1	2	9	1	1	2
	A21.1	2	6	4	1	1	3
	A22.2	1	29	28	2	1	4
	A23.4	11	2	21	2	1	3
	A30.2	6	4	1	1	1	2
	A32.3	1	5	8	1	1	2
	A40.1	1	2	9	1	1	2
	A41.3	1	3	8	1	1	1

[0328] Results from the ELISA pairing demonstrate that the anti-Cln248 MAbs detect several distinct epitopes. An epitope map of the Cln248 MAbs derived from the pairing results is shown in FIG. **1**. Antibody pairs with the highest signal/noise ratio were selected to test sensitivity for recombinant protein, reactivity towards native protein in cell lines and serum samples.

Cln248 Sandwich ELISA Formats and Standard Curves

[0329] Prior to screening ELISA antibody pairs, anti-Cln248 antibodies from clones 0.1 (Cln248.A22.1) and 0.2 (Cln248.A22.2) of hybridoma Cln248.A22 were evaluated in a sample set for use in ELISAs. As a result, the Cln248.A22.1 antibody was selected for use in the ELISA format and analyses below.

[0330] In the screening and pairing assays above, anti-Cln248 antibody pairs (capture/detect) A8.1/A6.4 and A22.1/A10.3 demonstrated high specificity and excellent sensitivity for detection of Cln248. These antibody pairs were selected for use in sandwich ELISA for detection of Cln248. Protocols for both sandwich ELISA assay are similar to the general protocol as described above except for minor modifications in concentrations of standards and detection system.

[0331] For the A8.1/A6.4 ELISA pair (FIG. **2**): standards were run at concentrations of 10, 2, 1, 0.5, 0.2 and 0 ng/ml in parallel with the samples. A sensitive detection system based on the use of alkaline phosphatase (AP, 1:2000 dilution) and a high sensitivity pNPP substrate (Pierce) was used.

[0332] For the A22.1/A10.3 ELISA pair (FIG. **3**): standards were run at concentrations of 5, 2, 0.5, 0.2, 0.05 and 0 ng/ml in parallel with the samples. A sensitive detection system based on the use of horseradish peroxidase (HRP, 1:40,000 dilution) and a high sensitivity TMB plus substrate (Dako) was used.

[0333] The minimal detectable concentration (MDC) for Cln248 in the two ELISA formats, A8.1/A6.4 and A22.1/A10.3, was determined to be 300 pg/ml and 35 pg/ml, respectively. For calculation of median values, samples with values below the MDC were defined as MDC. The MDC is defined as two standard deviations above the background signal of blank samples (n=4). Standard curves for A8.1/A6.4 and A22.1/A10.3 assay formats are depicted in FIGS. **2** and **3**, respectively.

[0334] The MDC for the Cln248 A22.1/A10.3 ELISA was further evaluated with a larger data set (N=96 blanks) across 2 plates for greater accuracy and determined to be 21 pg/mL. The MDC was calculated by the accepted industry standard method which takes into account the variability of the background of the assay in its detection ability and incorporates the systems noise into a fixed value, which allows one to say with confidence that the assay can detect a certain low end signal. For an overview see the MDC description at the MARSSIM home on the EPA website (epa with the extension .gov/radiation/marssim/faqsforusers.htm#faq3_1 of the world wide web). To ascertain a high level of confidence in the assays minimum measurement, the MDC was calculated as 2 standard deviations above the mean value of the blank. The MDC for the Cln248 A22.1/A10.3 ELISA was determined to be 21 pg/mL. The detection range of the Cln248 ELISA is 21 pg/mL to at least 5000 pg/mL.

[0335] The MDC of the A22.1/A10.3 Cln248 ELISA is 41% lower than that of the DcR3 ELISA described by Chen et al. (Journal of Immunological Methods 285 (2004) 63-70) which had a detection limit of 36 pg/mL. This increased ELISA sensitivity of the A22.1/A10.3 ELISA is advantageous for detecting smaller quantities of Cln48 in samples and discriminating between normal and elevated levels of Cln248 in low abundance samples or dilutions.

Example 2

Monoclonal Sandwich ELISA Detection of Cln248 in Human Serum Samples

Human Serum Samples

[0336] To evaluate the expression of Cln248 in individuals with cancer, serum sample panels were created.

[0337] Serum Sample Panel 1

[0338] For panel 1, human cancer and benign serum samples were obtained from IMPATH-BCP, Inc. (Franklin, Mass.), Diagnostic Support Services, Inc. (West Yarmouth, Mass.) and ProteoGenex (Culver City, Calif.). The serum samples from healthy men and women were obtained from ProMedDx LLC (Norton, Mass.). All samples were aliquoted upon arrival and stored at minus 80° C. until use.

[0339] The concentration of Cln248 was measured in more than 2685 serum samples from normal/healthy individuals, individuals with lung, breast, colon, prostate or ovarian cancer and individuals with non-cancerous, benign diseases. Benign diseases are grouped by tissue type and include: A. Hyperplasia, Fibroadenoma, and Fibrocystic Breasts for Breast; Crohn's, Diverticulitis, Ulcerative Colitis, and Polyps for Colon; Asthma, Chronic Bronchitis, Emphysema, Interstitial Lung Disease, and Pulmonary Hypertension for Lung; Endometriosis, Enlarged Ovaries, and Polycystic Ovaries for Ovarian; Benign Prostatic Hyperplasia, Prostatic Intraepithelial Neoplasia, and Prostatitis for Prostate. An overview of all samples tested is listed in the table 5 below.

TABLE 5

Summary of serum samples:

Sample Type	No. of Samples
Normal	684 (344-M, 340-F)
Breast Cancer	234
Breast Benign	180
Colon Cancer	176
Colon Benign	260
Lung Cancer	297
Lung Benign	250
Ovarian Cancer	223
Ovarian Benign	150
Prostate Cancer	299
Prostate Benign	335

[0340] Serum Sample Panel 2

[0341] For panel 2, eighty (80) human ovarian cancer and 80 normal female serum samples were obtained, handled and stored as outlined in serum panel 1 samples.

Detection of Cln248 in Serum with Sandwich ELISAs (panel 1)

[0342] The following tables demonstrate detection of Cln248 in various cancer, diseased and normal serum samples from panel 1.Samples are grouped by type and identified by tissue and disease state of the tissue. Tissue annotation includes: BR=Breast, CN=Colon, LN=Lung, OV=Ovarian, and PR=Prostate. Disease states may be specifically indicated or abbreviated into groups as: CAN=Cancer and BEN=Benign. Samples from non-diseased men and women are annotated as NRM Male (NRM M) and NRM Female (NRM F), respectively. For example, BR CAN indicates breast cancer samples and CN BEN indicates benign colon disease samples.

[0343] Benign Diseases are abbreviated as: A. Hyperplasia (AHYP), Fibroadenoma (FBAD), Fibrocystic Breasts (FBCY), Crohn's (CHRN), Diverticulitis (DVCT), Ulcerative Colitis (UCOL), Polyps (PLYP), Asthma (ASMA), Chronic Bronchitis (CBRN), Emphysema (EMPH), Interstitial Lung Disease (ILD), Pulmonary Hypertension (PLEBP), Endometriosis (ENDO), Enlarged Ovaries (ENOV), Polycystic Ovaries (PCYS), Benign Prostatic Hyperplasia (BPH), Prostatic Intraepithelial Neoplasia (PIN), and Prostatitis (PRST).

[0344] Cln248 A8.1/A 6.4 MAb ELISA Results

[0345] The concentration of Cln248 in serum from 684 healthy individuals and 1127 individuals with cancer was determined with the Cln248 A8.1/A6.4 MAb ELISA. Table 6 below shows the number of samples tested in each group of individuals, the minimum and maximum detected Cln248 concentration, the median Cln248 concentration, and the 25^{th} and 75^{th} percentile concentration of Cln248 in each group. Elevated levels of Cln248 were observed in individuals with colon, lung, ovarian and prostate cancer.

TABLE 6

Cln248 Levels (ng/mL) in Normal and Cancer Samples (A8.1/A6.4 MAb ELISA)

	NRM F	NRM M	BR CAN	CN CAN	LN CAN	OV CAN	PR CAN
Number of values	344	340	234	125	297	223	248
Minimum	0.08	0.14	0.04	0.27	0.15	0.31	0.38
25% Percentile	0.68	0.74	0.66	0.91	1.00	1.00	1.06
Median	1.03	1.07	1.01	1.55	1.52	1.45	1.47
75% Percentile	1.34	1.49	1.89	2.74	2.20	2.09	2.06
Maximum	26.53	24.49	42.70	16.14	12.31	7.03	24.39

[0346] The concentration of Cln248 was also measured in serum samples from individuals with various benign diseases with the Cln248 A8.1/A6.4 MAb ELISA. Tables 7A, 7B and 7C below show the number of samples tested in each group (listed above), the minimum and maximum detected Cln248 concentration, the median Cln248 concentration, and the 25^{th} and 75^{th} percentile concentration of Cln248 in each group.

TABLE 7A

Cln248 Levels (ng/mL) in Cancer and Benign Samples (A8.1/A6.4 MAb ELISA)

				BR BEN					CN BEN				
	NRM F	NRM M	BR CAN	All BR BEN	AHYP	FBAD	FBCY	CN CAN	All CN BEN	CHRN	DVCT	UCOL	PLYP
n #	344	340	234	180	56	62	62	125	200	50	50	50	50
Min	0.08	0.14	0.04	0.08	0.08	0.09	0.09	0.27	0.17	0.17	0.24	0.46	0.39
25^{th} %	0.68	0.774	0.66	0.114	0.1175	0.1105	0.1115	0.908	0.7625	0.5683	0.7908	0.8223	0.8148
Med	1.03	1.11	1.01	0.13	0.14	0.13	0.13	1.55	1.20	1.00	1.25	1.38	1.21
75^{th} %	1.40	1.595	1.89	0.15	0.16	0.15	0.15	2.74	1.71	1.42	1.69	1.95	1.79
Max	26.53	24.49	42.7	0.97	0.41	0.97	0.22	16.14	8.95	3.57	7.92	8.95	4.55

TABLE 7B

Cln248 Levels (ng/mL) in Cancer and Benign Samples (A8.1/A6.4 MAb ELISA)

				LN BEN					
	NRM F	NRM M	LN CAN	All LN BEN	ASMA	CBRN	EMPH	ILD	PLHP
n #	344	340	297	250	50	50	50	50	50
Min	0.08	0.14	0.15	0.17	0.36	0.34	0.17	0.33	0.36
25^{th} %	0.68	0.77	1.00	0.87	1.04	0.74	0.81	0.77	1.00
Med	1.03	1.11	1.52	1.28	1.29	1.58	1.12	1.03	1.94
75^{th} %	1.40	1.59	2.20	2.02	2.09	2.11	1.60	1.53	3.43
Max	26.53	24.49	12.31	9.98	9.98	3.66	4.53	2.67	5.25

TABLE 7C

Cln248 Levels (ng/mL) in Cancer and Benign Samples (A8.1/A6.4 MAb ELISA)

				OV BEN					PR BEN			
	NRM F	NRM M	OV CAN	All OVR BEN	ENDO	ENOV	PCYS	PR CAN	All PR BEN	BPH	PIN	PRST
n #	344	340	223	150	50	50	50	248	291	127	35	129
Min	0.08	0.14	0.31	0.26	0.39	0.54	0.26	0.38	0.16	0.23	0.74	0.16
25th %	0.68	0.77	1.00	0.73	0.63	0.87	0.72	1.06	0.86	0.86	0.88	0.82
Med	1.03	1.11	1.45	0.92	0.83	1.09	0.82	1.47	1.18	1.17	1.25	1.18
75th %	1.40	1.59	2.09	1.13	0.93	1.30	1.09	2.06	1.69	1.70	1.57	1.70
Max	26.53	24.49	7.03	3.47	2.20	3.47	1.86	24.39	46.62	27.96	46.62	31.82

[0347] Elevated levels of Cln248 were observed in individuals with colon, ovarian, lung and prostate cancer. Cln248 levels were not elevated significantly in individuals with colon, ovarian or prostate benign conditions. These results demonstrate that the Cln248A8.1/A6.4 MAb ELISA is able to determine Cln248 levels and discriminate individuals with colon, ovarian, lung and prostate cancers from individuals without disease and individuals with benign diseases.

[0348] Cln248A22.1/A10.3 MAb ELISA Results

[0349] Using the Cln248 A22.1/A10.3 MAb ELISA Cln248 levels were measured in serum from healthy individuals, individuals with colon or ovarian cancer and individuals with various colon or ovarian benign conditions (described above). Table 8 below shows the number of samples tested in each group (listed above), the minimum and maximum detected Cln248 concentration, the median Cln248 concentration, and the 25th and 75th percentile concentration of Cln248 in each group. Elevated levels of Cln248 were observed in individuals with colon and ovarian cancers.

TABLE 8

Cln248 Levels (ng/ml) in Colon and Ovarian Samples (A22.1/A10.3 MAb ELISA)

	NRM F	NRM M	CN CAN	CN BEN	OV CAN	OV BEN
Number of values	308	307	47	31	96	149
Minimum	0.09	0.06	0.16	0.19	0.24	0.09
25% Percentile	0.36	0.42	0.37	0.35	0.61	0.44
Median	0.48	0.53	0.62	0.44	0.85	0.59
75% Percentile	0.62	0.74	1.21	1.04	1.49	0.78
Maximum	3.84	5.72	2.64	16.56	2.93	1.71

[0350] The concentration of Cln248 was also measured in serum samples from individuals with various benign diseases with the Cln248 A22.1/A10.3 MAb ELISA. Tables 9A, 9B and 9C below show the number of samples tested in each group (listed above), the minimum and maximum detected Cln248 concentration, the median Cln248 concentration, and the 25th and 75th percentile concentration of Cln248 in each group.

TABLE 9A

Cln248 Levels (ng/ml) in Cancer and Benign Samples (A22.1/A10.3 MAb ELISA)

				CN BEN				
	NRM F	NRM M	CN CAN	All CN BEN	CHRN	DVCT	PLYP	UCOL
Number of values	308	307	47	31	7	8	8	8
Minimum	0.09	0.06	0.16	0.19	0.24	0.19	0.28	0.36
25% Percentile	0.36	0.42	0.37	0.35	0.29	0.31	0.34	0.43
Median	0.48	0.53	0.62	0.44	0.57	0.37	0.79	0.71
75% Percentile	0.62	0.74	1.21	1.04	1.00	0.42	1.85	1.30
Maximum	3.84	5.72	2.64	16.56	11.62	0.71	16.56	3.70

TABLE 9B

Cln248 Levels (ng/ml) in Cancer and Benign Samples (A22.1/A10.3 MAb ELISA)

			OV BEN			
	NRM F	OV CAN	All OV BEN	ENDO	ENOV	PCYS
Number of values	308	96	149	50	50	49
Minimum	0.09	0.24	0.09	0.09	0.23	0.22
25% Percentile	0.36	0.61	0.44	0.40	0.48	0.44
Median	0.48	0.85	0.59	0.56	0.64	0.60
75% Percentile	0.62	1.49	0.78	0.73	0.82	0.88
Maximum	3.84	2.93	1.71	1.57	1.71	1.37

TABLE 9C

Cln248 Levels (ng/ml) in Prostate Cancer and Benign Samples and Lung Cancer Samples (A22.1/A10.3 MAb ELISA)

				PR BEN			
	NRM F	NRM M	PR CAN	All PR BEN	BPH	PRST	LN CAN
Number of values	308	307	51	144	64	10	70
Minimum	0.09	0.06	0.06	0.04	0.04	0.76	0.04
25% Percentile	0.36	0.42	0.61	0.42	0.32	0.90	0.48
Median	0.48	0.53	0.82	0.62	0.56	1.14	0.67
75% Percentile	0.62	0.74	1.05	1.03	0.90	2.26	0.98
Maximum	3.84	5.72	8.45	9.02	8.62	2.45	9.02

[0351] Elevated levels of Cln248 were observed in individuals with colon, ovarian and prostate cancer but not those with colon, ovarian and prostate benign conditions. Elevated levels of Cln248 were also observed in individuals with lung cancer. These results obtained with the Cln248 A22.1/A10.3MAb ELISA are in agreement with the results using the Cln248 A8.1/A6.4 MAb ELISA. This demonstrates that the Cln248 A22.1/A10.3MAb ELISA as well as the Cln248 A8.1/A6.4 MAb ELISA is able to determine Cln248 levels in samples and identify individuals with cancer from individuals without disease and individuals with benign diseases.

Correlation between Cln248 MAb ELISA (A8.1/A6.4 and A22.1/A10.3).

[0352] The correlation between the Cln248 values observed in the serum samples from panel 1 using the two assay formats, A8.1/A6.4 ELISA and A22.1/A10.3 ELISA, was determined using standard methods. Plotting the values for the samples tested in each assay resulted in a line with the following equation: $y=1.874x-0.177$. The R^2 value was 0.917 indicating that the A22.1/A10.3 MAb Cln248 assay correlated well with the A8.1/A6.4 MAb Cln248 assay.

Detection of Cln248 in Serum with Sandwich ELISA (Panel 2)

[0353] The following tables demonstrate detection of Cln248 in ovarian cancer and normal serum female samples from panel 2.

[0354] Cln248 A22.1/10.3 MAb ELISA Results

[0355] Using the Cln248 A22.1/A10.3 MAb ELISA Cln248 levels were measured in serum from healthy women and individuals with ovarian cancer. Table 10 below shows the number of samples tested in each group, the minimum and maximum detected Cln248 concentration, the median Cln248 concentration, and the 25^{th} and 75^{th} percentile concentration of Cln248 in each group. Elevated levels of Cln248 were observed in individuals with ovarian cancers.

TABLE 10

Cln248 Levels (ng/ml) in Ovarian Samples (A22.1/A10.3 MAb ELISA)

	NRM F	OV CAN
Number of values	80	80
Minimum	0.048	0.278
25% Percentile	0.340	0.534
Median	0.428	0.737
75% Percentile	0.534	1.169
Maximum	4.027	7.383

[0356] Elevated levels of Cln248 were observed in individuals with ovarian cancer compared to normal female samples. These results obtained in samples from panel 2 using the Cln248 A22.1/A10.3MAb ELISA (Table 10) are in agreement with the results from normal female and ovarian cancer samples in pane 1 using the same Cln248 A22.1/A10.3 MAb ELISA (Table 8). This demonstrates that the Cln248 A22.1/A10.3MAb ELISA is able to determine Cln248 levels in various samples and identify individuals with cancer from individuals without disease and individuals with benign diseases.

Example 3

ROC Analysis of Cln248 Levels in Serum

[0357] The ability of a test or assay to discriminate diseased cases from normal cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis (Metz, 1978; Zweig & Campbell, 1993). ROC curves can also be used to compare the diagnostic performance of two or more laboratory or diagnostic tests (Griner et al., 1981).

[0358] ROC curve is generated by plotting sensitivity against specificity for each value. From the plot, the area under the curve (AUC) can be determined. The value for the area under the ROC curve (AUC) can be interpreted as follows: an area of 0.84, for example, means that a randomly selected positive result has a test value larger than that for a randomly chosen negative result 84% of the time (Zweig & Campbell, 1993). When the variable under study can not distinguish between the two result groups, i.e. where there is no difference between the two distributions, the area will be equal to 0.5 (the ROC curve will coincide with the diagonal). When there is a perfect separation of the values of the two groups, i.e. there no overlapping of the distributions, the area under the ROC curve equals 1 (the ROC curve will reach the upper left corner of the plot).

[0359] The 95% confidence interval for the area can be used to test the hypothesis that the theoretical area is 0.5. If the confidence interval does not include the 0.5 value, then there is evidence that the laboratory test has the ability to distinguish between the two groups (Hanley & McNeil, 1982; Zweig & Campbell, 1993).

ROC Analysis of Cln248 A8.1/A6.4 MAb ELISA

[0360] Serum Sample Panel 1 ROC Analysis

[0361] For the Cln248 A8.1/A6.4 MAb ELISA sensitivity and specificity for detecting cancer in samples from serum sample panel 1 was calculated through receiver operating characteristic (ROC) analysis. Table 11 below shows Area Under the Curve (AUC) from the ROC analysis of Cln248

levels in individual cancer samples versus the normal healthy samples and benign disease samples from the corresponding organ. AUC values were calculated with Cln248 concentration levels in serum panel 1 samples described above using the A8.1/A6.4 MAb ELISA.

TABLE 11

Cln248 (A8.1/A6.4 MAb ELISA) AUC Values for Various Cancers

Cancerous Tissue	AUC
Colon	0.68
Ovarian	0.69
Lung	0.65
Prostate	0.73
Breast	0.50

[0362] The AUC values for Cln248 from the A8.1/A6.4 MAb ELISA and carcinoembryonic antigen (CEA), a known cancer marker, were evaluated as individual markers in colon cancer samples versus all normal and colon benign samples. As shown in table 12 below, the AUC for Cln248 is comparable to the AUC of carcinoembryonic antigen (CEA) for all colon cancer samples (0.65). However, in early stage colon cancers (stages I and II), Cln248 showed significantly better sensitivity and specificity (AUC=0.74) than CEA (AUC=0.62) in detecting colon cancer. Additionally, when evaluated together in multivariate analysis, Cln248 and CEA were complementary and achieved an increased AUC of 0.72 in all colon cancer samples.

TABLE 12

AUC Values for Cln248 (A8.1/A6.4 MAb ELISA) and CEA

	Marker AUC Values		
Samples	Cln248	CEA	Cln248 + CEA
All Colon Cancer	0.65	0.65	0.72
Stage I/II Colon Cancer	0.74	0.62	n/a

ROC Analysis of Cln248 A22.1/A10.3 MAb ELISA

[0363] Serum Sample Panel 1 ROC Analysis

[0364] For the Cln248 A22.1/A10.3 MAb ELISA sensitivity and specificity for detecting cancer in samples from serum sample panel 1 was calculated through receiver operating characteristic (ROC) analysis. Table 13 below shows Area Under the Curve (AUC) from the ROC analysis of Cln248 levels in individual cancer samples versus the normal healthy samples and benign disease samples from the corresponding organ. AUC values were calculated with Cln248 concentration levels in serum panel 1 samples described above using the A22.1/A10.3 MAb ELISA.

TABLE 13

Cln248 (A22.1/A10.3 ELISA) AUC Values for ROC Analysis for Various Cancers

Cancerous Tissue	AUC
Colon	0.76
Ovarian	0.79
Lung	na

TABLE 13-continued

Cln248 (A22.1/A10.3 ELISA) AUC Values for ROC Analysis for Various Cancers

Cancerous Tissue	AUC
Prostate	0.71
Breast	na

[0365] The AUC values for Cln248 from the A22.1/A10.3 MAb ELISA and CEA a known cancer marker, were evaluated as individual markers and in combination in colon cancer samples versus all normal and colon benign samples. As shown in table 14 below, the AUC for Cln248 is significantly better than the AUC of CEA for all colon cancer samples. This holds true when comparing the markers in early stage colon cancers (stages I and II) to both the normal and colon benign samples.

TABLE 14

AUC Values for Cln248 (A22.1/A10.3 MAb ELISA) and CEA

	Marker AUC Values		
Samples	Cln248	CEA	Cln248 + CEA
All Colon Cancer	0.77	0.64	0.76
Stage I/II Colon Cancer	0.83	0.62	0.81

[0366] Serum Sample Panel 2 ROC Analysis

[0367] For the Cln248 A22.1/A10.3 MAb ELISA sensitivity and specificity for detecting ovarian cancer in samples from serum sample panel 2 was calculated through receiver operating characteristic (ROC) analysis. The AUC value was calculated with Cln248 concentration levels in serum panel 2 samples described above using the A22.1/A10.3 MAb ELISA. Additionally, the AUC value for CA125, a known ovarian cancer marker, was calculated with CA125 concentrations levels in serum panel 2 samples. CA125 concentration values were determined using a commercially available assay.

[0368] The AUC values for Cln248 from the A22.1/A10.3 MAb ELISA and CA125 were evaluated as individual markers and in combination in ovarian cancer samples versus all normal samples. As shown in table 15 below, the AUC for Cln248 is comparable to the AUC of CA125 for ovarian cancer samples indicating Cln248 is useful for the detection of ovarian cancer. When evaluated in combination, Cln248 and CA125 were complimentary and had a higher AUC than either marker alone. This demonstrates that evaluation of Cln248 and CA125 in combination increases the detection of ovarian cancer than evaluation of either marker alone.

TABLE 15

AUC Values for Cln248 (A22.1/A10.3 MAb ELISA) and CA125

	Marker AUC Values		
Samples	Cln248	CA125	Cln248 + CA125
Ovarian Cancer	0.774	0.720	0.818

Results of Cln248 ROC Analyses

[0369] The results from the ROC analyses of the Cln248 ELISAs demonstrate that Cln248 alone or in combination with other cancer makers, such as CEA and CA125, is useful for detecting cancer. Cln248 and CA125 in combination have a higher sensitivity and specificity for detecting ovarian cancer. Furthermore, Cln248 has a higher sensitivity and specificity, as represented by the AUC value, than CEA in detecting early stage colon cancers. These results demonstrate Cln248 is a useful marker for detecting early stage and low incident cancers. Detection and subsequent treatment of early stage cancers is advantageous since the 5-year survival rate of treated early stage cancers is greater than the 5-year survival rate treated late stage cancers.

Example 4

Western Blot Analysis of Cln248 in Normal and Cancer Cell Lines and Tissues

SDS-PAGE

[0370] For SDS-PAGE analysis, detection of Cln248 protein was evaluated in the following samples: Purified recombinant Cln248 protein, HCT116 cell supernatant, LoVo cell supernatant, Normal colon tissue, and Colon cancer tissue lysate. All samples were reduced with 20 mM DTT in 1×LDS Sample Buffer (Invitrogen) and heated at 70° C. for 10 minutes. 60 μg of cell or tissue lysate, 10 ul of sample supernatant and 1 ug of recombinant protein was loaded onto a 4-12% Bis-Tris gel (Invitrogen). The gel was transferred onto a PVDF membrane (Invitrogen) according to the manufacturer's protocol. After blocking, the membrane was incubated for 1 hour with anti-Cln248.A30.2 MAb. The membrane was then incubated for 30 minutes with a goat anti-mouse-HRP at 1:10,000 dilution (Jackson ImmunoResearch Laboratories). The blot was developed with ECL Plus developer (Amersham Biosciences). A clear band approximately 38 kDa representing Cln248 protein was observed in the lanes containing recombinant Cln248 protein, HCT116 cell supernatant and colon cancer tissue lysate.

[0371] These results demonstrate that Cln248 protein is expressed in colon cell lines as well as colon cancer tissue from individuals, but not found in normal colon tissue. Additionally, the anti-Cln248 antibodies are useful for detection of Cln248 in various samples.

Example 5

Deposits

Deposit of Cell Lines and DNA

[0372] The following hybridoma cell lines were deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., and accorded accession numbers.

TABLE 16

ATCC deposits		
Hybridoma	ATCC Accession No.	Deposit Date
Cln248.A22.1	PTA-7172	14 Oct. 2005
Cln248.A10.3	PTA-7175	18 Oct. 2005

[0373] Anti-Cln248 antibody hybridoma Cln248.A22.1 was shipped to the ATCC via FedEx Overnight on 13 Oct. 2005. The Tracking number for the shipment was 850829455417. FedEx confirmed delivery to ATCC on 14 Oct. 2005 by email. Additionally, a Patent Specialist at the ATCC Patent Depository sent a Material Receipt Form confirming receipt of 25 vials of hybridoma Cln248.A22.1 on 14 Oct. 2005.

[0374] Anti-Cln248 antibody hybridoma Cln248.A10.3 was shipped to the ATCC via FedEx Overnight on 17 Oct. 2005. The Tracking number for the shipment was 850829455440. FedEx confirmed delivery to ATCC on 18 Oct. 2005 by email. Additionally, a Patent Specialist at the ATCC Patent Depository sent a Material Receipt Form confirming receipt of 25 vials of hybridoma Cln248.A10.3 on 18 Oct. 2005.

[0375] The names of the deposited hybridoma cell lines above may be shortened for convenience of reference. E.g. A57.1 corresponds to Cln248.A57.1. These hybridomas correspond to the clones (with their full names) listed in Table 16.

[0376] These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations there under (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between diaDexus, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

[0377] The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strains are not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws. The making of these deposits is by no means an admission that deposits are required to enable the invention.

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Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His Tyr Thr Gln Phe Trp
65                  70                  75                  80

Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val Leu Cys Gly Glu Arg
                85                  90                  95

Glu Glu Glu Ala Arg Ala Cys His Ala Thr His Asn Arg Ala Cys Arg
            100                 105                 110

Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe Cys Leu Glu His Ala
        115                 120                 125

Ser Cys Pro Pro Gly Ala Gly Val Ile Ala Pro Gly Thr Pro Ser Gln
    130                 135                 140

Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala Ser Ser
145                 150                 155                 160

Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn Cys Thr Ala Leu Gly
                165                 170                 175

Leu Ala Leu Asn Val Pro Gly Ser Ser Ser His Asp Thr Leu Cys Thr
            180                 185                 190

Ser Cys Thr Gly Phe Pro Leu Ser Thr Arg Val Pro Gly Ala Glu Glu
        195                 200                 205
```

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Cys Glu Arg Ala Val Ile Asp Phe Val Ala Phe Gln Asp Ile Ser Ile
    210                 215                 220

Lys Arg Leu Gln Arg Leu Leu Gln Ala Leu Glu Ala Pro Glu Gly Trp
225                 230                 235                 240

Gly Pro Thr Pro Arg Ala Gly Arg Ala Ala Leu Gln Leu Lys Leu Arg
                245                 250                 255

Arg Arg Leu Thr Glu Leu Leu Gly Ala Gln Asp Gly Ala Leu Leu Val
            260                 265                 270

Arg Leu Leu Gln Ala Leu Arg Val Ala Arg Met Pro Gly Leu Glu Arg
        275                 280                 285

Ser Val Arg Glu Arg Phe Leu Pro Val His Ala Ser His His His His
    290                 295                 300

His His His His His His
305                 310


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<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<220> FEATURE:
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<400> SEQUENCE: 7

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<210> SEQ ID NO 8
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: ARTIFICIAL SEQUENCE
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 8

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1               5                   10                  15

Ala Thr His Glu Ala Glu Gln Asn Asp Ser Val Ser Pro Arg Lys Ser
            20                  25                  30

Arg Val Ala Ala Gln Asn Ser Ala Glu Val Val Arg Cys Leu Asn Ser
        35                  40                  45

Ala Leu Gln Val Gly Cys Gly Ala Phe Ala Cys Leu Glu Asn Ser Thr
    50                  55                  60

Cys Asp Thr Asp Gly Met Tyr Asp Ile Cys Lys Ser Phe Leu Tyr Ser
65                  70                  75                  80

Ala Ala Lys Phe Asp Thr Gln Gly Lys Ala Phe Val Lys Glu Ser Leu
                85                  90                  95

Lys Cys Ile Ala Asn Gly Val Thr Ser Lys Val Phe Leu Ala Ile Arg
            100                 105                 110
```

-continued

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Arg Cys Ser Thr Phe Gln Arg Met Ile Ala Glu Val Gln Glu Glu Cys
        115                 120                 125

Tyr Ser Lys Leu Asn Val Cys Ser Ile Ala Lys Arg Asn Pro Glu Ala
    130                 135                 140

Ile Thr Glu Val Val Gln Leu Pro Asn His Phe Ser Asn Arg Tyr Tyr
145                 150                 155                 160

Asn Arg Leu Val Arg Ser Leu Leu Glu Cys Asp Glu Asp Thr Val Ser
                165                 170                 175

Thr Ile Arg Asp Ser Leu Met Glu Lys Ile Gly Pro Asn Met Ala Ser
            180                 185                 190

Leu Phe His Ile Leu Gln Thr Asp His Cys Ala Gln Thr His Pro Arg
        195                 200                 205

Ala Asp Phe Asn Arg Arg Arg Thr Asn Glu Pro Gln Lys Leu Lys Val
    210                 215                 220

Leu Leu Arg Asn Leu Arg Gly Glu Glu Asp Ser Pro Ser His Ile Lys
225                 230                 235                 240

Arg Thr Ser His Glu Ser Ala Ala Ser His His His His His His His
                245                 250                 255

His His His
```

1. An antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma selected from the group of hybridomas in Table 1.

2. The antibody of claim **1** which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by hybridomas PTA-7172 or PTA-7175.

3. The antibody of claim **1** which is an antibody fragment, a monoclonal, a human, a chimeric or a humanized antibody.

4-5. (canceled)

6. The antibody of claim **1** which binds a Cln248 peptide, wherein said peptide comprises Val29 to His300 of Cln248, a post translational modification, motif, or domain.

7. (canceled)

8. The antibody of claim **6** wherein the post translational modification, motif, or domain is an EGF-like domain signature 2 or a TNFR/NGFR cysteine-rich region.

9. The antibody of claim **1** where the antibody competes for binding with FasL, LIGHT or TL1A.

10. The antibody of claim **3** which is produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175 or which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

11. (canceled)

12. The antibody of claim **3** which is conjugated to a growth inhibitory agent or a cytotoxic agent.

13. (canceled)

14. The antibody of claim **12** wherein the cytotoxic agent is selected from the group consisting of toxins, antibiotics, radioactive isotopes and nucleolytic enzymes.

15. The antibody of claim **3** wherein the antibody is detectably labeled.

16-17. (canceled)

18. The antibody of claim **1** where the antibody inhibits the growth of Cln248-expressing cancer cells.

19-21. (canceled)

22. The antibody of claim **18**, wherein the cancer cells are from a cancer selected from the group consisting of colon, ovarian, lung and prostate cancer or a metastatic colon, ovarian, lung or prostate cancer.

23. A cell that produces the antibody of claim **3**.

24. The cell of claim **23**, wherein the cell is selected from the group consisting of a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

25. (canceled)

26. A composition comprising the antibody of claim **3**, and a carrier.

27-29. (canceled)

30. The composition of claim **26**, wherein the antibody is an antibody produced by hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175 or an antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

31. A method of killing a Cln248-expressing cancer cell, comprising binding Cln248 with the antibody of claim **1**, thereby inhibiting Cln248 activity and killing the cancer cell.

32. The method of claim **31**, wherein the cancer cell is selected from the group consisting of a colon, ovarian, lung and prostate cancer cell or a metastatic colon, ovarian, lung or prostate cancer.

33-34. (canceled)

35. The method of claim **31**, wherein the antibody is an antibody fragment, a monoclonal, a human, a chimeric or a humanized antibody.

36. (canceled)

37. The method of claim **31**, wherein the antibody is conjugated to a cytotoxic agent or a growth inhibitory agent.

38-39. (canceled)

40. The method of claim **31**, wherein the antibody is a humanized form of the antibody produced by hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175 or an antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

41. A method of alleviating an Cln248-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the antibody of claim **18** to the mammal.

42. The method of claim **41**, wherein the cancer is selected from the group consisting of colon, ovarian, lung and prostate cancer or a metastatic colon, ovarian, lung or prostate cancer.

43-45. (canceled)

46. The method of claim **41**, wherein the antibody is administered in conjunction with at least one chemotherapeutic agent.

47. (canceled)

48. An article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody of claim **3**.

49. (canceled)

50. A method for determining if cells in a sample express Cln248 comprising

(a) contacting a sample of cells with an Cln248 antibody of claim **3** under conditions suitable for specific binding of the Cln248 antibody to Cln248, and

(b) determining the level of binding of the antibody to Cln248 in the sample, or the level of Cln248 antibody internalization by cells in said sample,

wherein Cln248 antibody binding to Cln248 in the sample or internalization of the Cln248 antibody by cells in the sample indicate cells in the sample express Cln248.

51. The method of claim **50** wherein said sample of cells are contacted with an antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175 or an antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

52. The method of claim **50** wherein

said sample of cells is from a subject who has a cancer, is suspected of having a cancer or who may have a predisposition for developing cancer.

53. The method of claim **52** wherein the cancer is a colon, ovarian, lung or prostate cancer or a metastatic colon, ovarian, lung or prostate cancer.

54-59. (canceled)

60. A method for detecting Cln248 overexpression in a subject in need thereof comprising,

(a) combining a sample from a subject with an Cln248 antibody of claim **3** under conditions suitable for specific binding of the Cln248 antibody to Cln248 in said bodily fluid sample,

(b) determining the level of Cln248 in the sample, and

(c) comparing the level of Cln248 determined in step (b) to the level of Cln248 in a control,

wherein an increase in the level of Cln248 in the sample from the subject as compared to the control is indicative of Cln248 overexpression in the subject.

61. The method of claim **60** wherein the subject has cancer.

62. The method of claim **61**

wherein the subject has colon, ovarian, lung or prostate cancer or a metastatic colon, ovarian, lung or prostate cancer.

63. (canceled)

64. The method of claim **60**, wherein the sample from the subject is selected from the group consisting of bodily fluids, cells, cancer cells, blood, serum, plasma, urine, ascites, peritoneal wash, saliva, sputum, seminal fluids, mucous membrane secretions, and other bodily excretions such as stool.

65. The method of claim **60** wherein the control is a bodily fluid sample or cell sample from a subject without a cancer overexpressing Cln248 or a sample of known concentration of Cln248.

66. (canceled)

67. The method of claim **60** wherein two Cln248 antibodies are utilized in a sandwich ELISA format.

68. The method of claim **67** wherein the ELISA has a MDC of 21 pg/mL.

69. The method of claim **67** wherein the Cln248 antibodies produced by hybridomas deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175 or an antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma deposited with the American Type Culture Collection selected from the group consisting of PTA-7172 and PTA-7175.

70. A screening method for antibodies that bind to an epitope which is bound by an antibody of claim **3** comprising,

(a) combining an Cln248-containing sample with a test antibody and an antibody of claim **3** to form a mixture,

(b) determining the level of Cln248 antibody bound to Cln248 in the mixture, and (c) comparing the level of Cln248 antibody bound in the mixture of step (a) to a control mixture,

wherein the level of Cln248 antibody binding to Cln248 in the mixture as compared to the control is indicative of the test antibody's binding to an epitope that is bound by the anti-Cln248 antibody of claim **3**.

71. (canceled)

72. The screening method of claim **70** wherein the control is a mixture of Cln248, a monoclonal antibody which competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma selected from the group of hybridomas in Table 1 and an antibody known to bind the epitope bound by said monoclonal antibody.

73-75. (canceled)

* * * * *

专利名称(译)	CLN248抗体组合物和使用方法		
公开(公告)号	US20090269345A1	公开(公告)日	2009-10-29
申请号	US12/158729	申请日	2006-12-22
[标]申请(专利权)人(译)	樊荣 金南DOO 沃尔弗特ROBERT大号		
申请(专利权)人(译)	樊荣 金南 沃尔弗特ROBERT大号		
当前申请(专利权)人(译)	DIADEXUS INC.		
[标]发明人	FAN RONG KIM NAM WOLFERT ROBERT L		
发明人	FAN, RONG KIM, NAM WOLFERT, ROBERT L.		
IPC分类号	A61K39/395 C07K16/00 C12N9/96 C12N5/16 C12N5/02 A61K39/00 G01N33/53 G01N33/566		
CPC分类号	C07K16/3046 C07K2317/92 C07K2317/73		
优先权	60/753993 2005-12-23 US		
外部链接	Espacenet USPTO		

摘要(译)

提供了与Cln248结合的分离的抗Cln248抗体和产生抗Cln248抗体的细胞。还提供了抗Cln248抗体和载体的组合物。另外，提供了编码抗Cln248抗体的分离的核酸，以及分离的核酸的表达载体。还提供了鉴定抗Cln248抗体的方法，产生抗Cln248抗体的方法，以及它们用于杀死表达Cln248的癌细胞和减轻或治疗哺乳动物中表达Cln248的癌症的方法。