Abstract: A method of diagnosing cancer or a pre-malignant lesion is disclosed. The method comprises determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein the level of CD24 above a predetermined threshold is indicative of the cancer or the pre-malignant lesion.
METHODS OF DIAGNOSING CANCER

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to methods for diagnosing cancer or pre-disposition to same by determining the expression level of CD24 in a sample of peripheral blood cells.

Colorectal cancer (CRC) is a major health concern in the Western world as it is the third most common cancer in both men and women in the United States and Israel. This form of cancer develops through a stepwise process that involves a variety of genetic and epigenetic changes that are acquired over several years and eventually culminate in the transformation of normal epithelium into neoplasm. Although the disease has a long latency period, the currently available markers for disease detection are limited to invasive tests such as colon or gastric endoscopy, which often detect the disease while it has already been spread.

Mutations in oncogenes and tumor suppressor genes, abnormal gene expression and genetic defects in a variety of genes are intimately involved in CRC carcinogenesis. On the basis of the allelotypes of a series of colon tumors, Vogelstein and colleagues [Vogelstein B, Fearon ER, Kern SE, et al.: Science 244 (4901): 207-11, 1989; Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 319(9): 525-32, 1988] have shown that the molecular steps that occur after the activation of the APC-β-catenin-Tcf pathway involve a nonlinear accumulation of specific genetic changes that accompany the transition from normal colonic mucosa to metastatic carcinoma. These include mutations in the k-Ras oncogene, changes in methylation patterns, loss of DCC (Deleted in Colorectal Cancer gene) and SMADs [homologs of drosophila Mothers Against Decapentaplegic (MAD) protein, and the C. elegans protein SMA] and mutations in p53.

The currently available screening methods for cancers of the gastrointestinal tract (Gl tract) such as colorectal cancer (CRC) include fecal occult blood testing (FOBT). However, although clinical trials have shown that screening with serial FOBT reduces CRC mortality (Mandel J, et al., 1993), the sensitivity of FOBT is limited [60%; McMahon PM, et al., 2001]. Several markers have been recently suggested as non-invasive diagnostic tools. These include proteins [e.g., fecal calprotectin, lactoferrin, lysozyme, albumin, alpha-1-antitrypsin, carcinoembryonic antigen (CEA), decay-accelerating factor (DAF), minichromosomal maintenance protein (MCM2)] or mRNA (e.g., fecal COX-2) (Kanaoka S., et al., 2004) which can be detected in stool samples, and proteins such as nicotinamide N-methyltransferase (NNMT) (Roessler M, et al., 2005) or proteasome activator complex subunit 3 (PSME3) (Roessler M., et al., 2006), which can be detected in serum samples. However, due to their low sensitivity and specificity, these markers are not in clinical use.

CD24, also known as heat-stable antigen (HSA) in mice, is a heavily glycosylated phosphatidylinositol-anchored mucin-like cell-surface protein. Physiologically, the CD24 protein is expressed mainly on hematopoietic subpopulations of B-lymphocytes, various epithelial cells, muscle and neural cells. It plays a crucial role in cell selection and maturation during
hematopoiesis and is expressed during the embryonic period, on developing neural and pancreatic cells. In addition, CD24 is a potential ligand for P-selectin which functions as an adhesion molecule that enhances platelets aggregation.

The cellular function of CD24 is still unknown, but recent reports have strengthened its involvement in the initiation of intracellular signal transduction. Schabath et al. (Schabath H, et al., 2006) have associated the expression of CD24 with downregulation in the CXCR-4 chemokine receptor. In addition, CD24 is overexpressed in various malignant tissues including B-cell lymphomas, gliomas, small-cell and non-small cell lung, hepatocellular, renal cell, nasopharyngeal, bladder, uterine, epithelial ovarian, breast, prostate and pancreatic carcinomas (reviewed by Kristiansen et al., 2004). Moreover, its expression was found to correlate with increased growth rate, motility and survival in carcinoma cell lines derived from several organs (Baumann P, et al., 2005; Smith SC, et al., 2006) and with a more aggressive course of cancer. Thus, Weichert W., et al. (2005), found that increased expression of CD24 in the cytoplasm correlates with higher tumor stage, grade and presence of metastasis and concluded that overexpression of CD24 in the cytoplasm (as a result of over production or disturbances in distribution in the cell) is a marker for poorer prognosis. In addition, the role of CD24 in platelet aggregation may explain the involvement with cancer metastases and worse prognosis (Sammar, M., et al., 1994; Aigner, S., et al., 1997; Aigner, S., et al., 1998).

U.S. Pat. Appl. 2004005596 to Li J., et al., discloses methods of diagnosing cancer by determining the level of CD24 in situ in tissue samples suspected to be precancerous or cancerous, thus again necessitating invasional procedures for cancer detection.


**SUMMARY OF THE INVENTION**

According to an aspect of some embodiments of the present invention there is provided a method of diagnosing cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein the level of CD24 above a predetermined threshold is indicative of the cancer or the pre-malignant lesion.

According to an aspect of some embodiments of the present invention there is provided a method of determining a predisposition to cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein the level of CD24 above a predetermined threshold is indicative of a predisposition to the cancer or the pre-malignant lesion.

According to some embodiments of the invention, the pre-malignant lesion is an adenoma.

According to some embodiments of the invention the determining is effectuated ex vivo.

According to some embodiments of the invention, the pre-malignant lesion is associated with a solid tumor.
According to some embodiments of the invention, the cancer is a solid tumor.

According to some embodiments of the invention, the pre-malignant lesion is associated with a gastrointestinal tract cancer.

According to some embodiments of the invention, the cancer is a gastrointestinal tract cancer.

According to some embodiments of the invention, the gastrointestinal tract cancer is colorectal cancer.

According to some embodiments of the invention, the gastrointestinal tract cancer is colorectal cancer.

According to some embodiments of the invention, the cancer is breast cancer.

According to some embodiments of the invention, the cancer is lung cancer.

According to some embodiments of the invention, the cancer is prostate cancer.

According to some embodiments of the invention, the cancer is a pancreatic cancer.

According to some embodiments of the invention, the cancer is a skin cancer.

According to some embodiments of the invention, the the cancer is a urinary tract cancer.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-G are scans of Western blot analyses of PBL cell lysates using anti-CD24 SWA11 antibody for immunodetection. Status numbers of individuals tested indicate the disease states as follows: 0- Normal healthy subjects; 1- patients with adenomas; 2- CRC; 3- Pancreatic carcinoma; 5- Barret's disease; 6- Gastric carcinoma; 8- Other tumors; 10- Breast cancer; IBD (4)- Inflammatory bowel disease. HT29 cell lysate served as positive control.

FIGs. 2A-B are bar graphs illustrating the results of a densitometry analysis of the blots shown in Figures 1A-G. The blots were analyzed by densitometry using ImageMaster 1D Gel.
Analysis v4.10. FIG. 2A: Normal vs. Adenomas, CRC, and IBD. FIG. 2B: Normal vs. various cancers. Bar-graphs represent band volumes as determined by optical density (OD) units. Total number of bands scanned was 56.

FIG. 3 is a graph of ROC analysis: Normal vs. Adenoma.

FIG. 4 is a graph of ROC analysis: Normal vs. CRC.

FIG. 5 is a bar graph illustrating the results of a densitometry analysis of Western blot analyses of PBL cell lysates using anti-CD24 SWA11 antibody for immunodetection.

FIG. 6 is a graph of ROC analysis: Normal vs. Adenoma.

FIG. 7 is a graph of ROC analysis: Normal vs. CRC.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods for diagnosing cancer or pre-disposition to same by determining the expression level of CD24 in a sample of peripheral blood cells.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Cancers of the gastrointestinal (GI) tract, including colorectal cancer (CRC), develop over several years through a stepwise process in which genetic and epigenetic changes are acquired and eventually culminate in the transformation of normal epithelium into neoplasm. For example, CRC carcinogenesis involves mutations in oncogenes (e.g., k-Ras) and tumor suppressor genes (e.g., p53), changes in methylation patterns and loss of DCC and SMADs. However, in spite of the current genetic knowledge and the currently available endoscopy procedures (e.g., colonoscopy), at the time of diagnosis most of GI tract-related cancers have already spread and are more difficult to treat.

While reducing the present invention to practice, the present inventors have uncovered that CD24 is over-expressed on the peripheral blood cells (PBCs) of a subject suffering from, or even predisposed to cancer.

As is shown in the Examples section which follows, patients suffering from colorectal cancer, adenomas, stomach cancer, pancreatic cancer, skin cancer, breast cancer, Barret's and other forms of cancer all have an elevated level of CD24 on their PBCs (Figures 2A-B and Figure 5).

The present inventors have shown that the CD24 levels for adenoma are such that the sensitivity and accuracy of a diagnostic method which is based on measuring the expression level of CD24 on PBCs is at least 75 % and 73 % respectively. The levels for colorectal cancer (CRC) are such that the sensitivity and specificity of a diagnostic method which is based on measuring the expression level of CD24 on PBCs is at least 70 % and 83 % respectively.
Thus, according to one aspect of the present invention, there is provided a method of diagnosing cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein the level of CD24 above a predetermined threshold is indicative of the cancer or the pre-malignant lesion.

As used herein, the term "diagnosing" refers to classifying a pathology (e.g., a CD24-associated cancer or a pre-malignant lesion) or a symptom, determining a severity of the pathology, monitoring pathology progression, forecasting an outcome of a pathology and/or prospects of recovery.

As used herein, the phrase "pre-malignant lesion" refers to a mass of cells and/or tissue having increased probability of transforming into a malignant tumor. Preferably, in the pre-malignant lesion of the present invention CD24 is over-expressed as compared to a non-malignant tissue or cell. Examples of pre-malignant lesions include, but are not limited to, adenomatous polyps, Barrett’s esophagus, IPMN (Intraductal Papillary Mucinous Neoplasia), DCIS (Ductal Carcinoma in Situ) in the breast, leukoplakia and erythroplakia. Thus, the pre-malignant lesion which is diagnosed according to the method of this aspect of the present invention can transform into a malignant solid or non-solid (e.g., hematological malignancies) CD24-associated cancer (or tumor). Preferably, the pre-malignant lesion which is diagnosed by the method of this aspect of the present invention is an adenomatous polyp of the colon, an adenomatous polyp of the rectum, an adenomatous polyp of the small bowel and Barrett’s esophagus.

Non-limiting examples of CD24-associated cancers which can be diagnosed by the method of this aspect of the present invention include tumors of the gastrointestinal tract (colon cancer, rectum cancer, anal region cancer, colorectal cancer, small and/or large bowel cancer, esophageal cancer, stomach cancer, pancreatic cancer, gastric cancer, small intestine cancer, adenocarcinoma arising in the small intestine, carcinoid tumors arising in the small intestine, lymphoma arising in the small intestine, mesenchymal tumors arising in the small intestine, gastrointestinal stromal tumors), gallbladder carcinoma, Biliary tract tumors, prostate cancer, kidney (renal) cancer (e.g., Wilms’ tumor), liver cancer (e.g., hepatoblastoma, hepatocellular carcinoma), hepatobiliary cancer, biliary tree cancer, tumors of the Gallbladder, bladder cancer, embryonal rhabdomyosarcoma, germ cell tumor, trophoblastic tumor, testicular germ cells tumor, immature teratoma of ovary, uterine, epithelial ovarian, sacrococcygeal tumor, choriocarcinoma, placental site trophoblastic tumor, epithelial adult tumor, ovarian cancer, cervical cancer, cancer of the vagina, cancer of the Vulva, lung cancer (e.g., small-cell and non-small cell lung carcinoma), nasopharyngeal, breast cancer, squamous cell carcinoma (e.g., in head and neck), neurogenic tumor, astrocytoma, ganglioblastoma, neuroblastoma, lymphomas (e.g., Hodgkin’s disease, non-Hodgkin’s lymphoma, B cell, Burkitt, cutaneous T cell, histiocytic, lymphoblastic, T cell, thymic, cutaneous T-cell lymphoma, primary central nervous system lymphoma), gliomas, medullary thyroid carcinoma, testicular cancer, brain and head/neck cancer, gynecologic cancer, endometrial cancer, germ cell tumors, mesenchymal
tumors, neurogenic tumors, cancer of the bladder, cancer of the ureter, cancer of the renal pelvis, cancer of the urethra, cancer of the penis, cancer of the testis, cancers of the uterine body, endometrial carcinoma, uterine sarcoma, peritoneal carcinoma and Fallopian Tube carcinoma, germ cell tumors of the ovary, sex cord-stromal tumors, cancer of the endocrine system, thyroid tumors, medullary thyroid carcinoma, thyroid lymphoma, parathyroid tumors, adrenal tumors, pancreatic endocrine tumors, sarcomas of the soft tissue and bone, benign and malignant mesothelioma, malignant peritoneal mesothelioma, malignant mesothelioma of the Tunica Vaginalis Testis, malignant mesothelioma of the Pericardium, skin cancer, cutaneous melanoma, intraocular melanoma, neoplasms of the central nervous system, medulloblastomas, meningiomas, peripheral nerve tumors, Pineal region tumors, pituitary adenomas, craniopharyngiomas, acoustic neuromas, Glomus Jugulare tumors, Chordomas and Chondrosarcomas, Hemangioblastomas, Choroid Plexus Papillomas and Carcinomas, spinal axis tumors, leukemia, and chronic leukemia.

As used herein the phrase "subject in need thereof" refers to a human subject who is at risk of having cancer [e.g., a genetically predisposed subject, a subject with medical and/or family history of cancer, a subject who has been exposed to carcinogens, occupational hazard, environmental hazard] and/or a subject who exhibits suspicious clinical signs of cancer [e.g., blood in the stool or melena, unexplained pain, sweating, unexplained fever, unexplained loss of weight up to anorexia, changes in bowel habits (constipation and/or diarrhea), tenesmus (sense of incomplete defecation, for rectal cancer specifically), anemia and/or general weakness]. Additionally or alternatively, the subject in need thereof can be a healthy human subject undergoing a routine well-being check up.

As used herein the term "CD24" refers to the nucleic acid sequence and/or the amino acid sequence of at least a functional portion of the phosphatidylinositol-anchored mucin-like cell-surface protein (e.g., CD24 protein - SEQ ID NO:2, GenBank Accession No. NP_037362.1; CD24 transcript - SEQ ID NO:1, GenBank Accession No. NM_013230.2) encoded by a genomic sequence on chromosome 6q21.

According to this aspect of the present invention, the CD24 is present in a peripheral blood cell sample.

According to one embodiment, the CD24 is anchored to peripheral blood cells (i.e. it does not refer to free, non-anchored CD24).

As used herein, the phrase "peripheral blood cell sample" refers to a sample taken from circulating blood as opposed to blood cells sequestered within the lymphatic system, spleen, liver, or bone marrow.

Peripheral blood cell samples are typically taken a syringe with a needle.

Methods of processing peripheral blood cell samples are known in the art and further described in the Examples section herein below.

It will be appreciated that determining the level of CD24 in peripheral blood can be effected ex vivo (on a sample derived from the subject) as well as in vivo (within the subject).
As used herein, the phrase "level of CD24" refers to the degree of gene expression and/or gene product activity of the CD24 gene in the biological sample. Accordingly, the level of CD24 can be determined at the amino acid level using protein detection methods.

Thus, the level of the CD24 amino acid sequence (CD24 protein) can be determined using a CD24 specific antibody via the formation of an immunocomplex [i.e., a complex formed between the CD24 antigen (a CD24 amino acid sequence) present in the biological sample and the CD24 specific antibody].

The immunocomplex of the present invention can be formed at a variety of temperatures, salt concentration and pH values which may vary depending on the method and the biological sample used and those of skills in the art are capable of adjusting the conditions suitable for the formation of each immunocomplex.

The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof, such as Fab, F(ab')2, Fv or single domain molecules such as VH and VL to an epitope of an antigen. These functional antibody fragments are defined as follows:

1. Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; 2. Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; 3. (Fab')2, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds; 4. Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; 5. Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule; and 6. Single domain antibodies are composed of a single VH or VL domains which exhibit sufficient affinity to the antigen.

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference).

Antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments.
Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647, and references contained therein, which patents are hereby incorporated by reference in their entirety. See also Porter, R. R. [Biochem. J. 73: 119-126 (1959)]. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Fv fragments comprise an association of VH and VL chains. This association may be noncovalent, as described in Inbar et al. [Proc. Nat'l Acad. Sci. USA 69:2659-62 (1972)]. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise VH and VL chains connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the VH and VL domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow and Filipula, Methods 2: 97-105 (1991); Bird et al., Science 242:423-426 (1988); Pack et al., Bio/Technology 11:1271-77 (1993); and U.S. Pat. No. 4,946,778, which is hereby incorporated by reference in its entirety.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick and Fry [Methods, 2: 106-10 (1991)].

Antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introduction of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368 812-13 (1994); Fishwild et al., Nature Biotechnology
According to the method of this aspect of the present invention, an amount of immunocomplex formation is indicative of a diagnosis of the cancer or the pre-malignant lesion. Various methods can be used to detect the formation of the CD24 immunocomplex of the present invention and those of skills in the art are capable of determining which method is suitable for each immunocomplex.

The CD24 antibody used in the immunocomplex of the present invention can be labeled using methods known in the art. It will be appreciated that the labeled antibodies can be either primary antibodies (i.e., which bind to the specific antigen, e.g., a CD24-specific antigen) or secondary antibodies (e.g., labeled goat anti rabbit antibodies, labeled mouse anti human antibody) which bind to the primary antibodies. The antibody can be directly conjugated to a label or can be conjugated to an enzyme.

Antibodies of the present invention can be fluorescently labeled (using a fluorescent dye conjugated to an antibody), radiolabeled (using radiolabeled e.g., $^{125}$I, antibodies), or conjugated to an enzyme (e.g., horseradish peroxidase or alkaline phosphatase) and used along with a chromogenic substrate to produce a colorimetric reaction. The chromogenic substrates utilized by the enzyme-conjugated antibodies of the present invention include, but are not limited to, AEC, Fast red, ELF-97 substrate [2-(5'-chloro-2-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone], p-nitrophenyl phosphate (PNPP), phenolphthalein diphosphate, and ELF 39-phosphate, BCIP/INT, Vector Red (VR), salmon and magenta phosphate (Avivi C, et al., 1994, J Histochem. Cytochem. 1994; 42: 551-4) for alkaline phosphatase enzyme and Nova Red, diaminobenzidine (DAB), Vector(R) SG substrate, luminol-based chemiluminescent substrate for the peroxidase enzyme. These enzymatic substrates are commercially available from Sigma (St Louis, MO, USA), Molecular Probes Inc. (Eugene, OR, USA), Vector Laboratories Inc. (Burlingame, CA, USA), Zymed Laboratories Inc. (San Francisco, CA, USA), Dako Cytomation (Denmark).

Detection of the CD24 immunocomplex in PBCs can be performed using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), Western blot and radio-immunoassay (RIA) analyses, immunoprecipitation (IP) or by a molecular weight-based approach.

For Western blot the proteins are extracted from a cell sample and are subjected to electrophoresis (e.g., SDS-PAGE) and blotting to a membrane (e.g., nitrocellulose or PVDF). The membrane is then interacted with a CD24 antibody which can be either directly labeled or further subjected to a secondary labeled antibody. Detection may be by autoradiography, colorimetric reaction or chemiluminescence. This method allows both quantitation of an amount of substrate and determination of its identity by a relative position on the membrane which is indicative of a migration distance in the acrylamide gel during electrophoresis.
In case the concentration of the antigen in the biological sample is low, detection of the antigen (CD24 amino acid sequence) can be performed by immunoprecipitation (IP). For immunoprecipitation analysis the CD24 antibody may directly interact with a sample (e.g., cell lysate) including CD24 and the formed complex can be further detected using a secondary antibody conjugated to beads (e.g., if the CD24 antibody is a mouse monoclonal antibody, the secondary antibody may be an anti-mouse antibody conjugated to e.g., Sepharose beads). The beads can be then precipitated by centrifugation, following which the precipitated proteins (e.g., CD24 and anti CD24 antibodies) can be detached from the beads (e.g., using denaturation at 95°C) and further subjected to Western blot analysis using the CD24 specific antibodies. Alternatively, the anti-CD24 antibody and the beads-conjugated secondary antibody may be added to the biological sample containing the antigen (CD24) to thereby form an immunocomplex. Alternatively, since CD24 is a highly glycosylated protein, it can be also precipitated using a substrate capable of binding glycosylated polypeptides such as Concanavalin A (GE Healthcare Bio-Sciences, Uppsala, Sweden) which may be also conjugated to beads, followed by Western blot analysis with anti-CD24 antibodies.

FACS analysis enables the detection of antigens present on cell membranes such as CD24. Briefly, CD24 specific antibodies are linked to fluorophores and detection is performed by means of a cell sorting machine which reads the wavelength of light emitted from each cell as it passes through a light beam. This method may employ two or more antibodies simultaneously.

The level of CD24 can be also determined using ELISA. Briefly, a sample containing CD24 antigen is fixed to a surface such as a well of a microtiter plate. An antigen specific antibody (a CD24 antibody) coupled to an enzyme is applied and allowed to bind to the antigen. Presence of the antibody is then detected and quantitated by a colorimetric reaction employing the enzyme coupled to the antibody. Enzymes commonly employed in this method include horseradish peroxidase and alkaline phosphatase. If well calibrated and within the linear range of response, the amount of substrate present in the sample is proportional to the amount of color produced. A substrate standard is generally employed to improve quantitative accuracy.

The level of CD24 can be also determined using radio-immunoassay (RIA). In one version, this method involves precipitation of the desired antigen (CD24) with a specific antibody and radiolabeled antibody binding protein (e.g., protein A labeled with $^{125}$I) immobilized on a precipitable carrier such as agarose beads. The number of counts in the precipitated pellet is proportional to the amount of antigen.

In an alternate version of the RIA, a labeled antigen and an unlabelled antibody binding protein are employed. A sample containing an unknown amount of antigen is added in varying amounts. The decrease in precipitated counts from the labeled antigen is proportional to the amount of antigen in the added sample.

The level of CD24 can be also determined using molecular weight-based approach. Since the immunocomplex exhibits a higher molecular weight than its components, methods capable of detecting such a change in the molecular weight can be also employed. For
example, the immunocomplex can be detected by a gel retardation assay. Briefly, a non-denaturing acrylamide gel is loaded with samples. A shift in the size (molecular weight) of the protein product as compared with its components is indicative of the presence of an immunocomplex. Such a shift to a higher molecular weight can be viewed using a non-specific protein staining such as silver stain or Coommassie blue stain.

It will be appreciated that analyzing an amount of CD24 in PBCs may also be effected on the polynucleotide level. RNA detection methods can be performed using an isolated polynucleotide (e.g., a polynucleotide probe, an oligonucleotide probe/primer) capable of hybridizing to a CD24 nucleic acid sequence such as the CD24 transcript set forth by SEQ ID NO:1 or a portion thereof. Such a polynucleotide can be at any size, such as a short polynucleotide (e.g., of 15-200 bases), an intermediate polynucleotide of 100-2000 bases and a long polynucleotide of more than 2000 bases.

The isolated polynucleotide probe used by the present invention can be any directly or indirectly labeled RNA molecule [e.g., RNA oligonucleotide (e.g., of 17-50 bases), an in vitro transcribed RNA molecule], DNA molecule (e.g., oligonucleotide, e.g., 15-50 bases, cDNA molecule, genomic molecule) and/or an analogue thereof [e.g., peptide nucleic acid (PNA)] which is specific to the CD24 RNA transcript of the present invention.

Oligonucleotides designed according to the teachings of the present invention can be generated according to any oligonucleotide synthesis method known in the art such as enzymatic synthesis or solid phase synthesis. Equipment and reagents for executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, for example, "Molecular Cloning: A Laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988) and Oligonucleotide Synthesis" Gait, M. J., ed. (1984) utilizing solid phase chemistry, e.g. cyanoethyl phosphoramidite followed by deprotection, desalting and purification by for example, an automated trityl-on method or HPLC.

The isolated polynucleotide used by the present invention can be labeled either directly or indirectly using a tag or label molecule. Such labels can be, for example, fluorescent molecules (e.g., fluorescein or Texas Red), radioactive molecule (e.g., 32P-γ-ATP or 32P-α-ATP) and chromogenic substrates [e.g., Fast Red, BCIP/INT, available from (ABCAM, Cambridge, MA)]. Direct labeling can be achieved by covalently conjugating a label molecule to the polynucleotide (e.g., using solid-phase synthesis) or by incorporation via polymerization (e.g., using an in vitro transcription reaction or random-primed labeling). Indirect labeling can be achieved by covalently conjugating or incorporating to the polynucleotide a non-labeled tag molecule (e.g., Digoxigenin or biotin) and subsequently subjecting the polynucleotide to a
labeled molecule (e.g., anti-Digoxigenin antibody or streptavidin) capable of specifically recognizing the non-labeled tag.

The above-described polynucleotides can be employed in a variety of RNA detection methods such as Northern blot analysis, reverse-transcribed PCR (RT-PCR) [e.g., a semi-quantitative RT-PCR, quantitative RT-PCR using e.g., the Light Cycler™ (Roche)], RNA in situ hybridization (RNA-ISH), in situ RT-PCR stain [e.g., as described in Nuovo GJ, et al. 1993, Intracellular localization of polymerase chain reaction (PCR)-amplified hepatitis C cDNA. Am J Surg Pathol. 17: 683-90, and Komminoth P, et al. 1994, Evaluation of methods for hepatitis C virus detection in archival liver biopsies. Comparison of histology, immunohistochemistry, in situ hybridization, reverse transcriptase polymerase chain reaction (RT-PCR) and in situ RT-PCR. Pathol Res Pract., 190: 1017-25] and oligonucleotide microarray analysis [e.g., using the Affymetrix microarray (Affymetrix®, Santa Clara, CA)].

As mentioned, a level of CD24 in a PBC sample above a predetermined threshold is indicative of the cancer or pre-malignant lesion.

The "predetermined threshold" may be experimentally determined by comparing normal PBC samples (e.g., samples obtained from healthy subjects) to PBC samples derived from subjects known to have carcinogenesis such as CRC. Preferably, a statistically significant number of samples are analyzed.

It will be appreciated that the presence of the cancer or the pre-malignant lesion can be further validated using additional assays. For example, in case the level of CD24 detected in a PBC sample of a subject is above a predetermined threshold, additional assays such as colon endoscopy followed by histological evaluations (including CD24 immunostaining) may be performed on the identified adenomas (in case adenomas are present).

The present inventors have also shown that subjects known to be at risk for cancer (e.g. have a family history), but do not actually have the cancer also show elevated levels of CDC24 in their blood - see Figure 5.

Thus, according to another aspect of the present invention there is provided a method of determining a predisposition to cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein the level of CD24 above a predetermined threshold is indicative of a predisposition to the cancer or the pre-malignant lesion.

It will be appreciated that the present teachings may also be used to determine treatment efficacy. Thus determining CD24 on PBC may be effected following and optionally prior to anti cancer treatment, whereby a reduction of CD24 on PBC is indicative of treatment efficacy.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".
The term "consisting essentially of means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.


EXAMPLE 1

Detection of CD24 in human samples - Study 1

In order to determine whether CD24 expressed in PBL cells might serve as a potential tumor biomarker in cancer, samples collected from 203 individuals, including healthy subjects, CRC patients, other tumors and disease states were screened. The "Other tumors" category includes lung, prostate, urinary tract and gynecological cancers.

MATERIALS AND METHODS

Patient Population: 203 consecutive subjects attending the Integrated Cancer Prevention Center at Tel Aviv Medical Center were consented. Each subject filled a personal questionnaire, gave a blood sample and was subjected to a physical examination. All patients underwent colonoscopy. A detailed epidemiological questionnaire was filled by an expert member of the staff. The study included healthy (normal), adenomas and CRC subjects.

Blood sample preparation: PBLs were isolated from whole blood samples by collecting white buffy coats obtained after blood centrifugation for 3 minutes at 3000 rpm and discarding the plasma supernatant. Residual erythrocytes were lysed by brief incubation in erythrocyte lysis buffer (ELB) containing, 155 mM NH₄Cl, 0.1 mM EDTA, and 10 mM KHCO₃ followed by washing of the cells in the same buffer. The resulting pellet was lysed in the presence of 1 % Triton X-100 and protease inhibitors (20 min on ice) and centrifuged at 15,000xg for 15 minutes, 4 °C. The protein concentration in lysates was determined by BioRad assay and protein extracts (20 µg) were subjected to SDS-PAGE and Western blotting using the monoclonal anti-CD24 SWA1. Detection was performed by enhanced chemiluminescence (ECL) using a commercial kit (Biological Industries, Beit HaEmek, Israel).
Band intensities were quantitated by densitometry analysis using the imaging TINA 2.0 software.

**RESULTS**

Representative results are presented in Figure 1. All results (203 subjects) are summarized in Table 1.

<table>
<thead>
<tr>
<th>Status</th>
<th>Description</th>
<th>CD24</th>
<th>Positive</th>
<th>CD24 Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Percentage</td>
<td>No.</td>
<td>Percentage</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
<td>54</td>
<td>47.4 %</td>
<td>60</td>
<td>52.6 %</td>
</tr>
<tr>
<td>1</td>
<td>Adenoma/s</td>
<td>12</td>
<td>63.2 %</td>
<td>7</td>
<td>36.8 %</td>
</tr>
<tr>
<td>2</td>
<td>CRC</td>
<td>33</td>
<td>71.7 %</td>
<td>13</td>
<td>28.3 %</td>
</tr>
<tr>
<td>3</td>
<td>Pancreatic Can.</td>
<td>2</td>
<td>66.7 %</td>
<td>1</td>
<td>33.3 %</td>
</tr>
<tr>
<td>4</td>
<td>IBD</td>
<td>2</td>
<td>50.0 %</td>
<td>2</td>
<td>50.0 %</td>
</tr>
<tr>
<td>5</td>
<td>Barret's</td>
<td>2</td>
<td>100.0 %</td>
<td>0</td>
<td>0.0 %</td>
</tr>
<tr>
<td>6</td>
<td>Stomach Can.</td>
<td>4</td>
<td>80.0 %</td>
<td>1</td>
<td>20.0 %</td>
</tr>
<tr>
<td>8</td>
<td>Other tumors</td>
<td>5</td>
<td>71.4 %</td>
<td>2</td>
<td>28.6 %</td>
</tr>
<tr>
<td>10</td>
<td>Breast Cancer</td>
<td>2</td>
<td>100.0 %</td>
<td>0</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>2,8</td>
<td>1</td>
<td>100.0 %</td>
<td>0</td>
<td>0.0 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>117</td>
<td></td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 summarizes the average age (Positive vs. Negative) of men and women screened.

<table>
<thead>
<tr>
<th>Status</th>
<th>Description</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F Av. Age</td>
<td>M Av. Age</td>
<td>F Av. Age</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
<td>26 56.4</td>
<td>28 55.5</td>
<td>36 49.7</td>
</tr>
<tr>
<td>1</td>
<td>Adenoma/s</td>
<td>3 74.3</td>
<td>9 61.1</td>
<td>1 58.0</td>
</tr>
<tr>
<td>2</td>
<td>CRC</td>
<td>13 67.1</td>
<td>20 58.3</td>
<td>6 57.5</td>
</tr>
<tr>
<td>3</td>
<td>Pancreatic Cancer</td>
<td>1 60.0</td>
<td>1 90.0</td>
<td>1 64.0</td>
</tr>
<tr>
<td>4</td>
<td>IBD</td>
<td>2 68.0</td>
<td>2 59.5</td>
<td>2 52.5</td>
</tr>
<tr>
<td>5</td>
<td>Barret's</td>
<td>2 71.0</td>
<td>3 63.0</td>
<td>1 52.0</td>
</tr>
<tr>
<td>6</td>
<td>Stomach Can.</td>
<td>2 53.0</td>
<td>3 76.0</td>
<td>1 59.0</td>
</tr>
<tr>
<td>8</td>
<td>Other tumors</td>
<td>2 62.5</td>
<td>3 55.0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Breast Cancer</td>
<td>2 55.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2,8</td>
<td></td>
<td>1 66</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>51</td>
<td>66</td>
<td>45</td>
</tr>
</tbody>
</table>

F- Females; M-Males
As shown in Table 1, the percentage of CD24 positives in normal samples was relatively high (~50%). In addition, a low percentage of CRC cases tested (28.3%) appear to be CD24 negative. Therefore, in order to facilitate the detection of a diagnostic cut off value of CD24, a densitometry analysis of the bands shown in Figure 1 was performed. Results are shown in Figure 2.

Differences between CRC patients, adenomas and the normal group were statistically significant (p<0.001). High CD24 levels were also validated in preliminary studies in other tumors (pancreatic, gastric, breast, lung, prostate, urinary tract and gynecological cancers and Barret's esophagus) as compared to healthy controls.

Receiver operating characteristic (ROC) analysis was used to determine the specificity and sensitivity of the CD24 test (Table 3, herein below), and its ability to discriminate patients with CRC (n=63) or adenoma (n=19) from normal individuals (n=68) with no clinical findings upon colonoscopy (Figures 3-4).

<table>
<thead>
<tr>
<th>Normal vs. Adenoma</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>84.2</td>
<td>73.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal vs. CRC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>70.5</td>
<td>83.3</td>
<td></td>
</tr>
</tbody>
</table>

**EXAMPLE 2**

**Detection of CD24 in human samples - Study 2**

In view of the above promising results a second (validation) study was conducted in which an additional 190 samples were tested.

**MATERIALS AND METHODS**

**Patient Population:** All patients underwent colonoscopy. Normal subjects with or without colonoscopy (status 0 or 00, respectively) were tested. Additional data was collected such as inflammation parameters (CRP levels and related clinical data), and family history of cancer. Thus, normal subjects with high CRP levels were not included, while normal subjects with a family history of cancer were included in a separated category.

**Blood sample preparation:** Briefly, PBLs were isolated from whole blood samples by collecting white buffy coats obtained after blood centrifugation for 3 minutes at 3000 rpm and discarding the plasma supernatant. Residual erythrocytes were lysed by brief incubation in erythrocyte lysis buffer (ELB) containing, 155 mM NH₄Cl, 0.1 mM EDTA, and 10 mM KHCO₃ following by washing of the cells in the same buffer. The resulting pellet was lysed in the presence of 1% Triton X-100 and protease inhibitors (20 minutes on ice) and centrifuged at 15,000×g for 15 minutes, 4 °C. The protein concentration in lysates was determined by BioRad assay and protein extracts (20 µg) were subjected to SDS-PAGE and Western blotting using anti-CD24 SWA11. Band intensities were quantitated by densitometry analysis using the imaging TINA 2.0 software as previously described. Densitometry results were plotted and subjected to statistical analysis for significance.
RESULTS

The results are presented in Figures 5-7 and Table 4, herein below.

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
</tr>
<tr>
<td>Normal vs. Adenoma</td>
</tr>
<tr>
<td>Normal vs. CRC</td>
</tr>
</tbody>
</table>

In conclusion, the present data further support that CD24 is able to detect CRC with high reliability and may serve as a potential CD24 biomarker for CRC and for early stages of colorectal neoplasia.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.
REFERENCES
(Additional References are cited in text)


WHAT IS CLAIMED IS:

1. A method of diagnosing cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein said level of CD24 above a predetermined threshold is indicative of the cancer or the pre-malignant lesion.

2. A method of determining a predisposition to cancer or a pre-malignant lesion, the method comprising determining a level of CD24 expressed on peripheral blood cells of a subject in need thereof, wherein said level of CD24 above a predetermined threshold is indicative of a predisposition to the cancer or the pre-malignant lesion.

3. The method of any one of claims 1 and 2, wherein said pre-malignant lesion is an adenoma.

4. The method of any one of claims 1 and 2, wherein said determining is effected ex vivo.

5. The method of any one of claims 1 and 2, wherein the pre-malignant lesion is associated with a solid tumor.

6. The method of any one of claims 1 and 2, wherein the cancer is a solid tumor.

7. The method of any one of claims 1 and 2, wherein the pre-malignant lesion is associated with a gastrointestinal tract cancer.

8. The method of any one of claims 1 and 2, wherein the cancer is a gastrointestinal tract cancer.

9. The method of claim 7, wherein said gastrointestinal tract cancer is colorectal cancer.

10. The method of claim 8, wherein said gastrointestinal tract cancer is colorectal cancer.

11. The method of any one of claims 1 and 2 wherein the cancer is breast cancer.

12. The method of any one of claims 1 and 2 wherein the cancer is lung cancer.
13. The method of any one of claims 1 and 2 wherein the cancer is prostate cancer.

14. The method of any one of claims 1 and 2 wherein the cancer is a pancreatic cancer.

15. The method of any one of claims 1 and 2 wherein the cancer is a skin cancer.

16. The method of any one of claims 1 and 2 wherein the cancer is a urinary tract cancer.
CD24 relative expression

Status

Normal

Adenoma

>14990.5
Sens:75.0
Spec:89.2

FIG. 6
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION UNDER SUBJECT MATTER**

**INV.** G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
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<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>WO 2007/088537 A (MEDICAL RES FUND OF TEL AVIV S [IL]; ARBER NADIR [IL]) 9 August 2007 (2007-08-09) abstract; claims</td>
<td>1-16</td>
</tr>
<tr>
<td>X</td>
<td>WO 03/079982 A (TULARIK INC [US]; LI JING [US]; POWERS SCOTT [US]; YANG QIANXIN [US]) 2 October 2003 (2003-10-02) abstract; claims 56-58</td>
<td>1-16</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

**Date of the actual completion of the international search**

4 March 2009

**Date of mailing of the international search report**

12/03/2009

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk

Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer

Gonçalves Mauger, M
<table>
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<th>Category</th>
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<td>Y</td>
<td>SAGIV EAYL ET AL: &quot;CD24 is a novel oncogene in colorectal cancer, detected by microarray profiling of cell transformation, and is a target for immunotherapy of cancer&quot; GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 130, no. 4, SUPPL. 2, 1 April 2006 (2006-04-01), page A680, XP008088622 ISSN: 0016-5085 abstract</td>
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<td>SAGIV ET AL: &quot;CD24 is a New Oncogene, Early at the Multistep Process of Colorectal Cancer Carcinogenesis&quot; GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 131, no. 2, 1 August 2006 (2006-08-01), pages 630-639, XP005587457 ISSN: 0016-5085 page 634 - page 638</td>
<td>1-16</td>
</tr>
<tr>
<td>Category</td>
<td>Citation of document, with indication, where appropriate, of the relevant passages</td>
<td>Relevant to claim No</td>
</tr>
<tr>
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</table>

Abstract:

The metastasis-associated gene CD24 is regulated by RaI GTPase and is a mediator of cell proliferation and survival in human cancer.
<table>
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<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
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</tr>
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<tr>
<td>MO 03079982 A</td>
<td>02-10-2003</td>
<td>AU 2003220387 A1</td>
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<td>02-10-2003</td>
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<td>EP 1497642 A2</td>
<td>19-01-2005</td>
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<td>US 2004097448 A1</td>
<td>20-05-2004</td>
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