

PROPHYLAXIS OF COLORECTAL AND GASTROINTESTINAL CANCER

1. REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of provisional application no. 61/317,245, filed March 24, 2010, the content of which is incorporated by reference in its entirety.

2. REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0002] The Sequence Listing is concurrently submitted herewith.

3. FIELD OF THE INVENTION

[0003] The present disclosure is directed to, among other things, methods of preventing colorectal and/or gastrointestinal cancer in subjects predisposed to develop adenomatous polyps by administering to the subject a composition comprising an antibody specific for progastrin.

4. BACKGROUND

[0004] Cancer of the gastrointestinal tract, including colorectal cancer ("CRC"), affects hundreds of thousands of individuals every year and tens of thousands of CRC-related deaths occur every year in the United States alone. *See*, Rustgi, 2010, "The genetics of hereditary colon cancer," *Genes & Development* 21:2525-2538. CRC can arise in different ways, one of which is the transformation of adenomatous polyps into malignant tumors. Adenomatous polyposis may be inherited, as is the case for individuals with familial adenomatous polyposis ("FAP"), or it may be sporadic. Individuals with FAP or sporadic adenomatous polyposis carry mutations of the Adenomatous Polyposis Coli ("APC") tumor suppressor gene which are associated with the formation of adenomatous polyps in the small intestine, colon and/or rectum. These polyps in turn can develop into colorectal and gastrointestinal cancer. In the case of sporadic adenomatous polyposis, a non-hereditary condition that underlies many instances of CRC, the APC gene is mutated

in somatic cells. Individuals with sporadic adenomatous polyposis develop benign polyps, a subset of which may subsequently transform into malignant carcinomas.

[0005] FAP accounts for around 1% of total CRC cases and affects one in 13,000 births.

Id. Mutation of APC in FAP patients is associated with the formation of hundreds to thousands of small adenomatous polyps throughout the colon. Progression of polyps to malignancy is virtually inevitable. On average, without prophylactic treatment, individuals with FAP develop CRC by age 39. Prophylactic treatment is the standard of care and involves radical surgery, including the removal of the colon, or of both the colon and the rectum, generally before the age of 25. While prophylaxis is preferable to no treatment, surgical resection of the colon (colectomy) in young patients severely impairs quality of life. In addition, surgical resection alone may be inadequate to keep patients cancer-free: patients who have colectomies have a high risk of developing polyps and cancer in the upper gastrointestinal tract. There is a serious need for effective prophylactic treatments, especially non-surgical treatments, that extend cancer-free life for individuals with FAP and individuals with sporadic adenomatous polyposis.

5. SUMMARY

[0006] The present disclosure provides methods and compositions useful for preventing gastrointestinal cancer, including CRC, in animals, including humans, predisposed to developing adenomatous polyps. As described below, the present application sets forth treatment regimens believed to bind progastrin ("PG"), with the apparent ability to neutralize PG's biological activity, which are useful in subjects who have an increased likelihood of developing, but have not yet developed, CRC or cancer in the upper gastrointestinal tract. The various inventions described in the application are based in part on the applicants' discovery that anti-PG antibodies prevent the development of gastrointestinal tumors in a mouse model of FAP. While not intending to be bound by any theory of operation, binding PG and interfering with its interaction with other proteins in the body is thought to prevent adenomatous polyps from developing into malignant tumors.

[0007] Accordingly, in one aspect, the present disclosure provides methods of preventing gastrointestinal cancer, including CRC, in subjects predisposed to developing adenomatous polyps by administering a composition comprising an anti-PG antibody. Generally, the methods comprise administering to a subject in need thereof an effective amount of an anti-PG antibody. Anti-PG antibodies, and compositions thereof, can be administered according to regimens known in the art for antibody-based therapy, at an effective dosage, *i.e.*, an amount effective to prevent or delay gastrointestinal cancer, including CRC, in a subject.

[0008] Suitable subjects for prophylactic anti-PG treatment are those predisposed to developing adenomatous polyps, including subjects with a family history of CRC, individuals with FAP, and those in whom adenomatous polyps have previously been found and/or removed. Typically, suitable subjects have one or more mutations in the APC gene, leading to FAP or sporadic adenomatous polyposis. Suitable subjects also include individuals who have previously had a colectomy and are at increased risk of developing polyps and cancer in the upper gastrointestinal tract.

[0009] Anti-PG antibodies of the present disclosure include antibodies capable of binding PG. Any antibody capable of binding PG may be used in the methods of the present disclosure, including, but not limited to, polyclonal and monoclonal anti-PG antibodies. Preferably, the anti-PG antibody is specific to the PG of the species being treated. For example, an anti-human PG (anti-hPG) antibody is administered to a human subject. Suitable anti-PG antibodies can range in binding affinity from at least about 5000 nM to at least about 0.001 nM, or higher, or any value in between.

[0010] Anti-PG antibodies described herein can be used in combination with, or adjunctive to, other treatments to prevent or delay gastrointestinal cancer, including CRC. Non-limiting examples of other treatments include surgical resection, chemotherapy, antibody therapy, radiation therapy, and treatment with a second agent as described herein. Anti-PG antibodies can be administered concurrently with, or at a time before or after, another treatment.

[0011] Compositions suitable for use in the methods of the present disclosure may comprise, in addition to an anti-PG antibody, a pharmaceutically acceptable carrier, excipient, and/or diluent. The compositions can be formulated for various routes of administration as described herein, comprising carriers, excipients, and/or diluents suitable for the chosen route. For treatment in humans and animals, compositions comprising anti-PG antibodies can be administered using any suitable route of administration, such as injection and other routes of administration known in the art for antibody-based clinical products. For treatment purposes, compositions can be packaged in unit doses for ease of use.

[0012] As shown herein, patients with multiple adenomatous polyps have elevated serum PG levels, whereas patients in whom polyps have been removed have low or undetectable serum PG levels. This discovery provides powerful new tools to diagnose and monitor the course of sporadic or familial adenomatous polyposis and its treatment.

[0013] Accordingly, in another aspect, the present disclosure provides methods of monitoring the efficacy of anti-PG treatment in an individual predisposed to developing adenomatous polyps. Generally, the methods comprise measuring a concentration, or level, of PG in a blood (serum, plasma, or whole blood) sample from the individual receiving anti-PG therapy, during or after a course of anti-PG therapy, and comparing the measured PG level to a baseline level of PG (*e.g.*, a PG level in the individual at the start of treatment), wherein a measured PG level below that of the baseline level is indicative of treatment efficacy and a measured PG level above that of the baseline level is indicative of a lack of efficacy. In some embodiments, the method further includes assessing the number and sizes of polyps in the subject by, for example, endoscopy.

[0014] In yet another aspect, the present disclosure provides methods for selecting individuals, in whom endoscopy or anti-PG treatment is indicated. The methods are intended to be carried out in individuals predisposed to developing adenomatous polyps. Generally, the method is carried out by measuring the level of PG in a blood sample from the individual, and comparing the measured level of PG to a baseline level, where a measured PG level higher than the baseline level indicates a need for endoscopy. In

some embodiments, a PG level above the baseline indicates a need for anti-PG treatment. The baseline can be obtained from one or more samples from the individual at an earlier point in time, or can be based upon PG levels measured in a population having characteristics similar to the individual.

6. BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1 provides amino acid sequences of human preprogastrin (SEQ ID NO:100), where the signal peptide sequence is underlined, mature human progastrin (SEQ ID NO:20) and certain products of progastrin processing, including G34 (SEQ ID NO:102), G34-Gly (SEQ ID NO:103), G17 (SEQ ID NO:104), G17-Gly (SEQ ID NO:105) and CTFP (SEQ ID NO:106).

[0016] FIG 2. provides polynucleotide and amino acid sequences of variable light and variable heavy chains of certain exemplary murine anti-hPG monoclonal antibodies. In each case, the three CDRs are shown in bolded-underlined text. Specifically:

FIG. 2A provides the polypeptide sequence of the V_H chain of murine anti-hPG MA b3 (SEQ ID NO:12) and a polynucleotide sequence encoding it (SEQ ID NO:16);

FIG. 2B provides the polypeptide sequence of the V_L chain of murine anti-hPG MA b3 (SEQ ID NO:13) and a polynucleotide sequence encoding it (SEQ ID NO:17);

FIG. 2C provides the polypeptide sequence of the V_H chain of murine anti-hPG MA b4 (SEQ ID NO:14) and a polynucleotide sequence encoding it (SEQ ID NO:18);

FIG. 2D provides the polypeptide sequence of the V_L chain of murine anti-hPG MA b4 (SEQ ID NO:15) and a polynucleotide sequence encoding it (SEQ ID NO:19);

FIG. 2E provides the polypeptide sequence of the V_H chain of murine anti-hPG MA b8 (SEQ ID NO:59) and a polynucleotide sequence encoding it (SEQ ID NO:67);

FIG. 2F provides the polypeptide sequence of the V_L chain of murine anti-hPG MA b8 (SEQ ID NO:63) and a polynucleotide sequence encoding it (SEQ ID NO:71);

FIG. 2G provides the polypeptide sequence of the V_H chain of murine anti-hPG MA b13 (SEQ ID NO:60) and a polynucleotide sequence encoding it (SEQ ID NO:68);

FIG. 2H provides the polypeptide sequence of the V_L chain of murine anti-hPG MAb13 (SEQ ID NO:64) and a polynucleotide sequence encoding it (SEQ ID NO:72);

FIG. 2I provides the polypeptide sequence of the V_H chain of murine anti-hPG MAb16 (SEQ ID NO:61) and a polynucleotide sequence encoding it (SEQ ID NO:69);

FIG. 2J provides the polypeptide sequence of the V_L chain of murine anti-hPG MAb16 (SEQ ID NO:65) and a polynucleotide sequence encoding it (SEQ ID NO:73);

FIG. 2K provides the polypeptide sequence of the V_H chain of murine anti-hPG MAb19 (SEQ ID NO:62) and a polynucleotide sequence encoding it (SEQ ID NO:70); and

FIG. 2L provides the polypeptide sequence of the V_L chain of murine anti-hPG MAb19 (SEQ ID NO:66) and a polynucleotide sequence encoding it (SEQ ID NO:74).

[0017] FIG. 3 provides projected polypeptide sequences for humanized variable heavy and light chains of selected anti-hPG monoclonal antibodies described herein. In each case, the three CDRs are shown in bolded-underlined text. Specifically:

FIG. 3A provides the projected amino acid sequence of the V_H chain of humanized MAb3 (SEQ ID NO:21);

FIG. 3B provides the projected amino acid sequence of the V_L chain of humanized MAb3 (SEQ ID NO:22);

FIG. 3C provides the projected amino acid sequence of the V_H chain of humanized MAb4 (SEQ ID NO:23);

FIG. 3D provides the projected amino acid sequence of the V_L chain of humanized MAb4 (SEQ ID NO:24);

FIG. 3E provides the projected amino acid sequence of the V_H chain of humanized MAb8(a) (SEQ ID NO:75);

FIG. 3F provides the projected amino acid sequence of the V_L chain of humanized MAb8(a) (SEQ ID NO:76);

FIG. 3G provides the projected amino acid sequence of the V_H chain of humanized MAb8(b) (SEQ ID NO:77);

FIG. 3H provides the projected amino acid sequence of the V_L chain of humanized MAb8(b) (SEQ ID NO:78);

FIG. 3I provides the projected amino acid sequence of the V_H chain of humanized MAb8(c) (SEQ ID NO:79);

FIG. 3J provides the projected amino acid sequence of the V_L chain of humanized MAb8(c) (SEQ ID NO:76);

FIG. 3K provides the projected amino acid sequence of the V_H chain of humanized MAb13(a) (SEQ ID NO:80);

FIG. 3L provides the projected amino acid sequence of the V_L chain of humanized MAb13(a) (SEQ ID NO:81);

FIG. 3M provides the projected amino acid sequence of the V_H chain of humanized MAb13(b) (SEQ ID NO:82);

FIG. 3N provides the projected amino acid sequence of the V_L chain of humanized MAb13(b) (SEQ ID NO:83);

FIG. 3O provides the projected amino acid sequence of the V_H chain of humanized MAb16(a) (SEQ ID NO:84);

FIG. 3P provides the projected amino acid sequence of the V_L chain of humanized MAb16(a) (SEQ ID NO:85);

FIG. 3Q provides the projected amino acid sequence of the V_H chain of humanized MAb16(b) (SEQ ID NO:86);

FIG. 3R provides the projected amino acid sequence of the V_L chain of humanized MAb16(b) (SEQ ID NO:87);

FIG. 3S provides the projected amino acid sequence of the V_H chain of humanized MAb16(c) (SEQ ID NO:88);

FIG. 3T provides the projected amino acid sequence of the V_L chain of humanized MAb16(c) (SEQ ID NO:89);

FIG. 3U provides the projected amino acid sequence of the V_H chain of humanized MAb19(a) (SEQ ID NO:90);

FIG. 3V provides the projected amino acid sequence of the V_L chain of humanized MAb19(a) (SEQ ID NO:91);

FIG. 3W provides the projected amino acid sequence of the V_H chain of humanized MAb19(b) (SEQ ID NO:92);

FIG. 3X provides the projected amino acid sequence of the V_L chain of humanized MAb19(b) (SEQ ID NO:93);

FIG. 3Y provides the projected amino acid sequence of the V_H chain of humanized MAb19(c) (SEQ ID NO:94); and

FIG. 3Z provides the projected amino acid sequence of the V_L chain of humanized MAb19(c) (SEQ ID NO:95).

7. DETAILED DESCRIPTION

7.1 Cancer In Familial And Sporadic Adenomatous Polyposis

[0018] Familial Adenomatous Polyposis (FAP) is a rare hereditary condition associated with a germinal mutation on one allele of the APC gene. Numerous mutations of the APC gene have been mapped in subjects with FAP, many of the mutations resulting in a truncated protein. *See, e.g.,* Rustgi, 2010, "The genetics of hereditary colon cancer," *Genes & Development* 21:2525-2538; Groves *et al.*, 2002, "Duodenal cancer in patients with familial adenomatous polyposis (FAP): results of a 10 year prospective study," *Gut* 50:636-641. These mutations in APC are associated with FAP of varying severity.

[0019] FAP is characterized by the appearance of multiple adenomas (polyps) in the intestine and colon of affected individuals, at a very young age. A subset of these polyps transform into colorectal cancer (CRC), and FAP-derived CRC cases represent approximately 1% of total CRC cases. Where there is a family history of CRC,

individuals typically undergo genetic testing at a very early age to detect the presence of a mutation in the APC gene. Classical follow-up in individuals found to have such mutations begins with colonoscopy, polyp resection where polyps are found, and if polyps are too numerous to remove endoscopically, partial or complete resection of the colon (colectomy). Most individuals with FAP will have undergone colectomy by the age of 25.

[0020] A large percentage of sporadic adenomatous polyps also harbor mutations in the APC gene. *See*, Rutsgi, 2010, "The genetics of hereditary colon cancer," *Genes & Development* 21:2525-2538. In the absence of any family history of CRC, individuals presenting with symptoms such as rectal bleeding are typically examined by colonoscopy. Individuals found to have large numbers of polyps, or in whom polyps recur after resection, will typically also be tested genetically. If a mutation in the APC gene is found, colectomy is the recommended treatment.

[0021] Even after colectomy, individuals predisposed to developing adenomatous polyps have an increased risk of developing adenomatous polyps in the unresected portion of their gastrointestinal tracts. Such individuals are regularly followed by endoscopy and assessed for adenomatosis in the upper gastrointestinal tract. Individuals are staged according to the Spigelman classification, which relies on four parameters to evaluate the degree or severity of adenomatosis: number of polyps, size of polyps, histology of polyps, and degree of polyp dysplasia (disordered growth). *See*, Spigelman *et al.*, 1989, "Upper gastrointestinal cancer in patients with Familial Adenomatous Polyposis," *Lancet* 2:783-785. The Spigelman classification categorizes individuals into one of five stages for duodenal polyposis, Stage 0 to IV, based on the four parameters, as shown in Table 1:

Table 1 Spigelman Classification				
Points	Number of polyps	Polyp size (mm)	Histology	Dysplasia
1	1-4	1-4	Tubular	Mild
2	5-20	5-10	Tubulovillous	Moderate
3	> 20	> 10	Villous	Severe

Stage I: 1-4 points; Stage II: 5-6 points; Stage III: 7-8 points; Stage IV: 9-12 points.

A ten-year study of 114 individuals with FAP revealed that Spigelman Stage IV patients had a 36.4% risk of developing duodenal cancer, as compared to a 0% to 2.4% risk for patients classified in Stages 0 to III. *See, Groves et al., 2002, "Duodenal cancer in patients with familial adenomatous polyposis (FAP): results of a 10 year prospective study," Gut 50:636-641.*

[0022] It has previously been shown that approximately 70% of patients with CRC have elevated levels of PG. As shown in the Examples below, Applicants have now discovered that blood levels of PG can be elevated in individuals with FAP who have not yet developed CRC, as well as in about 20% of patients exhibiting sporadic adenomatous polyposis. While not intending to be bound by any theory of operation, PG is thought to be part of the mechanism by which polyps transition to malignant tumors. Binding of PG by anti-PG antibodies is thought to interfere with this transition, as demonstrated in the mouse model of FAP, APC Δ 14. Prophylactic treatment with anti-PG antibodies presents the possibility of avoiding or delaying major surgery, significantly increasing quality of life.

7.2 Methods Of Prophylaxis

[0023] The present disclosure provides methods of preventing gastrointestinal cancer, including CRC, in patients predisposed to developing adenomatous polyps. Generally, the methods comprise administering to such patients an amount of one or more anti-PG antibody(ies) effective to provide a therapeutic benefit. Anti-PG antibodies generally, and specific anti-PG antibodies useful in the methods, are described in detail in a later section.

[0024] The "subject" or "patient" for prophylaxis is preferably a mammal such as a non-primate (*e.g.*, cow, pig, horse, cat, dog, rat, *etc.*) or a primate (*e.g.*, monkey or human). The anti-PG antibody administered should be specific for the species of animal being treated. For treatment of human subjects, the anti-PG antibody(ies) should specifically bind human progastrin (referred to herein as "anti-hPG antibodies," described in more detail below).

[0025] The subject or patient can be a human, such as an adult patient or a pediatric patient. Suitable subjects are individuals who are predisposed to developing adenomatous polyps and, as a result, have an increased likelihood of developing CRC or gastrointestinal cancer, including individuals with a family history of CRC, individuals in whom adenomatous polyps are or have been detected or removed, individuals with FAP, individuals who have had a colectomy to remove polyps, and individuals with mutation(s) in the APC gene.

[0026] Anti-PG treatment can be administered in combination with, or adjunctive to, one or more other treatments to prevent or delay gastrointestinal cancer, including CRC. Other treatments include, without limitation, chemotherapeutic treatment, radiation, surgical resection, antibody therapy, and treatment with a second agent, as described herein. Combination treatment as provided herein involves the administration of at least two treatments to a patient, the first of which is anti-PG treatment with at least one anti-PG antibody, and the second of which is treatment with a therapeutic or prophylactic agent or procedure.

[0027] Anti-PG treatment can be combined with surgical procedures, such as surgical resection. Anti-PG antibodies can be administered to subjects found to have, or predisposed to develop, pre-cancerous polyps, such as individuals with familial adenomatous polyposis, in combination with surgical resection of the affected portion(s) of the gastrointestinal tract. Anti-PG treatment can be initiated before, concurrently with, or after surgical resection.

[0028] Anti-PG treatment can also be combined with radiation therapy. Radiation therapy is the use of high-energy radiation from x-rays, gamma rays, neutrons, protons, and other sources to kill cancer cells and shrink tumors. Radiation may come from a machine outside the body (external-beam radiation therapy), or it may come from radioactive material placed in the body near cancer cells (internal radiation therapy, or brachytherapy). Systemic radiation therapy uses a radioactive substance, such as a radiolabeled monoclonal antibody, that travels in the blood to tissues throughout the body. Radiation therapy may also be called irradiation and radiotherapy. Other radiation

therapies include three-dimensional conformal radiation therapy (3D-CRT) and intensity modulated radiation therapy (IMRT). Other radiation therapies are also possible.

[0029] Where anti-PG antibody treatment is combined with a second agent, the second agent can be a chemotherapeutic agent. Chemotherapy is the use of small molecule drugs that kill (cytotoxic or cytocidal) or prevent the growth (cytostatic) of cancer cells.

Chemotherapeutic agents include, but are not limited to, toxins, also referred to as cytotoxins or cytotoxic agents, which includes any agent that is detrimental to the viability of cells, agents, and liposomes or other vesicles containing chemotherapeutic compounds. Examples of suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone, 5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, alkylating agents, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC), anti-mitotic agents, cis-dichlorodiamine platinum (II) (DDP) cisplatin, diamino dichloro platinum, anthracyclines, antibiotics, antimetabolites, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucovorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclophosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytosan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorubicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxy anthracin dione, Docetaxel, dolasetron mesylate, doxorubicin HCL, dronabinol, E. coli L-asparaginase, emetine, epoetin- α , Erwinia L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon α -2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptopurine, mesna, methotrexate,

methyletestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, oxaliplatin, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 with carmustine implant, porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, tegafur, teniposide, tenoposide, testolactone, tetracaine, thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate, trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

[0030] Anti-PG antibodies can also be administered with a combination of chemotherapeutic agents. Exemplary combinations of chemotherapeutic agents include 5-fluorouracil (5FU) in combination with leucovorin (folinic acid or LV); capecitabine, in combination with uracil (UFT) and leucovorin; tegafur in combination with uracil (UFT) and leucovorin; oxaliplatin in combination with 5FU, or in combination with capecitabine; irinotecan in combination with capecitabine, mitomycin C in combination with 5FU, irinotecan or capecitabine. Other combinations of chemotherapeutic agents disclosed herein is also possible.

[0031] Standard dosing regimens for chemotherapeutic agents used for patients suffering from CRC may be used in the methods of the present disclosure. As is known in the relevant art, chemotherapy regimes for colorectal cancer using combinations of different chemotherapeutic agents have been standardized in clinical trials. Such regimes are often known by acronyms and include 5FU Mayo, 5FU Roswell Park, LVFU2, FOLFOX, FOLFOX4, FOLFOX6, bFOL, FUFOX, FOLFIRI, IFL, XELOX, CAPOX, XELIRI, CAPIRI, FOLFOXIRI. *See, e.g., Chau, I. et al., 2009, Br. J., Cancer* 100:1704-19, and Field, K. *et al., 2007, World J. Gastroenterol.* 13:3806-15, both of which are incorporated by reference.

[0032] Anti-PG antibodies can also be used in combination with other antibodies, including but not limited to, monoclonal antibodies that directly or indirectly kill, slow or stop the growth of cancer cells. Such antibodies can function through a variety of distinct mechanisms. For example, certain antibodies can mark cancer cells for attack by the

patient's immune system *via* antibody-dependent cell-mediated cytotoxicity (ADCC) or other mechanisms. It is believed that rituximab (Rituxan®), which binds the CD20 antigen found on B cells, and edrecolomab, which binds the 17-1A antigen, function this way. Other antibodies bind to and alter or inhibit the function of antigens that cancer cells require for survival and/or growth. A number of antibodies are believed to function this way, including, for example, cetuximab (Erbix®) and panitumumab (Vectibix®), each of which binds to the EGF receptor (EGFR); and bevacizumab (Avastin®), which binds to the growth factor VEGF. Other mechanisms are also possible, and particular antibodies may be able to work *via* one or more mechanisms of action. Yet other antibodies can be conjugated to radioactive or chemotoxic moieties and target them to cancer cells which preferentially express antigens specifically recognized by the antibodies.

[0033] Anti-PG antibodies can also be administered in combination with non-steroidal anti-inflammatory drugs ("NSAIDs"). For example, celecoxib, or 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide, is an NSAID that has been shown to reduce adenomatous polyps in FAP patients.

[0034] The anti-PG antibody and a second agent can be administered simultaneously, successively, or separately. As used herein, the anti-PG antibody and the second agent are said to be administered successively if they are administered to the patient on the same day, for example during the same patient visit. Successive administration can occur 1, 2, 3, 4, 5, 6, 7 or 8 hours apart. In contrast, the anti-PG antibody and the second agent are said to be administered separately if they are administered to the patient on different days, for example, the anti-PG antibody and the second therapeutic agent can be administered at a 1-day, 2-day or 3-day, one-week, 2-week or monthly intervals. In the methods of the present disclosure, administration of the anti-PG antibody of the disclosure can precede or follow administration of the second agent.

[0035] As a non-limiting example, the anti-PG antibody and second agent can be administered concurrently for a period of time, followed by a second period of time in which the administration of anti-PG antibody and the second agent are alternated.

7.3 Pharmaceutical Compositions And Kits

[0036] Anti-PG antibodies useful in the methods of the present disclosure can be formulated in compositions. Optionally, the compositions can comprise one or more additional agent(s), such as the second agents described above. The compositions will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to an individual).

[0037] Anti-PG antibodies can be administered to an individual by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intraocularly, topically, intrathecally and intracerebroventricularly. The most suitable route for administration in any given case will depend on the particular antibody, the subject, and the nature and severity of the disease and the physical condition of the subject. Antibodies can be formulated as an aqueous solution and administered by subcutaneous injection. Pharmaceutically acceptable carriers for use in the disclosure can take a wide variety of forms depending, *e.g.*, on the condition to be treated or route of administration.

[0038] Pharmaceutical compositions can be conveniently presented in unit dose forms containing a predetermined amount of an anti-PG antibody per dose. Such a unit can contain for example 5 mg to 5 g, for example 10 mg to 1 g, or 20 to 50 mg of anti-PG antibody per unit dose. Pharmaceutical compositions can comprise anti-PG antibodies capable of binding more than one PG epitope. Alternatively, pharmaceutical compositions may comprise a combination of anti-PG antibodies, each capable of binding a different PG epitope.

[0039] Pharmaceutical compositions of the disclosure can be prepared for storage as lyophilized formulations or aqueous solutions by mixing the antibody having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as “carriers”), *i.e.*, buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. *See, e.g., Remington's Pharmaceutical*

Sciences, 16th edition (Osol, ed. 1980). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[0040] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They can be present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, *etc.*), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, *etc.*), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, *etc.*), gluconate buffers (*e.g.*, gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, *etc.*), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, *etc.*), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, *etc.*) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, *etc.*). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0041] Preservatives can be added to retard microbial growth, and can be added in amounts ranging from 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as “stabilizers” can be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol

and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*e.g.*, peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccharides such as raffinose; and polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[0042] Non-ionic surfactants or detergents (also known as “wetting agents”) can be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188, *etc.*), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, *etc.*). Non-ionic surfactants can be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, for example about 0.07 mg/ml to about 0.2 mg/ml.

[0043] Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

[0044] Anti-PG antibodies can be administered singly, as mixtures of one or more anti-PG antibodies, in mixture or combination with other agents useful in preventing CRC, or

adjunctive to therapy for CRC. Examples of suitable combination and adjunctive therapies are provided above.

[0045] Encompassed by the present disclosure are pharmaceutical kits containing the anti-PG antibodies (including antibody conjugates) of the disclosure. The pharmaceutical kit is a package comprising the anti-PG antibody composition (*e.g.*, either in lyophilized form or as an aqueous solution) and one or more of the following:

- A second agent, for example as described above;
- A device for administering an anti-PG antibody composition, for example a pen, needle and/or syringe; and
- Pharmaceutical grade water or buffer to re-suspend the antibody if the antibody is in lyophilized form.

[0046] Each unit dose of anti-PG antibody can be packaged separately, and a kit can contain one or more unit doses (*e.g.*, two unit doses, three unit doses, four unit doses, five unit doses, eight unit doses, ten unit doses, or more). In a specific embodiment, the one or more unit doses are each housed in a syringe or pen.

7.4 Effective Dosages And Treatment Regimens

[0047] The anti-PG antibodies of the present disclosure are administered to the subject in an amount sufficient or effective to provide a therapeutic benefit. In the context of preventing gastrointestinal cancer, including CRC, in a subject predisposed to develop adenomatous polyps, a therapeutic benefit can be inferred if one or more of the following is achieved: reduction or lack of increase in the number and/or size of polyps in a subject; absence of malignant tumors, including where a subject has or had polyps; reduction or lack of increase in plasma or serum PG level; regression from a more advanced stage of polyposis to a less advanced stage of polyposis, according to Spigelman's classification (*e.g.*, regression from Stage IV to Stage III, from Stage III to Stage II, from Stage II to Stage I); lack of progression from Spigelman Stage IV polyposis to gastrointestinal cancer. Pharmaceutical compositions comprising anti-PG antibodies can be administered to individuals (*e.g.*, human subjects) at effective dosages.

[0048] Complete prevention of gastrointestinal cancer, while desirable, is not required for therapeutic benefit to exist. Indeed, as most patients suffering from FAP require major surgery by the age of 25, slowing the progression of the disease such that surgery can be delayed improves quality of life. Furthermore, any delay in the onset of gastrointestinal cancer, such as CRC, provides a therapeutic benefit.

[0049] In some contexts, therapeutic benefit can be correlated with one or more surrogate end points, in accordance with the knowledge of one of ordinary skill in the art. By way of example and not limitation, plasma and/or serum PG concentrations can be measured in a subject over time, with a reduction in PG levels, or a level below a threshold level, for example, below about 50 pM, 40 pM, 30 pM, 20 pM, 10 pM, or 5 pM, being indicative of therapeutic benefit.

[0050] Polyp size and number can be measured using endoscopic techniques, such as colonoscopy, as well as other methods known to those of ordinary skill in the art.

[0051] Binding all free PG is not required to achieve therapeutic efficacy, although it may be desirable. Free PG means PG that is available to be bound by an anti-PG antibody. Rather, reducing the concentration of free PG within or around polyps, systemically, in particular body fluids, or elsewhere, to a more limited extent may also be effective. Exemplary tissues and body fluids in which free PG concentration may be reduced by administration of anti-PG antibody(ies) compositions include, but are not limited to, polyp or tumor samples removed from a patient, ascites fluid, fluid from pleural effusions, cerebrospinal fluid, lymph, blood, plasma, serum and others. The concentration of PG in one or more of these tissues or body fluids can be quantified using an ELISA technique or other techniques familiar to those of ordinary skill in the art.

[0052] In accordance with the knowledge of those ordinarily skilled in the art, the dose of an anti-PG antibody can be titrated in a patient so as to reduce the free PG concentration in a tissue or body fluid of interest at a predetermined time after administration at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90, or 100%, or about 5%-10%, about 10%-15%, about 15%-20%, about 20%-25%, about 25%-30%, about 30%-35%, about 35%-40%, about 40%-45%, about 45%-50%, about 50%-55%, about 55%-60%,

about 60%-65%, about 65%-70%, about 70%-75%, about 75%-80%, about 80%-85%, about 85%-90%, or about 90%-95%, or a percentage reduction in free PG concentration ranging between any of the foregoing values.

[0053] The amount of anti-PG antibody administered will depend on a variety of factors, including the number and size of adenomatous polyps found in the subject, the form, route and site of administration, the treatment regimen (*e.g.*, whether a second therapeutic agent is used), the age and condition of the particular subject being treated, the sensitivity of the patient to anti-PG antibodies. The appropriate dosage can be readily determined by a person skilled in the art. Ultimately, a physician will determine appropriate dosages to be used. This dosage can be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice. The proper dosage and treatment regimen can be established by monitoring the progress of treatment using conventional techniques known to the people skilled of the art.

[0054] Effective dosages can be estimated initially from *in vitro* assays. For example, an initial dose for use in animals may be formulated to achieve a circulating blood or serum concentration of anti-PG antibody that is at or above the binding affinity of the antibody for progastrin as measured *in vitro*. Calculating dosages to achieve such circulating blood or serum concentrations taking into account the bioavailability of the particular antibody is well within the capabilities of skilled artisans. For guidance, the reader is referred to Fingl & Woodbury, "General Principles" in *Goodman and Gilman's The Pharmaceutical Basis of Therapeutics*, Chapter 1, latest edition, Pagamonon Press, and the references cited therein.

[0055] Initial dosages can be estimated from *in vivo* data, such as animal models. Animal models useful for testing the efficacy of compounds to delay or prevent development of gastrointestinal tumors, including CRC tumors, are well known in the art. Additionally, an animal model of FAP is described in the Examples below. Ordinarily skilled artisans can routinely adapt such information to determine dosages suitable for human administration.

[0056] In specific embodiments, an *i.v.* dose may be determined for an individual subject by measuring the serum or plasma PG concentration of the individual a few times a few days to a few weeks prior to treatment and calculating an amount of anti-PG antibody that would be saturating, *i.e.*, an amount that would be sufficient to bind all of the PG. As will be appreciated by skilled artisans, the amount of any specific antibody necessary to achieve saturation for a given serum or plasma concentration of PG will depend, in part, on the affinity constant of the particular antibody. Methods for calculating saturating quantities for specific anti-PG antibodies of interest are well-known.

[0057] To insure saturation, an amount that is greater than the calculated saturating amount may be administered, for example, at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or even 10-fold greater than the calculated saturating amount may be administered. For modes of administration other than *i.v.*, the amount can be adjusted based upon pharmacokinetic and bioavailability, as is well known in the art.

[0058] The effective dose of an anti-PG antibody of the disclosure can range from about 0.001 to about 75 mg/kg per single (*e.g.* bolus) administration, multiple administrations or continuous (*e.g.* infusion) administration, or to achieve a serum concentration of 0.01-5000 µg/ml serum concentration per single administration, multiple administrations or continuous administration, or any effective range or value therein depending on the condition being treated, the route of administration and the age, weight and condition of the subject. In certain embodiments, each dose can range from about 0.1 mg/kg to about 0.5 mg/kg; about 0.25 mg/kg to about 0.75 mg/kg; about 0.5 mg/kg to about 1 mg/kg; about 1 mg/kg to about 2 mg/kg; about 1.5 mg/kg to about 2.5 mg/kg; about 2 mg/kg to about 3 mg/kg; about 2.5 mg/kg to about 3.5 mg/kg; about 3 mg/kg to about 4 mg/kg; about 3.5 mg/kg to about 4.5 mg/kg; about 4 mg/kg to about 5 mg/kg; about 5 mg/kg to about 7 mg/kg; about 6 mg/kg to about 8 mg/kg; about 7 mg/kg to about 9 mg/kg; about 8 mg/kg to about 10 mg/kg; about 10 mg/kg to about 15 mg/kg; about 12.5 mg/kg to about 17.5 mg/kg; about 15 mg/kg to about 20 mg/kg; about 17.5 mg/kg to about 22.5 mg/kg; about 20 mg/kg to about 25 mg/kg; about 22.5 mg/kg to about 27.5 mg/kg; about 25 mg/kg to about 30 mg/kg; about 30 mg/kg to about 40 mg/kg; about 35 mg/kg to about 45 mg/kg; about 40 mg/kg to about 50 mg/kg; about 45 mg/kg to about 55 mg/kg; about

50 mg/kg to about 60 mg/kg; about 55 mg/kg to about 65 mg/kg; about 60 mg/kg to about 70 mg/kg; about 65 mg/kg to about 75 mg/kg. Other dosage ranges are also possible.

[0059] Amount, frequency, and duration of administration will depend on a variety of factors, such as the patient's age, weight, and disease condition. Anti-PG treatment is indicated in subjects in whom pre-cancerous adenomatous polyps are detected and/or removed, and subjects diagnosed with FAP who have yet to manifest polyps. In humans with FAP, polyps generally begin to appear in the second decade. Anti-PG treatment can be initiated before or at the time polyps are detected in subjects with FAP. For subjects with sporadic adenomatous polyposis, anti-PG treatment can be initiated at the time polyps are detected. Anti-PG treatment can also be initiated in subjects who have had at least one polyp removed, and therefore are at increased risk of developing more polyps and gastrointestinal cancer, including CRC.

[0060] A treatment regimen for administration can continue for 2 weeks to indefinitely. Optionally, the treatment regimen provides for repeated administration, *e.g.*, once daily, twice daily, every two days, three days, five days, one week, two weeks, or one month. The repeated administration can be at the same dose or at a different dose. The administration can be repeated once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more. An effective amount of anti-PG antibody can be administered as a single dose or over the course of a treatment regimen. The duration of anti-PG treatment for patients predisposed to develop adenomatous polyposis is preferably long, *e.g.*, over the course of years, but may be shorter, *e.g.*, one to several months, to a year.

7.5 Methods Of Selecting Patients for Follow-Up or Treatment, and Patient Monitoring to Determine Treatment Efficacy

[0061] Without wishing to be bound by any particular theory of operation, it is believed that elevated levels of PG are associated with the transformation of adenomatous polyps from benign to malignant. As shown in Example 6 below, subjects with multiple polyps had elevated PG levels whereas subjects who did not have polyps had either low or

undetectable levels of serum PG. Based on this observation, plasma and/or serum levels of PG can be used to identify patients for follow-up or treatment, as well as to monitor the effectiveness of prophylaxis in patients undergoing treatment.

[0062] Monitoring PG levels in individuals with FAP or a history of sporadic adenomatous polyposis is useful for identifying subjects in whom follow up by colonoscopy is warranted, as well as patients in need of anti-PG treatment. Standard care for individuals predisposed to developing adenomatous polyps is endoscopy at an interval of 3 to 5 years. This interval between testing may mean that, in certain individuals, numerous polyps have developed or cancer has set in by the time follow up endoscopy is performed. A simple blood test for PG levels is readily performed at more frequent intervals and can identify those individuals who should undergo endoscopy (*e.g.*, colonoscopy) sooner or who are candidates for anti-PG treatment.

[0063] An individual diagnosed with FAP or in whom polyps have previously been detected can be monitored to determine PG level, or concentration, in a bodily fluid, such as whole blood, plasma, or serum, relative to an appropriate baseline. Accordingly, a PG level is measured in a sample from the individual and then compared to a baseline PG level.

[0064] Where the PG level in a subject with FAP or a history of sporadic adenomatous polyposis is unchanged relative to previous measurements in the subject or equal to a baseline level for the relevant population to which the subject is being compared, the subject is scored as not requiring further follow up. By contrast, where the PG concentration is above the baseline, or is seen to rise over a period of time in the subject, the subject is a candidate for further follow-up, including, for example, colonoscopy, and for anti-PG treatment.

[0065] For purposes of monitoring efficacy of the treatment, blood, plasma, or serum PG levels can be measured in the patient receiving anti-PG treatment at specified time points, and used as an indication of whether the treatment is effective based on whether the measured level is above or below a baseline PG level. This information can be used by care providers to decide whether to continue administering an anti-PG antibody or

modify treatment. These methods can be used to monitor anti-PG treatment, used alone, or in combination with other treatments, as described above.

[0066] In some embodiments of the methods, the PG level in one or more bodily fluids, such as whole blood, plasma, serum, of a patient receiving anti-PG antibody treatment can be measured and then compared to a baseline level. A decrease in concentration over time, and/or a measured level below a threshold value at a particular point in time, is indicative of efficacy. An increase in concentration over time and/or above-baseline PG level is indicative of lack of treatment efficacy. Typically, PG level is the concentration of PG in the sample, expressed in molar (M) amounts or moles/liter (mol/liter).

[0067] The baseline level can be a single number or a range of numbers. The baseline can be based on one or more measurements taken from the patient or based on measurements of PG in samples from a population of individuals. In some embodiments of the methods, the baseline is a PG level from the same patient, taken at one or more interval, for example, before the initiation of anti-PG treatment, during the course of treatment, or after treatment has been stopped. In some embodiments, the baseline can be an average PG level in a population of individuals with characteristics similar to those of the individual undergoing monitoring. Such characteristics may include, but are not necessarily limited to sex, age, location of mutation in APC gene, stage in Spigelman classification, history of surgery, anti-PG treatment, or other treatment. In some embodiments, the baseline is a specific PG level, such as about 50 pM, about 40 pM, about 30 pM, about 20 pM, about 10 pM, about 5 pM, about 2 pM, about 1 pM, or even lower. In some embodiments, the baseline is a range.

[0068] PG levels can be measured using techniques familiar to those of ordinary skill in the art, such as, but not limited to, RIA and ELISA. In a specific embodiment, PG levels may be measured using a sandwich ELISA with one anti-PG antibody targeting the N-terminus of progastrin and a second anti-PG antibody targeting the C-terminus of progastrin. Exemplary N- and C-terminal anti-PG antibodies useful for such a sandwich assay are described in a later section. In such an assay, a surface, such as the wells in a 96-well plate, is prepared to which a known quantity of a first, "capture," N-terminal or

C-terminal anti-PG antibody is bound. A test sample is then applied to the surface followed by an incubation period. The surface is then washed and a solution containing a second, "detection," anti-PG antibody is applied, where the detection antibody binds a different epitope of PG (for example, if the capture antibody is a C-terminal anti-PG antibody, an N-terminal anti-PG antibody is used as the detection antibody, and *vice versa*). PG levels are then measured either directly (if, for example, the detection antibody is conjugated to a detectable label) or indirectly (through a labeled secondary antibody that binds the detection anti-PG antibody). For this assay, antibodies should be used in excess such that all PG is bound and quantified. A specific sandwich assay for measuring plasma and/or serum PG levels is provided in Example 1.

[0069] Multiple measurements at different intervals may be taken, and then graphed to determine if a trend exists. In a non-limiting example, PG levels can be determined at weekly, monthly, or annual intervals while a patient is received anti-PG antibodies. Other intervals are also possible.

[0070] In an embodiment involving a round of therapy using an anti-PG antibody, one or more measurements may also be taken during the course of therapy so that the effect of the antibodies on PG levels can be estimated. In other such embodiments, where residual anti-PG antibodies are present in a patient during sampling, the data may show a reduction in PG levels, due to sequestration of PG by the antibodies, followed by a rise, as this effect abates, followed by a subsequent decline, if the treatment was effective. In yet other embodiments, post-therapy measurements can be taken after it is estimated that the anti-PG antibodies have been cleared from the patient so that binding of PG by such antibodies does not affect the accuracy of the measurement of PG concentration.

[0071] Because eating usually increases gastrin synthesis and secretion, it may also cause transient increases in blood PG levels, which may interfere with the accurate measurement of PG levels in patients being monitored. To avoid this effect, particularly where PG concentration in blood samples is to be determined, samples can be taken from the patient after fasting.

7.6 Anti-PG Antibodies

[0072] Antibodies useful in the methods disclosed herein are those that specifically bind progastrin over other products of the gastrin gene. Referring to FIG. 1, the human gastrin gene is translated into a 101-amino acid polypeptide, called pre-progastrin, which contains a signal sequence (underlined) that is cleaved, giving rise to human progastrin, an 80-amino-acid polypeptide. Progastrin, in turn, is cleaved to generate a 34-amino-acid product, corresponding in sequence to residues 38-71 of progastrin, which is then extended at its carboxy terminus with a glycine residue, generating glycine-extended G34 ("G34-Gly"). A by-product of this cleavage is a 6-amino-acid peptide, called the C-terminal flanking peptide, or CTFP, which corresponds in sequence to residues 75-80 of progastrin. G34-Gly is then further cleaved to generate a 17-residue polypeptide corresponding in sequence to residues 55-71 of progastrin and referred to as G17-Gly. Removal of the C-terminal glycines of G34-Gly and G17-Gly, followed by C-terminal amidation, yields G34 and G17, respectively, both of which are C-terminal amidated.

[0073] As used herein, an antibody is "highly specific for" hPG or "highly specifically binds" hPG if it binds to full-length progastrin but does not bind at all to CTFP, to amidated gastrin, or to glycine-extended gastrin, and is "specific for" hPG or "specifically binds" hPG if it exhibits at least about 5-fold greater binding of hPG than CTFP and the other products of the gastrin gene, as measured in standard binding assays. A specific ELISA assay that can be used to assess the specificity of a particular anti-hPG antibody is provided in Example 2.

[0074] Such highly specific and/or specific anti-hPG antibodies (referred to herein as "anti-hPG antibodies") may be polyclonal ("anti-hPG PABs") or monoclonal ("anti-hPG MABs"), although for therapeutic uses and, in some instances, diagnostic or other *in vitro* uses, monoclonal antibodies are preferred.

[0075] The epitope bound by the anti-hPG antibodies is not critical. Useful anti-hPG antibodies may bind an N-terminal region of hPG, a C-terminal region of hPG, or a different region of hPG. Recently, it has been discovered that, at least for monoclonal anti-hPG antibodies, the selection of antigen used to raise the anti-hPG antibodies may be

important (*see*, International Application No. PCT/EP2010/006329 filed October 15, 2010 and U.S. application No. 12/906,041 filed October 15, 2010, the disclosures and specifically disclosed anti-hPG antibodies of which are incorporated herein by reference; hereinafter referred to as the '329 and '041 applications, respectively). As disclosed in the '329 and '041 applications, not all antigens derived from hPG stimulate production of monoclonal antibodies that specifically bind hPG under physiological conditions.

Indeed, certain antigens that have been used to successfully raise polyclonal anti-hPG antibodies, such as full-length recombinant hPG (*see, e.g.*, WO 08/076454 to Singh) and a peptide corresponding to the last ten amino acids at the C-terminal end of hPG (*see* WO 07/135542 to Hollande *et al.*) failed to generate monoclonal antibodies. As noted in the '329 and '041 applications, antigenic N-terminal and C-terminal sequences within the hPG sequence have been identified that can be used to generate monoclonal antibodies that specifically bind hPG. Interestingly, the antigenic sequence need not be limited to regions of the hPG sequence that are unique to it. Peptide antigens having regions of sequence in common with other products of the gastrin gene, for example, G17, G34 and CTFP, yield monoclonal antibodies that not only bind hPG, but bind it specifically.

[0076] Anti-hPG antibodies obtainable using a peptide antigen having a sequence corresponding to an N-terminal region of hPG and/or that bind an N-terminal region of hPG are referred to herein as "N-terminal anti-PG antibodies." A specific exemplary antigenic region of hPG that can be used to construct an immunogen suitable for obtaining both polyclonal and monoclonal antibodies specific for hPG corresponds to residue 1 to 14 of hPG: SWKPRSQQPDAPLG (SEQ ID NO:25). Exemplary immunogens useful for obtaining N-terminal anti-hPG antibodies, as well as CDR and V_H and V_L sequences of N-terminal anti-hPG monoclonal antibodies obtained with these exemplary immunogens, are provided in TABLE 2A, below, and the Example sections:

TABLE 2A
N-Terminal Anti-hPG Monoclonal Antibodies

Immunogen	Hybridoma (Deposit #)	MAb	Murine CDR Sequences			Murine V _H and V _L Sequences	Humanized V _H and V _L Sequences (projected)
N1	43B9G11	MAb1					
N1	WE5H2G7	MAb2					
N2	6B5B11C10	MAb3	V _H CDR 1.3	GYFTSYW	(SEQ ID NO:1)	mV _H .3 (SEQ ID NO:12)	hV _H .3 (SEQ ID NO:21)
			V _H CDR 2.3	FYPGNSDS	(SEQ ID NO:2)		
			V _H CDR 3.3	TRRDSPQY	(SEQ ID NO:3)		
			V _L CDR 1.3	QSIVHSNGNTY	(SEQ ID NO:4)	mV _L .3 (SEQ ID NO:13)	hV _L .3 (SEQ ID NO:22)
			V _L CDR 2.3	KVS	(SEQ ID NO:5)		
			V _L CDR 3.3	FQGSHVPPT	(SEQ ID NO:6)		
N2	20D2C3G2	MAb4	V _H CDR 1.4	GYTFSSSW	(SEQ ID NO:7)	mV _H .4 (SEQ ID NO:14)	hV _H .4 (SEQ ID NO:23)
			V _H CDR 2.4	FLPGSGST	(SEQ ID NO:8)		
			V _H CDR 3.4	ATDGNYDWFAY	(SEQ ID NO:9)		
			V _L CDR 1.4	QSLVHSSGVTY	(SEQ ID NO:10)	mV _L .4 (SEQ ID NO:15)	hV _L .4 (SEQ ID NO:24)
			V _L CDR 2.4	KVS	(SEQ ID NO:5)		
			V _L CDR 3.4	SQSTHVPPT	(SEQ ID NO:11)		
N2	1E9A4A4 (I-4376)	MAb15					
N2	1E9D9B6	MAb16	V _H CDR 1.16	GYFTSYYY	(SEQ ID NO:39)	mV _H .16 (SEQ ID NO:61)	hV _H .16a (SEQ ID NO:84)
			V _H CDR 2.16	INPSNGGT	(SEQ ID NO:43)		
			V _H CDR 3.16	TRGGYYPFDY	(SEQ ID NO:47)		
			V _L CDR 1.16	QSLDSDGKTY	(SEQ ID NO:50)	mV _L .16 (SEQ ID NO:65)	hV _L .16a (SEQ ID NO:85)
			V _L CDR 2.16	LVS	(SEQ ID NO:53)		
			V _L CDR 3.16	WQGTSPYT	(SEQ ID NO:57)		
N2	1C8D10F5	MAb17					
N2	1A7C3F11	MAb18					

TABLE 2A N-Terminal Anti-hPG Monoclonal Antibodies							
Immunogen	Hybridoma (Deposit #)	MAb	Murine CDR Sequences			Murine V _H and V _L Sequences	Humanized V _H and V _L Sequences (projected)
N2	1B3B4F11	MAb19	V _H CDR 1.19	GYSITSDYA	(SEQ ID NO:40)	mV _H .19 (SEQ ID NO:62)	hV _H .19a (SEQ ID NO:90)
			V _H CDR 2.19	ISFSGYT	(SEQ ID NO:44)		hV _H .19b (SEQ ID NO:92)
			V _H CDR 3.19	AREVNYGDSYHFDY	(SEQ ID NO:48)		hV _H .19c (SEQ ID NO:94)
			V _L CDR 1.19	SQHRTYT	(SEQ ID NO:51)	mV _L .19 (SEQ ID NO:66)	hV _L .19a (SEQ ID NO:91)
			V _L CDR 2.19	VKKDGSH	(SEQ ID NO:54)		hV _L .19b (SEQ ID NO:93)
			V _L CDR 3.19	GVGDAIKGQSVFV	(SEQ ID NO:58)		hV _L .19c (SEQ ID NO:95)
N2	1C11F5E8	MAb20					
Immunogen N1 = SWKPRSQQPDAPLG-Ahx-Cys-BSA, also represented as (SEQ ID NO:25)-Ahx-Cys-BSA Immunogen N2 = SWKPRSQQPDAPLG-Ahx-Cys-KLH, also represented as (SEQ ID NO:25)-Ahx-Cys-KLH							

In TABLE 2A, all amino acid sequences are represented using conventional N→C orientation. For each immunogen, the progastrin peptide was synthesized with a C-terminal linker of one aminohexanoic acid (Ahx) residue followed by a cysteine (Cys) residue, which was then conjugated to a either a bovine serum albumin (“BSA”) or keyhole limpet hemocyanin (“KLH”) carrier *via* the Cys linker residue.

[0077] Anti-hPG antibodies obtainable using a peptide antigen having a sequence corresponding to a C-terminal region of hPG, and/or that bind a C-terminal region of hPG, are referred to herein as “C-terminal anti-hPG antibodies.” A specific exemplary antigenic region that can be used to construct an immunogen useful for obtaining both polyclonal and monoclonal C-terminal anti-hPG antibodies corresponds to residues 55 to 80 of hPG: QGPWLEEEEEEAYGWMDFGRRSAEDEN (SEQ ID NO:27). Exemplary immunogens including this antigen useful for obtaining C-terminal anti-hPG antibodies, as well as CDR and V_H and V_L sequences of C-terminal anti-hPG monoclonal antibodies obtained with these exemplary immunogens, are provided in TABLE 2B, below, and the Examples section.

TABLE 2B
C-Terminal Anti-hPG Monoclonal Antibodies

TABLE 2B C-Terminal Anti-hPG Monoclonal Antibodies									
Immunogen	Hybridoma (Deposit #)	MAb	Murine CDR Sequences			Murine V _H and V _L Sequences		Humanized V _H and V _L Sequences (projected)	
C1	1B4A11D11 (I-4371)	MAb5							
C1	1B6A11F2 (I-4372)	MAb6							
C1	1B11E4B11 (I-4373)	MAb7							
C1	1C10D3B9	MAb8	V _H CDR 1.8	GFTFTTYA	(SEQ ID NO:37)	mV _H .8	(SEQ ID NO:59)	hV _H .8a	(SEQ ID NO:75)
			V _H CDR 2.8	ISSGGTYT	(SEQ ID NO:41)			hV _H .8b	(SEQ ID NO:77)
			V _H CDR 3.8	ATQGNYSLDF	(SEQ ID NO:45)			hV _H .8c	(SEQ ID NO:79)
			V _L CDR 1.8	KSLRHTKGITF	(SEQ ID NO:49)	mV _L .8	(SEQ ID NO:63)	hV _L .8a	(SEQ ID NO:76)
			V _L CDR 2.8	QMS	(SEQ ID NO:52)			hV _L .8b	(SEQ ID NO:78)
			V _L CDR 3.8	AQNLELPLT	(SEQ ID NO:55)			hV _L .8c	(SEQ ID NO:76)
C1	1D8F5B3	MAb9							
C1	1E1C7B4	MAb10							
C1	2B4C8C8 (I-4374)	MAb11							
C1	2B11E6G4 (I-4375)	MAb12							
C1	2C6C3C7	MAb13	V _H CDR 1.13	GFISSYG	(SEQ ID NO:38)	mV _H .13	(SEQ ID NO:60)	hV _H .13a	(SEQ ID NO:80)
			V _H CDR 2.13	INTFGDRT	(SEQ ID NO:42)			hV _H .13b	(SEQ ID NO:82)
			V _H CDR 3.13	ARGTGTY	(SEQ ID NO:46)				
			V _L CDR 1.13	QSLDSDGKTY	(SEQ ID NO:50)	mV _L .13	(SEQ ID NO:64)	hV _L .13a	(SEQ ID NO:81)
			V _L CDR 2.13	LVS	(SEQ ID NO:53)			hV _L .13b	(SEQ ID NO:83)
			V _L CDR 3.13	WQGTHFPQT	(SEQ ID NO:56)				
C1	2H9F4B7	MAb14							

TABLE 2B C-Terminal Anti-hPG Monoclonal Antibodies					
Immunogen	Hybridoma (Deposit #)	MAb	Murine CDR Sequences	Murine V_H and V_L Sequences	Humanized V_H and V_L Sequences (projected)
C2	1F11F5E10	MAb21			
C2	1F11F5G9	MAb22			
C2	1A11F2C9	MAb23			
Immunogen C1 = KLH-Cys-Ahx-Ahx-QGPWLEEEEEAYGWMDFGRRSAEDEN, also represented as KLH-Cys-Ahx-Ahx-(SEQ ID NO:27) Immunogen C2 = DT-Cys-Ahx-Ahx-QGPWLEEEEEAYGWMDFGRRSAEDEN, also represented as DT-Cys-Ahx-Ahx-(SEQ ID NO:27)					

In TABLE 2B, all amino acid sequences are represented using conventional N→C orientation. For each immunogen, the progastrin peptide was synthesized with an N-terminal Ahx-Ahx-Cys linker, which was then conjugated to either a keyhole limpet hemocyanin (“KLH”) or a diphtheria toxin (“DT”) carrier via the Cys linker residue.

[0078] The specific epitopes bound by the exemplary anti-hPG monoclonal antibodies MAb1-MAb23 provided in TABLES 2A and 2B were mapped using the SPOT technique and alanine scanning, as described in Laune *et al.*, 2002, J. Immunol. Methods 267:53-70 and Laune, 1997, J. Biol. Chem. 272:30937-30944, respectively (*see also*, Example 6 of the '329 application).

[0079] In the SPOT technique, 15 amino acid peptide sequences spanning a putative epitope are generated and spotted onto a nitrocellulose membrane which is then probed with the test antibody to determine the minimal epitope sequence recognized by the antibody. Alanine scanning is used to determine residues within an epitope that are critical for antibody binding. Each residue within a putative epitope is mutated, one by one, to an alanine, and the alanine-containing peptides are then probed with the test antibody.

[0080] For N-terminal anti-hPG monoclonal antibodies MAbs1-4 and 15-20, epitopes comprise at least the following sequences: DAPLG (SEQ ID NO:28), PDAPLG (SEQ ID NO:29), PRSQQPD (SEQ ID NO:30), WKPRSQQPD (SEQ ID NO:31), or WKPRSQQPDAPLG (SEQ ID NO:32), as shown in TABLE 3A below.

TABLE 3A		
MAb#	PG peptide antigen: SWKPRSQQPDAPLG	SEQ ID NO
MAb2	WKPRSQQPDAPLG	32
MAb4	WKPRSQQPDAPLG	32
MAb1	PDAPLG	29
MAb3	DAPLG	28
MAb17	WKPRSQQPD	31
MAb18	WKPRSQQPD	31
MAb19	WKPRSQQPD	31
MAb20	WKPRSQQPD	31
MAb15	PRSQQPD	30
MAb16	PRSQQPD	30

[0081] For C-terminal anti-hPG monoclonal antibodies MAbs5-7, 9-12, 14 and 21-23, epitopes comprise at least the following sequences: FGRR (SEQ ID NO:33), MDFGR

(SEQ ID NO:34), AEDEN (SEQ ID NO:35), and GWMDFGRR (SEQ ID NO:36), as shown in TABLE 3B, below.

TABLE 3B		
MAb#	PG peptide antigen: QGPWLEEEEEAYGWMDFGRRSAEDEN	SEQ ID NO
MAb14	GWMDFGRR	36
MAb11	MDFGR	34
MAb5	FGRR	33
MAb6	FGRR	33
MAb7	FGRR	33
MAb9	FGRR	33
MAb10	FGRR . . E	33
MAb12	FGRR	33
MAb23	AEDEN	35

[0082] The epitope mapping experiments reveal that anti-hPG MAb2 and MAb4 bind the same epitope; anti-hPG MAb1 and MAb3 bind approximately the same epitope; MAb17, MAb18, MAb19, and MAb20 bind approximately the same epitope; MAb15 and MAb16 bind approximately the same epitope; anti-hPG MAb5, MAb6, MAb7, MAb9, and MAb12 bind the same epitope and bind approximately the same epitope as anti-hPG MAb10; and anti-hPG MAb11 and MAb14 bind approximately the same epitope.

[0083] Specific embodiments of N-terminal anti-PG antibodies useful in the methods and kits described herein include antibodies that bind an epitope that includes residues 10 to 14 of hPG (SEQ ID NO:28), residues 9 to 14 of hPG (SEQ ID NO:29), residues 4 to 10 of hPG (SEQ ID NO:30), residues 2 to 10 of hPG (SEQ ID NO:31), or residues 2 to 14 of hPG (SEQ ID NO:32).

[0084] Specific embodiments of C-terminal anti-PG antibodies useful in the methods and kits described herein include antibodies that bind an epitope that includes residues 71 to 74 of hPG (SEQ ID NO:33), residues 69 to 73 of hPG (SEQ ID NO:34), residues 76 to 80 of hPG (SEQ ID NO:35), or residues 67 to 74 of hPG (SEQ ID NO:36).

[0085] N-terminal and C-terminal anti-hPG antibodies useful in the methods and kits disclosed herein in addition to those provided in TABLES 2A & 2B can be identified in

competitive binding assays with exemplary MAbs 1-23, or with other reference antibodies that bind N- or C- terminal epitopes, as will be described in more detail in a later section.

[0086] As also reported in the '329 and '041 applications, not all anti-hPG antibodies, even those that exhibit a high degree of specificity and affinity for hPG, may neutralize the biological activity of hPG. For example, although anti-hPG MAb14 binds hPG with a K_D of about 6 pM, it did not inhibit the growth of colorectal cancer cells in an *in vitro* assay, whereas other anti-hPG monoclonal antibodies exhibited significant inhibitory activity (*see, e.g.*, Example 7 of the '329 application). While both non-neutralizing and neutralizing antibodies that specifically bind hPG are useful for the various diagnostic and monitoring methods described herein, anti-hPG antibodies useful for therapeutic methods should exhibit neutralizing activity.

[0087] As used herein, a "neutralizing anti-hPG antibody" is an anti-hPG antibody that yields a statistically significant reduction in the number of live LS174T in a test sample treated with the anti-hPG antibody as compared to a control sample treated with a non-specific antibody. A specific assay for assessing the ability of any particular anti-hPG antibody to neutralize hPG is described in Example 3. Those anti-hPG antibodies that exhibit at least about a 50% reduction in the number of live cells in this assay are believed to be especially useful in methods of preventing gastrointestinal cancer, including CRC, although anti-hPG antibodies exhibiting lower levels of neutralizing activity, for example, a statistically significant reduction of 40%, 30%, 20%, 15%, or even 10%, in the number of live cells in this assay, are expected to provide therapeutic benefits.

[0088] Accordingly, in some embodiments, for example therapeutic embodiments, useful anti-hPG antibodies are neutralizing. As disclosed in the '329 and '041 applications, the ability of an anti-hPG monoclonal antibody is not epitope-dependent, as both N-terminal and C-terminal anti-hPG monoclonal antibodies exhibited neutralizing activity in assays with colorectal cancer cells bearing a mutation in the APC gene. Thus, in some specific embodiments, the neutralizing anti-hPG antibodies are N-terminal neutralizing anti-hPG antibodies. In other embodiments, the neutralizing anti-hPG antibodies are C-terminal neutralizing anti-hPG antibodies.

[0089] The affinity of any specific anti-hPG antibody is not critical. However, for some uses, antibodies exhibiting affinities of at least about 1 μ M may be preferred. For therapeutic uses, an affinity of at least about 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 15 nM, 10 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.1 nM, 0.01 nM, or even greater, may be desirable. The measured affinities of the anti-hPG monoclonal antibodies identified in TABLES 2A & 2B range from 10^{-6} to 10^{-12} M, as noted in TABLE 4, below:

TABLE 4	
MAb#	Affinity (measured K_D)
MAb1	2.5 μ M (2.5×10^{-6} M)
MAb2	185 nM (1.85×10^{-7} M)
MAb3	6.4 nM (6.4×10^{-9} M)
MAb4	3.5 nM (3.5×10^{-9} M)
MAb5	13 pM (1.30×10^{-11} M)
MAb6	0.6 nM (6.38×10^{-10} M)
MAb7	58 pM (5.84×10^{-11} M)
MAb8	0.1 nM (1.08×10^{-10} M)
MAb10	3.6 nM (3.62×10^{-9} M)
MAb11	0.3 nM (3.12×10^{-10} M)
MAb12	0.4 nM (4.43×10^{-10} M)
MAb13	0.6 nM (6.12×10^{-10} M)
MAb14	6.8 pM (6.86×10^{-12} M)
MAb15	0.2 nM (2.11×10^{-10} M)
MAb16	0.2 nM (2.78×10^{-10} M)
MAb17	8.3 nM (8.29×10^{-9} M)
MAb18	1.2 nM (1.24×10^{-9} M)
MAb19	0.7 nM (7.79×10^{-10} M)
MAb20	0.2 nM (2.47×10^{-10} M)
MAb21	3.9 nM (3.90×10^{-9} M)
MAb22	5 nM (4.94×10^{-9} M)
MAb23	0.4 μ M (3.99×10^{-7} M)

[0090] An anti-PG monoclonal antibody having an affinity especially suited for a particular desired application can be readily selected from amongst these, or generated or designed using the various immunogens, complementarity determining region (CDR)

sequences, variable heavy (V_H) and variable light (V_L) chain sequences of anti-hPG antibodies described herein. The affinity of any particular anti-PG monoclonal antibody can be determined using techniques well known in the art or described herein, such as for example, ELISA, isothermal titration calorimetry (ITC), BIAcore, or fluorescent polarization assays. A specific assay is provided in Example 4.

[0091] As noted in TABLES 2A & 2B, several N-terminal and C-terminal monoclonal anti-hPG antibodies have been identified. All of these antibodies are specific for hPG, and, with the exception of MAb14, all exhibited neutralizing activity in tests with colorectal cancer cells. Several of the hybridomas useful for obtaining the antibodies were deposited on October 6, 2010 with the Collection Nationale de Cultures de Microorganismes (CNCM) in accordance with the Treaty of Budapest. The designated names of the hybridomas producing anti-hPG MAbs1-23 and the depository registration numbers of those hybridomas deposited are provided in TABLES 2A & 2B. In addition, for several of the antibodies, the amino acid sequences of their variable heavy chains (V_H), variable light chains (V_L), V_L complementarity determining regions (CDRs) and V_H CDRs have been determined. These amino acid sequences, and the shorthand nomenclature used to reference them throughout the disclosure, are also provided in TABLES 2A & 2B. Briefly, murine heavy and light chain variable domains are referred to herein as mV_H and mV_L followed by the number of the corresponding monoclonal antibody, for example $mV_H.3$ and $mV_L.3$ for the variable light and variable heavy chains of anti-hPG MAb3, respectively. Similarly, human heavy and light chain variable domains are referred to herein as hV_H and hV_L followed by the number of the corresponding monoclonal antibody. The three variable heavy chain CDRs and three variable light chain CDRs are referred to as V_H CDR 1, 2, or 3, and V_L CDR 1, 2, or 3, respectively, followed by the number of the specific anti-hPG monoclonal antibody. For example, V_H CDR 1 of MAb3 is denoted V_H CDR 1.3 and V_L CDR 1 of MAb3 is denoted V_L CDR 1.3. V_H CDR 2 of MAb3 is denoted V_H CDR 2.3, and V_L CDR 2 of MAb3 is denoted V_L CDR 2.3.

[0092] It is expected that corresponding CDRs and/or V_H and V_L chains of anti-hPG monoclonal antibodies that bind approximately the same epitopes could be interchanged to yield new anti-hPG monoclonal antibodies useful in the methods and kits described

herein. For example, as noted above, exemplary anti-hPG monoclonal antibodies MAb5 and MAb6 bind the same epitope. An anti-hPG monoclonal antibody can be designed that includes, in its V_L chain, various combinations of the V_L CDRs of these two antibodies, and/or in its V_H chain various combinations of the V_H CDRs of these two antibodies. As a specific non-limiting example to illustrate the various combinations possible, such an antibody could include in its V_L chain, CDRs 1 and 2 of MAb5 (V_L CDR 1.5 and V_L CDR 2.5, respectively) and CDR 3 of MAb6 (V_L CDR 3.6), and in its V_H chain, CDR 1 of MAb6 (V_H CDR 1.6) and CDRs 2 and 3 of MAb5 (V_H CDR 2.5 and V_H CDR 3.5, respectively). Amino acid sequences of CDRs of antibodies (also known as hypervariable regions) produced by hybridomas that have been deposited can be obtained using conventional means.

[0093] As is known in the art, the amino acid position/boundary delineating a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art. Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions. The anti-PG antibodies described herein may contain modifications in these hybrid hypervariable positions. The variable domains of native heavy and light chains each comprise four FR regions, largely by adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies (see Kabat *et al.*, 1987, *Sequences of Proteins of Immunological Interest*, National Institute of Health, Bethesda, Md.). As used herein, numbering of immunoglobulin amino acid residues is done according to the immunoglobulin amino acid residue numbering system of Kabat *et al.*, unless otherwise indicated.

[0094] With reference to TABLE 2A, specific embodiments of N-terminal anti-hPG antibodies useful in the methods and kits described herein include, but are not limited to, the following:

(a) antibodies having V_L CDRs that correspond in sequence to the V_L CDRs of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20, and V_H CDRs that correspond in sequence to the V_H CDRs of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20;

(b) antibodies having V_L CDRs and V_H CDRs that correspond in sequence to the V_L and V_H CDRs of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20;

(c) antibodies in which:

(i) V_L CDR 1 is selected from QSIVHSNGNTY ("V_L CDR 1.3"; SEQ ID NO:4), QSLVHSSGVITY ("V_L CDR 1.4"; SEQ ID NO:10), QSLDSDGKTY ("V_L CDR 1.16"; SEQ ID NO:50), and SQHRTYT ("V_L CDR 1.19"; SEQ ID NO:51);

(ii) V_L CDR 2 is selected from KVS ("V_L CDR 2.3" or "V_L CDR 2.4"; SEQ ID NO:5), LVS ("V_L CDR 2.16"; SEQ ID NO:53), and VKKDGS ("V_L CDR 2.19"; SEQ ID NO:54);

(iii) V_L CDR 3 is selected from FQGSHVPFT ("V_L CDR 3.3"; SEQ ID NO:6), SQSTHVPPT ("V_L CDR 3.4"; SEQ ID NO:11), WQGTHTSPYT ("V_L CDR 3.16"; SEQ ID NO:57), and GVGDAIKGQSVFV ("V_L CDR 3.19"; SEQ ID NO:58);

(iv) V_H CDR 1 is selected from GYIFTSYW ("V_H CDR 1.3"; SEQ ID NO:1), GYTFSSSW ("V_H CDR 1.4"; SEQ ID NO:7), GYTFTSY ("V_H CDR 1.16"; SEQ ID NO:39), and GYSITSDYA ("V_H CDR 1.19"; SEQ ID NO:40);

(v) V_H CDR 2 is selected from FYPGNSDS ("V_H CDR 2.3"; SEQ ID NO:2), FLPGSGST ("V_H CDR 2.4"; SEQ ID NO:8), INPSNGGT ("V_H CDR 2.16"; SEQ ID NO:43), and ISFSGYT ("V_H CDR 2.19"; SEQ ID NO:44); and

(vi) V_H CDR 3 is selected from TRRDSPQY ("V_H CDR 3.3"; SEQ ID NO:3), ATDGNYDWFAY ("V_H CDR 3.4" SEQ ID NO:9), TRGGYYPFDY ("V_H CDR 3.16"; SEQ ID NO:47), and AREVNYGDSYHFDY ("V_H CDR 3.19"; SEQ ID NO:48);

(d) antibodies having a V_L that corresponds in sequence to the V_L of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20 and a V_H

that corresponds in sequence to the V_H of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20; and

(e) antibodies having a V_L and a V_H that corresponds in sequence to the V_L and V_H of MAb1, MAb2, MAb3, MAb4, MAb15, MAb16, MAb17, MAb18, MAb19 or MAb20.

[0095] With reference to TABLE 2B, specific embodiments of C-terminal anti-hPG antibodies useful in the methods and kits described herein include, but are not limited to, the following:

(a) antibodies having V_L CDRs that correspond in sequence to the V_L CDRs of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23 and V_H CDRs that correspond in sequence to the V_H CDRs of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23;

(b) antibodies having V_L CDRs and V_H CDRs that correspond in sequence to the V_L and V_H CDRs of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23;

(c) antibodies in which:

(i) V_L CDR 1 is selected from KSLRHTKGITF ("V_L CDR 1.8"; SEQ ID NO:49) and QSLDSDGKTY ("V_L CDR 1.13"; SEQ ID NO:50);

(ii) V_L CDR 2 is selected from QMS ("V_L CDR 2.8"; SEQ ID NO:52) and LVS ("V_L CDR 2.13"; SEQ ID NO:53);

(iii) V_L CDR 3 is selected from AQNLELPLT ("V_L CDR 3.8"; SEQ ID NO:55) and WQGTHFPQT ("V_L CDR 3.13"; SEQ ID NO:56);

(iv) V_H CDR 1 is selected from GFTFTTYA ("V_H CDR 1.8"; SEQ ID NO:37) and GFIFSSYG ("V_H CDR 1.13"; SEQ ID NO:38);

(v) V_H CDR 2 is selected from ISSGGTYT ("V_H CDR 2.8"; SEQ ID NO:41) and INTFGDRT ("V_H CDR 2.13"; SEQ ID NO:42); and

(vi) V_H CDR 3 is selected from ATQGNYSLDF ("V_H CDR 3.8"; SEQ ID NO:45) and ARGTTGT ("V_H CDR 3.13"; SEQ ID NO:46);

(d) antibodies having a V_L that corresponds in sequence to the V_L of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23 and a V_H that corresponds in sequence to the V_H of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23; and

(e) antibodies having a V_L and a V_H that correspond in sequence to the V_L and V_H that correspond in sequence to the V_L and V_H of MAb5, MAb6, MAb7, MAb8, MAb9, MAb10, MAb11, MAb12, MAb13, MAb14, MAb21, MAb22 or MAb23.

[0096] As will be appreciated by skilled artisans, anti-hPG antibodies useful in the diagnostic methods can be of any origin, including, for example, mammalian (*e.g.*, human, primate, rodent, goat or rabbit), non-mammalian, or chimeric in nature (derived from more than one species of origin). Antibodies suitable for therapeutic uses in animals, including humans, are preferably derived from the same species intended to be treated, or have been modified or designed to be non-immunogenic or have reduced immunogenicity in the animal being treated. A specific class of anti-hPG antibodies useful for therapeutic uses in humans is the class of humanized antibodies, discussed in more detail, below. Anti-hPG antibodies useful in the methods and kits described herein can also be of, or derived from, any isotype, including, for example, IgA (*e.g.*, IgA1 or IgA2), IgD, IgE, IgG (*e.g.*, IgG1, IgG2, IgG3 or IgG4) or IgM. Anti-hPG antibodies designed for therapeutic uses are preferably of the IgG isotype.

[0097] In some embodiments, anti-hPG antibodies useful for therapeutic methods described herein are humanized. In general, humanized antibodies comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence, and can be referred to as “CDR-grafted.” The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence. Methods for humanizing antibodies, including methods for designing humanized antibodies, are well-known in the art. *See, e.g.*, Lefranc *et al.*, 2003, *Dev. Comp. Immunol.* 27:55-77; Lefranc *et al.*, 2009, *Nucl. Acids Res.* 37:D1006-1012; Lefranc, 2008, *Mol. Biotechnol.* 40: 101-111;

Riechmann *et al.*, 1988, Nature 332:323-7; U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,762 and 6,180,370 to Queen *et al.*; EP239400; PCT publication WO 91/09967; U.S. Patent No. 5,225,539; EP592106; EP519596; Padlan, 1991, Mol. Immunol. 28:489-498; Studnicka *et al.*, 1994, Prot. Eng. 7:805-814; Roguska *et al.*, 1994, Proc. Natl. Acad. Sci. 91:969-973; and U.S. Patent No. 5,565,332, the disclosures of which are hereby incorporated by reference in their entireties.

[0098] Humanized versions of antibodies having CDR sequences corresponding to the CDRs of non-human anti-hPG antibodies, including by way of example and not limitation, the various N-terminal anti-hPG monoclonal antibodies provided in TABLE 2A and the various C-terminal anti-hPG monoclonal antibodies provided in TABLE 2B, can be obtained using these well-known methods. Projected sequences for humanized V_L and V_H chains of selected anti-hPG antibodies are provided in TABLES 2A and 2B. Specific examples of humanized antibodies include antibodies comprising:

- (a) any three V_L CDRs and any three V_H CDRs disclosed herein;
- (b) a heavy chain variable region comprising an amino acid sequence corresponding to SEQ ID NO:21 and a light chain variable region comprising an amino acid sequence corresponding to SEQ ID NO:22;
- (c) a heavy chain variable region comprising an amino acid sequence corresponding to SEQ ID NO:23 and a light chain variable region comprising an amino acid sequence corresponding to SEQ ID NO:24;
- (d) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:75, 77, and 79 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:76 and 78;
- (e) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:80 and 82 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:81 and 83;
- (f) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:84, 86, and 88 and a light chain variable region

comprising an amino acid sequence selected from the group consisting of SEQ ID NO:85, 87, and 89; and

(g) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:90, 92, and 94 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:91, 93, and 95.

[0099] As will be recognized by skilled artisans, anti-hPG antibodies having specific binding properties, such as the ability to bind a specific epitope of interest, can be readily obtained using the various antigens and immunogens described herein and assessing their ability to compete for binding hPG with a reference antibody of interest. Any of the anti-hPG antibodies described herein can be utilized as a reference antibody in such a competition assay. A specific assay useful for assessing the ability of an antibody to compete for binding hPG with a biotinylated reference anti-hPG antibody of interest is provided in Example 5.

[0100] In conducting an antibody competition study between a reference anti-hPG antibody and any test antibody (irrespective of species or isotype), one may first label the reference with a label detectable either directly, such as, for example, a radioisotope or fluorophore, or indirectly, such as, for example biotin (detectable via binding with fluorescently-labeled streptavidin) or an enzyme (detectable via an enzymatic reaction), to enable subsequent identification. In this case, a labeled reference anti-hPG antibody (in fixed or increasing concentrations) is incubated with a known amount of hPG, forming an hPG:labeled anti-hPG antibody complex. The unlabeled test antibody is then added to the complex. The intensity of the complexed label is measured. If the test antibody competes with the labeled reference anti-hPG antibody for hPG by binding to an overlapping epitope, the intensity of the complexed label will be decrease relative to a control experiment carried out in the absence of test antibody.

[0101] Numerous methods for carrying out binding competition assays are known and can be adapted to yield results comparable to the assay described above and in Example 5.

[0102] An antibody is considered to compete for binding hPG with a reference anti-hPG antibody, and thus considered to bind approximately the same or an overlapping epitope of hPG as the reference anti-hPG antibody, if it reduces binding of the reference anti-hPG antibody to hPG in a competitive binding assay, and specifically the competitive binding assay of Example 5, by at least 50%, at a test antibody concentration in the range of 0.01-100 µg/mL (e.g., 0.01 µg/mL, 0.08 µg/mL, 0.4 µg/mL, 2 µg/mL, 10 µg/mL, 50 µg/mL or 100 µg/mL or other concentration within the stated range), although higher levels of reduction, for example, 60%, 70%, 80%, 90% or even 100%, may be desirable.

[0103] Skilled artisans will appreciate that in some contexts, for example, diagnostic and monitoring contexts, it may be desirable to label the anti-PG antibodies. Such labels are useful for detection and quantification. Suitable labels are well known in the art, and can be “direct” in that they are directly observable or detectable (for example, fluorophores or radioisotopes) or “indirect” in that they interact with something else that produces and observable or detectable signal (for example, an enzyme that acts on a substrate to produce a detectable signal, or a binding molecule such as biotin that binds a labeled, streptavidin molecule). Numerous labeling systems, as well as means for labeling antibodies with them, are known in the art, and are contemplated for use herein.

[0104] Although the various anti-hPG antibodies useful in the methods described herein have been exemplified with full length antibodies, skilled artisans will appreciate that binding fragments, or surrogate antibodies designed or derived from full-length antibodies or binding fragments, may also be used. Suitable fragments, surrogates, etc., include, but are not limited to, Fab', F(ab')₂, Fab, Fv, vIgG, scFv fragments and surrobodies. Unless specified otherwise, the term “antibody” as used herein is intended to include all forms of antibodies and “antibody-like” surrogate molecules, including single chain antibodies, surrobodies and binding fragments. Antibodies having structures typical of naturally occurring antibodies are referred to herein as “native antibodies.”

7.7 Methods of Producing Anti-PG Antibodies

[0105] Anti-PG antibodies useful in the methods described herein may be obtained using standard, well-known methods. To express anti-PG antibodies useful in the methods described herein, DNAs encoding partial or full-length light and heavy chains are inserted into expression vectors such that the genes are operatively linked to transcriptional and

translational control sequences. In this context, the term “operatively linked” is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector.

[0106] The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the anti-PG antibody light or heavy chain sequences, the expression vector can already carry antibody constant region sequences. For example, one approach to converting the anti-PG antibody V_H and V_L sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_L segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0107] In addition to the antibody chain genes, the recombinant expression vectors of the disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA, 1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of

regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Suitable regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, *see e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.*, and U.S. Patent No. 4,968,615 by Schaffner *et al.*

[0108] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors can carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (*see, e.g.*, U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, puromycin, blasticidin, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Suitable selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in DHFR⁻ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection). For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term “transfection” are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, lipofection, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

[0109] It is possible to express the antibodies described herein in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of antibodies is performed in eukaryotic cells, *e.g.*, mammalian host cells, for optimal secretion of a properly folded and immunologically active antibody. Exemplary mammalian host cells for expressing the recombinant antibodies of the disclosure include Chinese Hamster Ovary (CHO cells) (including DHFR⁻CHO cells, described in Urlaub & Chasin, 1980, Proc. Natl. Acad. Sci.

USA 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman & Sharp, 1982, Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells, 293 cells and SP2/0 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Host cells can also be used to produce portions of intact antibodies, such as F_{ab} fragments or scF_v molecules. It is understood that variations on the above procedure are within the scope of the present disclosure. For example, it can be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti-PG antibody described herein.

[0110] Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to PG. The molecules expressed from such truncated DNA molecules are also useful in the methods described herein.

[0111] For recombinant expression of an anti-PG antibody, the host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. Typically, the two vectors each contain a separate selectable marker. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides.

[0112] Anti-PG antibodies can also be produced by chemical synthesis (*e.g.*, by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.). Variant antibodies can also be generated using a cell-free platform (*see, e.g.*, Chu *et al.*, 2001, Biochemia No. 2 (Roche Molecular Biologicals)).

[0113] Once an anti-PG antibody has been produced by recombinant expression or synthetic means, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for PG after Protein A or Protein G selection, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the anti-PG antibodies or binding

fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

8. EXAMPLES

8.1 Example 1: Quantification of Plasma or Serum PG Levels

[0114] Plasma and/or serum levels of PG can be conveniently determined using the following assay. 96-well microtiter plates are coated with between 0.5 and 10 $\mu\text{g/mL}$ of a C-terminal anti-hPG antibody, for example, a rabbit C-terminal anti-hPG polyclonal antibody, or a C-terminal anti-hPG antibody described herein, and then incubated overnight. Plates are then washed three times in PBS-Tween (0.05%) and blocked with 2% (w/v) nonfat dried milk in PBS-Tween (0.05%). Separately, test samples, control samples (blank or PG-negative plasma or serum samples), and between about 5 pM (0.5×10^{-11} M) and about 0.1 nM (1×10^{-10} M) of an hPG reference standard (lyophilized hPG diluted in PG-negative plasma or serum) are prepared in an appropriate diluent (*e.g.*, PBS-Tween 0.05%). Samples are incubated on the coated plates for between 2 and 4 hours at 37°C, or alternatively between 12 and 16 hours at 21°C. After incubation, plates are washed three times with PBS-Tween (0.05%) and incubated with between 0.001 and 0.1 $\mu\text{g/mL}$ of an N-terminal anti-hPG antibody, for example, a polyclonal N-terminal anti-hPG antibody or an N-terminal monoclonal anti-hPG antibody as described herein, coupled to horseradish peroxidase (HRP) (*see*, Nakane *et al.*, 1974, J. Histochem. Cytochem. 22(12):1084–1091) for 30 minutes at 21°C. Plates are then washed three times in PBS-Tween (0.05%) and HRP substrate is added for 15 minutes at 21°C. The reaction is stopped by added 100 μL of 0.5M sulfuric acid and an optical density measurement is taken at 405 nm. Test sample hPG levels are determined by comparison to a standard curve constructed from the measurements derived from the hPG reference standard.

8.2 Example 2: ELISA Assay for Assessing Specificity of Anti-hPG Antibodies

[0115] Specificity of anti-hPG antibodies can be conveniently determined using an ELISA assays as follows. 96-well plates are incubated overnight at 4°C with appropriate concentration(s) of test polypeptide (*e.g.*, 25 and 50 ng recombinant human PG, and 50 and 250 ng CTFP or other gastrin-derived gene products) in Phosphate-Buffered Saline

(PBS), after which the wells are washed three times with wash solution (PBS and 0.1% Tween-20), and then incubated for 2 hours at 22°C with 100 µL blocking solution (PBS, 0.1% Tween-20, 0.1% Bovine Serum Albumin or casein hydrolysate) per well. After blocking, the wells are washed three times and the antibody to be assayed (test antibody) is added. 100 µL of the test antibody (at 0.3 to 1 ng/mL) in PBS and 0.1% Tween-20 are added to each well. Plates are then incubated for 2 hours at 22°C, after which the test antibody solution is discarded and replaced, after a wash step (3X 100 µL wash solution, as noted above), with blocking solution containing a secondary antibody, a goat anti-mouse IgG (Fc) antibody coupled to horseradish peroxidase. After a 1-hour incubation with secondary antibody, 100 µL of substrate solution (*e.g.* Fast OPD, or O-Phenylenediamine dihydrochloride, available from Sigma-Aldrich Co., prepared according to manufacturer's directions) is added to each well and incubated in the dark for 20 minutes at 22°C. The reaction is stopped by adding 50 µL of 4N sulfuric acid and the amount of substrate catalyzed determined by measuring the optical density (O.D.) at 492 nm. Substrate conversion is proportional to the amount of primary (test) antibody bound to the antigen. Experiments are run in duplicate and OD measurements plotted as a function of antigen concentration. Test antibodies are scored as specific for PG if the measured O.D. is between 0.2 and 1.5 for hPG and there is no statistically significant signal above background with CTFP or any of the other gastrin-gene derived peptides, where the background is the average signal from control wells containing only PBS.

8.3 Example 3: Assay for Assessing Neutralizing Activity of Anti-hPG Antibodies

[0116] A specific test for assessing whether a specific anti-hPG antibody is neutralizing can be performed as follows. LS174T cells are seeded in 6 wells of a 6-well plate, at approximately 50,000 cells per well. Cells are then treated at 12-hour intervals for 48 hours with the test anti-hPG antibody or a control antibody, at antibody concentrations of about 5 µg/mL. A test antibody is defined as neutralizing in the assay, if the number of cells treated with the test antibody shows a statistically significant reduction of at least 10% in the number of surviving cells compared to the number of cells treated with a control, non-specific antibody, using a two-tailed Mann-Whitney test (with differences considered as significant when $p < 0.05$). Total cell numbers are corrected for the number of cells at the start of the treatment period, referred to as T_0 .

8.4 Example 4: Assay for Assessing Affinity of an Anti-hPG Antibody

[0117] Affinity constants of anti-hPG antibodies can be measured using the Proteon Technique (BioRad), according to Nahshol *et al.*, 2008, Analytical Biochemistry 383:52-60, hereby incorporated by reference in its entirety. Briefly, for murine anti-PG antibodies, an anti-mouse IgG antibody (50 µg/ml) is first coated on a sensor chip, making sure that the signal detected by the chip after injection of the antibody falls between 10,000 and 11,500 response units (RU). The murine anti-hPG antibody of interest (test antibody) is then injected (at a typical concentration of 30 µg/ml). If the test antibody binds sufficiently, and additional signal of at least 500 RU will be observed. A time-course of binding between test antibody and hPG is then obtained by injecting varying concentrations of hPG, for example 200 nM, 100 nM, 50 nM, 25 nM, and 12.5 nM, and detecting the level of association. Typically, several channels are available to test multiple antibodies in parallel in a single experiment, making it possible to assay binding of a single test antibody at different concentrations of hPG in parallel. One channel should be injected with a murine monoclonal antibody that is not specific to hPG as a control for non-specific binding and another channel should be injected with dilution buffer alone as a baseline for the background signal. Generally, no binding is detectable in the channel injected with non-specific murine antibody. Antibodies displaying a high level of association in this setting, which may result in saturation of the trapped monoclonal antibody by hPG, can be tested against lower hPG concentrations (50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.125 nM), allowing for a more refined measurement.

[0118] Affinity constants (K_D) are calculated as the ratio between the dissociation constant (k_d) and the association constant (k_a). Experimental values can be validated by analyzing the statistically relevant similarity between experimental curves based on binding measurements and theoretical profiles.

[0119] Affinity constants of non-murine anti-hPG antibodies can be assessed in a similar format using an IgG specific for the species of origin of the anti-hPG test antibody.

8.5 Example 5: Assay for Assessing Competitive Binding With a Reference Anti-hPG Antibody

[0120] A specific assay for assessing whether an antibody of interest (test antibody) competes for binding hPG with a biotinylated reference anti-hPG antibody can be

performed as follows. 96-well plates are coated with a capture anti-hPG antibody (polyclonal or monoclonal antibody recognizing an N-or C-terminal region of hPG that differs from the epitope recognized by the biotinylated reference anti-hPG antibody), at a concentration to be chosen within the range of 1-10 µg/ml, overnight at 4°C (0.1 to 1 µg/well). After blocking with blocking buffer (0.1% Tween-20, 0.1% BSA in PBS) for 2 hr at 22°C, recombinant hPG is added at a concentration ranging between 10 pM to 1 nM (10 to 1000 pg/well) and incubated for 2 hr at 22°C. Thereafter, the biotinylated reference anti-hPG antibody (or a mixture containing the biotinylated reference anti-hPG antibody) is added, along with increasing concentrations of unlabeled test antibody, and incubated for 1 hr at 22°C. After washing to remove unbound antibodies, detection of bound labeled reference anti-hPG antibody is performed by incubating the mixture with 50 ng/ml streptavidin-HRP for 1 hr at 22°C, followed by incubation with a chemiluminescent substrate for horseradish peroxidase for 5 minutes at 22°C, and then quantifying the relative light units (RLU) in a luminometer. Assays are performed in duplicate.

[0121] Antibodies that compete with a reference anti-hPG antibody inhibit the binding of the reference antibody to hPG. An antibody that binds to substantially the same epitope, or with an overlapping epitope, as the reference antibody significantly reduces (for example, by at least 50%) the amount of reference anti-hPG antibody bound, as evidenced by a reduction observed RLUs.

[0122] A high control value is obtained from a control experiment carried out by incubating the labeled reference antibody with recombinant hPG without test antibody. A low control value is obtained from a control experiment carried out by incubating the labeled reference antibody with recombinant hPG in the presence of excess concentrations of the unlabeled reference antibody (the unlabeled reference antibody thus competing with the labeled antibody for binding to hPG). The capacity of test antibodies to compete with the reference anti-hPG antibody is then determined by incubating the labeled reference antibody with recombinant hPG in the presence of increasing concentrations of the unlabeled test antibody.

[0123] In a test assay, a significant reduction in the observed RLUs in the presence of a test antibody indicates that the test antibody recognizes substantially the same epitope as the reference anti-hPG antibody.

[0124] The inhibition of binding can be expressed as an inhibition constant, or K_i , which is calculated according to the following formula:

$$K_i = IC_{50} / [1 + (\text{reference anti-hPG Ab concentration} / K_D^{\text{reference anti-hPG Ab}})]$$

where “ IC_{50} ” is the concentration of test antibody that yields a 50% reduction in binding of the reference antibody and $K_D^{\text{reference anti-hPG Ab}}$ is the dissociation constant of the reference anti-hPG antibody, a measure of its affinity for hPG. Useful test antibodies that compete with a reference anti-hPG antibody (for example, one of the anti-hPG antibodies described herein) will typically have K_i s ranging from 10 pM to 100 nM under assay conditions described herein.

8.6 Example 6: Detection Of Serum PG In Samples from Patients With Familial Adenomatous Polyposis

[0125] This example shows that elevated serum PG levels can be correlated with the presence of polyps in individual with FAP.

8.6.1 Methods

[0126] Serum PG levels were quantified as described in Example 1 in samples from 6 patients with Familial Adenomatous Polyposis. Serum samples were obtained from patients with the following characteristics:

- Two individuals (A and B), both older than 55, having previously undergone colectomy, regularly monitored by endoscopy, in whom no polyps have been detected since surgery.
- One individual (C), aged 30, having previously undergone colectomy and follow up surgery to remove further polyps. The individual had surgery several months before the blood sample was collected.
- One individual (D), aged 27, having previously undergone colectomy, presenting with multiple polyps in the small intestine (but no cancer) at the time the blood sample was collected.

- One individual (E), aged 52, having previously undergone colectomy presenting with multiple polyps in the rectum at the time a blood sample was collected.
- One individual (F), aged 10, presenting with multiple colorectal polyps at the time a blood sample was collected.

8.6.2 Results

[0127] Results shown Table 5 below are expressed as mean PG concentration $\pm$ standard deviation (pM):

Table 5	
Patient	Mean PG Concentration (pM) $\pm$ s.d.
A	6.9 ± 3.3
B	0.0
C	0.0
D	167.5 ± 43.0
E	351.85 ± 96.0
F	233 ± 11.3

[0128] Results indicate that PG levels are particularly elevated in individuals bearing a high number of polyps at the time of sampling. In comparison, patients who have undergone surgery display very low or undetectable levels of PG.

8.7 Example 7: Detection Of Serum PG In Samples From Patients With Pre-Cancerous Sporadic Adenomatous Polyps

[0129] This example demonstrates that more than twenty-five percent of individuals with sporadic adenomatous polyposis have increased serum PG levels.

8.7.1 Methods

[0130] PG levels were measured in two different sample sets: a first set of samples obtained from twenty-five individuals having multiple adenomatous polyps, similar to the number that would be found in subjects with FAP, and a second set of samples from a plasma bank, collected from 104 individuals ranging in age from 45 to 65 years. Plasma

progastrin levels were quantified using an ELISA assay, as described above in Example 1.

8.7.2 Results

[0131] 23% (12/52) of subjects with adenomatous polyps studied had progastrin levels above 50 pM. By comparison, 16.3% (17/104) of subjects in from the blood bank group had PG levels above 50 pM. The medical history of the individuals whose samples were banked is unknown. Healthy individuals have low levels of PG, not typically exceeding 50 pM. *See, e.g., Siddheshwar et al., 2001, "Plasma levels of progastrin but not amidated gastrin or glycine extended gastrin are elevated in patients with colorectal carcinoma," Gut 48:47-52.* Levels above 50 pM are thought to be indicative of an underlying pathology.

[0132] Almost one quarter of the individuals in whom polyps were found also had elevated PG levels. This is consistent with the observation that about 20% of sporadic adenomatous polyps develop into malignant tumors, a transformation that is thought to be accompanied by elevated PG levels. Therefore, elevated PG levels in the presence of adenomatous polyps can serve as a useful metric in identifying patients for further follow-up or for prophylactic anti-PG treatment.

[0133] With respect to the samples from the plasma bank, it is likely that some or all of the banked samples with PG levels above 50 pM came from individuals with underlying conditions that caused elevated PG levels. Use of appropriately screened control samples would likely show a greater difference between the percentage of individuals with sporadic adenomatous polyps and those without polyps who have PG levels above 50 pM.

8.8 Example 8: Anti-PG Compositions Prevent Tumor Development In A Mouse Model Of FAP

[0134] This example demonstrates the ability of anti-hPG antibodies to prevent the formation of tumors *in vivo*.

8.8.1 Methods

[0135] Transgenic mice carrying a mutation in an allele of the Adenomatous Polyposis Coli (APC) gene similar to that found in individuals with Familial Adenomatous Polyposis (FAP), were treated with an anti-hPG antibody. These mice, referred to as

APC Δ 14 mice, spontaneously develop tumors in their intestines when the second (wild type) APC allele is lost *via* a “loss of heterozygosity” (LOH) mechanism, (Colnot *et al.*, 2004, “Colorectal cancers in a new mouse model of familial adenomatous polyposis: influence of genetic and environmental modifiers,” *Lab Investigation* 84:1619-1630). The first detectable tumors can be found around 2 months of age, and by 3.5 months, the number of tumors is generally around 15-20. These tumors have been shown to produce progastrin.

[0136] Four-month-old APC Δ 14 mice were treated twice a week for six weeks with either a control polyclonal antibody –a rabbit anti-human IgG antiserum (Jackson ImmunoResearch (reference no. 309-005-0089)– or an anti-PG polyclonal antibody, raised against (1) an N-terminal peptide and (2) a C-terminal peptide as described in Hollande *et al.*, WO 07/135542, by intra-peritoneal injection at a dose of 9 mg/kg. The mice were weighed once a week. At the end of the six-week treatment regimen, their intestines were photographed, the total number of tumors counted. There were six mice in the treatment and the control group. Genotyping identified two mice from the control group that did not carry the expected heterozygous APC mutation, and were excluded from the experiment.

8.8.2 Results

[0137] Results are shown below in Table 6. Mice treated with control antibody exhibited a total of 125 tumors, with 31.25 tumors on average per mouse. Anti-PG treated mice has 46 total tumors or, on average, 7.6 tumors per mouse. This difference is statistically significant (Mann-Whitney test, P=0.0095).

Table 6						
Treatment (no. of mice)	Number of tumors per mouse					
Control PAb (4)	23	48	28	26		
Anti-hPG PAb (6)	2	16	15	9	2	2

[0138] Results indicate that the number of tumors found in four out the six animals treated with anti-progastrin antibodies falls below the average number of fifteen to twenty tumors generally found in APC Δ 14 mice at 3.5 months of age. *See*, Table 6 below.

These data indicate that treatment with anti-progastrin antibodies prevents new tumors from developing in these animals.

[0139] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[0140] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s).

WHAT IS CLAIMED IS:

1. A method of preventing gastrointestinal cancer in a human subject, comprising administering to a human subject predisposed to develop adenomatous polyps an amount of an anti-PG antibody sufficient to provide a therapeutic benefit.
2. The method of **claim 1**, in which the anti-PG antibody is an anti-hPG monoclonal antibody.
3. The method of **claim 2** in which the anti-hPG monoclonal antibody is a humanized anti-hPG monoclonal antibody.
4. The method of **claim 2** in which the anti-hPG monoclonal antibody is an N-terminal anti-hPG monoclonal antibody.
5. The method of **claim 4** in which the N-terminal anti-hPG monoclonal antibody binds an epitope comprising a sequence selected from the group consisting of DAPLG (SEQ ID NO:28), PDAPLG (SEQ ID NO:29), PRSQPD (SEQ ID NO:30), WKPRSQPD (SEQ ID NO:31) and WKPRSQPDAPLG (SEQ ID NO:32).
6. The method of **claim 4** in which the N-terminal anti-hPG monoclonal antibody is raised against an immunogen comprising a peptide having the sequence SWKPRSQPDAPLG (SEQ ID NO:25).
7. The method of **claim 4** in which the N-terminal anti-hPG monoclonal antibody comprises three V_H CDRs corresponding in sequence to the V_H CDRs of MAb3, MAb4, MAb15, MAb16 or MAb19 and three V_L CDRs corresponding in sequence to the V_L CDRs of MAb3, MAb4, MAb15, MAb16 or MAb19.
8. The method of **claim 4** in which the N-terminal anti-hPG monoclonal antibody comprises CDRs corresponding in sequence to the CDRs of MAb3, MAb4, MAb15, MAb16 or MAb 19.
9. The method of **claim 4** in which the anti-hPG monoclonal antibody competes for binding hPG with a reference antibody selected from the group consisting of MAb3, MAb4, MAb15, MAb16 and MAb19.

10. The method of **claim 2** in which the anti-hPG monoclonal antibody is a C-terminal anti-hPG monoclonal antibody.

11. The method of **claim 10** in which the C-terminal anti-hPG monoclonal antibody binds an epitope comprising a sequence selected from the group consisting of FGRR (SEQ ID NO:33), MDFGR (SEQ ID NO:34), AEDEN (SEQ ID NO:35) and GWMDFGRR (SEQ ID NO:36).

12. The method of **claim 10** in which the C-terminal anti hPG monoclonal antibody is raised against an immunogen comprising a peptide having the sequence QGPWLEEEEEEAYGWMDFGRRSAEDEN (SEQ ID NO:27).

13. The method of **claim 10** in which the C-terminal anti-hPG monoclonal antibody comprises three V_H CDRs corresponding in sequence to the CDRs of MAb5, MAb6, MAb7, MAb8, MAb11, MAb12 or MAb13 and three V_L CDRs corresponding in sequence to the CDRs of MAb5, MAb6, MAb7, MAb8, MAb11, MAb12 or MAb13.

14. The method of **claim 10** in which the C-terminal anti hPG monoclonal antibody comprises CDRs corresponding in sequence to the CDRs of MAb5, MAb6, MAb7, MAb8, MAb11, MAb12 or MAb13.

15. The method of **claim 10** in which the C-terminal anti-hPG monoclonal antibody competes for binding hPG with a reference antibody selected from the group consisting of MAb5, MAb6, MAb7, MAb8, MAb11, MAb12 and MAb13.

16. The method of **claim 2**, wherein the subject has a mutation in the APC gene associated with adenomatous polyposis.

17. The method of **claim 16**, wherein the subject has familial adenomatous polyposis.

18. The method of **claim 16**, wherein the subject has sporadic adenomatous polyposis.

19. The method of **claim 2**, in which the anti-hPG monoclonal antibody is administered adjunctive to surgical resection of tissue comprising adenomatous polyps.

20. The method of **claim 2**, in which the anti-hPG monoclonal antibody is administered adjunctive to chemotherapy.

21. The method of **claim 2**, in which the anti-hPG monoclonal antibody is administered adjunctive to treatment with a non-steroidal anti-inflammatory drug.

22. A method of identifying a subject in need of colonoscopy comprising determining whether a subject predisposed to develop adenomatous polyps has a plasma or serum progastrin concentration at or above about 50 pM, where such a concentration in said subject is indicative of a need for follow-up colonoscopy.

23. A method of identifying a subject with increased probability of having adenomatous polyps comprising determining the level of PG in samples of a body fluid from a subject predisposed to develop adenomatous polyps, and comparing the level of PG in the sample to a baseline, wherein a level of PG in the sample above the baseline level is indicative of a need for anti-progastrin treatment.

24. The method of **claim 23**, wherein said baseline is the level of PG in a sample obtained at an earlier time from the subject.

25. A method of monitoring the effectiveness of anti-PG treatment comprising determining whether a subject with adenomatous polyposis has a plasma or serum progastrin concentration below about 10 pM, where such a concentration indicates the treatment is effective.