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(54) Title: COMBINATION OF PROGASTRIN DETECTION WITH OTHER CANCER BIOMARKERS IN CANCER DIAGNOSIS

(57) Abstract: The present invention relates to methods for the *in vitro* diagnosis of a specific cancer, wherein said methods comprise the use of an antibody binding to progastrin and of a biomarker specific for said cancer.



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COMBINATION OF PROGASTRIN DETECTION WITH OTHER CANCER BIOMARKERS IN CANCER DIAGNOSIS

INTRODUCTION

5 Cancer is a multi-faceted disease in which a group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumours, which do not invade or metastasise.

10 There are a number of methods currently used to treat each type of cancer, including surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy. Successful cancer therapy is directed to the primary tumour and to any metastases, whether clinically apparent or microscopic.

It is crucial for the patient to identify as early as possible the type of cancer to
15 be treated. Cancer that is diagnosed at an early stage, is more likely to be treated successfully. If cancer spreads, effective treatment becomes more difficult, and generally chances of surviving are much lower. So, it is essential to know when to use immediately a heavy and aggressive treatment protocol in order to prevent extension of an aggressive cancer.

20 Screening and monitoring assays are essential for the diagnosis and management of cancer. Cancer screening and monitoring tests, such as blood tests collected in a medical environment, can be useful for large scale screening of clinical healthy (or "asymptomatic") individuals, for diagnosis, for prediction tests or for disease monitoring in subjects. Blood based remote samples for such applications have
25 the advantage that it is convenient for a subject to provide a sample and the risk of side effects is extremely low. Therefore, compliance is improved in a test population.

Biomarkers are extensively used in oncology, notably for patient assessment in multiple clinical settings, including estimating risk of disease, screening for occult primary cancers, distinguishing benign from malignant findings or one type of
30 malignancy from another, determining prognosis and prediction for patients who have

been diagnosed with cancer, and monitoring status of the disease, either to detect recurrence or determine response or progression to therapy.

Biomarker selection for a specific cancer involves first the identification of markers that have a measurable and statistically significant difference in the cancer population compared to a control population for a specific medical application. Biomarkers can include secreted or shed molecules that parallel disease development or progression and readily diffuse into the blood stream from cancer tissue or from surrounding tissues and circulating cells in response to a tumour. The biomarker or set of biomarkers identified are generally clinically validated or shown to be a reliable indicator for the original intended use for which it was selected.

A diagnosis test based on the detection of progastrin has recently been developed by the applicant. Selected antibodies were used to set up an ELISA assay to detect progastrin in the blood of patients with various types of cancers and at various stages. This test, commercialized under the name CancerRead, is particularly efficient for detecting various types of cancer, including early stages (WO 2017/114973). Notably, the CancerRead test displays high sensitivity and specificity for early stage tumours.

However, even though the level of progastrin in blood is a reliable biomarker for early cancer screening, it gives no information regarding the origin of the cancer.

There is thus a real need for reagents allowing *in vivo* identification of a cancer, so that an appropriate therapy can be provided at the earliest possible stage.

SUMMARY OF THE INVENTION

The present invention relates to methods for the *in vitro* diagnosis of a specific cancer, wherein said methods comprise the use of an antibody binding to progastrin and of a biomarker specific for said cancer.

In a first embodiment, a method for the *in vitro* evaluation of a risk of the presence of a specific cancer is provided, wherein said method comprises a step of detecting progastrin and a biomarker of this specific cancer in a biological sample of a subject. The presence of progastrin and said cancer biomarker in the sample indicates that there is a risk of the presence of said cancer.

Another embodiment relates to a method of *in vitro* diagnosis of a specific cancer, wherein said method comprises a step of detecting progastrin and a biomarker of this specific cancer in a biological sample from a subject. The presence of progastrin and said cancer biomarker in the sample is a sign of said cancer.

5 In another embodiment, the concentration of progastrin is measured. Preferably, a concentration of progastrin above a threshold (e.g. 3 pM, 5 pM, 10 pM, 20 pM, 30 pM, 40 pM, 50 pM or 100 pM), in combination with the detection of said biomarker, is a sign of a risk of a specific cancer or of the presence of said cancer. Preferably, progastrin levels are measured by detecting the binding of a progastrin-
10 binding molecule (e.g., an anti-progastrin antibody) to progastrin in a biological sample of said subject.

In another embodiment, the cancer biomarker is a biomarker commonly used in clinic, such as any one of those listed by the National Cancer Institute on its web site. Preferably, the said cancer biomarker is calcitonin, alpha-fetoprotein, beta
15 chorionic gonadotropin hormone, prostate serum antigen (PSA), serotonin, catecholamines, monoclonal immunoglobulins, cancer antigen-125 (CA-125), cancer antigen 19.9 (CA19.9), cancer antigen 15.3 (CA 15.3), carcinoembryonic antigen (CEA), or thyroglobulin (Tg).

The present invention will become more fully understood from the detailed
20 description given herein and from the accompanying drawings, which are given by way of illustration only and do not limit the intended scope of the invention.

FIGURE LEGENDS

Figure 1: Combination of progastrin and PSA levels in the blood to detect prostate cancer.

25 **Figure 2:** Combination of progastrin and CA15-3 levels in the blood to detect breast cancer.

Figure 3: Combination of progastrin and CA19-9 levels in the blood to detect pancreatic cancer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a new method of diagnosing a cancer in a subject, wherein said method comprises the detection of progastrin and a cancer biomarker in a biological sample from a subject. Preferably, the amount of progastrin in said sample is determined, thus allowing quantification of progastrin.

Human pre-progastrin, a 101 amino acids peptide (Amino acid sequence reference: AAB19304.1), is the primary translation product of the gastrin gene. Progastrin is formed by cleavage of the first 21 amino acids (the signal peptide) from preprogastrin. The 80 amino-acid chain of progastrin is further processed by cleavage and modifying enzymes to several biologically active gastrin hormone forms: gastrin 34 (G34) and glycine-extended gastrin 34 (G34-Gly), comprising amino acids 38-71 of progastrin, gastrin 17 (G17) and glycine-extended gastrin 17 (G17-Gly), comprising amino acids 55 to 71 of progastrin.

Gastrin cells naturally produce progastrin, which is matured into gastrin. During digestion, 95% of progastrin is released as gastrin from the cell. A very small amount of progastrin is released as progastrin. Hence, except during digestion, healthy people have no progastrin in their blood.

On the other hand, in pathological conditions, progastrin becomes an early marker. In tumour cells, progastrin is not matured into gastrin and is consequently released from the tumoural cell. Progastrin can promote tumourigenesis (e.g. gastric [Burkitt et al., *World J Gastroenterol.* **15**(1): 1-16, 2009, WO 2017/114975], colon [Watson et al., *J Cancer.* **87**(5): 567-573, 2002], pancreatic [Harris et al., *Cancer Res.* **64**(16): 5624-5631, 2004, WO 2011/083091], ovarian [WO 2017/114972], prostate [WO 2018/178352], oesophageal [WO 2017/114976], and lung cancers [WO 2018/178354]) in an autocrine, paracrine or endocrine manner (Dimaline & Varro, *J Physiol* **592**(Pt.14): 2951-2958, 2014), which has also warranted progastrin as a preferred anti-tumour target in cancers expressing these stimulatory factors (see e.g., WO 2011/045080, WO 2011/083088, WO 2011/116954, WO 2012/013609, WO 2011/083090, WO 2011/083091, WO 2017/114975, WO 2017/114976, WO 2017/114972, WO 2018/178364). This process is independent of digestion.

Progastrin is in particular a reliable biomarker of cancer in asymptomatic patients (WO 2017/114973). However, even though the detection progastrin signs the presence of cancer, it does not indicate the origin of said cancer.

5 In a first aspect, the present invention relates to a method for the *in vitro* evaluation of a risk of the presence of a specific cancer, wherein said method comprises a step of detecting progastrin and a biomarker of this specific cancer in a biological sample from a subject. The presence of progastrin and said cancer biomarker in the sample indicates that there is a risk of the presence of said cancer.

10 Thus, in a first embodiment, the invention relates to an *in vitro* method for evaluating the risk of the presence of a specific cancer in a subject, said method comprising the steps of:

- a) contacting a biological sample from said subject with at least one progastrin-binding molecule,
- b) detecting the binding of said progastrin-binding molecule to progastrin in
15 said sample, and
- c) detecting at least one biomarker of said specific cancer in said sample,

wherein there is a risk of the presence of said cancer in said subject if said binding of said progastrin-binding molecule to progastrin and the presence of said biomarker are detected in said sample.

20 As used herein, the term “cancer” refers to or describes the physiological condition in mammals that is typically characterized by unregulated cell proliferation. The terms “cancer” and “cancerous” as used herein are meant to encompass all stages of the disease. A “cancer” as used herein is any malignant neoplasm resulting from the undesired growth, the invasion, and under certain conditions metastasis of
25 impaired cells in an organism. The cells giving rise to cancer are genetically impaired and have usually lost their ability to control cell division, cell migration behaviour, differentiation status and/or cell death machinery. Most cancers form a tumour but some hematopoietic cancers, such as leukaemia, do not.

30 Thus, a “cancer” as used herein may include both benign and malignant cancers. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukaemia or lymphoid malignancies. More specifically, a

cancer according to the present invention is selected from the group comprising squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, oropharyngeal cancer, nasopharyngeal cancer, laryngeal cancer, cancer of the peritoneum, oesophageal cancer, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, brain cancer, nervous system cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, gallbladder cancer, vulval cancer, testicular cancer, thyroid cancer, Kaposi sarcoma, hepatic carcinoma, anal carcinoma, penile carcinoma, non-melanoma skin cancer, melanoma, skin melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including Hodgkin lymphoma; non-Hodgkin lymphoma, such as e.g., low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukaemia (CLL); acute lymphoblastic leukaemia (ALL); hairy cell leukaemia; chronic myeloblastic leukaemia (CML); Acute Myeloblastic Leukaemia (AML); and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, oedema (such as that associated with brain tumours), Meigs' syndrome, brain, as well as head and neck cancer, including lip & oral cavity cancer, and associated metastases.

In a preferred embodiment, said cancer is lung cancer, lip & oral cavity cancer, oropharyngeal cancer, nasopharyngeal cancer, laryngeal cancer, prostate cancer, oesophageal cancer, gallbladder cancer, liver cancer, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, Hodgkin lymphoma, Non-Hodgkin lymphoma, leukaemia, multiple myeloma, Kaposi sarcoma, kidney cancer, bladder cancer, colon cancer, rectal cancer, colorectal cancer, hepatoma, hepatic carcinoma, anal carcinoma, thyroid cancer, non-melanoma skin cancer, skin melanoma, brain cancer, nervous

system cancer, testicular cancer, cervical cancer, uterine cancer, endometrial cancer, ovarian cancer, or breast cancer.

In a more preferred embodiment, said cancer is oesophageal cancer, liver cancer, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, Hodgkin lymphoma, 5 colon cancer, rectal cancer, colorectal cancer, hepatoma, hepatic carcinoma, anal carcinoma, non-melanoma skin cancer, skin melanoma, cervical cancer, uterine cancer, endometrial cancer, ovarian cancer, or breast cancer.

The binding of progastrin-binding molecule may be detected by various assays 10 available to the skilled artisan. Although any suitable means for carrying out the assays are included within the invention, it can be mentioned in particular FACS, ELISA, RIA, western-blot and IHC.

A great number of biomarkers are used in the clinic to identify specific cancers. Accordingly, the presence of said biomarker in said sample can thus be detected by 15 any of the relevant clinically approved assays. Cancer biomarkers are described in more details below.

In a preferred embodiment, the method according to the invention for the *in vitro* evaluation of a risk of the presence of a specific cancer in a subject, comprising the steps of:

- 20 a) contacting said biological sample from said subject with at least one progastrin-binding molecule,
- b) determining the concentration of progastrin in said biological sample, and
- c) detecting at least one biomarker of said specific cancer in said sample,

wherein there is a risk of the presence of said cancer in said subject if said 25 concentration of progastrin is above a threshold concentration and the presence of said biomarker are detected in said sample.

In a more particular embodiment, the threshold progastrin concentration is a concentration of progastrin of at least 3 pM, 5 pM, 10 pM, at least 20 pM, at least 30 pM, at least 40 pM, or at least 50 pM.

In another embodiment, the threshold progastrin concentration is provided by the progastrin concentration in a control sample. Accordingly, once the concentration of progastrin present in the sample is determined, the result can be compared with those of control sample(s), which is (are) obtained in a manner similar to the test samples but from individual(s) known not to suffer from the cancer. If the concentration of progastrin is significantly more elevated in the test sample, it may be concluded that there is an increased likelihood that the subject from whom it was derived has the specific cancer of interest.

Thus, in a more preferred embodiment, step b) of the method of the invention comprises the further steps of:

- determining a reference concentration of progastrin in a reference sample, and
- comparing the concentration of progastrin in said biological sample with said reference concentration of progastrin.

According to this specific embodiment, the comparison of the concentration of progastrin in the biological sample of the subject with the reference concentration is used in conjunction with the detection of said biomarker for assessing the risk of the presence of said cancer in said subject.

In another aspect, the invention relates to an *in vitro* method for diagnosing a specific cancer in a subject, said method comprising the steps of:

- a) contacting a biological sample from said subject with at least one progastrin-binding molecule,
 - b) detecting the binding of said progastrin-binding molecule to progastrin in said sample, and
 - c) detecting at least one biomarker of said specific cancer in said sample,
- wherein said specific cancer is present in said subject if said binding of said progastrin-binding molecule to progastrin and the presence of said biomarker are detected in said sample.

In a preferred embodiment, the present invention relates to a method for the *in vitro* diagnosis of a specific cancer in a subject, comprising the steps of:

- a) contacting said biological sample from said subject with at least one progastrin-binding molecule,
- b) determining the concentration of progastrin in said biological sample,
- c) detecting at least one biomarker of said specific cancer in said sample,

5 wherein said specific cancer is present in said subject if said concentration of progastrin is above a threshold concentration and the presence of said biomarker are detected in said sample.

 In a more particular embodiment of a method according to the invention, a concentration of progastrin of at least 3 pM, 5pM, 10 pM, at least 20 pM, at least 30
10 pM, at least 40 pM, or at least 50 pM in said biological sample is indicative of the presence of a cancer in said subject.

 In a more preferred embodiment, step b) of the method of the invention comprises the further steps of:

- determining a reference concentration of progastrin in a reference
15 sample, and
- comparing the concentration of progastrin in said biological sample with said reference concentration of progastrin.

 According to this specific embodiment, the comparison of the concentration of progastrin in the biological sample of the subject with the reference concentration is
20 used in conjunction with the detection of said biomarker for diagnosing said cancer in said subject.

 In a particular embodiment, the present invention relates to a method for the *in vitro* diagnosis of a specific cancer in a subject, comprising the determination of the concentration of progastrin in a biological sample and comparing said value
25 obtained to the concentration of progastrin in a reference sample.

 In a more particular embodiment, in a method for the diagnosis of a specific cancer according to the present invention, the biological sample of said subject is contacted with at least one progastrin-binding molecule, wherein said progastrin-binding molecule is an antibody, or an antigen-binding fragment thereof.

The expression “evaluation of a risk of the presence of a specific cancer in a subject” designates the determination of a relative probability for a given subject to suffer from said specific cancer, when compared to a reference subject or value. A method according to the invention represents a tool in the evaluation of said risk, in combination with other methods or indicators such as clinical examination, biopsy and imagery.

The expression “*in vitro* diagnosis” means to determine if a subject is suffering from a particular affection. Accordingly, a method for the *in vitro* diagnosis of a cancer, according to the present invention can be considered as a tool within a diagnosis process.

The term “progastrin” designates the mammalian progastrin peptide, and particularly human progastrin. For the avoidance of doubt, without any specification, the expression “human progastrin” refers to the human PG of sequence SEQ ID NO. 1. Human progastrin comprises notably a N-terminus and a C-terminus domain, which are both absent in the biologically active gastrin hormone forms mentioned above. Preferably, the sequence of said N-terminus domain is represented by SEQ ID NO. 2. In another preferred embodiment, the sequence of said C-terminus domain is represented by SEQ ID NO. 3.

The determination of the concentration of progastrin, in a method according to the invention, is performed by any method known by one skilled in the art of biochemistry.

Preferably, determining the levels of progastrin in a sample includes contacting said sample with a progastrin-binding molecule and measuring the binding of said progastrin-binding molecule to progastrin.

When expression levels are measured at the protein level, it may be notably performed using specific progastrin-binding molecules, such as e.g., antibodies, in particular using well known technologies such as cell membrane staining using biotinylation or other equivalent techniques followed by immunoprecipitation with specific antibodies, western blot, ELISA or ELISPOT, enzyme-linked immunosorbant assays (ELISA), radioimmunoassays (RIA), immunohistochemistry (IHC), immunofluorescence (IF), antibodies microarrays, or tissue microarrays coupled to immunohistochemistry. Other suitable techniques include FRET or BRET, single cell

microscopic or histochemistry methods using single or multiple excitation wavelength and applying any of the adapted optical methods, such as electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, e.g. multipolar resonance spectroscopy, confocal and non-
5 confocal, detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (e.g., surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry), cell ELISA, flow cytometry, radioisotopic, magnetic resonance imaging, analysis by polyacrylamide gel electrophoresis (SDS-
10 PAGE); HPLC-Mass Spectroscopy; Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC-MS/MS)). All these techniques are well known in the art and need not be further detailed here. These different techniques can be used to measure the progastrin levels.

Said method may in particular be chosen among: a method based on immuno-
15 detection, a method based on western blot, a method based on mass spectrometry, a method based on chromatography, and a method based on flow cytometry. Although any suitable means for carrying out the assays are included within the invention, methods such as FACS, ELISA, RIA, western-blot and IHC are particularly useful for carrying out the method of the invention.

20 In a more particular embodiment, a method for the *in vitro* diagnosis of a specific cancer according to the invention comprises contacting a biological sample from a subject with a progastrin binding molecule using an immunoenzymatic assay, preferably based on techniques chosen among RIA and ELISA.

A “biological sample” as used herein is a sample of biological tissue or fluid that
25 contains nucleic acids or polypeptides, e.g., of a protein, polynucleotide or transcript specific of the cancer of interest. Such a sample must allow for the determination of the expression levels of progastrin. Progastrin is known to be a secreted protein. Preferred biological samples for the determination of the level of the progastrin protein thus include biological fluids. A “biological fluid” as used herein means any
30 fluid that includes material of biological origin. Preferred biological fluids for use in the present invention include bodily fluids of an animal, e.g. a mammal, preferably a human subject. The bodily fluid may be any bodily fluid, including but not limited to blood, plasma, serum, lymph, cerebrospinal fluid (CSF), saliva, sweat and urine.

Preferably, said preferred liquid biological samples include samples such as a blood sample, a plasma sample, or a serum sample. More preferably, the biological sample is a blood sample. Indeed, such a blood sample may be obtained by a completely harmless blood collection from the patient and thus allows for a non-invasive
5 assessment of the risks that the subject will develop a tumour.

A “biological sample” as used herein also includes a solid cancer sample of the patient to be tested, when the cancer is a solid cancer. Such solid cancer sample allows the skilled person to perform any type of measurement of the level of the biomarker of the invention. In some cases, the methods according to the invention
10 may further comprise a preliminary step of taking a solid cancer sample from the patient. By a “solid cancer sample”, it is referred to a tumour tissue sample. Even in a cancerous patient, the tissue which is the site of the tumour still comprises non-tumour healthy tissue. The “cancer sample” should thus be limited to tumour tissue taken from the patient. Said “cancer sample” may be a biopsy sample or a sample
15 taken from a surgical resection therapy.

A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal, or a bird, reptile, or fish. Indeed, a “subject” which may be subjected to the method described herein may be any of mammalian animals including human, dog, cat, cattle, goat, pig, swine, sheep and monkey; or a bird; reptile; or
20 fish. Preferably, a subject is a human being; a human subject may be known as a “patient”. In one embodiment, the subject is asymptomatic, i.e. said subject does not show symptoms of cancer, or has been diagnosed with cancer. A subject has been “diagnosed with cancer” when a medical test conducted by a medical practitioner has revealed the presence of cancer. As used herein, a “symptom” is any subjective
25 evidence of disease, e.g., cancer. A “symptom” is a departure from normal function or feeling which is noticed by a patient, reflecting the presence of an unusual state, or of a disease, e.g., cancer. A disease is considered asymptomatic if a patient is a carrier for said disease, but experiences no symptom. Asymptomatic conditions may not be discovered until the patient undergoes medical tests, such as, e.g., measuring
30 the progastrin level.

By “obtaining a biological sample,” it is herein meant to obtain a biological sample for use in methods described in this invention. Most often, this will be done by removing a sample of cells from an animal, but can also be accomplished by using

previously isolated cells (e.g., isolated by another person, at another time, and/or for another purpose), or by performing the methods of the invention in vivo. Archival tissues, having treatment or outcome history, will be particularly useful.

This sample may be obtained and if necessary prepared according to methods known to a person skilled in the art. In particular, it is well known in the art that the sample should be taken from a fasting subject.

The determination of the concentration of progastrin relates to the determination of the quantity of progastrin in known volume of a sample. The concentration of progastrin may be expressed relatively to a reference sample, for example as a ratio or a percentage. The concentration may also be expressed as the intensity or localization of a signal, depending on the method used for the determination of said concentration. Preferably, the concentration of a compound in a sample is expressed after normalisation of the total concentration of related compounds in said sample, for example the level or concentration of a protein is expressed after normalisation of the total concentration of proteins in the sample.

Preferably, the risk that said subject suffers from a specific cancer is determined by comparing the level of progastrin measured in said biological sample with a reference level.

The term “reference level”, as used herein, refers to the expression level of the cancer marker under consideration, i.e. progastrin, in a reference sample. A “reference sample”, as used herein, means a sample obtained from subjects, preferably two or more subjects, known to be free of the disease or, alternatively, from the general population. The suitable reference expression levels of progastrin can be determined by measuring the expression levels of said marker in several suitable subjects, and such reference levels can be adjusted to specific subject populations. The reference value or reference level can be an absolute value; a relative value; a value that has an upper or a lower limit; a range of values; an average value; a median value, a mean value, or a value as compared to a particular control or baseline value. A reference value can be based on an individual sample value such as, for example, a value obtained from a sample from the subject being tested, but at an earlier point in time. The reference value can be based on a large number of samples, such as from population of subjects of the chronological age matched group, or based on a pool of samples including or excluding the sample to be tested.

Advantageously, a “reference level” is a predetermined progastrin level, obtained from a biological sample from a subject with a known particular status as regards cancer. In particular embodiments, the reference level used for comparison with the test sample in step (b) may have been obtained from a biological sample from a healthy subject, or from a biological sample from a subject suffering from cancer; it is understood that the reference expression profile can also be obtained from a pool of biological samples of healthy subjects or from a pool of samples from subjects having cancer.

In a particular embodiment of the method of the invention, the reference sample is collected from subjects exempt from any cancer, and preferably from any pathology. It is to be understood that, according to the nature of the biological sample collected from a patient, the reference sample will be a biological sample of the same nature of said biological sample.

The level of progastrin is determined in the present method by determining the amount of progastrin which is bound by a progastrin-binding molecule, preferably by an antibody recognising progastrin.

By “progastrin-binding molecule”, it is herein referred to any molecule that binds progastrin, but does not bind gastrin-17 (G17), gastrin-34 (G34), glycine-extended gastrin-17 (G17-Gly), or glycine-extended gastrin-34 (G34-Gly). The progastrin-binding molecule of the present invention may be any progastrin-binding molecule, such as, for instance, an antibody molecule or a receptor molecule. Preferably, the progastrin-binding molecule is an anti-progastrin antibody or an antigen-binding fragment thereof.

According to a particular embodiment, the present invention relates to an *in vitro* diagnosis method of a cancer comprising the determination of the concentration of progastrin in a biological sample from a subject, wherein said subject exhibits at least one clinical symptom of cancer.

According to another particular embodiment, the present invention relates to an *in vitro* diagnosis method of a cancer comprising the determination of the concentration of progastrin in a biological sample from a subject, wherein said subject exhibits at least one clinical symptom of cancer and/or of metastasis.

By “binding”, “binds”, or the like, it is intended that the antibody, or antigen binding fragment thereof, forms a complex with an antigen which, under physiologic conditions, is relatively stable. Methods for determining whether two molecules bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. In a particular embodiment, said antibody, or antigen-binding fragment thereof, binds to progastrin with an affinity that is at least two-fold greater than its affinity for binding to a non-specific molecule such as BSA or casein. In a more particular embodiment, said antibody, or antigen-binding fragment thereof, binds only to progastrin.

10 In a particular embodiment, in a method for the diagnosis of cancer according to the invention, a biological sample from the subject is contact with at least one progastrin-binding molecule, wherein the affinity of said molecule for progastrin is of at least 100 nM, at least 90 nM, at least 80 nM, at least 70 nM, at least 60 nM, at least 50 nM, at least 40 nM, at least 30 nM, at least 20 nM, at least 10 nM, at least 5 nM, at
15 least 1 nM, at least 100 pM, at least 10 pM, or at least 1 pM, as determined by a method such as above-described.

In a particular embodiment, the present invention relates to a method for the diagnosis of cancer, comprising the detection of the concentration of progastrin in a biological sample from a subject, wherein said biological sample is contacted with an
20 anti-hPG antibody, or an antigen-binding fragment thereof.

The term “antibody” as used herein is intended to include polyclonal and monoclonal antibodies. An antibody (or “immunoglobulin”) consists of a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (or domain)
25 (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed
30 “complementarity determining regions” (CDR) or “hypervariable regions”, which are primarily responsible for binding an epitope of an antigen, and which are interspersed with regions that are more conserved, termed framework regions (FR). Method for identifying the CDRs within light and heavy chains of an antibody and determining their

sequence are well known to the skilled person. For the avoidance of doubt, in the absence of any indication in the text to the contrary, the expression CDRs means the hypervariable regions of the heavy and light chains of an antibody as defined by IMGT, wherein the IMGT unique numbering provides a standardized delimitation of the framework regions and of the complementary determining regions, CDR1-IMGT: 27 to 38, CDR2.

The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species [Lefranc M.-P., Immunology Today 18, 509 (1997) / Lefranc M.-P., The Immunologist, 7, 132-136 (1999) / Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, Dev. Comp. Immunol., 27, 55-77 (2003)]. In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cystein 23 (1st-CYS), tryptophan 41 (CONSERVED-TRP), hydrophobic amino acid 89, cystein 104 (2nd-CYS), phenylalanine or tryptophan 118 (J-PHE or J-TRP). The IMGT unique numbering provides a standardized delimitation of the framework regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65 and CDR3-IMGT: 105 to 117. As gaps represent unoccupied positions, the CDR-IMGT lengths (shown between brackets and separated by dots, e.g. [8.8.13]) become crucial information. The IMGT unique numbering is used in 2D graphical representations, designated as IMGT Colliers de Perles [Ruiz, M. and Lefranc, M.-P., Immunogenetics, 53, 857-883 (2002) / Kaas, Q. and Lefranc, M.-P., Current Bioinformatics, 2, 21-30 (2007)], and in 3D structures in IMGT/3Dstructure-DB [Kaas, Q., Ruiz, M. and Lefranc, M.-P., T cell receptor and MHC structural data. Nucl. Acids. Res., 32, D208-D210 (2004)].

Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g. effector cells) and the first component (Clq) of the classical complement system. Antibodies can be of different isotypes (namely IgA, IgD, IgE, IgG or IgM).

In a particular embodiment, said progastrin-binding antibody, or an antigen-binding fragment thereof, is selected from the group consisting of: polyclonal antibodies, monoclonal antibodies, chimeric antibodies, single chain antibodies, camelised antibodies, IgA1 antibodies, IgA2 antibodies, IgD antibodies, IgE antibodies, IgG1 antibodies, IgG2 antibodies, IgG3 antibodies, IgG4 antibodies and IgM antibodies.

A “polyclonal antibody” is an antibody which was produced among or in the presence of one or more other, non-identical antibodies. In general, polyclonal antibodies are produced from a B-lymphocyte in the presence of several other B-lymphocytes producing non-identical antibodies. Usually, polyclonal antibodies are obtained directly from an immunised animal.

The term “monoclonal antibody” designates an antibody arising from a nearly homogeneous antibody population, wherein population comprises identical antibodies except for a few possible naturally-occurring mutations which can be found in minimal proportions. A monoclonal antibody arises from the growth of a single cell clone, such as a hybridoma, and is characterized by heavy chains of one class and subclass, and light chains of one type.

By the expression “antigen-binding fragment” of an antibody, it is intended to indicate any peptide, polypeptide, or protein retaining the ability to bind to the target (also generally referred to as antigen) of the said antibody, generally the same epitope, and comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, or at least 200 contiguous amino acid residues, of the amino acid sequence of the antibody.

In a particular embodiment, the said antigen-binding fragment comprises at least one CDR of the antibody from which it is derived. Still in a preferred embodiment, the said antigen binding fragment comprises 2, 3, 4 or 5 CDRs, more preferably the 6 CDRs of the antibody from which it is derived.

The “antigen-binding fragments” can be selected, without limitation, in the group consisting of Fv, scFv (sc for single chain), Fab, F(ab')₂, Fab', scFv-Fc fragments or diabodies, or fusion proteins with disordered peptides such as XTEN (extended recombinant polypeptide) or PAS motifs, or any fragment of which the half-life time would be increased by chemical modification, such as the addition of poly(alkylene) glycol such as poly(ethylene) glycol (“PEGylation”) (pegylated fragments called Fv-PEG, scFv-PEG, Fab-PEG, F(ab')₂-PEG or Fab'-PEG) (“PEG” for Poly(Ethylene) Glycol), or by incorporation in a liposome, said fragments having at least one of the characteristic CDRs of the antibody according to the invention. Preferably, said “antigen-binding fragments” will be constituted or will comprise a partial sequence of the heavy or light variable chain of the antibody from which they are derived, said partial sequence being sufficient to retain the same specificity of binding as the antibody from which it is descended and a sufficient affinity, preferably at least equal to 1/100, in a more preferred manner to at least 1/10, of the affinity of the antibody from which it is descended, with respect to the target.

In another particular embodiment, in a method for the diagnosis of cancer according to the invention, a biological sample from a subject is contacted with an antibody binding to progastrin, wherein said antibody has been obtained by an immunisation method known by a person skilled in the art, wherein using as an immunogen a peptide which amino acid sequence comprises the totality or a part of the amino-acid sequence of progastrin. More particularly, said immunogen comprises a peptide chosen among:

- a peptide which amino acid sequence comprises, or consists of, the amino acid sequence of full length progastrin, and particularly full length human progastrin of SEQ ID N°1,
- a peptide which amino acid sequence corresponds to a part of the amino acid sequence of progastrin, and particularly full length human progastrin of SEQ ID N°1,
- a peptide which amino acid sequence corresponds to a part or to the whole amino acid sequence of the N-terminal part of progastrin, and in particular peptides comprising, or consisting of, the amino acid sequence: SWKPRSQQPDAPLG (SEQ ID N°2), and
- a peptide which amino acid sequence corresponds to a part or to the whole amino acid sequence of the C-terminal part of progastrin, and in particular

peptides comprising, or consisting of, the amino acid sequence: QGPWLEEEEEAYGWMDFGRRSAEDEN (SEQ ID N° 3),

- a peptide which amino acid sequence corresponds to a part of the amino acid sequence of the C-terminal part of progastrin, and in particular peptides comprising the amino acid sequence FGRRSAEDEN (SEQ ID N° 40) corresponding to amino acids 71-80 of progastrin

The skilled person will realize that such immunisation may be used to generate either polyclonal or monoclonal antibodies, as desired. Methods for obtaining each of these types of antibodies are well known in the art. The skilled person will thus easily select and implement a method for generating polyclonal and/or monoclonal antibodies against any given antigen.

Examples of monoclonal antibodies which were generated by using an immunogen comprising the amino-acid sequence “SWKPRSQQPDAPLG”, corresponding to the amino acid sequence 1-14 of human progastrin (N-terminal extremity) include, but are not restricted to, monoclonal antibodies designated as: mAb3, mAb4, mAb16, and mAb19 and mAb20, as described in the following Table 1 to Table 4. Other monoclonal antibodies have been described, although it is not clear whether these antibodies actually bind progastrin (WO 2006/032980). Experimental results of epitope mapping show that mAb3, mAb4, mAb16, and mAb19 and mAb20 do specifically bind an epitope within said hPG N-terminal amino acid sequence. Polyclonal antibodies recognizing specifically an epitope within the N-terminus of progastrin represented by SEQ ID NO. 2, have been described in the art (see e.g., WO 2011/083088).

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
6B5B11C10	mAb3	VH CDR 1	GYIFTSYW	SEQ ID N° 4
		VH CDR 2	FYPGNSDS	SEQ ID N° 5
		VH CDR 3	TRRDSPQY	SEQ ID N° 6
		VL CDR 1	QSIVHSNGNTY	SEQ ID N° 7
		VL CDR 2	KVS	SEQ ID N° 8
		VL CDR 3	FQGSHVPFT	SEQ ID N° 9

		mVH 3	EVQLQQSGTVLARPGASVKMSCK ASGYIFTSYVWHVVKQRPGQGLE WIGGFYPGNSDSRYNQKFKGKAT LTAVTSASTAYMDLSSLTNEDSAV YFCTRRDSPQYWGQGTTTLTVSS	SEQ ID N° 41
		mVL 3	DVLMTQTPLSLPVSLGDQASISCR SSQSIVHSNGNTYLEWYLQKPGQS PKLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRLEAEDLGVIYCFQG SHVPFTFGGGTKLEIK	SEQ ID N° 42
		huVH 3	QVQLVQSGAEVKKPGASVKVSK ASGYIFTSYVWHVVRQAPGQRLE WMGGFYPGNSDSRYSQKFQGRV TITRDTASTAYMELSSLRSEDVAV YYCTRRDSPQYWGQGTTLTVSS	SEQ ID N° 53
		huVL 3	DVVMTQSPLSLPVTLGQPASISCR SSQSIVHSNGNTYLEWFQQRPGQ SPRRLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYCFQG SHVPFTFGGGTKVEIK	SEQ ID N° 54

Table 1

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
20D2C3G2	mAb4	VH CDR 1	GYTFSSW	SEQ ID N° 10
		VH CDR 2	FLPGSGST	SEQ ID N° 11
		VH CDR 3	ATDGNYDWFAY	SEQ ID N° 12
		VL CDR 1	QSLVHSSGVTY	SEQ ID N° 13
		VL CDR 2	KVS	SEQ ID N° 14
		VL CDR 3	SQSTHVPPT	SEQ ID N° 15
		mVH 4	QVQLQQSGAELMKPGASVKISCK ATGYTFSSSWIEWLKQRPGHGLE WIGEFLLPGSGSTDYNEKFKGKATF TADTSSDTAYMLLSSLTSEDSAVY	SEQ ID N° 43

			YCATDGNVDWFAYWGQGLTVT SA	
		mVL 4	DLVMTQTPLSLPVSLGDQASISCR SSQSLVHSSGVTYLHWYLQKPGQ SPKLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDLGVYFCSQS THVPPTFGSGTKLEIK	SEQ ID N° 44
		huVH 4	QVQLVQSGAEVKKPGASVKVSCK ASGYTFSSWMHWVRQAPGQGL EWMGIFLPGSGSTDYAQKFQGRV TMTRDTSTSTVYMESSLRSEDTA VYYCATDGNVDWFAYWGQGLTV TVSS	SEQ ID N° 55
		huVL 4	DIVMTQTPLSLVTPGQPASISCKS SQSLVHSSGVTYLYWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPPTFGQGTKLEIK	SEQ ID N° 56

Table 2

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
1E9D9B6	mAb16	VH CDR 1	GYTFTSY	SEQ ID N° 16
		VH CDR 2	INPSNGGT	SEQ ID N° 17
		VH CDR 3	TRGGYYPFDY	SEQ ID N° 18
		VL CDR 1	QSLLDSGKTY	SEQ ID N° 19
		VL CDR 2	LVS	SEQ ID N° 20
		VL CDR 3	WQGTHTSPYT	SEQ ID N° 21
		mVH 16	QVQLQQSGAELVKPGASVKLSCK ASGYTFTSYMYWVKQRPQGGL WIGEINPSNGGTNFNEKFKSKATL TVDKSSSTAYMQLSSLTSEDSAVY YCTRGGYYPFDYWGQGTTLTVSS	SEQ ID N° 45

	mVL 16	DVVMQTQPLTSLVTIGRPASISCKS SQSLDSDGKTYLYWLLQRPQGS PKRLIYLVSELDGVPDRITGSGSG TDFTLKISRVEAEDLGVYYCWQG THSPYTFGGGTKLEIK	SEQ ID N° 46
	huVH 16a	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTSYMYWVRQAPGQGLE WMGIINPSNGGTSYAQKFQGRVT MTRDTSTSTVYMELSSLRSEDVAV YYCTRGGYYPFDYWGQGTTVT SS	SEQ ID N° 57
	huVH 16b	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTSYMHWRQAPGQGL EWMGIINPSNGGTSYAQKFQGRV TMTRDTSTSTVYMELSSLRSEDVAV VYYCTRGGYYPFDYWGQGTTVT VSS	SEQ ID N° 58
	huVH 16c	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTSYMYWVRQAPGQGLE WMGEINPSNGGTNYAQKFQGRV TMTRDTSTSTVYMELSSLRSEDVAV VYYCTRGGYYPFDYWGQGTTVT VSS	SEQ ID N° 59
	huVL 16a	DVVMTQSPLSLPVTLGQPASISCR SSQSLDSDGKTYLYWFQQRPGQ SPRRLIYLVSNRDSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCWQ GTHSPYTFGGGTKLEIK	SEQ ID N° 60
	huVL 16b	DVVMTQSPLSLPVTLGQPASISCR SSQSLDSDGKTYLNWFQQRPGQ SPRRLIYLVSNRDSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCWQ GTHSPYTFGGGTKLEIK	SEQ ID N° 61

		huVL 16c	DVVMTQSPLSLPVTLGQPASISCR SSQSLLDSDGKTYLYWFQQRPGQ SPRRLIYLVSERDSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCWQ GTHSPYTFGQGKLEIK	SEQ ID N° 62
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Table 3

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
1B3B4F11	mAb19	VH CDR 1	GYSITSDYA	SEQ ID N° 22
		VH CDR 2	ISFSGYT	SEQ ID N° 23
		VH CDR 3	AREVNYGDSYHFDY	SEQ ID N° 24
		VL CDR 1	SQHRTYT	SEQ ID N° 25
		VL CDR 2	VKKDGSH	SEQ ID N° 26
		VL CDR 3	GVGDAIKGQSVFV	SEQ ID N° 27
		mVH 19	DVQLQESGPGLVKPSQSLTCTV TGYSITSDYAWNWIWIRQFPGNKLE WMGYISFSGYTSYNPSLKSRI SVTR DTSRNQFFLQLTSVTTEDTATYYC AREVNYGDSYHFDYWGQGTVTV SS	SEQ ID N° 47
		mVL 19	QLALTQSSSASFSLGASAKLTCTLS SQHRTYTIEWYQQQSLKPPKYVM EVKKDGSHSTGHGIPDRFSGSSSG ADRYLSISNIQPEDEAIYICGVGDAI KGQSVFVFGGGTKVTVL	SEQ ID N° 48
		huVH 19a	QVQLQESGPGLVKPSQTLTCT VSGYSITSDYAWNWIWIRQHPGKGL EWIGYISFSGYTYNPSLKSRTIS VDTSKNQFSLKLSSVTAADTAVYY CAREVNYGDSYHFDYWGQGTLV TVSS	SEQ ID N° 63

	huVH 19b	QVQLQESGPGLVKPSQTLSTCT VSGYSITSDYAWSWIRQHPGKGLE WIGYISFSGYTYYNPSLKSRTISV DTSKNQFSLKLSSVTAADTAVYYC AREVNYGDSYHFDYWGGQTLVT VSS	SEQ ID N° 64
	huVH 19c	QVQLQESGPGLVKPSQTLSTCT VSGYSITSDYAWNIRQHPGKGL EWIGYISFSGYTSYNPSLKSRTIS VDTSKNQFSLKLSSVTAADTAVYY CAREVNYGDSYHFDYWGGQTLV TVSS	SEQ ID N° 65
	huVL 19a	QLVLTQSPSASASLGASVKLTCTL SSQHRITYIEWHQQPEKGPRYL MKVKKDGSHSKGDGIPDRFSGSSS GAERYLTISLQSEDEADYYCGVG DAIKGQSVFVFGGGTKVEIK	SEQ ID N° 66
	huVL 19b	QLVLTQSPSASASLGASVKLTCTL SSQHRITYIAWHQQPEKGPRYL MKVKKDGSHSKGDGIPDRFSGSSS GAERYLTISLQSEDEADYYCGVG DAIKGQSVFVFGGGTKVEIK	SEQ ID N° 67
	huVL 19c	QLVLTQSPSASASLGASVKLTCTL SSQHRITYIEWHQQPEKGPRYL MEVKKDGSHSKGDGIPDRFSGSSS GAERYLTISLQSEDEADYYCGVG DAIKGQSVFVFGGGTKVEIK	SEQ ID N° 68

Table 4

Examples of monoclonal antibodies that can be generated by using an immunogen comprising the amino-acid sequence “QGPWLEEEEEAYGWMDFGRRSAEDEN”, (C-terminal part of progastrin) corresponding to the amino acid sequence 55-80 of human progastrin include, but are not restricted to antibodies designated as: mAb8 and mAb13 in the following Table 5 and 6. Experimental results of epitope mapping show that mAb13 do specifically bind an

- epitope within said hPG C-terminal amino acid sequence. Another example of a monoclonal antibody that can thus be generated by is the antibody Mab14, produced by hybridoma 2H9F4B7, described in WO 2011/083088. Hybridoma 2H9F4B7 was deposited under the Budapest Treaty at the CNCM, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris CEDEX 15, France, on 27 December 2016, under reference I-5158.

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
1C10D3B9	mAb8	VH CDR 1	GFTFTTYA	SEQ ID N° 28
		VH CDR 2	ISSGGTYT	SEQ ID N° 29
		VH CDR 3	ATQGNYSLDF	SEQ ID N° 30
		VL CDR 1	KSLRHTKGITF	SEQ ID N° 31
		VL CDR 2	QMS	SEQ ID N° 32
		VL CDR 3	AQNLELPLT	SEQ ID N° 33
		mVH 8	EVQLVESGGGLVKPGGSLRLSC AASGFTFTTYAMSWVRQAPGK GLEWVATISSGGTYTYADSVK GRFTISRDNANKNSLYLQMNSLRA EDTAVYYCATQGNYSLDFWGQ GTTVTVSS	SEQ ID N° 49
		mVL 8	DIVMTQSPLSLPVTPGEPASISCR SSKSLRHTKGITFLYWYLQKPGQ SPQLLIYQMSNLASGVPRFSSS GSGTDFTLKISRVEAEDVGVYYC AQNLELPLTFGGGTKVEIK	SEQ ID N° 50
		VH hZ8CV1	EVQLVESGGGLVKPGGSLRLSC AASGFTFTTYAMSWVRQAPGK GLEWVSSISSGGTYTYADSVKG RFTISRDNANKNSLYLQMNSLRAE	SEQ ID N° 69

			DTAVYYCATQGNYSLDFWGQG TTVTVSS	
		VL hZ8CV1	DIVMTQSPLSLPVTGPGEASISCR SSKSLRHTKGITFLYWYLQKPGQ SPQLLIYQMSNRASGVDPDRFSGS GSGTDFTLKISRVEAEDVGVYYC AQNLELPLTFGGGKVEIK	SEQ ID N° 70
		VH hZ8CV2	EVQLVESGGGLVKPGGSLRLSC AASGFTFTTYAMSWVRQAPGK GLEWVATISSGGTYTYADSVK GRFTISRDNANKSLYLQMNSLRA EDTAVYYCATQGNYSLDFWGQ GTTVTVSS	SEQ ID N° 71
		VL hZ8CV2	DIVMTQSPLSLPVTGPGEASISCR SSKSLRHTKGITFLYWYLQKPGQ SPQLLIYQMSNLASGVDPDRFSSS GSGTDFTLKISRVEAEDVGVYYC AQNLELPLTFGGGKVEIK	SEQ ID N° 72
		CH hZ8CV2	EVQLVESGGGLVKPGGSLRLSC AASGFTFTTYAMSWVRQAPGK GLEWVATISSGGTYTYADSVK GRFTISRDNANKSLYLQMNSLRA EDTAVYYCATQGNYSLDFWGQ GTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQS SGLYSLSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREE	SEQ ID N° 73

			MTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGD SFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG K	
		CL hZ8CV2	DIVMTQ\$PLSLPVTGPASISCR SSKSLRHTKGITFLYWYLQKPGQ SPQLLIYQMSNLASGVPDRFSSS GSGTDFTLKISRVEAEDVGVYYC AQNLELPLTFGGGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLT LSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	SEQ ID N° 74

Table 5

Hybridoma deposit	mAb	Amino acid sequences		SEQ ID N°
2C6C3C7	mAb13	VH CDR 1	GFIFSSYG	SEQ ID N° 34
		VH CDR 2	INTFGDRT	SEQ ID N° 35
		VH CDR 3	ARGTGTY	SEQ ID N° 36
		VL CDR 1	QSLLDSDGKTY	SEQ ID N° 37
		VL CDR 2	LVS	SEQ ID N° 38
		VL CDR 3	WQGTHFPQT	SEQ ID N° 39
		mVH 13	EVQLVESGGGLVQPGGSLKLSC AASGFIFSSYGMSWVRQSPDRRL ELVASINTFGDRTYYPDSVKGRF TISRDNANKNTLYLQMTSLKSEDT	SEQ ID N° 51

			AIYYCARGTGTWYGQGTTLTVS S	
		mVL 13	DVVLQTPLTSLVTIGQPASISCK SSQSLLDSDGKTYLNWLLQRPG QSPKRLIYLVSKLDSGVPDRFTG SGSGTDFTLKISRVEAEDLGVYY CWQGTHFPQTFGGGTKLEIK	SEQ ID N° 52
		huVH 13a	EVQLVESGGGLVQPGGSLRLSC AASGFIFSSYGMSWVRQAPGKG LEWVANINTFGDRTYYVDSVKG RFTISRDNAKNSLYLQMNSLRAE DTAVYYCARGTGTWYGQGTTLV TVSS	SEQ ID N° 75
		huVH 13b	EVQLVESGGGLVQPGGSLRLSC AASGFIFSSYGMSWVRQAPGKG LEWVASINTFGDRTYYVDSVKG RFTISRDNAKNSLYLQMNSLRAE DTAVYYCARGTGTWYGQGTTLV TVSS	SEQ ID N° 76
		huVL 13a	DVVMTQSPLSLPVTLGQPASISC RSSQSLLDSDGKTYLNWFQQR GQSPRRLIYLVSNRDSGVPDRFS SGSGTDFTLKISRVEAEDVGVY YCWQGTHFPQTFGGGTKVEIK	SEQ ID N° 77
		huVL 13b	DVVMTQSPLSLPVTLGQPASISC RSSQSLLDSDGKTYLNWFQQR GQSPRRLIYLVSKRDSGVPDRFS SGSGTDFTLKISRVEAEDVGVY YCWQGTHFPQTFGGGTKVEIK	SEQ ID N° 78

Table 6

Other examples include anti-hPG monoclonal and/or polyclonal antibodies generated by using an immunogen comprising an amino acid sequence of SEQ ID N° 40.

In a more particular embodiment, in a method according to the invention said
5 biological sample is contacted with an anti-hPG antibody or antigen-binding fragment

thereof, wherein said anti-hPG antibody is chosen among N-terminal anti-hPG antibodies and C-terminal anti-hPG antibodies.

The terms “N-terminal anti-hPG antibodies” and “C-terminal anti-hPG antibodies” designate antibodies binding to an epitope comprising amino acids located in the N-terminal part of hPG or to an epitope comprising amino acids located in the C-terminal part of hPG, respectively. Preferably, the term “N-terminal anti-hPG antibodies” refers to antibodies binding to an epitope located in a domain of progastrin whose sequence is represented by SEQ ID NO. 2. In another preferred embodiment, the term “C-terminal anti-hPG antibodies” refers to antibodies binding to an epitope located in a domain of progastrin whose sequence is represented by SEQ ID NO. 3.

The term “epitope” refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those amino acids that directly contribute to the affinity of the interaction. Epitopes may also be conformational. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. The determination of the epitope bound by an antibody may be performed by any epitope mapping technique, known by a man skilled in the art. An epitope may comprise different amino acids which located sequentially within the amino acid sequence of a protein. An epitope may also comprise amino acids which are not located sequentially within the amino acid sequence of a protein.

In a particular embodiment, said antibody is a monoclonal antibody selected in the group consisting of:

- A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 4, 5 and 6, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 4, 5 and 6, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 7, 8 and 9, respectively, or sequences with at least 80%,

preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 7, 8 and 9, respectively,

- 5 • A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 10, 11 and 12, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 10, 11 and 12, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
10 sequences SEQ ID N° 13, 14 and 15, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 13, 14 and 15, respectively,
- 15 • A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 16, 17 and 18, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 16, 17 and 18, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
20 sequences SEQ ID N° 19, 20 and 21, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 19, 20 and 21, respectively,
- 25 • A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 22, 23 and 24, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 22, 23 and 24, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
30 sequences SEQ ID N° 25, 26 and 27, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 25, 26 and 27, respectively,
- 35 • A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially at least three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 28, 29 and 30,

- 5 respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 28, 29 and 30, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 31, 32 and 33, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 31, 32 and 33, respectively, and
- 10 • A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 34, 35 and 36, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 34, 35 and 36, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 37, 38 and 39, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 37, 38 and 39, respectively.

20 In another embodiment, the antibody is a monoclonal antibody produced by the hybridoma deposited at the CNCM, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris CEDEX 15, France, on 27 December 2016, under reference I-5158.

25 In the sense of the present invention, the “percentage identity” or “% identity” between two sequences of nucleic acids or amino acids means the percentage of identical nucleotides or amino acid residues between the two sequences to be compared, obtained after optimal alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly along their length. The comparison of two nucleic acid or amino acid sequences is traditionally carried out by comparing the sequences after having optimally aligned them, said comparison being able to be conducted by segment or by using an “alignment window”. Optimal alignment of the sequences for comparison can be carried out, in addition to 30 comparison by hand, by means of methods known by a man skilled in the art.

For the amino acid sequence exhibiting at least 80%, preferably 85%, 90%, 95% and 98% identity with a reference amino acid sequence, preferred examples include those containing the reference sequence, certain modifications, notably a deletion,

addition or substitution of at least one amino acid, truncation or extension. In the case of substitution of one or more consecutive or non-consecutive amino acids, substitutions are preferred in which the substituted amino acids are replaced by “equivalent” amino acids. Here, the expression “equivalent amino acids” is meant to indicate any amino acids likely to be substituted for one of the structural amino acids without however modifying the biological activities of the corresponding antibodies and of those specific examples defined below.

Equivalent amino acids can be determined either on their structural homology with the amino acids for which they are substituted or on the results of comparative tests of biological activity between the various antibodies likely to be generated.

In a more particular embodiment, said antibody is a monoclonal antibody selected in the group consisting of:

- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 41 and a light chain of amino acid sequence SEQ ID N° 42;
- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 43 and a light chain of amino acid sequence SEQ ID N° 44;
- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 45 and a light chain of amino acid sequence SEQ ID N° 46;
- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 47 and a light chain of amino acid sequence SEQ ID N° 48;
- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 49 and a light chain of amino acid sequence SEQ ID N° 50; and
- A monoclonal antibody comprising a heavy chain of amino acid sequence SEQ ID N° 51 and a light chain of amino acid sequence SEQ ID N° 52.

In another particular embodiment, the antibody used in the method of the invention is a humanised antibody.

As used herein, the expression “humanised antibody” means an antibody that contains CDR regions derived from an antibody of nonhuman origin, the other parts of the antibody molecule being derived from one or several human antibodies. In

addition, some of the skeleton segment residues (called FR for framework) can be modified to preserve binding affinity, according to techniques known by a man skilled in the art (Jones *et al.*, Nature, 321:522-525, 1986). The goal of humanisation is a reduction in the immunogenicity of a xenogenic antibody, such as a murine antibody, for introduction into a human, while maintaining the full antigen binding affinity and specificity of the antibody.

The humanised antibodies of the invention or fragments of same can be prepared by techniques known to a person skilled in the art (such as, for example, those described in the documents Singer *et al.*, J. Immun., 150:2844-2857, 1992). Such humanised antibodies are preferred for their use in methods involving *in vitro* diagnoses or preventive and/or therapeutic treatment *in vivo*. Other humanisation techniques are also known to the person skilled in the art. Indeed, Antibodies can be humanised using a variety of techniques including CDR- grafting (EP 0 451 261; EP 0 682 040; EP 0 939 127; EP 0 566 647; US 5,530,101; US 6,180,370; US 5,585,089; US 5,693,761; US 5,639,641; US 6,054,297; US 5,886,152; and US 5,877,293), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., 1991, Molecular Immunology 28(4/5): 489-498; Studnicka G. M. et al., 1994, Protein Engineering 7(6): 805-814; Roguska M.A. et al., 1994, Proc. Natl. Acad. Sci. U.S.A., 91:969-973), and chain shuffling (U.S. Pat. No. 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods. See also U.S. Pat. Nos. 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and international patent application publication numbers WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741.

In a more particular embodiment, said antibody is a humanised antibody selected in the group consisting of:

- A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 4, 5 and 6, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 4, 5 and 6, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 7, 8 and 9, respectively, or sequences with at least 80%,

preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 7, 8 and 9, respectively,

- 5 • A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 10, 11 and 12, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 10, 11 and 12, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
10 sequences SEQ ID N° 13, 14 and 15, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 13, 14 and 15, respectively,
- 15 • A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 16, 17 and 18, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 16, 17 and 18, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
20 sequences SEQ ID N° 19, 20 and 21, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 19, 20 and 21, respectively,
- 25 • A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 22, 23 and 24, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 22, 23 and 24, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid
30 sequences SEQ ID N° 25, 26 and 27, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 25, 26 and 27, respectively,
- 35 • A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 28, 29 and 30, respectively, or

- 5 sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 28, 29 and 30, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 31, 32 and 33, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 31, 32 and 33, respectively, and
- 10
- A humanised antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 34, 35 and 36, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 34, 35 and 36, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 37, 38 and 39, respectively, or sequences with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID N° 37, 38 and 39, respectively,
- 15

wherein said antibody also comprises constant regions of the light-chain and the heavy-chain derived from a human antibody.

- 20 In another more particular embodiment, said antibody is a humanised antibody selected in the group consisting of:
- A humanised antibody comprising a heavy chain variable region of amino acid sequence SEQ ID N° 53, and a light chain variable region of amino acid sequence SEQ ID N° 54;
- 25
- A humanised antibody comprising a heavy chain variable region of amino acid sequence SEQ ID N° 55, and a light chain variable region of amino acid sequence SEQ ID N° 56;
 - A humanised antibody comprising a heavy chain variable region of amino acid sequence selected between SEQ ID N° 57, 58, and 59, and a light chain variable region of amino acid sequence selected between SEQ ID N° 60, 61, and 62;
- 30
- A humanised antibody comprising a heavy chain variable region of amino acid sequence selected between SEQ ID N° 63, 64, and 65, and a light chain

variable region of amino acid sequence selected between SEQ ID N° 66, 67, and 68;

- A humanised antibody comprising a heavy chain variable region of amino acid sequence selected between SEQ ID N° 69 and 71, and a light chain variable region of amino acid sequence selected between SEQ ID N° 70 and 72; and
- A humanised antibody comprising a heavy chain variable region of amino acid sequence selected between SEQ ID N° 75 and 76, and a light chain variable region of amino acid sequence selected between SEQ ID N° 77 and 78;

wherein said antibody also comprises constant regions of the light-chain and the heavy-chain derived from a human antibody.

In a first embodiment, a method according to the invention comprises contacting a biological sample with an anti-hPG antibody binding to an epitope of hPG, wherein said epitope is located within the C-terminal part of hPG or to an epitope located within the N-terminal part of hPG.

In a more specific embodiment, a method according to the invention comprises contacting a biological sample with an anti-hPG antibody binding to an epitope of hPG, wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the N-terminal part of progastrin chosen among an amino acid sequence corresponding to amino acids 10 to 14 of hPG, amino acids 9 to 14 of hPG, amino acids 4 to 10 of hPG, amino acids 2 to 10 of hPG and amino acids 2 to 14 of hPG, wherein the amino acid sequence of hPG is SEQ ID N° 1.

In a more specific embodiment, a method according to the invention comprises contacting a biological sample with an anti-hPG antibody binding to an epitope of hPG, wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the C-terminal part of progastrin, chosen among an amino acid sequence corresponding to amino acids 71 to 74 of hPG, amino acids 69 to 73 of hPG, amino acids 71 to 80 of hPG (SEQ ID N° 40), amino acids 76 to 80 of hPG, and amino acids 67 to 74 of hPG, wherein the amino acid sequence of hPG is SEQ ID N° 1.

In a first embodiment, a composition according to the invention comprises an antibody recognizing an epitope including an amino acid sequence corresponding to an amino acid sequence of progastrin.

In a more specific embodiment, a composition according to the invention comprises an antibody recognizing an epitope of progastrin wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the N-terminal part of progastrin, wherein said amino acid sequence may include residues 10 to 14 of hPG, residues 9 to 14 of hPG, residues 4 to 10 of hPG, residues 2 to 10 of hPG or residues 2 to 14 of hPG, wherein the amino acid sequence of hPG is SEQ ID N° 1.

In a more specific embodiment, a composition according to the invention comprises an antibody recognizing an epitope of progastrin wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the C-terminal part of progastrin, wherein said amino acid sequence may include residues 71 to 74 of hPG, residues 69 to 73 of hPG, residues 71 to 80 of hPG (SEQ ID N° 40), residues 76 to 80 of hPG, or residues 67 to 74 of hPG, wherein the amino acid sequence of hPG is SEQ ID N° 1.

In a particular embodiment of a method for the *in vitro* diagnosis of cancer according to the invention, said method comprises a step of contacting a biological sample from a subject with a first molecule which binds to a first part of progastrin and with a second molecule which binds to a second part of progastrin. In a more particular embodiment, wherein said progastrin-binding molecule is an antibody, a biological sample from a subject is contacted with an antibody which binds to a first epitope of progastrin and with a second antibody which binds to a second epitope of progastrin.

In a particular embodiment of the method of the invention, said method comprises a step of contacting a biological sample from a subject with a first agent which binds to a first part of progastrin and with a second agent which binds to a second part of progastrin. In a more particular embodiment, wherein said progastrin-binding molecule is an antibody, a biological sample from a subject is contacted with an antibody which binds to a first epitope of progastrin and with a second antibody which binds to a second epitope of progastrin.

According to a preferred embodiment, said first antibody is bound to an insoluble or partly soluble carrier. Binding of progastrin by said first antibody results in capture of progastrin from said biological sample. Preferably, said first antibody is an antibody binding to an epitope of hPG, wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the C-terminal part of

progastrin, as described above. More preferably, said first antibody is monoclonal antibody Mab14, produced by hybridoma 2H9F4B7, described in WO 2011/083088. Hybridoma 2H9F4B7 was deposited under the Budapest Treaty at the CNCM, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris CEDEX 15, France, on 27 December 5 2016, under reference I-5158.

According to another preferred embodiment, said second antibody is labelled with a detectable moiety, as described below. Binding of progastrin by second antibody enables the detection of the progastrin molecules which were present in the biological sample. Further, binding of progastrin by second antibody enables the 10 quantification of the progastrin molecules which were present in the biological sample. Preferably, said second antibody is an antibody binding to an epitope of hPG, wherein said epitope includes an amino acid sequence corresponding to an amino acid sequence of the N-terminal part of progastrin, as described above. More preferably, said N-terminal antibody is a polyclonal antibody, as described above. Alternatively, it is also 15 possible to use a monoclonal antibody binding an epitope within the N-terminus of progastrin, such as e.g. the N-terminus monoclonal antibodies described above, notably a monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 16, 17 and 18, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 19, 20 20 and 21.

In a particularly preferred embodiment, the first antibody is bound to an insoluble or partly soluble carrier and the second antibody is labelled with a detectable moiety.

Detection of progastrin in a biological sample signals the presence of cancer, 25 but does not give any information on the type of cancer. This question can be addressed by detecting specific biomarkers in addition to assaying progastrin.

As used herein, a “biomarker” is a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. Biomarkers typically differentiate an affected patient from a person 30 without the disease. There is tremendous variety of biomarkers, which can include proteins (e.g., an enzyme or receptor), nucleic acids (e.g., a microRNA or other non-coding RNA), antibodies, peptides, hormones, and metabolites, among other categories. A biomarker can also be a collection of alterations, such as gene

expression, proteomic, and metabolomic signatures. Biomarkers can be detected in the circulation (whole blood, serum, or plasma) or excretions or secretions (stool, urine, sputum, or nipple discharge), and thus easily assessed non-invasively and serially, or can be tissue-derived, and require either biopsy or special imaging for
5 evaluation.

As used herein, a “cancer biomarker” is a biomarker which indicates the presence of cancer in a subject. Accordingly, a cancer biomarker differentiates between a cancer patient and a person who does not have a cancer. A cancer biomarker is thus any type of proteins (e.g., an enzyme or receptor), nucleic acids
10 (e.g., a microRNA or other non-coding RNA), antibodies, and peptides, which can differentiate between a cancer patient and a subject who does not have cancer. Cancer biomarkers can be specific for a type of cancer. A list of cancer biomarkers currently used in clinic and the cancer which they can be used to detect can be found on the web site of the National Cancer Institute. They include ALK gene
15 rearrangements and overexpression (non-small cell lung cancer and anaplastic large cell lymphoma), alpha-fetoprotein or AFP (liver cancer and germ cell tumours), beta-2-microglobulin or B2M (multiple myeloma, chronic lymphocytic leukaemia, and some lymphomas), beta-human chorionic gonadotropin or beta-hCG (choriocarcinoma and germ cell tumours), BRCA1 and BRCA2 gene mutations (ovarian cancer), BCR-ABL fusion
20 gene or Philadelphia chromosome (chronic myeloid leukaemia, acute lymphoblastic leukaemia, and acute myelogenous leukaemia), BRAF V600 mutations (cutaneous melanoma and colorectal cancer), C-kit/CD117 (gastrointestinal stromal tumour and mucosal melanoma), CA15-3/CA27.29 (breast cancer), CA19-9 (pancreatic cancer, gallbladder cancer, bile duct cancer, and gastric cancer), CA-125 (ovarian cancer),
25 calcitonin (medullary thyroid cancer), carcinoembryonic antigen or CEA (colorectal cancer and some other cancers), CD20 (non-Hodgkin lymphoma), chromogranin A or CgA (neuroendocrine tumours), chromosomes 3, 7, 17, and 9p21 (bladder cancer), circulating tumour cells of epithelial origin (CELLSEARCH®), metastatic breast, prostate, and colorectal cancers), mytokeratin fragment 21-1 (lung cancer), EGFR gene
30 mutation analysis (non-small cell lung cancer), oestrogen receptor (ER)/progesterone receptor (PR) (breast cancer), fibrin/fibrinogen (bladder cancer), HE4 (ovarian cancer), HER2/neu gene amplification or protein overexpression (breast cancer, gastric cancer, and gastroesophageal junction adenocarcinoma), monoclonal immunoglobulins (multiple myeloma and Waldenström macroglobulinemia),

catecholamines (pheochromocytomas and paragangliomas.), KRAS gene mutation analysis (colorectal cancer and non-small cell lung cancer), lactate dehydrogenase (germ cell tumours, lymphoma, leukaemia, melanoma, and neuroblastoma), neuron-specific enolase or NSE (small cell lung cancer and neuroblastoma), nuclear matrix protein 22 (bladder cancer), programmed death ligand 1 or PD-L1 (non-small cell lung cancer), prostate-specific antigen or PSA (prostate cancer), thyroglobulin or Tg (thyroid cancer), urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) (breast cancer), 5-Protein signature (OVA1®, ovarian cancer), 21-gene signature (Oncotype DX®, breast cancer), 70-gene signature (Mammaprint®, breast cancer)

All the cancer biomarkers listed are currently in common use in the clinic. Therefore, the skilled person would not have any problem assaying any of these biomarkers.

Preferably, the cancer biomarker is selected in the group consisting of calcitonin, alpha-fetoprotein, beta chorionic gonadotropin hormone, prostate serum antigen (PSA), serotonin, catecholamines, monoclonal immunoglobulins, CA125, CA19.9, CA 15.3, CEA, Tg.

The skilled person will easily realise that a specific cancer can be identified by detecting the cancer biomarkers sequentially. According to this embodiment, the methods provided herein comprise detecting an individual biomarker. If the presence of said biomarker is not detected in the sample tested, then a further biomarker can be detected, until the presence of one biomarker is detected and the cancer identified.

Alternatively, it is possible to detect more than one biomarker at the same time. Such an approach may save time, as well as being more economical. Preferably, the present methods comprise detecting 2, preferably 3, preferably 4, preferably 5, preferably 6, preferably 7, preferably 8, preferably 9, preferably 10, preferably 11, or preferably 12 biomarkers.

The present invention also relates to methods for monitoring the efficacy of a treatment for a specific cancer in a patient, such as chemotherapy, biological therapy, immunotherapy or antibody therapy, by determining the concentration of progastrin and detecting at least one biomarker in a first sample, such as a bodily fluid or biopsy

of said specific cancer, obtained from a patient before treatment for said specific cancer, and then comparing the concentration of progastrin and the biomarker in the first sample to that in a second sample obtained from the same patient after treatment, where a reduction in the concentration of progastrin in said second sample
5 compared to said first sample and/or a reduction or an absence of the biomarker indicates that the treatment was effective.

In a particular embodiment, a method according to the invention comprises comparing the concentration of progastrin in a biological sample obtained from a patient with a predetermined value of concentration of progastrin in the sample, in a
10 more particular embodiment, said predetermined value is chosen among: an mean, or average, of sample values based on the mean, or average, determination of the value in a population free of said specific cancer, a progastrin concentration value obtained when the patient was known to be free of said specific cancer.

In a particular embodiment of the invention, a method according to the present
15 invention comprises the determination of the level of progastrin over time in samples from a patient who has been or is being treated for said specific cancer.

The characteristics of the embodiments of the invention will become further apparent from the following detailed description of examples below.

EXAMPLES

20 Example 1: Detection of prostate cancer

Materials and Methods

Plasma PSA levels were measured with the kit RAB0331A-EA (Sigma-Aldrich), in accordance with the manufacturer's instructions.

Progastrin levels in the plasma were measured with the CancerRead kit (ECS
25 Progastrin, CH). Briefly, progastrin levels were quantified by ELISA through the use of two specific anti-progastrin antibodies: a capture polyclonal anti-hPG antibody coated on the wells of the plate, and a revelation monoclonal anti-hPG antibody which is used to detect progastrin and mediates revelation of the signal.

Results

45 male patients (18 prostate cancer + 12 pancreatic cancer + 15 colorectal cancer) were tested for progastrin levels (using cancer read kit) and for PSA levels (using the kit RAB0331A-EA from Sigma-Aldrich). All the cancer patients were positive for progastrin (concentration over 3 pM). 14/18 (78%) prostate cancer, 3/12 (25%)
5 pancreatic cancer and 1/15 (7%) colorectal cancer (CRC) had a level of PSA over 600 pg/ml.

Combined progastrin and PSA positive levels allowed to identified 18 cancer patients including 14 prostate cancer patients showing an accuracy of the combination of 78% to identify prostate cancer patients among a population of unknown cancer type
10 patients when the population contains 40% of prostate cancer patients.

Example 2: Detection of breast cancer

Materials and Methods

CA15-3 levels were assayed in the plasma of patients with the kit RAB0375A-EA (Sigma-Aldrich), according to the manufacturer's instructions.

15 Plasma progastrin levels were measured with the CancerRead kit (ECS Progastrin, CH).

Results

48 female patients (25 breast cancer + 11 pancreatic cancer + 12 colorectal cancer) were tested for progastrin levels (using cancer read kit) and for CA15-3 levels
20 (using the kit RAB0375A-EA from Sigma-Aldrich). All the cancer patients were positive for progastrin (concentration over 3 pM). 21/24 (88%) breast cancer patients, 7/11 (64%) pancreatic cancer and 6/12 colorectal cancer (CRC) had a level of CA15-3 over 13000 mU/ml.

Combined progastrin and CA15-3 positive levels allowed to identified 34 cancer
25 patients including 21 breast cancer patients showing an accuracy of the combination of 62% to identify breast cancer patients among a population of unknown cancer type patients when the population contains 52% of breast cancer patients.

Example 3: Detection of pancreatic cancer

Material and Methods

CA19-9 levels in the plasma were measured with the kit RAB0035A-EA (Sigma-Aldrich), in agreement with the manufacturer's instructions.

Plasma progastrin levels were measured with the CancerRead kit (ECS Progastrin, CH).

5 Results

93 patients (18 prostate cancer + 25 breast cancer + 23 pancreatic cancer + 27 colorectal cancer) were tested for progastrin levels (using cancer read kit) and for CA19-9 levels (using the kit RAB0035A-EA from Sigma-Aldrich). All the cancer patients were positive for progastrin (concentration over 3 pM). 12/18 (67%) prostate cancer, 10 9/25 (36%) breast cancer, 18/23 (43%) pancreatic cancer and 17/27 (63%) colorectal cancer (CRC) had a level of CA19-9 over 2 U/ml.

Combined progastrin and CA19-9 positive levels allowed to identified 56 cancer patients including 18 pancreatic cancer patients showing an accuracy of the combination of 32% to identify pancreatic cancer patients among a population of 15 unknown cancer type patients when the population contains 19% of breast cancer patients.

CLAIMS

1. A method for the in vitro diagnosis of a cancer in a subject, comprising the steps of:
 - a) contacting said biological sample from said subject with at least one progastrin-binding molecule,
 - b) determining the concentration of progastrin in said biological sample, and
 - c) detecting at least one biomarker of said specific cancer in said sample,wherein there is a risk of the presence of said cancer in said subject if said concentration of progastrin is above a threshold concentration and the presence of said biomarker are detected in said sample.
2. The method of claim 1, wherein step b) further comprises determining the concentration of progastrin and wherein a concentration of progastrin of at least 3 pM, 5pM, 10 pM, at least 20 pM, at least 30 pM, at least 40 pM, or at least 50 pM in said biological sample is indicative of the presence of said cancer in said subject.
3. The method of any one of claims 1 or 2, wherein step b) comprises the further steps of:
 - determining a reference concentration of progastrin in a reference sample, and
 - comparing the concentration of progastrin in said biological sample with said reference concentration of progastrin.
4. The method of any one of claims 1 to 3, wherein said progastrin-binding molecule is an antibody, or an antigen-binding fragment thereof.
5. The method of any of claims 1 to 4, wherein said antibody, or antigen-binding fragment thereof, is selected among N-terminal anti-progastrin monoclonal antibodies and C-terminal anti-progastrin monoclonal antibodies.
6. The method of any of claims 1 to 5, wherein said antibody binding to progastrin is a monoclonal antibody chosen in the group consisting of:
 - A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and

CDR-H3 of amino acid sequences SEQ ID N° 4, 5 and 6, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 7, 8 and 9, respectively,

- 5 - A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 10, 11 and 12, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences
- 10 SEQ ID N° 13, 14 and 15, respectively,
- A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 16, 17 and 18, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences
- 15 SEQ ID N° 19, 20 and 21, respectively,
- A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 22, 23 and 24, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences
- 20 SEQ ID N° 25, 26 and 27, respectively,
- A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 28, 29 and 30, respectively, and a light chain comprising at least one, preferentially at least two, preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences
- 25 SEQ ID N° 31, 32 and 33, respectively,
- A monoclonal antibody comprising a heavy chain comprising at least one, preferentially at least two, preferentially three, of CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 34, 35 and 36, respectively, and a light chain comprising at least one, preferentially at least two,
- 30

preferentially three, of CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 37, 38 and 39, respectively, and

5 - A monoclonal antibody produced by the hybridoma deposited at the CNCM, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris CEDEX 15, France, on 27 December 2016, under reference I-5158.

7. The method of any one of claim 1 to 6, wherein the determination of step a) includes:

(i) contacting said sample with a first progastrin-binding molecule which binds to a first part of progastrin, and

10 (ii) contacting said sample with a second progastrin-binding molecule which binds to a second part of progastrin.

8. The method of claim 7, wherein the first progastrin-binding molecule binds an epitope within the C-terminus of progastrin.

15 9. The method of any one of claims 7 or 8, wherein said progastrin-binding molecule is a monoclonal antibody produced by the hybridoma deposited at the CNCM, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris CEDEX 15, France, on 27 December 2016, under reference I-5158.

10. The method of any one of claims 7 to 9, wherein the second progastrin-binding molecule binds an epitope within the N-terminus of progastrin.

20 11. The method of any one of claims 7 to 10, wherein said second progastrin-binding molecule is a polyclonal antibody binding an epitope within the N-terminus of progastrin or a monoclonal antibody comprising a heavy chain comprising the following three CDRs, CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N° 16, 17 and 18, respectively, and a light chain comprising the following
25 three CDRs, CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N° 19, 20 and 21, respectively.

12. The method of any one of claims 1 to 11, wherein the level of progastrin is determined in step a) with an ELISA.

30 13. The method of any one of claims 1 to 12, wherein said cancer biomarker is selected in the group consisting of calcitonin, alpha-fetoprotein, beta chorionic

gonadotropin hormone, prostate serum antigen (PSA), serotonin, catecholamines, monoclonal immunoglobulins, cancer antigen-125 (CA-125), cancer antigen 19.9 (CA19.9), cancer antigen 15.3 (CA 15.3), carcinoembryonic antigen (CEA), thyroglobulin (Tg).

- 5 14. The method of any one of claims 1 to 13, wherein step c) comprises detecting
2, preferably 3, preferably 4, preferably 5, preferably 6, preferably 7,
preferably 8, preferably 9, preferably 10, preferably 11, or preferably 12
biomarkers.
- 10 15. The method of any one of claim 1 to 14, wherein said biological sample is
chosen among: blood, serum and plasma.

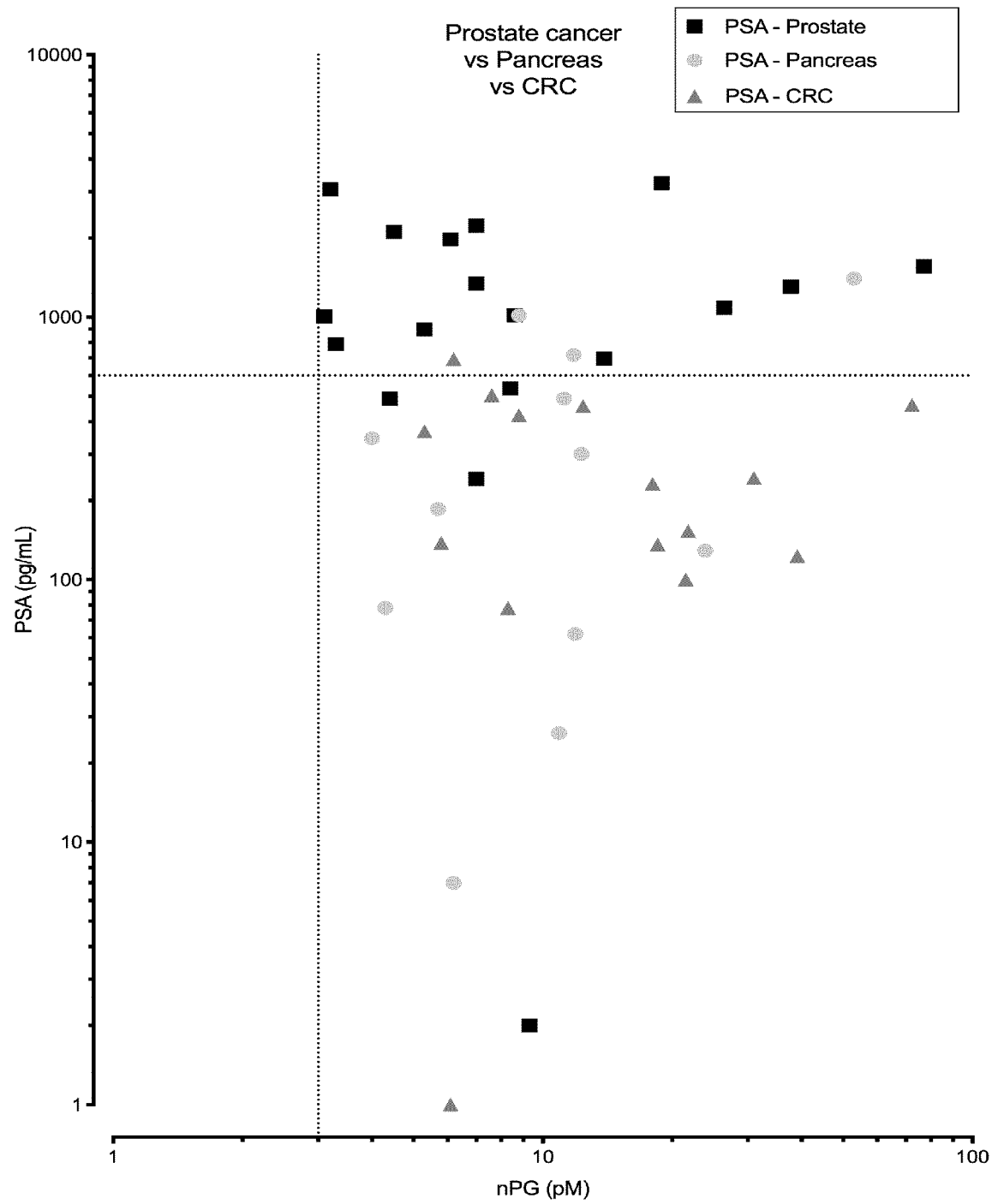
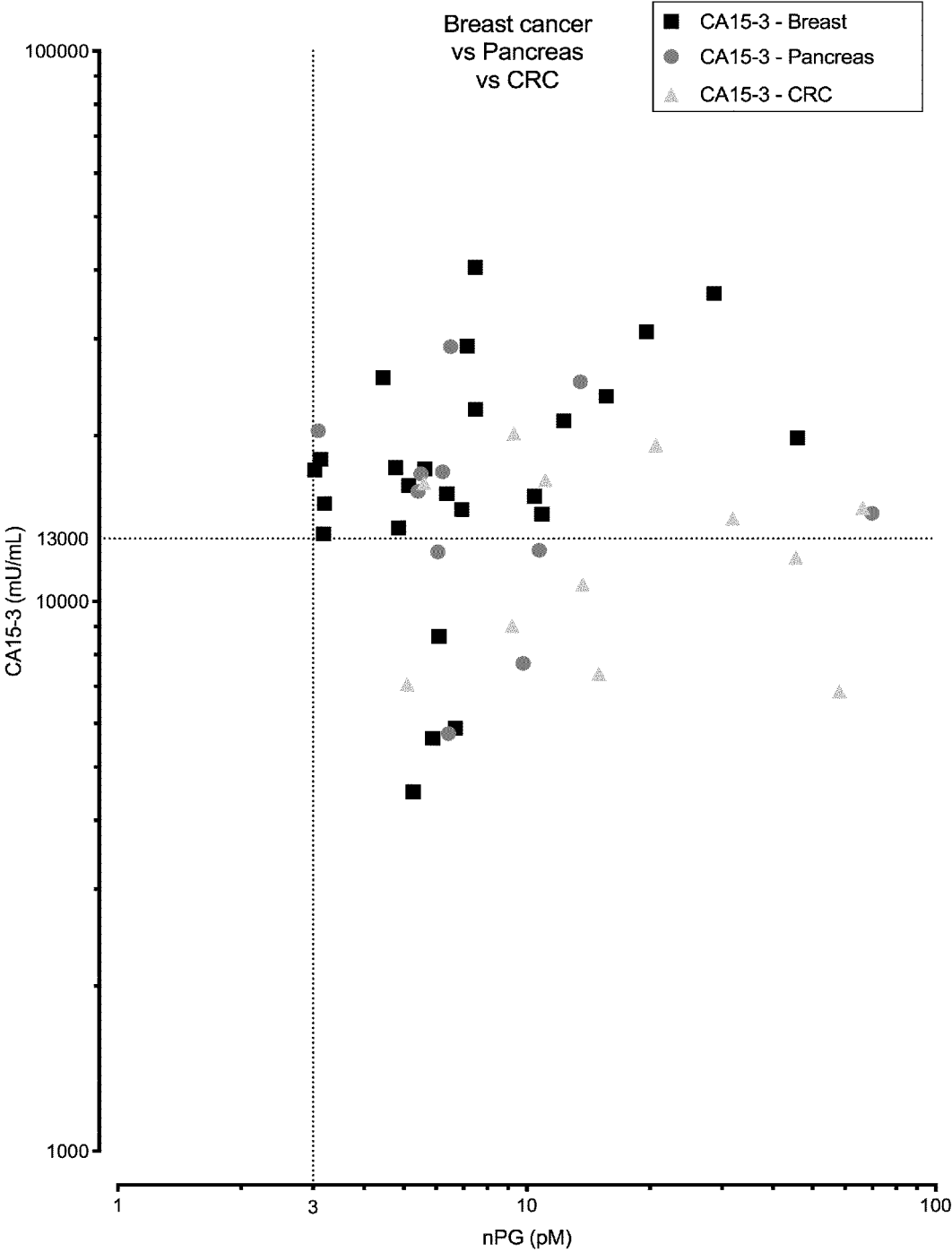


Figure 1



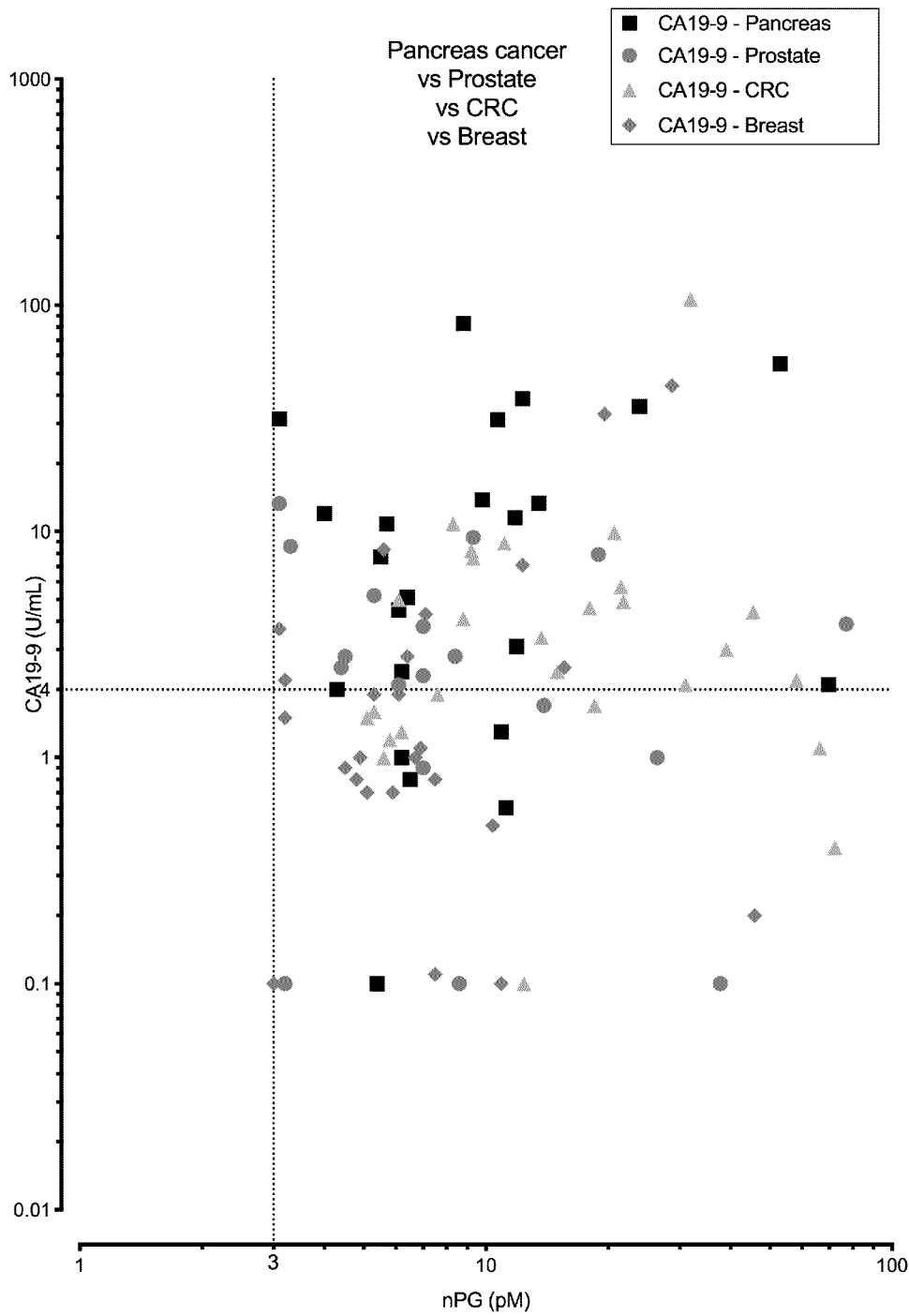


Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/052005

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574 G01N33/74
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE, COMPENDEX, FSTA, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/114973 A1 (SYNCERUS S À R L [LU]) 6 July 2017 (2017-07-06) cited in the application the whole document abstract page 3, line 16 - line 19 page 8, line 3 - line 30 page 27, line 5 - line 18 figures 4,6,8,10,12,15,16 claims 1, 3, 5-13 ----- -/--	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 April 2019

Date of mailing of the international search report

25/04/2019

Name and mailing address of the ISA/

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Authorized officer

Gall-Truchot, A

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/052005

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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