The data demonstrate that patients with prostate cancer have detectable levels of progastrin in their plasma whereas healthy control individuals have none.

Example 4: Detection of plasmatic progastrin concentration using DECODE Lab Kit

The test allows a measurement of hPG in plasma EDTA by ELISA.

The kit utilizes a capture antibody specific for hPG pre-coated on a 96-well plate. hPG present in standards and samples added to the wells was bound by the immobilized capture antibody. The wells were washed and an anti-hPG detection antibody horseradish peroxidase (HRP) conjugate was added, resulting in an antibody-antigen-antibody complex. After a second wash, a 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was added to the well, producing a blue color in direct proportion to the amount of hPG present in the initial sample. The color of the Stop Solution changed from blue to yellow, and the wells were read at 450 nm with a microplate reader.

Methods and results

40 plasma samples from prostrate cancer patients and 119 plasma samples from healthy donors were used to measure the concentration of progastrin using the ELISA Kit DECODE Lab (capture antibody: Mab14, detection antibody: anti-hPG polyclonal) following manufacturer's recommendation.

Briefly:

1. Prepare all reagents, controls, and samples as directed in the previous section except the 1x Conjugate.
2. Remove excess strip from the microtiter plate frame, return them to the plate packet and store at 2-8°C.
3. Samples and controls must be tested in duplicate. Prepare the pre-loading of controls and samples by adding 65 µl/replicate in wells of the 96-Well DeepWell Polypropylene Microplates.
4. Add 50 µl of Sample dilution buffer to all the wells that will be used from the 96 pre-coated well plate strips included in the kit.
5. Transfer 50 µl of the controls and samples with a multi-channel pipette (8 channels) from the pre-loading 96-Well DeepWell Polypropylene Microplates

CLAIMS

1. A method for the *in vitro* diagnosis of prostate cancer in a subject, comprising the steps of:

   a) contacting a biological sample from said subject with at least one progastrin-binding antibody, or an antigen-binding fragment thereof,
   b) detecting the binding of said progastrin-binding antibody, or an antigen-binding fragment thereof to progastrin in said sample, and
   detecting in said sample the presence of at least one prostate cancer biomarker,
   wherein the binding of said progastrin-binding antibody, or an antigen-binding fragment thereof to progastrin and the detection of said prostate cancer biomarker in said sample indicate the presence of prostate cancer in said subject,

   wherein said biological sample is chosen among: blood, serum, and plasma, and

   wherein said antibody binding to progastrin is a monoclonal antibody chosen from the group consisting of:

   - A monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N°4, 5 and 6, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N°7, 8 and 9, respectively,

   - A monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N°10, 11 and 12, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N°13, 14 and 15, respectively,

   - 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 and 21, respectively,

- A monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N°22, 23 and 24, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N°25, 26 and 27, respectively,

- A monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N°28, 29 and 30, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N°31, 32 and 33, respectively, and

- A monoclonal antibody comprising a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 of amino acid sequences SEQ ID N°34, 35 and 36, respectively, and a light chain comprising CDR-L1, CDR-L2 and CDR-L3 of amino acid sequences SEQ ID N°37, 38 and 39, respectively,

and wherein said antigen binding fragment thereof comprises the 6 CDRs of the antibody it derives from.

2. The method of claim 1, wherein said prostate cancer biomarker is selected from the list consisting in: PSA, kallikreins, PCa antigen 3 (PCa3), TMPRSS2-ERG, SChLAP1, three exosomes genes (ERG, PCA3, SPDEF), a urinary two gene combination (HOXC6 and DLX1).

3. The method of claim 1 or 2, wherein step b) further comprises determining the concentration of progastrin.

4. The method of claim 3, comprising the further steps of:

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

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 one of claims 1 to 5, wherein said antibody is 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.

7. The method of any one of claims 1 to 6, wherein step (a) comprises contacting said biological sample with:

(i) a first antibody or an antigen-binding fragment thereof, which binds to the C-terminal part of progastrin, and

(ii) with a second antibody or an antigen-binding fragment thereof, which binds to the N-terminal part of progastrin,

wherein the C-terminal part of progastrin is defined by SEQ ID No 3 and wherein the N-terminal part of progastrin is defined by SEQ ID No 2.

8. The method of claim 7, wherein the first progastrin-binding antibody, or an antigen-binding fragment thereof binds an epitope selected from the group consisting of:

- an amino acid sequence corresponding to residues 71-74 of SEQ ID No 1,
- an amino acid sequence corresponding to residues 69-73 of SEQ ID No 1,
- an amino acid sequence corresponding to residues 71-80 of SEQ ID No 1,
- an amino acid sequence corresponding to residues 76-80 of SEQ ID No 1, and

- an amino acid sequence corresponding to residues 67-74 of SEQ ID No 1.

9. The method of claim 7 or 8, wherein the second progastrin-binding antibody, or an antigen-binding fragment thereof binds an epitope selected from the group consisting of:
   - an amino acid sequence corresponding to residues 10-14 of SEQ ID No 1,
   - an amino acid sequence corresponding to residues 9-14 of SEQ ID No 1,
   - an amino acid sequence corresponding to residues 4-10 of SEQ ID No 1,
   - an amino acid sequence corresponding to residues 2-10 of SEQ ID No 1, and
   - an amino acid sequence corresponding to residues 2-14 of SEQ ID No 1.

10. The method of any one of claims 7 to 9, wherein said first progastrin-binding 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.

11. The method of any one of claims 7 to 10, wherein said second progastrin-binding antibody is a polyclonal antibody 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 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 7 to 11, wherein the first antibody is bound to an insoluble or partly soluble carrier and the second antibody is labelled with a detectable moiety.

13. Use of a progastrin-binding antibody, or an antigen-binding fragment thereof, as in any one of claims 1 to 12 for the *in vitro* diagnosis of prostate cancer.