NEW METHOD AND COMPOUND FOR PROSTATE CANCER DIAGNOSIS

TECHNICAL FIELD

The present disclosure relates human antibodies, or antigen binding fragments thereof, able to bind prostasomes, and to prostate cancer diagnosis and prognosis. More specifically, the proposed technique relates to methods for diagnosing prostate cancer using human antibodies, or antigen binding fragments thereof, for detecting prostasomes in body fluids. The disclosure comprises human antibodies able to specifically and selectively detect prostasomes in body fluids, and methods for diagnosing prostate cancer using the human antibodies. The disclosure further comprises providing prognosis, evaluating the severity of the prostate cancer and determining the efficacy of a medical treatment of the prostate cancer.

BACKGROUND

Cancer is one of the most prevalent deadly diseases, which despite recent advances in diagnosis and treatment still accounts for a substantial number of deaths each year. Prostate cancer, as an example, is the most prevalent cancer disease in men exhibiting symptoms derived from a local tumor or metastatic spread of a tumor, such as dysfunctional voiding or bone pain, and the disease is often at an advanced stage at the time of diagnosis.

Measurement of prostate specific antigen (PSA) has changed the pattern of diagnosis of prostate cancer with more cases detected at an early stage and fewer cases at advanced stages. However, since serum PSA is not a prostate cancer specific marker in serum it is not the ideal diagnostic marker and therefore not accommodated for screening of prostate cancer. The PSA test cannot discriminate between benign prostatic hyperplasia and prostate cancer at an early stage and, what is more, between prostate cancer with high metastasizing potential (aggressive prostate cancer) and such cancer with no or weak aggressiveness (indolent prostate cancer).

It has previously been shown that cells excrete small vesicles called exosomes, released from the cell when multivesicular bodies fuse with the plasma membrane. Such vesicles are derived mainly from the raft part of the membrane of the cell of origin with a protein conformation specific for the cell of origin. Exosomes are present in many eukaryotic fluids, including blood, urine, and medium of cell cultures, etc. Exosomes contain various molecular constituents of their cell of origin, including proteins and RNA. Although the exosomal protein

composition varies with the cell and tissue of origin, most exosomes contain an evolutionarily-conserved common set of protein molecules. The protein content of a single exosome can be about 20,000 molecules, and may be representative of the cell from which it originated, i.e. it may be possible to conclude which cell type that secreted an exosome by looking at its protein content. Exosomes have also been shown to carry double-stranded DNA. Evidence further suggest that exosomes have specialized functions and play a key role in processes such as coagulation, intercellular signaling, and waste management. Consequently, there is a growing interest in the clinical applications of exosomes. Exosomes can potentially be used for prognosis, for therapy, and as biomarkers for health and disease.

It has previously been discovered that submicron membranous vesicles are secreted by the prostate gland acinar cells into seminal fluid. These submicron membranous vesicles are a type of exosomes called prostasomes. Altered tissue architecture in malignancy facilitates the release of prostasomes to the interstitial space rather than to the acinar lumen of the prostate. Thus, prostasomes also leak into the external blood stream. It has been demonstrated that malignant prostate cells secrete prostasomes, and that presence of malignant prostate tumors increase the number of prostasomes present in peripheral blood (Tavoosidana *et al. PNAS*, May 24, 2011, vol. 108, no. 21, 8809–8814). It has thus been speculated that prostasomes may be a potential biomarker of prostate cancer.

SUMMARY

An object of the present disclosure is to provide methods and antibodies or fragments thereof which seek to mitigate, alleviate, or eliminate the above-identified deficiencies in the art and disadvantages singly or in any combination. This object is obtained by novel human antibodies or antigen binding fragments thereof, including synthetic fragments, for use in determining prostasome levels in a fluid body sample of a subject in need thereof.

In one embodiment is provided a human monoclonal antibody or antigen binding fragment thereof, including synthetic fragments, which selectively binds prostasomes. In some embodiments, the human monoclonal antibody or antigen binding fragment thereof is a full-length antibody, an antigen binding (Fab) fragment, or an antigen binding single chain Fv (scFv) fragment, such as a human synthetic scFv fragment.

Disclosed herein is a human monoclonal antibody or antigen binding fragment thereof, which selectively binds prostasomes, wherein the human monoclonal antibody or antigen binding fragment thereof is a full-length antibody, a Fab fragment, or an antigen binding single chain Fv (scFv) fragment, such as a human synthetic scFv fragment, wherein the monoclonal antibody or antigen binding fragment thereof comprises a heavy chain complementary determining region (CDR) being selected from SEQ ID NO: 1-12, and a light chain CDR being selected from SEQ ID NO: 13-24, wherein the antibody or antigen binding fragment thereof comprises at least six complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3, wherein the CDRs are selected from the group comprising:
CDR-H1 selected from SEQ ID NO: 25, 27 and 28;
CDR-H2 selected from SEQ ID NO: 26, 29 and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L1 is selected from SEQ ID NO: 56 and 57;
CDR-L2 is selected from SEQ ID NO: 58 and 59;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, and wherein the monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding a plurality of prostasome surface antigens selected from the group consisting of SEQ ID NO: 60-104, and wherein the prostasome surface antigens form a conglomerate on the prostasome membrane, which conglomerate is identified and bound by the monoclonal antibody or antigen binding fragment thereof.

In a further embodiment, the human monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding one or more prostasome surface antigens, the prostasome surface antigens being selected from the group consisting of SEQ ID NO: 60-104, as defined in table 3.

In some embodiments, the monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding a plurality of prostasome surface antigens. In some embodiments, the monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding a plurality of prostasome surface antigens, wherein the prostasome

surface antigens form a cluster or conglomerate on the prostasome membrane, which cluster is identified and bound by the monoclonal antibody or antigen binding fragment thereof.

In one embodiment, the human monoclonal antibody or antigen binding fragment thereof bind a conglomerate of proteins/antigens, wherein the conglomerate comprise at least five antigens selected from the proteins in table 11. For each respective scFv fragment 1-12, at least five proteins/antigens are present in the conglomerate bound, as defined for each respective fragment in table 11.

In some embodiments, the antibody or antigen binding fragment thereof enable a sensitivity of at least 10 nano grams per milli liter (ng/mL) in an immunoassay using the human monoclonal antibody or antigen binding fragment thereof as a capturing antibody in the immunoassay.

In some embodiments, the human monoclonal antibody or antigen binding fragment thereof comprises at least six complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3, wherein the CDRs are selected from the group comprising:

CDR-H1 selected from SEQ ID NO: 25, 27 and 28;
CDR-H2 selected from SEQ ID NO: 26, 29 and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L1 is selected from any variant of SEQ ID NO: 56 and 57;
CDR-L2 is selected from any variant of SEQ ID NO: 58 and 59;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24.

In a related embodiment the human monoclonal antibody or antigen binding fragment thereof may comprise at least six CDRs as described above, where CDR-H1, CDR-H2 and CDR-H3 are comprised in a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO: 32-43 and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto, and CDR-L1, CDR-L2 and CDR-L3 is comprised in a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO: 44-55 and 56-59, and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto.

In some embodiments, the human monoclonal antibody or antigen binding fragment thereof comprises at least four complementary determining regions (CDRs) in any combination of

CDR-H1, CDR-H2, CDR-H3 and CDR-L3, wherein the CDRs are selected from the group comprising:

CDR-H1 selected from SEQ ID NO: 25, 27 and 28;

CDR-H2 selected from SEQ ID NO: 26, 29 and 30;

CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;

CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.

In a related embodiment the human monoclonal antibody or antigen binding fragment thereof may comprise at least four CDRs as described above, where CDR-H1, CDR-H2 and CDR-H3 are comprised in a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO: 32-43 and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto, and CDR-L3 is comprised in a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO: 44-55 and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto.

In one embodiment, the human antibody or antigen binding fragment thereof is a synthetic scFv fragment selected from the group comprising:

i) an scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26

CDR-H3 as defined by SEQ ID NO:4 and

CDR-L3 as defined by SEQ ID NO:16,

ii) an scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26

CDR-H3 as defined by SEQ ID NO:7 and

CDR-L3 as defined by SEQ ID NO:19,

iii) an scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26

CDR-H3 as defined by SEQ ID NO:1 and

CDR-L3 as defined by SEQ ID NO:13,

iv) an scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:2 and
CDR-L3 as defined by SEQ ID NO:14,

v) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:3 and
CDR-L3 as defined by SEQ ID NO:15,

vi) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:5 and
CDR-L3 as defined by SEQ ID NO:17,

vii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:6 and
CDR-L3 as defined by SEQ ID NO:18,

viii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:8 and
CDR-L3 as defined by SEQ ID NO:20,

ix) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:9 and
CDR-L3 as defined by SEQ ID NO:21,

x) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:10 and
CDR-L3 as defined by SEQ ID NO:22,

xi) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:11 and

CDR-L3 as defined by SEQ ID NO:23,

xii) an scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26

CDR-H3 as defined by SEQ ID NO:12 and

CDR-L3 as defined by SEQ ID NO:24.

In one embodiment, the human antibody or antigen binding fragment thereof is a synthetic scFv fragment comprising the CDR:s as above, the fragment having a variable heavy chain, VH, and a variable light chain, VL, connected via a linker, wherein the fragments are selected from the group comprising and further selected from the group comprising:

i) an scFv fragment having
a VH as defined by SEQ ID NO:35 and
a VL as defined by SEQ ID NO:47,
ii) an scFv fragment having
a VH as defined by SEQ ID NO:38 and
a VL as defined by SEQ ID NO:50,
iii) an scFv fragment having
a VH as defined by SEQ ID NO:32 and
a VL as defined by SEQ ID NO:44,
iv) an scFv fragment having
a VH as defined by SEQ ID NO:33 and
a VL as defined by SEQ ID NO:45,
v) an scFv fragment having
a VH as defined by SEQ ID NO:34 and
a VL as defined by SEQ ID NO:46,
vi) an scFv fragment having
a VH as defined by SEQ ID NO:36 and
a VL as defined by SEQ ID NO:48,
vii) an scFv fragment having
a VH as defined by SEQ ID NO:37 and
a VL as defined by SEQ ID NO:49,
viii) an scFv fragment having
a VH as defined by SEQ ID NO:39 and
a VL as defined by SEQ ID NO:51,
ix) an scFv fragment having

a VH as defined by SEQ ID NO:40 and
a VL as defined by SEQ ID NO:52,
x) an scFv fragment having
a VH as defined by SEQ ID NO:41 and
a VL as defined by SEQ ID NO:53,
xi) an scFv fragment having
a VH as defined by SEQ ID NO:42 and
a VL as defined by SEQ ID NO:54,
xii) an scFv fragment having
a VH as defined by SEQ ID NO:43 and
a VL as defined by SEQ ID NO:55.

In one embodiment is provided an *in vitro* method for determining whether prostate cancer is present in a subject, the method comprising: providing a human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes; reacting the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject; detecting any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing said level of prostasomes detected with a predetermined threshold; and determining that prostate cancer is present in the subject if the detected level of prostasomes is higher than the predetermined threshold.

Disclosed herein is an *in vitro* method for determining whether prostate cancer is present in a subject, the method comprising: providing (S1) the human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes as defined herein; reacting (S2) the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject; detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes by detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof; comparing (S4) said level of prostasomes detected with a predetermined threshold; and determining (S5A) that prostate cancer is present in the subject if the detected level of prostasomes is higher than the predetermined threshold; determining (S5B) that prostate cancer is not clinically detectable in the subject if the detected level of prostasomes is lower than the predetermined threshold, wherein the sample from the subject is a body fluid sample, selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension, and wherein the predetermined threshold is 10 ng prostasomes per mL of body fluid sample.

In a further embodiment, detecting any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof.

In yet a further embodiment, the method is a sandwich immunoassay and detecting prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the anti-prostasome detection antibody using a further detection antibody, wherein the human monoclonal antibody or binding fragment thereof is a capture antibody, the anti-prostasome detection antibody is a primary detection antibody and the further detection antibody is a secondary detection antibody.

In one embodiment is provided a method *in vitro* for providing a prognosis of a prostate cancer in a subject in need thereof, the method comprising: providing a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes; reacting the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; detecting prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing said level of prostasomes detected with a first and second predetermined threshold; and providing a prognosis of the prostate cancer, wherein prognosis of the prostate cancer is provided to be poor if the detected level of prostasomes are above a first predetermined threshold, and provided to be good if the detected level of prostasomes are below a second threshold.

Disclosed herein is a method *in vitro* for providing a prognosis of a prostate cancer in a subject in need thereof, the method comprising: providing (S11) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes as defined herein; reacting (S12) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; detecting (S13) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing (S14) said level of prostasomes detected with a first and second predetermined threshold; and providing (S15) a prognosis of the prostate cancer, wherein prognosis of the prostate cancer is provided (S15a) to be poor if the detected level of prostasomes are above a first predetermined threshold, and provided (S15b) to be good if the detected level of prostasomes are below a second threshold, wherein the sample from the subject is a body fluid sample and wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng

prostasomes per mL of body fluid sample.

In one embodiment is provided a method *in vitro* for evaluating severity of a prostate cancer in a subject in need thereof, the method comprising: providing a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes; reacting the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; detecting prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing said level of prostasomes detected with a first and second predetermined threshold; and evaluating the severity of the prostate cancer, wherein the prostate cancer is evaluated to be severe if the detected levels of prostasomes are above a first threshold, evaluated to be moderate if the detected levels of prostasomes are below a first threshold but above a second threshold, and evaluated to be light if the detected levels of prostasomes are below a second threshold.

Disclosed herein is a method *in vitro* for evaluating severity of a prostate cancer in a subject in need thereof, the method comprising: providing (S21) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes as defined herein; reacting (S22) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; detecting (S23) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing (S24) said level of prostasomes detected with a first and second predetermined threshold; and evaluating (S25) the severity of the prostate cancer, wherein the prostate cancer is evaluated (S25a) to be severe if the detected levels of prostasomes are above a first threshold, evaluated (S25b) to be moderate if the detected levels of prostasomes are below a first threshold but above a second threshold, and evaluated (S25c) to be light if the detected levels of prostasomes are below a second threshold, wherein the sample from the subject is a body fluid sample and wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample.

In one embodiment is provided a method *in vitro* of evaluating the efficacy of a prostate cancer treatment in a subject in need thereof, the method comprising: detecting a level of prostasomes in a sample from a subject before a prostate cancer treatment; providing an anti-prostate cancer treatment to the subject; detecting a level of prostasomes in a sample from the subject after said prostate cancer treatment; comparing the level of prostasomes before the treatment to the levels after the treatment; and determining the efficacy of the

treatment, where the treatment is determined to be effective if the level of prostasomes after the treatment have decreased compared to the level before the treatment, and determined to be ineffective if the level of prostasomes have remained the same or increased. In some embodiments, detecting a level of prostasomes in a sample from a subject comprises: providing a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes; reacting the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; and detecting prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes.

Disclosed herein is a method in vitro of evaluating the efficacy of a prostate cancer treatment in a subject in need thereof, the method comprising: detecting (S31) a level of prostasomes in a sample from a subject before a prostate cancer treatment; providing (S32) an anti-prostate cancer treatment to the subject; detecting (S33) a level of prostasomes in a sample from the subject after said prostate cancer treatment; comparing (S34) the level of prostasomes before the treatment to the levels after the treatment; and determining (S35) the efficacy of the treatment, where the treatment is determined (S35a) to be effective if the level of prostasomes after the treatment have decreased compared to the level before the treatment, and determined (S35b) to be ineffective if the level of prostasomes have remained the same or increased, wherein detecting (S31, S33) a level of prostasomes in a sample from a subject comprises: providing (S31a, S33a) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes as defined herein; reacting (S31b, S33b) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; and detecting (S31c, S33c) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes, wherein the sample from the subject is a body fluid sample, selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

In some embodiments, the sample from the subject is a body fluid sample, selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

In some embodiments, the predetermined threshold used in the diagnostic method is 10 ng prostasomes per mL of body fluid sample, and the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample, as used in the method for providing a prognosis or severity of a prostate cancer disease.

In some embodiments, the human monoclonal antibody or antigen binding fragment thereof used in the methods is an antibody or antigen binding fragment as described above.

In another embodiment is provided the antibody or antigen binding fragment thereof according as above for use in the methods above.

In one embodiment is provided the use of an antibody or antigen binding fragment thereof as defined herein in the methods as defined herein.

It is noted that the invention relates to all possible combinations of features recited in the claims. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing detailed description, which proceeds with reference to the following illustrative drawings, and the attendant claims.

A feature described in relation to one aspect may also be incorporated in other aspects, and the advantage of the feature is applicable to all aspects in which it is incorporated.

Other objectives, features and advantages of the present inventive concept will appear from the following detailed disclosure, from the attached claims as well as from the drawings.

Generally, all terms used in the claims are to be interpreted according to their ordinary meaning in the technical field, unless explicitly defined otherwise herein. Further, the use of terms "first", "second", and the like, herein do not denote any order, quantity, or importance, but rather are used to distinguish one element from another. All references to "a/an/the [element, device, component, means, step, etc.]" are to be interpreted openly as referring to at least one instance of said element, device, component, means, step, etc., unless explicitly stated otherwise. The steps of any method disclosed herein do not have to be performed in the exact order disclosed, unless explicitly stated.

BRIEF DESCRIPTION OF THE DRAWINGS

The above, as well as additional objects, features and advantages of the present inventive concept, will be better understood through the following illustrative and non-limiting detailed

description of different embodiments of the present inventive concept, with reference to the appended drawings, wherein:

Figure 1 illustrates an exemplary assay using an antibody of the invention.

Figure 2 illustrates an scFv fragment of the invention.

Figure 3 shows an example of the HelL-11 and HelL-13 synthetic scFv library designs, where figure 3a shows the CDRs and figure 3b shows the amino acid contents of the CDRs from example 1.

Figure 4 illustrates the HelL-11 and HelL-13 library constructions where figure 4a shows the amino acid sequence of the HelL library scaffolds, and figure 4b shows the defining positions that are carriers of diversity (X) in the design of VH and VL of HelL-11 and HelL-13 antibody libraries.

Figure 5 shows a flowchart of a method of the present disclosure for diagnosing a prostate cancer in a subject.

Figure 6 shows a flowchart of a method of the present disclosure for prognosing prostate cancer in a subject.

Figure 7 shows a flowchart of a method of the present disclosure for evaluating the severity of a prostate cancer in a subject.

Figure 8 shows a flowchart of a method of the present disclosure for determining the efficacy of a prostate cancer treatment of a subject.

The figures are not necessarily to scale, and generally only show parts that are necessary in order to elucidate the inventive concept, wherein other parts may be omitted or merely suggested.

DETAILED DESCRIPTION

The present disclosure relates to new monoclonal antibodies or antigen binding fragments which selectively bind prostasomes, and to prostate cancer diagnosis and prognosis using these monoclonal antibodies or fragments specific for prostasomes. Aspects of the present disclosure will be described more fully hereinafter with reference to the accompanying drawings. The antibodies and method disclosed herein can, however, be realized in many different forms and should not be construed as being limited to the aspects set forth herein. Like numbers in the drawings refer to like elements throughout.

The terminology used herein is for the purpose of describing particular aspects of the disclosure only, and is not intended to limit the disclosure. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly

indicates otherwise. Initially, some terminology may be defined to provide clarification for the following disclosure.

In some embodiments a non-limiting terms “antibody” or “antigen binding fragment thereof” is used. The term “antibody” is used herein in its broadest sense, including both monoclonal and polyclonal antibodies. As is well known, antibodies are immunoglobulin molecules capable of specific binding to a target (an antigen), such as a protein, carbohydrate, polynucleotide, lipid, polypeptide or other, through at least one antigen recognition site located in the variable region of the immunoglobulin molecule. The human monoclonal antibodies herein, typically phage display antibodies, can be any type of human antibodies or human antigen binding fragments of antibodies which are capable of selectively binding the prostasomes.

As used herein, the term ”antibody” or “an antigen binding fragment thereof” encompasses not only full-length or intact polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof, such as Fab, Fab’, F(ab’)2, Fab3, Fv and variants thereof, fusion proteins comprising one or more antibody portions, humanized antibodies, chimeric antibodies, minibodies, diabodies, triabodies, tetrabodies, linear antibodies, single chain antibodies, multispecific antibodies (e.g. bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies and covalently modified antibodies. A full-length antibody comprises two heavy chains and two light chains. Each heavy chain contains a heavy chain variable region (VH) and first, second and third constant regions (CH1, CH2 and CH3). Each light chain contains a light chain variable region (VL) and a light chain constant region (CL). Depending on the amino acid sequence of the constant domain of its heavy chains, antibodies are assigned to different classes. There are six major classes of antibodies: IgA, IgD, IgE, IgG, IgM and IgY, and several of these may be further divided into subclasses, e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The term ”full-length antibody” as used herein, refers to an antibody of any class, such as IgD, IgE, IgG, IgA, IgM or IgY (or any sub-class thereof). The subunit structures and three-dimensional configurations of different classes of antibodies are well known. The term ”antigen binding fragment” refers to a portion or region of an antibody molecule, or a derivative thereof, that retains all or a significant part of the antigen binding of the corresponding full-length antibody. An antigen binding fragment may comprise the heavy chain variable region (VH), the light chain variable region (VL), or both. Each of the VH and VL typically contains three complementarity determining regions CDR1, CDR2 and CDR3, denoted CDR-H1, CDR-H2 and CDR-H3 for the CDRs from the VH domain and CDR-L1,

CDR-L2 and CDR-L3 for the CDRs from the VL domain. The three CDRs in VH or VL are flanked by framework regions (FR1, FR2, FR3 and FR4). As briefly listed above, examples of antigen binding fragments include, but are not limited to: (1) a Fab fragment, which is a monovalent fragment having a VL-CL chain and a VH-CH chain; (2) a Fab' fragment, which is a Fab fragment with the heavy chain hinge region, (3) a F(ab')2 fragment, which is a dimer of Fab' fragments joined by the heavy chain hinge region, for example linked by a disulfide bridge at the hinge region; (4) an Fc fragment; (5) an Fv fragment, which is the minimum antibody fragment having the VL and VH domains of a single arm of an antibody; (6) a single chain Fv (scFv) fragment, which is a single polypeptide chain in which the VH and VL domains of an scFv are linked by a peptide linker; (7) an (scFv)2, which comprises two VH domains and two VL domains, which are associated through the two VH domains via disulfide bridges and (8) domain antibodies, which can be antibody single variable domain (VH or VL) polypeptides that specifically bind antigens. Antigen binding fragments can be prepared via routine methods. For example, F(ab')2 fragments can be produced by pepsin digestion of a full- length antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of F(ab')2 fragments. Alternatively, fragments can be prepared via recombinant technology by expressing the heavy and light chain fragments in suitable host cells (e.g., E. coli, yeast, mammalian, plant or insect cells) and having them assembled to form the desired antigen-binding fragments either in vivo or in vitro. A single-chain antibody can be prepared via recombinant technology by linking a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. For example, a flexible linker may be incorporated between the two variable regions. In one embodiment, CDRs present on a library scaffold are linked via a linker to form a synthetic scFv fragment. The term "antibody or antigen binding fragment thereof" as used herein thus also encompasses synthetic binding fragments, such as human synthetic scFv fragments. Accordingly, throughout the description the generic term "antibody" or "human antibody" is used. These terms are used in their broadest sense and thus also incorporate all variants and fragments described above and below. Thus, the term "human antibody" also encompass a human binding fragment of an antibody.

The term "human monoclonal antibodies" or "human antibodies" refers to fully human sequence derived antibodies or related fragments that have no murine sequence, and encompasses also fragments of antibodies, and synthetic scFv fragments. The human antibodies largely produced via two sources: phage display technologies and transgenic mice. The human antibodies of the invention do not encompass humanized antibodies, where for example a murine-monoclonal antibody has been treated to become more human. Humanization is the replacement of mouse constant regions and variable (V) framework

regions for human sequences, and results in an antibody where only the complementarity determining regions (CDRs) of the variable (V) regions are of mouse-sequence origin. In contrast, human monoclonal antibodies are fully human, compared to humanized antibodies which still comprises a binding epitope which is non-human.

As used herein, the term ”capable of binding X”, wherein X is an antigen, refer to a property of an antibody or binding fragment thereof which may be tested for example by ELISA, by use of surface plasmon resonance (SPR) technology, by use of the Kinetic Exclusion Assay (KinExA®) or by bio-layer interferometry (BLI). The skilled person is aware of said methods and others.

Also in some embodiments generic terminology “immunoassay”, is used. It can be any kind of immunoassay, such as Lateral Flow Immunochromatographic assays, Immunomagnetic Separation and Electrochemiluminescence (IMS-ECL) assays, Fluorescence immunoassays, Time-resolved Fluorescence (TRF) assays, Radioimmunoassays (RIA) or Enzyme-linked Immunosorbent (ELISA) assays, which use the monoclonal antibodies of the invention for detecting levels or amounts of prostasomes in a sample from a subject, where the “sample” may be a body fluid sample from a subject, such as a blood sample, plasma, serum, urine, cerebrospinal fluid or a cell suspension, wherein the cell suspension may be derived e.g. from a biopsy.

The term “conglomerate” may be used herein as referring to an entity comprising a plurality of prostasome antigens forming a three dimensional conformation of antigens bound to a prostate membrane or prostate membrane fragment. The conglomerate of protein antigens is present on the surface of the prostate membrane. In one embodiment, the antibodies or fragments thereof according to the invention bind a plurality of prostasome surface antigens, wherein the prostasome surface antigens form a conglomerate on the prostasome membrane, which conglomerate is identified by the monoclonal antibody or antigen binding fragment thereof. In some embodiments, monoclonal antibody or antigen binding fragment thereof bind a plurality of antigens, wherein the antigens are selected from Table 11. In some embodiments, the term “cluster”, “structure”, or “agglomerate” may be used synonymously when referring to an entity comprising a plurality of prostasome antigens forming a three dimensional conformation of antigens.

The term “sensitivity” refers to, in the context of a diagnostic assay for example, the true positive rate of the condition of interest, which is the probability of detection, and measures the proportion of actual positives that are correctly identified as such (e.g., the percentage of

sick people who are correctly identified as having the condition). A high sensitivity will give a low number of false negatives, i.e. subject identified as not having the condition while they in reality do. “Sensitivity” in the context of antibodies, such as a “sensitive antibody” is an antibody which is able to detect also low amounts of an intended antigen. The sensitivity could thus be defined in relation to the lowest concentration of the antigen that may be detected in an assay using the antibody.

The term “specificity”, sometimes referred to as “selectivity”, refers to the true negative rate of the condition of interest and measures the proportion of actual negatives that are correctly identified as such (e.g., the percentage of healthy people who are correctly identified as not having the condition). A high specificity in an assay will give a low number of false positives, i.e. subject identified as having the condition while they in reality do not. A selective or specific antibody will not, or to a low extent, cross-react with other targets than the intended antigen. Thus, a prostasome specific antibody or binding fragment thereof of the invention will specifically or selectively bind to prostasomes or prostasome antigens, but not to other antigens such as antigens present on other types of exosomes or cells.
With the term “diagnosis” or “diagnosing” as used herein is meant a process of determining if a disease or condition, in this case prostate cancer, is present in a subject tested. A diagnosis, in the sense of diagnostic procedure, can be regarded as an attempt at classification of an individual's condition into separate and distinct categories that allow medical decisions about treatment and prognosis to be made. Subsequently, a diagnostic opinion is often described in terms of a disease or other condition. The initial task is to detect a medical indication to perform a diagnostic procedure, such as detection of any deviation from what is known to be normal, in this case the levels of prostasomes in peripheral blood. Typically, an aggressive prostate cancer will be detected and diagnosed in the present assays, in comparison with an indolent one.

With the term “prognosis” or “prognosing” as used herein is meant a prediction or estimate of the chance of recovery or survival from a disease. Prognosis with cancer can depend on several factors, such as the stage of disease at diagnosis, type and subtype of cancer, the molecular profile of the tumor, and even gender. The prognosis of the prostate cancer of the invention may be correlated to the levels of the prostasomes in the sample from the subject being tested, where high levels are indicative of a poor prognosis (i.e. a high likelihood of a negative outcome of the disease) and low levels are indicative of a good prognosis (i.e. high likelihood of a positive outcome of the disease).

With “prostasome” is meant submicron membranous vesicles, also called exosomes, which are secreted by the prostate gland epithelial cells into seminal fluid. Thus, the prostasome is a type of exosome. The prostasome is characterized by a size between 50 and 500 nm, surrounded by a lipid plasma membrane bilayer with exceptionally high cholesterol/phospholipid ratio and characteristic proteins like caveolin-1, prostate stem cell antigen, prostate specific membrane antigen (PSMA), CD 10, CD 13, and CD 26 on said surface membrane, while comprising RNA and DNA within (Beneficial effects of seminal prostasomes on sperm functional parameters, Amit Kumar, Sujata Pandita, Subha Ganguly, Simson Soren and Nileshkumar Pagrut J Entomology and Zoology Studies 2008;6(5):2464-2471). Based on this information and the information of the cited paper, the skilled person would be able to distinguish a prostasome from a micro vesicle or from any other type of exosome if encountered in isolation or in peripheral blood. Due to the specific characteristics of prostasomes, prostasome specific antibodies not cross reacting with other exosomes are possible to obtain. The prostasomes typically display one or more protein antigens, as listed in table 2 in the specification below.

The term “prostate cancer”, in the context of a subject suffering from prostate cancer is meant in the broadest sense the presence of one prostate cancer cell. Even though one cell will not be enough for having detectable prostasomes levels in body fluids outside of the seminal fluid above a threshold, early diagnosis of prostate cancer is possible. A detected or diagnosed prostate cancer of the invention could typically be at a stage where it could be possible to make a biopsy to retrieve cancer cells, and is preferably not metastasized but only present in the prostate, and thus possible to remove surgically. Accordingly, early detection of prostate cancer using the method gives a technical effect of an increased amount of cancers that will be possible to treat with surgery, and could also be used as a decision support for determining which cancers that will be possible to perform surgery on. Typically, a detected level of prostasomes in a sample of a subject being 10 ng/mL or less would be considered as treatable by surgery, while a level above 100 ng/mL would be considered as untreatable by surgery. A detected level of prostasomes between 10 ng/mL and 100 ng/mL could be used as an indication that further characterization of the prostate cancer is needed before deciding on the method of treatment to be performed. To be able to perform surgery the prostate cancer must remain in the prostate, and should not have metastasized, and high levels detected in the assays of the invention can thus predict that it is no longer possible to perform surgery.
PSA is the most used marker for detection of prostate cancer today, but unfortunately this biomarker is not specific enough. Thus, there is a need for a better marker for screening of prostate cancer. The preferred marker should be specific both for the organ prostate and for

the cancer disease. One promising marker is the prostasome, a small vesicle emanating from acinar cells in the prostate.

The inventors have previously demonstrated that malignant prostate cells secrete prostasomes, and that presence of malignant prostate tumors increase the number of prostasomes present in peripheral blood, which indicates that prostasomes may be a potential biomarker of prostate cancer. However, developing a method which is simple, robust, reliable, selective and sensitive enough to diagnose prostate cancer using prostasomes as a biomarker has been difficult.

Methods of using monoclonal antibodies against prostasomes for prostate cancer diagnosis have been previously described, however none of these methods seem to have the sensitivity to detect small amounts of prostasomes in blood, serum or plasma using a single capture antibody. Also, the specificity to detect prostasomes and not exosomes in general seems to be lacking. Hence, there is a need for new robust methods which may be used for prostate cancer diagnosis, which are both sensitive and specific enough to avoid false negatives and positives when performing the diagnosis.

As presented in the current disclosure, the above-mentioned problems and drawbacks of the prior art have been overcome by the use of fully human monoclonal antibodies or binding fragments thereof in the diagnosis of prostate cancer. The fully human monoclonal antibodies or binding fragments of the invention will achieve the desired sensitivity and specificity/selectivity to actually be able to perform prostate cancer diagnosis on serum and plasma samples from a patient using a single capture antibody. The current disclosure thus relates to a method for diagnosing or providing a prognosis of a subject suspected of suffering from prostate cancer, comprising in vitro detection of prostasomes in a peripheral blood sample from the subject, where the detected levels of prostasomes may be compared to a threshold or to a reference value derived from healthy subject(s). The thresholds and reference values may be predetermined, or may be calibrated for a specific test.

Previous methods for detecting prostasomes for prostate cancer diagnosis have been using monoclonal antibodies which are typically murine or, at best, humanized. These methods have failed to provide the specificity and sensitivity needed to be able to efficiently diagnose and prognose prostate cancer. The biggest problem is probably the sensitivity, where the antibodies cannot detect small amounts of prostasomes in a sample, hence generating false negatives. For example, in a previous study an enzyme-linked immunosorbent assay (ELISA) was set up to test a number of antibodies for their ability to work as suitable capture

or detection antibodies for prostasomes (E. Thermaenius *et al,* DiVA, id: diva2:544923). Two surface proteins specific for prostasomes were used in the study as capture antigens, and a prostasomal surface-bound protein was used as antigen for the secondary antibody in the assay. With this experimental setup the detection limit was 2500ng/mL, which was not considered enough to detect prostasomes in cancer. In another previous study (Tavoosidana *et al. PNAS*, May 24, 2011, vol. 108, no. 21, 8809–8814) prostasome levels were detected in serum, which requested using five antibodies combined for reaching a fairly sufficient level of sensitivity and specificity for use in diagnosis. In contrast, the current invention provides method which only requires using one monoclonal antibody in the detection assay to specifically and selectively detect prostasomes for diagnosis. The use of the antibodies of the invention thus provides a far simpler assay, which can easily be scaled up and which are cheaper to produce.

There could also be a problem in the assays with false positives if the antibodies used is not specific enough. The antibodies used in an assay may for example react with other exosomes, since all exosomes share common epitopes. As shown by the Human Protein Atlas, a program aimed at mapping all the human proteins in cells, tissues and organs, a random antibody shows a large cross-reactivity with different tissues. It has previously been shown that prostasome surface antigens are the primary auto-antigens causing immunologic infertility and anti-sperm antibodies (see e.g. L. Carlsson *et al.* Journal of Andrology, Vol. 25, No. 5, September/October 2004 and A. Minelli *et al*. ANTICANCER RESEARCH 25: 4399-4402 (2005)), i.e. auto-antibodies against prostasomes are common within men with clinical infertility. One thing that characterizes immunologic infertility is that the man affected is infertile, but generally without any further diseases. This has been interpreted by the inventors as that these auto-antibodies, which are antibodies that the human subject himself are producing, are specific for prostasomes and do not react with other types of cells or tissues. Hence, using human antibodies from human DNA libraries will attain better and more specific/selective prostasome antibodies than using murine monoclonal antibodies or humanized antibodies. Thus, using human antibodies or antibody fragments based on human antigen-binding epitopes for detecting prostasomes as a biomarker for cancer will achieve a higher specificity and selectivity compared to the antibodies suggested in the prior art.

In contrast to the prior art, the current disclosure provides new monoclonal antibodies or binding fragments, which are fully human, and which are specific enough to avoid false positives and sensitive enough to detect small amounts of prostasomes in body fluids. These prostasome specific antibodies may be used in different detection assays for detecting levels

of prostasomes in body fluids, such as blood, serum or plasma. Upon performing a biopsy or prostate massage, also a cell suspension from the biopsy or urine comprising prostasomes due to the massage may be used as sample body fluids. Prostasomes are secreted by the prostate gland cells into seminal fluid, hence, in a healthy subject the prostasomes are generally present in seminal fluid, with only a very small fraction leaking out into the external blood stream. However, prostasomes are also secreted by malignant prostate cells, which increases the number of prostasomes present in peripheral blood. It has been found that the reason that the levels of prostasomes in peripheral blood rises fast when cancer cells secrete them is not only that the number and thus leakage increases, but in fact that the prostate cells loses their polarity when they become cancer cells, hence secreting prostasomes into the blood. Thus, also a slightly elevated level of prostasomes in the peripheral blood is indicative of prostate cancer. To be able to efficiently detect and diagnose and prognose prostate cancer, including also early stage prostate cancer which might have only slightly increased levels of prostasomes in peripheral blood, a very sensitive assay is needed.

The assays of the invention use fully human monoclonal antibodies or binding fragments thereof for detecting the presence of elevated levels of prostasomes in the blood, and thus the presence of malignant prostate cancer cells. A body fluid sample, such as a blood sample, is taken from a subject potentially in need of a diagnosis, such as a patient in a hospital or at a clinic, and the sample is either directly screened for elevated amounts of prostasomes, or first treated to obtain a serum or plasma sample which is then screened in the assay. The amount of detected prostasomes in the body fluid is compared to a predetermined threshold or reference value, where an increased detected value above the threshold or compared to the reference value is indicative of the presence of prostate cancer in the subject being tested. Typically, a level (concentration) of prostasomes of about 1-2 nanogram (ng) per milli liter (mL or ml) body fluid sample is indicative of cancer, and a level above 10 ng/mL may be used for definite diagnosis. The level of detected prostasomes compared to one or more thresholds or one or more reference values may also be used in prognosis of prostate cancer, where for example a higher detected level compared to a reference value is indicative of a later stage prostate cancer and thus a worse prognosis, while a lower detected value compared to the reference value is indicative of an earlier stage prostate cancer and thus a better prognosis. The prognosis could also be defined by certain thresholds, where being above or below the certain thresholds indicates different prognosis. For example, if the levels of prostasomes are above a certain threshold, such as a first predetermined threshold, the prognosis of the prostate cancer is prognosed to be poor, while if the level is below a certain threshold, such as a second predetermined threshold, the

prognosis of the prostate cancer is prognosed to be good. The first threshold may be 10ng prostasomes per mL of sample, and the second threshold may be 1ng prostasomes per mL of sample. The level of prostasomes may also be indicative of the severity of the prostate cancer, which may be linked to possible treatments, such as surgery or no surgery. The cancer progression and treatment efficacy may also be determined, by making measurements of the prostasome levels in the same subject at different points in time, where a decrease in the amount of prostasomes is indicative of tumor regression and high treatment efficacy while an increase of the amount is indicative of tumor progression and low treatment efficacy. The efficacy of a treatment may thus be determined by measuring the levels of prostasomes before and after a treatment regime, where a lowering of the prostasome levels is indicative of an effective treatment. The treatment may be for example a surgery, administration of a medical substance to the subject, or radiation therapy. The medical substance may be for example an oral drug or a substance administered through injections or infusions.

The assays of the invention may be any assay suitable for detecting an analyte within a fluid sample using a monoclonal antibody. The assays may be immunoassays which rely on the ability of an antibody to recognize and bind a specific macromolecule in a sample which comprise a complex mixture of macromolecules. In immunology the particular macromolecule bound by an antibody is referred to as an antigen and the area on an antigen to which the antibody binds is called an epitope. In addition to the binding of an antibody to its antigen, the other key feature of all immunoassays is a means to produce a measurable signal in response to the binding. Most, though not all, immunoassays involve chemically linking antibodies or antigens with some kind of detectable label. A large number of labels exist in modern immunoassays, and they allow for detection through different means. Many labels are detectable because they either emit radiation, produce a color change in a solution, fluoresce under light, or can be induced to emit light. The assays of the invention may be for example Lateral Flow Immunochromatographic assays, Immunomagnetic Separation and Electrochemiluminescence (IMS-ECL) assays, Time-resolved Fluorescence (TRF) assays, or Enzyme-linked Immunosorbent (ELISA) assays. In one embodiment, the assay is an immunoassay or immunocapturing assay, such as a sandwich immunoassay, where the monoclonal antibodies of the invention are used to capture the prostasomes within the fluid sample (the body fluid extracted from the subject potentially in need of a diagnosis or prognosis), and then detected. For example, the assay may be a Sandwich ELISA, where a surface is prepared to which a known quantity of the capture antibody is bound, i.e. one of the monoclonal antibodies of the invention, to which the fluid sample comprising the prostasomes is applied. The prostasomes are captured by

the capture antibody, the surface is washed and a primary detection antibody is applied, which binds to the prostasomes. Enzyme-linked secondary antibodies are applied as detection antibodies that also bind specifically to the antibody's Fc region, thereby detecting the presence of the primary detection antibodies and thus the bound prostasomes. The surface is washed to remove the unbound antibody-enzyme conjugates, and a chemical is added to be converted by the enzyme into a color or fluorescent or electrochemical signal. The absorbance or fluorescence or electrochemical signal (e.g., current) of the plate wells is measured to determine the presence and quantity of the analyte, i.e. prostasomes. The quantity of the prostasomes may then be compared to a reference value, and an increased level be linked to the presence of malignant prostate cancer cells in the subject being diagnosed. The primary detection antibodies may be for example chicken anti-PSA or anti-prostasome antibodies, and the secondary detection antibody may be an anti-chicken antibody, which may be HRP (Horseradish Peroxide) labelled. A commonly used enzymatic marker in ELISA assays is for example OPD (o-phenylenediamine dihydrochloride), which turns amber to detect HRP, which is often used to as a conjugated protein. Also, TMB (3,3',5,5'-tetramethylbenzidine) may be used, which turns blue when detecting HRP and turns yellow after the addition of sulfuric or phosphoric acid, or ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt), which turns green when detecting HRP. Figure 1 shows an example where the human monoclonal antibody or antigen binding fragment thereof of the present disclosure (101) is used as a capture entity (capture antibody), which captures prostasomes (200), after which bound prostasomes (200) may be detected using a primary detection antibody (102) binding the prostasomes and being detected by a secondary detection antibody (103). The primary detection antibody (102) may be an anti-prostasome or anti-PSA antibody and may be of chicken origin (chicken anti-prostasome or chicken anti-PSA), and the secondary detection antibody (103) may be a Horseradish peroxidase (HRP) labelled antibody and will be anti the species of the primary detection antibody, i.e. anti-chicken if the primary detection antibody is chicken (HRP labelled anti-chicken antibody). The current invention thus provides assays for detecting prostasome levels and diagnosing prostate cancer, which are sensitive enough to use a single capturing antibody and a single primary detection antibody, together with a secondary detection antibody, which has not been possible in the prior art. Thus, in one embodiment a single capturing antibody is used in an immunoassay for detecting levels of prostasomes in a sample. In another embodiment, a plurality of capture antibodies are used, such as two, three or four capture antibodies of the invention, but less than five capture antibodies.

The antibodies or fragments of the invention are fully human monoclonal antibodies of fragments which are prostasome specific (selective for prostasomes compared to other cells

or exosomes), and sensitive enough to be able to detect small levels of prostasomes in a sample. The sensitivity needed in an assay for an antibody to work as a single capture antibody in said assay, such as an ELISA, is typically at least 10 nanogram (ng) prostasomes per milli liter (mL). Thus, the assays of the invention makes it possible to detect levels of prostasomes of 10 ng/mL or lower. This sensitivity may be obtained using the antibodies or antigen binding fragment thereof of the invention. The sensitivity may be even higher than 10 ng/mL, where a sensitivity range of 1-2 ng /mL may be attained. No antibodies present in the prior art has been shown to enable this sensitivity in similar assays, hence, there is no antibodies in the art that could be used as a single capture antibody in a detection assay to detect prostasomes for adequate prostate cancer diagnosis.

The antibodies or fragments thereof according to the present invention bind clusters of protein antigens present on the surface of the prostate membrane. The agglomerated antigens form a three dimensional structure, a conglomerate of antigens and prostate membrane, which is identified by the antibodies or fragments thereof according to the present invention. Accordingly, the present antibodies or fragments thereof are ideal for measuring the presence of whole prostasomes in a sample, compared to prior art antibodies. The antibodies or fragments according to the present invention thus both have greater specificity and sensitivity compared to other antibodies or binding fragments. Accordingly, an assay sensitivity of detecting prostasomes in a sample, such as a blood or serum sample, of 10 ng prostasomes per mL, or even higher, such as 1-2 ng /mL, using a single one of these antibodies and fragments may be attained. This is not possible using any of the antibodies in the prior art.

Thus, in contrast to the prior art antibodies for use in prostate cancer diagnosis, the present antibodies are fully human and bind several prostasome antigens, such as a cluster of antigens. Further, the specificity and sensitivity is higher than in the prior art. For example, in Tavoosidana *et al.*, as shown in figure 1, several antibodies (five more specifically) must be used in combination in an assay to attain a sensitivity in the range of what is attained using a single antibody or fragment of the invention. To state that an anti-CD 13 antibody achieves a detection sensitivity of 0,5 mg/mL is simply not correct. 5 antibodies and DNA fragments are used in Tavoosidana *et al.* (see figure 1), hence also requiring a further PCR step in the method. As several antibodies and a DNA fragment are needed to attain the sensitivity, it cannot be said that the antibodies in Tavoosidana *et al,* enable a sensitivity of at least 10 ng/mL in an immunoassay using the antibody as a single capturing antibody in the immunoassay. Hence, the individual antibodies of Tavoosidana *et al,* are not fully human, do not bind to several prostasome antigens, or a cluster of antigens on the prostasome

membrane, nor do they individually achieve the specificity of the antibodies/fragments of the invention. The methods of the present invention also have further advantages over the method in Tavoosidana *et al,* since they are simpler, cheaper, and more efficient.

Regarding the method in document Yu *et al.*, ("Electrical and Label-Free 1-18 Quantification of Exosomes with a Reduced Graphene Oxide Field Effect Transistor Biosensor", ANALYTICAL CHEMISTRY, vol. 91, no. 16, 23 July 2019 (2019-07-23) , pages 10679-10686), also this method as Tavoosidana *et al.* is built on analysis of DNA (PNA), which is a much more complex outline than a simple immune assay, and further DNA analyses and immunological analyses are fundamentally different from each other. Usually, DNA tests may achieve a high sensitivity, but immunological, e.g. antibody based, tests are simpler and cheaper. Further, regarding Yu *et al.,* the anti-CD 63 antibody used have very low tissue specificity (see e.g. the protein atlas, URL https://www.proteinatlas.org/ENSG00000135404-CD63), which means that it cannot distinguish prostasomes from other types of exosomes, nor prostasomes from whole cells (as the anti-CD 63 antibody will react to whole cells). The antibody in Yu *et al.* is used to detect exosomes in a pure sample, and to detect non-specific occurrence of cancer, based merely on the fact that cancer produce more exosomes. There is no specificity in a diagnose based on just number of unspecified exosomes in blood. Further, in a sample, such as a blood sample of the invention, the antibody would not only detect all exosomes present, which may signify the presence of a broad variation of cancers or other disorders, but would also detect whole cells. Thus, the antibody would not be able to distinguish prostasomes or prostate cancer, nor would it be able to distinguish exosomes in a blood sample. Accordingly, the antibody in Yu *et al.* would not be able to be used in a method for detecting prostate cancer in patient samples. Thus, the antibody in Yu *et al.* is not fully human, it does not selectively bind prostasomes, nor does it bind to several prostasome antigens, or a cluster of antigens on the prostasome membrane, nor achieve the sensitivity of the antibodies/fragments of the invention in a simple immunoassay.

A human monoclonal antibody or antigen binding fragment thereof according to the invention is selected from the group consisting of full-length antibodies, Fab fragments, Fab' fragments, F(ab')2 fragments, Fc fragments, Fv fragments, single chain Fv fragments, (scFv)2 and domain antibodies. In one embodiment, said antibody or antigen binding fragment thereof is selected from full-length antibodies, Fab fragments and scFv fragments. In one embodiment, the antibody comprises twelve complementary determining regions (CDRs). In another embodiment, the antibody or antigen binding fragment thereof comprises six CDRs, CDR-H1 / CDR-H2 / CDR-H3 / CDRL1 / CDR-L2 / CDR-L3. In a more specific such embodiment, the antibody or antigen binding fragment thereof comprises at least three

complementarity determining regions (CDRs), where the CDRs may be for example from the heavy chain, CDR-H1, CDR-H2 and CDR-H3, or from the light chain, CDR-L1, CDR-L2 and CDR-L3.

In one embodiment, the human monoclonal antibody of the invention is an antigen-binding fragment (Fab) of an antibody. The antigen-binding fragment (Fab) is a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and the light chain. The variable domain contains the paratope (the antigen-binding site), comprising a set of complementarity determining regions, at the amino terminal end of the monomer.

In one embodiment, the human monoclonal antibody of the invention is a single-chain variable fragment (scFv) of an antibody. The single-chain variable fragments lack the constant Fc region found in complete antibody molecules, and is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. These molecules maintain the same binding functionality that full-length antibodies but possess several advantageous features as quickness to penetrate the tissues, easy manipulation, fast elimination of their immunocomplex and the possibility of being produced in simple expression systems like bacteria and yeast. Figure 2 shows a schematic figure of an scFv fragment of the invention, having a VH part comprising the heavy CDRs (CDR-H1, CDR-H2 and/or CDR-H3) and a VL part comprising the light CDR(s) (CDR-L1, CDR-L2 and/or CDR-L3), jointed together using a linker (dotted line of figure 2).

In one embodiment, the human monoclonal antibody of the invention is a human synthetic single-chain variable fragment (scFv) of an antibody. The scFv fragments may have a molecule weight of about 30 kDa, and comprise a heavy and a light chain linked via a linker. The heavy and light chains may have constant and variable parts, where the loops may be referred to as CDRs, which may be variable. In some embodiments, at least four variable CDRs are present, where typically the CDR-H3 and CDR-L3 are most variable since they form the most exposed part of the binding molecule or fragment. CDR-L1 and CDR-L2 may be lacking (may be constant), while CDR-H1 and CDR-H2 may have slight variations. In some embodiments, the synthetic scFv fragments comprise short heavy chain sequences of CDR-H1, CDR-H2 and/or CDR-H3 on a VH scaffold in combination with a short light chain sequence from CDR-L3 on a VL scaffold, which are linked using a linker to form a scFv fragment. The synthetic scFv fragments may arise from human synthetic scFv libraries, where the CDRs are present on a library scaffold, such as from a HelL-11 or HelL-13

scaffold library (Säll *et al*., Generation and analyses of human synthetic antibody libraries and their application for protein microarrays. Protein Eng Des Sel. 2016 Oct;29(10):427-437) or from a similar MAW-16 library. The length of the CDR-H3 is typically 8 to 22 amino acids, and the lengths of the CDR-L3 8-12 amino acids. The total scFv fragment comprising both the library scaffold, the heavy and light CDR parts and the linker is typically around 250-300 amino acids, such as 273 amino acids. The scFv fragments may be modified by attaching the fragment to another structure. These other structures may be e.g. an antibody or part of an antibody, a HIS-tag, FLAG or biotin etc. The CDRs may also be mounted on a full-size antibody, using the constant regions of CDR-L1 and CDR-L2 as corresponding antibody CDRs.

The human antibodies or binding fragments of the invention may be produced using different methods, such as using phage display and libraries. Monoclonal antibody production based on phage display technology represents an attractive alternative to traditional hybridoma technology. A gene sequence coding for a particular antibody is integrated into the DNA sequence of a filamentous bacteriophage, which allows its expression on the surface of the bacteriophage capsid. This peculiarity establishes a link between the genotype and the phenotype. The phage infects Escherichia coli and uses its inner replication system to continuously display new phage, without killing the host cell. This enables fast production of antibodies, in large numbers. A library of naïve or immune phage is therefore constituted and can be used to detect an antigen-antibody interaction of interest thanks to screening methods. Libraries can be generated from humans giving access to the human antibody repertoire.

For specific antibodies to prostasomes, the human genome offers clear advantages. Autoantibodies to prostasome are frequently occurring and when present they cause immunological infertility. Apart from the infertility the antibodies do not cause damage to other organs in the body. This is a strong indication that human antibodies to prostasomes are highly specific. The high selectivity is a clear advantage when detecting prostasomes in e.g. blood where other cell types will be present. One way of producing the antibodies or fragments of the invention is thus to use phage display technology to develop human scFv antibodies to human prostasomes. Another advantage of using antibodies from the same species is the immunological tolerance that should reduce the number of antibodies to antigens generally presented in the body. Immunological tolerance is a complex series of mechanisms that impair the immune system to mount responses against self-antigens. Studies of the scFv antibodies also indicates that they are superior to mouse antibodies as binders when used to detect prostasomes, allowing for the detection of much lower amounts

of prostasomes. This is surprising since it is contrary to general beliefs that claim that the affinity of phage display antibodies often have lower affinity than hybridoma antibodies. A hypothesis for this finding is that it could be related to the fact that the antibodies are directed to prostasomes.

In one embodiment, the antibody or binding fragment thereof originates from a library which has been screened for binding capacities towards prostasomes (full size whole prostasomes). There exist numerous approaches to create antibody libraries from which binders can be selected, and these can be divided into natural or synthetic, depending on the source of the genetic diversity. Natural antibody repertoires take advantage of the ability of the immune system to generate diversity, while diversity in synthetic repertoires is introduced artificially at defined regions of the antibody sequence. Synthetic libraries generate high genetic diversity, but these have not been selected for function. In order to maximize function, the trend within synthetic library design has therefore been to try to mimic the diversity found in natural antibodies while still trying to boost the genetic diversity.

In the examples, below, we describe the generation of synthetic scFv libraries, which may be used to screen for the synthetic scFv fragments described herein. Two human synthetic antibody fragment libraries have previously been developed, made as part of a two-step process through which initial experience with the first-generation library (HelL-11) was utilized to design a second-generation library (HelL-13) (see Säll *et al*. Generation and analyses of human synthetic antibody libraries and their application for protein microarrays. Protein Eng Des Sel. 2016 Oct;29(10):427-437). Although differing slightly in the design of CDR-H3 and CDR-L3 (Figure 3), both template scFv genes used as scaffolds for the HelL-11 and HelL-13 libraries were built on the human IGHV3-23 and IGKV1-39 genes, in which kappa light chain IGKV1-39 and heavy chain IGHV3-23 were used as scaffolds for library construction. Antibodies consisting of these genes are among the most frequently found in natural antibody repertoires and this particular VH/VL combination has been shown favorable. Alternatively to HelL-11 and HelL-13, a MAW-16 library may be used, in which the variable heavy chain is also based on IGHV3-23, and the light variable chain is also based on IGKV1-39.

Diversity was introduced at defined positions in four of the six CDR loops; CDR-H1, CDR-H2, CDR-H3 and CDR-L3, and residues distributed in CDR-H1, CDR-H2, CDR-H3 and CDR-L3 loops were chosen for diversification. The scFv fragments of the different libraries are shown in figure 4a, and the VH and VL parts are shown in figure 4b. One primer was used each for CDR-H1 and CDR-H2, while three or five and 10 or 15 primers, one primer for

each of the allowed lengths of these loops, were used to diversify CDR-L3 and CDR-H3 of HelL-13 and HelL-11, respectively. The introduced diversity was biased for tyrosine, serine and glycine since these amino acids are highly abundant in antigen-binding sites and their preferred role in antigen recognition has been demonstrated in several studies. Targeted residues of CDR-H1 and CDRH2 were restricted to this trimer code. Although biased for tyrosine, serine and glycine, CDR-H3 and CDR-L3 were allowed a much more complex chemical diversity based on the knowledge of what is commonly found in natural antibodies of CDR-H3. Solvent accessible residues of CDR-H1 and CDR-H2 were restricted to tyrosine, serine and glycine, whereas a more complex diversity scheme was allowed in CDR-H3 and CDR-L3. The allowed lengths of CDR-H3 and CDR-L3 of HelL-11 were 8-22 and 8-12 residues, respectively. In HelL-13 the corresponding lengths were 8–17 and 8–10 residues. The variability introduced here was mainly restricted to amino acids commonly found in natural antibodies at these positions. In HelL-11 the lengths of CDR-H3 and CDR-L3 were allowed to vary from 8 to 22 and 8 to 12, respectively, thereby covering >90% of the most frequently occurring lengths of natural antibodies. The HelL-13 library was designed to contain CDR-H3 loop lengths of 8–17 and CDR-L3 of 8–10, thereby covering ~90% of the functional selected binders derived from HelL-11. To further increase the fraction of functional binders in the naïve repertoire of HelL-13 a CDR-L3 loop without stop-codons was included. The IGKV1-39 germline sequence was chosen as template for CDR-L3 (position 105–115). In position 116, which is naturally derived from the J-segment, a tyrosine was included because of its general favorable binding properties.

In one embodiment, the human monoclonal antibody of the invention is a human synthetic single-chain variable fragment (scFv) of an antibody. The technology for developing the library and screening for the antibody fragments utilizes a focused single chain antibody fragment (scFv) repertoire (Persson *et al*., (2006) Creating a focused antibody library for improved hapten recognition. J Mol Biol 357, 607-620. (Abstract) (GenBank)). The template scFv genes used as scaffolds for the HelL-11 and HelL-13 libraries were built on the human Immunoglobulin heavy variable 3-23 precursor (IGHV3-23) and Immunoglobulin kappa variable 1-39 precursor (IGKV1-39) genes. The phagemid vector backbone was pFab5c. Residues distributed in CDR-H1, CDR-H2, CDR-H3 and CDR-L3 were cloned and the sequences were introduced into the library scaffold genes, as shown in figure 3a. The lengths of CDR-H1 and H2 were 8 amino acid residues for both HelL-11 and HelL-13. The lengths of CDR-H3 and CDR-L3 of HelL-11 were 8-22 and 8-12 amino acid residues, respectively. In HelL-13 the corresponding lengths were 8–17 and 8–10 residues. (Säll *et al*., Generation and analyses of human synthetic antibody libraries and their application for protein microarrays. Protein Eng Des Sel. 2016 Oct;29(10):427-437).

The library was screened, using phage display, against a panel of different prostasomes, yielding diverse and highly specific binders to prostasomes. Analysis has shown that members selected from such populations are excellent sources to develop high affinity binders following affinity maturation by random mutagenesis and stringent phage display selection. Mutations in high affinity variants were scattered throughout the scaffold and could not easily have been predicted.

The synthetic scFv fragments of the invention may include one VH part comprising CDR H1, CDR H2, and CDR H3, and one VL part comprising CDR L3 or CDR L1, CDR L2 and CDR L3. The CDR-L1 and CDR-L2 could be seen as CDR:s present in the synthetic scFv fragments, the location of the CDR-L1 and CDR-L2 being shown in figure 4b. But as they do not differ between the different fragments of the invention, they could also be seen as part of the scaffold. Thus, in one embodiment only CDR H1, CDR H2, CDR H3 and CDR L3 is defined for each fragment. In other embodiments, such as when using the CDR:s in an antibody, all six CDR:s may be defined, including CDR L1 and CDR L2.

The synthetic scFv fragments of the invention may include one VH part comprising CDR H1, CDR H2, and CDR H3, and one VL part comprising CDR L3, which VH and VL parts are combined using a linker. This may be a gly-ser sequence of about 15 amino acids, such as GGGGSGGGGSGGGGS as shown in SEQ ID NO: 31. In some embodiments, the VH fragments is 128 amino acids, the VL fragment is 128 amino acids and the total scFv fragment is 273 amino acids. In some embodiments, the fragments may be shorter due to omission of some amino acids, as shown as dotted circles in figure 4b.

In one embodiment the antibody or antigen binding fragment may comprise a CDR of the VH part, such as a heavy chain CDR which may be referred to as CDR-H3, and a CDR of the VL part, such as a light chain CDR which may be referred to as CDR-L3, wherein:
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24,
and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.
The most important variable parts constitutes the CDR-H3 and CDR-L3 parts. The CDR-H1, CDR- H2, CDR-L1 and CDR-L2 parts may be constant or vary, as show in table 1 below.

Table 1

SEQUENCE	VL CDR L1
SEQ ID NO: 56	SQSISSYL
SEQ ID NO: 57	SQSI$X_5X_6X_7X_8X_9X_{10}$SSYL
SEQUENCE	**VL CDR L2**
SEQ ID NO: 58	YAASS
SEQ ID NO: 59	YAA$X_4X_5X_6X_7X_8X_9X_{10}$SS
SEQUENCE	**VH CDR H1**
SEQ ID NO: 25	GFTFSSYA
SEQ ID NO: 27	GFTF $X_5X_6X_7X_8$MX_{10}
SEQ ID NO: 28	GFTF$X_5X_6X_7X_8$SSYA
SEQUENCE	**VH CDR H2**
SEQ ID NO: 26	ISGSGGST
SEQ ID NO: 29	GFTF $X_5X_6X_7X_8$TX_{10}
SEQ ID NO: 30	ISGS X_5X_6GGST

The amino acids denoted as X may be any natural occurring amino acids. However, in a preferred embodiment the amino acids X are chosen from Y, S or G.

In one embodiment the antibody or antigen binding fragment comprises at least three heavy CDRs (CDR-H1, CDR-H2, CDR-H3) and one light CDR (CDR-L3), in any combination thereof, wherein:
CDR-H1 is selected from any variant of SEQ ID NO:25 and 27-28;
CDR-H2 is selected from any variant of SEQ ID NO:26 and 28;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24,
and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.

In some embodiments, the antibody or antigen binding fragment comprises at least two CDRs; CDR-H3 and CDR-L3, or at least four CDRs; CDR-H1, CDR-H2, CDR-H3 and CDR-L3, or at least six CDRs; CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3, wherein:
CDR-H1 is selected from any variant of SEQ ID NO: 25, 27 and 28;
CDR-H2 is selected from any variant of SEQ ID NO: 26, 29, and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L1 is selected from any variant of SEQ ID NO: 56 and 57;
CDR-L2 is selected from any variant of SEQ ID NO: 58 and 59;

CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto. The CDRs may be grafted on any suitable human antibody or antibody scaffold, such as built on the human IGHV3-23 and IGKV1-39 genes, in which kappa light chain IGKV1-39 and heavy chain IGHV3-23 are used as scaffolds.

In another embodiment, the antibody or antigen binding fragment thereof comprises a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO: 32-43 and sequences having 80 % or more, such as 85 %, 90 %, 95 % or more, identity thereto. In another embodiment, the antibody or antigen binding fragment thereof comprises a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO: 44-55 and sequences having 80 % or more, such as 85 %, 90 %, 95 % or more, identity thereto.

In some embodiments, the antigen binding fragment is an scFv fragment comprising a VH part selected from SEQ ID NO: 32-43 and a VL part selected from SEQ ID NO: 44-55, joined via a linker, such as the linker of SEQ ID NO: 31.

In one embodiment, the human monoclonal antibody or binding fragment thereof is a synthetic scFv fragment , built e.g. on the HeIL-11 or HeIL.13 scaffold, and comprising the CDRs chosen from the 12 scFv fragments of table 2, where VH CDR-H1 for fragment scFv 1 to scFv 12 corresponds to SEQ ID NO: 25, VH CDR-H2 for fragment scFv 1 to scFv 12 corresponds to SEQ ID NO: 26, VH CDR-H3 for fragment scFv 1 to scFv 12 corresponds to SEQ ID NO: 1-12, VL CDR-L3 for fragment scFv 1 to scFv 12 corresponds to SEQ ID NO: 13-24, and the linker between the fragments correspond to SEQ ID NO: 31.

Table 2

Synthetic scFv fragment	**VH CDR H1**	**VH CDR H2**	**VH CDR H3**	**VL CDR L3**
scFv 1	GFTFSSYA	ISGSGGST	CARHYYNWASGYFDYW	CQQGWYPPTF
scFv 2	GFTFSSYA	ISGSGGST	CARTTSYYGYSAHFIDYW	CQQGYGPFTF
scFv 3	GFTFSSYA	ISGSGGST	CARVYYHYYYYGSFDYW	CQQSTHLSTF
scFv 4	GFTFSSYA	ISGSGGST	CARYSYYYAGSYYFDYW	CQQSYSTPYTF
scFv 5	GFTFSSYA	ISGSGGST	CARSYSWYSRGPSLDYW	CQQAFYAHLLTF
scFv 6	GFTFSSYA	ISGSGGST	CARYVRGYGYGYLDYW	CQQAPAYSPFTF
scFv 7	GFTFSSYA	ISGSGGST	CARYTHSTYGYRLDYW	CQQDAYHPPTF
scFv 8	GFTFSSYA	ISGSGGST	CARVFYYGPYYFSYHFDYW	CQQDDRGLPTF
scFv 9	GFTFSSYA	ISGSGGST	CARSHYGSLDYW	CQQFSYTLSTF
scFv 10	GFTFSSYA	ISGSGGST	CARYSYYYTWLDYW	CQQFYPSLHTF

scFv 11	GFTFSSYA	ISGSGGST	CARYTYYVGGFDYW	CQQGFVLSTF
scFv 12	GFTFSSYA	ISGSGGST	CARTYYYFYPYGYYFDYW	CQQGFYLPTF

The human antibodies and binding fragments of the invention are capable of binding prostasomes, and thus to be used as a capture antibody in an assay for diagnosing prostate cancer. The antibodies or binding fragments thereof may recognize and bind any possible epitope of the prostasomes, which epitopes are known in the art. Some of the relevant epitopes are summarized in table 3 below.

Table 3

Protein	**Uniprot code**	**SEQ ID NO**
14-3-3 protein	P62258	SEQ ID NO: 60
Actin,	P60709	SEQ ID NO: 61
Alpha-actinin-2	P35609	SEQ ID NO: 62
Aminopeptidase N	P15144	SEQ ID NO: 63
Annexin A2	P07355	SEQ ID NO: 64
Clusterin	P10909	SEQ ID NO: 65
Desmoglein-1	Q02413	SEQ ID NO: 66
Desmoplakin	P15924	SEQ ID NO: 67
Dipeptidyl peptidase 4	P27487	SEQ ID NO: 68
Epiplakin	P58107	SEQ ID NO: 69
Ezrin	P15311	SEQ ID NO: 70
Filaggrin-2	Q5D862	SEQ ID NO: 71
Flotillin-1	O75955	SEQ ID NO: 72
Flotillin-2	Q14254	SEQ ID NO: 73
Glyceraldehyde-3-phosphate dehydrogenase	P04406	SEQ ID NO: 74
G-protein coupled receptor	Q8IZP9	SEQ ID NO: 75
Heat shock 70 kDa protein	P0DMV8	SEQ ID NO: 76
Heat shock cognate 71 kDa protein	P11142	SEQ ID NO: 77
Heat shock protein HSP 90-alpha	P07900	SEQ ID NO: 78
Heat shock protein HSP 90-beta	P08238	SEQ ID NO: 79
High affinity immunoglobulin gamma Fc receptor I	P12314	SEQ ID NO: 80
Histidine-rich glycoprotein	P04196	SEQ ID NO: 81
Histidine--tRNA ligase	P12081	SEQ ID NO: 82
Immunoglobulin lambda-like polypeptide	B9A064	SEQ ID NO: 83
Intelectin-1	Q8WWA0	SEQ ID NO: 84
Intelectin-2	Q8WWU7	SEQ ID NO: 85
Junction plakoglobin	P14923	SEQ ID NO: 86
Kallikrein-2	P20151	SEQ ID NO: 87
Myosin-1	P12882	SEQ ID NO: 88
Myosin-2	Q9UKX2	SEQ ID NO: 89
Nebulin	P20929	SEQ ID NO: 90
Programmed cell death 6-interacting protein	Q8WUM4	SEQ ID NO: 91

Prolactin-inducible protein (PIP)	P12273	SEQ ID NO: 92
Prostate-specific antigen	P07288	SEQ ID NO: 93
Prostatic acid phosphatase	P15309	SEQ ID NO: 94
Protein-glutamine gamma-glutamyltransferase	Q08188	SEQ ID NO: 95
Ras-related protein Rab	Q9BZG1	SEQ ID NO: 96
Semenogelin-1	P04279	SEQ ID NO: 97
Semenogelin-2	Q02383	SEQ ID NO: 98
Serine--tRNA ligase	P49591	SEQ ID NO: 99
Sorbitol dehydrogenase	Q00796	SEQ ID NO: 100
Titin	Q8WZ42	SEQ ID NO: 101
Tubulin alpha 1C	Q9BQE3	SEQ ID NO: 102
Tubulin alpha 4A	P68366	SEQ ID NO: 103
Tubulin beta	P07437	SEQ ID NO: 104

A method for diagnosing, prognosing, evaluating severity or treatment efficacy of a prostate cancer according to the inventive concept will now be described with reference to Figures 5-8. For clarity and simplicity, the method will be described in terms of ‘steps’. It is emphasized that steps are not necessarily processes that are delimited in time or separate from each other, and more than one ‘step’ may be performed at the same time in a parallel fashion.

The inventive concept has mainly been described above with reference to a few embodiments. However, as is readily appreciated by a person skilled in the art, other embodiments than the ones disclosed above are equally possible within the scope of the inventive concept, as defined by the appended patent claims.

Example operations

The proposed methods will now be described in more detail referring to Figures 5- 8. It should be appreciated that the operations do not need to be performed in order. Furthermore, it should be appreciated that not all of the operations need to be performed.

Figure 5 illustrates an *in vitro* method for determining whether prostate cancer is present in a subject, the method comprising the steps of: providing (S1) a human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes, reacting (S2) the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject, detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes, comparing (S4) said level of prostasomes detected with a predetermined threshold; and determining (S5) that prostate cancer is present in the subject if the detected level of prostasomes is higher than the predetermined threshold. Determining that prostate

cancer is not present in this regard includes determining that no clinically detectable prostate cancer is present, i.e. that any potentially existing cancer cells are too few to be detected or labelled as an existing prostate cancer. However, a few cancer cells may of course be present, which will develop into a detectable prostate cancer eventually. However, these low amounts will be considered as that prostate cancer is not present at the time of diagnosis. The predetermined threshold may correspond to a level of 10ng prostasomes per mL sample. In another embodiment, the predetermined threshold is as low as 1-2 ng prostasomes per mL sample. The threshold may also be 3, 4, 5, 6, 7, 8, or 9 ng/mL.

In one aspect, detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof. Typically, a low amount of prostasomes are always present in the sample if the subject is male. In a further aspect, the method is a sandwich immunoassay and detecting (S3) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody. In one further aspect, the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody. In one aspect, the sample from the subject is a body fluid sample. In a further aspect, the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine cerebrospinal fluid and a cell suspension. The blood, serum and plasma arise from a blood sample of peripheral blood taken from the patient, while the cell suspension typically arise from a biopsy. The urine may be used after e.g. a prostate massage has been performed, where after prostasomes may leak into the urine. In one aspect, the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment of the present disclosure.

Figure 6 illustrates an *in vitro* method for providing a prognosis of a prostate cancer in a subject in need thereof, the method comprising the steps of: providing (S11) a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes, reacting (S12) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes, detecting (S13) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes, comparing (S14) said level of prostasomes detected with a first and second predetermined threshold, and providing (S15) a prognosis of the prostate cancer,

wherein prognosis of the prostate cancer is provided (S15a) to be poor if the detected level of prostasomes are above a first predetermined threshold, and provided (S15b) to be good if the detected level of prostasomes are below a second threshold. In one embodiment, the first threshold is 10ng prostasomes per mL sample and the second threshold is 1ng prostasomes per mL sample.

In one aspect, the detecting (S13) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof. In a further aspect, the method is a sandwich immunoassay and detecting (S13) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody. In one further aspect, the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody. In one aspect, the sample from the subject is a body fluid sample. In a further aspect, the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension. In one aspect, the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment of the present disclosure.

Figure 7 illustrates an *in vitro* method for evaluating severity of a prostate cancer in a subject in need thereof, the method comprising the steps of: providing (S21) a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes; reacting (S22) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; detecting (S23) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes; comparing (S24) said level of prostasomes detected with a first and second predetermined threshold; and evaluating (S25) the severity of the prostate cancer, wherein the prostate cancer is evaluated (S25a) to be severe if the detected levels of prostasomes are above a first threshold, evaluated (S25b) to be moderate if the detected levels of prostasomes are below a first threshold but above a second threshold, and evaluated (S25c) to be light if the detected levels of prostasomes are below a second threshold. In one embodiment, the first threshold is 10ng prostasomes per mL sample and the second threshold is 1ng prostasomes per mL sample.

In one aspect, the detecting (S23) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof. In a further aspect, the method is a sandwich immunoassay and detecting (S23) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody. In one further aspect, the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody. In one aspect, the sample from the subject is a body fluid sample. In a further aspect, the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension. In one aspect, the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment of the present disclosure. The severity of the prostate cancer is usually described in the art in relation to a Gleason score, which may be linked to both aggressiveness and severity, as well as prognosis. Men with a biochemical recurrence and a PSA doubling time <12 months and/or a biopsy Gleason score of 8-10 are considered to have an indication for androgen deprivation therapy (ADT) according to the European Association of Urology (EAU) guidelines. The prostasome assay of the invention distinguished patients with high and medium Gleason scores (8/9 and 7, respectively) from those with low score (≤6), thus reflecting disease aggressiveness. Thus, a determined level of prostasomes above 1ng/mL could be said to correspond to a Gleason score of above 6.

Figure 8 illustrates an *in vitro* method for evaluating the efficacy of a prostate cancer treatment in a subject in need thereof, the method comprising the steps of: detecting (S31) a level of prostasomes in a sample from a subject before a prostate cancer treatment, providing (S32) an anti-prostate cancer treatment to the subject, detecting (S33) a level of prostasomes in a sample from the subject after said prostate cancer treatment, comparing (S34) the level of prostasomes before the treatment to the levels after the treatment, and determining (S35) the efficacy of the treatment, where the treatment is determined to be effective (S35a) if the level of prostasomes after the treatment have decreased compared to the level before the treatment, and determined as ineffective (S35b) if the level of prostasomes have remained the same or increased.

In one aspect, the detecting (S33) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-

prostasome detection antibody or antigen binding fragment thereof. In a further aspect, the method is a sandwich immunoassay and detecting (S33) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody. In one further aspect, the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody. In one aspect, the sample from the subject is a body fluid sample. In a further aspect, the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension. In one aspect, the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment of the present disclosure.

The content of this disclosure thus enables to diagnose prostate cancer, to determine the prognosis of the prostate cancer, which is linked to the stage and aggressiveness of the cancer, to evaluate the severity of the cancer, which may be used as a decision support for selecting treatment, such as performing surgery or not, and to evaluate a treatment regimen of the prostate cancer in a subject, the subject typically being a male human being. The treatment regimen may be evaluating the efficacy of the treatment by measuring the levels of prostasomes in a sample before and after said treatment, the treatment including both surgery, radiation therapy and administration of a medical substance. Thus, the method may comprise measuring the prostasome levels according to the invention, administering a therapeutic drug, and measuring the levels of prostasomes again after the drug has been administered and has had time to affect the cancer, the time depending on the drug used, which would be apparent to a skilled person depending on the treatment used.

In the drawings and specification, there have been disclosed exemplary aspects of the disclosure. However, many variations and modifications can be made to these aspects without substantially departing from the principles of the present disclosure. Thus, the disclosure should be regarded as illustrative rather than restrictive, and not as being limited to the particular aspects discussed above. Accordingly, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation.
The description of the example embodiments provided herein have been presented for purposes of illustration. The description is not intended to be exhaustive or to limit example embodiments to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of various alternatives to the

provided embodiments. The examples discussed herein were chosen and described in order to explain the principles and the nature of various example embodiments and its practical application to enable one skilled in the art to utilize the example embodiments in various manners and with various modifications as are suited to the particular use contemplated. The features of the embodiments described herein may be combined in all possible combinations of methods, products, and systems. It should be appreciated that the example embodiments presented herein may be practiced in any combination with each other.

It should be noted that the word “comprising” does not necessarily exclude the presence of other elements or steps than those listed and the words “a” or “an” preceding an element do not exclude the presence of a plurality of such elements. It should further be noted that any reference signs do not limit the scope of the claims, that the example embodiments may be realized in the broadest sense of the claims.

While the invention has been described with reference to various exemplary aspects and embodiments, it will be understood by those skilled in the art that various changes may be made, and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation to the teachings of the invention without departing from the essential scope thereof. Therefore, it is intended that the invention not be limited to any particular embodiment, but that the invention will include all embodiments falling within the scope of the appended claims. The invention will be further illustrated by the following non-limiting Examples.

EXAMPLES

Example 1 Synthetic antibody fragment library construction

The synthetical scFv fragments scFv 1-12 of the invention originates from the human synthetic antibody fragment libraries HeIL-11 and HeIL-13 produced by Säll *et al*. (Säll *et al*., Generation and analyses of human synthetic antibody libraries and their application for protein microarrays. Protein Eng Des Sel. 2016 Oct;29(10):427-437), aspects of the method are summarized below.

Residues distributed in CDR-H1, CDR-H2, CDR-H3 and CDR-L3 were chosen for diversification and oligonucleotides targeting these loops were made using custom-made trimer phosphoramidite mixes (TriLink BioTechnologies, San Diego, CA, USA), encoding the amino acid compositions shown in Fig. 3. One primer was used each for CDR-H1 and CDR-

H2, while three or five and 10 or 15 primers, one primer for each of the allowed lengths of these loops, were used to diversify CDR-L3 and CDR-H3 of HelL-13 and HelL-11, respectively. The primer encoded sequences were introduced into the library scaffold genes using an optimized Kunkel mutagenesis methodology, and the Kunkel DNA was subsequently electrocorporated into E-coli cells. Ultimately the scFv displaying phages were harvested. Phage selection based on specific antigens could then be performed to attain specific antigen binding fragments.

Thus, figure 3 shows the HelL-11 and HelL-13 library designs. The two libraries have similar designs and are both build on the human scFv scaffold based on heavy chain variable gene IGHV3-23 and kappa light chain variable gene IGKV1-39 (for full sequences of these, see Säll *et al*., Generation and analyses of human synthetic antibody libraries and their application for protein microarrays. Protein Eng Des Sel. 2016 Oct;29(10):427-437), as shown in figure 3a. The libraries were constructed to contain diversity, denoted with the standard one letter code, in selected positions within and in immediate proximity to CDR-H1, CDR-H2, CDR-H3 and CDR-L3. Solvent accessible residues of CDR-H1 and CDR-H2 were restricted to tyrosine, serine and glycine, whereas a more complex diversity scheme was allowed in CDR-H3 and CDR-L3. X and J each denote a mixture of 13 amino acids introduced at proportions as described in figure 3b. The allowed lengths of CDR-H3 and CDR-L3 of HelL-11 were 8-22 and 8-12 residues, respectively. In HelL-13 the corresponding lengths were 8–17 and 8–10 residues. (Boundaries for CDRs and residue numbering are as defined by the IMGT nomenclature).

The library design and construction is further depicted in figure 4. Figure 4a shows the amino acid sequence of the HelL library scaffolds. HelL-11 and HelL-13 are synthetic scFv libraries based on the human scaffold based on heavy chain variable gene IGHV3-23 and kappa light chain variable gene IGKV1-39, connected by a 15 amino acid long Gly-Ser linker (underlined). CDR-H1, H2, H3 and L3 are marked with a box, and randomized positions are highlighted in grey. Since the Kunkel cloning procedure is not 100% efficient, stop-codons were introduced in CDR-H3 and CDR-L3 of the HelL-11 template and in the CDR-H3 of the HelL-13 template gene (indicated with *). This ensures that only clones mutagenized in these regions are displayed on phage. Stop-codons were not introduced in any of the other CDR loops and hence non-mutated versions of these loops will be found in the displayed library. The template sequence of the first and second CDRs of both the heavy and light chains were chosen to mimic that found in the germline genes. Likewise, the sequence of IGKV1-39 was chosen for CDR-L3 (position 105-115) of the HelL-13 scaffold gene. In position 116 of VL, which is naturally derived from the J-segment, a tyrosine was incorporated because of the general favorable binding properties of this residue.

In figure 4b is shown Collier de Perles defining positions that are carriers of diversity (X) in the design of VH and VL of HelL-11 and HelL-13 antibody libraries. The present illustrations demonstrate sequences with CDR3 lengths of 13 (VH) and 8 (VL) residues, but the library design allows for length variation in these hypervariable loops. Residues defined by the IMGT numbering scheme but not present in these VH and VL sequences are represented by dashed circles.

Examples 2-5 below show different prostasome detection tests. Human monoclonal synthetic scFv fragments were produced (see example 1 above) and screened against micro titer plates coated with prostasomes. 64 positive clones were sequenced and according to the DNA sequences the positive selected clones represented 60 different human monoclonals.

Example 2 Testing of human monoclonals as detectors, scFv fragments 1-12

Purified prostasomes (1 mg/mL) were diluted 1:250 in PBS (phosphate buffered saline) and 100 µL were added per well in a microtitre plate. It was incubated for 2 h at room temperature, and washed three times with PBS containing 0.05% Tween 20. 120 µL 1% bovine serum albumin (BSA) was added to the wells, which were incubated over night at room temperature. The wells were washed three times with PBS containing 0.05% Tween 20. Addition of biotinylated human synthetic scFv fragments 1:250 in row A were made and then 1:5 dilutions downwards. The plates were then incubated for 2 h at room temperature and then washed three times with PBS containing 0.05% Tween 20. 100 µL Streptavidine-HRP was added to the wells and incubated for 2 h at room temperature. The wells were washed three times with PBS containing 0.05% Tween 20. 100 µL TMB (tetramethylbenzidine) substrate was added to the wells, and incubated for 20 min and 25 µL 1 M H2SO4 was added to the plate. The plate was read at 450 nm.

Seminal plasma was collected from the Reproduction Centre at Akademiska Hospital (Uppsala, Sweden) following previously described routines (Ronquist, G.K., et al., Prostasomal DNA characterization and transfer into human sperm. Mol Reprod Dev, 2011. 78(7): p. 467-76.). The seminal plasma was thawed and centrifuged at 3,000 g for 12 min. The supernatants were collected and centrifuged at 10,000 g for 30 min. The supernatants were then transferred to new tubes and ultra-centrifuged using a 90Ti rotor (Beckman Coulter, Brea, CA, USA) at 100,000 g for 2 h. The pellets were resuspended in 0.02 M NaH2PO4, 0.15 M NaCl, pH 7.2 (PBS), overnight at 4 °C. The resuspended pellets were then loaded on a chromatography column (XK 60/70, GE Healthcare, Uppsala, Sweden)

packed with Superdex 200 gel. Fractions were collected at a flow rate of 5 mL/h. The fractions were then measured with a spectrophotometer where peaks at both 260 nm (nucleic acid) and 280 nm (proteins) corresponds to prostasomes. These fractions were collected and pooled and ultra-centrifuged at 100,000 g. The obtained pellet was resuspended in PBS. The purified prostasomes (1 mg/ml were diluted 1:250 in PBS and 100 µL were added per well in a F96 Polysorb NUNC Immunoplate (Thermo Fisher Scientific, Uppsala, Sweden). The plates were incubated for 2 h at ambient temperature and then washed three times with PBS containing 0.05% Tween 20 (P1379, Sigma-Aldrich). 120 µL 1% bovine serum albumin (BSA, Sigma-Aldrich) in PBS were added to the wells and the plate was incubated over night at ambient temperature. The plate was washed three times with PBS containing 0.05% Tween 20. 100 µL of the biotinylated monoclonal antibodies were added in dilution of 4 µg/mL in PBS to the wells. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL Streptavidine-HRP (43-8322, Thermo Fisher Scientific) diluted 1:2000 in PBS was added to the plates. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate (EC-Blue Enhanced TMB substrate, Medicago, Uppsala, Sweden) was added to the plates and the plates were incubated for 20 min at ambient temperature in darkness. 25 µL of 1 M H2SO4 was added to each well and the plate was read at 450 nm in a SpectraMax 250 ELISA reader.

The results for individual human monoclonal scFv fragments 1-12 are presented in table 4 below.

Table 4

scFv fragment no:	**Dilution**	**Absorbance**
1	4 µg/mL	0.643
2	4 µg/mL	0.394
3	4 µg/mL	0.903
4	4 µg/mL	0.653
5	4 µg/mL	0.617
6	4 µg/mL	0.556
7	4 µg/mL	1.36
8	4 µg/mL	0.653
9	4 µg/mL	1.291
10	4 µg/mL	1.643

11	4 µg/mL	0.066
12	4 µg/mL	0.954

A conclusion from the experiment is that all synthetic scFv fragments give a positive response as detector antibodies when tested against purified prostasomes.

Example 3 Testing of chicken polyclonals as detectors

Purified prostasomes (1 mg/mL) were diluted 1:250 in PBS and 100 µL were added per well in a microtitre plate. The plate was incubated for 2 h at room temperature, and washed three times with PBS containing 0.05% Tween 20. 120 µL 1% bovine serum albumin (BSA) was added to the wells, which were incubated over night at room temperature. The wells were washed three times with PBS containing 0.05% Tween 20. Addition of chicken anti-prostasome antibodies to row 1 and 2 and chicken anti-PSA antibodies to row 3 and 4 was made. Initial dilution 1:125 in row A and then 1:5 dilutions downwards were used, where the dilution was made in PBS-tween. The plates were then incubated for 1 h at room temperature and then washed three times with PBS containing 0.05% Tween 20. 100 µL anti-chicken IgY-HRP was added to the wells diluted in PBS-tween, and incubated for 1 h at room temperature. The wells were washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate was added to the wells, and incubated for 10 min and thereafter 25 µL 1 M H2SO4 was added to the plate. The plate was read at 450 nm.

Purified prostasomes (1 mg/mL) were diluted 1:250 in PBS and 100 µL were added per well in a F96 Polysorb NUNC Immunoplate (Thermo Fisher Scientific, Uppsala, Sweden). The plates were incubated for 2 h at ambient temperature and then washed three times with PBS containing 0.05% Tween 20 (P1379, Sigma-Aldrich). 120 µL 1% bovine serum albumin (BSA, Sigma-Aldrich) in PBS were added to the wells and the plate was incubated over night at ambient temperature. The plate was washed three times with PBS containing 0.05% Tween 20. 100 µL of chicken anti-prostasome and chicken anti-PSA antibodies (Immunsystem AB, Uppsala, Sweden) were added to the wells in dilution 1:125 in PBS containing 0.05% tween 20. The plate was incubated for one hour at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL chicken anti-IgY-HRP (A16130, Novex, Frederick, MD, USA) diluted 1:2000 in PBS-tween was added to the plates. The plate was incubated for one hour at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate (EC-Blue Enhanced TMB substrate, Medicago, Uppsala, Sweden) was added to

the plates and the plates were incubated for 20 min at ambient temperature in darkness. 25 µL of 1 M H2SO4 was added to each well and the plate was read at 450 nm in a SpectraMax 250 ELISA reader. The results for the antibodies are presented in table 5 below.

Table 5

Chicken Antibody	Dilution	Absorbance
anti-prostasome	125 x	3.685
anti-prostasome	125 x	3.915
anti-PSA	125 x	3.364
anti-PSA	125 x	3.217

A conclusion from the experiment is that both chicken antibodies give a positive response as detector antibodies when tested against purified prostasomes. The tested antibodies give a strong response due to polyclonal antibodies having many epitopes, which gives a strong signal. Thus, the polyclonal chicken antibodies are well suited for use as a primary detection antibody for detection prostasomes bound to a capture antibody of the inventions.

Example 4 Testing of purified prostasomes added to normal plasma

Human synthetic scFv fragment 4 (2 µg/mL) and human synthetic scFv fragment 7 (2 µg/mL) was coated in PBS. 100 µL were added per well and incubated for 2 h at room temperature, and then washed three times with PBS containing 0.05% Tween 20. 120 µL 1% bovine serum albumin (BSA) was added to the wells, which were incubated over night at room temperature. After incubation the wells were washed three times with PBS containing 0.05% Tween 20. Addition of 100 µL prostasomes 1 ng/mL diluted in plasma from female blood donor was made. 100 µL was added to row 1 and incubated for 2 h at room temperature. Addition of chicken anti-prostasome antibodies and chicken anti-PSA antibodies both in dilution 1:1000 were made, diluted in PBS-tween. The plate was incubated for 2 h at room temperature and washed three times with PBS containing 0.05% Tween 20. 100 µL anti-chicken IgY-HRP was added to the wells diluted in PBS-tween and incubated for 2 h at room temperature and then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate was added to the wells and incubated for 10 min where after 20 µL 1 M H2SO4 was added to the plate. The plate was read at 450 nm.
100 µL of human synthetic scFv fragment 4 (final concentration 2 µg/mL in PBS) and scFv fragment 7 (final concentration 2 µg/mL in PBS) were added separately per well in a F96 Polysorb NUNC Immunoplate (Thermo Fisher Scientific, Uppsala, Sweden). The plates were incubated for 2 h at ambient temperature and then washed three times with PBS containing

0.05% Tween 20 (P1379, Sigma-Aldrich). 120 µL 1% bovine serum albumin (BSA, Sigma-Aldrich) in PBS were added to the wells and the plate was incubated over night at ambient temperature. The plate was washed three times with PBS containing 0.05% Tween 20. 1 ng/mL purified prostasomes (final concentration) were diluted in female human blood donor plasma and 100 µL were added to the wells. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL of chicken anti-prostasome and chicken anti-PSA antibodies (Immunsystem AB, Uppsala, Sweden) were added separately to the wells in dilution 1:1000 in PBS containing 0.05% tween 20. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL anti-IgY-HRP (A16130, Novex, Frederick, MD, USA) diluted 1:2000 in PBS-tween was added to the plates. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate (EC-Blue Enhanced TMB substrate, Medicago, Uppsala, Sweden) was added to the plates and the plates were incubated for 20 min at ambient temperature in darkness. 25 µL of 1 M H2SO4 was added to each well and the plate was read at 450 nm in a SpectraMax 250 ELISA reader. The results for the scFv fragments are presented in table 6 below.

Table 6

Capture Antibody	**Chicken ab**	**Absorbance**
scFv fragment 4	anti-prostasome	1.212
scFv fragment 7	anti-prostasome	1.053
scFv fragment 4	anti-PSA	1.41
scFv fragment 7	anti-PSA	1.346

In the previous experiment we tested the use of the chicken detection antibodies using purified prostasomes. In the present example we tested how well the antibodies work when the prostasomes are diluted in a sample, such as serum or plasma. A conclusion from the experiment is that both chicken antibodies tested give a positive response also in the presence of human plasma indicating that they can be used to detect prostasomes also in plasma.

Example 5 Testing of patient samples, patients with elevated PSA (>50), female blood donors and male blood donors <35 years.

Human synthetic scFv fragment 4 (2 µg/mL) and human synthetic scFv fragment 7 (2 µg/mL), human synthetic scFv fragment 10 (2 µg/mL) was coated in PBS. 100 µL was added

per well and incubated for 2 h at room temperature, and then washed three times with PBS containing 0.05% Tween 20. 120 µL 1% bovine serum albumin (BSA) was added to the wells, and incubated over night at room temperature, and then washed three times with PBS containing 0.05% Tween 20. Addition of 100 µL patient samples was made, and the plates then incubated for 2 h at room temperature. Addition of chicken anti-PSA both in dilution 1:1000 was made, diluted in PBS-tween, and incubated for 2 h at room temperature and then washed three times with PBS containing 0.05% Tween 20. 100 µL anti-chicken IgY-HRP 1:2000 was added to the wells diluted in PBS-tween, and then incubated for 2 h at room temperature and then and washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate was added to the wells and incubated for 10 min where after 20 µL 1 M H2SO4 was added to the plate. The plate was read at 450 nm.

100 µL of human synthetic scFv fragment 4 (final concentration 2 µg/mL in PBS), human synthetic scFv fragment 7 (final concentration 2 µg/mL in PBS) and human synthetic scFv fragment 10 (final concentration 2 µg/mL in PBS) were added per well in a F96 Polysorb NUNC Immunoplate (Thermo Fisher Scientific, Uppsala, Sweden). The plates were incubated for 2 h at ambient temperature and then washed three times with PBS containing 0.05% Tween 20 (P1379, Sigma-Aldrich). 120 µL 1% bovine serum albumin (BSA, Sigma-Aldrich) in PBS were added to the wells and the plate was incubated over night at ambient temperature. The plate was washed three times with PBS containing 0.05% Tween 20. 100 µL of samples from patients with elevated PSA (>50 µg/L, labeled PSA in the table), female blood donors (control) and male blood donors <35 years (young male blood donors very rarely have prostate cancer and thus served as a negative control) were added to the wells. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL of chicken anti-prostasome and chicken anti-PSA antibodies (Immunsystem AB, Uppsala, Sweden) were added to the wells in dilution 1:1000 in PBS containing 0.05% tween 20. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL anti-IgY-HRP (A16130, Novex, Frederick, MD, USA) diluted 1:2000 in PBS-tween was added to the plates. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate (EC-Blue Enhanced TMB substrate, Medicago, Uppsala, Sweden) was added to the plates and the plates were incubated for 20 min at ambient temperature in darkness. 25 µL of 1 M H2SO4 was added to each well and the plate was read at 450 nm in a SpectraMax 250 ELISA reader. The results for the scFv fragments are presented in tables 7-9 below.

Table 7

Capture Antibody	Chicken ab	Sample	Absorbance
scFv fragment 4	anti-PSA	PSA	1.414
scFv fragment 4	anti-PSA	PSA	1.409
scFv fragment 4	anti-PSA	PSA	0.985
scFv fragment 4	anti-PSA	PSA	1.762
scFv fragment 4	anti-PSA	Female	0.781
scFv fragment 4	anti-PSA	Female	0.782
scFv fragment 4	anti-PSA	Female	0.800
scFv fragment 4	anti-PSA	Female	0.714
scFv fragment 4	anti-PSA	Male<35	0.678
scFv fragment 4	anti-PSA	Male<35	0.934
scFv fragment 4	anti-PSA	Male<35	0.751
scFv fragment 4	anti-PSA	Male<35	0.823

Table 8

Capture Antibody	Chicken ab	Sample	Absorbance
scFv fragment 7	anti-PSA	PSA	0.795
scFv fragment 7	anti-PSA	PSA	0.833
scFv fragment 7	anti-PSA	PSA	0.671
scFv fragment 7	anti-PSA	PSA	1.094
scFv fragment 7	anti-PSA	Female	0.527
scFv fragment 7	anti-PSA	Female	0.543
scFv fragment 7	anti-PSA	Female	0.597
scFv fragment 7	anti-PSA	Female	0.546
scFv fragment 7	anti-PSA	Male<35	0.518
scFv fragment 7	anti-PSA	Male<35	0.641
scFv fragment 7	anti-PSA	Male<35	0.623
scFv fragment 7	Anti-PSA	Male<35	0.526

Table 9

Capture Antibody	Chicken ab	Sample	Absorbance
scFv fragment 10	anti-PSA	PSA	0.502
scFv fragment 10	anti-PSA	PSA	0.508

scFv fragment 10	anti-PSA	PSA	0.36
scFv fragment 10	anti-PSA	PSA	0.783
scFv fragment 10	anti-PSA	Female	0.364
scFv fragment 10	anti-PSA	Female	0.404
scFv fragment 10	anti-PSA	Female	0.431
scFv fragment 10	anti-PSA	Female	0.416
scFv fragment 10	anti-PSA	Male<35	0.333
scFv fragment 10	anti-PSA	Male<35	0.433
scFv fragment 10	anti-PSA	Male<35	0.365
scFv fragment 10	anti-PSA	Male<35	0.377

A conclusion from the experiment is that we get higher values for PSA positive plasma samples compared to females and younger male blood donors, indicating it as a marker for prostate cancer.

Example 6 Testing of mouse monoclonals as detectors in the prostasome ELISA

Seminal plasma was collected from the Reproduction Centre at Akademiska Hospital (Uppsala, Sweden) following previously described routines (Ronquist, G.K., et al., Prostasomal DNA characterization and transfer into human sperm. Mol Reprod Dev, 2011. 78(7): p. 467-76.). The seminal plasma was thawed and centrifuged at 3,000 g for 12 min. The supernatants were collected and centrifuged at 10,000 g for 30 min. The supernatants were then transferred to new tubes and ultra-centrifuged using a 90Ti rotor (Beckman Coulter, Brea, CA, USA) at 100,000 g for 2 h. The pellets were resuspended in 0.02 M NaH2PO4, 0.15 M NaCl, pH 7.2 (PBS), overnight at 4 °C.

The resuspended pellets were then loaded on a chromatography column (XK 60/70, GE Healthcare, Uppsala, Sweden) packed with Superdex 200 gel. Fractions were collected at a flowrate of 5 mL/h. The fractions were then measured with a spectrophotometer where peaks at both 260 nm (nucleic acid) and 280 nm (proteins) corresponds to prostasomes. These fractions were collected and pooled and ultra-centrifuged at 100,000 g. The obtained pellet was resuspended in PBS.
100 µL of monoclonal antibodies at a final concentration of 1 µg/mL in PBS were added per well in duplicates in a F96 Polysorb NUNC Immunoplate (Thermo Fisher Scientific, Uppsala, Sweden). The mouse monoclonal antibodies used for coating were anti-CD10, (0112, Immunotech, Marseille, France), anti-CD46 (0846, Immunotech), anti-CD38 (Sanquin, Amsterdam, The Netherlands), anti-CD59 (Sanquin), anti-CD13, (C8589, Sigma-Aldrich, St

Louis, MI, USA) and anti-PSMA (Invitrogen, Carlsbad, CA).The plates were incubated for 2 h at ambient temperature and then washed three times with PBS containing 0.05% Tween 20 (P1379, Sigma-Aldrich). 120 µL 1% bovine serum albumin (BSA, Sigma-Aldrich) in PBS were added to the wells and the plate was incubated over night at ambient temperature. The plate was washed three times with PBS containing 0.05% Tween 20. 100 µL of the purified prostasomes, diluted to 100 ng/mL in PBS, were added to the wells. According to a previous study (Tavoosidana *et al. PNAS*, May 24, 2011, vol. 108, no. 21, 8809–8814), the sensitivity for detecting prostasomes in the blood from prostate cancer patients is around 1 ng/mL. The tested concentration is thus well above this level.

The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL of chicken anti-PSA antibodies (Immunsystem AB, Uppsala, Sweden) were added to the wells in dilution 1:1000 in PBS containing 0.05% Tween 20. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL anti-IgY-HRP (A16130, Novex, Frederick, MD, USA) diluted 1:2000 in PBS-Tween was added to the plates. The plate was incubated for two hours at ambient temperature and the wells were then washed three times with PBS containing 0.05% Tween 20. 100 µL TMB substrate (EC-Blue Enhanced TMB substrate, Medicago, Uppsala, Sweden) was added to the plates and the plates were incubated for 20 min at ambient temperature in darkness. 25 µL of 1 M H2SO4 was added to each well and the plate was read at 450 nm in a SpectraMax 250 ELISA reader. None of the tested antibodies gave a positive reaction.

Thus, the examples contained herein proves the efficacy of the proposed human monoclonal antibodies or binding fragments thereof in selectively detecting prostasomes at low concentrations in a sample, while murine counterparts are shown not to be able to detect low levels of prostasomes, thus not being suitable for use in prostate cancer diagnosis.

<u>Example 7 Testing of prognostic efficacy of fragment 4 and 7</u>

A study was performed to evaluate the potential to prognosticate severity and outcome for a patient/subject based on the measured levels of prostasomes performed according to an embodiment of the present disclosure. The efficacy of fragment 4 and 7 were evaluated.

Blood samples were collected prior to surgery from patients with prostate cancer. The samples were collected in the years 1991-2001. After centrifugation, the obtained serum samples were stored at -70C. Follow-up data on death and cause of death were extracted

from the patient files in February 2021. The endpoint used was death at the time of the follow up after exclusion of documented lack of tumor recurrence. Eighty-one samples were thawed and analyzed using ScFv fragment 4 (AK4) and ScFv fragment 7 (AK7) as capture antibodies (capture ScFv fragments) and chicken anti-prostasome antibody as detector. Fifty-one of these samples were also analyzed using AK4 and AK7 as capture antibodies (capture ScFv fragments) and chicken anti-PSA antibody as detector.

For both experiments using AK4 and AK7 in combination with the anti-prostasome antibody (Pros) as detector, measured relatively higher levels of prostasomes were correlated with death, and relatively lower levels with survival after 20 years or more. AK7 in combination with the anti-PSA antibody (PSA) as detector was significantly associated with predicted death or survival after 20 years or more. The results are presented in table 10 below.

Table 10

Fragment and Antibody used	**p-value**	**N alive**	**N dead**
scFv 4 -Pros	0.007063	42	39
scFv 4 -Pros	0.040716	42	39
scFv 4 -PSA	0.650606	27	24
scFv 7 -PSA	0.000018	27	24

Example 8 Evaluation of fragment binding targets using immunoprecipitation mass spectrometry

By using immunoprecipitation in combination with mass spectrometry (IP-MS) it is possible to verify that an antibody interacts specifically with an intended target. To determine the targets on the prostasomes to which the respective fragments 1-12 bind, the binding of the fragments to lysed prostasomes were studied using liquid chromatography (LC) -IP-MS analysis.

Purified prostasomes were lysed in high-salt buffer by a freeze-thaw and sonication procedure. Debris was removed by centrifugation and the prostasome solution was mixed with magnetic beads each coated with one of antibody fragments 1-12. After incubation for 2 h at 4 °C the beads were washed three times with buffer containing 10 mM Tris-HCl, pH 7.9, 100 mM NaCl, 0.1% (v/v) Nonidet P-40 and two times with the same buffer without detergent. The samples were eluted from the beads with 0.5 M NH4OH in water. The eluates were dried in a Speed-Vac and then reconstituted in 50 µL of 50 mM ammonium bicarbonate. The samples were subsequently reduced with 100 mM TCEP-HCl and alkylated with 500 mM iodoacetamide followed by digestion with trypsin.

Subsequently, the samples were subjected to liquid chromatography (LC) -MS analysis. The enzyme specificity was set to trypsin and the search was performed against human proteins. The top 5 identified proteins (Homo sapiens) that were present in the complexes bound by each antibody fragment (1-12) are listed in table 11 below.

Table 11

scFv fragment	Accession No.	Name
1	O75955	Flotillin-1
	P10909-2	Isoform 2 of Clusterin
	P15309-2	Isoform 2 of Prostatic acid phosphatase
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
2	P60709	Actin, cytoplasmic 1
	P02751	Fibronectin
	Q8WWA0	Intelectin-1
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
3	P15144	Aminopeptidase N
	P02751	Fibronectin
	P10909-2	Isoform 2 of Clusterin
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
4	Q8WZ42-12	Isoform 12 of Titin
	P12882	Myosin-1
	Q9UKX2	Myosin-2
	Q9Y623	Myosin-4
	P13535	Myosin-8
5	P58107	Epiplakin
	E9PPU0	Epiplakin
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
	Q5T5C7	Serine--tRNA ligase, cytoplasmic
6	P68133	Actin, alpha skeletal muscle
	P12882	Myosin-1
	Q9UKX2	Myosin-2
6	Q9Y623	Myosin-4
	P04279	Semenogelin-1
7	P02751	Fibronectin
	Q8WWA0	Intelectin-1
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
	P02768	Serum albumin
8	P02751	Fibronectin

	Q8WWA0	Intelectin-1
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
	P02768	Serum albumin
9	P60709	Actin, cytoplasmic 1
	P15924	Desmoplakin
	P10909-2	Isoform 2 of Clusterin
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
10	P02751	Fibronectin
	Q8WWA0	Intelectin-1
	P10909-2	Isoform 2 of Clusterin
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
11	P60709	Actin, cytoplasmic 1
	P08107	Heat shock 70 kDa protein 1A/1B
	P08238	Heat shock protein HSP 90-beta
	P04279	Semenogelin-1
	Q02383	Semenogelin-2
12	P60709	Actin, cytoplasmic 1
	P12314	High affinity immunoglobulin gamma Fc receptor I
	P15309-2	Isoform 2 of Prostatic acid phosphatase
	P04279	Semenogelin-1
	Q02383	Semenogelin-2

The result showed that each individual fragment 1-12 of the invention, respectively, does not bind one single target protein/lipid/carbohydrate, but binds several targets. The identified top targets in the IP-MS analysis revealed that the targets bound by the fragments were not individual targets, but complexes formed by several individual proteins/lipids/carbohydrates. The binding strength to the pure antigens, the respective individual targets in the lysed prostasome samples, were much weaker than the binding to the complexes, where the complexes were comprised of several individual targets agglomerated or bound to a prostasome membrane fragment, indicating that the scFv fragments indeed bind to a three-dimensional structure or three-dimensional conformation of target proteins on the surface of the prostasome. Thus, the antigens bound by the scFv fragments of the invention are structures on the surface of the prostasome membrane, conglomerates or complexes of antigens bound to a prostasome membrane fragment, instead of binding individual isolated antigens, thereby being ideal for measuring whole prostasomes in a sample. Hence, the proteins of table 11 should not be viewed as individual targets/antigens that the respective fragment binds, but as being part of a complex that the respective fragment binds.

ITEMIZED LIST OF EMBODIMENTS

1. A human monoclonal antibody or antigen binding fragment thereof, which selectively binds prostasomes.

2. The human monoclonal antibody or antigen binding fragment thereof according to item 1, wherein the human monoclonal antibody or antigen binding fragment thereof is a full-length antibody, an antigen binding (Fab) fragment, or an antigen binding single chain Fv (scFv) fragment.

3. The human monoclonal antibody or antigen binding fragment thereof according to items 1 or 2, wherein the human monoclonal antibody or antigen binding fragment thereof is a human synthetic scFv fragment.

4. The human monoclonal antibody or antigen binding fragment thereof according to any one of items1-3, wherein the antibody or antigen binding fragment thereof enable a sensitivity of at least 10 ng/mL in an immunoassay using the human monoclonal antibody or antigen binding fragment thereof as a capturing antibody in the immunoassay.

5. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-4, wherein the monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding one or more prostasome surface antigens, the prostasome surface antigens being selected from SEQ ID NO: 60-104.

6. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-5, wherein the monoclonal antibody or antigen binding fragment thereof comprises a heavy chain complementary determining region (CDR) being selected from SEQ ID NO: 1-12, and a light chain CDR being selected from SEQ ID NO: 13-24, and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.

7. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-6, wherein the antibody or antigen binding fragment thereof comprises at least four complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3 and CDR-L3, wherein the CDRs are selected from the group comprising:

CDR-H1 selected from SEQ ID NO: 25, 27 and 28;
CDR-H2 selected from SEQ ID NO: 26, 29 and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;

CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.

8. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-7, wherein the antibody or antigen binding fragment thereof comprises at least six complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3, wherein the CDRs are selected from the group comprising:

CDR-H1 selected from SEQ ID NO: 25, 27 and 28;

CDR-H2 selected from SEQ ID NO: 26, 29 and 30;

CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;

CDR-L1 is selected from any variant of SEQ ID NO: 56 and 57;

CDR-L2 is selected from any variant of SEQ ID NO: 58 and 59;

CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, and CDR sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 % or more, identity thereto.

9. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-8, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO: 32-43 and sequences having 80 % or more, such as 85 %, 90 %, 95 % or more, identity thereto.

10. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-8, wherein the antibody or antigen binding fragment thereof comprises a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO:44-55 and sequences having 80 % or more, such as 85 %, 90 %, 95 % or more, identity thereto.

11. The human monoclonal antibody or antigen binding fragment thereof according to items 9 and 10, wherein the antibody or antigen binding fragment thereof is a synthetical scFv fragment comprising a VH fragment according to item 9, a VL fragment according to item 10, and a linker.

12. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-11, wherein the antibody or antigen binding fragment thereof is a synthetic scFv fragment selected from the group of scFv fragment 4, scFv fragment 1, scFv fragment 2, scFv fragment 3, scFv fragment 5, scFv fragment 6, scFv fragment 7, scFv fragment 8, scFv fragment 9, scFv fragment 10¸ scFv fragment 11 and scFv fragment 12.

13. The human monoclonal antibody or antigen binding fragment thereof according to any one of items 1-12, wherein the antibody or antigen binding fragment thereof is scFv fragment 4.

14. An *in vitro* method for determining whether prostate cancer is present in a subject, the method comprising:

providing (S1) a human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes;

reacting (S2) the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject;

detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;

comparing (S4) said level of prostasomes detected with a predetermined threshold; and

determining (S5) that prostate cancer is present in the subject if the detected level of prostasomes is higher than the predetermined threshold.

15. The method according to item 14, further comprising:

determining (S5) that prostate cancer is not present in the subject if the detected level of prostasomes is lower than the predetermined threshold.

16. The method according to items 14 or 15, wherein detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof comprises detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof.

17. The method according to item 16, wherein the method is a sandwich immunoassay and detecting (S3) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody.

18. The method according to item 17, wherein the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody.

19. The method according to any one of items 14-18, wherein the sample from the subject is a body fluid sample.

20. The method according to item 19, wherein the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

21. The method according to any one of items 19 or 20, wherein the predetermined threshold is 10 ng prostasomes per mL of body fluid sample.

22. The method according to any one of items 14-21, wherein the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment thereof according to any one of items 1-13.

23. A method *in vitro* for providing a prognosis of a prostate cancer in a subject in need thereof, the method comprising:

providing (S11) a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes;

reacting (S12) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes;

detecting (S13) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;

comparing (S14) said level of prostasomes detected with a first and second predetermined threshold; and

providing (S15) a prognosis of the prostate cancer, wherein prognosis of the prostate cancer is provided (S15a) to be poor if the detected level of prostasomes

are above a first predetermined threshold, and provided (S15b) to be good if the detected level of prostasomes are below a second threshold.

24. The method according to item 23, wherein the sample from the subject is a body fluid sample

25. The method according to item 24, wherein the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

26. The method according to any one of items 24-25, wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample.

27. The method according to any one of claims 23-26, wherein the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment thereof according to any one of items 1-13.

28. A method *in vitro* for evaluating severity of a prostate cancer in a subject in need thereof, the method comprising:

providing (S21) a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes;

reacting (S22) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes;

detecting (S23) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;

comparing (S24) said level of prostasomes detected with a first and second predetermined threshold; and

evaluating (S25) the severity of the prostate cancer, wherein the prostate cancer is evaluated (S25a) to be severe if the detected levels of prostasomes are above a first threshold, evaluated (S25b) to be moderate if the detected levels of prostasomes are below a first threshold but above a second threshold, and evaluated (S25c) to be light if the detected levels of prostasomes are below a second threshold.

29. The method according to item 28, wherein the sample from the subject is a body fluid sample.

30. The method according to item 29 wherein the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

31. The method according to any one of items 29-30, wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample.

32. The method according to any one of items 28-31, wherein the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment thereof according to any one of items 1-14.

33. A method *in vitro* of evaluating the efficacy of a prostate cancer treatment in a subject in need thereof, the method comprising:

detecting (S31) a level of prostasomes in a sample from a subject before a prostate cancer treatment;

providing (S32) an anti-prostate cancer treatment to the subject;

detecting (S33) a level of prostasomes in a sample from the subject after said prostate cancer treatment;

comparing (S34) the level of prostasomes before the treatment to the levels after the treatment; and

determining (S35) the efficacy of the treatment, where the treatment is determined to be effective (S35a) if the level of prostasomes after the treatment have decreased compared to the level before the treatment, and determined as ineffective (S35b) if the level of prostasomes have remained the same or increased.

34. The method according to item 33, wherein detecting (S31, S33) a level of prostasomes in a sample from a subject comprises:

providing (S31a, S33a) a human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes;

reacting (S31b, S33b) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; and

detecting (S31c, S33c) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes.

35. The method according to any one of items 33 or 34, wherein the sample from the subject is a body fluid sample.

36. The method according to item 35, wherein the body fluid sample from the subject is selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.

37. The method according to any one of claims 34-36, wherein the human monoclonal antibody or antigen binding fragment thereof is an antibody or antigen binding fragment thereof according to any one of items 1-13.

38. The antibody or antigen binding fragment thereof according to any one of claims 1-13 for use in the methods according to any one of items 14-37.

39. A method of treating a subject having prostate cancer, the method comprising:

having provided a human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes;

having reacted the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject;

having detected prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;

having compared said level of prostasomes detected with a predetermined first threshold;

having determined that prostate cancer is present in the subject if the detected level of prostasomes is higher than the first predetermined threshold;

having determined that the level of prostasomes are lower than a second predetermined threshold;

treating the subject in need thereof by performing surgery on the prostate cancer.

All references cited herein are incorporated by reference to the extent allowed.

CLAIMS

1. A human monoclonal antibody or antigen binding fragment thereof, which selectively binds prostasomes, wherein the human monoclonal antibody or antigen binding fragment thereof is a full-length antibody, a Fab fragment, or an antigen binding single chain Fv (scFv) fragment, such as a human synthetic scFv fragment,
wherein the monoclonal antibody or antigen binding fragment thereof comprises a heavy chain complementary determining region (CDR) being selected from SEQ ID NO: 1-12, and a light chain CDR being selected from SEQ ID NO: 13-24,
wherein the antibody or antigen binding fragment thereof comprises at least six complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3, wherein the CDRs are selected from the group comprising:
CDR-H1 selected from SEQ ID NO: 25, 27 and 28;
CDR-H2 selected from SEQ ID NO: 26, 29 and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L1 is selected from SEQ ID NO: 56 and 57;
CDR-L2 is selected from SEQ ID NO: 58 and 59;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, and
wherein the monoclonal antibody or antigen binding fragment thereof selectively binds prostasomes by binding a plurality of prostasome surface antigens selected from the group consisting of SEQ ID NO: 60-104, and wherein the prostasome surface antigens form a conglomerate on the prostasome membrane, which conglomerate is identified and bound by the monoclonal antibody or antigen binding fragment thereof.

2. The human monoclonal antibody or antigen binding fragment thereof according to claim 1, wherein the conglomerate comprise at least five antigens selected from table 11, and
wherein the antibody or antigen binding fragment thereof enable a sensitivity of at least 10 ng/mL in an immunoassay using the human monoclonal antibody or antigen binding fragment thereof as a capturing antibody in the immunoassay.

3. The human monoclonal antibody or antigen binding fragment thereof according to claim 1 or 2, wherein the antibody or antigen binding fragment thereof is an antigen binding single chain Fv (scFv) fragment, such as a human synthetic scFv fragment, and wherein the scFv fragment comprises at least four complementary determining regions (CDRs) in any combination of CDR-H1, CDR-H2, CDR-H3 and CDR-L3, wherein the CDRs are selected from the group comprising:
CDR-H1 selected from SEQ ID NO: 25, 27 and 28;
CDR-H2 selected from SEQ ID NO: 26, 29 and 30;
CDR-H3 selected from SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12;
CDR-L3 selected from SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24.

4. The human monoclonal antibody or antigen binding fragment thereof according to claims 1 or 3, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO: 32-43 and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto, and a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO: 44-55 and sequences having 70 % or more, such as 75 %, 80 %, 85 %, 90 %, 95 % or more, identity thereto.

5. The human monoclonal antibody or antigen binding fragment thereof according to any one of claims 1-4, wherein the antibody or antigen binding fragment thereof is a synthetic scFv fragment selected from the group comprising:
i) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:4 and
CDR-L3 as defined by SEQ ID NO:16,
ii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:7 and
CDR-L3 as defined by SEQ ID NO:19,
iii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:1 and
CDR-L3 as defined by SEQ ID NO:13,

iv) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:2 and
CDR-L3 as defined by SEQ ID NO:14,

v) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:3 and
CDR-L3 as defined by SEQ ID NO:15,

vi) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:5 and
CDR-L3 as defined by SEQ ID NO:17,

vii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:6 and
CDR-L3 as defined by SEQ ID NO:18,

viii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:8 and
CDR-L3 as defined by SEQ ID NO:20,

ix) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:9 and
CDR-L3 as defined by SEQ ID NO:21,

x) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:10 and
CDR-L3 as defined by SEQ ID NO:22,

xi) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25

CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:11 and
CDR-L3 as defined by SEQ ID NO:23,

xii) an scFv fragment having
CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:12 and
CDR-L3 as defined by SEQ ID NO:24.

6. The human monoclonal antibody or antigen binding fragment thereof according to claim 5, wherein the antibody or antigen binding fragment thereof is a synthetic scFv fragment having a variable heavy chain, VH, and a variable light chain, VL, connected via a linker, wherein the fragments are selected from the group comprising:

i) an scFv fragment having
a VH as defined by SEQ ID NO:35 and
a VL as defined by SEQ ID NO:47,
ii) an scFv fragment having
a VH as defined by SEQ ID NO:38 and
a VL as defined by SEQ ID NO:50,
iii) an scFv fragment having
a VH as defined by SEQ ID NO:32 and
a VL as defined by SEQ ID NO:44,
iv) an scFv fragment having
a VH as defined by SEQ ID NO:33 and
a VL as defined by SEQ ID NO:45,
v) an scFv fragment having
a VH as defined by SEQ ID NO:34 and
a VL as defined by SEQ ID NO:46,
vi) an scFv fragment having
a VH as defined by SEQ ID NO:36 and
a VL as defined by SEQ ID NO:48,
vii) an scFv fragment having
a VH as defined by SEQ ID NO:37 and
a VL as defined by SEQ ID NO:49,
viii) an scFv fragment having
a VH as defined by SEQ ID NO:39 and
a VL as defined by SEQ ID NO:51,

ix) an scFv fragment having
a VH as defined by SEQ ID NO:40 and
a VL as defined by SEQ ID NO:52,
x) an scFv fragment having
a VH as defined by SEQ ID NO:41 and
a VL as defined by SEQ ID NO:53,
xi) an scFv fragment having
a VH as defined by SEQ ID NO:42 and
a VL as defined by SEQ ID NO:54,
xii) an scFv fragment having
a VH as defined by SEQ ID NO:43 and
a VL as defined by SEQ ID NO:55.

7. The human monoclonal antibody or antigen binding fragment thereof according to any one of claims 3-6, wherein the antibody or antigen binding fragment thereof is a synthetic scFv fragment having

CDR-H1 as defined by SEQ ID NO: 25
CDR-H2 as defined by SEQ ID NO: 26
CDR-H3 as defined by SEQ ID NO:4 and
CDR-L3 as defined by SEQ ID NO:16,

and wherein the scFv fragment bind a conglomerate comprising the proteins Isoform 12 of Titin, Myosin-1, Myosin-2, Myosin-4, and Myosin-8.

8. An *in vitro* method for determining whether prostate cancer is present in a subject, the method comprising:
providing (S1) the human monoclonal antibody or antigen binding fragment thereof which selectively binds human prostasomes according to any one of claims 1-7;
reacting (S2) the human monoclonal antibody or antigen binding fragment thereof with a sample comprising prostasomes from a subject;
detecting (S3) any prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes by detecting the prostasomes using an anti-prostasome detection antibody or antigen binding fragment thereof;
comparing (S4) said level of prostasomes detected with a predetermined threshold; and
determining (S5A) that prostate cancer is present in the subject if the detected level of prostasomes is higher than the predetermined threshold;
determining (S5B) that prostate cancer is not clinically detectable in the subject if the detected level of prostasomes is lower than the predetermined threshold,

wherein the sample from the subject is a body fluid sample, selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension, and

wherein the predetermined threshold is 10 ng prostasomes per mL of body fluid sample.

9. The method according to claim 8, wherein the method is a sandwich immunoassay and detecting (S3) prostasomes (200) bound by the human monoclonal antibody or antigen binding fragment thereof (101) comprises detecting the anti-prostasome detection antibody (102) using a further detection antibody (103), wherein the human monoclonal antibody or binding fragment thereof (101) is a capture antibody, the anti-prostasome detection antibody (102) is a primary detection antibody and the further detection antibody (103) is a secondary detection antibody, and wherein the primary detection antibody (102) is a chicken antibody and the secondary detection antibody (103) is an anti-chicken antibody.

10. A method *in vitro* for providing a prognosis of a prostate cancer in a subject in need thereof, the method comprising:
providing (S11) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes according to any one of claims 1-7;
reacting (S12) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes;
detecting (S13) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;
comparing (S14) said level of prostasomes detected with a first and second predetermined threshold; and
providing (S15) a prognosis of the prostate cancer, wherein prognosis of the prostate cancer is provided (S15a) to be poor if the detected level of prostasomes are above a first predetermined threshold, and provided (S15b) to be good if the detected level of prostasomes are below a second threshold, wherein the sample from the subject is a body fluid sample and wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample.

11. A method *in vitro* for evaluating severity of a prostate cancer in a subject in need thereof, the method comprising:

providing (S21) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes according to any one of claims 1-7;

reacting (S22) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes;
detecting (S23) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes;
comparing (S24) said level of prostasomes detected with a first and second predetermined threshold; and
evaluating (S25) the severity of the prostate cancer, wherein the prostate cancer is evaluated (S25a) to be severe if the detected levels of prostasomes are above a first threshold, evaluated (S25b) to be moderate if the detected levels of prostasomes are below a first threshold but above a second threshold, and evaluated (S25c) to be light if the detected levels of prostasomes are below a second threshold, wherein the sample from the subject is a body fluid sample and wherein the first predetermined threshold is 10 ng prostasomes per mL of body fluid sample and the second predetermined threshold is 1 ng prostasomes per mL of body fluid sample.

12. A method *in vitro* of evaluating the efficacy of a prostate cancer treatment in a subject in need thereof, the method comprising:

detecting (S31) a level of prostasomes in a sample from a subject before a prostate cancer treatment;
providing (S32) an anti-prostate cancer treatment to the subject;
detecting (S33) a level of prostasomes in a sample from the subject after said prostate cancer treatment;
comparing (S34) the level of prostasomes before the treatment to the levels after the treatment; and
determining (S35) the efficacy of the treatment, where the treatment is determined (S35a) to be effective if the level of prostasomes after the treatment have decreased compared to the level before the treatment, and determined (S35b) to be ineffective if the level of prostasomes have remained the same or increased, wherein detecting (S31, S33) a level of prostasomes in a sample from a subject comprises:
providing (S31a, S33a) the human monoclonal antibody or antigen binding fragment thereof which selectively binds prostasomes according to any one of claims 1-7;
reacting (S31b, S33b) the human monoclonal antibody or antigen binding fragment thereof with a sample from a subject comprising prostasomes; and

detecting (S31c, S33c) prostasomes bound by the human monoclonal antibody or antigen binding fragment thereof to obtain a level of prostasomes, wherein the sample from the subject is a body fluid sample, selected from the group consisting of blood, serum, plasma, urine, cerebrospinal fluid and a cell suspension.