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(54) Benævnelse: **PHOSPHODIESTERASE-4D7 SOM MARKØR FOR PROSTATACANCER**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to phosphodiesterase 4D7 (PDE4D7) for use as a marker for prostate cancer, and the use of PDE4D7 as a marker for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer. The present invention also relates to a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer, a corresponding method and immunoassay, a method for diagnosing, monitoring or prognosticating hormone-resistant prostate cancer vs. hormone-sensitive prostate cancer, a corresponding immunoassay, a method of data acquisition, an immunoassay for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer, a method of identifying an individual for eligibility for prostate cancer therapy, an immunoassay for stratifying an individual or cohort of individuals with a prostate cancer disease, and an immunoassay for stratifying an individual with prostate cancer.

BACKGROUND OF THE INVENTION

[0002] Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Among men, the three most commonly diagnosed cancers are prostate, lung and colorectal cancer in developed countries. Particularly prostate cancer is the most common malignancy in European males. In 2002 in Europe, an estimated 225,000 men were newly diagnosed with prostate cancer and about 83,000 died from this disease.

[0003] Certain phosphodiesterases have been associated with cancer development. For instance, phosphodiesterase PDE7 has been shown to be linked to chronic lymphocytic leukemia (Zhang L et al., PNAS, 2008, 105(49): 19532-7). Yet, for many cancer types or cancer progression forms there is no adequate marker molecule available.

[0004] Prostate cancer, for example, is traditionally diagnosed via the serum level of prostate-specific antigen (PSA). However, PSA is not prostate cancer-specific and can be raised in other circumstances, leading to a large number of false-positives (cancer is not found in around 70% of men with raised PSA levels who undergo biopsy). Furthermore, there will be an unpredictable number of false-negatives who later develop prostate cancer in the presence of a "normal" PSA test.

[0005] Therefore, there is a need for the provision of a new and effective, alternative diagnosis perspective for the detection, monitoring and prognostication of prostate cancer.

[0006] International publication WO 2003044170 A2 discloses phosphodiesterase PDE4D7 isoforms from human, mouse and rat. The full length cloning of the human PDE4D7 occurred by polymerase chain reaction using human hippocampus cDNA. The homologs from rat and mice were also cloned from hippocampus mRNA or brain cDNA respectively. It is disclosed that specific isoforms exhibit tissue specificity and that the PDE4D7 protein is highly expressed in kidney, testis and brain tissue, but is not highly expressed in heart, lung, liver, spleen, thymus or pancreas. Whether PDE4D7 is expressed in prostate is not disclosed.

[0007] Phosphodiesterase PDE4D is described as proliferation promoting factor in prostate cancer (Rahrmann EP et al., Cancer Res., 2009, 69(10): 4388-97). It is disclosed that expression profiling studies generally observed no change for PDE4D mRNA expression or a decrease in expression with increasing pathologic grade of prostate cancer. However, the predominant RLM-5'-RACE product obtained from pooled prostatic adenocarcinoma RNA identified a cDNA with a first exon that began at base pair 349 of the Genebank deposited sequence for the PDE4D5 isoform. Hence, in the experimental approach allowing amplification of all known PDE4D isoforms, the PDE4D5 was identified as the predominantly expressed PDE4D isoform in prostate cancer suggesting that it is the PDE4D5 isoform that may be associated with prostate cancer.

SUMMARY OF THE INVENTION

[0008] The present invention addresses this need and provides means and methods which allow the diagnosis and detection of prostate cancer.

[0009] The above objective is accomplished by phosphodiesterase 4D7 (PDE4D7) for use as a prostate cancer marker.

[0010] Phosphodiesterase 4D7 is shown by the present inventors to be down-regulated in prostate cancer cell lines and patient derived prostate tissue. PDE4D7 is, thus, considered as a biomarker for prostate cancer prediction and a decision tool for the stratification of certain cancer surveillance regimes, as well as the prognosis and monitoring of prostate cancer progression. In particular, it was demonstrated by the present inventors that PDE4D7 is down-regulated in hormone-resistant human-derived prostate cell lines as well as corresponding human tissue samples. Diagnostic methods and uses based on PDE4D7 as a prostate cancer marker can, thus, advantageously be employed for (i) detecting and diagnosing life-threatening prostate cancer forms, (ii) prognosticating life-threatening prostate cancer forms, (iii) monitoring of cancer progression towards life-threatening prostate cancer forms, and (iv) distinguishing between indolent and life-threatening cancer forms.

[0011] In another aspect the present invention relates to a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or for diagnosing, detecting, monitoring or prognosticating the progression of prostate cancer, comprising a nucleic acid

affinity ligand and/or a peptide affinity ligand for the PDE4D7 expression product or protein.

[0012] In a preferred embodiment of the present invention said composition comprises a nucleic acid affinity ligand or peptide affinity ligand which is modified to function as a contrast agent.

[0013] In a further preferred embodiment of the present invention said composition comprises a set of oligonucleotides specific for the PDE4D7 expression product, a probe specific for the PDE4D7 expression product, an aptamer specific for the PDE4D7 expression product or protein, an antibody specific for the PDE4D7 protein and/or an antibody variant specific for the PDE4D7 protein.

[0014] In a further aspect the present invention relates to the use of PDE4D7 as a marker for diagnosing, detecting, monitoring or prognosticating prostate cancer or for diagnosing, detecting, monitoring or prognosticating the progression of prostate cancer. In another aspect the present invention relates to a method for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer comprising the step of determining the level of PDE4D7.

[0015] In another aspect the present invention relates to a method for diagnosing, monitoring or prognosticating hormone-resistant prostate cancer or for diagnosing, detecting, monitoring or prognosticating the progression towards hormone-resistant prostate cancer, wherein said method discriminates between a hormone-sensitive and a hormone-resistant prostate cancer, comprising the steps of

1. (a) determining the level of PDE4D7 in a sample;
2. (b) determining the level of expression of a reference gene in a sample;
3. (c) normalizing the measured expression level of PDE4D7 to the expression of the reference gene; and

comparing the normalized expression level with a predetermined cutoff value chosen to exclude hormone-sensitive prostate cancer, wherein a normalized expression level below the cutoff value is indicative of a hormone-resistant prostate cancer, wherein said cutoff value is between about 1 and 7, preferably about 5.

[0016] In another aspect the present invention relates to a method of data acquisition for diagnosing, detecting, monitoring or prognosticating prostate cancer or for diagnosing, detecting, monitoring or prognosticating the progression of prostate cancer comprising at least the steps of:

1. (a) testing in an individual for expression of PDE4D7; and
2. (b) comparing the expression as determined in step (a) to a control level.

[0017] In a further preferred embodiment of the present invention the diagnosing, detecting, monitoring prognosticating or data acquisition is to be carried out on a sample obtained from an individual.

[0018] In another aspect the present invention relates to an immunoassay for detecting, diagnosing, monitoring or prognosticating prostate cancer or for diagnosing, detecting, monitoring or prognosticating the progression of prostate cancer comprising at least the steps of:

1. (a) testing in a sample for the expression of PDE4D7,
2. (b) testing in a control sample for the expression of PDE4D7,
3. (c) determining the difference in expression of PDE4D7 of steps (a) and (b); and
4. (d) deciding on the presence or stage of prostate cancer or the progression of prostate cancer based on the results obtained in step (c),

wherein said testing steps are based on the use of an antibody specifically binding to PDE4D7.

[0019] In another aspect the present invention relates to an immunoassay for discriminating between a hormone-sensitive and a hormone-resistant prostate cancer, comprising the steps of:

1. (a) determining the level of PDE4D7 in a sample;
2. (b) determining the level of expression of a reference gene in a sample;
3. (c) normalizing the measured expression level of PDE4D7 to the expression of the reference gene; and
4. (d) comparing the normalized expression level with a predetermined cutoff value to exclude hormone-sensitive prostate cancer, wherein a normalized expression level below the cutoff value is indicative of a hormone-resistant prostate cancer, wherein said cutoff value is between about 1 and 7, preferably about 5.

[0020] In another aspect the present invention relates to a method of identifying an individual for eligibility for prostate cancer therapy comprising:

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7;
2. (b) testing in said sample for the expression of a reference gene and/or testing in a control sample for the expression of PDE4D7;
3. (c) classifying the levels of expression of step (a) relative to levels in control samples of PDE4D7 of step (b); and
4. (d) identifying the individual as eligible to receive a prostate cancer therapy where the individual's sample is classified as having a reduced level of PDE4D7 expression.

[0021] In yet another aspect the present invention relates to an immunoassay for stratifying an

individual or cohort of individuals with a prostate cancer disease comprising:

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7;
2. (b) testing in said sample for the expression of a reference gene and/or testing in a control sample for the expression of PDE4D7;
3. (c) determining the difference in expression of PDE4D7 of step (a) and the expression of PDE4D7 and/or the reference gene in step (b); and
4. (d) stratifying an individual or cohort of individuals to prostate cancer therapy based on the results obtained in step (c), where the individual's sample has a reduced level of PDE4D7 expression.

[0022] In a further preferred embodiment of the present invention said testing or determining of the expression is accomplished, or additionally accomplished, by the measurement of nucleic acid or protein levels or by the determination of the biological activity of PDE4D7, or of the reference gene.

[0023] In a further preferred embodiment of the present invention said method or immunoassay comprises the additional step of comparing the measured nucleic acid or protein levels or the measured biological activity to a control level.

[0024] In a further preferred embodiment of the present invention said reference gene is a housekeeping gene, particularly preferred GAPDH or PBGD, or a different phosphodiesterase, particularly preferred PDE4D5.

[0025] In a further preferred embodiment of the present invention said method or immunoassay comprises the additional step of determining the level of prostate specific antigen (PSA).

[0026] In a further preferred embodiment of the present invention the sample as mentioned above is a tissue sample, a urine sample, a urine sediment sample, a blood sample, a saliva sample, a semen sample, a sample comprising circulating tumor cells or a sample containing prostate secreted exosomes.

[0027] In yet another preferred embodiment of the present invention said prostate cancer is hormone-resistant prostate cancer.

[0028] These and other characteristics, features and objectives of the present invention will become apparent from the following detailed description, taken in conjunction with the accompanying figures and examples, which demonstrate by way of illustration the principles of the invention.

[0029] The description is given for the sake of example only, without limiting the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS**[0030]****Fig. 1**

gives an overview over the samples tested on expression levels. AD means "androgen dependent", AS stands for "androgen sensitive" and AI means "androgen independent". Samples "PC346P xenograft" through "346Flu2" are cell lines, samples "PC295" through "PC374" are xenografts.

Fig. 2

depicts the relative PDE4D7 mRNA expression in several prostate cancer cell lines and xenografts normalized to GAPDH compared to LNCaP. The figure provides an overview over all investigated samples, including cell lines and xenograft material.

Fig. 3

depicts the relative PDE4D7 mRNA expression in prostate cancer cell lines normalized to GAPDH compared to LNCaP.

Fig. 4

depicts the relative PDE4D7 mRNA expression in prostate cancer xenografts normalized to GAPDH compared to LNCaP.

Fig. 5

shows the PDE4D7 content expressed as a percentage of total PDE4D mRNA in prostate cancer cell lines and xenografts. The figure provides an overview over all investigated samples, including cell lines and xenograft material.

Fig. 6

shows the PDE4D7 content expressed as a percentage of total PDE4D mRNA in prostate cancer cell lines.

Fig. 7

shows the PDE4D7 content expressed as percentage of total PDE4D mRNA in prostate cancer xenografts.

Fig. 8

shows the relative gene expression of human PDE4D7 in human patient tissue samples. Information is derived from 16 different samples in total, as depicted in Table 1 (including the lymph node resected tissues). Sample group "1=no" is defined as hormone-responsive primary prostate tumors, Sample group "2=yes" is defined as hormone-resistant prostate tumors. Indicated are the individual relative expression values for human PDE4D7 on human prostate tissues. The results were normalized to the expression of GAPDH and PBGD. The median of the data relative data measurements is indicated for each patient group.

Fig. 9

shows the relative gene expression of human PDE4D7 in human patient tissue samples. Information is derived from 16 different samples in total, as depicted in Table 1 (including the lymph node resected tissues). Sample group "1" is defined as hormone-responsive

primary prostate tumors, Sample group "2" is defined as hormone-resistant prostate tumors. The results were normalized to the expression of GAPDH and PBGD. The figure shows a box plot of the individual data relative expression measurements for human PDE4D7, whereby the box includes 75% of all measurements. The median relative expression value is indicated as the border between the two colored boxes.

Fig. 10

shows the relative gene expression of human PDE4D7 in human patient tissue samples. Information is derived from 12 different samples in total, as depicted in Table 1 (excluding the lymph node resected tissues). Sample group "1=no" is defined as hormone-responsive primary prostate tumors, Sample group "2=yes" is defined as hormone-resistant prostate tumors. The results were normalized to the expression of GAPDH and PBGD. Indicated are the individual relative expression values for human PDE4D7 on human prostate tissues. The median of the data relative data measurements is given for each patient group.

Fig. 11

shows the relative gene expression of human PDE4D7 in human patient tissue samples. Information is derived from 12 different samples in total depicted in Table 1 (excluding the lymph node resected tissues). Sample group "1" is defined as hormone-responsive primary prostate tumors, Sample group "2" is defined as hormone-resistant prostate tumors. The results were normalized to the expression of GAPDH and PBGD. The figure shows a box plot of the individual data relative expression measurements for human PDE4D7, whereby the box includes 75% of all measurements. The median relative expression value is indicated as the border between the two colored boxes.

Fig. 12

shows the effect of human PDE4D7 in vivo expression on the proliferation of PC3 prostate cancer cells.

DETAILED DESCRIPTION OF EMBODIMENTS

[0031] The inventors have found that PDE4D7 is strongly down-regulated in certain prostate cancer-associated cell types and human patient tissues and can, hence, be used as biomarker for prostate cancer. PDE4D7 as well as agents modifying PDE4D7 or modifying PDE4D7 expression can further be used as medicaments, in particular for the treatment of prostate cancer.

[0032] Although the present invention will be described with respect to particular embodiments, this description is not to be construed in a limiting sense.

[0033] Before describing in detail exemplary embodiments of the present invention, definitions important for understanding the present invention are given.

[0034] As used in this specification and in the appended claims, the singular forms of "a" and "an" also include the respective plurals unless the context clearly dictates otherwise.

[0035] In the context of the present invention, the terms "about" and "approximately" denote an interval of accuracy that a person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates a deviation from the indicated numerical value of $\pm 20\%$, preferably $\pm 15\%$, more preferably $\pm 10\%$, and even more preferably $\pm 5\%$.

[0036] It is to be understood that the term "comprising" is not limiting. For the purposes of the present invention the term "consisting of" is considered to be a preferred embodiment of the term "comprising of". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is meant to also encompass a group which preferably consists of these embodiments only.

[0037] Furthermore, the terms "first", "second", "third" or "(a)", "(b)", "(c)", "(d)" etc. and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

[0038] In case the terms "first", "second", "third" or "(a)", "(b)", "(c)", "(d)" etc. relate to steps of a method or use there is no time or time interval coherence between the steps, i.e. the steps may be carried out simultaneously or there may be time intervals of seconds, minutes, hours, days, weeks, months or even years between such steps, unless otherwise indicated in the application as set forth herein above or below.

[0039] It is to be understood that this invention is not limited to the particular methodology, protocols, proteins, bacteria, vectors, reagents etc. described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention that will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0040] As has been set out above, the present invention concerns in one aspect phosphodiesterase 4D7 (PDE4D7) for use as a prostate cancer marker. The term "phosphodiesterase 4D7" or "PDE4D7" relates to the splice variant 7 of the human phosphodiesterase PDE4D, i.e. the human phosphodiesterase PDE4D7 gene, preferably to the sequence as defined in Genbank Accession No: AF536976 (version AF536976.1, GI:22901883 as of 3 March 2009), more preferably to the nucleotide sequence as set forth in SEQ ID NO: 1, which corresponds to the sequence of the above indicated Genbank Accession number of the PDE4D7 transcript, and also relates to the corresponding amino acid sequence as set forth in

SEQ ID NO: 2, which corresponds to the sequence of the above indicated Genbank Accession number of the PDE4D7 polypeptide encoded by the PDE4D7 transcript. The term also comprises nucleotide sequences showing a high degree of homology to PDE4D7, e.g. nucleic acid sequences being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1, or amino acid sequences being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2, or nucleic acid sequences encoding amino acid sequences being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2, or amino acid sequences being encoded by nucleic acid sequences being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1.

[0041] The term "human phosphodiesterase PDE4D7 gene", "PDE4D7 gene" or "PDE4D7 marker gene" as used herein relates to the gene encoding phosphodiesterase 4D. Preferably, the term relates to a gene expressing phosphodiesterase 4D as splice variant 7, e.g. the specific exon combination as defined in Genbank Accession No: AF536976 (version AF536976.1, GI:22901883 as of 3 March 2009) or as set forth in SEQ ID NO: 1. The term also relates to DNA molecules derived from mRNA transcripts encoding phosphodiesterase 4D spliced as variant 7, preferably cDNA molecules.

[0042] The term "marker" or "PDE4D7 marker", as used herein, relates to a gene, genetic unit or sequence (a nucleotide sequence or amino acid or protein sequence) as defined herein above, whose expression level is modified, preferably decreased, in a cancerous cell or in cancerous tissue or in any type of sample comprising cancerous cells or cancerous tissues or portions or fragments thereof, in comparison to a control level or state. The term also refers to any expression product of said genetic unit or sequence, in particular to a PDE4D7 mRNA transcript, a polypeptide encoded by the PDE4D7 transcript or variants or fragments thereof, as well as homologous derivatives thereof as described herein above. The term "expression level" as used herein refers to the amount of PDE4D7 transcript and/or PDE4D7 protein derivable from a defined number of cells or a defined tissue portion, preferably to the amount of PDE4D7 transcript and/or PDE4D7 protein obtainable in a standard nucleic acid (e.g. RNA) or protein extraction procedure. Suitable extraction methods are known to the person skilled in the art.

[0043] The term "control level" (or "control state"), as used herein, relates to an expression level which may be determined at the same time and/or under similar or comparable conditions as the test sample by using (a) sample(s) previously collected and stored from a subject/subjects whose disease state, e.g. non-cancerous, is/are known. The term "disease state" or "cancerous disease state" relates to any state or type of cellular or molecular condition between a non-cancerous cell state and (including) a terminal cancerous cell state. Preferably, the term includes different cancerous proliferation/developmental stages or levels of tumor development in the organism between (and excluding) a non-cancerous cell state and (including) a terminal cancerous cell state. Such developmental stages may include all stages

of the TNM (Tumor, Node, Metastasis) classification system of malignant tumors as defined by the UICC, e.g. stages 0 and I to IV. The term also includes stages before TNM stage 0, e.g. developmental stages in which cancer biomarkers known to the person skilled in the art show a modified expression or expression pattern.

[0044] The expression level as mentioned above may preferably be the expression level of PDE4D7 as defined herein above. Alternatively or additionally, the expression level may also be the expression level of any other suitable gene or genetic element expressed in a cell, preferably in the context of PDE4D7, e.g. the expression level of another phosphodiesterase, the expression level of a housekeeping gene, e.g. GAPDH or PBGD.

[0045] The term "cancerous" relates in the context of the present invention to a cancerous disease state as defined herein above. A preferred control level in the context of cancerous controls is the expression of PDE4D7 in malignant, hormone-sensitive tumors.

[0046] The term "non-cancerous" relates in the context of the present invention to a condition in which neither benign nor malign proliferation can be detected. Suitable means for said detection are known in the art. A preferred control level in the context of non-cancerous controls is the expression of PDE4D7 in normal, i.e. healthy or non-cancerous tissue or the expression of PDE4D7 in benign prostate tumor tissue. The term "benign prostate tumor" as used herein refers to a prostate tumor which lacks all three of the malignant properties of a cancer, i.e. does not grow in an unlimited, aggressive manner, does not invade surrounding tissues, and does not metastasize. Typically, a benign prostate tumor implies a mild and non-progressive prostate neoplastic or swelling disease lacking the invasive properties of a cancer. Furthermore, benign prostate tumors are typically encapsulated, and thus inhibited in their ability to behave in a malignant manner. A benign tumor or a healthy condition may be determined by any suitable, independent molecular, histological or physiological method known to the person skilled in the art.

[0047] Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of the PDE4D7 marker gene of the present invention in samples from subjects whose disease state is known. Furthermore, the control level can be derived from a database of expression patterns from previously tested subjects or cells. Moreover, the expression level of the marker genes of the present invention in a biological sample to be tested may be compared to multiple control levels, whose control levels are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the patient-derived biological sample. It is particularly preferred to use sample(s) derived from a subject/subjects whose disease state is non-cancerous as defined herein above, i.e. which present a health condition in which neither benign nor malign proliferation can be detected. In another embodiment of the present invention, the control level can be determined from a reference sample derived from a subject who has been diagnosed to suffer from prostate cancer, e.g. from hormone-independent or hormone-resistant prostate cancer.

[0048] Alternatively, reference samples may comprise material derived from cell lines, e.g. immortalized cancer cell lines, or be derived from tissue xenografts. Preferably, material derived from prostate cancer cell lines or material derived from tissue xenografts with human prostate tissue, in particular with benign and tumor-derived human prostate tissue, may be comprised in a reference sample according to the present invention. Examples of preferred cancer cell lines comprise cells lines PC346P, PC346B, LNCaP, VCaP, DuCaP, PC346C, PC3, DU145, PC346CDD, PC346Flu1, PC346Flu2. Examples of preferred xenografts comprise PC295, PC310, PC-EW, PC82, PC133, PC135, PC324 and PC374. Preferably an entire panel of cell lines and xenografts may be used, e.g. the human PC346 panel. Further preferred are cell lines and xenografts as described in Marques et al., 2006, Eur. Urol., 49(2):245-57.

[0049] In a further, preferred alternative, reference samples may be derived from patient tissues, or tissue panels or tissue collections obtained in clinical environments. The samples may, for example, be obtained from male patients undergoing surgery. The samples may be derived from any suitable tissue type, e.g. from prostate tissue or lymph nodes. Preferred examples of patient tissue collections are from surgical prostate procedures (e.g., prostatectomy).

[0050] Moreover, it is preferred to use the standard value of the expression levels of the PDE4D7 marker of the present invention in a population with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean \pm 2 SD (standard deviation) or mean \pm 3 SD may be used as standard value.

[0051] Furthermore, the control level may also be determined at the same time and/or under similar or comparable conditions as the test sample by using (a) sample(s) previously collected and stored from a subject/subjects whose disease state is/are known to be cancerous, i.e. who have independently been diagnosed to suffer from a certain cancer type, e.g. from prostate cancer, in particular hormone-dependent, hormone-sensitive or hormone-resistant prostate cancer.

[0052] In the context of the present invention, a control level determined from a biological sample that is known not to be cancerous is called "normal control level". If the control level is determined from a cancerous biological sample, e.g. a sample from a subject for which prostate cancer, in particular hormone-dependent, hormone-sensitive or hormone-resistant cancer was diagnosed independently, it may be designated as "cancerous control level".

[0053] The term "prostate cancer" relates to a cancer of the prostate gland in the male reproductive system, which occurs when cells of the prostate mutate and begin to multiply out of control. Typically, prostate cancer is linked to an elevated level of prostate-specific antigen (PSA). In one embodiment of the present invention the term "prostate cancer" relates to a cancer showing PSA levels above 4.0. In another embodiment the term relates to cancer showing PSA levels above 2.0. The term "PSA level" refers to the concentration of prostate specific antigen (PSA) in the blood in ng/ml.

[0054] The term "hormone-dependent prostate cancer" means that the growth and/or proliferation of prostate cancer or prostate cancer cell lines is dependent on male sex hormone stimulation.

[0055] The term "hormone-sensitive prostate cancer" means that the growth and proliferation of prostate cancer or prostate cancer cell lines is sensitive on male sex hormone stimulation. The term "sensitive" relates to situations in which the prostate cancer or prostate cancer cell line shows a biochemical or cellular reaction pattern in the presence of male sex hormones, but does need a male sex hormone for growth and/or proliferation.

[0056] The term "hormone-resistant prostate cancer" means that the growth and proliferation of prostate cancer or prostate cancer cell lines is resistant to male sex hormone stimulation. The term also relates to a late prostate cancer developmental stage which is no longer amenable to an administration of anti-hormones, preferably anti-androgens as defined herein above. The term "male sex hormone" as used herein refers to an androgen, preferably to testosterone, androstenedione, dihydrotestosterone, dehydroepiandrosterone, androstanediol or androsterone.

[0057] In a further aspect the present invention relates to the use of PDE4D7 as a marker for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer.

[0058] The term "diagnosing prostate cancer" as used herein means that a subject or individual may be considered to be suffering from prostate cancer, when the expression level of the PDE4D7 marker of the present invention is modified, preferably reduced or down-regulated, compared to a control level as defined herein above, preferably if compared to the normal control level as defined herein above. The term "diagnosing" also refers to the conclusion reached through that comparison process.

[0059] The term "modified" or "modified expression level" in the context of the present invention thus denotes a change in the expression level. Expression levels are deemed to be "changed" when the PDE4D7 gene expression, e.g. in a sample to be analyzed, differs by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than 50% from a control level, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more in comparison to a control level. The control level may either be a normal control level or a cancerous control level as defined herein above. If a comparison with a cancerous control level is to be carried out, an additional comparison with a normal control level is preferred. Such an additional comparison allows for the determination of a tendency of the modification, i.e. an increase or a decrease of the expression level is observed.

[0060] The term "modified" relates preferably to a decrease or down-regulation of the expression level of the PDE4D7 marker or a complete inhibition of the PDE4D7 marker expression if a test sample is compared to a control level. The control level may either be a normal control level or a cancerous control level as defined herein above. In a preferred

embodiment of the present invention the control level is a cancerous control level derived from, or associated with hormone-dependent prostate tumors or tissues, more preferably derived from or associated with hormone-sensitive prostate tumors or tissues. The term "reduced expression level" or "down-regulated expression level" or "decrease of expression level" (which may be used synonymously) in the context of the present invention thus denotes a reduction of the expression level of PDE4D7 between a situation to be analyzed, e.g. a situation derivable from a patient's sample, and a reference point, which could either be a normal control level or cancerous control level derivable from any suitable cancer stage known to the person skilled in the art, preferably a hormone-dependent, more preferably a hormone-sensitive prostate tumor stage. Expression levels are deemed to be "reduced" or "down-regulated" when the PDE4D7 gene expression decreases by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than 50% from a control level, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more in comparison to a control level, preferably in comparison to a hormone-dependent, or to a hormone-sensitive prostate tumor control.

[0061] In a further embodiment, an additional similarity in the overall gene expression pattern between a sample obtained from a subject and a reference as defined herein above, which is cancerous, indicates that the subject is suffering from a cancer. In another embodiment of the present invention, the diagnosis may be combined with the elucidation of additional cancer biomarker expression levels. For example, the expression of biomarkers like PSA may be tested.

[0062] A cancer, in particular a prostate cancer, may be considered as being diagnosed when the expression level of the PDE4D7 marker of the present invention is modified, preferably reduced or down-regulated, compared to a control level as defined herein above, e.g. the normal control level as defined herein above.

[0063] In a particularly preferred embodiment a prostate cancer may be considered as being diagnosed if the PDE4D7 expression level, as defined herein above, is decreased by a value of between 20% to 80%, preferably by a value of 30%, 40%, 50%, 60% or 70% in a test sample in comparison to a control level, preferably in comparison to a control expression level derived from a hormone-dependent tumor control or a hormone-sensitive prostate tumor control. In a further preferred embodiment a hormone-resistant prostate cancer may be considered as being diagnosed if the PDE4D7 expression level, as defined herein above, is decreased by a value of between 20% to 90%, preferably by a value of 30%, 40%, 50%, 60%, 70% or 80% in a test sample in comparison to a control level. The control level may either be a normal control level or a cancerous control level, preferably derivable from a hormone-dependent or hormone-sensitive prostate cancer.

[0064] The term "detecting prostate cancer" as used herein means that the presence of a cancerous disease or disorder in an organism may be determined or that a cancerous disease or disorder may be identified in an organism. The determination or identification of a cancerous disease or disorder may be accomplished by a comparison of the expression level of the

PDE4D7 marker of the present invention and the normal control level as defined herein above. A cancer, in particular a prostate cancer, may be detected when the expression level of the PDE4D7 marker is similar to a cancerous control level as defined herein above. In a preferred embodiment of the present invention a prostate cancer may be detected if the expression level of the PDE4D7 marker is similar to a cancerous control level of an established prostate cancer cell or cell line, e.g. a prostate cancer cell line as mentioned herein above.

[0065] The term "monitoring prostate cancer" as used herein relates to the accompaniment of a diagnosed or detected cancerous disease or disorder, e.g. during a treatment procedure or during a certain period of time, typically during 2 months, 3 months, 4 months, 6 months, 1 year, 2 years, 3 years, 5 years, 10 years, or any other period of time. The term "accompaniment" means that states of disease as defined herein above and, in particular, changes of these states of disease may be detected by comparing the expression level of the PDE4D7 marker of the present invention in a sample to a normal or a cancerous control level as defined herein above, preferably a control expression level derived from a hormone-dependent tumor control or a hormone-sensitive prostate tumor control in any type of periodical time segment, e.g. every week, every 2 weeks, every month, every 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 month, every 1.5 year, every 2, 3, 4, 5, 6, 7, 8, 9 or 10 years, during any period of time, e.g. during 2 weeks, 3 weeks, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 years, respectively. The cancerous control level maybe derived from samples corresponding to different stages of cancer development, e.g. stages 0 and I to IV of the TNM classification system. In a preferred embodiment of the present invention the term relates to the accompaniment of a diagnosed prostate cancer, more preferably of a hormone-dependent and a hormone-sensitive prostate cancer. In a further embodiment the monitoring may also be used for the accompaniment of hormone-resistant prostate cancer, e.g. during a treatment procedure. The monitoring may also include the detection of the expression of additional genes or genetic elements, e.g. housekeeping genes like GAPDH or PBGD, or other phosphodiesterases, preferably PDE4D5.

[0066] The term "prognosticating prostate cancer" as used herein refers to the prediction of the course or outcome of a diagnosed or detected cancerous disease, e.g. during a certain period of time, during a treatment or after a treatment. The term also refers to a determination of chance of survival or recovery from a disease, as well as to a prediction of the expected survival time of a subject. A prognosis may, specifically, involve establishing the likelihood for survival of a subject during a period of time into the future, such as 6 months, 1 year, 2 years, 3 years, 5 years, 10 years or any other period of time.

[0067] The term "progression of prostate cancer" as used herein relates to a switch between different stages of prostate cancer development, e.g. stages 0 and I to IV of the TNM classification, or any other stage or sub-stage, starting from a healthy condition up to a terminal cancer scenario. Typically such switches are accompanied by a modification of the expression level of PDE4D7, preferably a decrease, in a test sample in comparison to a previous test sample from the same individual, e.g. in comparison to a sample derived from a hormone-dependent prostate tumor or tumor control or a hormone-sensitive prostate tumor or

tumor control. A progression of prostate cancer may be considered as being detected or diagnosed if the PDE4D7 expression level, as defined herein above, is decreased by a value of between 3% to 50%, preferably by a value of 10%, 15%, 20% or 25% in a test sample in comparison to a previous test sample from the same individual. The modification may be detected over any period of time, preferably over 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 years, i.e. the value indicated above maybe calculated by comparing the expression level of PDE4D7 at a first point in time and at a second point in time after the above indicated period of time. The progression may, in a specific embodiment, be a progression towards hormone-resistant prostate cancer.

[0068] In a particularly preferred embodiment of the present invention the term "progression of prostate cancer" relates to a switch from a hormone-dependent or hormone-sensitive prostate cancer state to a hormone-resistant prostate cancer state.

[0069] A progression from a hormone-dependent or hormone-sensitive prostate cancer state to a hormone-resistant prostate cancer state may be considered as being detected or diagnosed if the PDE4D7 expression level, as defined herein above, is decreased by a value of between 3% to 50%, preferably by a value of 10%, 15%, 20% or 25% in a test sample in comparison to a previous test sample from the same individual, which has been diagnosed as suffering from a hormone-sensitive or hormone-dependent prostate cancer. The progression may also be considered to be detected if the comparison is carried out with test samples from other individuals, test samples from tissue collections, values from databases etc.

[0070] The modification may be detected over any period of time, preferably over 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 years, i.e. the value indicated above may be calculated by comparing the expression level of PDE4D7 at a first point in time and at a second point in time after the above indicated period of time.

[0071] In a further embodiment the present invention relates to the diagnosis and detection of a predisposition for developing prostate cancer, more preferably hormone-resistant prostate cancer. A "predisposition for developing prostate cancer" and in particular a "predisposition for developing hormone-resistant prostate cancer" in the context of the present invention is a state of risk of developing prostate cancer, in particular hormone-resistant prostate cancer. Preferably a predisposition for developing hormone-resistant prostate cancer may be present in cases in which the PDE4D7 expression level as defined herein above is below a cancerous control level as defined herein above, e.g. a reference expression level derived from tissues or samples of a subject which evidently suffers from hormone-sensitive prostate cancer. The term "below" as used herein means that the expression level of PDE4D7 is decreased by about 40 % to 80% in comparison to a cancerous control level, preferably decreased by about 50%.

[0072] Alternatively, a predisposition for developing cancer in the context of the present invention may be present in situations in which the PDE4D7 expression level as defined herein above is given and in which further, alternative cancer markers, e.g. PSA, show no modification of expression level or the expression pattern. Suitable further cancer markers are known to the

person skilled in the art.

[0073] Thus, a predisposition for prostate cancer, in particular hormone-resistant prostate cancer, may be considered as being diagnosed or detected if one of the above depicted situations is observed.

[0074] The difference between the expression levels of a test biological sample and a control level can be normalized to the expression level of further control nucleic acids, e.g. housekeeping genes whose expression levels are known not to differ depending on the cancerous or non-cancerous state of the cell. Exemplary control genes include *inter alia* β -actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), porphobilinogen deaminase (PBGD) and ribosomal protein P1. The normalization may also be carried out with other phosphodiesterases, preferably with a human phosphodiesterase showing an unaltered expression pattern in different tumor stages. A preferred phosphodiesterase is PDE4D5 or any other isoform of the PDE4D family, e.g. PDE4D1, PDE4D2, PDE4D3, PDE4D4, PDE4D6, PDE4D8 or PDE4D9.

[0075] In the context of the present invention, the terms "diagnosing" and "prognosticating" are also intended to encompass predictions and likelihood analyses. PDE4D7 as a marker may accordingly be used clinically in making decisions concerning treatment modalities, including therapeutic intervention or diagnostic criteria such as a surveillance for the disease. According to the present invention, an intermediate result for examining the condition of a subject may be provided. Such intermediate result may be combined with additional information to assist a doctor, nurse, or other practitioner to diagnose that a subject suffers from the disease. Alternatively, the present invention may be used to detect cancerous cells in a subject-derived tissue, and provide a doctor with useful information to diagnose that the subject suffers from the disease.

[0076] A subject or individual to be diagnosed, monitored or in which a prostate cancer, a progression of prostate cancer or predisposition for prostate cancer is to be detected or prognosticated according to the present invention is an animal, preferably a mammal, more preferably a human being.

[0077] Particularly preferred is the use of molecular imaging tools as known to the person skilled in the art, e.g. magnetic resonance imaging (MRI) and/or magnetic photon resonance imaging (MPI) technology in the context of using PDE4D7 as a marker for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer. For example, PDE4D7 may be used as a marker for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer in approaches like MRI or MPI that allows for online detection of the diagnostic marker within a human subject.

[0078] In a further aspect the present invention relates to a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer in an individual. The composition according to the

present invention may comprise a nucleic acid or peptide affinity ligand for the PDE4D7 expression product or protein.

[0079] The term "nucleic acid affinity ligand for the PDE4D7 expression product" as used herein refers to a nucleic acid molecule being able to specifically bind to a PDE4D7 transcript or a DNA molecule derived from splice variant 7 of PDE4D, even more preferably to the DNA sequence depicted in SEQ ID NO: 1 or to the complementary DNA sequence of the sequence depicted in SEQ ID NO: 1 or a corresponding RNA molecule. The nucleic acid affinity ligand may also be able to specifically bind to a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or a DNA sequence encoding an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2 or to any fragments of said sequences.

[0080] The term "peptide affinity ligand for the PDE4D7 protein" as used herein refers to a peptide molecule being able to specifically bind to the PDE4D7 protein. The peptide molecule may preferably be able to specifically bind to a protein or polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2. The peptide affinity ligand may also be able to specifically bind to a protein or polypeptide comprising an amino acid sequence encoded by a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or to a protein or polypeptide comprising an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2 or to any fragments of said sequences. The term "peptide" refers to any type of amino acid sequence comprising more than 2 amino acids, e.g. polypeptide structures, protein structures or functional derivatives thereof. Furthermore, the peptide may be combined with further chemical moieties or functionalities.

[0081] The term "expression product" as used herein refers to a PDE4D7 transcript or an mRNA molecule generated by the expression of the PDE4D gene. More preferably, the term relates to a processed PDE4D transcript as defined herein above, e.g. via the sequence as set forth in SEQ ID NO: 1.

[0082] In a preferred embodiment of the present invention the composition of the present invention comprises nucleic acid and peptide affinity ligands selected from the group consisting of a set of oligonucleotides specific for the PDE4D7 expression product, a probe specific for the PDE4D7 expression product, an aptamer specific for the PDE4D7 expression product or for the PDE4D7 protein, an antibody specific for the PDE4D7 protein and an antibody variant specific for the PDE4D7 protein.

[0083] The composition of the present invention may, for example, comprise a set of oligonucleotides specific for the PDE4D7 expression product and/or a probe specific for the PDE4D7 expression product. The term "oligonucleotide specific for the PDE4D7 expression product" as used herein refers to a nucleotide sequence which is complementary to the sense-

or antisense-strand of splice variant 7 of PDE4D. Preferably, the oligonucleotide is complementary to the DNA sequence depicted in SEQ ID NO: 1 or to the complementary DNA sequence of the sequence depicted in SEQ ID NO: 1. The oligonucleotide sequence may also be complementary to a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or a DNA sequence encoding an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2.

[0084] The oligonucleotide may have any suitable length and sequence known to the person skilled in the art, as derivable from the sequence of SEQ ID NO: 1 or its complement. Typically, the oligonucleotide may have a length of between 8 and 60 nucleotides, preferably of between 10 and 35 nucleotides, more preferably a length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 or 33 nucleotides. Oligonucleotide sequences specific for the PDE4D7 expression product may be defined with the help of software tools known to the person skilled in the art.

[0085] In a further embodiment of the present invention the oligonucleotide sequences may be complementary to sequences localized in exon 1 or exon 2 of the PDE4D7 gene, to sequences localized in the boundary between exon 1 and exon 2 of the PDE4D7 gene or to sequences localized in exon 2 of the PDE4D7 gene solely, preferably to a stretch of 271 unique nucleotides of PDE4D7, i.e. 42 nucleotides at the 3' end of exon 1 and 229 5'-terminal nucleotides of exon 2 of PDE4D. For instance, an oligonucleotide usable as a forward primer may be localized at the boundary between exon 1 and exon 2 of the PDE4D7 gene and the oligonucleotide usable as a reverse primer may be localized in exon 2 of the PDE4D7 gene.

[0086] In a preferred embodiment of the present invention the set of oligonucleotides has the sequences as set forth in SEQ ID NO: 3 and SEQ ID NO: 4. Further preferred are the oligonucleotides having or comprising the sequence as set forth in SEQ ID NO: 7 and/or SEQ ID NO: 8.

[0087] The term "probe specific for the PDE4D7 expression product" as used herein means a nucleotide sequence which is complementary to the sense- or antisense-strand of splice variant 7 of PDE4D. Preferably, the probe is complementary to the DNA sequence depicted in SEQ ID NO: 1 or to the complementary DNA sequence of the sequence depicted in SEQ ID NO: 1. The probe sequence may also be complementary to a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or a DNA sequence encoding an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2.

[0088] The probe may have any suitable length and sequence known to the person skilled in the, as derivable from the sequence of SEQ ID NO: 1 or its complement. Typically, the probe may have a length of between 6 and 300 nucleotides, preferably of between 15 and 60

nucleotides, more preferably a length of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides. Probe sequences specific for the PDE4D7 expression product may be defined with the help of software tools known to the person skilled in the art.

[0089] In a further embodiment of the present invention the probe sequence may be complementary to a sequence localized in exon 1 or exon 2 of the PDE4D7 gene, preferably to a stretch of 271 unique nucleotides of PDE4D7, i.e. 42 nucleotides at the 3' end of exon 1 and 229 5'-terminal nucleotides of exon 2 of PDE4D. If the probe is to be used for quantitative PCR reactions, e.g. real time PCR, the probe may be designed such that it is localized at a position in between the binding positions of a forward and reverse primer. Preferably, the probe may be designed such that it is localized in the proximity of one of the primer oligonucleotides. More preferably, it may be localized in the proximity of the forward primer.

[0090] In a preferred embodiment of the present invention the probe has the sequence as set forth in SEQ ID NO: 5 or SEQ ID NO: 9.

[0091] The composition of the present invention may additionally or alternatively comprise an aptamer specific for the PDE4D7 expression product or protein. The term "aptamer specific for the PDE4D7 expression product" as used herein refers to a short nucleic acid molecule, e.g. RNA, DNA, PNA, CNA, HNA, LNA or ANA or any other suitable nucleic acid format known to the person skilled in the art, being capable of specifically binding to splice variant 7 of PDE4D, preferably the DNA molecule derived from splice variant 7 of PDE4D. More preferably, the nucleic acid aptamer molecule may specifically bind to a DNA sequence depicted in SEQ ID NO: 1 or a double stranded derivative thereof. The nucleic acid aptamer according to the present invention may also bind to an RNA molecule corresponding to the PDE4D7 transcript, preferably an RNA molecule corresponding to the DNA sequence as set forth in SEQ ID NO: 1.

[0092] The nucleic acid aptamer may further be capable of specifically binding to a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or a DNA sequence encoding an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2 or RNA molecules corresponding to these sequences.

[0093] Specificity of the nucleic acid aptamer to splice variant 7 of PDE4D may be conferred by a specific binding to sequences solely present in said splice variant, e.g. exon 2 or the exon boundary between exon 1 and exon 2 of PDE4D. In a particular embodiment of the present invention specificity of the nucleic acid aptamer to splice variant 7 of PDE4D may be conferred by a specific binding to a sequence located within a stretch of 271 unique nucleotides of PDE4D7, i.e. 42 nucleotides at the 3' end of exon 1 and 229 5'-terminal nucleotides of exon 2 of PDE4D. Nucleic acid aptamers may be generated according to any suitable method known to the person skilled in the art, e.g. by in vitro selection or SELEX methods. Preferably, nucleic acid aptamers may be generated and/or designed according to the guidance provided in

Ellington and Szostak, 1990, *Nature*, 346:818-822. A nucleic acid aptamer according to the present invention may further be combined with additional moieties, e.g. with interacting portions like biotin or enzymatic functionalities like ribozyme elements.

[0094] The term "aptamer specific for the PDE4D7 protein" as used herein refers to a short peptide capable of interacting and specifically binding the PDE4D7 protein. The peptide aptamer may preferably be able to specifically bind to a protein or polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2. The peptide aptamer may also be able to specifically bind to a protein or polypeptide comprising an amino acid sequence encoded by a DNA sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 1 or to a protein or polypeptide comprising an amino acid sequence being at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence as set forth in SEQ ID NO: 2. Typically, a peptide aptamer is a variable peptide loop, comprising for example, 10 to 20 amino acids. In the context of the present invention the peptide aptamer may preferably be attached at one or both ends to a scaffold structure. The scaffold structure may be any molecule, preferably a protein, which has good solubility properties. Suitable scaffold molecules would be known to the person skilled in the art. A preferred scaffold molecule to be used in the context of the present invention is the bacterial protein thioredoxin-A. The aptamer peptide loop may preferably be inserted within a reducing active site of the scaffold molecule. Alternatively, staphylococcal protein A and domains thereof and derivatives of these domains, such as protein Z or lipocalins may be used as scaffold structures in the context of the present invention.

[0095] Peptide aptamers may be generated according to any suitable method known to the person skilled in the art, e.g. via yeast two-hybrid approaches.

[0096] In another preferred embodiment of the present invention the composition may comprise, or may additionally comprise, an antibody specific for the PDE4D7 protein, preferably a monoclonal or polyclonal antibody. Also preferred are antibody variants or fragments like a single chain antibody, a diabody, a minibody, a single chain Fv fragment (sc(Fv)), a sc(Fv)₂ antibody, a Fab fragment or a F(ab')₂ fragment based on a monoclonal PDE4D7 specific antibody, a small modular immunopharmaceutical (SMIP), a binding-domain immunoglobulin fusion protein, a camelized antibody, a V_{HH} containing antibody etc. The antibody may be mono-, bi-, tri- or multivalent. The antibody may be of any origin, e.g. a murine, human, or chimeric, or a humanized murine antibody. In a specific embodiment of the present invention commercially available anti-PDE4D7 antibodies like NB300-652 (Novus Biologicals, Inc.) or GTX14629 (GeneTex, Inc.) maybe comprised in the composition or may be used diagnostically.

[0097] Antibodies may be produced according to any suitable method known to the person skilled in the art. Polyclonal antibodies may be produced by immunization of animals with the antigen of choice, whereas monoclonal antibodies of defined specificity may be produced using, for instance, the hybridoma technology developed by Köhler and Milstein (Köhler and

Milstein, 1976, Eur. J. Immunol., 6:511-519).

[0098] An affinity ligand, as described herein above, may be labeled with various markers or may be detected by a secondary affinity ligand, labeled with various markers, to allow detection, visualization and/or quantification. This can be accomplished using any suitable labels, which can be conjugated to the affinity ligand capable of interaction with the PDE4D7 expression product or the PDE4D7 protein or to any secondary affinity ligand, using any suitable technique or methods known to the person skilled in the art. The term "secondary affinity ligand" refers to a molecule which is capable of binding to the affinity ligand as defined herein above (i.e. a "primary affinity ligand" if used in the context of a system with two interacting affinity ligands). The binding interaction is preferably a specific binding.

[0099] Examples of labels that can be conjugated to a primary and/or secondary affinity ligands include fluorescent dyes or metals (e.g. fluorescein, rhodamine, phycoerythrin, fluorescamine), chromophoric dyes (e.g. rhodopsin), chemiluminescent compounds (e.g. luminal, imidazole) and bioluminescent proteins (e.g. luciferin, luciferase), haptens (e.g. biotin).

[0100] In a particularly preferred embodiment an affinity ligand to be used as a probe, in particular a probe specific for the PDE4D7 expression product as defined herein above, maybe labeled with a fluorescent label like 6-FAM, HEX, TET, ROX, Cy3, Cy5, Texas Red or Rhodamine, and/or at the same time with a quenching label like TAMRA, Dabcyl, Black Hole Quencher, BHQ-1 or BHQ-2. A variety of other useful fluorescents and chromophores are described in Stryer, 1968, Science, 162:526-533. Affinity ligands may also be labeled with enzymes (e.g. horseradish peroxidase, alkaline phosphatase, beta-lactamase), radioisotopes (e.g. ³H, ¹⁴C, ³²P, ³³P, ³⁵S, ¹²⁵I, ¹¹C, ¹³N, ¹⁵O, ¹⁸F, ⁶⁴Cu, ⁶²Cu, ¹²⁴I, ⁷⁶Br, ⁸²Rb, ⁶⁸Ga or ¹⁸F) or particles (e.g. gold).

[0101] The different types of labels may be conjugated to an affinity ligand using various chemistries, e.g. the amine reaction or the thiol reaction. However, other reactive groups than amines and thiols can also be used, e.g. aldehydes, carboxylic acids and glutamine.

[0102] In a preferred embodiment of the present invention the nucleic acid affinity ligand or peptide affinity ligand of the present invention may be modified to function as a contrast agent.

[0103] The term "contrast agent" as used herein refers to a molecular compound that is capable of specifically interacting with the PDE4D7 marker and which can be detected by an apparatus positioned outside the human or animal body. Preferably, such contrast agents are suitable for use in magnetic resonance imaging (MRI) or magnetic photon imaging (MPI). The term "specifically interacting" refer to the property of a molecular compound to preferentially interact with the PDE4D7 marker on the cell surface of cells being present within the human or animal body over other proteins that are expressed by such cells. Preferred contrast agents which may also be designated as contrast agent compositions will be capable of specifically detecting molecules having the nucleotide sequence of SEQ ID NO: 1 or the amino acid sequence of SEQ ID NO: 2 or derivatives or homologous variants thereof as defined herein

above. Preferred contrast agents are aptamers specific for the PDE4D7 expression product or for the PDE4D7 protein as defined herein above as well as antibodies specific for the PDE4D7 protein as defined herein above.

[0104] Contrast agents, aside from their property of being capable of specifically recognizing the PDE4D7 marker will in addition typically comprise a further molecule which is detectable by the specific detection technology used. The term "modified to function" as used herein thus refers to any suitable modifications known to the person skilled in the art, which may be necessary in order to allow the use of the contrast agent in molecular imaging methods, in particular in MRI or MPI. For example, if fluorescent spectroscopy is used as a detection means, such molecules may comprise fluorophores as detectable marker molecules that can be excited at a specific wavelength. Alternatively, a radioactive label, e.g. a radioisotope as described herein above may be employed. With respect to preferred contrast agents in accordance with the invention that are suitable for MRI, the contrast agents such as the above described antibodies may comprise a marker molecule which is detectable by MRI. Such detectable labels include e.g. USPIOS and 19Fluor.

[0105] In a specific embodiment of the present invention a composition may additionally comprise accessory ingredients like PCR buffers, dNTPs, a polymerase, ions like bivalent cations or monovalent cations, hybridization solutions, secondary affinity ligands like, e.g. secondary antibodies, detection dyes and any other suitable compound or liquid necessary for the performance of a detection based on any of the affinity ligands or contrast agents as defined herein above, which is known to the person skilled in the art.

[0106] In another aspect the present invention relates to the use of a nucleic acid or peptide affinity ligand for the PDE4D7 expression product or protein, as defined herein above, for the preparation of a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer in an individual, as described herein above.

[0107] In a preferred embodiment the present invention relates to the use of a set of oligonucleotides specific for the PDE4D7 expression product and/or a probe specific for the PDE4D7 expression product, as defined herein above, for the preparation of a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer in an individual, as described herein above. In another preferred embodiment the present invention relates to the use of an aptamer specific for the PDE4D7 expression product or protein, as defined herein above, for the preparation of a composition for diagnosing, detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer in an individual, as described herein above.

[0108] In a further preferred embodiment the present invention relates to the use of an antibody specific for the PDE4D7 protein or an antibody variant specific for the PDE4D7 protein, as defined herein above, for the preparation of a composition for diagnosing,

detecting, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer in an individual, as described herein above.

[0109] In a preferred embodiment of the present invention a composition as defined herein above is a diagnostic composition.

[0110] In another aspect the present invention relates to a diagnostic kit for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer or a predisposition for prostate cancer, comprising a set of oligonucleotides specific for the PDE4D7 expression product, a probe specific for the PDE4D7 expression product and/or an aptamer specific for the PDE4D7 expression product or protein and/or an antibody specific for the PDE4D7 protein and an antibody variant specific for the PDE4D7 protein.

[0111] Typically, the diagnostic kit of the present invention contains one or more agents allowing the specific detection of PDE4D7 as defined herein above. The agents or ingredients of a diagnostic kit may, according to the present invention, be comprised in one or more containers or separate entities. The nature of the agents is determined by the method of detection for which the kit is intended. Where detection at the PDE4D7 mRNA expression level, i.e. via the PDE4D7 expression product, is intended, the agents to be comprised may be a set of oligonucleotides specific for the PDE4D7 expression product and/or a probe specific for the PDE4D7 expression product as defined herein above, which may be optionally labeled according to methods known in the art, e.g. with labels described herein above. In addition or alternatively an aptamer specific for the PDE4D7 expression production may be comprised. Where detection is at the PDE4D7 protein level is intended, the agents to be comprised may be antibodies or compounds containing an antigen-binding fragment of an antibody or antibody variants specific for the PDE4D7 protein, as described herein above. In addition or alternatively an aptamer specific for the PDE4D7 protein may be comprised. Alternatively, a diagnostic kit may comprise a contrast agent as defined herein above.

[0112] The presence of specific proteins may also be detected using other compounds that specifically interact with PDE4D7, e.g. specific substrates or ligands.

[0113] Preferably, a diagnostic kit of the present invention contains detection reagents for PDE4D7 expression product or the PDE4D7 protein. Such detection reagents comprise, for example, buffer solutions, labels or washing liquids etc. Furthermore, the kit may comprise an amount of a known nucleic acid molecule or protein, which can be used for a calibration of the kit or as an internal control. Typically, a diagnostic kit for the detection of PDE4D7 expression products may comprise accessory ingredients like a PCR buffers, dNTPs, a polymerase, ions like bivalent cations or monovalent cations, hybridization solutions etc. A diagnostic kit for the detection of PDE4D7 proteins may also comprise accessory ingredients like secondary affinity ligands, e.g. secondary antibodies, detection dyes and any other suitable compound or liquid necessary for the performance of a protein detection based known to the person skilled in the art. Such ingredients are known to the person skilled in the art and may vary depending on the detection method carried out. Additionally, the kit may comprise an instruction leaflet and/or

may provide information as to the relevance of the obtained results.

[0114] In another aspect the present invention relates to a method for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer in an individual comprising at least the step of determining the level of PDE4D7 in a sample. The term "determining the level of PDE4D7" refers to the determination of the presence or amount of PDE4D7 expression products, e.g. PDE4D7 transcript(s), and/or the determination of the presence and/or amount of PDE4D7 protein(s). The term "level of PDE4D7" thus means the presence or amount of PDE4D7 expression products, e.g. PDE4D7 transcript(s), and/or the determination of the presence or amount of PDE4D7 protein(s). The determination of the presence or amount of PDE4D7 expression products, e.g. PDE4D7 transcript(s) or PDE4D7 protein(s) may be accomplished by any means known in the art.

[0115] In a preferred embodiment of the present invention the determination of the presence or amount of PDE4D7 expression products, e.g. PDE4D7 transcript(s) and/or of PDE4D7 protein(s), is accomplished by the measurement of nucleic acid or protein levels or by the determination of the biological activity of PDE4D7. Thus, the PDE4D7 expression level(s) may be determined by a method involving the detection of an mRNA encoded by the PDE4D7 gene, the detection of the PDE4D7 protein encoded by the PDE4D7 transcript and/or the detection of the biological activity of the PDE4D7 protein.

[0116] For example, the measurement of the nucleic acid level of PDE4D7 expression may be assessed by separation of nucleic acid molecules (e.g. RNA or cDNA) obtained from the sample in agarose or polyacrylamide gels, followed by hybridization with PDE4D7 specific oligonucleotide probes as defined herein above. Alternatively, the expression level may be determined by the labeling of nucleic acid obtained from the sample followed by separation on a sequencing gel. Nucleic acid samples may be placed on the gel such that patient and control or standard nucleic acid are in adjacent lanes. Comparison of expression levels may be accomplished visually or by means of a densitometer. Methods for the detection of mRNA or expression products are known to the person skilled in the art. Typically, Northern blot analysis may be used for such a purpose.

[0117] Alternatively, the nucleic acid level of PDE4D7 expression may be detected in a DNA array or micro array approach. Typically, sample nucleic acids derived from subjects to be tested are processed and labeled, preferably with a fluorescent label. Subsequently, such nucleic acid molecules may be used in a hybridization approach with immobilized capture probes corresponding to the PDE4D7 marker gene of the present invention or known biomarker or cancer marker genes. Suitable means for carrying out microarray analyses are known to the person skilled in the art.

[0118] In a standard setup a DNA array or microarray comprises immobilized high-density probes to detect a number of genes. The probes on the array are complementary to one or more parts of the sequence of the marker gene, or to the entire coding region of the marker gene. In the present invention, any type of PDE4D7 associated polynucleotide may be used as

probe for the DNA array, as long as the polynucleotide allows for a specific distinction between PDE4D7 expression and the expression of other genes. Typically, cDNAs, PCR products, and oligonucleotides are useful as probes. Preferably, a probe involving the specific portions of splice variant 7 of PDE4D may be used as a probe. In addition to the determination of the PDE4D7 expression also the determination of the expression of other genes, e.g. additional biomarker or cancer marker genes may be accomplished.

[0119] A DNA array- or microarray-based detection method typically comprises the following steps: (1) Isolating mRNA from a sample and optionally converting the mRNA to cDNA, and subsequently labeling this RNA or cDNA. Methods for isolating RNA, converting it into cDNA and for labeling nucleic acids are described in manuals for micro array technology. (2) Hybridizing the nucleic acids from step 1 with probes for the marker genes. The nucleic acids from a sample can be labeled with a dye, such as the fluorescent dyes Cy3 (red) or Cy5 (blue). Generally a control sample is labeled with a different dye. (3) Detecting the hybridization of the nucleic acids from the sample with the probes and determining at least qualitatively, and more particularly quantitatively, the amounts of mRNA in the sample for PDE4D7 and/or additional marker genes investigated. The difference in the expression level between sample and control can be estimated based on a difference in the signal intensity. These can be measured and analyzed by appropriate software such as, but not limited to the software provided for example by Affymetrix.

[0120] There is no limitation on the number of probes corresponding to the marker genes used, which are spotted on a DNA array. Also, a marker gene can be represented by two or more probes, the probes hybridizing to different parts of a gene. Probes are designed for each selected marker gene. Such a probe is typically an oligonucleotide comprising 5-50 nucleotide residues. Longer DNAs can be synthesized by PCR or chemically. Methods for synthesizing such oligonucleotides and applying them on a substrate are well known in the field of micro-arrays. Genes other than the marker genes may be also spotted on the DNA array. For example, a probe for a gene whose expression level is not significantly altered may be spotted on the DNA array to normalize assay results or to compare assay results of multiple arrays or different assays.

[0121] Alternatively, the nucleic acid level of PDE4D7 expression may be detected in a quantitative RT-PCR approach, preferably in a real-time PCR approach following the reverse transcription of the PDE4D7 mRNA transcript. Typically, as first step, a transcript is reverse transcribed into a cDNA molecule according to any suitable method known to the person skilled in the art. A quantitative or real-time PCR approach may subsequently be carried out based on a first DNA strand obtained as described above.

[0122] Preferably, Taqman or Molecular Beacon probes as principal FRET-based probes of this type may be used for quantitative PCR detection. In both cases, the probes, preferably PDE4D7 probes as defined herein above, serve as internal probes which are used in conjunction with a pair of opposing primers that flank the target region of interest, preferably a set of PDE4D7 oligonucleotides as defined herein above. Upon amplification of a target

segment, the probe may selectively bind to the products at an identifying sequence in between the primer sites, thereby causing increases in FRET signaling relative to increases in target frequency.

[0123] Preferably, a Taqman probe to be used for a quantitative PCR approach according to the present invention may comprises a PDE4D7 oligonucleotide as defined above of about 22 to 30 bases that is labeled on both ends with a FRET pair. Typically, the 5' end will have a shorter wavelength fluorophore such as fluorescein (e.g. FAM) and the 3' end is commonly labeled with a longer wavelength fluorescent quencher (e.g. TAMRA) or a non-fluorescent quencher compound (e.g. Black Hole Quencher). It is preferred that the probes to be used for quantitative PCR, in particular the PDE4D7 probes as defined herein above, have no guanine (G) at the 5' end adjacent to the reporter dye in order to avoid quenching of the reporter fluorescence after the probe is degraded.

[0124] A Molecular Beacon probe to be used for a quantitative PCR approach according to the present invention preferably uses FRET interactions to detect and quantify a PCR product, with each probe having a 5' fluorescent-labeled end and a 3' quencher-labeled end. This hairpin or stem-loop configuration of the probe structure comprises preferably a stem with two short self-binding ends and a loop with a long internal target-specific region of about 20 to 30 bases.

[0125] Alternative detection mechanisms which may also be employed in the context of the present invention are directed to a probe fabricated with only a loop structure and without a short complementary stem region. An alternative FRET-based approach for quantitative PCR which may also be used in the context of the present invention is based on the use of two hybridization probes that bind to adjacent sites on the target wherein the first probe has a fluorescent donor label at the 3' end and the second probe has a fluorescent acceptor label at its 5' end.

[0126] The measurement of protein levels of the PDE4D7 protein or of any fragments, homologues or derivates derived thereof may be carried out via any suitable detection technique known in the art. Preferably, the protein level of PDE4D7 and derivatives thereof may be determined immunologically, e.g. by using an antibody specific for the PDE4D7 protein, preferably an antibody as defined herein above. Alternatively, antibody variants or fragments as defined herein above may be used. The present invention also envisages the use of peptide affinity ligands like aptamers specific for the PDE4D7 protein as defined herein above.

[0127] Determination of the protein levels of the PDE4D7 protein can be accomplished, for example, by the separation of proteins from a sample on a polyacrylamide gel, followed by identification of the PDE4D7 protein using specifically binding antibodies in a Western blot analysis. Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well known in the art and typically involves iso-electric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. The analysis of 2D SDS-PAGE gels can be performed by determining the intensity of protein spots on the gel, or can be performed using immune detection. In other

embodiments, protein samples are analyzed by mass spectroscopy.

[0128] Within the context of the present invention PDE4D7 specific antibodies may be placed on a support and be immobilized. Proteins derived from samples or tissues to be analyzed may subsequently be mixed with the antibodies. A detection reaction may then be carried out, e.g. with a second affinity ligand as defined herein above, preferably with a specific antibody.

[0129] Immunological tests which may be used in the context of the present invention, in particular for the diagnostic purposes of the present invention, include, for example, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassay like RIA (radio-linked immunoassay), ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, e.g. latex agglutination, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, e.g. FIA (fluorescence-linked immunoassay), chemiluminescence immunoassays, electrochemiluminescence immunoassay (ECLIA) and protein A immunoassays. Such assays are routine and well known to the person skilled in the art.

[0130] Furthermore, the binding affinity of an antibody to an antigen and the off-rate of an antibody- antigen interaction may be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ³H or ¹²⁵I) with a suitable antibody in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off- rates may be determined from the data by any suitable analysis approach, e.g. by a scatchard plot analysis. Competition with a second antibody may also be determined using radioimmunoassays. In this case, the antigen may be incubated with a suitable antibody conjugated to a labeled compound (e.g., ³H or ¹²⁵I) in the presence of increasing amounts of an unlabeled second antibody.

[0131] In addition, aptamers specific for the PDE4D7 protein, preferably as defined herein above, may be used in a method of detecting PDE4D7 proteins. Such aptamers may preferably be labeled in order to allow the detection of a protein-ligand interaction.

[0132] The determination of the biological activity of PDE4D7 may be carried out by employing molecular or enzymatic assays specific to the corresponding function or functions of PDE4D7. Preferably, a readout system based on the conversion of cAMP by phosphodiesterase may be used. Suitable techniques would be known to the person skilled in the art. In a further preferred embodiment, an assay for the determination of the biological activity of PDE4D7 may be carried out in combination with the inhibition of the activity of other PDE4D splice variants, other PDE4 isoforms and/or other PDEs, preferably other PDEs capable of performing the conversion of cAMP. Such an inhibition of the activity may be carried out by any suitable means known to the person skilled in the art, preferably via the use of suitable antisense nucleotides, siRNA molecules or miRNA molecules, more preferably via specifically hybridizing antisense

nucleotides, specific siRNA or miRNA molecules.

[0133] In a further preferred embodiment the biological activity of PDE4D7 may be tested with the help of specific PDE4D7 inhibitors. The use of such inhibitors may, for example, be combined with a readout system based on the conversion of the cAMP substrate. Typical PDE4D7 inhibitors to be used comprise antisense molecules, siRNA molecules or miRNA molecules.

[0134] The level of PDE4D7 may also be detected in methods involving histological or cell-biological procedures. Typically, visual techniques, such as light microscopy or immunofluorescence microscopy, as well as flow cytometry or luminometry may be used. The presence of PDE4D7 protein in a cell may, for instance, be detected or determined by removing cells to be tested from samples as defined herein above. Also tissue sections or biopsy samples may be used for these methods. Subsequently, affinity ligands for PDE4D7 may be applied, preferably antibodies or aptamers. Typically, such affinity ligands are labeled, preferably with fluorescent labels as defined herein above. Such a procedure allows for the detection of PDE4D7, for its quantification and, in addition, allows to determine the distribution and relative level of expression thereof.

[0135] Such procedures involve the use of visualization methods. Suitable visualization methods are known to the person skilled in the art. Typical methods to be used comprise fluorometric, luminometric and/or enzymatic techniques. Fluorescence is normally detected and/or quantified by exposing fluorescent labels to light of a specific wavelength and thereafter detecting and/or quantifying the emitted light of a specific wavelength. The presence of a luminescently tagged affinity ligand may be detected and/or quantified by luminescence developed during a chemical reaction. Detection of an enzymatic reaction is due to a color shift in the sample arising from chemical reaction.

[0136] In a further, preferred embodiment the level of PDE4D7 may be determined by suitable molecular imaging techniques, e.g. magnetic resonance imaging (MRI) or magnetic photon imaging (MPI), and/or by using suitable contrast agents, e.g. contrast agents as defined herein above.

[0137] In a further, preferred embodiment a method for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer of the present invention comprises the additional step of comparing the measured nucleic acid or protein levels or the measured biological activity to a control level. The term "control level" as used herein refers to the expression of the PDE4D7 marker or other suitable markers in a cancerous control or non-cancerous control, as defined herein above. The status, nature, amount and condition of the control level may be adjusted according to the necessities. Preferably a non-cancerous control level may be used. The term "comparing" as used herein refers to any suitable method of assessing, calculating, evaluating or processing of data.

[0138] In yet another embodiment as a further, additional step a decision on the presence or

stage of prostate cancer or the progression of prostate cancer may be based on the results of the comparison step. A prostate cancer may be diagnosed or prognosticated or a progression of prostate cancer may be diagnosed or prognosticated in said method according to the corresponding definitions provided herein above in the context of PDE4D7 as prostate cancer marker.

[0139] In another embodiment the present invention relates to a method for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer comprising at least the steps of:

1. (a) testing in at least one sample obtained from at least one individual suspected to suffer from prostate cancer for expression of the PDE4D7 expression product or the PDE4D7 protein;
2. (b) testing in at least one control sample obtained from at least one individual not suffering from cancer for the expression of the PDE4D7 expression product or the PDE4D7 protein;
3. (c) determining the difference in the expression of steps (a) and (b); and
4. (d) deciding on the presence or stage of prostate cancer or the progression of prostate cancer based on the results obtained in step (c).

[0140] In one embodiment, steps a), b), c) and/or d) of this method of diagnosis may be performed outside the human or animal body, e.g. in samples obtained from a patient or individual.

[0141] In another aspect the present invention relates to a method for diagnosing, monitoring or prognosticating hormone-resistant prostate cancer or the progression towards hormone-resistant prostate cancer, wherein said method discriminates between a hormone-sensitive and a hormone-resistant prostate cancer, comprising the steps of

1. (a) determining the level of PDE4D7 in a sample;
2. (b) determining the level of expression of a reference gene in a sample;
3. (c) normalizing the measured expression level of PDE4D7 to the expression of the reference gene; and
4. (d) comparing the normalized expression level with a predetermined cutoff value chosen to exclude hormone-sensitive prostate cancer, wherein a normalized expression level below the cutoff value is indicative of a hormone-resistant prostate cancer, wherein said cutoff value between about 1 and 7, preferably about 5.

[0142] The level of PDE4D7 may be determined on the nucleic acid, protein or activity level as described herein above. Preferred is the determination of the amount of PDE4D7 transcript(s) and/or protein. In addition the level of a reference gene in a sample may be determined. The

term "reference gene" as used herein refers to any suitable gene, e.g. to any steadily expressed and continuously detectable gene, gene product, expression product, protein or protein variant in the organism of choice. The term also includes gene products such as expressed proteins, peptides, polypeptides, as well as modified variants thereof. The invention hence also includes reference proteins derived from a reference gene. Also encompassed are all kinds of transcripts derivable from the reference gene as well as modifications thereof or secondary parameters linked thereto. Alternatively or additionally, other reference parameters may also be used for reference purposes, e.g. metabolic concentrations, cell sizes etc.

[0143] The expression may be preferably be carried out in the same sample, i.e. the level of PDE4D7 and of the reference gene is determined in the same sample. If the testing is carried out in the same sample, a single detection or a multiplex detection approach as described herein may be performed. Preferably, for a multiplex detection the oligonucleotides and probes having the sequence of SEQ ID NO: 7, 8 and 9 may be used. For the performance of the multiplex detection the concentration of primers and/or probe oligonucleotides may be modified. Furthermore, the concentration and presence of further ingredients like buffers, ions etc. may be modified, e.g. increased or decreased in comparison to manufacturers' indications.

[0144] In a specific embodiment of the present invention, the expression of more than one reference gene or steadily expressed gene may be determined. E.g. the expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 30 or more reference genes may be determined. The results of such measurements may be either calculated separately, or may be combined in order to obtain an average expression index. Furthermore, pattern of reference gene expression may be determined and/or used as basis for subsequent steps. Such pattern may be based on known expression behaviors of genes in certain cancer, in particular prostate cancer stages or states.

[0145] Furthermore, expression results may be compared to already known results from reference cases or databases. The comparison may additionally include a normalization procedure in order to improve the statistical relevance of the results.

[0146] In an alternative embodiment of the present invention, instead of determining the level of expression of a reference gene in a sample, the expression of a further cancer marker or non-steadily expressed gene may be determined. For example, the expression of a gene, which is known to be reduced during hormone-resistant prostate cancer, or which is known to be increased during hormone-sensitive prostate cancer, may be determined.

[0147] In a further embodiment, also both expression determinations may be carried out, i.e. the determination of expression of a reference gene and of a further cancer or biomarker gene.

[0148] Expression results may be normalized according to any suitable method known to the person skilled in the art, e.g. according to normalization statistical methods like the standard score, Student's T-test, studentized residual test, standardized moment test, or coefficient

variation test. Typically, such tests or corresponding formula, which would be known to the person skilled in the art, would be used to standardize expression data to enable differentiation between real variations in gene expression levels and variations due to the measurement processes.

[0149] Based on the expression results obtained in steps (a) and (b) and/or the normalized results obtained in step (c) a comparison with a cutoff value for PDE4D7 expression may be carried out. The cutoff value below which the expression level of PDE4D7 is indicative of a hormone-resistant prostate cancer, thereby excluding hormone-sensitive prostate cancer or tumor forms, is between about 0.75 and 8, 0.75 and 7.5, 0.75 and 7, 0.75 and 6.5, 0.75 and 6, 0.75 and 6, 0.75 and 5.5, 0.75 and 5.5, 1.0 and 8, 1.25 and 8, 1.5 and 8, 1.75 and 8, 2 and 8, 2.25 and 8, 2.5 and 8, 2.75 and 8, 3 and 8, 3.25 and 8, 3.5 and 8, 3.75 and 8, 4 and 8, 4.25 and 8, 4.5 and 8, 4.75 and 8 or 5 and 8. More preferred is a cutoff value of about 5, e.g. 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1 or 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, or 5.1. In a particularly preferred embodiment, said cutoff is to be used with a housekeeping gene as reference gene. Even more preferably, said cutoff is to be used with GAPDH and/or PGBD as reference gene.

[0150] In a preferred embodiment of the present invention the cutoff value is a cutoff value for PDE4D7 in blood samples, e.g. serum or plasma samples, urine samples or urine sediment samples. In a particularly preferred embodiment of the present invention the cutoff value is a cutoff value for the PDE4D7 protein or polypeptide or any derivative thereof as defined herein above in a urine sample. In another particularly preferred embodiment of the present invention the cutoff value is a cutoff value for the PDE4D7 protein or polypeptide or any derivative thereof as defined herein above in cells contained in urine or exosomes secreted from cells contained in urine. In an even more preferred embodiment of the present invention the cutoff value is a cutoff value for the PDE4D7 protein or polypeptide or any derivative thereof as defined herein above in a urine sediment sample and cells contained in a urine sediment sample, or exosomes secreted from cells contained in a urine sediment sample.

[0151] If the measured and/or normalized PDE4D7 expression is above the indicated cutoff value this may be seen as an indication that the individual does not suffer from a hormone-resistant prostate cancer. The value may additionally indicate that the individual suffers from a prostate cancer other than hormone-resistant prostate cancer, in particular hormone-dependent prostate cancer or hormone-sensitive prostate cancer.

[0152] In another aspect the present invention relates to a method of data acquisition comprising at least the steps of:

1. (a) testing in an individual for expression of PDE4D7; and
2. (b) comparing the expression as determined in step (a) to a control level.

[0153] The testing for expression of PDE4D7 maybe carried out according to steps as defined

herein above. Preferably the testing may be carried out as measurement of nucleic acid or protein levels of PDE4D7 or by determining the biological activity of PDE4D7, more preferably according to the herein above described options for such measurements. The testing may be carried out in an individual, i.e. in vivo, or outside the individual, i.e. ex vivo or in vitro. The term "control level" as used in the context of the method of data acquisition refers to the expression of the PDE4D7 marker or other suitable markers in a cancerous control or non-cancerous control, as defined herein above. The status, nature, amount and condition of the control level may be adjusted according to the necessities. Preferably a non-cancerous control level may be used. More preferably, a control level derived from hormone-sensitive prostate cancer stages may be used. A comparison of the expression to a control level may be carried out according to any suitable method of assessing, calculating, evaluating or processing of data and particularly aims at the detection of differences between two data sets. A statistical evaluation of the significance of the difference may further be carried out. Suitable statistical methods are known to the person skilled in the art. Obtained data and information may be stored, accumulated or processed by suitable informatics or computer methods or tools known to the person skilled in the art and/or be presented in an appropriate manner in order to allow the practitioner to use the data for one or more subsequent deduction or conclusion steps.

[0154] In another aspect the present invention relates to an immunoassay for detecting, diagnosing, monitoring or prognosticating prostate cancer or the progression of prostate cancer comprising at least the steps of:

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7,
2. (b) testing in a control sample for the expression of PDE4D7,
3. (c) determining the difference in expression of PDE4D7 of steps (a) and (b); and
4. (d) deciding on the presence or stage of prostate cancer or the progression of prostate cancer based on the results obtained in step (c).

The immunoassay is preferably based on the use of an antibody specifically binding to PDE4D7, e.g. one or more of the PDE4D7 antibodies mentioned herein. Alternatively, the immunoassay may be carried out or combined with any other suitable agent. For example, the assay may be combined with the detection of nucleic acids, or enzymatic testing methods as described herein.

[0155] In a further aspect the present invention relates to an immunoassay for discriminating between a hormone-sensitive and a hormone-resistant prostate cancer, comprising the steps of

1. (a) determining the level of PDE4D7 in a sample;
2. (b) determining the level of expression of a reference gene in a sample;
3. (c) normalizing the measured expression level of PDE4D7 to the expression of the reference gene; and
4. (d) comparing the normalized expression level with a predetermined cutoff value chosen to exclude hormone-sensitive prostate cancer, wherein a normalized expression level below the cutoff value is indicative of a hormone-resistant prostate cancer, wherein said

cutoff value is between about 1 and 7. Preferably, the cutoff value is about 5.

[0156] The level of PDE4D7 may preferably be determined on the protein or activity level as described herein above. Preferred is the determination of the amount of PDE4D7 protein with the help of PDE4D7 specific antibodies, e.g. one or more of the PDE4D7 antibodies mentioned herein. Alternatively, the immunoassay may be carried out with any other suitable agent or be combined with the determination of other entities. For example, the assay may be combined with the detection of the presence or amount of nucleic acids, or enzymatic testing methods as described herein.

[0157] In addition the level of a reference gene as defined herein above in a sample may be determined. For the detection of a reference gene the amount of the gene's expression product (i.e. protein) may be determined, preferably with the help of one or more suitable antibodies known to the person skilled in the art. Alternatively, the determination of the reference gene may be carried out with any other suitable agent or be combined with the detection of the presence or amount of nucleic acids, or enzymatic testing methods as described herein.

[0158] Based on the expression results obtained in steps (a) and (b) and/or the normalized results obtained in step (c) a comparison with a cutoff value for PDE4D7 expression may be carried out. The cutoff value below which the expression level of PDE4D7 is indicative of a hormone-resistant prostate cancer, thereby excluding hormone-sensitive prostate cancer or tumor forms in the immunoassay is between about 0.75 and 8, 0.75 and 7.5, 0.75 and 7, 0.75 and 6.5, 0.75 and 6, 0.75 and 5.5, 0.75 and 5.5, 1.0 and 8, 1.25 and 8, 1.5 and 8, 1.75 and 8, 2 and 8, 2.25 and 8, 2.5 and 8, 2.75 and 8, 3 and 8, 3.25 and 8, 3.5 and 8, 3.75 and 8, 4 and 8, 4.25 and 8, 4.5 and 8, 4.75 and 8 or 5 and 8. More preferred is a cutoff value of about 5, e.g. 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1 or 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, or 5.1

[0159] The cutoff value may be a cutoff value for PDE4D7 in blood samples, e.g. serum or plasma samples, urine samples or urine sediment samples etc. as described herein below.

[0160] If the measured and/or normalized PDE4D7 expression is above the indicated cutoff value this may be seen as an indication that the individual is does not suffer from a hormone-resistant prostate cancer. The value may additionally indicate that the individual suffers from a prostate cancer other than hormone-resistant prostate cancer, in particular hormone-dependent prostate cancer or hormone-sensitive prostate cancer.

[0161] In a further aspect the present invention relates to a method of identifying an individual for eligibility for prostate cancer therapy comprising:

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7;
2. (b) testing in said sample for the expression of a reference gene and/or testing in a

control sample for the expression of PDE4D7;

3. (c) classifying the levels of expression of step (a) relative to levels of step (b); and
4. (d) identifying the individual as eligible to receive a prostate cancer therapy where the individual's sample is classified as having a reduced level of PDE4D7 expression.

[0162] The level of PDE4D7 may be determined on the nucleic acid, protein or activity level as described herein above. Preferred is the determination of the amount of PDE4D7 transcript(s) and/or protein. In addition the level of a reference gene as described herein above in a sample may be determined. Testing for the expression of a reference gene may be carried out in the same sample used for the determination of PDE4D7. If the testing is carried out in the same sample, a single detection or a multiplex detection approach may be performed. Preferably, for a multiplex detection the oligonucleotides and probes having the sequence of SEQ ID NO: 7, 8 and 9 may be used. For the performance of the multiplex detection the concentration of primers and/or probe oligonucleotides may be modified. Furthermore, the concentration and presence of further ingredients like buffers, ions etc. may be modified, e.g. increased or decreased in comparison to manufacturers' indications. Alternatively, the testing for the expression of a reference gene may be carried out in a different sample, preferably a control sample as defined herein above. Preferably, such a control sample may be a control sample from the same individual as the test sample, or a control sample derived from a different source or individual. The control sample may further be either a sample derived from the same tissue, preferably prostate tissue, or be derived from a different tissue type. Examples of preferred alternative tissue types are stromal prostate tissue, bladder epithelial tissue and urethra epithelial tissue.

[0163] Furthermore, the testing of the test sample for the expression of a reference gene and the testing of control sample for the expression of PDE4D7 may be combined.

[0164] In a further embodiment the control sample may also be tested for the expression of the reference gene. In case more than one sample was tested for the expression of a reference gene, the obtained expression results may be compared and/or averaged or normalized according to any suitable statistical method known to the person skilled in the art.

[0165] The term "classifying the levels of expression of step (a) relative to levels of step (b)" as used herein means that the expression in a test sample for PDE4D7 and the expression in a control sample for PDE4D7 are compared, e.g. after normalization against a suitable normalization references. According to the outcome of the comparison the test sample is indicated as providing a similar expression as the control sample, an increased expression in comparison to the control sample, or an reduced expression in comparison to the control sample. The term further means that the expression in a test sample for PDE4D7 and the expression in the same test sample for a reference gene are compared, e.g. after normalization against a further gene as normalization reference. According to the outcome of the comparison the test sample is indicated as providing a similar expression as the reference

gene, an increased expression in comparison to the reference gene, or an reduced expression in comparison to the reference gene.

[0166] According to the classification of the expression results an individual may be considered to be eligible for a prostate cancer therapy when the PDE4D7 expression levels are reduced. The expression level is deemed to be "reduced" when the PDE4D7 gene expression in the test sample is decreased by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than 50% in comparison to the PDE4D7 expression in a control sample, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more in comparison to the PDE4D7 expression in a control sample; or when the PDE4D7 gene expression is decreased by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than 50% in comparison to the expression of a reference gene in a control sample, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more decreased in comparison to the expression of a reference gene. In a specific embodiment, the expression of a reference gene may also be normalized or adjusted to the expression of additional genes or markers, e.g. housekeeping genes.

[0167] In a further aspect the present invention relates to an immunoassay for stratifying an individual or cohort of individuals with a prostate cancer disease comprising:

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7;
2. (b) testing in said sample for the expression of a reference gene and/or testing in a control sample for the expression of PDE4D7;
3. (c) determining the difference in expression of PDE4D7 of step (a) and the expression of PDE4D7 and/or the reference gene in step (b); and
4. (d) stratifying an individual or cohort of individuals to prostate cancer therapy based on the results obtained in step (c), where the individual's sample has a reduced level of PDE4D7 expression.

[0168] The testing of the expression of PDE4D7 may preferably be carried out via the determination of the amount of PDE4D7 protein or the determination of the PDE4D7 activity level as described herein above. Preferred is the determination of the amount of PDE4D7 protein with the help of PDE4D7 specific antibodies, e.g. one or more of the PDE4D7 antibodies mentioned herein. Alternatively, the immunoassay may be carried out with any other suitable agent or be combined with the determination of other entities. For example, the assay may be combined with the detection of the presence or amount of nucleic acids, or enzymatic testing methods as described herein. In addition the level of a reference gene as described herein above in a sample may be determined. Testing for the expression of a reference gene may be carried out in the same sample used for the determination of PDE4D7. If the testing is carried out in the same sample, a single detection or a parallel or multiplex detection approach may be performed. Preferably, for a parallel or multiplex detection differently labeled primary or secondary antibodies may be used.

[0169] Alternatively, the testing for the expression of a reference gene may be carried out in a different sample, preferably a control sample as defined herein above. Preferably, such a control sample may be a control sample from the same individual as the test sample, or a control sample derived from a different source or individual. The control sample may further be either a sample derived from the same tissue, preferably prostate tissue, or be derived from a different tissue type. Examples of preferred alternative tissue types are stromal prostate tissue, bladder epithelial tissue and urethra epithelial tissue. Furthermore, the testing of the test sample for the expression of a reference gene and the testing of control sample for the expression of PDE4D7 maybe combined.

[0170] In a further embodiment the control sample may also be tested for the expression of the reference gene. In case more than one sample was tested for the expression of a reference gene, the obtained expression results may be compared and/or averaged or normalized according to any suitable statistical method known to the person skilled in the art.

[0171] The term "determining the difference in expression of PDE4D7 of step (a) and the expression of PDE4D7 and/or the reference gene in step (b)" as used herein means that the expression in a test sample for PDE4D7 and the expression in a control sample for PDE4D7 are compared, e.g. after normalization against a suitable normalization references. According to the outcome of the comparison the test sample is indicated as providing a similar expression as the control sample, an increased expression in comparison to the control sample, or an reduced expression in comparison to the control sample. The term further means that alternatively or additionally the expression in a test sample for PDE4D7 and the expression in the same test sample for a reference gene are compared, e.g. after normalization against a further gene as normalization reference. According to the outcome of the comparison the test sample is indicated as providing a similar expression as the reference gene, or a difference in the expression. The difference may be either an increased expression in comparison to the reference gene, or a reduced expression in comparison to the reference gene.

[0172] The term "stratifying an individual or cohort of individuals to prostate cancer therapy" as used herein means that an individual is identified as pertaining to a group of similar individuals, whose optimal therapy form is a prostate cancer therapy, preferably a therapy against hormone-resistant prostate cancer in accordance with the outcome of the expression test as described herein above, in particular in accordance with encountered difference in the PDE4D7 expression level and a reference gene or the PDE4D7 expression level in different samples. According to the determination of the expression difference an individual may be identified as pertaining to a group of similar individuals whose optimal therapy form is prostate cancer therapy when the PDE4D7 expression levels are reduced. The expression level is deemed to be "reduced" when the PDE4D7 gene expression in the test sample is decreased by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than 50% in comparison to the PDE4D7 expression in a control sample, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more in comparison to the PDE4D7 expression in a control sample; or when the PDE4D7 gene expression is decreased by, for example, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more than

50% in comparison to the expression of a reference gene in a control sample, or at least 0.1 fold, at least 0.2 fold, at least 1 fold, at least 2 fold, at least 5 fold, or at least 10 fold or more decreased in comparison to the expression of a reference gene. In a specific embodiment, the expression of a reference gene may also be normalized or adjusted to the expression of additional genes or markers, e.g. housekeeping genes.

[0173] An individual being considered to be eligible for a prostate cancer therapy or being stratified to prostate cancer therapy as described herein above may receive any suitable therapeutic prostate cancer treatment known to the person skilled the art. Typically, an individual considered to be eligible for prostate cancer therapy, or stratified to a corresponding treatment group, due to reduced PDE4D7 expression may be deemed to be suffering from a hormone-resistant prostate cancer or be prone to develop a hormone-resistant prostate cancer in the future, e.g. within the next 1 to 24 months. A correspondingly identified or stratified individual may be treated with a pharmaceutical composition. In a further embodiment a correspondingly identified individual may be treated with a pharmaceutical composition in combination with an additional cancer therapy. The term "additional cancer therapy" refers to any types of cancer therapy known to the person skilled in the art. Preferred are cancer therapy forms known for hormone-resistant prostate cancer. The term includes, for example, all suitable forms of chemotherapy, radiation therapy, surgery, antibody therapies etc.

[0174] Alternatively, a correspondingly identified or stratified individual may also be treated solely with one or more cancer therapies such as a chemotherapy, radiation therapy, surgery, antibody therapies etc. Preferred are cancer therapies typically used for prostate cancer, more preferred cancer therapies used for hormone-resistant prostate cancer.

[0175] In a further embodiment of the present invention the classification method for eligibility or the immunoassay for stratification as described herein above may also be used for monitoring the treatment of an individual, e.g. an individual being classified as suffering from a hormone-resistant prostate cancer. The monitoring process may be carried out as expression determination over a prolonged period of time, e.g. during or after treatment sessions, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 months, or 1, 2, 3 or more years. The determination steps may be carried out in suitable intervals, e.g. every week, 2 weeks, 3 weeks, every month, 2 months, 3 months, 6 months, 12 months etc. In a further embodiment of the present invention any treatment scheme as mentioned herein above may be adjusted, e.g. enforced or attenuated, or altered in any suitable manner in correspondence with the results of the monitoring process.

[0176] The testing for expression of PDE4D7 maybe carried out according to steps as defined herein above. Preferably, the testing may be carried out as measurement of protein levels of PDE4D7, more preferably according to the herein above described options for such measurements. As controls or control samples controls as defined herein above may be used. In a particularly preferred embodiment the testing steps may be based on the use of an antibody specifically binding to PDE4D7, e.g. a commercially available anti-PDE4D7 antibody like NB300-652 or GTX14629. A cancer may be diagnosed or prognosticated or a progression

of cancer may be diagnosed or prognosticated in said immunoassay or an individual may be identified for eligibility for prostate cancer, or an individual or cohort of individuals may be stratified in an immunoassay according to the corresponding definitions provided herein above in the context of the PDE4D7 as cancer marker. Accordingly, said testing or determining of the expression of PDE4D7 may be accomplished, or may additionally be accomplished, by the measurement of nucleic acid or protein levels or by the determination of the biological activity of PDE4D7. Similar measurements may be carried out with respect to the reference gene.

[0177] In a particularly preferred embodiment of the present invention the reference gene is a housekeeping gene or a different phosphodiesterase. In human organisms, examples of "housekeeping genes" include *inter alia* β -actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), porphobilinogen deaminase (PBGD), and ribosomal protein PI. Apart from these genes any other suitable gene may be used as a house-keeping gene, as long as the gene shows an expression or transcription on a steady, non-modified level, in particular during different stages of cancer development, more preferably during different stages of prostate cancer development, more preferably during the transition of hormone-sensitive prostate cancer to hormone-resistant prostate cancer states. Particularly preferred is the gene or transcript or expression product or protein of GAPDH. Further particularly preferred is the gene or transcript or expression product or protein of PBGD. Expression data of a house-keeping gene may be obtained from one or more samples of the same individual or from more individuals, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 100, 1000, 5.000, 10.000 or more. Expression data may also be obtained from databases or from data collections available to the person skilled in the art.

[0178] The term "different phosphodiesterase" as used herein refers to other phosphodiesterases which are not PDE4D7. Such phosphodiesterases, to be suitable as reference genes, should be steadily expressed and provide a continuously detectable gene product, expression product, protein or protein variant in the organism of choice. Particularly preferred are phosphodiesterases of the PDE4D family, e.g. PDE4D1, PDE4D2, PDE4D3, PDE4D4, PDE4D5, PDE4D6, PDE4D8 and PDE4D9. More preferred is the PDE4D5 phosphodiesterase.

[0179] Accordingly normalization and/or comparison with GAPDH, PBGD and in particular PDE4D5 may preferably be used for the above described cutoff based diagnosis methods and immunoassays, the methods of identifying or the immunoassays for discriminating or stratifying individuals. Corresponding determination steps may either be carried out in separate reactions, or, particularly preferred in multiplex reactions. For the performance of the multiplex detection the concentration of primers and/or probe oligonucleotides may be modified. Furthermore, the concentration and presence of further ingredients like buffers, ions etc. may be modified, e.g. increased or decreased in comparison to manufacturers' indications. In a further embodiment of the present invention the method of identifying an individual for eligibility for prostate cancer therapy based on the expression of PDE4D7 as described herein above may further be combined with one or more similar identification methods, based on the expression of one or more different biomarkers. Preferred is the determination of the level of prostate specific

antigen (PSA). Thus, if the level of PSA is encountered to 10, 20, 30 or preferably above 5.0, an individual may be considered to be suffering from hormone-resistant prostate cancer, or be likely to develop hormone-resistant prostate cancer in the near future, i.e. within the next 1, 2, 3, 4, 5, 6, 12 months

[0180] In a preferred embodiment of the present invention the diagnosing, detecting, monitoring or prognosticating as mentioned above is to be carried out on a sample obtained from an individual. The term "sample obtained from an individual" as used herein relates to any biological material obtained via suitable methods known to the person skilled in the art from an individual. The sample used in the context of the present invention should preferably be collected in a clinically acceptable manner, more preferably in a way that nucleic acids (in particular RNA) or proteins are preserved.

[0181] The biological samples may include body tissues and fluids, such as blood, sweat, sputum or saliva, semen and urine, as well as feces or stool samples. Furthermore, the biological sample may contain a cell extract derived from or a cell population including an epithelial cell, preferably a cancerous epithelial cell or an epithelial cell derived from tissue suspected to be cancerous. Even more preferably the biological sample may contain a cell population derived from a glandular tissue, e.g. the sample may be derived from the prostate of a male individual. Additionally, cells may be purified from obtained body tissues and fluids if necessary, and then used as the biological sample.

[0182] Samples, in particular after initial processing, may be pooled. However, also non-pooled samples may be used.

[0183] In a specific embodiment of the present invention the content of a biological sample may also be submitted to an enrichment step. For instance, a sample may be contacted with ligands specific for the cell membrane or organelles of certain cell types, e.g. prostate cells, functionalized for example with magnetic particles. The material concentrated by the magnetic particles may subsequently be used for detection and analysis steps as described herein above or below.

[0184] In a specific embodiment of the invention, biopsy or resections samples may be obtained and/or used. Such samples may comprise cells or cell lysates.

[0185] Furthermore, cells, e.g. tumor cells, may be enriched via filtration processes of fluid or liquid samples, e.g. blood, urine, sweat etc. Such filtration processes may also be combined with enrichment steps based on ligand specific interactions as described herein above.

[0186] In a particularly preferred embodiment of the present invention a sample may be a tissue sample, a urine sample, a urine sediment sample, a blood sample, a saliva sample, a semen sample, a sample comprising circulating tumor cells, or a sample containing prostate secreted exosomes.

[0187] The cancer to be diagnosed, detected, monitored or prognosticated or whose progression is diagnosed, detected, monitored or prognosticated is prostate cancer.

[0188] In another particularly preferred embodiment of the present invention the cancer to be diagnosed, detected, monitored or prognosticated or whose progression is diagnosed, detected, monitored or prognosticated is hormone-resistant prostate cancer. The term "hormone-resistant prostate cancer" means that the growth and proliferation of prostate cancer or prostate cancer cell lines is resistant to male sex hormone stimulation. The term also relates to a late prostate cancer developmental stage which is no longer amenable to an administration of anti-hormones, preferably anti-androgens as defined herein above.

[0189] Typically, prostate cancer progression is accompanied by a shift in reliance on endocrine controls to paracrine and eventually autocrine controls and that this complex process is believed to be the result of changes which occur at molecular levels of cellular control. Due to the possibility of metastatic spread of tumors at this stage hormone-resistant prostate cancers are a prime target for diagnosis and treatments according to the present invention, in particular according to the above provided embodiments.

[0190] The following examples and figures are provided for illustrative purposes. It is thus understood that the example and figures are not to be construed as limiting. The skilled person in the art will clearly be able to envisage further modifications of the principles laid out herein.

EXAMPLES:

Example 1 - quantitative RT-PCR assay human cell lines and human tissue xenografts

[0191] From the cell lines and human tissue xenografts depicted in Fig. 1 (see also for further details Marques et al., 2006, Eur. Urol., 49(2):245-57) RNA was isolated and transcribed by standard procedures into cDNA. The prepared cDNA's of samples "PC346P xenograft" through "346Flu2" which are cells lines and samples "PC295" through "PC374" which are xenografts were tested on expression levels of PDE4D7.

qRT-PCR: Materials and Methods

[0192] RNA samples were treated with DNase to ensure there was no DNA contamination. Prior to cDNA synthesis RNA samples were treated with DnaseI (In Vitrogen) for 30 min at 37°C. 1µg of the RNA sample was then treated with Superscript Vilo (In Vitrogen) to synthesize the first strand DNA for qPCR analysis as per manufacturer's guidelines. DNA samples were then treated with RnaseH1 30 min at 37°C.

[0193] Resulting DNA was diluted to a final concentration of 50 - 60 ng/µl, of which 5µl was added to each reaction well of a 96-well optical reaction plate.

[0194] Quantitative PCR reactions were performed using an ABI Prism 7300 machine in a reaction volume of 15µl according to the following protocol:

7.5µl Platinum qPCR SuperMix-UDG with ROX (InVitrogen)

2.2µl nuclease free water

0.1µl 100 pmol / µl Probe

0.1 µl 100 pmol / µl Forward Primer

0.1 µl 100 pmol / µl Reverse Primer

[0195] Total volume in each reaction well was 15 µl including cDNA.

[0196] The PCR itself was run over 40 cycles under the following program:

Stage	Repetitions	Temperature (°C)	Time
1	1	50	2 seconds
2	1	95	2 minutes
3	40	95	15 seconds
		60	1 minute

qRT-PCR Primers and Probes (TAQMAN)

[0197] The following oligonucleotide primers and probes were used for RT-PCR on PDE4D7: Forward Primer 5'-TGCCTCTGAGGAAACACTAC-3' (SEQ ID NO: 3), Reverse Primer 5'-GCTGAATATTGCGACATGAAAG-3' (SEQ ID NO: 4) giving rise to a product of the length 101.

[0198] As probe the sequence 5'-CCAGTAATGAAGAGGAAGACCCTTCCGC-3' (SEQ ID NO: 5) was used.

[0199] The probe-sets was designed to target the unique N-terminal regions of the PDE isoform. The amplicon was designed to be within the optimal range for Taqman assays on ABI Prism technology. All assays were performed in quadruplicate to maximize data integrity. A GAPDH reference probe was also included to which all consecutive data were referenced against.

qRT-PCR: data analysis

[0200] A -ddCt approach was carried out in order to normalize and compare different RT-PCR experiments. Ct values were obtained by manual threshold observation where each probe-set was amplifying exponentially at a comparable efficiency. In particular, the following steps were carried out:

1. 1.) The difference in cycle number (Ct) between reference and gene of interest (GAPDH subtracted from Gene of interest) was calculated to give the experimental sample (ES) dCt.
2. 2.) One sample was selected as standard to be compared against (LNCaP) (C) and its dCt was calculated.
3. 3.) The change in cycle number difference could be derived by $dCt(ES) - dCt(C) = ddCt$
4. 4.) The final comparable expression values could be derived by 2^{-ddCt} in order to take into account the doubling of DNA after each cycle, hence showing the amount of mRNA in comparison to LNCaP.

[0201] This operation gave a value in comparison to LNCaP (which will have the value of 1), i.e. any value > 1 was considered to be an increase in expression, a value of < 1 was considered to be a decrease in expression.

[0202] It was accordingly assumed that the extension efficiencies of all the PCR reactions are within a certain range, resulting in a value of 1.

Percentage approach to normalize and compare different RT-PCR experiments

[0203] For each probe-set a Ct (cycle number) value was obtained. This was generated by finding a baseline which intersected the amplification curves during their exponential phase. The baselines were generated dynamically according to the curves obtained in each experiment. The Ct (intersect or cycle) values of the GOIs were then subtracted from the Ct value of the GAPDH standard.

[0204] According to the formula $Ct(GAPDH) - Ct(GOI) = dCt$, given that GOI Ct values are always larger than the reference gene the dCt value resulted in negative numbers, i.e. a -dCt value.

[0205] Based on the doubling effect of each cycle and the absolute values were determined according to the Comparative Expression Value = 2^{-dCt} . Due to the very small values gained from this calculation the value multiplied by 1000 for handling purposes.

[0206] Expression levels for PDE4D7 obtained by this approach are depicted in Figs. 2 to 7. In

particular, Figures 2 to 4 cover the relative expression of PDE4D7 cDNA in different human prostate cancer cell lines and xenografts (see Fig. 1) normalized according to the -ddCt approach, whereas Figures 5 to 7 cover the relative expression of PDE4D7 cDNA in different human prostate cancer cell lines and xenografts (see Fig. 1) normalized relative to total PDE4D expression in the individual cell lines or xenograft tissue material.

[0207] As can be derived from Fig. 2-7 the expression (or transcription) level of PDE4D7 is significantly different between most androgen-dependent or androgen-sensitive vs. androgen-independent cell lines (Figs 3 and 6) or human xenografts (Figs 4 and 7). Figs. 2 and 5 show the combined results of cell lines and xenografts.

[0208] According to the results presented in Figs. 2 to 7 the transcription of PDE4D7 in human prostate cancer cell lines and tissues is dependent on the status of the Androgen Receptor activity in a given cell type. In case of presence of AR and sensitivity of a cell to androgen/hormone stimulation significant PDE4D7 transcription can be observed whereas on the absence of active AR the PDE4D7 transcription is very minimal, i.e. virtually absent.

Example 2 - quantitative RT-PCR assay with human tissue samples

[0209] The relative gene expression of human PDE4D7 was evaluated in prostate cancer tissues derived from patients with hormone-sensitive/responsive vs. hormone-refractory/castration-resistant patients.

Materials and Methods

[0210] Details on the samples used in the qPCR measurements of PDE gene expression experiment are given in Table 1, below:

Table 1: Patient information used in the experiment

Tissue	Type of Tissue	Gleason Score	Age at Treatment	Hormone Refractory Status
Prostate	TURP	4+4	66	no
Lymphnode		4+4	63	no
Prostate	TURP	3+3	67	no
Prostate	TURP	4+4	82	no
Prostate	TURP	5+4	76	yes
Prostate	TURP	3+4	60	yes
Lymphnode		4+4	66	no
Prostate	TURP	5+3	90	yes
Prostate	TURP	4+3	66	yes

Tissue	Type of Tissue	Gleason Score	Age at Treatment	Hormone Refractory Status
Prostate	TURP	3+4	60	yes
Prostate	TURP	4+4	64	no
Prostate	TURP	6	71	yes
Lymphnode		4+4	62	no
Prostate	TURP	7	75	yes
Prostate	TURP	3+5	70	yes
Lymphnode		4+4	52	no

[0211] All samples were derived from male patients (ages 52-82). The column "Tissue" defines the tissue that has been taken during surgery, either prostate tissue, or lymph nodes for staging. The column "Type of Tissue" describes the approach of tissue resection. If not otherwise indicated the tissue was resected during prostate surgery (prostatectomy). "TURP" is defined as Trans Urethral Resection of the Prostate. The cDNA panel includes 4 samples derived from patients with hormone-sensitive prostate cancer, and 8 samples derived from patients with hormone-refractory prostate cancer (indicated by "yes" in the column "Hormone Refractory Status") and 4 samples from lymph node metastases

[0212] Primer and probe sequences used for human PDE4D7:

sense primer sequence: CGGAATGGAACCCCTA TCTTGTC (SEQ ID NO: 7)

antisense primer sequence: TTGGTCGTTGAATGTTCTCTGAT (SEQ ID NO: 8)

probe sequence: CCTCTCGCCTTCAGACAGTTGGAACAAGGAG AGG (FAM-labeled) (SEQ ID NO: 9).

[0213] The PDE4D7 specific primers (SEQ ID NO: 7 and 8) were premixed with the FAM probes (SEQ ID NO: 9) to perform quantitative, real-time PCR (qPCR), and used in a 1:20 dilution according to manufacturer's description (PrimerDesign, UK). The human cDNA samples are arranged in standard, qPCR-ready, 96-well microtiter (MT) plates.

[0214] 16 tissue samples, derived from 16 different patients were arranged per 96-well MT plate, with each of the 16 wells used per plate containing ca 2-3 ng of RNA reverse transcribed cDNA.

[0215] To each of the used well of the MT plate 15 µL Applied Biosystems' GeneAmp mastermix (2x), 13.5 µL RNase/DNAse free water and 2 µL PrimerDesign PerfectProbe primermix (PrimerDesign, UK) were added.

[0216] All samples were analyzed with the following PCR protocol: 2 min at 50°C, 10 min at 95°C, 15 sec at 95°C, 30 sec at 50°C while recording fluorescence, 15 sec at 72°C and the last three steps repeated 50 times.

[0217] For all calculations relative gene expression values, the following procedure was used: C_T values of 40 or higher or below 16 were excluded for poor quality reasons. (The genes examined here had an average C_T -value of ~33).

[0218] To normalize the C_T values, the following approach was used: the C_T values were converted to relative gene copy number based on calibration curves. The calibration curves were independently measured on different dilutions of cDNA. Subsequently the PDE4D7 expression by dividing the PDE4D7 copy number by the average copy number of the household genes (Glycerinaldehyde-3-phosphate-Dehydrogenase (GAPDH), and Porphobilinogen Deaminase (PBGD)).

Relative Expression of human PDE4D7 in human prostate tissues (hormone-responsive vs. hormone-resistant) including lymph node resected tissue samples

[0219] The gene expression level of the human PDE4D7 isoform was determined on human prostate tissues as described above. The relative expression levels were determined in two defined prostate tissues: "hormone-responsive" and "hormone-refractory". For an initial investigation of the human PDE4D7 expression status lymph node resected tissue samples together with primary prostate cancer samples were included.

[0220] Lymph node resected tissue samples are characterized by invasive growth as they have been disseminating from the primary tumor into the sentinel lymph nodes. In that sense these samples are from a molecular perspective different from the primary tumor cells, which are characterized by uncontrolled proliferation but are still growing within the primary organ confines. Invasive growth implicates more aggressive growth behavior as also found in the hormone-refractory prostate cancer tissue.

[0221] A Student's T-test was performed to see whether human PDE4D7 gene expression is on average slightly decreased in hormone-resistant tumor tissues compared to hormone-responsive human prostate tissue.

[0222] As can be derived from Figures 8 and 9, the expression in the hormone-resistant prostate tumors is generally decreased but cannot easily be distinguished from the expression in the hormone-sensitive prostate tumor groups. The average PDE4D7 expression difference between the tested tissue types was correspondingly found to be not significant (p-value = 0.22).

[0223] This result could be possibly explained by the more aggressive behavior of part of the

samples included in the analysis of group 1. It has been speculated whether aggressive disease harbors already tumor cells that have lost their responsiveness to androgen, such that the characteristics of hormone-refractory or hormone-resistant behavior would be inherently contained within an aggressive tumor. These cells would consequently be selected under androgen deprivation conditions. This could explain why such tissue types already tend to have reduced expression levels of human PDE4D7 leading to less significant p-values in a standard T-test. Thus, the difficulty in correctly determining the status of lymph node resected tissue samples, i.e. the fact that it could not be excluded that these tumors already contained to some extent hormone-resistant tumor cell populations could possibly have lead to the obtained results.

Relative Expression of human PDE4D7 in human prostate tissues (hormone-responsive vs. hormone-resistant) excluding lymph node resected tissue samples

[0224] In order to circumvent the encountered problems, the relative expression levels were determined in two defined prostate tissues: "hormone-responsive" and "hormone-refractory", whereby for the investigation of the human PDE4D7 expression status only primary prostate cancer samples were included, i.e. all samples derived from lymph node resections were dismissed.

[0225] A Student's T-test was performed to see whether human PDE4D7 gene expression is significantly decreased on average in hormone-resistant tumor tissues compared to hormone-responsive human prostate tissue.

[0226] As can be derived from Figures 10 and 11, the expression in the hormone-resistant prostate tumors is decreased and can easily be distinguished from the expression in the hormone-sensitive prostate tumor groups. The average PDE4D7 expression difference between the two tissue types (hormone-resistant tumor tissues vs. hormone-sensitive tumor tissues) is significant ($p=0.032$).

[0227] Figures 10 and 11 show that the PDE4D7 expression in the hormone-resistant prostate tumors is generally decreased compared to hormone-responsive prostate tumors. It is hence concluded that a decreased level of cAMP-PDE activity is advantageous for enhanced cell proliferation. It has been long speculated that next to Androgen Receptor gene mutations or gene amplification, the activation of other cellular signaling pathways can support the transition of hormone-responsive to hormone-independent cell growth. The cAMP-PKA pathway is one of the pathways that have been implicated in that transition of hormone related growth. The change in PDE4D7 expression from hormone-sensitive to hormone-refractory human prostate tissue supports this view.

Example 3 - The effect of PDE4D7 expression on PC3 Proliferation

[0228] To test the effect of heterologous expression of human PDE4D7 on the proliferation of androgen-independent cell lines several gene constructs were transfected into PC3 cells and tested cell growth compared to control treated cell lines. As controls untreated PC3 cells, PC3 cell transfected with a dominant negative form of PDE4D7 wherein the catalytic activity of PDE4D7 has been knocked out by genetic mutation of the coding sequence of PDE4D7, as well as PC3 cells transfected with a different isoform of the PDE4D family, namely PDE4D1, were used.

[0229] PC3 Cells were grown at 5% CO₂, 37C, 5% Foetal Bovine Serum in RPMI 1640 supplemented with L-Glutamine (2mM final concentration) and PenStrep (1%). The PC3 cells were seeded at a density of 5000 cells per well of a 96 well plate (MTP). The Transfection complexes for the gene of interest (PDE4D7, PDE4D7 dominant Negative and PDE4D1) were formed using Fugene 6 Transfection reagent as per the manufacturer's instructions. For each gene of interest -25 independent transfections were performed to increase the statistical power of the test (each bar represents -25 independent measurements).

[0230] After initial plating time of 4 hours the transfection complexes were added to the growth media and the cells were allowed to proliferate for 60 hours before the addition of the Promega MTT compound. The MTT assay procedure to investigate cell proliferation was followed as per manufacturer's instructions.

[0231] Absorbance readings were obtained using the Mithras LB940 plate reader system. 1 second readings were taken from each well.

[0232] As demonstrated in the above experiment human PDE4D7 heterologous expression in the androgen-independent prostate cancer cell line PC3 showed a negative effect on proliferation in vivo. Cell proliferation under the chosen conditions was overall inhibited ~15% by expression of human PDE4D7 compared to untreated cells, cells transfected with an inactive form of PDE4D7 as well as compared to PDE4D1 expression. The effect is statistically significant (p <0.001 compared to untreated PC3).

[0233] This experiment demonstrates that the activation of human PDE4D7 has an inhibitory effect on cell proliferation of androgen-independent prostate cancer cells. The observed effect on cell proliferation is statistically highly significant.

[0234] Due to the transient manner of the transfection used for the experiment (i.e., depending on the transfection efficiency more or less PC3 less were transfected and subsequently overexpress the PDE enzyme) it is expected that an even higher level of inhibition of cell proliferation can be reached once a higher transient transfection efficiency is achieved in PC3 cells.

[0235] The present disclosure comprises the following additional embodiments:

Item 1: Phosphodiesterase 4D7 (PDE4D7) for use as a marker for cancer.

Item 2: A composition for diagnosing, detecting, monitoring or prognosticating cancer or the progression of cancer, comprising a nucleic acid affinity ligand and/or a peptide affinity ligand for the PDE4D7 expression product or protein.

Item 3: The composition of item 2, wherein said nucleic acid affinity ligand or peptide affinity ligand is modified to function as a contrast agent.

Item 4: The composition of item 2, wherein said affinity ligand is a set of oligonucleotides specific for the PDE4D7 expression product, a probe specific for the PDE4D7 expression product, an aptamer specific for the PDE4D7 expression product or for the PDE4D7 protein, an antibody specific for the PDE4D7 protein and/or an antibody variant specific for the PDE4D7 protein.

Item 5: Use of PDE4D7 as a marker for diagnosing, detecting, monitoring or prognosticating cancer or the progression of cancer.

Item 6: A method for detecting, diagnosing, monitoring or prognosticating cancer or the progression of cancer comprising at least the step of determining the level of PDE4D7 in a sample.

Item 7: The method of item 6, wherein the determining step is accomplished by the measurement of nucleic acid or protein levels or by the determination of the biological activity of PDE4D7.

Item 8: The method of item 7, wherein said method comprises the additional step of comparing the measured nucleic acid or protein levels or the measured biological activity to a control level.

Item 9: A method of data acquisition comprising at least the steps of:

1. (a) testing in an individual for expression of PDE4D7; and
2. (b) comparing the expression as determined in step (a) to a control level.

Item 10: The use of item 2 or the method of any one of items 6 to 9, wherein the diagnosing, detecting, monitoring, prognosticating or data acquisition is to be carried out on a sample obtained from an individual.

Item 11: An immunoassay for detecting, diagnosing, monitoring or prognosticating cancer or the progression of cancer comprising at least the steps

1. (a) testing in a sample obtained from an individual for the expression of PDE4D7,
2. (b) testing in a control sample for the expression of PDE4D7,
3. (c) determining the difference in expression of PDE4D7 of steps (a) and (b); and
4. (d) deciding on the presence or stage of cancer or the progression of cancer based on the results obtained in step (c),

wherein said testing steps are based on the use of an antibody specifically binding to PDE4D7.

Item 12: The use or method of item 10 or the immunoassay of item 11, wherein said sample is a tissue sample, a urine sample, a urine sediment sample, a blood sample, a saliva sample, a semen sample, or a sample comprising circulating tumor cells.

Item 13: A pharmaceutical composition comprising at least one element selected from the group of:

1. (a) a compound directly stimulating or modulating the activity of PDE4D7, preferably an allosteric agonist of PDE4D7 enzymatic activity;
2. (b) a compound indirectly stimulating or modulating the activity of PDE4D7;
3. (c) the PDE4D7 protein or a biologically active equivalent thereof;
4. (d) a nucleic acid encoding and expressing PDE4D7;
5. (e) a miRNA inhibitor specific for PDE4D7 miRNAs;
6. (f) a demethylation agent; and
7. (g) a phosphodiesterase displacement factor, preferably a peptide, a peptidomimetic, a small molecule, an antibody or an aptamer.

Item 14: A pharmaceutical composition for the treatment or prevention of cancer comprising at least one element selected from the group of:

1. (a) a compound directly stimulating or modulating the activity of PDE4D7, preferably an allosteric agonist of PDE4D7 enzymatic activity;
2. (b) a compound indirectly stimulating or modulating the activity of PDE4D7;
3. (c) the PDE4D7 protein or a biologically active equivalent thereof;
4. (d) a nucleic acid encoding and expressing PDE4D7;
5. (e) a miRNA inhibitor specific for PDE4D7 miRNAs;
6. (f) a demethylation agent; and
7. (g) a phosphodiesterase displacement factor preferably a peptide, a peptidomimetic, a small molecule, an antibody or an aptamer.

Item 15: Use of

1. (a) a compound directly stimulating or modulating the activity of PDE4D7, preferably an allosteric agonist of PDE4D7 enzymatic activity;
2. (b) a compound indirectly stimulating or modulating the activity of PDE4D7;
3. (c) the PDE4D7 protein or a biologically active equivalent thereof;
4. (d) a nucleic acid encoding and expressing PDE4D7;
5. (e) a miRNA inhibitor specific for PDE4D7 miRNAs;
6. (f) a demethylation agent; and/or
7. (g) a phosphodiesterase displacement factor, preferably a peptide, a peptidomimetic, a small molecule, an antibody or an aptamer,

for the preparation of a pharmaceutical composition for the treatment or prevention of cancer.

Item 16: The phosphodiesterase of item 1, the composition of any one of items 2 to 4, the use of item 5, 10 or 12, the method of any one of item 6 to 10 or 12, the immunoassay of item 11 or 12, the pharmaceutical composition of item 13 or 14, or the use of item 15, wherein said

cancer is prostate cancer.

Item 17: The phosphodiesterase, use, composition, method, immunoassay, or pharmaceutical composition of item 16, wherein said prostate cancer is hormone-resistant prostate cancer.

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

5 **1.** Phosphodiesterase-4D7 (PDE4D7) til anvendelse i en fremgangsmåde til *in vivo*-
diagnosticering, -påvisning, -overvågning eller -prognosticering af prostatacancer
eller *in vivo*-diagnosticering, -påvisning, -overvågning eller -prognosticering af
progressionen af prostatacancer.

10 **2.** Sammensætning til anvendelse i en fremgangsmåde til *in vivo*-
diagnosticering, -påvisning, -overvågning eller -prognosticering af prostatacancer
eller *in vivo*-diagnosticering, -påvisning, -overvågning eller -prognosticering af
progressionen af prostatacancer, hvilken sammensætning omfatter en
nukleinsyreaffinitetsligand og/eller en peptidaffinitetsligand for PDE4D7-
ekspressionsproduktet eller -proteinet.

15 **3.** Sammensætning til anvendelse ifølge krav 2, hvor nukleinsyreaffinitetsliganden
eller peptidaffinitetsliganden er modificeret for at fungere som et kontrastmiddel.

20 **4.** Sammensætning til anvendelse ifølge krav 2, hvor affinitetsliganden er et sæt af
oligonukleotider, som er specifikke for PDE4D7-ekspressionsproduktet, en probe,
som er specifik for PDE4D7-ekspressionsproduktet, en aptamer, som er specifik for
PDE4D7-ekspressionsproduktet eller for PDE4D7-proteinet, et antistof, som er
specifikt for PDE4D7-proteinet, og/eller en antistofvariant, som er specifik for
25 PDE4D7-proteinet.

30 **5.** Anvendelse af PDE4D7 som markør til diagnosticering, påvisning, overvågning
eller prognosticering af prostatacancer eller til diagnosticering, påvisning,
overvågning eller prognosticering af progressionen af prostatacancer.

35 **6.** *In vitro*- eller *ex vivo*-anvendelse af en sammensætning, som omfatter en
nukleinsyreaffinitetsligand og/eller peptidaffinitetsligand for PDE4D7-
ekspressionsproduktet eller -proteinet, til diagnosticering, påvisning, overvågning
eller prognosticering af prostatacancer eller til diagnosticering, påvisning,

overvågning eller prognosticering af progressionen af prostatacancer.

7. *In vitro*- eller *ex vivo*-anvendelse ifølge krav 6, hvor nukleinsy reaffinitetsliganden eller peptidaffinitetsliganden er modificeret for at fungere som et kontrastmiddel.

5

8. *In vitro*- eller *ex vivo*-anvendelse ifølge krav 6, hvor affinitetsliganden er et sæt af oligonukleotider, som er specifikke for PDE4D7-ekspressionsproduktet, en probe, som er specifik for PDE4D7-ekspressionsproduktet, en aptamer, som er specifik for PDE4D7-ekspressionsproduktet eller for PDE4D7-proteinet, et antistof, som er specifikt for PDE4D7-proteinet, og/eller en antistofvariant, som er specifik for PDE4D7-proteinet.

10

9. Fremgangsmåde til diagnosticering, påvisning, overvågning eller prognosticering af prostatacancer eller til diagnosticering, påvisning, overvågning eller

15 prognosticering af progressionen af prostatacancer, hvilken fremgangsmåde mindst omfatter trinnet, der går ud på at bestemme koncentrationen af PDE4D7 i en prøve.

10. Fremgangsmåde til diagnosticering, påvisning, overvågning eller prognosticering af hormonresistent prostatacancer eller til diagnosticering, påvisning, overvågning eller prognosticering af progressionen af hormonresistent prostatacancer, hvor fremgangsmåde skelner mellem hormonfølsom og hormonresistent prostatacancer, hvilken fremgangsmåde omfatter trinnene, der går ud på

20

(a) at bestemme koncentrationen af PDE4D7 i en prøve;

(b) at bestemme ekspressionsniveauet af et referencegen i en prøve;

25

(c) at normalisere det målte ekspressionsniveau af PDE4D7 i forhold til ekspressionen af referencegenet; og

(d) at sammenligne det normaliserede ekspressionsniveau med en forudbestemt afskæringsværdi, der er valgt for at udelukke hormonfølsom prostatacancer, hvor et normaliseret ekspressionsniveau under

30

afskæringsværdien er tegn på hormonresistent prostatacancer, hvor afskæringsværdien ligger på mellem ca. 1 og 7, fortrinsvis på ca. 5.

11. Fremgangsmåde til dataindsamling til diagnosticering, påvisning, overvågning eller prognosticering af prostatacancer eller til påvisning, diagnosticering,

overvågning eller prognosticering af progressionen af prostatacancer, hvilken fremgangsmåde mindst omfatter trinnene, der går ud på:

- (a) at teste et individ for ekspressionen af PDE4D7 og
- (b) at sammenligne ekspressionen som bestemt i trin (a) med et

5 kontrolniveau.

12. Immunoassay til diagnosticering, påvisning, overvågning eller prognosticering af prostatacancer eller til påvisning, diagnosticering, overvågning eller prognosticering af progressionen af prostatacancer, hvilket immunoassay mindst omfatter trinnene:

- 10 (a) teste en prøve for ekspressionen af PDE4D7,
- (b) at teste en kontrolprøve for ekspressionen af PDE4D7,
- (c) at bestemme forskellen i ekspressionen af PDE4D7 i trin (a) og (b), og
- (d) at fastslå tilstedeværelsen eller stadiet af prostatacancer eller progressionen af prostatacancer på basis af de i trin (c) opnåede resultater,

15 hvor testtrinnene er baseret på anvendelsen af et antistof, der binder specifikt til PDE4D7.

13. Immunoassay til at skelne mellem hormonfølsom og hormonresistent prostatacancer, hvilket immunoassay mindst omfatter trinnene, der går ud på:

- 20 (a) at bestemme koncentrationen af PDE4D7 i en prøve;
- (b) at bestemme ekspressionsniveauet af et referencegen i en prøve;
- (c) at normalisere det målte ekspressionsniveau af PDE4D7 i forhold til ekspressionen af referencegenet; og
- (d) at sammenligne det normaliserede ekspressionsniveau med en

25 forudbestemt afskæringsværdi, der er valgt for at udelukke hormonfølsom prostatacancer, hvor et normaliseret ekspressionsniveau under afskæringsværdien er tegn på hormonresistent prostatacancer, hvor afskæringsværdien ligger på mellem ca. 1 og 7, fortrinsvis på ca. 5.

30 **14.** Fremgangsmåde til identificering af et individ med hensyn til egnethed for prostatacancerbehandling, hvilken fremgangsmåde omfatter:

- (a) at teste en prøve, der er opnået fra et individ, for ekspressionen af PDE4D7;

- (b) at teste prøven for ekspressionen af et referencegen og/eller at teste en kontrolprøve for ekspressionen af PDE4D7;
- (c) at klassificere ekspressionsniveauerne i trin (a) i forhold til niveauerne i trin (b); og

5 (d) at identificere individet som egnet til at modtage prostatacancerbehandling, hvor individets prøve er klassificeret som havende et reduceret niveau af PDE4D7-ekspression.

15. Immunoassay til stratificering af et individ eller en kohorte af individer med

10 **prostatacancersydom, hvilket immunoassay omfatter:**

- (a) at teste en prøve, der er opnået fra et individ, for ekspressionen af PDE4D7;
- (b) at teste prøven for ekspressionen af et referencegen og/eller at teste en kontrolprøve for ekspressionen af PDE4D7;

15 (c) at bestemme forskellen i ekspressionen af PDE4D7 i trin (a) og ekspressionen af PDE4D7 og/eller referencegenet i trin (b); og

(d) at stratificere et individ eller en kohorte af individer i forhold til prostatacancerbehandling på basis af de i trin (c) opnåede resultater, hvor individets prøve har et reduceret niveau af PDE4D7-ekspression.

20

16. Fremgangsmåde ifølge et hvilket som helst af kravene 9 til 11 eller 14 eller immunoassay ifølge krav 12, 13 eller 15, hvor testen eller bestemmelsen af ekspressionen udføres eller yderligere udføres ved måling af koncentrationerne af nukleinsyre eller protein eller ved bestemmelse af den biologiske aktivitet af PDE4D7 eller referencegenet.

25

17. Fremgangsmåde eller immunoassay ifølge krav 16, hvor fremgangsmåden eller immunoassayet omfatter det yderlige trin, der går ud på at sammenligne de målte koncentrationer af nukleinsyre eller protein eller den målte biologiske aktivitet med et

30 **kontrolniveau.**

18. Fremgangsmåde ifølge et hvilket som helst af kravene 10, 14, 16 eller 17 eller immunoassay ifølge et hvilket som helst af kravene 13 eller 15 til 17, hvor referencegenet er et husholdningsgen, fortrinsvis GAPDH eller PBGD, eller en

anden phosphodiesterase, fortrinsvis PDE4D5.

19. Fremgangsmåde ifølge et hvilket som helst af kravene 10, 14, 16, 17 eller 18 eller immunoassay ifølge et hvilket som helst af kravene 13, 15, 16, 17 eller 18, hvor

5 fremgangsmåden eller immunoassayet omfatter det yderligere trin, der går ud på at bestemme koncentrationen af prostataspecifikt antigen (PSA).

20. Fremgangsmåde ifølge et hvilket som helst af kravene 9, 10, 14, 16, 17 eller 18 eller immunoassay ifølge et hvilket som helst af kravene 12, 13, 15, 16, 17 eller 18,

10 hvor prøven er en vævsprøve, en urinprøve, en urinsedimentprøve, en blodprøve, en spytprøve, en sædprøve, en prøve omfattende cirkulerende tumorceller eller en prøve indeholdende exosomer, som udskilles af prostata.

21. Fremgangsmåde ifølge et hvilket som helst af kravene 14 eller 16 til 19 eller

15 immunoassay ifølge et hvilket som helst af kravene 15 to 19, hvor prostatacancerbehandlingen omfatter indgivelse af den farmaceutiske sammensætning ifølge krav 19 eller indgivelse af den farmaceutiske sammensætning ifølge krav 19 i kombination med en yderligere cancerbehandling, fortrinsvis strålebehandling eller kemoterapi.

20

22. Phosphodiesterase til anvendelse ifølge krav 1, sammensætning til anvendelse ifølge et hvilket som helst af kravene 2 til 4, anvendelse ifølge et hvilket som helst af kravene 5 til 8, fremgangsmåde ifølge et hvilket som helst af kravene 9, 14, 16 til 20 eller 23 eller immunoassay ifølge et hvilket som helst af kravene 12, 15 til 20 eller

25 21, hvor prostatacanceren er hormonresistent prostatacancer.

DRAWINGS

FIGURE 1

Sample	Origine	Androgen Response	AR expression	PSA expression	AR sequence
PC348P xenograft (PC348c cell line)	Lymph node	AD	Yes	Yes	WT
PC348B xenograft (from same patient as PC348P)	Primary	AD	Yes	Yes	WT
LuCaP 8 hr - (no R1881 treatment)	Lymph node	AD/AS	Yes	Yes	T877A
LuCaP 8 hr + R1881	Lymph node	AD/AS	Yes	Yes	T877A
LuCaP 8 hr - (no R1881 treatment)	Vertebra	AI/AS	Yes	Yes	WT
LuCaP 8 hr + R1881	Vertebra	AI/AS	Yes	Yes	WT
LuCaP 8 hr - (no R1881 treatment)	Vertebra	AI/AS	Yes	Yes	WT
LuCaP 8 hr + R1881	Vertebra	AI/AS	Yes	Yes	WT
PC348c 2 hr - (no R1881 treatment)	Vertebra	AI/AS	Yes	Yes	WT
PC348c 8 hr + R1881	Vertebra	AI/AS	Yes	Yes	WT
PC3	Bone	AI	No	No	n/a
GB448	Brain	AI	No	No	n/a
PC3489CC	Vertebra	AI	Yes	Yes	WT
PC348Flu1	Vertebra	AI	Yes	Yes	WT
PC348Flu2	Vertebra	AI	Yes	Yes	WT
PC265	Primary	AD	Yes	Yes	WT
PC348	Lymph node	AD	Yes	Yes	WT
PC-EW	Primary	AS	Yes	Yes	WT
PC32	Primary	AS	Yes	Yes	WT
PC133	Primary	AI	No	No	n/a
PC135	Primary	AI	No	No	n/a
PC324	Primary	AI	No	No	n/a
PC374	Primary	AI	No	No	n/a

FIGURE 2

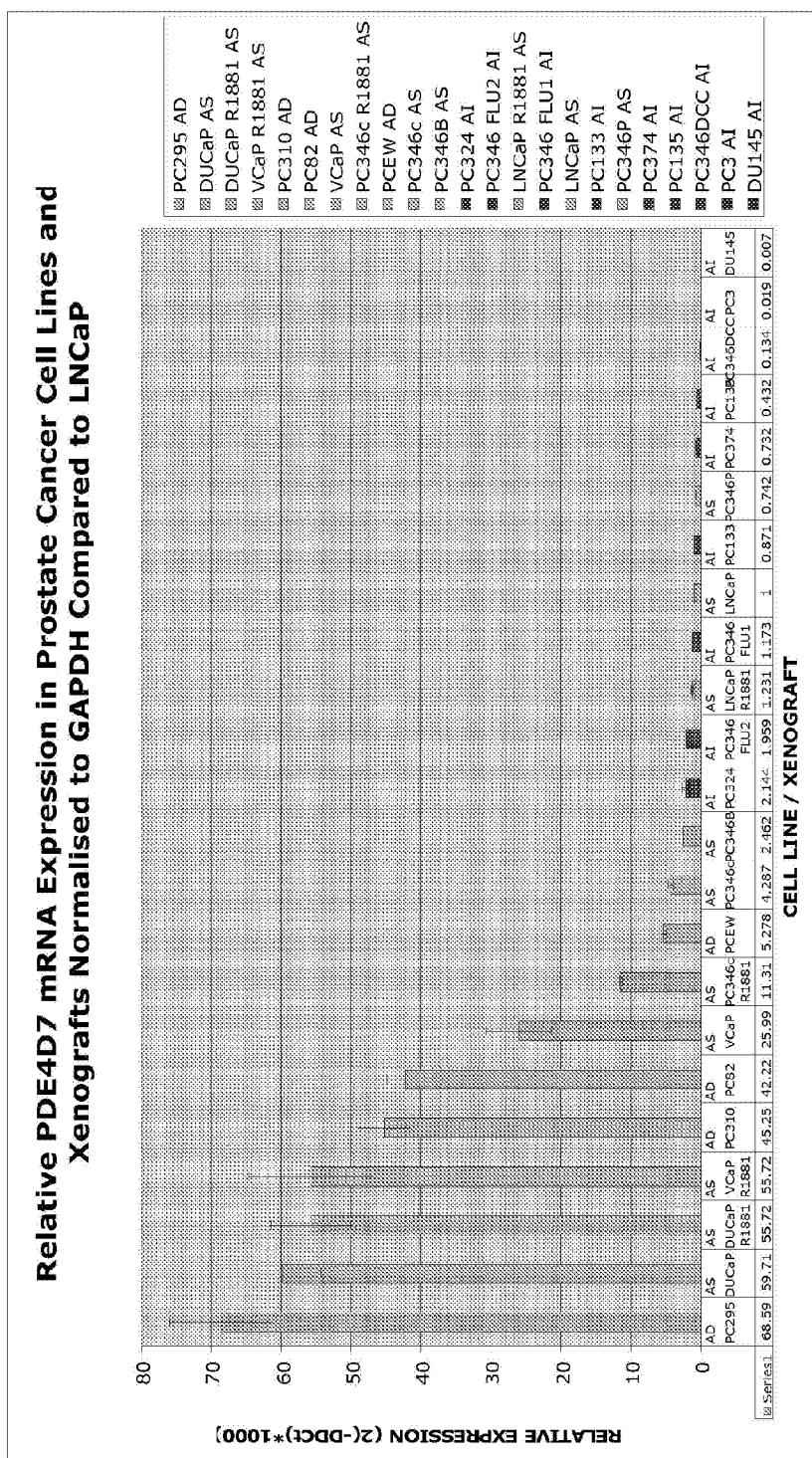


FIGURE 3

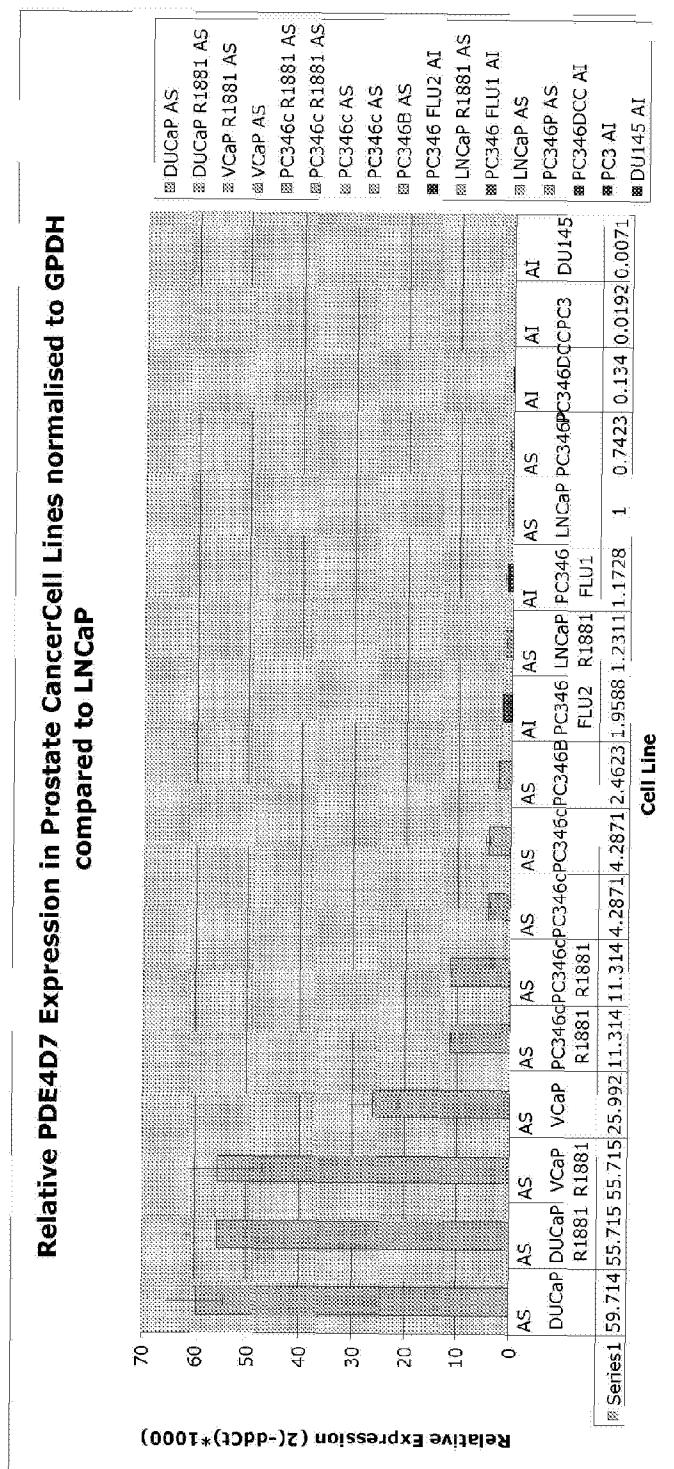


FIGURE 4

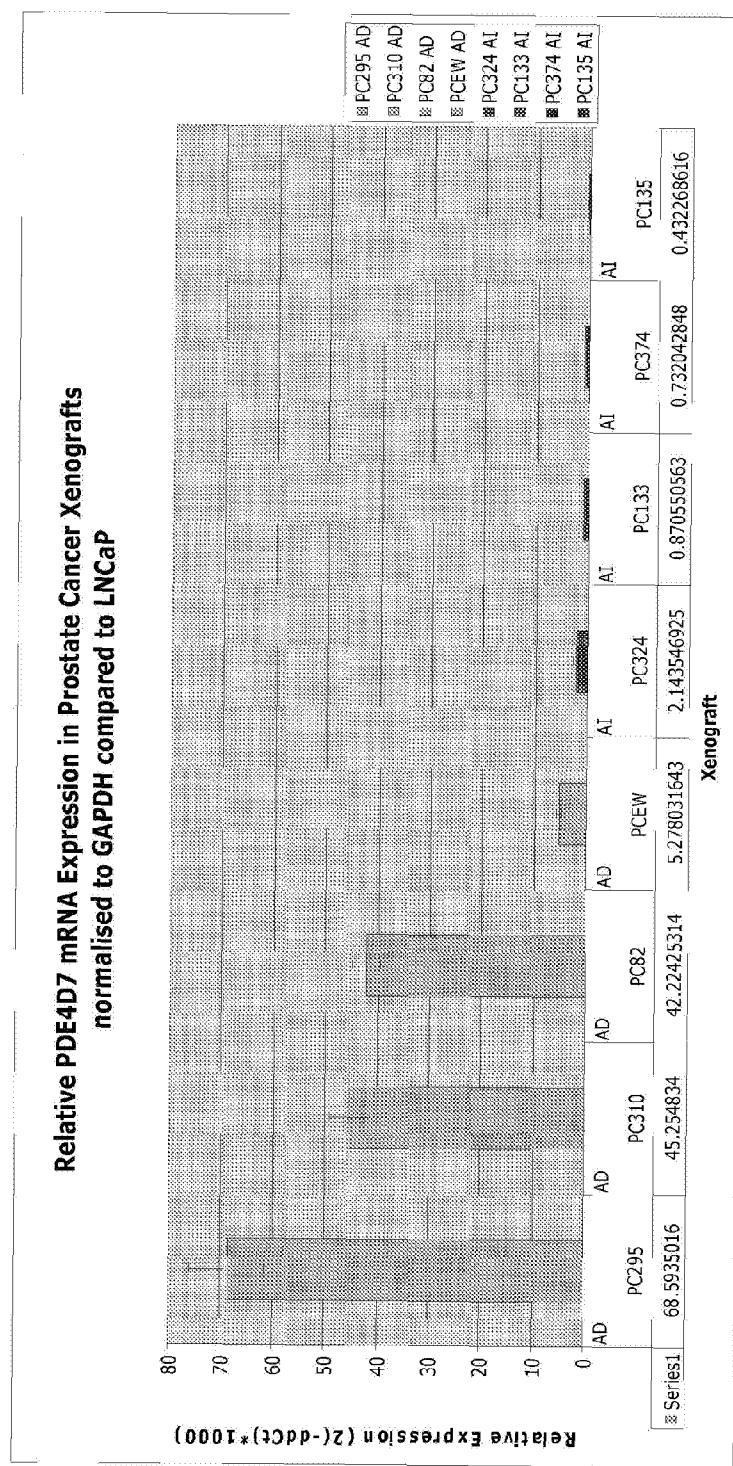


FIGURE 5

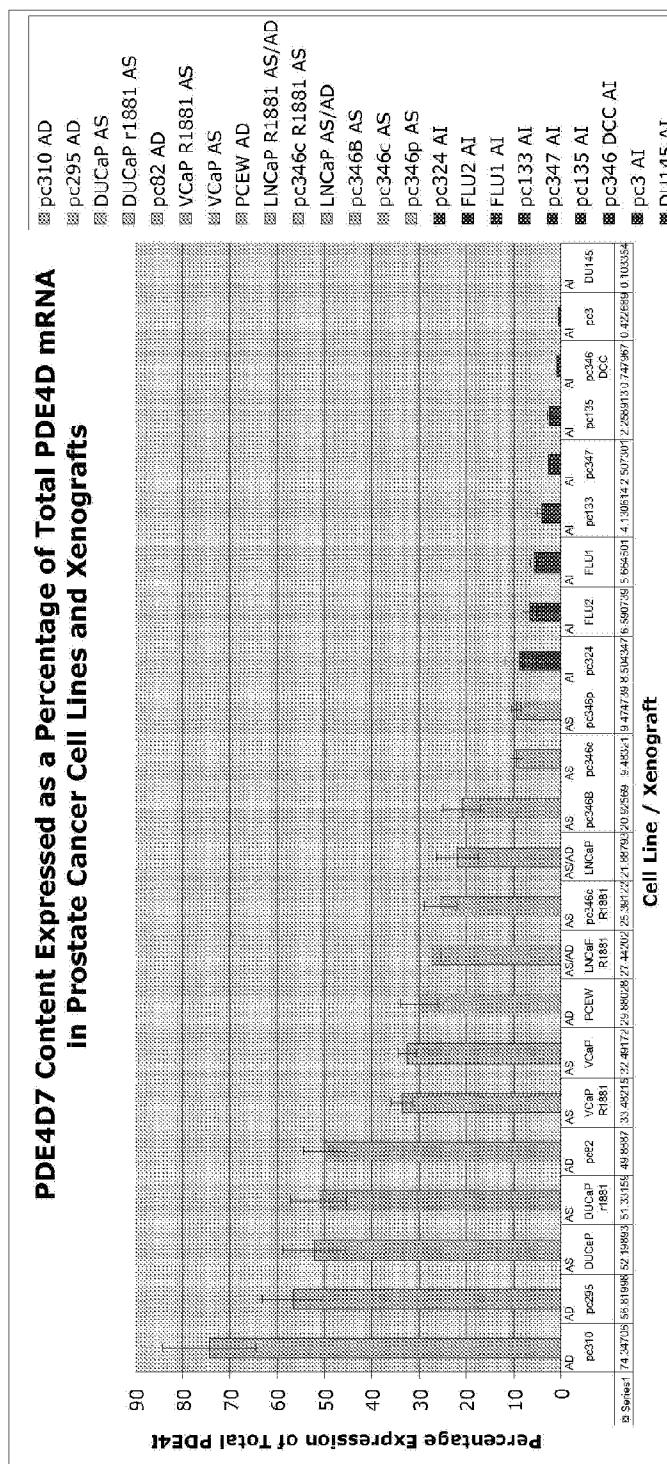


FIGURE 6

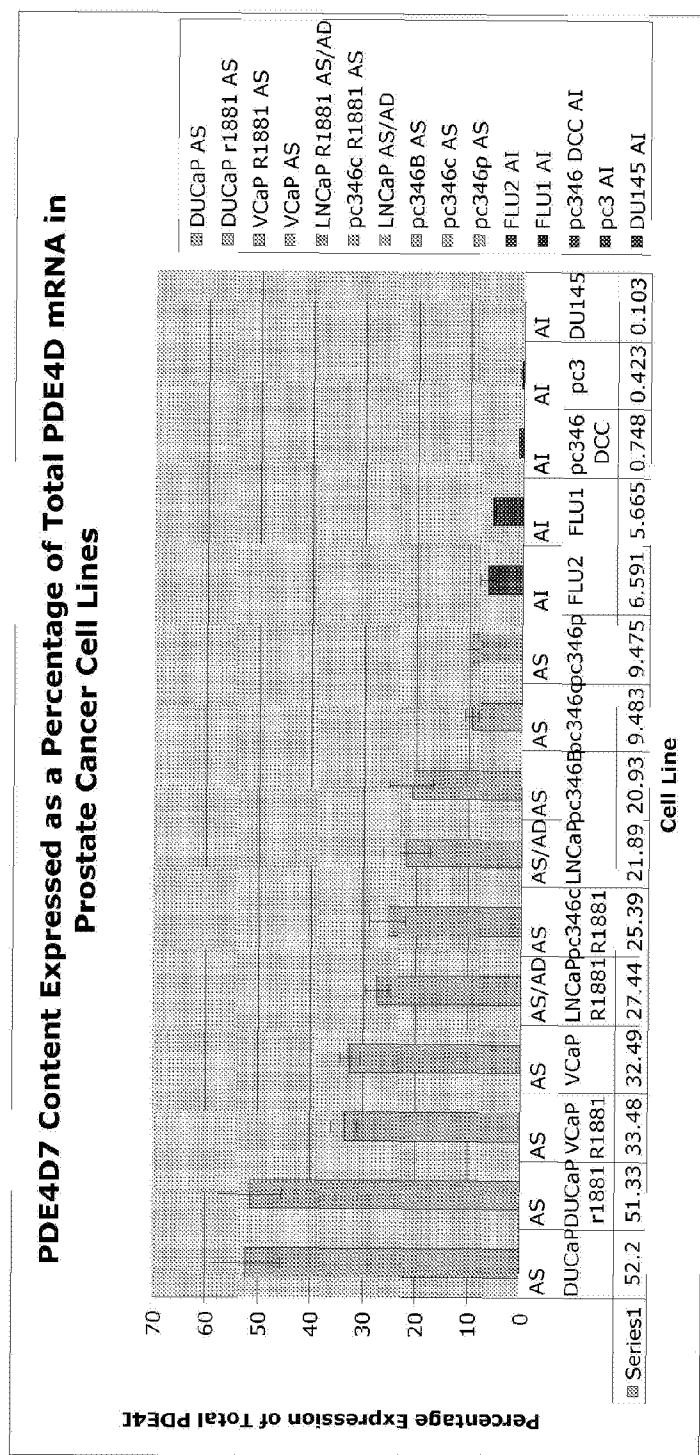


FIGURE 7

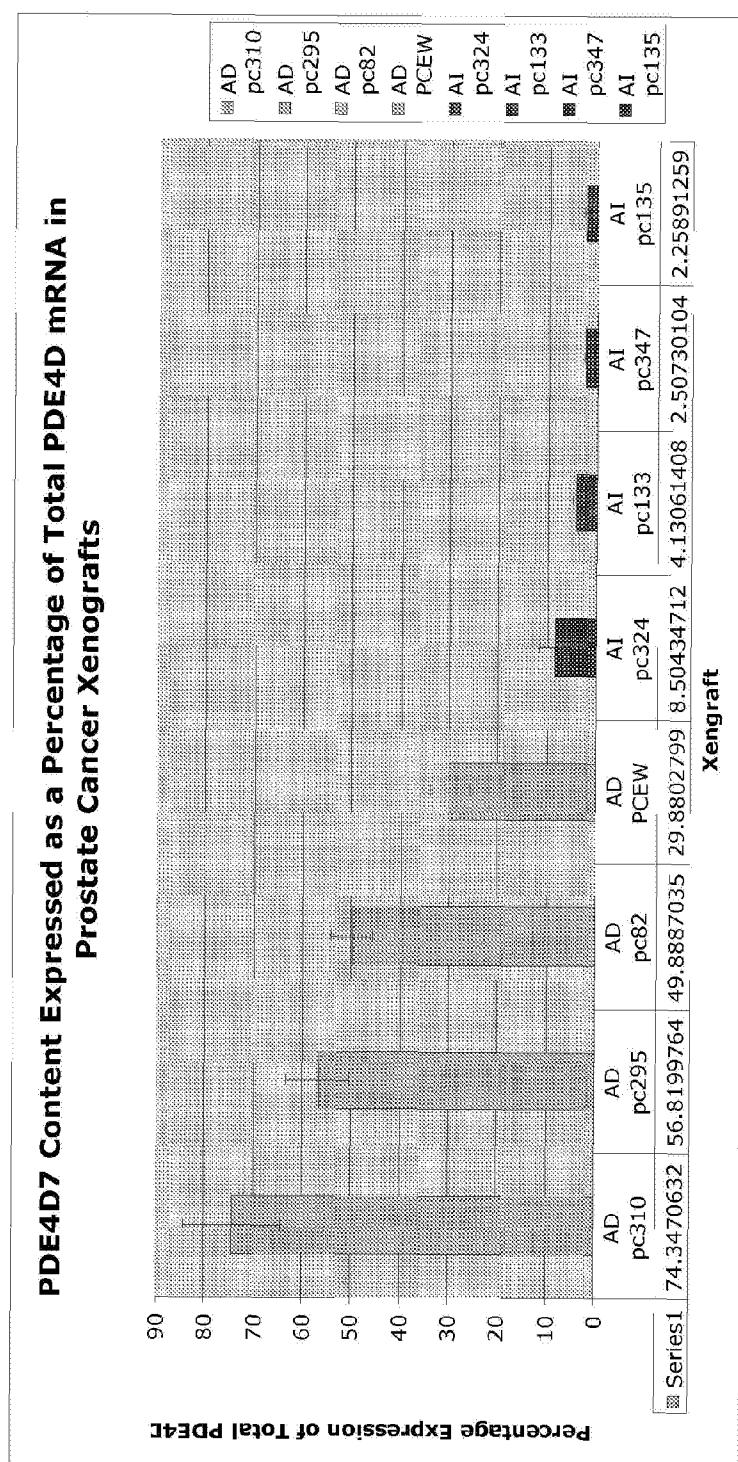


FIGURE 8

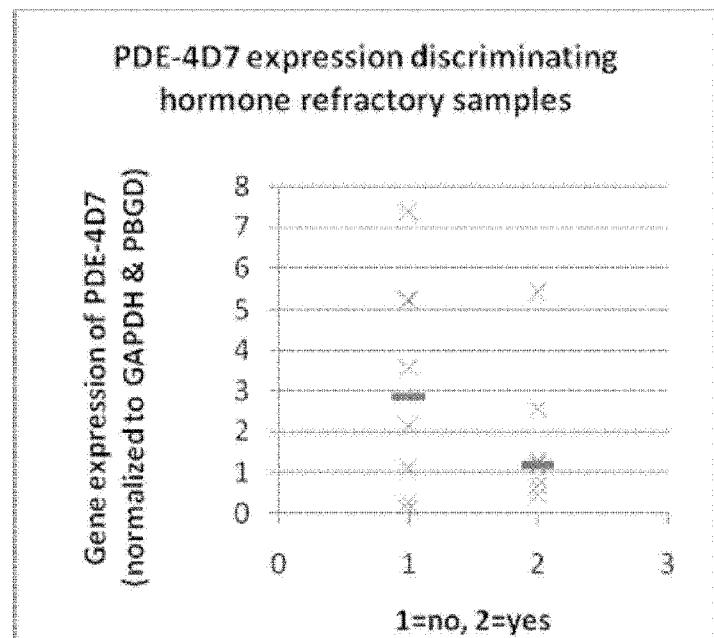


FIGURE 9

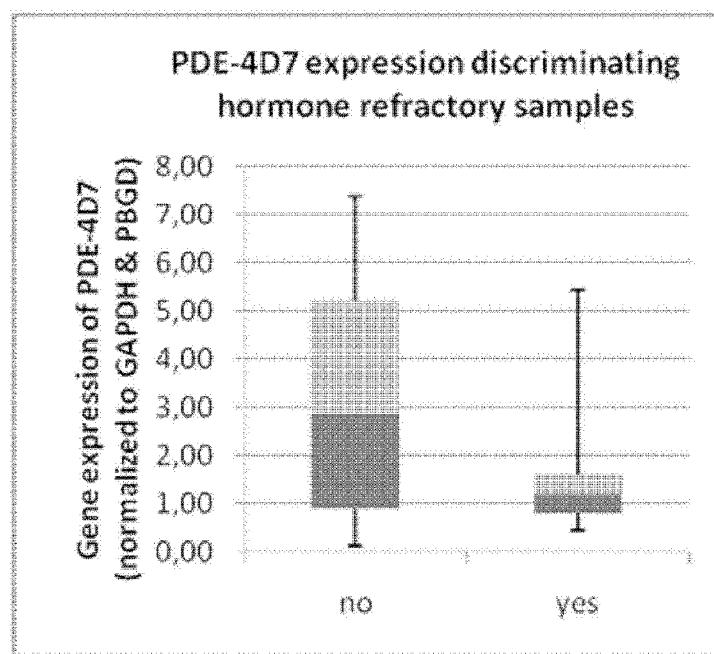


FIGURE 10

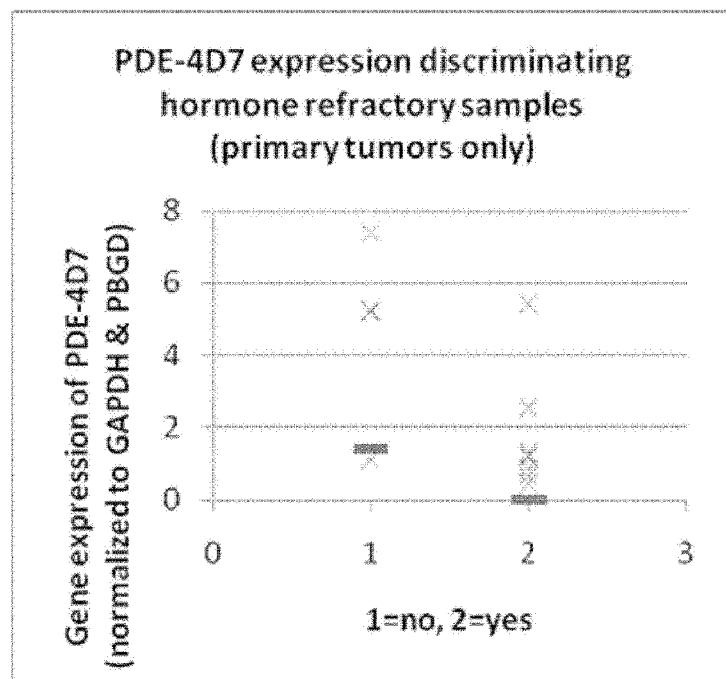


FIGURE 11

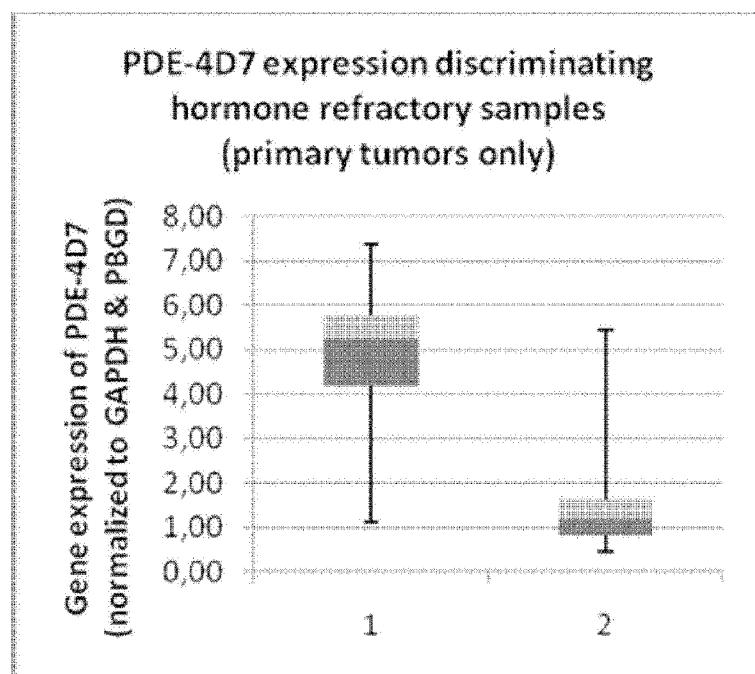


FIGURE 12

