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(54) **Title**: THERAPEUTIC EFFECTS OF ACE INHIBITORS IN PATIENTS WITH DIABETES MELLITUS

(57) **Abstract**: The present invention relates to the field of diagnostics. Specifically, it discloses means and methods for the monitoring of a treatment with ACE-inhibitors for the prevention of nephropathy in diabetes patients. Antifibrotic therapy with ACE-inhibitors requires higher dosages than necessary for treating high blood pressure. However, high dosages of ACE-inhibitors may lead to renal dysfunction. The present invention provides means and methods for determining the therapeutically effective amount of ACE-inhibitors for antifibrotic therapy. By monitoring the antifibrotic effect of ACE-inhibitors the administered dosages can be limited to the necessary dosages, thus reducing the danger of renal dysfunction as a side effect of antifibrotic therapy.

Therapeutic effects of ACE inhibitors in patients with diabetes mellitus

The present invention relates to the field of diagnostics. Specifically, it discloses means and methods for the monitoring of a treatment with ACE-inhibitors for the prevention of nephropathy in diabetes patients. The present invention provides means and methods for determining the therapeutically effective amount of ACE-inhibitors for antifibrotic therapy.

An aim of modern medicine is to provide personalized or individualized treatment regimens. Those are treatment regimens which take into account a patient's individual needs or risks. Individualized treatment regimens promise to maximize the benefits for the patient while minimizing undesired side effects of the treatment. A prerequisite for individualized treatment regimens are diagnostic tests which allow the monitoring of the effects and side effects for a given treatment.

Fibrosis is a pathophysiological mechanism which underlies many diseases, amongst others cardiac fibrosis and diabetic nephropathy. Fibrosis is a consequence of local or systemic inflammatory processes. Generally, in the course of fibrosis functional cells in an organ are replaced by mesenchymal cells which form connective tissue. The replacement of functional cells by connective tissue compromises the function of the affected organ. One important mechanism underlying fibrosis is the emergence of fibroblasts from endothelial cells, a process known as "endothelial-mesenchymal transition" (EndMT). Transforming growth factor-β1 (TGF-β) promotes EndMT, while bone morphogenetic protein 7 (BMP-7) inhibits it. BMP-7 has been shown to have a protective effect against fibrosis in heart and kidney (Dussaule JC and Chatziantoniou C, 2007, Cell Death and Differentiation 14: 1343-1349; Zeisberg EM et al., 2007, Nature Medicine 13: 952-961).

Cardiac fibrosis is caused by inflammatory processes in the heart muscle. Inflammation leads to an excessive deposition of extracellular matrix in the muscle. Thus, the walls of the heart become more rigid. Due to the increased stiffness of the walls of the heart the heart chambers do not fill properly with blood during diastole. The consequence is heart failure, i.e. the inability of the heart to supply the body with enough oxygenated blood

(Vasan et al., 2001, N Engl J Med, 344: 56-59). The inflammatory processes leading to cardiac fibrosis may be caused by high blood pressure or heart failure. Inflammation may, however, also occur without an identifiable cause.

Diabetic nephropathy is a consequence of longstanding diabetes, especially if blood glucose levels are not controlled tightly. It is caused by fibrotic processes. Anatomically it is characterized by a dropout of podocytes, thickening of the basal lamina and sclerosis of the capillaries in the glomerulus. As a consequence the glomeruli lose their ability to filtrate the primary urine properly. Normal glomeruli retain large molecules such as albumin in the blood. The first sign of sclerotic processes is microalbuminuria, i.e. the passing of small amounts of albumin from the blood into the urine. Microalbuminuria can be detected by sensitive albumin tests. A key mediator of diabetic nephropathy is TGF-β. In diabetic nephropathy TGF-β-levels are increased, possibly as a consequence of high blood glucose levels and excessive activation of glucose dependent metabolic pathways in the kidney (Gnudi et al., 2007, J Am Soc Nephrol, 18: 2226-2232). Bone morphogenic protein 7 (BMP-7), in contrast, is down-regulated. It has been shown that increased BMP-7-expression can protect mice against diabetic nephropathy (Wang et al., 2006, J Am Soc Nephrol, 17: 2504-2512). If left untreated, diabetic nephropathy progresses within a few years to terminal renal failure and necessitates renal replacement therapy.

It has been demonstrated that ACE-inhibitors have antifibrotic and anti-inflammatory effects in liver diseases, although AT_1-receptor inhibitors are more effective for this purpose.

The nephroprotective effect of a treatment with ACE-inhibitors in diabetes patients has been shown (Gæde P et al., 2008, Effect of a Multifactorial Intervention in Type 2 Diabetes" The New England Journal of Medicine, 358: 580-591). There are strong indications that abnormalities in the renin-angiotensin system precede the onset of renal damage in diabetes patients (Lely AT et al., 2007 "Angiotensin I-Converting Enzyme: A Pathogenic role in Diabetic Renal Damage?" Current Diabetes Reviews 3: 41-52).

ACE-inhibitors inhibit the angiotensin converting enzyme which converts angiotensin I into angiotensin II. They reduce cardiac myocyte growth, myocyte apoptosis, aldosterone release, norepinephrine release, fibroblast proliferation, smooth muscle proliferation and vasoconstriction. ACE-inhibitors are frequently used for the treatment or prevention of heart failure.

ACE inhibitors are also used for the treatment of heart failure in diabetes patients. Nesto (Reviews in Cardiovascular Medicine, 2004, vol. 5, no. 1, pages 1 to 8), e.g. describes that trandolapril reduced the rate of progression to severe heart failure by 62% in diabetic patients. This effect, however, was not observed in patients without diabetes.

The effects of ACE-inhibitors differ between different patients due to environmental factors or polymorphisms of the angiotensin receptor (Lely et al., loc. cit.). Consequently, it is difficult to determine the required dosage for antifibrotic therapy in an individual patient. However, such an individual assessment of each patient is highly desirable, because renal impairment is a significant adverse effect of ACE inhibitors. Due to the threat of renal failure, it is sensible to limit the administered dosages of ACE-inhibitors strictly to the required dosages for antifibrotic therapy. The nephrotoxic effects of ACE-inhibitors are linked to their influence on angiotensin II-mediated functions such as renal blood flow. Angiotensin II vasoconstricts the efferent arterioles of the glomeruli of the kidney, and thereby increases glomerular filtration rate (GFR), a marker for renal function. Inhibiton of angiotensin II, thus, potentially impairs renal function. The adverse effect on renal function may even be increased if ACE-inhibitors are combined with other pharmaceuticals such as non-steroidal anti-inflammatory drugs.

The problem underlying the present invention can be seen as the provision of means and methods for adjusting and monitoring the use of ACE-inhibitors in antifibrotic therapy. The dosage of ACE-inhibitors has to be high enough to prevent fibrotic processes. On the other hand the dosage should not be higher than strictly necessary in order to avoid renal impairment. Since the therapeutically effective dosages as well as the toxic dosages of drugs differ between different patients, means and methods for determining the required dosage for individual patients are desirable.

The technical problem is solved by the embodiments characterized in the claims and herein below.

The present invention relates to a method for monitoring the therapy with at least one ACE-inhibitor in a patient comprising the steps of

a) determining the amount of BMP-7 in a sample of a patient;
b) comparing the determined amount of BMP-7 to a reference amount.

In a preferred embodiment, the aforementioned method according to the present invention, the sample in step a) is a first sample of said patient, and the reference amount in step b) is the amount of BMP-7 in at least one further sample (preferably a second sample) from said patient. Preferably, said at least one further sample has been obtained after said first sample.

Thus, the method for monitoring the therapy with at least one ACE-inhibitor in a patient, preferably, comprises the steps of

a) determining the amount of BMP-7 in a first sample of a patient;
b) determining the amount of BMP-7 in at least one further sample of a patient; and
c) comparing the level of BMP-7 in the at least one further sample to the level in the first sample.

Preferably, the antifibrotic therapy with at least one ACE inhibitor is monitored. The term "antifibrotic therapy", preferably, refers to a pharmacotherapy with one or more ACE-inhibitors. As described above, fibrosis is a pathophysiological process which leads to the replacement of functional endothelial cells in an organ by mesenchymal cells which form connective tissue. Fibrotic processes can be inhibited or even reversed by bone morphogenetic protein 7 (BMP-7). Surprisingly, it has been found in the study underlying the present invention that high dosages of ACE-inhibitors increase the levels of BMP-7 in a patient. The link between the administration of ACE-inhibitors and the prevention of fibrosis makes it possible to use BMP-7 as a marker for the antifibrotic effects of the treatment with ACE-inhibitors.

ACE-inhibitors have two distinct modes of action: (i) They lower the blood pressure, thereby reducing pressure induced damage to tissues and organs. The effect of ACE-inhibitors on blood pressure was indirectly assessed by measuring the aldosterone levels. Increases of the administered dosages above 40 mg lisinopril had only small effects on the aldosterone levels of the patients (see example 1). (ii) Additionally, ACE-inhibitors interfere directly with fibrotic processes because they increase the levels of BMP-7. For antifibrotic therapy with ACE-inhibitors higher dosages are useful as compared to the control of blood pressure. While dosages of more than 40 mg lisinopril did not influence the levels of aldosterone strongly, higher dosages (60 mg) still increased the levels of BMP-7 in the patients (see example 1).

The term "ACE-inhibitor", preferably refers to benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, and trandolapril.

The term "monitoring the antifibrotic therapy" refers to the control of the success of antifibrotic therapy. This is, preferably, achieved by comparison of the amount of BMP-7 measured in a patient with a reference amount. The reference amount shall allow for monitoring the antifibrotic therapy with at least one ACE inhibitor in a patient. Accordingly, the reference may either be derived from a patient known to have been successfully treated with an antifibrotic therapy with at least one ACE inhibitor or from a patient known to have not been successfully treated with said antifibrotic therapy.

More preferably, the reference amount is the amount of BMP-7 in the at least one further sample (preferably, in a second sample) from the patient to be tested. Preferably, said at least one further sample has been obtained after said first sample.

Accordingly, control of the success of antifibrotic therapy with at least one ACE inhibitor (and, thus, the monitoring of said therapy) is, preferably, achieved by comparing the amount of BMP-7 in a first sample of a patient to the amount of BMP-7 in at least one further sample (preferably, a second sample) from said patient.

Based on the results of the comparison step described herein above, the antifibrotic therapy may be adjusted. The term "adjusting the antifibrotic therapy", preferably, refers to a change in the administered dosage(s) of the administered ACE-inhibitor(s) (based on the results of said comparison). Also preferably, "adjusting the antifibrotic therapy" includes changes of the combination of ACE-inhibitors, i.e. the administration of an additional ACE-inhibitor or the removal of an ACE-inhibitor from the combination.

Preferably, a measured amount of BMP-7 higher than the reference amount indicates that the dosage(s) may be kept constant or even lowered while a measured amount of BMP-7 lower than the reference amount indicates that the dosage has to be increased.

Also preferably, if ACE-inhibitors are insufficient to increase the level of BMP-7, BMP-7 receptor agonists are, preferably, administered, instead of ACE inhibitors.. Preferred BMP-7 receptor agonists are kielin and chordin like protein. Also preferably, other pharmaceuticals are administered in addition to or instead of the at least one ACE-inhibitor if the at least one ACE-inhibitor fails to increase the BMP-7 level. Preferably, these pharmaceuticals are aldosterone receptor antagonists or renin antagonists. Preferred

aldosterone receptor antagonists are spironolactone and eplerenone. A preferred renin antagonist is aliskiren. Also preferably, recombinant BMP-7 is administered to the patient.

The combination of ACE-inhibitors and AT_1–receptor inhibitors has been shown to have a deleterious effect on kidney function, especially in patients with heart failure. Thus, if the ACE-inhibitor fails to increase the amount of BMP-7, the aforementioned combination has to be especially avoided in said patients. The use of AT_1-receptor inhibitors alone has also to be considered carefully in these patients. The term "AT_1–receptor inhibitors", preferably refers to telmisartan, losartan, irbesartan, lalsartan, cadesartan and trandolarpril.

Bone morphogenic protein 7 (BMP-7) is a member of the TGF-β superfamily. It serves as a paracrine signalling molecule, i.e. it is secreted by cells to influence the surrounding cells. It plays a key role in the transformation of mesenchymal cells into bone and cartilage. In addition to this, it plays a role in liver development and in the growth and differentiation of many other cell types. The mature protein is 139 amino acids long. The precursor protein comprises 431 amino acids. The mature BMP-7 protein is derived from this precursor by sequential cleavage (Celeste AJ et al., 1990, PNAS, 87: 9843-9847; Ozkaynak et al., 1990, EMBO J, 9: 2085-2093). The term "BMP-7" encompasses the mature protein as well as its precursors. In the present application the terms "protein" and "polypeptide" are used interchangeably.

BMP-7 as used herein also encompasses variants of the aforementioned specific BMP-7 polypeptide. Such variants have at least the same essential biological and immunological properties as the specific BMP-7 polypeptide. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA assays using polyclonal or monoclonal antibodies specifically recognizing the said BMP-7 polypeptide. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific BMP-7 polypeptide (preferably over the full-lenght of said BMP-7 polypeptide). The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not

comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Add. APL. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman Proc. Natl. Acad Sci. (USA) 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments or subunits of the specific BMP-7 polypeptide or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Such fragments may be, e.g., degradation products of the BMP-7 peptide. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

Determining the amount of BMP-7 or any other peptide or polypeptide referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal - sometimes referred to herein as intensity signal - may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay devices and methods which may utilize labelled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on Elecsys™ analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-Hitachi™ analyzers), and latex agglutination assays (available for example on Roche-Hitachi™ analyzers).

Preferably, determining the amount of a peptide or polypeptide comprises the steps of (a) contacting a cell capable of eliciting a cellular response the intensity of which is indicative of the amount of the peptide or polypeptide with the said peptide or polypeptide for an adequate period of time, (b) measuring the cellular response. For measuring cellular responses, the sample or processed sample is, preferably, added to a cell culture and an internal or external cellular response is measured. The cellular response may include the measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule. The expression or substance shall generate an intensity signal which correlates to the amount of the peptide or polypeptide.

Also preferably, determining the amount of a peptide or polypeptide comprises the step of measuring a specific intensity signal obtainable from the peptide or polypeptide in the sample. As described above, such a signal may be the signal intensity observed at an m/z variable specific for the peptide or polypeptide observed in mass spectra or a NMR spectrum specific for the peptide or polypeptide.

Determining the amount of a peptide or polypeptide, preferably, comprises the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. A particularly preferred ligand of BMP-7 is a polyclonal or monoclonal antibody that specifically binds BMP-7. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and $F(ab)_2$ fragments that are capable of binding antigen or hapten. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.

Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for an detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labelling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels

("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemoluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include ^{35}S, ^{125}I, ^{32}P, ^{33}P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

The amount of a peptide or polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes,

wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

The term "amount" or "level" (the terms are used interchangeably herein) as used herein encompasses the absolute amount of a polypeptide or peptide, the relative amount or concentration of the said polypeptide or peptide as well as any value or parameter which correlates thereto or can be derived therefrom. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., response levels determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, or urine, more preferably, samples of blood, plasma or serum. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein.

The term "patient" (herein also referred to as "subject") refers to an individual that is receiving ACE-inhibitors as antifibrotic therapy or for whom antifibrotic therapy with ACE-inhibitors is considered. Because poorly controlled blood glucose levels lead to diabetic nephropathy, all diabetes patients are at risk of diabetic nephropathy, no matter whether they suffer from type 1 or type 2 diabetes. Thus, the patient, preferably, suffers from diabetes. More preferably, the patient suffers from type 1 diabetes.

The term "diabetes", preferably, refers to type 1 or type 2 diabetes. In type 1 diabetes (previously called iuvenile-onset or insulin-dependent diabetes), insulin production is absent because of autoimmune pancreatic beta-cell destruction, possibly triggered by environmental exposure in genetically susceptible people. Destruction progresses subclinically over months or years until beta-cell mass decreases to the point that insulin concentrations are no longer adequate to control plasma glucose levels. The type 1 diabetes generally develops in childhood or adolescence and until recently was the most common form diagnosed before age 30; however, it can also develop in adults.

In type 2 diabetes (previously called adult-onset or non-insulin-dependent diabetes), insulin secretion is inadequate. Often insulin levels are very high, especially early in the disease, but peripheral insulin resistance and increased hepatic production of glucose makes insulin levels inadequate to normalized plasma glucose levels. Insulin production then falls, further exacerbating hyperglycemia. The disease generally develops in adults and becomes more common with age. Plasma glucose levels reach higher levels after eating in older than in younger adults, especially after high carbohydrate loads, and take longer to return to normal, in part because of increased accumulation of visceral and abdominal fat and decreased muscle mass.

The term "comparing" as used herein encompasses comparing the amount of the peptide or polypeptide comprised by the sample to be analyzed with an amount of a suitable reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (c) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by

a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount determined in step a) and the reference amount, it is possible to determine the effective dosage of at least one ACE-inhibitor that has to be administered to a patient. Furthermore, during the therapy with at least one ACE-inhibitor the comparison of the measured amount of BMP-7 with the desired amount enables the adjustment of the antifibrotic therapy to the patient's needs.

Preferably, the first sample is taken before or at the onset (i.e. the initiation) of antifibrotic therapy. The at least one further sample is taken after the first sample, preferably after 6 weeks to give the patient time to adapt to the therapy. If an ongoing antifibrotic therapy with ACE-inhibitors is to be monitored, samples are, preferably, taken every 3 months, every 6 months or every 12 months.

Thus, it is particularly contemplated to obtain the at least one further sample (preferably, the second sample) 6 weeks, 3 months, 6 months or 12 months after the previous sample (preferably, the first sample).

Preferably, antifibrotic therapy is considered successful if the level of BMP-7 in the patient rises after initiation of antifibrotic therapy. Thus, an increased amount of BMP-7 in the at least one further sample (preferably, the second sample) as compared to the amount in the first sample, preferably, indicates that the antifibrotic therapy with at least one ACE-inhibitor is successful and, thus, has an antifibrotic effect. Preferably, a therapy has an antifibrotic effect if the therapy reduces fibrosis or prevents progression of fibrosis in a patient. Whether a therapy reduces fibrosis or prevents progression can be determined by well known methods.

More preferably, the BMP-7 level increases significantly. Thus, a significantly increased amount of BMP-7 in the at least one further sample (preferably, the second sample) as compared to the amount in the first sample, preferably, indicates that the antifibrotic therapy with at least one ACE-inhibitor is successful.

Whether an increase is statistically significant can be determined by the person skilled in the art without further ado by using the statistical tests described below. Even more preferably, in successful antifibrotic therapy the BMP-7 level increases by at least 20 %, 30 %; 40 %; 50 %, 60 %, 70 %, 80 %, 90 %, 100 % or 200 % as compared to the reference

amount (which is, preferably, the amount of BMP-7 in the at least one further sample). Most preferably, it increases by at least 100 % as compared to the reference amount. Conversely, if BMP-7 levels remain close to the lower limit of detection despite treatment with an ACE-inhibitor, the antifibrotic therapy with ACE-inhibitors is, preferably, considered a failure. This was the case with at least 25 % of the patients in the study underlying the present invention even at the highest dosage of lisinopril (see example 1, table 2).

Thus, preferably, a decrease of the amount of BMP-7 in said at least one further sample (preferably the second sample) as compared to the amount of BMP-7 in said first sample, or an unchanged amount of of BMP-7 in said at least one further sample (preferably the second sample) as compared to the amount of BMP-7 in said first sample, preferably, indicates the antifibrotic therapy with at least one ACE inhibitor is not successful, and, thus. A therapy is considered as not successful if it has no or negligible antifibrotic effects. A therapy is considered as not successful if fibrosis progresses during said therapy.

Preferably, a significant decrease of the amount of BMP-7 in the at least one further sample as compared to the amount of BMP-7 in the first sample indicates that the antifibrotic therapy with the at least one ACE inhibitor is not successful. Preferred significant decreases are 5% , 10%, 15%, 20% or, preferably, 25% of the amount of BMP-7 in the at least one further sample as compared to the amount of BMP-7 in the first sample indicates that the antifibrotic therapy with the at least one ACE inhibitor is not successful.

Thus, the present invention provides a method for monitoring the success of the antifibrotic therapy with ACE-inhibitors in a patient.

Depending on the results of the comparison step, i.e. depending on whether the amount of BMP increases or decreases during therapy, the therapy with the at least one ACE-inhibitor can be adjusted (for an explanation of the term, see herein above).

To achieve a maximal antifibrotic effect it would be, in principle, desirable to maximize the dosages of ACE-inhibitors. Unfortunately, this is impossible, because high dosages of ACE-inhibitors lead to renal dysfunction. Thus, the dosage of the ACE-inhibitor for antifibrotic therapy has to be carefully adjusted. According to the method of the present invention a dosage of an ACE-inhibitor can be determined that is sufficient for antifibrotic therapy, i.e. a therapeutically effective amount of at least one ACE-inhibitor. Consequently, the administered dosage of one or more ACE-inhibitors can be strictly

limited to the therapeutically effective amount. The dangers of excessive dosages are, thus, minimized. In principle, the method of the present invention enables the determination of a therapeutically effective amount for individual patients, thus allowing individualized treatment regimens.

Accordingly, the present invention relates to a method for deciding about adjusting the antifibrotic therapy with at least one ACE-inhibitor in a patient comprising the steps of

a) determining the amount of BMP-7 in a first sample of a patient;
b) determining the amount of BMP-7 in at least one further sample of a patient;
c) comparing the level of BMP-7 in the at least one further sample to the level in the first sample.

The aforementioned method may further comprise the step d) of deciding about adjusting the antifibrotic therapy with at least one ACE-inhibitor according to the result of c).

The term "adjusting the antifibrotic therapy" with at least one ACE-inhibitor has been explained elsewhere herein.

Preferably, a measured amount of BMP-7 in the first sample higher than the reference amount indicates that the dosage(s) may be kept constant or even lowered while a measured amount of BMP-7 lower than the reference amount indicates that the dosage shall be increased.

Preferably, an increase of the amount of BMP-7 in the at least one further sample as compared to the first sample indicates that the dosage (s) of said at least one ACE inhibitor may be kept constant or even decreased.

Preferably, a decrease of the amount of BMP-7 in the at least one further sample as compared to the first sample indicates that the dosage (s) of said at least one ACE inhibitor shall be/has to be increased. Also preferably, if ACE-inhibitors are insufficient to increase the level of BMP-7, BMP-7 receptor agonists are, preferably, administered instead of ACE inhibitos. Preferred BMP-7 receptor agonists are described elsewhere. Also preferably, other pharmaceuticals may be administered in addition to or instead of the at least one ACE-inhibitor if the at least one ACE-inhibitor fails to increase the BMP-7 level.

Preferably, these pharmaceuticals are aldosterone receptor antagonists or renin antagonists. Preferred aldosterone receptor antagonists and renin antagonist are described elsewhere. Also preferably, recombinant BMP-7 is administered to the patient.

Furthermore, the present invention relates to method for determining the effective dosage of one or more ACE-inhibitors for antifibrotic therapy in a patient comprising the steps of

a) determining the amount of BMP-7 in a first sample of a patient;
b) determining the amount of BMP-7 in at least one further sample of a patient;
c) comparing the amount of BMP-7 in the at least one further sample to the level in the first sample; and
d) determining the dosage of the at least on ACE-inhibitor for antifibrotic therapy according to the result of c).

The term "effective dosage of one or more ACE-inhibitors" refers to the amount(s) of one or more ACE-inhibitors that has/have to be given to a patient in order to inhibit fibrotic processes. Preferably, an effective dosage is the dosage of a single ACE-inhibitor or of a combination of at least two ACE-inhibitors which leads to an increase of the BMP-7-level in a patient, by at least 20 %, 30 %; 40 %; 50 %, 60 %, 70 %, 80 %, 90 %, 100 % or 200 %. Thus, an increase by at least 20 %, 30 %; 40 %; 50 %, 60 %, 70 %, 80 %, 90 %, 100 % or 200 % of the amount BMP-7 in the at least one further sample (preferably, the second sample) as compared to the amount in said first sample, preferably, indicates that the dosage of the of a single ACE-inhibitor or of a combination of at least two ACE-inhibitors is effective, and, thus, inhibits fibrotic processes. A particularly preferred increase is 30%.

In a preferred embodiment of the aforementioned method, instead of the amount of BMP-7, the amount of BNP (brain natriuretic peptide) or ANP (atrial natriuretic peptide) is determined in the first and at least one second sample (steps (a) and (b). In step (c), the, thus, determined amount of BNP or ANP in the first sample is compared the amount of BNP or ANP in the at least one further sample.

It is to be understood that an effective dosage as used in accordance with the present invention may not have the desired effect in all subjects to be treated. However, the term

shall require that a statistically significant portion of subjects suffering from a disease or disorder referred to herein can be successfully treated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student´s t-test, Mann-Whitney test etc. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the treatment shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

In a preferred embodiment of the methods of the present invention the antifibrotic therapy aims at treating or preventing diabetic nephropathy or cardiac fibrosis.

The term "treating" refers to ameliorating the diseases or disorders referred to herein or the symptoms accompanied therewith to a significant extent. Said treating as used herein also includes an entire restoration of the health with respect to the diseases or disorders referred to herein.

The term "preventing" means that the onset of the diseases or disorders or their symptoms is delayed as compared to an untreated patient. Preferably, antifibrotic therapy completely prevents the onset of the diseases or disorders referred to herein.

It is to be understood that treating and preventing as used in accordance with the present invention may not be effective in all subjects to be treated. However, the term shall require that a statistically significant portion of subjects suffering from a disease or disorder referred to herein can be successfully treated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools described above.

Diabetic nephropathy is a leading cause of chronic renal failure. It is characterized by thickening of the glomerular basement membrane, mesangial expansion, and glomerular sclerosis. These changes cause glomerular hypertension and progressive decline. Systemic hypertension may accelerate progression. The disease is usually asymptomatic until a nephrotic syndrome or renal failure develops.

Chronic renal failure can be roughly categorized as diminished renal reserve, renal insufficiency, or renal failure (end-stage renal disease). Initially, as renal tissue loses function, there are few abnormabilities because the remaining tissue increases its performance. Decreased renal function interferes with the kidneys' ability to maintain fluid and electrolyte homeostasis.

The diagnosis of renal failure includes the determination of serum creatinin levels. When creatinin levels rise, chronic renal failure is usually first suspected. The initial step is to determine whether the renal failure is acute, chronic, or acute superimposed or chronic (i.e. an acute disease that further compromises renal function in a patient with chronic renal failure). The cause of renal failure is also determined. Sometimes determining the duration of renal failure helps determine the cause. Testing includes urine analysis with examination of the urinary sediment, electrolytes, urea nitrogen, and creatinin, phosphate, calcium. Sometimes specific serologic tests are necessary to determine the cause. Urine analysis findings depend on the nature of the underlying disorder, but broad or especially waxy casts often are prominent in advanced renal failure of any cause. An ultrasound examination of the kidneys is usually helpful in evaluating for obstructive uropathy and in distinguishing acute from chronic renal failure based on kidney size. Except in certain conditions, patients with chronic renal failure have small shrunken kidneys with thinned, hyperechoic cortex. Obtaining a precise diagnosis becomes increasingly difficult as renal function reaches values close to those of end-stage renal disease. The definite diagnostic tool is renal biopsy, but it is not recommended when ultrasonography indicates small, or fibrotic kidneys.

Progression of chronic renal failure is predicted in most cases by the degree of proteinuria. Patients with nephrotic-range proteinuria usually have a poorer prognosis and progress to renal failure more rapidly. Progression may occur even if the underlying disorder is not active. Hypertension is associated with more rapid progression as well.

The term "cardiac fibrosis" refers to an inflammatory condition of the heart muscle, wherein extracellular matrix is deposited excessively. This leads to an increased stiffness of the walls of the heart chambers and to impaired filling of the chambers during diastole. The consequence of this condition is diastolic heart failure. Cardiac fibrosis may be caused by high blood pressure. However, it may also occur without an identifiable reason.

In a further preferred embodiment of the present invention, the methods of the present invention are used in a patient who suffers from diabetes.

Preferably, the methods of the present invention are in-vitro methods. That means that the determination of BMP-7 is carried out in vitro.

The present invention further relates to a device for determining the effective dosage of one or more ACE-inhibitors for antifibrotic therapy in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount; and
c) (optionally) means for deciding on the effective dosage of the ACE-inhibitor(s).

Furthermore, the present invention relates to a device for monitoring the antifibrotic therapy in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount.

Preferably, the means in a) are for measuring the amount of BMP-7 in a first sample and in at least one further sample of the patient. Preferably, the means in b) are for comparing the amount of BMP-7 in said first sample to the amount of BMP-7 in at least one further sample (preferably, a second sample).

The "device" as used herein, preferably, relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow the prediction. Preferred means for determining the amount of BMP-7, and means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of the peptides are applied, the data obtained by said automatically operating means can be

processed by, e.g., a computer program in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the peptides or polypeptides in an applied sample and a computer unit for processing the resulting data for the evaluation. Alternatively, where means such as test stripes are used for determining the amount of the peptides or polypeptides, the means for comparison may comprise control strips or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides or polypeptides referred to herein. The strip or device, preferably, comprises means for detection of the binding of said peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the peptide, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

The present invention also pertains to a device adapted for carrying out the methods of the present invention disclosed above comprising:

a) an analyzing unit comprising a detection agent which specifically binds BMP-7 adapted for determining the amount of BMP-7 in a sample of a patient; and

b) an evaluation unit for comparing the determined amount in said sample (preferably, the second sample) with the amount(s) of BMP-7 from at least one previous sample of the patient (preferably, from the first sample) whereby an antifibrotic therapy in at least one patient can be monitored or the effective dose of at least one ACE inhibitor can be determined, said unit comprising a database with a value (s) of the amount of

BMP-7 from at least one previous sample (preferably, from the first sample) and a computer-implemented algorithm for carrying out a comparison.

The analyzing unit, preferably, comprises said detection agents in immobilized form on a solid support which is to be contacted to the sample comprising the biomarkers the amount of which is to be determined. The term "detection agent" as used herein refers to an agent which is capable of specifically recognizing and binding to the biomarker polypeptide(s) present in a sample. Moreover, the analyzing unit can also comprise a detector which determines the amount of detection agent which is specifically bound to the biomarker(s). The determined amount can be transmitted to the evaluation unit. Said evaluation unit comprises a data processing element, such as a computer, with an implemented algorithm for carrying out a comparison between the determined amount and a suitable reference.

Moreover, the present invention relates to a kit for determining the effective dosage of one or more ACE-inhibitors for antifibrotic therapy a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount; and
c) (optionally) means for deciding on the effective dosage of the ACE-inhibitor(s).

Moreover, the present invention relates to a kit for monitoring the antifibrotic therapy in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount.

Preferably, the means in a) are for measuring the amount of BMP-7 in a first sample and in at least one further sample of the patient. Preferably, the means in b) are for comparing the amount of BMP-7 in said first sample to the amount of BMP-7 in at least one further sample (preferably, a second sample).

The term "kit" as used herein refers to a collection of the aforementioned compounds, means or reagents of the present invention which may or may not be packaged together. The components of the kit may be comprised by separate vials (i.e. as a kit of separate

parts) or provided in a single vial. Moreover, it is to be understood that the kit of the present invention is to be used for practising the methods referred to herein above. It is, preferably, envisaged that all components are provided in a ready-to-use manner for practising the methods referred to above. Further, the kit preferably contains instructions for carrying out the said methods. The instructions can be provided by a users manual in paper- or electronic form. For example, the manual may comprise instructions for interpreting the results obtained when carrying out the aforementioned methods using the kit of the present invention.

Moreover, the present invention also relates to the in vitro use of the biomarker BMP-7, or a ligand thereof, preferably, in a sample of a patient, for monitoring the antifibrotic therapy with at least one ACE inhibitor, for deciding on adjusting the antifibrotic therapy with at least one ACE inhibitor or for determining the effective dosage of one or more ACE inhibitors for antifibrotic therapy.

Finally, present invention relates to BMP7, or a ligand thereof for monitoring the antifibrotic therapy with at least one ACE inhibitor, for deciding on adjusting the antifibrotic therapy with at least one ACE inhibitor or for determining the effective dosage of one or more ACE inhibitors for antifibrotic therapy.

Preferably, the ligand of BMP-7 specifically binds BMP-7. Preferred ligands of BMP-7 such as antibodies that bind BMP-7 are disclosed herein elsewhere.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

The examples merely illustrate the invention and are not intended to limit its scope in any way.

Example 1:

The study was performed in a collective of 49 type 1 diabetes patients with diabetic nephropathy characterized by albuminuria but otherwise unimpaired renal function. The characteristics of the collective were as shown in table 1:

Table 1: Characteristics of the patient collective

Sex (male/female):	16/33
Age[1] (years):	49 (± 10)
Diabetes duration[1] (years):	33 (± 10)
24-hour ambulatory blood pressure[1] (mm Hg):	142 (± 14)/ 74 (± 8)
UAER[2] (mg/24 hours):	362 (240-545)
GFR[1] (mL min^{-1} 1.73 m^{-2}):	81 (30)

[1]data given with standard deviation

[2]geometric mean (95 % CI)

In an initial wash-out period all antihypertensive medication except for slow-release furosemide was withdrawn. After the wash-out period patients were given 20 mg lisinopril daily. After 6 weeks the dosage was escalated to 40 mg and after another 6 weeks it was escalated to 60 mg. Before each escalation aldosterone and bone morphogenic protein 7 (BMP-7) were measured. Aldosterone was measured by DRG with a radio immunoassay. BMP-7 was determined with the Quantikine human BMP-7 assay (Catalogue # DBP700) produced by R&D Systems. Table 2 gives the results for all patients while table 3 shows the BMP-7 levels of those who responded to treatment with lisinopril (the "responders"). Table 4 shows the BMP-7 levels of those did not responded to treatment with lisinopril (the "non-responders"). A patient was considered to respond to the therapy, if the aldosterone level decreased more than 25% during treatment. A decrease of the aldosterone level of less than 25% indicated that the patient did not respond to the therapy.

It can be seen that several patients in the complete collective did not respond to lisinopril at all. Thus, even at the highest dosage (60 mg/d) at least 25 % of all patients had BMP-7 levels of considerably less than 1 pg/ml. For patients whose BMP-7 levels failed to increase in response to lisinopril this pharmaceutical is not suitable for antifibrotic therapy. BMP-7 levels in almost all of the responders, in contrast, rose by at least 100 % as evidenced by the increase of the median, the 25th percentile and the 75th percentile between the beginning of the study and the last escalation of lisinopril to 60 mg/d.

Table 2: influence of lisinopril on the levels of aldosterone and BMP-7

		Lisinopril		
	Baseline	20 mg	40 mg	60 mg
Aldosterone[1] (pg/ml)	132.90 (100.10/197.90)	99.80 (76.0/124.05)	77.2 (56.55/127.70)	71.35 (54.05/108.50)
BMP-7[1] (pg/ml)	9.72 (0.01/20.71)	13.21 (3.48/26.59)	11.83 (0.38/28.04)	23.81 (0.05/56.74)

[1]Data are given as median (25^{th} perc./75^{th} perc.)

Table 3: Levels of BMP-7 in responders

		Lisinopril		
	Baseline	20 mg	40 mg	60 mg
BMP-7[1] (pg/ml)	13.47 (7.26/31.07)	16.88 (6.46/26.59)	16.88 (9.72/36.18)	36.18 (19.23/63.66)

[1]Data are given as median (25^{th} perc./75^{th} perc.)

Table 4: Levels of BMP-7 in non-responders

		Lisinopril		
	Baseline	20 mg	40 mg	60 mg
BMP-7 (pg/ml)	9.72	8.69	9.02	9.56

Example 2:

A patient with albuminuria is treated with ACE-inhibitors. The treatment starts with 20 mg lisinopril per day. The level of BMP-7 at the onset of treatment is 8.35 pg/ml. After two months the level rises to 8.40 pg/ml. Consequently the dosage is increased to 40 mg lisinopril. Three moths later the level of BMP-7 is 16.90 pg/ml. This level is deemed sufficient so that a further escalation of the dosage is not necessary.

Example 3:

A patient with diabetes mellitus and albuminuria has a BMP-7 level of 7.20 pg/ml. Therapy with 20 mg/d lisinopril is initiated. Despite three increases of the dosage of lisinopril the BMP-7 level in several controls remains in the range between 6.8 and 9.2 pg/ml indicating that the ACE-inhibitor is not effective against the fibrotic processes in the kidney. It is considered to change the medication to AT_1–receptor inhibitors agents or aldosterone antagonists or to an rennin antagonist.

Claims

1. A method for monitoring the antifibrotic therapy with at least one ACE-inhibitor in a patient comprising the steps of

   a) determining the amount of BMP-7 in a sample of a patient;
   b) comparing the determined amount of BMP-7 to a reference amount.

2. The method of claim 1, wherein the sample in step a) is a first sample of said patient, and wherein the reference amount in step b) is the amount of BMP-7 in at least one further sample from said patient, and wherein said at least one further sample has been obtained after said first sample.

3. The method of claim 2, wherein the first sample has been obtained from said patient before or at initiation of the antifibrotic therapy, and wherein the at least one further sample has been obtained 6 weeks after said first sample.

4. The method of claim 3, wherein an increase of the amount of amount of BMP-7 after initiation of the said antifibrotic therapy indicates that the antifibrotic therapy is successful.

5. The method of claim 2, wherein an ongoing antifibrotic therapy is monitored, and wherein the at least one further sample has been obtained 6 months or 12 months after said first sample.

6. The method of any one of claims 1 to 5 , wherein the antifibrotic therapy aims at treating or preventing diabetic nephropathy or cardiac fibrosis.

7. The method of any of claims 1 to 6, wherein the patient suffers from diabetes.

8. The method of any of claims 1 to 7, wherein the at least one ACE-inhibitor is selected from the group consisting of benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, and trandolapril.

9. A method for deciding about adjusting the antifibrotic therapy in a patient comprising the steps of

a) determining the amount of BMP-7 in a first sample of a patient;
b) determining the amount of BMP-7 in at least one further sample of a patient;
c) comparing the amount of BMP-7 in the at least one further sample to the level in the first sample; and
d) deciding about adjusting the antifibrotic therapy with at least one ACE-inhibitor according to the result of c).

10. The method of claim 9, wherein the antifibrotic therapy aims at treating or preventing diabetic nephropathy or cardiac fibrosis, and/or wherein the patient suffers from diabetes.

11. The method of claims 9 or 10, wherein an increase of the amount of BMP-7 in the at least one further sample as compared to the first sample indicates that the dosage (s) of said at least one ACE inhibitor may be kept constant or even decreased.

12. The method of any one of claims 9 to 11, wherein instead of the amount of BMP-7 the amount of BNP is determined in the first and the at least one further sample, and wherein the amount of BNP in the at least one further sample is compared to the amount in the first sample.

13. A device for determining the effective dosage of one or more ACE-inhibitors for antifibrotic therapy in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount; and
c) (optionally) means for determining on the effective dosage of the ACE-inhibitor(s).

14. A kit for determining the effective dosage of one or more ACE-inhibitors for antifibrotic therapy in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient;
b) means for comparing the measured amount of BMP-7 to a reference amount; and
c) (optionally) means for determining on the effective dosage of the ACE-inhibitor(s).

15. A device for monitoring the antifibrotic therapy with at least one ACE-inhibitor in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient; and
b) means for comparing the measured amount of BMP-7 to a reference amount.

16. A kit for monitoring the antifibrotic therapy with at least one ACE-inhibitor in a patient comprising

a) means for measuring the amount of BMP-7 in a sample of the patient; and
b) means for comparing the measured amount of BMP-7 to a reference amount.

17. In vitro use of BMP-7, or a ligand thereof, in a sample of a patient, for monitoring the antifibrotic therapy with at least one ACE inhibitor, for deciding on adjusting the antifibrotic therapy with at least one ACE inhibitor, or for determining the effective dosage of one or more ACE inhibitors for antifibrotic therapy.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/058702

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/74

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 707 810 A (SMART J.E. [US] ET AL.) 13 January 1998 (1998-01-13)	13-16
Y	abstract column 2, line 60 - column 3, line 7 column 7, lines 57-58 column 27, line 3 - column 28, line 14 claims 1-27	1-11,17

X	TUGLULAR S. ET AL.: "Cyclosporine-A induced nephrotoxicity is associated with decreased renal bone morphogenetic protein-7 expression in rats." TRANSPLANTATION PROCEEDINGS, vol. 36, no. 1, January 2004 (2004-01), pages 131-133, XP002555567	1-11,17
Y	abstract page 131, left-hand column page 132, right-hand column	1-11,17

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2010	18/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giry, Murielle

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZEISBERG M. ET AL.: "Reversal of experimental renal fibrosis by BMP7 provides insights into novel therapeutic strategies for chronic kidney disease" PEDIATR. NEPHROL., vol. 23, no. 9, September 2008 (2008-09), pages 1395-1398, XP002555568 abstract page 1395, right-hand column page 1397, left-hand column - right-hand column	1-11,17
A	WO 2005/123070 A1 (FIBROGEN, INC. [US]) 29 December 2005 (2005-12-29) abstract; claims 1-10	1-17
A	WANG G. ET AL.: "Urinary messenger RNA expression of podocyte-associated molecules in patients with diabetic nephropathy treated by angiotensin-converting enzyme inhibitor and angiotensin receptor blocker" EUR. J. ENDOCRINOL., vol. 158, no. 3, March 2008 (2008-03), pages 317-322, XP002555569 abstract page 321, right-hand column, last paragraph	1-17
A	WO 2008/070117 A1 (PROMEDIOR, INC. [US]) 12 June 2008 (2008-06-12) abstract claims 1,19,21,32,34	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/058702

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-8, 13-17(completely); 9-11(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8, 13-17(completely); 9-11(partially)

   Method for monitoring or adjusting antifibrotic therapy with ACE inhibitor(s) aiming at treating for example diabetic nephropathy or cardiac fibrosis of patients suffering from diabetes, said method comprising the determination of bone morphogenic protein 7 (BMP-7).

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2. claims: 12(completely); 9-11(partially)

   Method for adjusting antifibrotic therapy with ACE inhibitor(s) aiming at treating for example diabetic nephropathy or cardiac fibrosis of patients suffering from diabetes, said method comprising the determination of BNP.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/058702

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5707810 A	13-01-1998	NONE	
WO 2005123070 A1	29-12-2005	NONE	
WO 2008070117 A1	12-06-2008	AU 2007328206 A1	12-06-2008
		CA 2708004 A1	12-06-2008
		EP 2094289 A1	02-09-2009
		JP 2010511712 T	15-04-2010
		US 2010111898 A1	06-05-2010