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(72) Inventeurs/Inventors:

BOBADILLA, MARIA, FR;
BADI, LAURA, CH;
DUCHATEAU-NGUYEN, GUILLEMETTE, FR;
ESSIOUX, LAURENT, FR;
LANGEN, HANNO, DE;
MAGNONE, MARIA CHIARA, CH;
SCHINDLER, THOMAS, DE;

...

(73) Propriétaire/Owner:

THE REGENTS OF THE UNIVERSITY OF MICHIGAN,
US

(74) Agent: GOWLING WLG (CANADA) LLP

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CHRONIQUE

(54) Title: BIOMARKERS AND METHODS FOR PROGRESSION PREDICTION FOR CHRONIC KIDNEY DISEASE

(57) Abrégé/Abstract:

Subject of the present invention are biomarkers and methods for the identification of an increased risk of the progression of chronic kidney disease (CKD), or for monitoring chronic kidney disease therapy, comprising the detecting the level of one or more of NTpro BNP, EGF, Apo H, GDF-15, and albumin-to-creatinine ratio.



(72) **Inventeurs(suite)/Inventors(continued):** THIER, MARTINA, CH; FORMENTINI, IVAN, CH;
DURAN PACHECO, GONZALO CHRISTIAN, CH; SOLIER, CORINNE, FR; KRETZLER, MATTHIAS, US; NAIR, VIJI, US;
JU, WENJU, US

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- (71) **Applicant (for all designated States except US):** **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH).
- (71) **Applicant (for US only):** **HOFFMANN-LA ROCHE INC.** [US/US]; 340 Kingsland Street, Nutley, New Jersey 07110 (US).
- (71) **Applicant:** **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 1600 Huron Parkway, 2nd Floor, Ann Arbor, Michigan 48109-2590 (US).
- (72) **Inventors:** **BOBADILLA, Maria**; 50b, rue de Village-Neuf, F-68128 Rosenau (FR). **BADI, Laura**; Klingentalgraben 17, CH-4057 Basel (CH). **DUCHAT-EAU-NGUYEN, Guillemette**; 11, rue Bellevue, F-68400 Riedisheim (FR). **ESSIOUX, Laurent**; 6, rue Charles de Gaulle, F-68220 Attenschwiller (FR). **LANGEN, Hanno**; Rebweg 1, 79540 Loerrach (DE). **MAGNONE, Maria Chiara**; Leimenstrasse 30, CH-4051 Basel (CH). **SCHINDLER, Thomas**; Lisa-Rees-Strasse 10, 79540 Loerrach (DE). **THIER, Martina**; Nonnenweg 64D, CH-4055 Basel (CH). **FORMENTINI, Ivan**; Spalenring 160, CH-4055 Basel (CH). **DURAN PACHECO, Gonzalo Christian**; Baeumliweg 18, CH-4125 Riehen (CH). **SOLLIER, Corinne**; 10, rue Lina Ritter, F-68510 Sierentz (FR). **KRETZLER, Matthias**; The Regents of the University of Michigan, 1600 Huron Parkway, 2nd Floor, Ann Arbor, Michigan 48109-2590 (US). **NAIR, Viji**; The Regents of the University of Michigan, 1600 Huron Parkway,

2nd Floor, Ann Arbor, Michigan 48109-2590 (US). **JU, Wenju**; The Regents of the University of Michigan, 1600 Huron Parkway, 2nd Floor, Ann Arbor, Michigan 48109-2590 (US).

(74) **Agent:** **BRODBECK, Michel**; Grenzacherstrasse 124, CH-4070 Basel (CH).

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(54) **Title:** BIOMARKERS AND METHODS FOR PROGRESSION PREDICTION FOR CHRONIC KIDNEY DISEASE

(57) **Abstract:** Subject of the present invention are biomarkers and methods for the identification of an increased risk of the progression of chronic kidney disease (CKD), or for monitoring chronic kidney disease therapy, comprising the detecting the level of one or more of NTpro BNP, EGF, Apo H, GDF-15, and albumin-to-creatinine ratio.



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BIOMARKERS AND METHODS FOR PROGRESSION PREDICTION
FOR CHRONIC KIDNEY DISEASE

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FIELD OF THE INVENTION

10 The present disclosure relates to the field of diagnostic measures.

BACKGROUND

Epidemiologic studies and clinical registries have reported that chronic kidney disease (CKD) has a discrete increasing prevalence but it is clear that patients with CKD are a heterogeneous group with
15 different diagnosis, diverse disease etiologies, variable prognosis and markedly diverse rate of progressive decline (1, 2) (3). CKD is thought traditionally to follow an unremittingly progressive decline over time, with often an inconstant rate of decline, and even increases in baseline renal function (glomerular filtration rate (GFR)) may also be common(4) (5). Several studies identified risk factors for CKD progression or increased renal function loss, however it is also recognized that only a relatively small
20 percentage of the individuals with CKD eventually progress to end-stage renal disease (ESRD).

Renal disease progression may follow linear and non-linear trajectories with only a minority accelerating swiftly to end-stage kidney disease (6). The prediction of speed or change in disease progression is a challenging disease characteristic to forecast. Nevertheless the ability to identify those individuals at greatest risk of progression that may require intensification of standard therapy from those at low risk to
25 be spared unnecessary intervention may well be the cornerstone strategy to overcome the current limitations in renal clinical development. From a clinical and therapeutic point of view, early detection of fast progressing patients allows a closer monitoring of both adherence and therapeutic efficacy and may guide intensification of standard therapy (7-9). From a drug development perspective, being able to distinguish patients with an increased risk of disease progression from those patients with a lower risk is
30 an essential step for increasing the probability of success and impact of a novel therapeutic approach.

Such a patient stratification protocol would result in increased efficacy with acceptable safety within a desirable development timeframe. Clinical nephrology has suffered so far of the lack of those concrete tools to identify distinct disease dynamic changes that inevitably would help clinical decision making, promote novel therapeutic approaches and bring innovation in the design of Proof-of-Concept (PoC) clinical studies.

Therefore there is a compelling clinical need for novel risk scores, clinical predictors and/or biomarkers to identify individuals with an increased risk of CKD disease progression at the earliest possible stage (11) (12). The need for risk stratification within CKD is particularly great among patients in the general population or primary care because the majority of patients with CKD are first identified in this setting and most are never referred to a nephrologist.

BRIEF SUMMARY OF THE DISCLOSURE

The present disclosure provides biomarkers and methods for the identification of an increased risk of the progression of chronic kidney disease (CKD), or for monitoring chronic kidney disease therapy.

One aspect of the invention provides for a method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising a) detecting the amount of one, two, three, four or five biomarkers selected from the group consisting of GDF-15, EGF, NT-proBNP, ApoH, and albumin to creatinine ratio (AUCR) in a sample of the subject; b) comparing the amount of the one, two, three, four or five biomarkers to a reference amount of the one, two, three, four or five biomarkers; and c) identifying the subject as having an increased risk for disease progression if the amount of the one, two, three, four or five biomarkers in the sample is greater than the reference amount of the one, two, three, four or five biomarkers.

In one embodiment, the method comprises a) detecting the amount of GDF-15 in the sample of the subject; b) comparing the amount of GDF-15 to a reference amount of GDF-15; and c) identifying the subject as having an increased risk for disease progression if the amount of the GDF-15 in the sample is greater than the reference amount of GDF-15. In one embodiment, the method comprises a) detecting the amount of EGF in the sample of the subject; b) comparing the amount of EGF to a reference amount of EGF; and c) identifying the subject as having an increased risk for disease progression if the amount of the EGF in the sample is greater than the reference amount of EGF. In one embodiment, the method comprises a) detecting the amount of NT-proBNP in the sample of the subject; b) comparing the amount of NT-proBNP to a reference amount of NT-proBNP; and c) identifying the subject as having an increased risk for disease progression if the amount of the NT-proBNP in the sample is greater than the

reference amount of NT-proBNP. In one embodiment, the method comprises a) detecting the amount of ApoH in the sample of the subject; b) comparing the amount of ApoH to a reference amount of ApoH; and c) identifying the subject as having an increased risk for disease progression if the amount of the ApoH in the sample is greater than the reference amount of ApoH. In one embodiment, the method

5 comprises a) detecting the amount of AUCR in the sample of the subject; b) comparing the amount of AUCR to a reference amount of AUCR; and c) identifying the subject as having an increased risk for disease progression if the amount of the AUCR in the sample is greater than the reference amount of AUCR.

Another aspect of the invention provides for a method for identifying a subject suffering from chronic

10 kidney disease as having an increased risk for disease progression, the method comprising a) detecting the amount of ApoH and one or more additional biomarkers selected from the group consisting of GDF-15, EGF, NT-proBNP, and albumin to creatinine ratio (AUCR) in a sample of the subject; b) comparing the amount of ApoH and the one or more additional biomarkers to a reference amount of ApoH and the one or more additional biomarkers; and c) identifying the subject as having an increased risk for disease

15 progression if the amount of ApoH in the sample and the amount of the one or more additional biomarkers is greater than the reference amount of ApoH and the one or more additional biomarkers.

In one embodiment, the one or more additional biomarkers is GDF-15. In one embodiment, the one or more additional biomarkers is EGF. In one embodiment, the one or more additional biomarkers is NT-proBNP. In one embodiment, the one or more additional biomarkers is AUCR. In one embodiment, the

20 one or more additional biomarkers is GDF-15 and EGF. In one embodiment, the one or more additional biomarkers is GDF-15 and NT-proBNP. In one embodiment, the one or more additional biomarkers is GDF-15 and AUCR. In one embodiment, the one or more additional biomarkers is EGF and NT-proBNP. In one embodiment, the one or more additional biomarkers is EGF and AUCR. In one embodiment, the one or more additional biomarkers is NT-proBNP and AUCR. In one embodiment, the one or more

25 additional biomarkers is EGF and AUCR. In one embodiment, the one or more additional biomarkers is GDF-15, NT-proBNP and AUCR. In one embodiment, the one or more additional biomarkers is EGF, NT-proBNP and AUCR. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and AUCR. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and NT-proBNP. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, NT-proBNP, and

30 AUCR.

Another aspect of the invention provides for a method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising a) detecting the amount of albumin to creatinine ratio (AUCR) and one or more additional biomarkers selected from the

group consisting of GDF-15, EGF, NT-proBNP, and ApoH in a sample of the subject; b) comparing the amount of AUCR and the one or more additional biomarkers to a reference amount of AUCR and the one or more additional biomarkers; and c) identifying the subject as having an increased risk for disease progression if the amount of AUCR in the sample and the amount of the one or more additional

5 biomarkers is greater than the reference amount of AUCR and the one or more additional biomarkers.

In one embodiment, the one or more additional biomarkers is GDF-15. In one embodiment, the one or more additional biomarkers is EGF. In one embodiment, the one or more additional biomarkers is NT-proBNP. In one embodiment, the one or more additional biomarkers is ApoH. In one embodiment, the one or more additional biomarkers is GDF-15 and EGF. In one embodiment, the one or more additional
10 biomarkers is GDF-15 and NT-proBNP. In one embodiment, the one or more additional biomarkers is GDF-15 and ApoH. In one embodiment, the one or more additional biomarkers is EGF and NT-proBNP. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is NT-proBNP and ApoH. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15, NT-proBNP and ApoH. In one embodiment, the one or more additional biomarkers is EGF,
15 NT-proBNP and ApoH. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and ApoH. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and NT-proBNP. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, NT-proBNP, and ApoH.

20 Another aspect of the invention provides for a method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising detecting the amount of NT-proBNP and one or more additional biomarkers selected from the group consisting of GDF-15, EGF, albumin to creatinine ratio (AUCR), and ApoH in a sample of the subject; b) comparing the amount of NT-proBNP and the one or more additional biomarkers to a reference amount of NT-
25 proBNP and the one or more additional biomarkers; and c) identifying the subject as having an increased risk for disease progression if the amount of NT-proBNP in the sample and the amount of the one or more additional biomarkers is greater than the reference amount of NT-proBNP and the one or more additional biomarkers.

In one embodiment, the one or more additional biomarkers is GDF-15. In one embodiment, the one or more additional biomarkers is EGF. In one embodiment, the one or more additional biomarkers is
30 AUCR. In one embodiment, the one or more additional biomarkers is ApoH. In one embodiment, the one or more additional biomarkers is GDF-15 and EGF. In one embodiment, the one or more additional biomarkers is GDF-15 and AUCR. In one embodiment, the one or more additional biomarkers is GDF-15

and ApoH. In one embodiment, the the one or more additional biomarkers is EGF and AUCR. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is AUCR and ApoH. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15, AUCR and ApoH. In one embodiment, the one or more additional biomarkers is EGF, AUCR, and ApoH. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and ApoH. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, and AUCR. In one embodiment, the one or more additional biomarkers is EGF, GDF-15, AUCR, and ApoH.

Another aspect of the invention provides for a method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising a) detecting the amount of GDF-15 and one or more additional biomarkers selected from the group consisting of NT-proBNP, EGF, albumin to creatinine ratio (AUCR), and ApoH in a sample of the subject; b) comparing the amount of GDF-15 and the one or more additional biomarkers to a reference amount of GDF-15 and the one or more additional biomarkers; and c) identifying the subject as having an increased risk for disease progression if the amount of GDF-15 in the sample and the amount of the one or more additional biomarkers is greater than the reference amount of GDF-15 and the one or more additional biomarkers.

In one embodiment, the one or more additional biomarkers is NT-proBNP. In one embodiment, the one or more additional biomarkers is EGF. In one embodiment, the one or more additional biomarkers is AUCR. In one embodiment, the one or more additional biomarkers is ApoH. In one embodiment, the one or more additional biomarkers is NT-proBNP and EGF. In one embodiment, the one or more additional biomarkers is NT-proBNP and AUCR. In one embodiment, the the one or more additional biomarkers is NT-proBNP and ApoH. In one embodiment, the one or more additional biomarkers is EGF and AUCR. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is AUCR and ApoH. In one embodiment, the one or more additional biomarkers is EGF and ApoH. In one embodiment, the one or more additional biomarkers is NT-proBNP, AUCR and ApoH. In one embodiment, the one or more additional biomarkers is EGF, AUCR, and ApoH. In one embodiment, the one or more additional biomarkers is EGF, NT-proBNP, and ApoH. In one embodiment, the one or more additional biomarkers is EGF, NT-proBNP, and AUCR. In one embodiment, the one or more additional biomarkers is EGF, NT-proBNP, AUCR, and ApoH.

Another aspect of the invention provides for a method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising a) detecting the amount of EGF and one or more additional biomarkers selected from the group consisting of NT-proBNP, GDF-15, albumin to creatinine ratio (AUCR), and ApoH in a sample of the subject; b)

comparing the amount of EGF and the one or more additional biomarkers to a reference amount of EGF and the one or more additional biomarkers; and c) identifying the subject as having an increased risk for disease progression if the amount of EGF in the sample and the amount of the one or more additional biomarkers is greater than the reference amount of EGF and the one or more additional biomarkers.

- 5 In one embodiment, the one or more additional biomarkers is NT-proBNP. In one embodiment, the one or more additional biomarkers is GDF-15. In one embodiment, the one or more additional biomarkers is AUCR. In one embodiment, the one or more additional biomarkers is ApoH. In one embodiment, the one or more additional biomarkers is NT-proBNP and GDF-15. In one embodiment, the one or more additional biomarkers is NT-proBNP and AUCR. In one embodiment, the one or more additional biomarkers is NT-proBNP and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15 and AUCR. In one embodiment, the one or more additional biomarkers is GDF-15 and ApoH. In one embodiment, the one or more additional biomarkers is AUCR and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15 and ApoH. In one embodiment, the one or more additional biomarkers is NT-proBNP, AUCR and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15, AUCR, and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15, NT-proBNP, and ApoH. In one embodiment, the one or more additional biomarkers is GDF-15, NT-proBNP, and AUCR. In one embodiment, the one or more additional biomarkers is GDF-15, NT-proBNP, AUCR, and ApoH.

- In certain embodiments of the above aspects, the detecting comprises contacting, in vitro, the sample with a combination of detection agents, each agent having specific binding affinity for one of the biomarkers. In certain embodiments, the agent is antibody or fragment thereof.

In certain embodiments of the above aspects, the sample is a serum or urine sample.

- In certain embodiments of the above aspects, the subject is identified as having an increased risk of disease progression when the amount of the biomarkers in the sample is greater than the median of the reference amount. In certain embodiments of the above aspects, the subject is identified as having an increased risk of disease progression when the amount of the biomarkers in the sample is in the fourth quartile range of the reference amount.

In certain embodiments, the method further comprises the step of recommending a therapy to treat the chronic kidney disease, if the subject is identified as having an increased risk for disease progression.

- 30 In certain embodiments, the method further comprises the step of administering to the subject a pharmaceutical agent to treat the chronic kidney disease, if the subject is identified as having an increased

risk for disease progression. The therapy can comprises, for example, an investigational new drug therapy.

Another aspect of the invention provides for a device adapted for carrying out the method, comprising: a) an analysing unit comprising a combination of detection agents which specifically bind to the biomarkers,
5 the analysing unit adapted for contacting, in vitro, the sample from the subject with the detection agent;
b) an evaluation unit including a computing device having a database and a computer-implemented algorithm on the database, the computer-implemented algorithm when executed by the computing device determines an amount of the biomarker in the sample from the subject and compares the determined
10 amount of the biomarker with a biomarker reference amount and provides a diagnosis of at increased risk
for disease progression if the amount of the biomarker determined in the step of determining is greater
than the biomarker reference amount. In one embodiment, the database further includes the biomarker
reference amount.

Another aspect of the invention provides for a kit adapted for carrying out the method, comprising a
detection agent for the biomarkers and instructions for carrying out the method. In one embodiment, the
15 kit further comprises a combination of detection agents for the biomarkers.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-2 shows the results of the statistical analysis of each biomarker and combinations thereof in
relation to time to CKD event.

20 Figure 3 shows Table A which provides a listing of CKD events evaluated from the C-PROBE study
cohort of patients.

Figure 4 shows Table B which provides a listing of patient characteristics and summary statistics
evaluated from the C-PROBE study cohort of patients.

Figure 5 shows exemplary amino acid sequences of GDF-15 (SEQ ID NO: 1).

25 Figure 6 shows an exemplary amino acid sequence of EGF (SEQ ID NO: 2).

Figure 7 shows an exemplary amino acid sequence of NT-proBNP (SEQ ID NO: 3).

Figure 8 shows an exemplary amino acid sequence of ApoH (SEQ ID NO: 4).

Figure 9 shows an exemplary amino acid sequence of Albumin (SEQ ID NO: 5)

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DETAILED DESCRIPTION OF THE DISCLOSURE

Definitions

The term "chronic kidney disease" (CKD) refers to a condition defined as abnormalities of kidney structure or function, present for months, with implications for health which can occur abruptly, and either resolve or become chronic (Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease Guidelines (KDIGO 2012). CKD is a general term for heterogeneous disorders affecting kidney structure and function with variable clinical presentation, in part related to cause, severity and the rate of progression (Kidney International Supplements (2013) 3, vii).

Definition and identification of CKD is defined with the following criteria:

1. For individuals at higher risk of progression, and/or where measurement will impact therapeutic decisions
2. Recognize that small fluctuations in GFR are common and are not necessarily indicative of progression.
3. Define CKD progression based on one of more of the following (Not Graded):
 - a. Decline in GFR category (Z90 [G1], 60–89 [G2], 45–59 [G3a], 30–44 [G3b], 15–29 [G4], ≤ 15 [G5] ml/min/1.73 m²). A certain drop in eGFR is defined as a drop in GFR category accompanied by a 50% or greater drop in eGFR from baseline or End-Stage Renal Disease (ESRD, eGFR < 15 ml/min/1.73 m², Renal Replacement Therapy or death or composite of the above parameters.
 - b. Rapid progression is defined as a sustained decline in eGFR of more than -3.3% per year.
 - c. The confidence in assessing progression is increased with increasing number of serum creatinine measurements and duration of follow-up

This damage can cause wastes to build up in the body and lead to other health problems, including cardiovascular disease (CVD), anemia, and bone disease. CKD is usually an irreversible and progressive disease and can lead to kidney failure, also called End Stage Renal Disease (ESRD), over time if it is not treated

The term "GDF-15" refers to Growth-Differentiation Factor-15, also known as MIC-1 (Macrophage inhibitory cytokine 1), a member of the transforming growth factor beta (TGF-beta.) cytokine superfamily, exemplified by SEQ ID NO:1, shown in FIGURE 5 (Swiss Prot Accession Number NP_004855, Gene ID NCBI 9518); WO99/06445, WO00/70051, WO2005/113585. "GDF-15"

encompasses the protein having the amino acid sequence of SEQ ID NO: 1 as well as GDF-15 variants, homologues and isoforms thereof. Such variants, homologues and isoforms have at least the same essential biological and immunological properties as the specific GDF-15. For example, 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 GDF-15 polypeptides. Exemplary assays are described in the accompanying Examples. 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 of the specific GDF-15 polypeptides 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 GDF-15 polypeptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

The term "EGF" refers the peptide growth factor, exemplified by SEQ ID NO: 2, shown in FIGURE 6 (Swiss Prot Accession Number NP_001954, Gene ID NCBI 1950). "EGF" encompasses the protein having the amino acid sequence of SEQ ID NO: 2 as well as variants, homologues and isoforms thereof. Such variants, homologues and isoforms have at least the same essential biological and immunological properties as the specific EGF. For example, 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 EGF polypeptides. Exemplary assays are described in the accompanying Examples. 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 of the specific EGF polypeptides 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 EGF polypeptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

The term "NT-proBNP" refers to Amino-terminal proBNP, exemplified by SEQ ID NO: 3, shown in FIGURE 7 (Swiss Prot Accession Number NP_002512.1, Gene ID NCBI 4879), WO 02/089657, WO 02/083913, EP 0 648 228. "NT-proBNP" encompasses the protein having the amino acid sequence of SEQ ID NO: 3 as well as variants, homologues and isoforms thereof. Such variants, homologues and isoforms have at least the same essential biological and immunological properties as the specific NT-proBNP. For example, 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 NT-proBNP polypeptides. Exemplary

assays are described in the accompanying Examples. 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 of the specific NT-proBNP polypeptides or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

5 Such fragments may be, e.g., degradation products of the NT-proBNP polypeptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

The term "ApoH" refers to Apolipoprotein H, exemplified by SEQ ID NO: 4, shown in FIGURE 8 (Swiss Prot Accession Number NP_000033, Gene ID NCBI 350). "ApoH" encompasses the protein having the amino acid sequence of SEQ ID NO: 4 as well as variants, homologues and isoforms thereof. Such
10 variants, homologues and isoforms have at least the same essential biological and immunological properties as the specific ApoH. For example, 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 ApoH polypeptides. Exemplary assays are described in the accompanying Examples. Variants referred to above
15 may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific ApoH polypeptides 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 ApoH polypeptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or
20 myristylation.

The term "AUCR" refers to the ratio of albumin to creatinine in a sample. This ratio is a known measure of kidney function (KDIGO 2012), albumin SEQ ID NO: 5, shown in FIGURE 9 (Swiss Prot Accession Number NP_000468; Gene ID NCBI 213). "Albumin" encompasses the protein having the amino acid sequence of SEQ ID NO: 5 as well as variants, homologues and isoforms thereof. Such variants,
25 homologues and isoforms have at least the same essential biological and immunological properties as the specific Albumin. For example, 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 albumin polypeptides. Exemplary assays are described in the accompanying Examples. Variants referred to above may be allelic variants or
30 any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific albumin polypeptides 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 albumin polypeptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

The term "increased risk for disease progression" as used herein means that the subject to be analyzed by the method of the present disclosure is allocated either into the group of subjects of a population having a normal (i.e., non-elevated) risk for disease progression or into a group of subjects having a significantly elevated risk. An increased risk as referred to in accordance with the present disclosure means that the risk of disease progression within a predetermined predictive window is elevated significantly for a subject with respect to the average risk for disease progression in a population of subjects.

The term "diagnosing" or "identifying" or "assessing" as used herein means predicting whether the risk for disease progression is increased in a subject suffering from chronic kidney disease, or not. As will be understood by those skilled in the art, such a prediction is usually not intended to be correct for 100% of the subjects to be diagnosed. The term, however, requires that the prediction to be at increased risk for disease progression, or not, is correct for a statistically significant portion of the subjects (e.g. a cohort in a cohort study). 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. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Example confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. The p-values include 0.1, 0.05, 0.01, 0.005, or 0.0001.

The phrase "providing a diagnosis/assessment" as used herein refers to using the information or data generated relating to the level or presence of the biomarker(s) in a sample of a patient to diagnose/assess the risk of CKD disease progression in the patient. The information or data may be in any form, written, oral or electronic. In some embodiments, using the information or data generated includes communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof. In some embodiments, communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof are performed by a computing device, analyzer unit or combination thereof. In some further embodiments, communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof are performed by a laboratory or medical professional. In some embodiments, the information or data includes a comparison of the level of the biomarker(s) to a reference level. In some embodiments, the information or data includes an indication that the biomarker(s) is present or absent in the sample. In some embodiments, the information or data includes an indication that the patient is diagnosed/assessed with an increased risk of CKD disease progression.

The term "subject" as used herein relates to animals, such as mammals (for example, humans). The subject according to the present disclosure shall suffer from chronic kidney disease as described elsewhere herein.

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, 5 samples of blood, plasma, serum, urine, lymphatic fluid, sputum, ascites, bronchial lavage or any other bodily secretion or derivative thereof. 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. E.g., cell-, tissue- or organ samples may be 10 obtained from those cells, tissues or organs which express or produce the biomarker. The sample may be frozen, fresh, fixed (*e.g.* formalin fixed), centrifuged, and/or embedded (*e.g.* paraffin embedded), *etc.* The cell sample can, of course, be subjected to a variety of well-known post-collection preparative and storage techniques (*e.g.*, nucleic acid and/or protein extraction, fixation, storage, freezing, ultrafiltration, concentration, evaporation, centrifugation, *etc.*) prior to assessing the amount of the marker in the sample. 15 Likewise, biopsies may also be subjected to post-collection preparative and storage techniques, *e.g.*, fixation.

The term "detecting" the amount of a biomarker peptide or polypeptide as used herein refers to measuring the amount or concentration, semi-quantitatively or quantitatively for example. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or 20 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 25 peptide or polypeptide itself) or a biological read out system, *e.g.*, measurable cellular responses, ligands, labels, or enzymatic reaction products.

The term "comparing" as used herein refers to comparing the level of the biomarker in the sample from the individual or patient with the reference level of the biomarker specified elsewhere in this description. It is to be understood that comparing as used herein usually refers to a comparison of corresponding 30 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 the biomarker in a sample is compared to the same type of intensity signal obtained from a reference sample. The comparison may be carried out manually or computer assisted. Thus, the comparison may be carried out

by a computing device (e.g., of a system disclosed herein). The value of the measured or detected level of the biomarker in the sample from the individual or patient and the reference level can be, e.g., compared to each other and the said comparison can be automatically carried out by a computer program executing an algorithm for the comparison. The computer program carrying out the said evaluation will provide the
5 desired assessment in a suitable output format. 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. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to
10 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 provides the desired assessment in a suitable output format.

Accordingly, the term "reference amount" as used herein refers to an amount which allows assessing whether a subject suffering from CKD has an increased risk disease progression. The reference may e.g.
15 be derived from a pool of subjects suffering from CKD or a pool of subjects from the general population. Moreover, the reference amount may define a threshold amount or range, whereby dependent on the type of reference a change in the determined amount with respect to the threshold is either indicative for an increased risk for disease progression or a normal risk. Alternatively, an essentially identical amount may be either indicative for an increased risk for disease progression or a normal risk as well, if a suitable
20 reference amount is used. The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age, gender, or subpopulation, as well as on the means used for the determination of the polypeptide or peptide referred to herein. A suitable reference amount may be determined from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample.

25 The term "monitoring" as referred to above relates to keeping track of the status of the disease, i.e. chronic kidney disease. Monitoring includes comparing the status of the disease as reflected by the amount of the biomarker in a first sample taken at a first time point to the status of the disease reflected by the amount of the biomarker in a second sample taken at a second time point. The status of the disease may become worse and, thus, there will be progression of the disease, if the amount of the biomarker
30 increases whereas there is amelioration and, thus, improvement of the status of the disease if the biomarker decreases. If no change is observed, i.e. an essentially identical amount is determined in the first and the second sample, the status of the disease is unchanged and the disease, thus, is stagnating. An essentially identical amount is determined if no statistically significant change in the amount is

determined between the first and the second sample. Whether the amounts are essentially identical can be determined by the skilled artisan without further ado. A change, i.e. increase or decrease is statistically significant if the amounts differ by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25% or at least about 50%. Again, it is to be understood that the aforementioned

5 method allows monitoring in a statistically significant portion of subjects to investigated but not necessarily in all analyzed subjects.

The term "binding agent" refers to a molecule that comprises a binding moiety which specifically binds the corresponding target biomarker molecule. Examples of "binding agent" are a nucleic acid probe, nucleic acid primer, DNA molecule, RNA molecule, aptamer, antibody, antibody fragment, peptide,
10 peptide nucleic acid (PNA) or chemical compound.

The term "aptamer" refers to oligonucleotides, including RNA, DNA and RNA/DNA molecules, or peptide molecules, which exhibit the desired biological activity, in particular, binding to the corresponding target molecule.

The term "probe" or "nucleic acid probe" refers to a nucleic acid molecule that is capable of hybridizing
15 with a target nucleic acid molecule (e.g., genomic target nucleic acid molecule) and, when hybridized to the target, is capable of being detected either directly or indirectly. Thus probes permit the detection, and in some examples quantification, of a target nucleic acid molecule. In particular examples, a probe includes a plurality of nucleic acid molecules, which include binding regions derived from the target nucleic acid molecule and are thus capable of specifically hybridizing to at least a portion of the target
20 nucleic acid molecule. A probe can be referred to as a "labeled nucleic acid probe," indicating that the probe is coupled directly or indirectly to a detectable moiety or "label," which renders the probe detectable.

The term "primer" or "nucleic acid primer" refers to a short single stranded polynucleotide, generally with a free 3'-OH group, which binds to a target molecule potentially present in a sample of interest by
25 hybridizing with a target sequence, and thereafter promotes polymerization of a polynucleotide complementary to the target.

The term "specific binding" or "specifically bind" refers to a binding reaction wherein binding pair molecules exhibit a binding to each other under conditions where they do not significantly bind to other molecules.

30 The term "specific binding" or "specifically binds", when referring to a protein or peptide as a binding agent, refers to a binding reaction wherein a binding agent binds to the corresponding target molecule with an affinity of at least 10^{-7} M. The term "specific binding" or "specifically binds" preferably refers

to an affinity of at least 10^{-8} M or even more preferred of at least 10^{-9} M for its target molecule. The term “specific” or “specifically” is used to indicate that other molecules present in the sample do not significantly bind to the binding agent specific for the target molecule. Preferably, the level of binding to a molecule other than the target molecule results in a binding affinity which is only 10% or less, more preferably only 5% or less of the affinity to the target molecule.

The term “specific binding” or “specifically binds”, when referring to a nucleic acid as a binding agent, refers to a hybridization reaction wherein a binding agent or a probe contains a hybridizing region exactly or substantially complementary to the target sequence of interest. A hybridization assay carried out using the binding agent or probe under sufficiently stringent hybridization conditions enables the selective detection of the specific target sequence. The hybridizing region is preferably from about 10 to about 35 nucleotides in length, more preferably from about 15 to about 35 nucleotides in length. The use of modified bases or base analogues which affect the hybridization stability, which are well known in the art, may enable the use of shorter or longer probes with comparable stability. A binding agent or a probe can either consist entirely of the hybridizing region or can contain additional features which allow for the detection or immobilization of the probe, but which do not significantly alter the hybridization characteristics of the hybridizing region.

The term “specific binding” or “specifically binds”, when referring to a nucleic acid aptamer as a binding agent, refers to a binding reaction wherein a nucleic acid aptamer binds to the corresponding target molecule with an affinity in the low nM to pM range.

The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

The term “amount” 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 "device" as used herein relates to a system comprising the aforementioned units operatively linked to each other as to allow the diagnosis or monitoring according to the methods of the disclosure. Example detection agents which can be used for the analyzing unit are disclosed elsewhere herein. The analyzing unit may comprise said detection agents in immobilized form on a solid support which is to be
5 contacted to the sample comprising the biomarkers the amount of which is to be determined. 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.

10

The term "kit" as used herein refers to a collection of the aforementioned components which may be provided separately or within a single container. The container also comprises instructions for carrying out the method of the present disclosure. These instructions may be in the form of a manual or may be provided by a computer program code which is capable of carrying out the comparisons referred to in the
15 methods of the present disclosure and to establish a diagnosis accordingly when implemented on a computer or a data processing device. The computer program code may be provided on a data storage medium or device such as an optical storage medium (e.g., a Compact Disc) or directly on a computer or data processing device.

20 Illustrative Embodiments

Clinical risk prediction models incorporate multiple variables to prognosticate the risk of adverse events for an individual patient and should be able to predict renal endpoints and well as all-cause mortality and cardiovascular disease in CKD patients. Proteinuria, hypertension, diabetes, race, and ethnicity are strong risk factors for progression from CKD to ESRD (13). The biomarker approach described herein reflects
25 the various pathways involved in the pathogenesis of CKD and provides a surrogate representation of disease course of progression and prediction of long term outcomes. The use and implementation of this approach can be used to identify segments of the CKD population that would benefit most from a novel treatment. Clinical use of the biomarker and methods described herein is useful in identifying which patients may need a more aggressive treatment and avoid additional therapeutic options or dose escalation
30 in patients with a lowest risk of progression. Embodiments of the instant disclosure also encompass diagnostic devices and kits for carrying out the aforementioned methods.

One aspect of the present disclosure relates to methods for diagnosing whether a subject suffering from chronic kidney disease (CKD) is at increased risk for disease progression. In one embodiment, the method comprises detecting the amount of one or more of the biomarkers GDF-15, EGF, NT-proBNP, ApoH, and albumin to creatinine ratio (AUCR) in a sample of the subject and comparing the amount to a reference.

- 5 The subject is identified as having an increased risk of disease progression if the amount of one, two, three, four or all five biomarkers in the sample is greater than the reference amount of the one, two, three, four or five biomarkers.

Another aspect of the present disclosure relates to methods for monitoring whether a subject suffering from chronic kidney disease (CKD) is at increased risk for disease progression during the course of
10 treatment for CKD. In one embodiment, the method comprises detecting the amount of one or more of the biomarkers GDF-15, EGF, NT-proBNP, ApoH, and albumin to creatinine ratio (AUCR) in a sample of the subject and comparing the amount to a reference. The subject is identified as having an increased risk of disease progression if the amount of one, two, three, four or all five biomarkers in the sample is greater than the reference amount of the one, two, three, four or five biomarkers. In one embodiment, the
15 reference is from a sample of subjects suffering from CKD. In another embodiment, the reference is sample taken from the subject prior to beginning treatment for CKD or a sample taken from the subject at a timepoint during the treatment process. The treatment may be modified based on the results of this method. For example, the treatment may be continued if the subject exhibits a decrease in the amount of biomarker(s) as compared to the reference. Conversely, the treatment may be substituted for an
20 alternative treatment if the subject exhibits an increase in the amount of biomarker(s) as compared to the reference.

Another aspect of the invention relates to a device adapted for carrying out the methods provided above and herein is provided. Exemplary embodiments of the device comprise a) an analysing unit comprising a detection agent which specifically binds to a biomarker of the invention, said analysing unit adapted for
25 contacting, in vitro, a portion of a sample from the subject with the detection agent; b) an evaluation unit including a computing device having a database and a computer-implemented algorithm on the database, the computer-implemented algorithm when executed by the computing device determines an amount of the biomarker in the sample from the subject and compares the determined amount of the biomarker with a biomarker reference amount and provides a diagnosis of at increased risk for disease progression if the
30 amount of the biomarker determined in said step of determining is greater than the biomarker reference amount. According to some embodiments, the database further includes the biomarker reference amount.

Another aspect of the invention provides for a kit adapted for carrying out the above disclosed methods of the present disclosure comprising a detection agent for the biomarker(s) as well as instructions for

carrying out the method. In one embodiment, the kit is for diagnosing whether a subject suffering from chronic kidney disease (CKD) is at increased risk for disease progression.

In any of the above aspects and methods, the biomarker or biomarkers are selected from among GDF-15, EGF, NT-proBNP, ApoH, and albumin to creatinine ratio (AUCR). In one embodiment of the aspects and methods, one, two, three, four or five biomarkers are selected from the group consisting of GDF-15, EGF, NT-proBNP, ApoH, and AUCR. In certain embodiments, the following combinations are specifically contemplated :

- AUCR+ApoH
- AUCR+NT-proBNP
- 10 AUCR+GDF-15
- AUCR+EGF
- ApoH+NT-proBNP
- ApoH+GDF-15
- ApoH+EGF
- 15 NT-proBNP +GDF-15
- NT-proBNP +EGF
- GDF-15+EGF
- AUCR+ApoH+ NT-proBNP
- AUCR+ApoH+GDF
- 20 AUCR+ApoH+EGF
- AUCR+ NT-proBNP +GDF-15
- AUCR+ NT-proBNP +EGF
- AUCR+GDF-15+EGF
- ApoH+ NT-proBNP +GDF-15
- 25 ApoH+ NT-proBNP +EGF
- ApoH+GDF-15
- NT-proBNP +GDF-15+EGF
- AUCR+ApoH+ NT-proBNP +GDF-15
- AUCR+ApoH+ NT-proBNP +EGF
- 30 AUCR+ApoH+GDF-15+EGF
- AUCR+ NT-proBNP +GDF-15+EGF
- ApoH+ NT-proBNP +GDF-15+EGF
- AUCR+ApoH+ NT-proBNP +GDF-15+EGF

In one embodiment, the amounts of at least one, at least two, at least three, at least four, or all five of the biomarkers determined in the test sample are increased as compared to the reference amounts for the biomarkers is indicative for a subject who has an increased risk of disease progression.

In one embodiment, the amounts of all biomarkers markers determined in the test sample are increased as compared to the reference amounts for the biomarkers is indicative for a subject who has an increased risk of disease progression.

In one embodiment, the subject is identified as having an increased risk of disease progression if the amount of at least one, at least two, at least three, at least four, or all five of the biomarkers determined in the test sample is greater than the reference amount. In one embodiment, the reference amount is the median amount derived from a cohort of patients suffering from CKD.

- 5 In one embodiment, the subject is identified as having an increased risk of disease progression if the amount of at least one, at least two, at least three, at least four, or all five of the biomarkers determined in the test sample is at, or greater, than the second, third, or fourth quartile based on the quartiles derived from a cohort of patients suffering from CKD. In one embodiment, the reference amount is in the fourth quartile based on the quartiles derived from a cohort of patients suffering from CKD.

10 **Methods of detecting the biomarkers**

- Biomarkers, including proteins or nucleic acids, can be detected using methods generally known in the art. Methods of detection generally encompass methods to quantify the level of a biomarker in the sample (quantitative method) or that determine whether or not a biomarker is present in the sample (qualitative method). It is generally known to the skilled artisan which of the following methods are suitable for
- 15 qualitative and/or for quantitative detection of a biomarker. Samples can be conveniently assayed for, e.g., proteins using Westerns and immunoassays, like ELISAs, RIAs, fluorescence-based immunoassays, as well as mRNAs or DNAs from a genetic biomarker of interest using Northern, dot-blot, polymerase chain reaction (PCR) analysis, array hybridization, RNase protection assay, or using DNA SNP chip microarrays, which are commercially available, including DNA microarray snapshots. Further suitable
- 20 methods to detect biomarker include measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, e.g., biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-spectrometers, NMR- analyzers, or chromatography devices. Further, methods include microplate ELISA-based methods, fully-automated or robotic immunoassays (available for example on Elecsys™
- 25 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).

- For the detection of biomarker proteins a wide range of immunoassay techniques using such an assay format are available, see, e.g., U.S. Pat. Nos. 4,016,043, 4,424,279, and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional
- 30 competitive binding assays. These assays also include direct binding of a labeled antibody to a target biomarker.

Sandwich assays are among the most useful and commonly used immunoassays.

Methods for measuring electrochemiluminescent phenomena are well-known. Such methods make use of the ability of special metal complexes to achieve, by means of oxidation, an excited state from which they decay to ground state, emitting electrochemiluminescence. For review see Richter, M.M., Chem. Rev. 104 (2004) 3003-3036.

5 Biomarkers can also be detected by generally known methods including magnetic resonance spectroscopy (NMR spectroscopy), Gas chromatography–mass spectrometry (GC-MS), Liquid chromatography–mass spectrometry (LC-MS), High and ultra-HPLC HPLC such as reverse phase HPLC, for example, ion-pairing HPLC with dual UV-wavelength detection, capillary electrophoresis with laser-induced fluorescence detection, anion exchange chromatography and fluorescent detection, thin layer
10 chromatography.

In accordance with the present disclosure, detecting the amount of a biomarker peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Examples of such means include immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. These assays will develop a signal which is indicative for
15 the presence or absence of the peptide or polypeptide. Moreover, the signal strength can 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. These methods may comprise biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-
20 spectrometers, NMR-analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on Elecsys.TM. analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-Hitachi.TM. analyzers), and latex agglutination assays (available for example on Roche-Hitachi.TM. analyzers).

25 According to the instant disclosure, determining the amount of a biomarker peptide or polypeptide may comprise 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 may be added to a cell culture and an internal or external cellular response is
30 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, detecting the amount of a biomarker 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.

5 Detecting the amount of a biomarker peptide or polypeptide may comprise 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 disclosure includes both covalent and non-covalent binding. A ligand according to the present disclosure can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Exemplary ligands include antibodies, nucleic acids, peptides or
10 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. 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
15 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).sub.2 fragments that are capable of binding antigen or hapten.

20 The present disclosure 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
25 several methods well known in the art. The ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present disclosure 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. The specifically bound peptide or polypeptide should be bound with at least 3 times higher, and in some embodiments at least 10 times higher or even at least 50 times higher affinity
30 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. Said method may be 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

5 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, the amount of substrate may be saturating. The substrate may also be labeled with a detectable label prior to the reaction. For example, the sample is contacted with the substrate for an adequate period of time. An

10 adequate period of time refers to the time necessary for a detectable, and in some embodiments 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. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label

15 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

20 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, digoxigenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and

25 the like. In the case of a peptide or polypeptide, the tag may be 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,

30 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.TM. (Amersham Biosciences), ECF.TM. (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 ³⁵S, ¹²⁵I, ³²P, ³³P 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 to the present disclosure 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.

According to embodiments of the instant disclosure, the amount of a peptide or polypeptide may be detected 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 may be chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers. In some embodiments, the ligand is 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 disclosure. 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 disclosure (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 (U.S. Pat. No. 5,744,305).

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Reference amounts

Reference amounts can be calculated for a cohort of subjects (i.e. subjects which are known to have CKD) based on the average or mean values for a given biomarker by applying standard statistically methods. In one embodiment, the reference is determined in a cohort of subjects suffering from CKD using multivariable Proportional Hazard (Cox) Regression analysis (Cox DR. Regression models and life tables. J R Stat Soc (B). 1972; 34(series B):187-220). Techniques and assays useful in this type of analysis are described in the Examples and Figures referenced therein.

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The median values for the biomarker(s) determined in a cohort of patients may be also used as a basis for establishing reference levels

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In certain embodiments, the term “reference level” herein refers to a predetermined value. In this context “level” encompasses the absolute amount, the relative amount or concentration as well as any value or parameter which correlates thereto or can be derived therefrom. As the skilled artisan will appreciate the reference level is predetermined and set to meet routine requirements in terms of e.g. specificity and/or sensitivity. These requirements can vary, e.g. from regulatory body to regulatory body. It may for example be that assay sensitivity or specificity, respectively, has to be set to certain limits, e.g. 80%, 90%, 95% or 98%, respectively. These requirements may also be defined in terms of positive or negative predictive values. Nonetheless, based on the teaching given in the present invention it will always be possible for a skilled artisan to arrive at the reference level meeting those requirements. In one embodiment the reference level is determined in reference samples from healthy individuals. The reference level in one embodiment has been predetermined in reference samples from the disease entity to which the patient belongs. In certain embodiments the reference level can e.g. be set to any percentage between 25% and 75% of the overall distribution of the values in a disease entity investigated. In other embodiments the reference level can e.g. be set to the median, tertiles or quartiles as determined from the overall distribution of the values in reference samples from a disease entity investigated.

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In one embodiment the reference level is set to the median value as determined from the overall distribution of the values in a disease entity investigated. The reference level may vary depending on various physiological parameters such as age, gender or subpopulation, as well as on the means used for

the determination of the biomarker Y referred to herein. In one embodiment, the reference sample is from essentially the same type of cells, tissue, organ or body fluid source as the sample from the individual or patient subjected to the method of the invention, e.g. if according to the invention blood is used as a sample to determine the level of biomarker Y in the individual, the reference level is also determined in blood or a part thereof.

In certain embodiments, the term “at the reference level” refers to a level of the biomarker in the sample from the individual or patient that is essentially identical to the reference level or to a level that differs from the reference level by up to 1%, up to 2%, up to 3%, up to 4%, up to 5%.

In certain embodiments, the term “greater than the reference level” refers to a level of the biomarker in the sample from the individual or patient above the reference level or to an overall increase of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 100% or greater, determined by the methods described herein, as compared to the reference level. In certain embodiments, the term increase refers to the increase in biomarker level in the sample from the individual or patient wherein, the increase is at least about 1.5-, 1.75-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 25-, 30-, 40-, 50-, 60-, 70-, 75-, 80-, 90-, or 100- fold higher as compared to the reference level, e.g. predetermined from a reference sample.

In certain embodiments, the term “decrease” or “below” herein refers to a level of the biomarker in the sample from the individual or patient below the reference level or to an overall reduction of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater, determined by the methods described herein, as compared to the reference level. In certain embodiments, the term decrease in biomarker level in the sample from the individual or patient wherein the decreased level is at most about 0.9-, 0.8-, 0.7-, 0.6-, 0.5-, 0.4-, 0.3-, 0.2-, 0.1-, 0.05-, or 0.01- fold of the reference level, e.g. predetermined from a reference sample, or lower.

Methods of Treatment

Some methods of the invention further comprise administering a pharmaceutical agent or composition for treating chronic kidney disease to a subject with an increased risk of disease progression. Such pharmaceutical agents include for example agents of the class of angiotensin converting enzyme inhibitors (ACEi) or of the class of the angiotensin receptor blockers (ARBs) . Additional pharmaceutical agents include those agents undergoing clinical trial studies with a regulatory agency, such as the FDA or EMA. Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a dose may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by exigencies of the therapeutic situation.

A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. The effectiveness of a given dose or treatment regimen of the antagonist can be determined, for example, by assessing signs and symptoms in the patient using standard

In yet another aspect, the invention provides, after the identification step, a method of determining
 5 whether to continue administering the pharmaceutical agent or composition to a subject diagnosed with CKD comprising measuring current status of the CKD disease via standard techniques, such as GFR.

EXAMPLES

10 Sample collection

Patient cohort description

The following study utilizes patients and samples from the Clinical Phenotyping Resource and Biobank research core within the NIH sponsored George M. O'Brien Renal Center (C-PROBE) study, identified as NCT01016613 in clinicaltrials.gov.

15 C-Probe is an observational time prospective multicenter, actively enrolling study in which 221 patients has simultaneously recorded for clinical and demographic parameters, clinical chemistry analysis and longitudinal regular medical follow ups.

Clinical and biomarker data were available for 393 patients of this cohort. Outcome measured data and time to the outcome occurrence was available for 221 patients. CKD events were defined based on the
 20 existing outcome categories according to Table A (Figure 3). Patient characteristics and summary statistics of this cohort are presented in Table B (Figure 4).

Patient samples were assayed to evaluate the utility of several biomarkers to aid in assigning an increased likelihood of CKD progression and increase incidence of outcome to a patient diagnosed with CKD.

Samples obtained from each patient were analyzed by immunoassay to determine the level of each
 25 biomarker. Immunoassays were operated in a sandwich assay format or, for NT-proBNP, using the Elecsys® proBNP platform (Elecsys 20.10 immunoanalyzer).

Assay

Concentrations of ApoH (cat. no. ab108814, Abcam, Cambridge, United Kingdom) and EGF (cat. no. DEG00, R&D Systems, Minneapolis, MN, U.S.A.) were measured in duplicate in urine samples using
 30 commercial enzyme-linked immunosorbent assay kits according to the manufacturer's protocol. Urine

samples were diluted respectively 16 fold and 150 fold. The Lower Limits of Quantification were determined and respectively set to 0.96 pg/ml and 4.5 pg/ml.

Concentrations of GDF-15 (cat. no. DGD150, R&D Systems, Minneapolis, MN, U.S.A.) were measured in duplicate in EDTA-plasma samples using commercial enzyme-linked immunosorbent assay kits according to the manufacturer's protocol. Plasma samples were diluted 10 fold. The Lower Limit of Quantification was determined and set to 93.8 pg/ml.

Albumin concentrations (cat. no. 11970569216, Roche Diagnostics, Mannheim, Germany) and NT-proBNP (cat. no. 04842464190, Roche Diagnostics, Mannheim, Germany) were measured respectively in urine and in EDTA-plasma using commercial CE certified test kits following the manufacturer's instructions.

The following characteristics were evaluated:

Dynamic concentration range; Lower and upper limits of quantification; Matrix effects; Precision; Accuracy; Stability; Selectivity and specificity; Dilution parallelism; and Interfering agents.

15 Statistical analysis

Time to event analysis

The association of each of the five biomarkers GDF-15, EGF, NT-proBNP, ApoH, and albumin to creatinine ratio (AUCR), and combinations thereof, with the risk of CKD events was conducted by multivariable Proportional Hazard (Cox) Regression. Quantitative covariates were reduced and scaled.

Each biomarker was added on top of available clinical parameters (age, gender and eGFR levels at baseline, henceforth denoted as basal model). All possible biomarker combinations were fitted (on top of the basal model) and their respective predictive models compared. Summary statistics for each model are shown in Figures 1-2. (goodness of fit information criteria). AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) are two measures of quality of a statistical model for a set of data, which represent the amount of information lost when a given model is used to represent the data. Thus, the smaller value the better. They deal with the trade-off of goodness of fit and the complexity of the model. BIC is a version of AIC adjusted for the number of parameters in the model; BIC therefore penalizes complex models (with larger number of predictors).

While this disclosure has been described as having an exemplary design, the present disclosure may be further modified within the spirit and scope of this disclosure. This application is therefore intended to cover any variations, uses, or adaptations of the disclosure using its general principles. Further, this application is intended to cover such departures from the present disclosure as come within the known or customary practice in the art to which this disclosure pertains.

References

1. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis.* 2003 Jan;41(1):1-12.
2. Xue JL, Eggers PW, Agodoa LY, Foley RN, Collins AJ. Longitudinal study of racial and ethnic differences in developing end-stage renal disease among aged medicare beneficiaries. *J Am Soc Nephrol.* 2007 Apr;18(4):1299-306.
3. Keith DS, Nichols GA, Gullion CM, Brown JB, Smith DH. Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Archives of internal medicine.* 2004 Mar 22;164(6):659-63.
4. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *Journal of the American Geriatrics Society.* 1985 Apr;33(4):278-85.
5. Hu B, Gadegbeku C, Lipkowitz MS, Rostand S, Lewis J, Wright JT, et al. Kidney function can improve in patients with hypertensive CKD. *J Am Soc Nephrol.* 2012 Apr;23(4):706-13.
6. Li L, Astor BC, Lewis J, Hu B, Appel LJ, Lipkowitz MS, et al. Longitudinal progression trajectory of GFR among patients with CKD. *Am J Kidney Dis.* 2012 Apr;59(4):504-12.
7. Taal MW, Brenner BM. Renal risk scores: progress and prospects. *Kidney international.* 2008 Jun;73(11):1216-9.
8. Taal MW, Brenner BM. Defining renal risk. Current opinion in nephrology and hypertension. 2007 Nov;16(6):554-6.

9. Taal MW, Brenner BM. Predicting initiation and progression of chronic kidney disease: Developing renal risk scores. *Kidney international*. 2006 Nov;70(10):1694-705.
10. Clark WF, Macnab JJ, Sontrop JM, Jain AK, Moist L, Salvadori M, et al. Dipstick proteinuria as a screening strategy to identify rapid renal decline. *J Am Soc Nephrol*. 2011 Sep;22(9):1729-36.
- 5 11. Hallan SI, Ritz E, Lydersen S, Romundstad S, Kvenild K, Orth SR. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. *J Am Soc Nephrol*. 2009 May;20(5):1069-77.
12. Halbesma N, Jansen DF, Heymans MW, Stolk RP, de Jong PE, Gansevoort RT. Development and validation of a general population renal risk score. *Clin J Am Soc Nephrol*. 2011 Jul;6(7):1731-8.
13. Echouffo-Tcheugui JB, Kengne AP. Risk models to predict chronic kidney disease and its progression: a systematic review. *PLoS medicine*. 2012;9(11):e1001344.
- 10 14. O'Seaghdha CM, Hwang SJ, Ho JE, Vasan RS, Levy D, Fox CS. Elevated Galectin-3 Precedes the Development of CKD. *J Am Soc Nephrol*. 2013 Sep;24(9):1470-7.
15. O'Seaghdha CM, Hwang SJ, Larson MG, Meigs JB, Vasan RS, Fox CS. Analysis of a Urinary Biomarker Panel for Incident Kidney Disease and Clinical Outcomes. *J Am Soc Nephrol*. 2013 Aug 29.
- 15 16. Fox CS, Gona P, Larson MG, Selhub J, Tofler G, Hwang SJ, et al. A multi-marker approach to predict incident CKD and microalbuminuria. *J Am Soc Nephrol*. 2010 Dec;21(12):2143-9.
17. Formentini I, Bobadilla M, Haefliger C, Hartmann G, Loghman-Adham M, Mizrahi J, et al. Current drug development challenges in chronic kidney disease (CKD)--identification of individualized determinants of renal progression and premature cardiovascular disease (CVD). *Nephrol Dial Transplant*. 2012 Oct;27 Suppl 3:iii81-8.
- 20 18. Austin WJ, Bhalla V, Hernandez-Arce I, Isakson SR, Beede J, Clopton P, et al. Correlation and prognostic utility of B-type natriuretic peptide and its amino-terminal fragment in patients with chronic kidney disease. *American journal of clinical pathology*. 2006 Oct;126(4):506-12.
19. Spanaus KS, Kronenberg F, Ritz E, Schlapbach R, Fliser D, Hersberger M, et al. B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: the Mild-to-Moderate Kidney Disease Study. *Clinical chemistry*. 2007 Jul;53(7):1264-72.
- 25 20. Sato Y. Diagnostic and prognostic property of NT-proBNP in patients with renal dysfunction. *Journal of cardiology*. 2013 Jun;61(6):446-7.

21. Yasuda K, Kimura T, Sasaki K, Obi Y, Iio K, Yamato M, et al. Plasma B-type natriuretic peptide level predicts kidney prognosis in patients with predialysis chronic kidney disease. *Nephrol Dial Transplant*. 2012 Oct;27(10):3885-91.
22. Desai AS, Toto R, Jarolim P, Uno H, Eckardt KU, Kewalramani R, et al. Association between
5 cardiac biomarkers and the development of ESRD in patients with type 2 diabetes mellitus, anemia, and CKD. *Am J Kidney Dis*. 2011 Nov;58(5):717-28.
23. Svensson M, Gorst-Rasmussen A, Schmidt EB, Jorgensen KA, Christensen JH. NT-pro-BNP is an independent predictor of mortality in patients with end-stage renal disease. *Clinical nephrology*. 2009 Apr;71(4):380-6.
- 10 24. Wang AY, Lam CW, Yu CM, Wang M, Chan IH, Zhang Y, et al. N-terminal pro-brain natriuretic peptide: an independent risk predictor of cardiovascular congestion, mortality, and adverse cardiovascular outcomes in chronic peritoneal dialysis patients. *J Am Soc Nephrol*. 2007 Jan;18(1):321-30.
25. Breit SN, Carrero JJ, Tsai VW, Yagoutifam N, Luo W, Kuffner T, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15) and mortality in end-stage renal disease. *Nephrol Dial Transplant*. 2012
15 Jan;27(1):70-5.
26. Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Plasma growth differentiation factor-15 independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy. *Diabetes care*. 2010 Jul;33(7):1567-72.
27. Hellemons ME, Mazagova M, Gansevoort RT, Henning RH, de Zeeuw D, Bakker SJ, et al.
20 Growth-differentiation factor 15 predicts worsening of albuminuria in patients with type 2 diabetes. *Diabetes care*. 2012 Nov;35(11):2340-6.
28. Ranieri E, Gesualdo L, Petrarulo F, Schena FP. Urinary IL-6/EGF ratio: a useful prognostic marker for the progression of renal damage in IgA nephropathy. *Kidney international*. 1996 Dec;50(6):1990-2001.
- 25 29. Stangou M, Papagianni A, Bantis C, Liakou H, Pliakos K, Giamalis P, et al. Detection of multiple cytokines in the urine of patients with focal necrotising glomerulonephritis may predict short and long term outcome of renal function. *Cytokine*. 2012 Jan;57(1):120-6.
- 30 30. Stangou M, Alexopoulos E, Papagianni A, Pantzaki A, Bantis C, Dovas S, et al. Urinary levels of epidermal growth factor, interleukin-6 and monocyte chemoattractant protein-1 may act as predictor
30 markers of renal function outcome in immunoglobulin A nephropathy. *Nephrology (Carlton, Vic)*. 2009 Sep;14(6):613-20.

31. Torres DD, Rossini M, Manno C, Mattace-Raso F, D'Altri C, Ranieri E, et al. The ratio of epidermal growth factor to monocyte chemotactic peptide-1 in the urine predicts renal prognosis in IgA nephropathy. *Kidney international*. 2008 Feb;73(3):327-33.
32. Lapsley M, Flynn FV, Sansom PA. Beta 2-glycoprotein-1 (apolipoprotein H) excretion and renal tubular malfunction in diabetic patients without clinical proteinuria. *Journal of clinical pathology*. 1993 May;46(5):465-9.
33. Flynn FV, Lapsley M, Sansom PA, Cohen SL. Urinary excretion of beta 2-glycoprotein-1 (apolipoprotein H) and other markers of tubular malfunction in "non-tubular" renal disease. *Journal of clinical pathology*. 1992 Jul;45(7):561-7.
- 10 34. Lapsley M, Sansom PA, Marlow CT, Flynn FV, Norden AG. Beta 2-glycoprotein-1 (apolipoprotein H) excretion in chronic renal tubular disorders: comparison with other protein markers of tubular malfunction. *Journal of clinical pathology*. 1991 Oct;44(10):812-6.
35. Flynn FV, Lapsley M, Sansom PA, Norden AG. Absence of increased urinary excretion of adenosine-deaminase-binding protein by patients with chronic renal tubular malfunction. *Clinica chimica acta; international journal of clinical chemistry*. 1991 Aug 30;200(2-3):183-9.
- 15 36. Norden AG, Fulcher LM, Lapsley M, Flynn FV. Excretion of beta 2-glycoprotein I (apolipoprotein H) in renal tubular disease. *Clinical chemistry*. 1991 Jan;37(1):74-7.

We Claim:

1. A method for identifying a subject suffering from chronic kidney disease as having an increased risk for disease progression, the method comprising:
 - a) detecting the amount of biomarkers comprising EGF, as an EGF to creatinine ratio, and albumin as an albumin to creatinine ratio (AUCR) and one, two, or three additional biomarkers selected from the group consisting of GDF-15, NT-proBNP, glomerular filtration rate (GFR) and ApoH in a urine sample of the subject;
 - b) comparing the amount of the biomarkers to a reference amount of the biomarkers; and
 - c) identifying the subject as having an increased risk for disease progression if the EGF to creatinine ratio is lower relative to the reference amount thereof, and the amount of least one of the AUCR or additional biomarkers in the sample is increased relative to the reference amount thereof.
2. The method of claim 1, wherein the detecting comprises contacting, in vitro, the sample with a combination of detection agents, each agent having specific binding affinity for one of the biomarkers.
3. The method of claim 2, wherein at least one of the detection agents is an antibody or fragment thereof.
4. The method of any one of claims 1-3, wherein the subject is identified as having an increased risk of disease progression when the amount of the AUCR or additional biomarkers in the sample is greater than the median of the reference amount.
5. The method of any one of claims 1-3, wherein the subject is identified as having an increased risk of disease progression when the amount of the AUCR or additional biomarkers in the sample is in the first or fourth quartile range of the reference amount thereof, as determined from the overall distribution of the reference amount from a cohort of patients suffering from chronic kidney disease.

6. The method of any one of claims 1 to 5, further comprising the step of recommending a therapy to treat the chronic kidney disease, if the subject is identified as having an increased risk for disease progression.
7. A device adapted for carrying out the method as defined by any one of claims 1 to 5 comprising:
- a) an analysing unit comprising a combination of detection agents which specifically bind to the biomarkers, the analysing unit adapted for contacting, in vitro, the sample from the subject with the detection agents; and,
 - b) an evaluation unit including a computing device having a database and a computer-implemented algorithm on the database, the computer-implemented algorithm when executed by the computing device determines an amount of the biomarkers in the sample from the subject and compares the determined amount of the biomarkers with a reference amount of the biomarkers and provides a diagnosis of at increased risk for disease progression if the EGF to creatinine ratio is lower relative to the reference amount thereof and the amount of one or more of the biomarkers selected from the group consisting of GDF-15, NT-proBNP, glomerular filtration rate (GFR) and ApoH determined in the step of determining is greater than the reference amount of the biomarkers.
8. The device of claim 7, wherein the database further includes the biomarker reference amount.
9. A kit adapted for carrying out the method as defined by claim 2 or 3, comprising the combination of detection agents for the biomarkers.
10. The kit of claim 9, further comprising instructions for carrying out the method.

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Figure 1: Information Criteria and Goodness of Fit

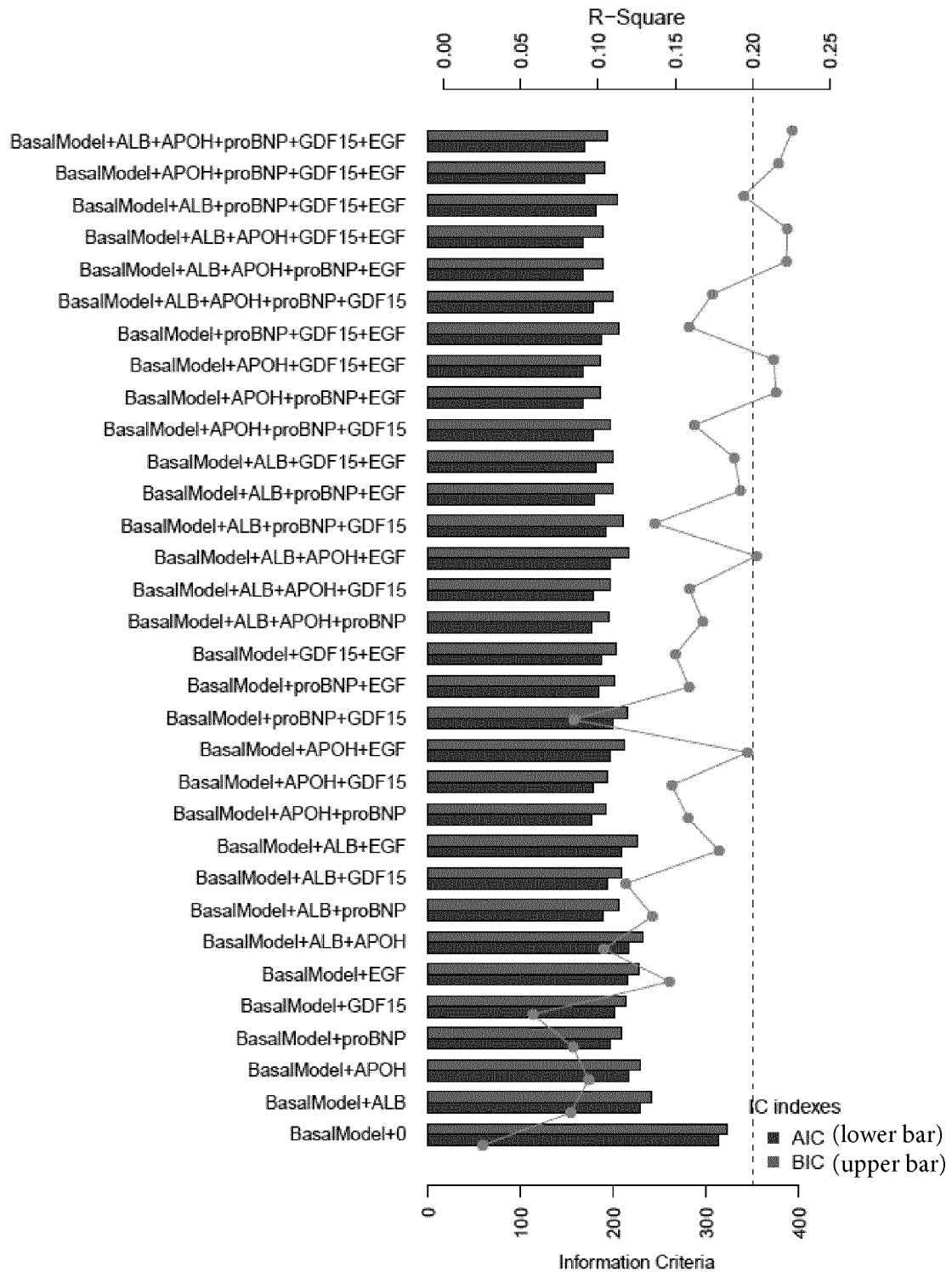


Figure 2: Goodness of Fit All Models

	NrParm	Rsqr	AIC	BIC	N
BasalModel+0	3	0.025	312.68	322.86	220
BasalModel+ALB	4	0.082	228.15	241.14	190
BasalModel+APOH	4	0.094	216.55	229.43	185
BasalModel+proBNP	4	0.084	197.09	209.54	166
BasalModel+GDF15	4	0.058	201.70	214.15	166
BasalModel+EGF	4	0.146	214.39	227.38	190
BasalModel+ALB+APOH	5	0.104	216.52	232.62	185
BasalModel+ALB+proBNP	5	0.135	189.47	205.03	166
BasalModel+ALB+GDF15	5	0.118	192.74	208.30	166
BasalModel+ALB+EGF	5	0.178	209.17	225.41	190
BasalModel+APOH+proBNP	5	0.158	176.55	191.96	161
BasalModel+APOH+GDF15	5	0.148	178.52	193.92	161
BasalModel+APOH+EGF	5	0.197	196.31	212.41	185
BasalModel+proBNP+GDF15	5	0.084	199.00	214.56	166
BasalModel+proBNP+EGF	5	0.159	184.88	200.44	166
BasalModel+GDF15+EGF	5	0.150	186.62	202.18	166
BasalModel+ALB+APOH+proBNP	6	0.168	176.70	195.19	161
BasalModel+ALB+APOH+GDF15	6	0.159	178.35	196.84	161
BasalModel+ALB+APOH+EGF	6	0.203	196.94	216.26	185
BasalModel+ALB+proBNP+GDF15	6	0.137	191.20	209.88	166
BasalModel+ALB+proBNP+EGF	6	0.192	180.17	198.85	166
BasalModel+ALB+GDF15+EGF	6	0.188	181.03	199.70	166
BasalModel+APOH+proBNP+GDF15	6	0.162	177.76	196.25	161
BasalModel+APOH+proBNP+EGF	6	0.215	167.25	185.74	161
BasalModel+APOH+GDF15+EGF	6	0.214	167.55	186.04	161
BasalModel+proBNP+GDF15+EGF	6	0.159	186.85	205.52	166
BasalModel+ALB+APOH+proBNP+GDF15	7	0.174	177.51	199.08	161
BasalModel+ALB+APOH+proBNP+EGF	7	0.222	167.83	189.40	161
BasalModel+ALB+APOH+GDF15+EGF	7	0.222	167.79	189.36	161
BasalModel+ALB+proBNP+GDF15+EGF	7	0.194	181.77	203.55	166
BasalModel+APOH+proBNP+GDF15+EGF	7	0.217	168.94	190.51	161
BasalModel+ALB+APOH+proBNP+GDF15+EGF	8	0.226	169.07	193.72	161

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Figure 3

Table A

Outcome	Code	Event
Non Case	0	No
Transplant	1	Yes
Dialysis	2	Yes
Disease Progression	3	No
Deceased	4	Yes
eGFR < 15	5	No
40% eGFR loss	6	Yes
50% eGFR loss	7	Yes

Figure 4

Table B

	N	Non-Case N = 181	ESRD at entrance N = 4	Progr. Decline N = 8	CKD Hard EP N = 28
Age	221	42.0 59.0 67.0	53.8 58.0 63.2	31.2 48.5 64.5	43.0 58.0 67.0
Gender : 2	221	65% (117)	25% (1)	62% (5)	46% (13)
eGFR at BL	221	35.7 48.5 67.2	20.3 21.1 21.9	40.3 53.5 101.2	22.6 31.4 37.5
CKD-stage at BL : 1 - 2	221	33% (60)	0% (0)	38% (3)	11% (3)
3		51% (93)	0% (0)	62% (5)	39% (11)
4 - 5		15% (28)	100% (4)	0% (0)	50% (14)
initial egfr	221	42.8 58.3 80.9	25.8 35.4 48.3	52.1 70.5 92.3	38.3 60.3 77.2
Nr of CKD events	221	0% (0)	0% (0)	88% (7)	100% (28)
log(APOH/Cr ng/mg)	185	7.16 8.06 9.27	11.06 11.76 12.13	8.95 9.14 9.24	8.82 12.25 13.68
log(UrineAlb/Cr µg/mg)	191	3.71 7.03 9.46	9.97 11.40 11.70	7.97 11.36 11.68	8.57 10.74 11.16
log(ProBNP pg/ml)	167	5.86 6.88 8.57	9.48 10.66 10.77	6.62 6.72 6.78	7.00 8.89 10.17
log(GDF15plasma pg/ml)	167	10.05 10.58 11.20	11.32 11.50 12.27	9.61 9.94 10.65	10.72 11.18 11.64
log(EGF/Cr ng/mg)	191	2.22 2.66 3.26	0.57 1.00 1.00	2.40 2.81 3.53	0.76 1.30 2.17

^a ^b ^c represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables.
N is the number of non-missing values.
 Numbers after percents are frequencies.

Figure 5

SEQ ID NO:1 GDF-15 NP_004855

1 MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRAS F PGPSELHSED
51 SRFREL RKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH
101 LHLRISRAAL PEGLP EASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP
151 QAPALHLRLS PPFSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG
201 DHCPLGPGR C CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA
251 ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTD T GVSLQTYDDL
301 LAKDCHCI

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Figure 6

SEQ ID NO: 2 EGF NP_001954

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1  MLLTLIILLP VVSKFSFVSL SAPQHWSCPE GTLAGNGNST CVGPAPFLIF
51 SHGNSIFRID TEGTNYEQLV VDAGVSVIMD FHYNEKRIYW VDLERQLLQR
101 VFLNGSRQER VCNIEKNVSG MAINWINEEV IWSNQQEGII TVTDMKGNNs
151 HILLSALKYP ANVAVDPPER FIFWSSEVAG SLYRADLDGV GVKALLETSE
201 KITAVSLDVL DKRLFWIQYN REGSNSLICS CDYDGGSVHI SKHPTQHNLF
251 AMSLFGDRIF YSTWKMKTIW IANKHTGKDM VRINLHSSFV PLGELKVVHP
301 LAQPKAEDDT WEPEQKLCKL RKGNCSSVC GQDLQSHLCM CAEGYALSRD
351 RKYCEDVNEC AFWNHGCTLG CKNTPGSYYC TCPVGFVLLP DGKRCHQLVS
401 CPRNVSECSH DCVLTSEGPL CFCPEGSVLE RDGKTCGCS SPDNGGCSQL
451 CVPLSPVSWE CDCFPGYDLQ LDEKSCAASG PQPFLLFANS QDIRHMHFDG
501 TDYGTLLSQQ MGMVYALDHD PVENKIYFAH TALKWIERAN MDGSQRERLI
551 EEGVDVPEGL AVDWIGRRFY WDRGKSLIG RSDLNGKRSK IITKENISQP
601 RGIAVHPMAK RLFWTDGTIN PRIESSSLQG LGRLVIASSD LIWPSGITID
651 FLTDKLYWCD AKQSVIEMAN LDGSKRRRLT QNDVGHPFAV AVFEDYVWFS
701 DWAMPSVMRV NKRTGKDRVR LQGSMLKPSS LVVVHPLAKP GADPCLYQNG
751 GCEHICKKRL GTAWCSCREG FMKASDGKTC LALDGHQLLA GGEVDLKNQV
801 TPLDILSKTR VSEDNITESQ HMLVAEIMVS DQDDCAPVGC SMYARCISEG
851 EDATCQCLKG FAGDGKLCSD IDECEMGVPV CPPASSKCIN TEGGYVCRCS
901 EGYQGDGIHC LDIDECQLGE HSCGENASCT NTEGGYTCMC AGRLEPGLI
951 CPDSTPPPHL REDDHHYSVR NSDSECPLSH DGYCLHDGVC MYIEALDKYA
1001 CNCVVG YIGE RCQYRDLKWW ELRHAGHGQQ QKVIVVAVCV VVLVMLLLLS
1051 LWGAHYIRTQ KLLSKNPKNP YEESRDVRS RRPADTEDGM SSCPQPFV
1101 IKEHQDLKNG GQPVAGEDGQ AADGSMQPTS WRQEPQLCGM GTEQGCWIPV
1151 SSDKGSCPQV MERSFHMPsy GTQTLEGGVE KPHSLLSANP LWQQRALDPP
1201 HQMELTQ
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Figure 7

SEQ ID NO: 3 NT-proBNP NP_002512

1 MDPQTAPSRA LLLLLFLHLA FLGGRSHPLG SPGSASDLET SGLQEQRNHL
51 QGKLSELQVE QTSLEPLQES PRPTGVWKS R EVATEGIRGH RKMVLYTLRA
101 PRSPKMVQGS GCFGRKMDRI SSSSGLGCKV LRRH

Figure 8

SEQ ID NO: 4 ApoH NP_000033

1 MISPVLILFS SFLCHVAIAG RTCPKPDDL P FSTVVPLKTF YEPGEEITYS
51 CKPGYVSRGG MRKFICPLTG LWPINTLKCT PRVCPFAGIL ENGAVRYTTF
101 EYPNTISFSC NTGFYLNAD SAKCTEEGKW SPELPVCAPI ICPPPSIPTF
151 ATLRVYKPSA GNNSLYRDTA VFECLPQHAM FGNDTITCTT HGNWTKLPEC
201 REVKCPFPSR PDNGFVNYP A KPTLYYKDKA TFGCHDGYS L DGPEEIECTK
251 LGNWSAMPSC KASCKVPVKK ATVVYQGERV KIQEKFKNGM LHGDKVSFFC
301 KNKEKKCSYT EDAQCIDGTI EVPKCFKEHS SLAFWKTDAS DVKPC

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Figure 9

SEQ ID NO: 5 ALBUMIN NP_000468

1 MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA
51 FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD ESAENCCKSL HTLFGDKLCT
101 VATLRETYGE MADCCAKQEP ERNECFLLQHK DDNPNLPRLV RPEVDVMCTA
151 FHDNEETFLK KYLYEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA
201 CLLPKLDELRL DEGKASSAKQ RLKCASLQKF GERAFAKAWAV ARLSQRFPKA
251 EFAEVSKLVT DLTQVHTECC HGDLLLECADD RADLAKYICE NQDSISSKLK
301 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF
351 LGMFLYEYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
401 FKPLVEEPQN LIKQNCSELF QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV
451 SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTKC
501 CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
551 TALVELVKHK PKATKEQLKA VMDDFAAFVE KCKKADDKET CFAEEGKKLV
601 AASQAALGL