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(54) Title: BIOMARKERS FOR THE DIAGNOSIS OF RENAL ALLOGRAFT AND KIDNEY STATUS

(57) Abstract: The present invention relates to the identification and use of protein biomarkers with clinical relevance to kidney status and chronic renal injury or disorder. In particular, the invention provides the identity of marker proteins which are recognized by antibodies present in patients suffering from end-stage renal disorder, stable renal transplant, renal transplant glomerulopathy (TG), and interstitial fibrosis and tubular atrophy (IFTA). Methods and kits are described for using these proteins in the study and diagnosis of chronic renal transplant injury, and in the selection and/or monitoring of treatment regimens.

Biomarkers for the Diagnosis of Renal Allograft and Kidney Status

Related Applications

The present application claims priority to European Patent Application No. EP 08 305 988.1 filed on December 19, 2008. The European patent application is incorporated herein by reference in its entirety.

Background of the Invention

Although end-stage disease patients can be treated through other renal replacement therapies such as hemodialysis and peritoneal dialysis, kidney transplantation is generally accepted as the best treatment because it increases patients survival, improves quality of life and presents cost effectiveness. Renal transplantation is by far the most frequently carried out transplantation globally. The World Health Organization estimates that about 66,000 kidney transplants (from living and deceased donors) are performed annually compared to 21,000 liver transplants and 6,000 heart transplants. Renal transplantation is also the most successful and therapeutically advanced of all organ transplant procedures. Recent reports of patient survival in renal transplantation show a 91.3% 1-year and 69.6% 5-year survival for recipients of cadaveric grafts, and a 97.3% 1-year and 80.3% 5-year survival for those receiving living donor transplants (2007 Annual Report of the Scientific Registry of Transplant Recipients and U.S. Organ Procurement and Transplantation Network: Transplant Data 1997-2006). In France, patient survival in renal transplantation shows a 79.1% at 5-years and 62.4% at 10-year survival for recipients of cadaveric grafts, and 89.6% at 5-year and 76.7% at 10-year for those receiving a living donor transplant (2007 Report of "Agence de Biomédecine": www.agence-biomedicine.fr). With improved immunosuppression and early transplant survival, chronic allograft rejection has become the most prevalent cause of kidney transplant failure. Up to 40% of grafts develop progressive dysfunction after the initial few post-transplant months and ultimately fail within a decade, despite the use of immunosuppressive drugs in doses sufficient to prevent acute rejection.

Several pathophysiological processes contribute to chronic renal transplant injury, including renal transplant glomerulopathy (TG) and interstitial fibrosis and tubular atrophy (IFTA) according to the newly revised Banff classification system (Solez *et al.*, Am. J. Transplant, 2007, 7: 518-526). Renal transplant glomerulopathy

is associated with graft dysfunction, hypertension, proteinurea and shortened graft survival. It is a unique pathologic and pathogenic entity distinct from other forms of chronic allograft injury (Suri *et al.*, Am. J. Kidney Dis., 2000, 35: 674-680). Transplant glomerulopathy is characterized by duplication of the glomerular basement membrane, mesangial interposition, and electron luscent widening of the sub-endothelial space with accumulation of flocculent material. The pathogenesis of transplant glomerulopathy is unclear, but, increasingly, investigators hypothesize immune-mediated mechanisms with a strong emphasis on humoral immunity as defined by detection of donor-specific anti-HLA antibodies in patients' serum (Hourmant *et al.*, J. Am. Soc. Nephrol., 2005, 16: 2804-2812) and/or positive C4d staining of the allograft.

The chronic allograft nephropathy (CAN) encompasses all late scarring of the graft due to immunologic and non-immunologic injury. The last Banff classification eliminated the CAN denomination and replaced it with Interstitial Fibrosis and Tubular Atrophy (IFTA). The etiologies of this disease are multiple including chronic hypertension, drug toxicity, viral infection, and chronic obstruction (Solez *et al.*, Am. J. Transplantation, 2007, 7: 518-526).

Biopsies have revealed that renal transplant glomerulopathy and IFTA can develop during the first few months after transplantation (Wavamunno *et al.*, Am. J. Transplantation, 2007, 7: 2757-2768). However, currently available diagnostic methods often fail to detect chronic rejection until late stages of progression, when lesions and damage to the transplant have already occurred. Clinical manifestations such as creatinine and altered resistance indexes on Doppler ultrasonography are not specific. At present, biopsy of the renal allograft is considered as the standard for the diagnosis of chronic rejection. However, percutaneous renal biopsy is a costly, invasive procedure, which carries the risk of procedural complications including allograft thrombosis, sepsis, hematuria, and anuria. Consequently, serial renal biopsies are avoided, which limits the use of this procedure in the follow-up of the disease activity, treatment response and recurrence. This situation explains the need for a non-invasive diagnostic tool that can be used to monitor renal allograft, even before graft dysfunction occurs, and to improve treatment success through early and reliable detection.

Several studies have been undertaken with the goal of identifying biomarkers of chronic renal transplant rejection. Gene expression profiles of chronically rejected renal transplants (Donauer *et al.*, Transplantation, 2003, 76: 539-547; Sarwal, New Engl. J. Medicine, 2003, 349: 125-138), operationally tolerant kidney graft recipients and patients with chronic rejection (WO 2005/070086); kidney biopsies and peripheral blood lymphocytes (Flechner *et al.*, Am. J. Transplantation, 2004, 4: 1475-1489) have been determined using microarrays. A study investigated kidney biopsies performed 6 months post transplant using an Affymetrix GeneChip and identified 10 genes for which expression correlated with the risk of developing chronic rejection defined by biopsy at 12 months post transplant (Scherer *et al.*, Transplantation, 2003, 75: 1323-1330). Recently, efforts are focussing on the characterization of renal rejection by urinary proteomic analysis (Clarke *et al.*, Annals of Surg., 2003, 237: 660-665; WO 2004/030521; Schaub *et al.*, J. Am. Soc. Nephrol., 2004, 15: 219-227; Reichelt *et al.*, Urology, 2006, 67: 472-475; O'Riordan *et al.*, Am. J. Transplant., 2007, 7: 930-340, Thongboonkerd, Proteomics Clin. Appl., 2008, 2: 1413-1421).

Summary of the Invention

The present invention relates to improved systems and strategies for the diagnosis of chronic renal transplant rejection. In particular, the invention provides the identity of proteins that reflect clinically relevant processes in dialysis patients with end-stage kidney disorder and in renal transplant patients. More specifically, the invention provides biomarkers that can be used for detecting the presence of antibodies that are selectively indicative of end-stage kidney disorder, renal transplant glomerulopathy, interstitial fibrosis and tubular atrophy (IFTA), and stable renal transplant, in *in vitro* biological samples (in particular blood samples) obtained from patients. Compared to existing methods of diagnosis, the inventive methods are non-invasive, and the protein profiles disclosed herein constitute a more robust signature of each of the different pathologies, and provide a more reliable basis for the selection and monitoring of appropriate therapeutic regimens.

More specifically, in one aspect, the present invention provides a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof; a set of biomarkers indicative of stable renal

transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof, a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof; and a set of biomarkers indicative of IFTA consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

The present invention also provides for the use of at least one of these biomarkers, preferably a plurality of these biomarkers, for the *in vitro* determination of kidney status or renal transplant status in a subject.

In certain embodiments, the biomarkers are provided immobilized on a solid carrier or support, such as beads or array.

Accordingly, in a related aspect, the present invention provides an array for the *in vitro* determination of kidney status or renal transplant status in a subject, the array essentially having, attached to its surface, a plurality of biomarkers selected from those described herein.

In another aspect, the present invention provides a method for diagnosing or determining kidney status or renal transplant status in a subject, the method comprising steps of contacting a blood sample obtained from the subject with at least one biomarker for a time and under conditions allowing a biomarker-antibody complex to form between the at least one biomarker and an antibody present in the blood sample; and detecting any biomarker-antibody complex formed. In this method, the at least one biomarker is selected from the group consisting of:

a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of stable renal transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof, and

5 a set of biomarkers indicative of IFTA consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

In certain embodiments, the at least one biomarker comprises a plurality of biomarkers, *i.e.*, in other words, the method of the invention comprises steps of: 10 contacting the blood sample with a plurality of biomarkers for a time and under conditions allowing biomarker-antibody complexes to form between the biomarkers and antibodies present in the blood sample; and detecting any biomarker-antibody complex formed.

A blood sample, obtained from the subject and suitable for use in a method of 15 diagnosis of the present invention, may be selected from the group consisting of whole blood, plasma, and serum. Generally, the subject from whom the blood sample is obtained is a dialysis patient or a renal transplant patient.

In certain embodiments, the biomarkers used in the methods of the invention are immobilized on a solid carrier or support, such as beads or array. In certain preferred 20 embodiments, the biomarkers are immobilized on an array.

In the methods provided herein, detection of a biomarker-antibody complex formed between the at least one biomarker and an antibody present in the blood sample may be performed by any suitable method. In certain embodiments, the detection is by immunoassay. For example, the step of detecting a biomarker-25 antibody complex may comprise contacting the blood sample obtained from the subject with at least one reagent for the detection of the biomarker-antibody complex, such as a labeled anti-human antibody.

In yet another aspect, the present invention provides a kit for the *in vitro* determination of kidney status or renal transplant status in a subject, the kit 30 comprising at least one biomarker described herein. In certain embodiments, the kit comprises a plurality of biomarkers. Preferably, determination of kidney status or renal transplant status is performed using a blood sample obtained from the subject.

In certain embodiments, the at least one biomarker or the plurality of biomarkers is immobilized on a solid support, such as beads or array. Preferably, the at least one biomarker or the plurality of biomarkers is immobilized on an array.

In certain embodiments, a kit of the present invention further comprises at least one reagent for the detection of a biomarker-antibody complex formed between the at least one biomarker included in the kit and an antibody present in a blood sample obtained from a subject. Such a reagent may be a labeled anti-human antibody.

The kit may further comprise instructions for carrying out any of the methods of diagnosis provided herein.

In certain embodiments, one or both of CCL19 and CDC20 is/are not used in the methods of the present invention and/or is/are not present on an inventive array or an inventive kit.

These and other objects, advantages and features of the present invention will become apparent to those of ordinary skill in the art having read the following detailed description of the preferred embodiments.

Brief Description of the Drawing

Figure 1 is a table presenting the characteristics of the patients who were tested in the study described in Example 1. Patients 1 to 6 were found to exhibit both TG and IFTA features based on biopsy analysis. Patients 7 to 19 only showed signs of TG. Patients 20 to 29 had IFTA only without C4d deposits in the graft. Patients 30 to 35 were found to have no renal function degradation and had normal biopsies. Dialysis (DIA) patients (who were also tested in the study described in Example 1) are not presented in this table since they had not received kidney graft and therefore did no have any biopsy data.

Definitions

Throughout the specification, several terms are employed that are defined in the following paragraphs.

As used herein, the term "***subject***" refers to a human or another mammal (*e.g.*, primate, dog, cat, goat, horse, pig, mouse, rat, rabbit, and the like) that can undergo kidney transplantation, but may or may not have undergone kidney

transplantation. In many embodiments of the present invention, the subject is a human being. In such embodiments, the subject is often referred to as an "***individual***" or a "***patient***". As used herein, the term "***renal transplant patient***" refers to an individual that has undergone kidney transplantation. The terms "individual" and
5 "patient" do not denote a particular age.

As used herein, the term "***diagnosis***" refers to a process aimed at determining if an individual is afflicted with a disease or ailment. The terms "***diagnosis of renal transplant status***" and "***determination of renal transplant status***" are used herein interchangeably. They refer to a process aimed at one or more of: determining if the
10 renal transplant of a patient is stable, determining if a renal transplant patient has a chronic renal transplant rejection disorder, and determining if the chronic renal transplant rejection disorder is renal transplant glomerulopathy or IFTA. The terms "***diagnosis of kidney status***" and "***determination of kidney status***" are used herein interchangeably. They refer to a process aimed at determining if the kidney of a
15 patient is afflicted with end-stage chronic kidney disease.

The term "***biological sample***" is used herein in its broadest sense. A biological sample is generally obtained from a subject. A sample may be of any biological tissue or fluid with which biomarkers of the present invention may be assayed. Frequently, a sample will be a "clinical sample", *i.e.*, a sample derived from a patient. Such
20 samples include, but are not limited to, bodily fluids which may or may not contain cells, *e.g.*, blood (*e.g.*, whole blood, serum or plasma), urine, saliva, tissue or fine needle biopsy samples, and archival samples with known diagnosis, treatment and/or outcome history. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. The term "biological sample" also
25 encompasses any material derived by processing a biological sample. Derived materials include, but are not limited to, cells (or their progeny) isolated from the sample, proteins or nucleic acid molecules extracted from the sample. Processing of a biological sample may involve one or more of: filtration, distillation, extraction, concentration, inactivation of interfering components, addition of reagents, and the
30 like. In preferred embodiments of the invention, the biological sample is (or is derived from) whole blood, serum or plasma obtained from a subject.

The terms "***normal***" and "***healthy***" are used herein interchangeably. They refer to an individual or group of individuals who have not undergone kidney

transplantation and who have not shown any symptoms of kidney injury, damage or dysfunction. The term "normal" is also used herein to qualify a sample (*e.g.*, a blood sample) obtained from a healthy individual.

In the context of the present invention, the term "***control***", when used to
5 characterize a subject, refers to a subject that is healthy, to a patient that has been diagnosed with chronic renal disease (*e.g.*, end-stage chronic kidney disease), or to a renal transplant patient that has been diagnosed with a stable renal transplant, with renal transplant glomerulopathy or with IFTA. The term "control sample" refers to one, or more than one, sample that has been obtained from a healthy subject or from a
10 patient diagnosed with a particular kidney status or renal transplant status as described above.

The terms "***biomarker***" and "***marker***" are used herein interchangeably. They refer to a substance that is a distinctive indicator of a biological process, biological event and/or pathologic condition. The term "***biomarker of kidney status***" refers to a
15 protein selected from the set of proteins provided by the present invention and which is indicative of or specifically recognized by dialysis patients waiting for renal transplantation. The term "***biomarker of renal transplant status***" refers to a protein selected from the set of protein provided by the present invention and which is indicative of or specifically recognized by renal transplant patients with stable renal
20 transplant, renal transplant glomerulopathy or IFTA.

As used herein, the terms "***indicative of kidney status***" and "***indicative of renal transplant status***", when applied to a process or event, refers to a process or event which is diagnostic of a kidney status or a renal transplant status, such that the process or event is found significantly more often in subjects with a given kidney status or a
25 given renal transplant status than in subjects with a different kidney status or a different renal transplant status (as determined using routine statistical methods). Preferably, a protein biomarker which is indicative of a given kidney status (*e.g.*, end-stage kidney disease) or a given renal transplant status (*e.g.*, stable renal transplant, renal transplant glomerulopathy or IFTA) is recognized by at least 60% of subjects
30 who exhibit the kidney status or the renal transplant status, respectively and is recognized by less than 10% of subjects who do not exhibit the kidney status or the renal transplant status. More preferably, a protein biomarker which is indicative of a given kidney status or of a given renal transplant status is recognized by at least 70%,

at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more patients who exhibit the same kidney status or the same renal transplant status and is recognized by less than 10%, less than 8%, less than 5%, less than 2% or less than 1% subjects who do not exhibit the same kidney status or the same renal transplant status.

5 The terms "***protein***", "***polypeptide***", and "***peptide***" are used herein interchangeably, and refer to amino acid sequences of a variety of lengths, either in their neutral (uncharged) forms or as salts, and either unmodified or modified by glycosylation, side chain oxidation, or phosphorylation. In certain embodiments, the amino acid sequence is a full-length native protein. In other embodiments, the amino 10 acid sequence is a smaller fragment of the full-length protein. In still other embodiments, the amino acid sequence is modified by additional substituents attached to the amino acid side chains, such as glycosyl units, lipids, or inorganic ions such as phosphates, as well as modifications relating to chemical conversion of the chains such as oxidation of sulfhydryl groups. Thus, the term "protein" (or its equivalent 15 terms) is intended to include the amino acid sequence of the full-length native protein or a fragment thereof, subject to those modifications that do not significantly change its specific properties. In particular, the term "protein" encompasses protein isoforms, *i.e.*, variants that are encoded by the same gene, but that differ in their pI or MW, or both. Such isoforms can differ in their amino acid sequence (*e.g.*, as a result of 20 alternative splicing or limited proteolysis), or in the alternative, may arise from differential post-translational modification (*e.g.*, glycosylation, acylation, phosphorylation).

The term "***protein analog***", as used herein, refers to a polypeptide that possesses a similar or identical function as the protein but need not necessarily comprise an 25 amino acid sequence that is similar or identical to the amino acid sequence of the protein or a structure that is similar or identical to that of the protein. Preferably, in the context of the present invention, a protein analog has an amino acid sequence that is at least about 30%, more preferably at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 30 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or at least bout 99%, identical to the amino acid sequence of the protein.

The term "***protein fragment***", as used herein, refers to a polypeptide comprising an amino acid sequence of at least 5 consecutive amino acid residues (preferably at least about: 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, or 250 consecutive amino acid residues) of the amino acid sequence of the protein. The
5 fragment of a protein may or may not possess a functional activity of the protein.

The term "***antibody-binding fragment***", when used herein in connection with a protein (in particular a protein biomarker), refers to a fragment of the protein that retains the ability of the protein to bind an antibody to form an antigen-antibody complex. In particular, an antibody-binding fragment of a protein biomarker of the
10 invention retains the ability to bind an antibody specifically found in patients with one type of kidney status or renal transplant status. Suitable antibody-binding fragments of a protein biomarker may be identified by one skilled in the art by simple trials to ascertain their ability to bind kidney status-specific antibodies or renal transplant status-specific antibodies.

15 The term "***homologous***" (or "homology"), as used herein is synonymous with the term "***identity***" and refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both compared sequences is occupied by the same base or same amino acid residue, then the respective molecules are homologous at that position. The percentage of homology
20 between two sequences corresponds to the number of matching or homologous positions shared by the two sequences divided by the number of positions compared and multiplied by 100. Generally, a comparison is made when two sequences are aligned to give maximum homology. Homologous amino acid sequences share identical or similar amino acid sequences. Similar residues are conservative
25 substitutions for, or "allowed point mutations" of, corresponding amino acid residues in a reference sequence residue. "Conservative substitutions" of a residue in a reference sequence are substitutions that are physically or functionally similar to the corresponding reference residue, *e.g.*, they have a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the
30 like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for "accepted point mutation" by Dayhoff *et al.* ("Atlas of Protein Sequence and Structure", 1978, Nat. Biomed. Res. Foundation, Washington, DC, Suppl. 3, 22: 354-352).

The terms "***protein array***" and "***protein chip***" are used herein interchangeably. They refer to a substrate surface on which different proteins (or protein fragments) have been immobilized, in an ordered manner, at discrete spots on the substrate. Protein arrays may be used to identify protein/protein interactions (*e.g.*, antigen/antibody interactions), to identify substrates of enzymes, or to identify the targets of biologically active small molecules. The term "microarray" more specifically refers to an array that is miniaturized so as to require microscopic examination for visual evaluation.

The term "***protein bead suspension array***" refers to a suspension of one or more identifiable distinct particles whereby each particle contains coding features relating to its size and color or fluorescence signature and to which each of the beads of a particular combination is coated with a protein (*e.g.*, a protein biomarker of the invention). Like protein arrays, protein bead suspension arrays may be used to identify protein/protein interactions (*e.g.*, antigen/antibody interactions), to identify substrates of enzymes, or to identify the targets of biologically active small molecules. Examples of bead suspension arrays include thexMAP® bead suspension array (Luminex Corporation).

The terms "***labeled***", "***labeled with a detectable agent***" and "***labeled with a detectable moiety***" are used herein interchangeably. These terms are used to specify that an entity (*e.g.*, an antibody) can be visualized, for example, following binding to another entity (*e.g.*, a protein biomarker). Preferably, a detectable agent or moiety is selected such that it generates a signal which can be measured and whose intensity is related to the amount of bound entity. In array-based methods, a detectable agent or moiety is also preferably selected such that it generates a localized signal, thereby allowing spatial resolution of the signal from each spot on the array. Methods for labeling proteins and polypeptides are well known in the art. Labeled polypeptides (*e.g.*, antibodies) can be prepared by incorporation of or conjugation to a label, that is directly or indirectly detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, or chemical means, or any suitable means. Suitable detectable agents include, but are not limited to, various ligands, radionuclides, fluorescent dyes, chemiluminescent agents, microparticles, enzymes, colorimetric labels, magnetic labels, and haptens.

The term "***treatment***" is used herein to characterize a method that is aimed at (1) delaying or preventing the onset of a disease or condition; or (2) slowing down or stopping the progression, aggravation, or deteriorations of the symptoms of the condition; or (3) bringing about ameliorations or the symptoms of the condition; or
5 (4) curing the condition. A treatment may be administered prior to the onset of the disease, for a prophylactic or preventive action. It may also be administered after initiation of the disease, for a therapeutic action.

Detailed Description of Certain Preferred Embodiments

As mentioned above, the present invention provides biomarkers that can be used
10 for detecting the presence of antibodies that are selectively and specifically indicative of chronic renal disease, stable renal transplant, renal transplant glomerulopathy (TG), and IFTA, in *in vitro* biological samples obtained from patients. Also provided are methods, arrays and kits for using these biomarkers for determining renal transplant or kidney status in a subject.

15 **I – Biomarkers**

As described in the Examples Section below, the present Applicants have identified protein biomarkers relevant to chronic renal transplant rejection disorders using the ProtoArray Human Protein Microarray commercially available from Invitrogen. This microarray contains more than 8000 human proteins, including
20 proteases/peptidases, secreted proteins, transcription factors, cell death proteins, protein kinases, nuclear proteins, membrane proteins, and metabolism proteins. More specifically, the Applicants have screened samples of serum obtained from dialysis individuals diagnosed with end-stage renal disorder, patients with stable renal transplant, patients diagnosed with renal transplant glomerulopathy, and patients
25 diagnosed with IFTA; and then compared the binding patterns of each group tested with each one of the other three groups. This work led to the identification of highly specific protein biomarkers, *i.e.*, protein biomarkers that are recognized only by one group of the patients tested and that are not recognized by any of the three other groups of patients tested.

30 The proteins that were selectively recognized by the dialysis patients with end-stage renal disorder (and not by the other groups of patients) are: RAB20 (member RAS oncogene family, which is encoded by a gene whose GenBank Accession

Number is NM_017817.1), OAS2 (2',5'-oligoadenylate synthetase 2 69/71 kDA, which is encoded by a gene whose GenBank Accession Number is BC049215.1); IL1RL1(3) (interleukin 1 receptor-like 1, transcript variant 3, which is encoded by a gene whose GenBank Accession Number is NM_173459.1); GLRA2 (glycine receptor, alpha 2, which is encoded by a gene whose GenBank Accession Number is BC032864.2); SMAD3 (SMAD family member 3, which is encoded by a gene whose GenBank Accession Number is NM_005902); ING4(2) (inhibitor of growth family, member 4, transcript variant 2, which is encoded by a gene whose GenBank Accession Number is NM_198287.1); CCL19 (chemokine (C-C motif) ligand 19, which is encoded by a gene whose GenBank Accession Number is NM_006274.2); CENTD2 (centaurin, delta 2, which is encoded by a gene whose GenBank Accession Number is BC056401.1); ECE1 (endothelin converting enzyme 1, which is encoded by a gene whose GenBank Accession Number is NM_001397.1); and LGALS3 (lectin, galactoside-binding, soluble, 3, which is encoded by a gene whose GenBank Accession Number is BC001120.2).

The proteins that were selectively recognized by the patients with stable renal transplant (and not by the other groups of patients) are: ARPC1B (actin related protein 2/3 complex, subunit 1B, 41kDa, which is encoded by a gene whose GenBank Accession Number is NM_005720.2); CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1, which is encoded by a gene whose GenBank Accession Number is NM_000773.3); CDC20 (cell division cycle 20 homolog (S. cerevisiae), which is encoded by a gene whose GenBank Accession Number is BC001088.2); RIT1 (Ras-like without CAAX 1, which is encoded by a gene whose GenBank Accession Number is NM_006912.4); PHF21B (PHD finger protein 21B, which is encoded by a gene whose GenBank Accession Number is NM_138415.2); RBM34 (RNA binding motif protein 34, which is encoded by a gene whose GenBank Accession Number is NM_015014.1); MS4A6E (membrane-spanning 4-domains, subfamily A, member 6E, which is encoded by a gene whose GenBank Accession Number is NM_139249.2); C20orf132 (chromosome 20 open reading frame 132, which is encoded by a gene whose GenBank Accession Number is BC057767.1); LRRFIP1 (leucine rich repeat (in FLII) interacting protein 1, which is encoded by a gene whose GenBank Accession Number is BC010662.1); and CKLF(1) (chemokine-

like factor, transcript variant 1, which is encoded by a gene whose GenBank Accession Number is NM_016951.2).

The proteins that were selectively recognized by the patients with renal transplant glomerulopathy (TG) (and not by the other groups of patients) are: PODN (podocan, which is encoded by a gene whose GenBank Accession Number is BC030608.2); HM13(4) (histocompatibility (minor) 13, transcript variant 4, which is encoded by a gene whose GenBank Accession Number is NM_178582.1); TSC22D3(2) (TSC22 domain family, member 3, transcript variant 2, which is encoded by a gene whose GenBank Accession Number is NM_004089.3); EPN1 (epsin 1, which is encoded by a gene whose GenBank Accession Number is NM_013333.3); COL23A1 (collagen, type XXIII, alpha 1, which is encoded by a gene whose GenBank Accession Number is BC042428.1); GIMAP6 (GTPase, IMAP family member 6, which is encoded by a gene whose GenBank Accession Number is BC060760.1); ZNF174 (zinc finger protein 174, which is encoded by a gene whose GenBank Accession Number is BC000876.1); MAP3K11 (mitogen-activated protein kinase kinase kinase 11, which is encoded by a gene whose GenBank Accession Number is NM_002419.3); CRB3(2) (crumbs homolog 3 (Drosophila), transcript variant 2, which is encoded by a gene whose GenBank Accession Number is NM_139161.3); PRKCDBP (protein kinase C, delta binding protein, which is encoded by a gene whose GenBank Accession Number is BC011585.1); DCLK1 (doublecortin-like kinase 1, which is encoded by a gene whose GenBank Accession Number is NM_004734.2); FEN1 (flap structure-specific endonuclease 1, which is encoded by a gene whose GenBank Accession Number is NM_004111.4); TANK(2) (TRAF family member-associated NFKB activator, transcript variant 2, which is encoded by a gene whose GenBank Accession Number is NM_133484.1); GLRX5 (glutaredoxin 5, which is encoded by a gene whose GenBank Accession Number is NM_016417.2); RAB22A (RAB22A, member RAS oncogene family, which is encoded by a gene whose GenBank Accession Number is NM_020673.2); MAEL (maelstrom homolog (Drosophila), which is encoded by a gene whose GenBank Accession Number is NM_032858.1); FAM71D (family with sequence similarity 71, member D, which is encoded by a gene whose GenBank Accession Number is BC050401.1); and TTC35 (tetratricopeptide repeat domain 35, which is encoded by a gene whose GenBank Accession Number is NM_014673.3).

The proteins that were selectively recognized by the patients with IFTA (and not by the other groups of patients) are: IL16 (interleukin 16 (lymphocyte chemoattractant factor), which is encoded by a gene whose GenBank Accession Number is BC040272.1); MLF1 (myeloid leukemia factor 1, which is encoded by a gene whose GenBank Accession Number is NM_022443.3); IFT81(2) (intraflagellar transport 81 homolog (Chlamydomonas), transcript variant 2, which is encoded by a gene whose GenBank Accession Number is NM_031473.2); DYRK2 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2, which is encoded by a gene whose GenBank Accession Number is BC006375.2); NDFIP2 (Nedd4 family interacting protein 2, which is encoded by a gene whose GenBank Accession Number is BC021988.1); CCNE2 (cyclin E2, which is encoded by a gene whose GenBank Accession Number is BC020729.1); SNX21 (sorting nexin family member 21, which is encoded by a gene whose GenBank Accession Number is BC019823.1); TUBB2C (tubulin, beta 2C, which is encoded by a gene whose GenBank Accession Number is BC029529.1); MUC20 (mucin 20, cell surface associated, which is encoded by a gene whose GenBank Accession Number is BC029267.1); ATP1A3 (ATPase, Na+/K+ transporting, alpha 3 polypeptide, which is encoded by a gene whose GenBank Accession Number is NM_152296.3); and F11R(1) (F11 receptor, transcript variant 1, which is encoded by a gene whose GenBank Accession Number is NM_016946.4).

Inventive Protein Biomarkers

Accordingly, the present invention provides a set of protein biomarkers that are specifically recognized by dialysis patients diagnosed with end-stage renal disorder, and that can be used for detecting the presence of antibodies that are selectively indicative of end-stage renal disorder in *in vitro* biological samples (in particular blood samples) obtained from patients. This set of protein biomarkers consists of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof.

The present invention also provides a set of protein biomarkers that are specifically recognized by renal transplant patients with stable renal implants, and that can be used for detecting the presence of antibodies that are selectively indicative of stable renal transplant in *in vitro* biological samples (in particular blood samples) obtained from patients. This set of protein biomarkers consists of ARPC1B, CYP2E1,

CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof.

The present invention also provides a set of protein biomarkers that are specifically recognized by renal transplant patients diagnosed with renal transplant
5 glomerulopathy, and that can be used for detecting the presence of antibodies that are specifically indicative of renal transplant glomerulopathy in *in vitro* biological samples (in particular blood samples) obtained from patients. This set of protein biomarlers consists of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5,
10 RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof.

The present invention also provides a set of protein biomarkers that are specifically recognized by renal transplant patients diagnosed with IFTA, and that can be used for detecting the presence of antibodies that are specifically indicative of
15 IFTA in *in vitro* biological samples (in particular blood samples) obtained from patients. This set of protein biomarkers consists of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

The present invention also provides a set of protein biomarkers that can be used
20 to discriminate between patients with end-stage renal disorder, patients with stable renal transplant, patients with renal transplant glomerulopathy, and patients with IFTA. The set of protein biomarkers consists of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), PODN,
25 HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, IL16 MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof. The pattern of recognition of these proteins by a patient can be used to diagnose a
30 kidney status or a renal transplant status.

Thus, the present invention further provides for the use of at least one of the protein biomarkers described herein to diagnose kidney status or renal transplant

status in a subject. The present invention also provides for the use of any combination (*i.e.*, at least two) of the protein biomarkers described herein to diagnose kidney status or renal transplant status in a subject. The present invention also provides for the use of all of the protein biomarkers described herein to diagnose kidney status or renal
5 transplant status in a subject. The present invention further provides for the use of all of the protein biomarkers indicative of stable renal transplant, renal transplant glomerulopathy, and IFTA to diagnose renal transplant status. The present invention further provides for the use of all of the protein biomarkers indicative of end-stage kidney disorder to diagnose kidney status.

10 Other biomarkers provided by the present invention include nucleic acid molecules comprising polynucleotide sequences coding for the inventive protein biomarkers described herein (or analogs and fragments thereof) and polynucleotides that hybridize with portions of these nucleic acid molecules.

Preparation of Protein Biomarkers

15 Protein biomarkers to be used in the methods of diagnosis of the present invention may be prepared by any suitable method, including chemical synthesis and recombinant methods.

For example, the biomarkers of the invention may be prepared using standard chemical methods. Solid-phase peptide synthesis, which was initially described by
20 R.B. Merrifield (J. Am. Chem. Soc. 1963, 85: 2149-2154) is a quick and easy approach to synthesizing peptides and peptidic molecules of known sequences. A compilation of such solid-state techniques may be found, for example, in "*Solid Phase Peptide Synthesis*" (Methods in Enzymology, G.B. Fields (Ed.), 1997, Academic Press: San Diego, CA, which is incorporated herein by reference in its entirety). Most
25 of these synthetic procedures involve the sequential addition of one or more amino acid residues or suitable protected amino acid residues to a growing peptide chain. For example, the carboxy group of the first amino acid is attached to a solid support *via* a labile bond, and reacted with the second amino acid, whose amino group has, beforehand, been chemically protected to avoid self-condensation. After coupling, the
30 amino acid group is deprotected, and the process is repeated with the following amino acid. Once the desired peptide is assembled, it is cleaved off from the solid support, precipitated, and the resulting free peptide may be analyzed and/or purified as desired.

Solution methods, as described, for example, in "*The Proteins*" (Vol. II, 3rd Ed., H. Neurath *et al.* (Eds.), 1976, Academic Press: New York, NY, pp. 105-237), may also be used to synthesize the biomarkers of the invention.

Alternatively, the protein biomarkers provided herein can be produced by
5 recombinant DNA methods. These methods generally involve isolation of the gene encoding the desired protein, transfer of the gene into a suitable vector, and bulk expression in a cell culture system. The DNA coding sequences for the polypeptides of the invention may be readily prepared synthetically using methods known in the art (see, for example, M.P. Edge *et al.*, Nature, 1981, 292: 756-762).

10 After synthesis, the DNA encoding the desired peptide is inserted into a recombinant expression vector, which may be a plasmid, phage, viral particle, or other nucleic acid molecule-containing vectors or nucleic acid molecule-containing vehicles which, when introduced into an appropriate host cell, contains the necessary genetic elements to direct expression of the coding sequence of interest. Standard techniques
15 well known in the art can be used to insert the nucleic acid molecule into the expression vector. The insertion results in the coding sequence being operatively linked to the necessary regulatory sequences.

Host cells for use in the production of proteins are well known and readily available. Examples of host cells include bacteria cells such as *Escherichia coli*,
20 *Bacillus subtilis*, attenuated strains of *Salmonella typhimurium*, and the like; yeast cells such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins; insect cells such as *Spodoptera frugiperda*; non-human mammalian tissue culture cells such as Chinese Hamster Ovary (CHO) cells, monkey COS cells, and mouse 3T3
25 cells; and human tissue culture cells such as HeLa cells, HL-60 cells, kidney 293 cells and epidermal S431 cells.

Several expression vectors to produce polypeptides in well known expression systems are commercially available. For example, the plasmids pSE420 (available from Invitrogen, San Diego, CA) and pBR322 (available from New England Biolabs,
30 Beverly, MA) may be used for the production of the inventive peptides in *E. coli.* Similarly, the plasmid pYES2 (Invitrogen) may be used for peptide production in *S. cerevisiae* strains of yeast. The commercially available MacBacR™ kit (Invitrogen)

for baculovirus expression system or the BaculoGold™ Transfection Kit available from PharMingen (San Diego, CA) may be used for production in insect cells, while the plasmids pcDNA I, pcDNA 3, and pRc/RSV, commercially available from Invitrogen, may be used for the production of the peptides of the invention in mammalian cells such as Chinese Hamster Ovary (CHO) cells.

Other expression vectors and systems can be obtained or produced using methods well known to those skilled in the art. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers are readily available for a variety of hosts (see, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, 2nd Ed., 1989, Cold Spring Harbor Press: Cold Spring, NY; and R. Kaufman, Methods in Enzymology, 1990, 185: 537-566).

The expression vector including DNA that encodes the desired protein is used to transform the compatible host cell. The host cell is then cultured and maintained under conditions favoring expression of the desired protein. The protein thus produced is recovered and isolated, either directly from the culture medium or by lysis of the cells. If desired, it can then be characterized by different methods such as Nuclear Magnetic Resonance (NMR) or X-ray crystallography.

As understood by one skilled in the art, a protein biomarker of the present invention may be produced as a fusion protein (*i.e.*, a molecule in which an antibody-binding moiety is linked to a polypeptide entity). Such a polypeptide entity may be selected to confer any of a number of advantageous properties to the resulting fusion protein. For example, the polypeptide entity may be selected to provide increased expression of the recombinant fusion protein. Alternatively or additionally, the polypeptide entity may facilitate purification of the fusion protein for example, by acting as a ligand in affinity purification. A proteolytic cleavage site may be added to the recombinant protein so that the desired sequence can ultimately be separated from the polypeptide entity after purification. The polypeptide entity may also be selected to confer an improved stability to the fusion protein, when stability is a goal. Examples of suitable polypeptide entities include, for example, polyhistidine tags, that allow for the easy purification of the resulting fusion protein on a nickel chelating column. Glutathione-S-transferase (GST), maltose B binding protein, or protein A are

other examples of suitable polypeptide entities that can be fused to a protein biomarker of the invention using commercial fusion expression vectors.

Alternatively, protein biomarkers of the invention may be prepared using commercially available proteins.

5 In certain embodiments, a protein biomarker of the invention is provided immobilized onto a solid carrier or support (*e.g.*, a bead or an array). Methods for immobilizing polypeptide molecules onto a solid surface are known in the art. In particular, the invention provides an array for the diagnosis of kidney status or renal transplant status, consisting essentially of biomarkers described herein, immobilized 10 to its surface. In certain embodiments, the array has, immobilized to its surface, at least one (*i.e.*, one or more than one) of the inventive protein biomarkers that are specific for dialysis patients with end-stage renal disorder, at least one of the inventive protein biomarkers that are specific for patients with stable renal transplant, at least one of the inventive protein biomarkers that are specific for patients with renal 15 transplant glomerulopathy, and at least one of the inventive protein biomarkers that are specific for patients with IFTA. In a particular embodiment, the array has, immobilized to its surface, all of the inventive protein biomarkers that are specific for dialysis patients with end-stage renal disorder, all of the inventive protein biomarkers that are specific for patients with stable renal transplant, all of the inventive protein 20 biomarkers that are specific for patients with renal transplant glomerulopathy, and all of the inventive protein biomarkers that are specific for patients with IFTA. An array of the invention may further comprise various proteins and other controls to allow verifications of reagents background, and detection conditions during probing, assessment of the performance of an assay and/or normalization. Examples of such 25 controls include anti-biotin antibodies which are recognized by the detection IgG antibody, and the Influenza A antigen, which is recognized by approximately 95% of the total population.

The terms “array for diagnosing kidney status or renal transplant status” and “array for determining kidney status or renal transplant status” are used herein 30 interchangeably. They refer to an array for the detection, in an *in vitro* biological sample, of antibodies that are indicative of one and only one kidney status or renal transplant status: end-stage renal disorder, stable renal transplant, renal transplant glomerulopathy or IFTA.

A protein biomarker may be immobilized by being either covalently or passively bound to the surface of a solid carrier or support. Examples of suitable carrier or support materials include, but are not limited to, agarose, cellulose, nitrocellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose, polyacrylamides, polystyrene, polyvinyl chloride, polypropylene, gabbros, filter paper, magnetite, ion-exchange resin, glass, polyamine-methyl-vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, and the like. Immobilization of a protein biomarker on the surface of a solid carrier or support may involve crosslinking, covalent binding or physical adsorption, using methods well known in the art. The solid carrier or support may be in the form of a bead, a particle, a microplate well, an array, a cuvette, a tube, a membrane, or any other shape suitable for conducting a diagnostic method according to the invention (*e.g.*, using an immunoassay). In certain embodiments, immobilization of a protein biomarker to a solid carrier or support includes gel electrophoresis followed by transfer to a membrane (typically nitrocellulose or PVDF) in a process called western blotting (or immunoblot well known in the art).

II – Diagnosis Methods

The inventive protein biomarkers may be used to detect, in a biological sample obtained from a subject, the presence of antibodies that are indicative of kidney status or renal transplant status.

Accordingly, the present invention provides methods for diagnosing or determining kidney status or renal transplant status in a subject. Such methods comprise contacting a biological sample obtained from the subject (in particular a blood sample) with at least one protein biomarker described herein for a time and under conditions allowing a biomarker-antibody complex to form between the at least one biomarker and an antibody present in the biological sample; and detecting any biomarker-antibody complex formed. Kidney status or renal transplant status may be determined based on the nature of the at least one biomarker used and the detection (or not) of a biomarker-antibody complex.

In certain embodiments, the method comprises contacting a biological sample obtained from the subject (in particular a blood sample) with a plurality of protein biomarkers described herein for a time and under conditions allowing biomarker-

antibody complexes to form between the biomarkers and antibodies present in the biological sample; and detecting any biomarker-antibody complex formed. Kidney status or renal transplant status may be determined based on the binding pattern obtained (*i.e.*, the nature of the biomarkers used and the detection (or not) of biomarker-antibody complexes).

The plurality of biomarkers may be any combination of the biomarkers described herein. For example, the plurality of biomarkers may consist of only biomarkers that are indicative of one of the 4 pathologies (*e.g.*, only biomarkers indicative of IFTA or only biomarkers indicative of renal transplant glomerulopathy). Alternatively, the plurality of biomarkers may consist of a combination of biomarkers of the 4 different pathologies. In one embodiment, the plurality of biomarkers consists of all the biomarkers described herein. Alternatively, the plurality of biomarkers may consist of only biomarkers that are indicative of renal transplant status (*i.e.*, biomarkers that are indicative of stable renal transplant, biomarkers that are indicative of renal transplant glomerulopathy and biomarkers that are indicative of IFTA).

Biological Samples

The methods of diagnosis of the present invention may be applied to the study of any type of biological samples allowing one or more inventive biomarkers to be assayed. Examples of suitable biological samples include, but are not limited to, blood samples, and urine. Preferred biological samples are blood samples, *i.e.*, whole blood, serum or plasma. Biological samples used in the practice of the invention may be fresh or frozen samples collected from a subject, or archival samples with known diagnosis, treatment and/or outcome history. Biological samples may be collected by any non-invasive means, such as, for example, by drawing blood from a subject.

In preferred embodiments, the inventive methods are performed on the biological sample itself without, or with limited, processing of the sample.

However, alternatively, the inventive methods may be performed on a protein extract prepared from the biological sample. In this case, the protein extract preferably contains the total protein content. Methods of protein extraction are well known in the art (see, for example, "*Protein Methods*", D.M. Bollag et al., 2nd Ed., 1996, Wiley-Liss; "*Protein Purification Methods: A Practical Approach*", E.L. Harris

and S. Angal (Eds.), 1989; "*Protein Purification Techniques: A Practical Approach*", S. Roe, 2nd Ed., 2001, Oxford University Press; "*Principles and Reactions of Protein Extraction, Purification, and Characterization*", H. Ahmed, 2005, CRC Press: Boca Raton, FL). Various kits can be used to extract proteins from bodily fluids and tissues. Such kits are commercially available from, for example, BioRad Laboratories (Hercules, CA), BD Biosciences Clontech (Mountain View, CA), Chemicon International, Inc. (Temecula, CA), Calbiochem (San Diego, CA), Pierce Biotechnology (Rockford, IL), and Invitrogen Corp. (Carlsbad, CA). User Guides that describe in great detail the protocol to be followed are usually included in all these kits. Sensitivity, processing time and costs may be different from one kit to another. One of ordinary skill in the art can easily select the kit(s) most appropriate for a particular situation.

Detection of Biomarker-Antibody Complexes

The diagnostic methods of the present invention generally involve detection of at least one complex formed between a protein biomarker and an antibody present in a biological sample. In the practice of the invention, detection of such a biomarker-antibody complex may be performed by any suitable method (see, for example, E. Harlow and A. Lane, "*Antibodies: A Laboratories Manual*", 1988, Cold Spring Harbor Laboratory: Cold Spring Harbor, NY).

For example, detection of a biomarker-antibody complex may be performed using an immunoassay. A wide range of immunoassay techniques is available, including radioimmunoassays, enzyme immunoassays (EIA), enzyme-linked immunosorbent assays (ELISA), and immunofluorescence immunoprecipitation. Immunoassays are well known in the art. Methods for carrying out such assays as well as practical applications and procedures are summarized in textbooks. Examples of such textbooks include P. Tijssen, In: Practice and theory of enzyme immunoassays, eds. R.H. Burdon and v. P.H. Knippenberg, Elsevier, Amsterdam (1990), pp. 221-278 and various volumes of Methods in Enzymology, Eds. S.P. Colowick *et al.*., Academic Press, dealing with immunological detection methods, especially volumes 70, 73, 74, 84, 92 and 121. Immunoassays may be competitive or non-competitive.

For example, any of a number of variations of the sandwich assay technique may be used to perform an immunoassay. Briefly, in a typical sandwich assay applied to the detection of antibodies indicative of a kidney status or a renal transplant status according to the present invention, an unlabeled protein biomarker is immobilized on 5 a solid substrate and the sample to be tested is brought into contact with the bound biomarker for a time and under conditions allowing formation of a biomarker-antibody complex. Following incubation, a secondary antibody that is labeled with a detectable moiety and that specifically recognizes antibodies from the species tested (*e.g.*, an anti-human IgG for human subjects) is added and incubated under conditions 10 allowing the formation of a ternary complex between any biomarker-bound antibody and the labeled secondary antibody. Any unbound material is washed away, and the presence of any antibody indicative of a kidney status or a renal transplant status is determined by observation of the signal directly or indirectly produced by the detectable moiety. Variations in this assay include an assay in which both the 15 biological sample and the labeled secondary antibody are added simultaneously to the immobilized biomarker (or biomarkers).

The secondary antibody may be labeled with any suitable detectable moiety, *i.e.*, any entity which, by its chemical nature, provides an analytical identifiable signal allowing detection of the ternary complex, and consequently detection of the 20 biomarker-antibody complex.

Detection may be either qualitative or quantitative. Methods for labeling biological molecules such as antibodies are well-known in the art (see, for example, "*Affinity Techniques. Enzyme Purification: Part B*", Methods in Enzymol., 1974, Vol. 34, W.B. Jakoby and M. Wilneck (Eds.), Academic Press: New York, NY; and M. 25 Wilchek and E.A. Bayer, Anal. Biochem., 1988, 171: 1-32).

The most commonly used detectable moieties in immunoassays are enzymes and fluorophores. In the case of an enzyme immunoassay (EIA), an enzyme such as horseradish peroxidase, glucose oxidase, beta-galactosidase, alkaline phosphatase, and the like, is conjugated to the second antibody, generally by means of glutaraldehyde or 30 periodate. The substrate to be used with the specific enzymes is generally chosen for the production of a detectable color change, upon hydrolysis of the corresponding enzyme. In the case of immunofluorescence, the second antibody is chemically coupled to a fluorescent moiety without alteration of its binding capacity. After

binding of the fluorescently labeled antibody to the biomarker-antibody complex and removal of any unbound material, the fluorescent signal generated by the fluorescent moiety is detected, and optionally quantified. Alternatively, the second antibody may be labeled with a radioisotope, a chemiluminescent moiety, or a bioluminescent moiety.

Determination of Kidney Status or Renal Transplant Status

In the methods of the present invention, detection of a biomarker-antibody complex is indicative of the presence of specific antibodies in the biological sample tested and is therefore indicative of the kidney status or renal transplant status of the subject from whom the biological sample has been obtained. Thus, methods of the present invention may be used for determining (or diagnosing) kidney status (*i.e.*, end-stage kidney disorder) or renal transplant status (*i.e.*, stable renal transplant, renal transplant glomerulopathy, or IFTA). In particular, methods of the invention may be used for testing subjects after renal transplant as a way of monitoring renal transplant status.

It will be appreciated by one skilled in the art that diagnosis (or determination) of kidney status or renal transplant status may be made solely on the results provided by a method described herein. Alternatively, a physician may also consider other clinical or pathological parameters used in existing methods to diagnose renal transplant status. Thus, results obtained using methods of the present invention may be compared to and/or combined with results from other tests, assays or procedures performed for the diagnosis of kidney status or renal transplant status. Such comparison and/or combination may help provide a more refine diagnosis.

Alternatively or additionally, results from diagnosis methods of the present invention may be used in combination with results from one or more assays that employ other biomarkers of renal transplant status. Thus, in certain embodiments, diagnostics of renal transplant status may be based on results from a method of the invention and results from one or more additional assays using different biomarkers.

Selection of Appropriate Treatment

The biomarkers and methods of diagnostic provided by the present invention have the potential to radically change the way in which transplant patients are managed. The methods are non-invasive, and have no associated morbidity or

mortality. They require only small volumes of samples, and are highly cost-effective when compared to other clinical and biochemical modalities such as biopsies. They are rapid, and can easily be repeated over time allowing a frequent surveillance and monitoring of renal transplant status and function. In addition, the assays have the
5 potential for development of automated analysis for the clinical laboratory.

Thus, using methods described herein, skilled physicians may select and prescribe treatments adapted to each individual patient based on the diagnosis (or determination) of the renal transplant status. In particular, the present invention provides physicians with a means to diagnose early renal transplant glomerulopathy,
10 which will allow for early treatment, when intervention is likely to have its greatest effect. Frequent non-invasive renal transplant monitoring according to the invention could allow for immunosuppression doses to be increased or decreased according to specific individual needs. For example, immunosuppression could be lowered, even gradually weaned off, in patients with stable renal transplant. Decision to reduce or
15 eliminate immunosuppressive drugs could be made with a strategy to safely monitor the results before clinically apparent changes in kidney function occur. Alternatively, for patients in whom early rejection is detected, immunosuppression could be increased until a satisfactory response is achieved. Adequate response to treatment and resolution of rejection could be rapidly verified, preventing over-
20 immunosuppression and its associated consequences.

The methods of the present invention may also find applications in the development of new therapeutics, such as new immunosuppressive drugs, for the management or treatment of renal transplant patients.

III - Kits

25 In another aspect, the present invention provides kits comprising materials useful for carrying out the diagnostic methods of the invention. The diagnostic procedures described herein may be performed by clinical laboratories, experimental laboratories, or practitioners. The invention provides kits which can be used in these different settings.

30 In certain embodiments, an inventive kit comprises at least one biomarker described herein, preferably in an amount that is suitable for detecting antibodies in a biological sample, and optionally, instructions for using the kit according to a method

of the invention. The biomarkers may or may not be immobilized on a substrate surface (*e.g.*, beads, array and the like).

In preferred embodiments, an inventive kit comprises a plurality of biomarkers described herein, preferably each one in an amount that is suitable for detecting antibodies in a biological sample. The plurality of biomarkers may be any combination of the biomarkers described herein. For example, kits that are specifically designed for the diagnostic of one of the 4 pathologies will preferably comprise only biomarkers that are indicative of the pathology of interest (*e.g.*, only biomarkers indicative of IFTA, or only biomarkers indicative of renal transplant glomerulopathy). Alternatively, kits that are designed for the determination of kidney status or renal transplant status will preferably comprise a combination of biomarkers of the 4 different pathologies. In one embodiment, the plurality of biomarkers included in the kit will consist of all the biomarkers described herein. Alternatively, kits that are designed for the determination of renal transplant status only will preferably comprise a combination of biomarkers that are indicative of renal transplant status (*i.e.*, biomarkers that are indicative of stable renal transplant, biomarkers that are indicative of renal transplant glomerulopathy and biomarkers that are indicative of IFTA).

In addition, an inventive kit may further comprise at least one reagent for the detection of a biomarker-antibody complex formed between a biomarker included in the kit and an antibody present in a biological sample obtained from a patient. Such a reagent may be, for example, a labeled antibody that specifically recognizes antibodies from the species tested (*e.g.*, an anti-human IgG), as described above. If the biomarkers are provided attached to the surface of an array, a kit of the invention may comprise only one reagent for the detection of biomarker-antibody complexes (*e.g.*, a fluorescently-labeled anti-human antibody).

Depending on the procedure, the kit may further comprise one or more of: extraction buffers and/or reagents, western blotting buffers and/or reagents, immunodetection buffers and/or reagents, labeling buffers and/or reagents, and detection means. Protocols for using these buffers and reagents for performing different steps of the procedure may be included in the kit.

The different reagents included in an inventive kit may be supplied in a solid (*e.g.*, lyophilized) or liquid form. The kits of the present invention may optionally comprise different containers (*e.g.*, vial, ampoule, test tube, flask or bottle) for each individual buffer and/or reagent. Each component will generally be suitable as aliquoted in its respective container or provided in a concentrated form. Other containers suitable for conducting certain steps of the disclosed methods may also be provided. The individual containers of the kit are preferably maintained in close confinement for commercial sale.

In certain embodiments, a kit further comprises instructions for using its components for the diagnosis of kidney status or renal transplant status in a subject according to a method of the invention. Instructions for using the kit according to methods of the invention may comprise instructions for processing the biological sample obtained for the subject and/or for performing the test, and/or instructions for interpreting the results. A kit may also contain a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products.

Examples

The following examples describe some of the preferred modes of making and practicing the present invention. However, it should be understood that the examples are for illustrative purposes only and are not meant to limit the scope of the invention. Furthermore, unless the description in an Example is presented in the past tense, the text, like the rest of the specification, is not intended to suggest that experiments were actually performed or data were actually obtained.

Example 1: Biomarkers of Kidney or Renal Transplant Status

Materials and Methods

Patients. 45 patients who received a renal transplant in the Nantes University Hospital (Nantes, France) between 1986 and 2007, or who are waiting for one, were retrospectively included in the present study and divided into four groups according to the outcome of their kidney graft. The first group, Transplant Glomerulopathy (TG), comprised 19 patients among whom 6 were in late stage TG and 13 were in early stage TG. The diagnostic of TG is based on the histology of the graft biopsy such as

C4d deposits and glomerular basement membrane duplication. The IFTA group was composed of 10 patients who showed evidence of Interstitial Fibrosis and Tubular Atrophy, but no presence of antibody or C4d deposits, and this was also diagnosed histologically after the biopsy. 10 patients who were undergoing dialysis (DIA) while waiting for renal transplantation were included in the third group. The last group was made of renal transplant patients considered as stable (STA), *i.e.*, patients under immunosuppressive treatment showing a stable renal function.

Sera were available from all these patients at the Etablissement Français du Sang (Nantes, France). Patients' characteristics are summarized in the table presented on Figure 1. Sera were collected at the time of biopsy from patients of the TG, STA and IFTA groups, and a few days before transplantation from DIA patients. Sera were stored at -80°C prior to being tested.

Sera Screening on Protein Microarrays (ProtoArrays® Invitrogen). Sera were sent to Invitrogen Corporation (Brandford, Connecticut, US) for analysis.

Human Protein Collection and Protein Microarray Manufacture. Over 8000 human clones obtained from Invitrogen's Ultimate ORF collection and Gateway collection of kinase clones were transferred into insect cells *via* baculovirus infection to produce recombinant proteins tagged with Glutathione-S-Transferase (GST). Recombinant proteins were then purified using GST affinity, under native conditions. Because of their production in insect cells and of the particular purification conditions used, the proteins thus obtained are expected to contain appropriate post-translational modifications and to maintain their native conformations. A contact-type printer equipped with 48 matched quill-type pins printed the recombinant proteins in duplicates on a glass slide coated with a thin layer of nitrocellulose.

Protein Microarray Probing. Once printed with duplicates of recombinant proteins and appropriate negative and positive controls, microarray slides were blocked using a blocking buffer containing 50 mM HEPES, 200 mM NaCl, 0.08% Triton X-100, 25% glycerol, 20 mM reduced glutathione, 1.0 mM DTT and 1% BSA, at 4°C for 1 hour. Microarrays were then probed with a 1:500 dilution of serum sample (one microarray per serum) diluted in 5 mL of freshly prepared PBST buffer and incubated for 90 minutes at 4°C with gentle agitation. Microarrays were washed five times in 5 mL PBST buffer. Slides were incubated with an Alexa Fluor®647-conjugated goat anti-

human IgG antibody diluted in 5 mL probe buffer to a 1 mg/mL final concentration at 4°C for 90 minutes. After incubation, slides were washed as described above and dried by spinning in a table top centrifuge equipped with a plate rotor at 1000 rpm for 2 minutes. Arrays were then scanned using an Axon GenePix-4000B fluorescent microarray scanner.

Data Acquisition. The GenePix 6.0 software was used for data acquisition. This software overlays the mapping of human proteins in the array list file to each array image with a fixed feature size of 130 µm (diameter). AlexaFluor®647-conjugated goat anti-human IgG antibody printed on each subarray helps aligning the spots. Other proteins are systematically used as control spots, *e.g.*, anti-biotin antibodies which are recognized by the detection IgG antibody and the Influenza A antigen, which is recognized by approximately 95% of the total population. Scanner settings were selected such that maximal signals on the array were sub-saturated thus ensuring that the full dynamic range of the scanner was utilized.

Data Analysis. Data analysis was performed using Invitrogen's ProtoArray Prospector software. This software follows a 3-step process: (1) Single array analysis: for each protein on each array, a series of values are calculated including background subtracted signals, CI-P value, and replicate spot coefficient of variation; (2) Group characterization: signals for each individual protein across all samples from a given population are aligned for downstream analysis; and (3) Identification of the differences between treated and untreated sample populations. Utilizing M-statistics, proteins are identified for which the differential signals between two populations result in a significant p-value. The output from the comparative analysis includes information on the number of patients in each population that exhibited an immune response against each ProtoArray® protein that was above the M-statistic threshold established for that protein.

Identification of Candidate Biomarkers. All the possible two group-comparisons were performed, which led to lists of total biomarkers for each pathology, at a p-value threshold of 0.05. These lists were compared and biomarkers that were found for more than one pathology were removed from the lists in order to obtain final lists containing only specific biomarkers, *i.e.*, biomarkers unique to one of the 4 pathologies.

Other Embodiments

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, 5 with the true scope of the invention being indicated by the following claims.

Claims

What is claimed is:

1. An *in vitro* method for determining kidney status or renal transplant status in a subject, said method comprising steps of:

    contacting a blood sample obtained from the subject with at least one biomarker for a time and under conditions allowing a biomarker-antibody complex to form between the at least one biomarker and an antibody present in the blood sample; and

    detecting any biomarker-antibody complex formed,

    wherein the at least one biomarker is selected from the group consisting of:

    a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof,

    a set of biomarkers indicative of stable renal transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof,

    a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof, and

    a set of biomarkers indicative of interstitial fibrosis and tubular atrophy consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

2. Method according to claim 1, wherein the blood sample is contacted with a plurality of biomarkers for a time and under conditions allowing biomarker-antibody complexes to form between the biomarkers and antibodies present in the blood sample.

3. Method according to claim 1 or claim 2, wherein the blood sample is selected from the group consisting of whole blood, serum and plasma.

4. Method according to any one of claims 1 to 3, wherein the at least one biomarker or the plurality of biomarkers is immobilized on a solid carrier or support, preferably on an array.

5. Method according to any one of claims 1 to 4, wherein detecting the biomarker-antibody complex comprises contacting the blood sample with at least one reagent for the detection of the biomarker-antibody complex formed between the at least one biomarker and an antibody present in the blood sample.

6. Method according to any one of claims 1 to 5, wherein detecting the biomarker-antibody complex is performed by immunoassay.

7. A kit for the *in vitro* determination of kidney status or renal transplant status in a subject, the kit comprising at least one biomarker selected from the group consisting of:

a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of stable renal transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof, and

a set of biomarkers indicative of interstitial fibrosis and tubular atrophy consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

8. Kit according to claim 7, wherein the kit comprises a plurality of biomarkers.

9. Kit according to claim 7 or claim 8, wherein the at least one biomarker or the plurality of biomarkers is immobilized on a solid carrier or support, preferably on an array.

10. Kit according to any one of claims 7 to 9, further comprising at least one reagent for the detection of a biomarker-antibody complex formed between the at least one biomarker included in the kit and an antibody present in a blood sample obtained from a subject.

11. Kit according to claim 10, wherein the at least one reagent is a labeled anti-human antibody.

12. An array for the *in vitro* determination of kidney status or renal transplant status in a subject, the array essentially having, attached to its surface, a plurality of biomarkers selected from the group consisting of:

a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of stable renal transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof, and

a set of biomarkers indicative of interstitial fibrosis and tubular atrophy consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof.

13. Use of at least one biomarker selected from the group consisting of:

a set of biomarkers indicative of kidney status consisting of RAB20, OAS2, IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of stable renal transplant consisting of ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), analogs thereof and antibody-binding fragments thereof,

a set of biomarkers indicative of renal transplant glomerulopathy consisting of PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, analogs thereof, and antibody-binding fragments thereof, and

a set of biomarkers indicative of interstitial fibrosis and tubular atrophy consisting of IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1), analogs thereof and antibody-binding fragments thereof,

for the *in vitro* determination of kidney status or renal transplant status in a subject.

14. Use according to claim 13, wherein the at least one biomarker comprises a plurality of biomarkers.

Patient	Graft year	time of biopsy (year after graft)	C4d	GBM duplication	IFTA	Diagnostic
1	1996	7.00	NA	+	+	TG + IFTA
2	1997	4.58	NA	+	+	TG + IFTA
3	1984	8.58	NA	-/+	+	TG + IFTA
4	1991	10.33	NA	+	+	TG + IFTA
5	2003	2.25	NA	-/+	+	TG + IFTA
6	2001	3.83	NA	+	+	TG + IFTA
7	2000	2.5	NA	+	-/+	TG
8	1999	3.25	NA	+	-/+	TG
9	1999	2.92	NA	+	-/+	TG
10	1988	15.25				TG
11	2004	2.50	+	+	-	TG
12	1995	11.75	+	+	-	TG
13	1998	8.33	+	+	-	TG
14	2006	1.42	+	+	-	TG
15	1998	9.25	+	+	-	TG
16	2004	3.25	+	+	-	TG
17	2004	0.75	+	+	-	TG
18	2004	2.58	+	+	-	TG
19	2002	3.33	+	+	-	TG
20	1998	8.50	-	-	+	IFTA
21	2005	1.00	-	-	+	IFTA
22	1986	10.83	-	-	+	IFTA
23	2005	1.16	-	-	+	IFTA
24	2005	1.00	-	-	+	IFTA
25	1997	9.91	-	-	+	IFTA
26	1996	10.00	-	-	+	IFTA
27	1994	11.92	-	-	+	IFTA
28	2002	5.08	-	-	+	IFTA
29	2001	5.00	-	-	+	IFTA
30	2006	0.92	-	-	-	STA
31	2006	1.08	-	-	-	STA
32	2006	0.66	-	-	-	STA
33	2006	0.83	-	-	-	STA
34	2006	1.33	-	-	-	STA
35	2006	1.00	-	-	-	STA

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/067398

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/53 G01N33/68
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/070086 A (UNIV LELAND STANFORD JUNIOR [US]; INST NAT SANTE RECH MED [FR]; MANSFI) 4 August 2005 (2005-08-04) cited in the application page 7, line 30- page 8, line 33; page 13, line 32-page 14, line 3; page 22, line 10-page 23, line 20; claims 1, 3-9; Table1.	1-14
A	WO 2007/124419 A1 (CHILDRENS HOSP MEDICAL CENTER [US]; DEVARAJAN PRASAD [US]) 1 November 2007 (2007-11-01) par 0002, 0014, 0015, 0017-0018, 0020, 0091, 0093; claims 1,2,5.	1-14

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2010	06/05/2010

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/067398

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/099209 A1 (CLARKE MICHAEL F [US] ET AL) 3 May 2007 (2007-05-03) par 0004, 0061, 0233-0236, page 38, table 7D	7-12
X	WO 2008/008846 A2 (US GOVERNMENT [US]; BAIRD ALISON E [US]; MOORE DAVID F [US]; GOLDIN EH) 17 January 2008 (2008-01-17) page 1, lines 10-14; page 161, table 8; page 212, example 15; page 218, lines 14-32; page 220, line 18-page 221, line 14.	7-12
X	LUETCKE A ET AL: "CLONING AND SUBCELLULAR LOCALIZATION OF NOVEL RAB PROTEINS REVEALS POLARIZED AND CELL TYPE-SPECIFIC EXPRESSION" JOURNAL OF CELL SCIENCE, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB, vol. 107, no. PART 12, 1 December 1994 (1994-12-01), pages 3437-3448, XP000604112 ISSN: 0021-9533 pages 3439, 3441	7-9
A	US 2008/166741 A1 (TSENG TZU-LING [TW] ET AL) 10 July 2008 (2008-07-10) the whole document, in particular claim 1	1-12
A	SUMITRAN-HOLGERSSON ET AL: "Relevance of MICA and other non-HLA antibodies in clinical transplantation" CURRENT OPINION IN IMMUNOLOGY, vol. 20, no. 5, 1 October 2008 (2008-10-01), pages 607-613, XP024527300 [retrieved on 2008-08-12] the whole document, in particular table 1	1-12
X	Li L et al.: "Integrative Genomics To Identify Non-HLA Allogenic Kidney-SpecificTargets after Kidney Transplantation" American Journal of Transplantation vol. 8, no. s2, 258, 23 April 2008 (2008-04-23), page 247, XP002576074 Retrieved from the Internet: URL:http://www3.interscience.wiley.com/cgi-bin/fulltext/120089600/PDFSTART> [retrieved on 2010-03-30]	7-12
A	the whole abstract	1-6

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROCHE S ET AL: "Autoantibody profiling on high-density protein microarrays for biomarker discovery in the cerebrospinal fluid" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 338, no. 1-2, September 2008 (2008-09), pages 75-78, XP002576075 the whole document, in particular page 76, paragraph 2.1	7-12
A	VAKALIKOS J ET AL: "A NEW APPROACH TO MONITOR PATIENTS WITH RENAL CELL CARCINOMA TREATED WITH IFN-A" JOURNAL OF TUMOR MARKER ONCOLOGY, vol. 5, no. 1, 1990, pages 101-106, XP009131677 the whole document, in particular abstract	13,14
A	HOVNANIAN A ET AL: "The Human 2',5'-Oligoadenylate Synthetase Locus Is Composed of Three Distinct Genes Clustered on Chromosome 12q24.2 Encoding the 100-, 69-, and 40-kDa Forms" GENOMICS, vol. 52, no. 3, 15 September 1998 (1998-09-15), pages 267-277, XP004449027 the whole document, in particular abstract; figure 1	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/067398

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

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

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

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

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

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

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

see additional sheet

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

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

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

1-14(partially)

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

Remark on Protest

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

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

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-14(partially)

This International Search Report covers Inventions 1 and 2.

1.1. claims: 1-14(partially)

Invention 1: An in vitro method for determining kidney status or renal transplant status in a subject comprising the steps of contacting a blood sample obtained from a subject with the biomarker RAB20 thus allowing a RAB20-antibody complex to be formed between RAB20 and the antibody present in the blood sample; detecting the antibody-RAB20 complex. A kit for determination of kidney status or renal transplant status in a subject comprising as biomarker RAB20. An array for the determination of kidney status or renal transplant status in a subject the array having attached to its surface a plurality of biomarkers including the RAB20 biomarker. Use of RAB20 for the determination of kidney status or renal status in a subject.

---

2. claims: 1-14(partially)

Invention 2: An in vitro method for determining kidney status or renal transplant status in a subject comprising the steps of contacting a blood sample obtained from a subject with the biomarker OAS2 thus allowing a OAS2-antibody complex to be formed between OAS2 and the antibody present in the blood sample; detecting the antibody-OAS2 complex. A kit for determination of kidney status or renal transplant status in a subject comprising as biomarker OAS2. An array for the determination of kidney status or renal transplant status in a subject the array having attached to its surface a plurality of biomarkers including the OAS2 biomarker. Use of OAS2 for the determination of kidney status or renal status in a subject.

3-49. claims: 1-14(partially)

Inventions 3-49: The same as the invention number 1 wherein the biomarker is one of the following: IL1RL1(3), GLRA2, SMAD3, ING4(2), CCL19, CENTD2, ECE1, LGALS3, ARPC1B, CYP2E1, CDC20, RIT1, PHF21B, RBM34, MS4A6E, C20orf132, LRRFIP1, CKLF(1), PODN, HM13(4), TSC22D3(2), EPN1, COL23A1, GIMAP6, ZNF174, MAP3K11, CRB3(2), PRKCDBP, DCLK1, FEN1, TANK(2), GLRX5, RAB22A, MAEL, FAM71D, TTC35, IL16, MLF1, IFT81(2), DYRK2, NDFIP2, CCNE2, SNX21, TUBB2C, MUC20, ATP1A3, F11R(1).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2009/067398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005070086 A	04-08-2005	CA 2553551 A1 EP 1718768 A2	04-08-2005 08-11-2006
WO 2007124419 A1	01-11-2007	US 2007248989 A1	25-10-2007
US 2007099209 A1	03-05-2007	NONE	
WO 2008008846 A2	17-01-2008	NONE	
US 2008166741 A1	10-07-2008	NONE	

patsnap

专利名称(译)	用于诊断肾同种异体移植和肾脏状态的生物标志物		
公开(公告)号	EP2368113A1	公开(公告)日	2011-09-28
申请号	EP2009775218	申请日	2009-12-17
[标]申请(专利权)人(译)	法国国家健康医学研究院 褚南特 南特大学		
申请(专利权)人(译)	INSERM（INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE） 褚南特 Üniversite电南特		
当前申请(专利权)人(译)	INSERM（INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE） 褚南特 Üniversite电南特		
[标]发明人	HARB JEAN HOURMANT MARYVONNE LE ROUX SANDRINE		
发明人	HARB, JEAN HOURMANT, MARYVONNE LE ROUX, SANDRINE		
IPC分类号	G01N33/53 G01N33/68		
CPC分类号	G01N33/6854 G01N33/6893 G01N2800/245 G01N2800/347		
代理机构(译)	柜PLASSERAUD		
优先权	2008305988 2008-12-19 EP		
外部链接	Espacenet		

摘要(译)

本发明涉及与肾状态和慢性肾损伤或病症临床相关的蛋白质生物标志物的鉴定和用途。特别地，本发明提供了被存在于患有终末期肾病，稳定肾移植，肾移植肾小球病（TG）和间质纤维化和肾小管萎缩（IFTA）的患者中的抗体识别的标记蛋白的身份。描述了在慢性肾移植损伤的研究和诊断中以及在治疗方案的选择和/或监测中使用这些蛋白质的方法和试剂盒。