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(54) **PREDICTIVE BIOMARKERS FOR CHRONIC ALLOGRAFT NEPHROPATHY**

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(52) **U.S. Cl.** ..... **514/44 R**; 435/6; 435/7.1

(57) **ABSTRACT**

The invention relates to the analysis and identification of genes that are modulated in transplant rejection. This alteration of gene expression provides a molecular signature to accurately detect transplant rejection.

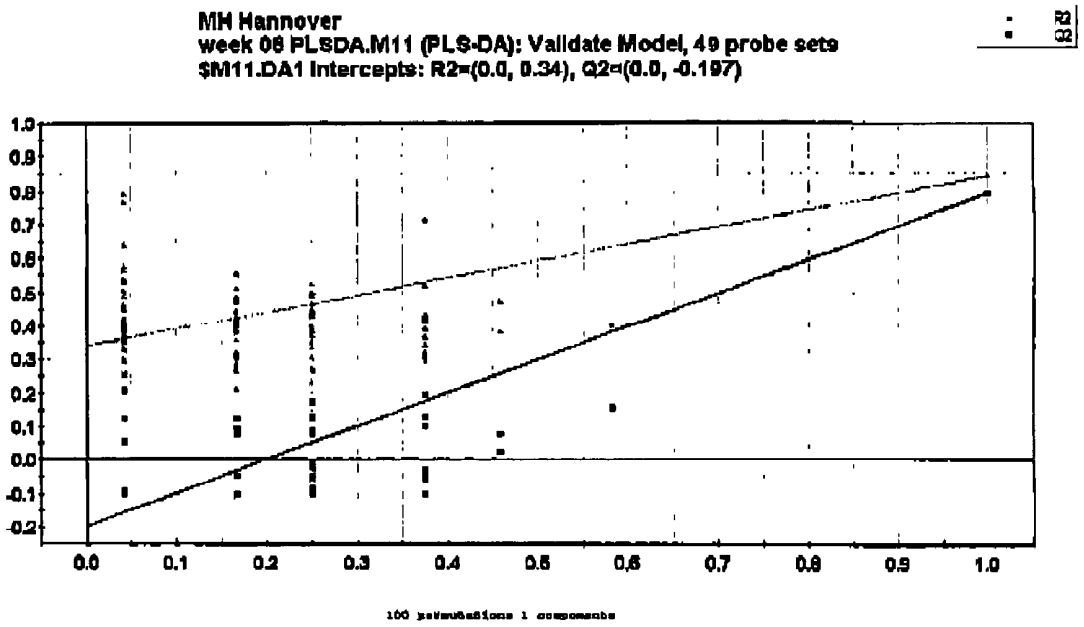


FIG. 1

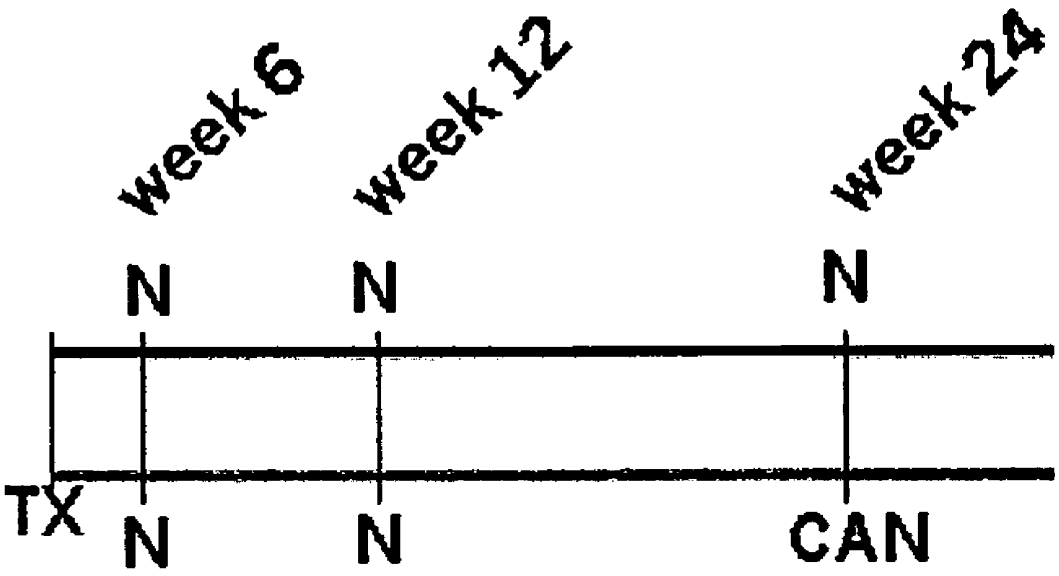


FIG. 2

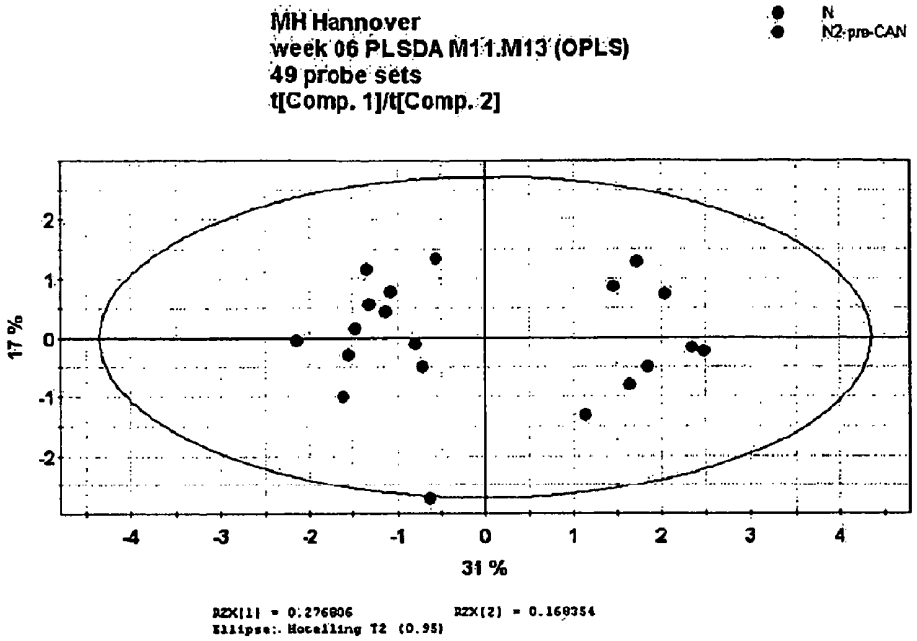

MH Hannover
week 06 PLSDA M11.M13 (OPLS)
49 probe sets
t[Comp. 1]/t[Comp. 2]
N
N2-pre-CAN
17 %
31 %
2
1
0
-1
-2
-4
-3
-2
-1
0
1
2
3
4
R2X[1] = 0.276806
R2X[2] = 0.169354
Ellipse: Hotelling T2 (0.95)

FIG. 3

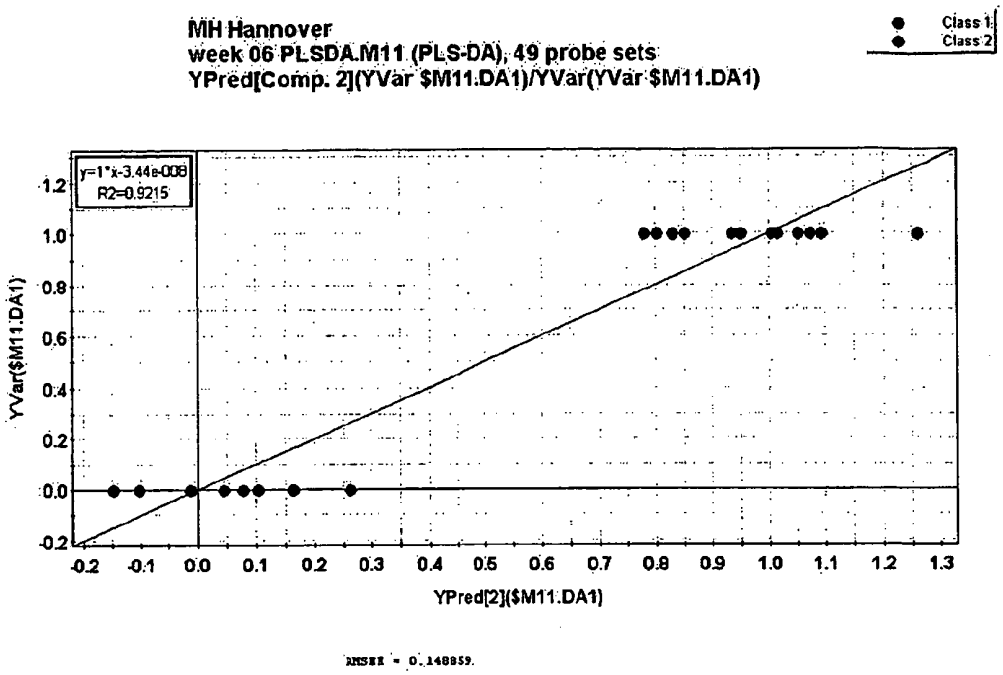

MH Hannover
week 06 PLSDA.M11 (PLS-DA), 49 probe sets
YPred[Comp. 2](YVar $M11.DA1)/YVar(YVar $M11.DA1)
Class 1
Class 2
y=1*x-3.44e-008
R2=0.9215
YVar($M11.DA1)
YPred[2]($M11.DA1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3

FIG. 4

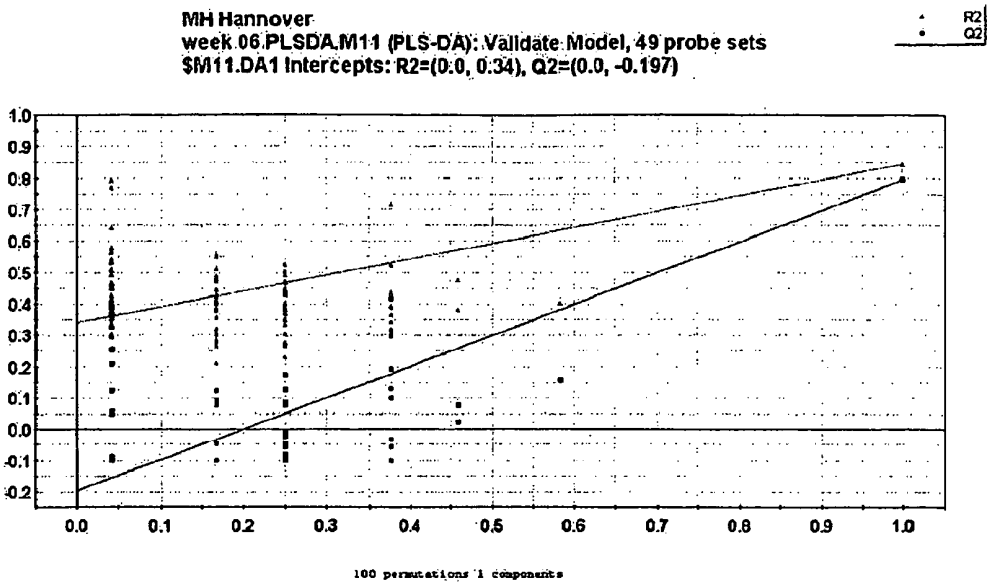

MH Hannover
week.06.PLSDA.M11 (PLS-DA): Validate Model, 49 probe sets
$M11.DA1 Intercepts: R2=(0.0, 0.34), Q2=(0.0, -0.197)
R2
Q2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
100 permutations 1 components

FIG. 5

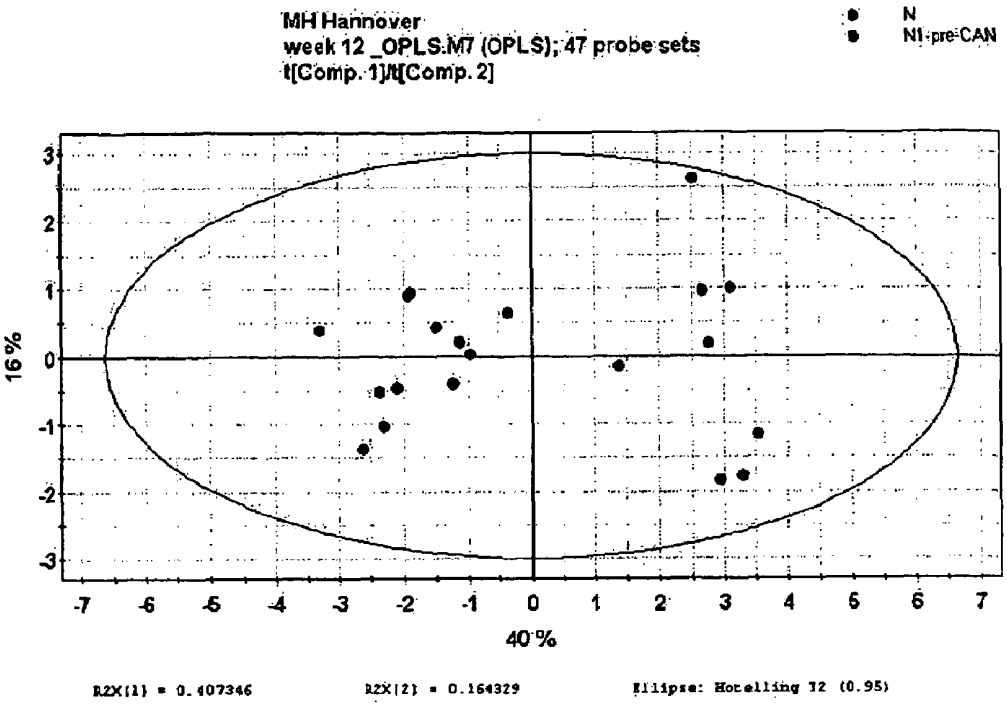

MH Hannover
week 12 _OPLS:M7 (OPLS); 47 probe sets
t[Comp. 1]/t[Comp. 2]
N
N1-pre-CAN
16 %
40 %
R2X[1] = 0.407346
R2X[2] = 0.164329
Ellipse: Hotelling T2 (0.95)

FIG. 6

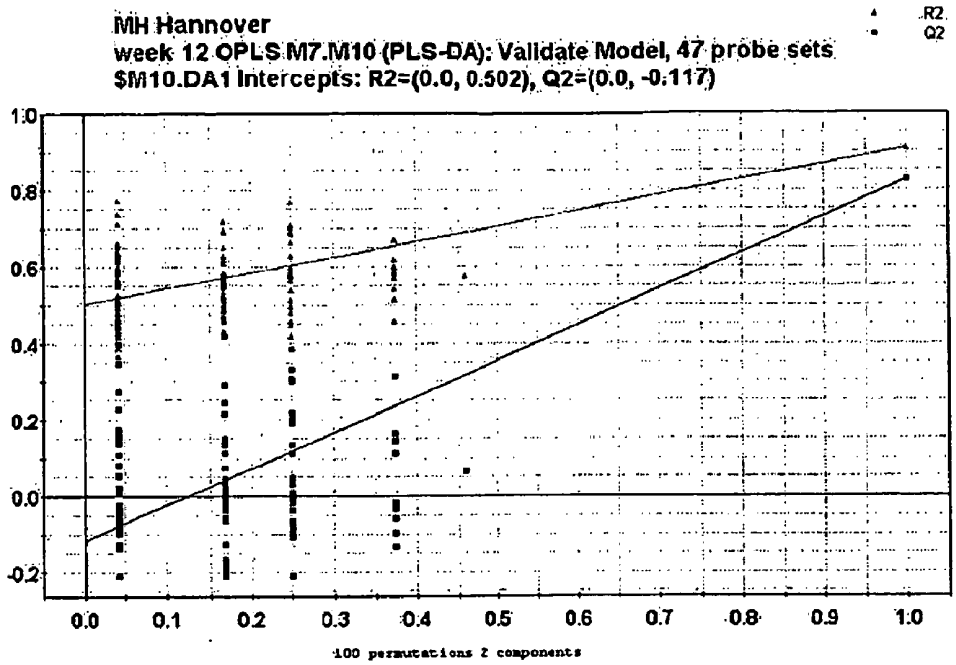

MH Hannover
week 12 OPLS M7.M10 (PLS-DA): Validate Model, 47 probe sets
$M10.DA1 Intercepts: R2=(0.0, 0.502), Q2=(0.0, -0.117)
R2
Q2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
100 permutations 2 components

FIG. 7

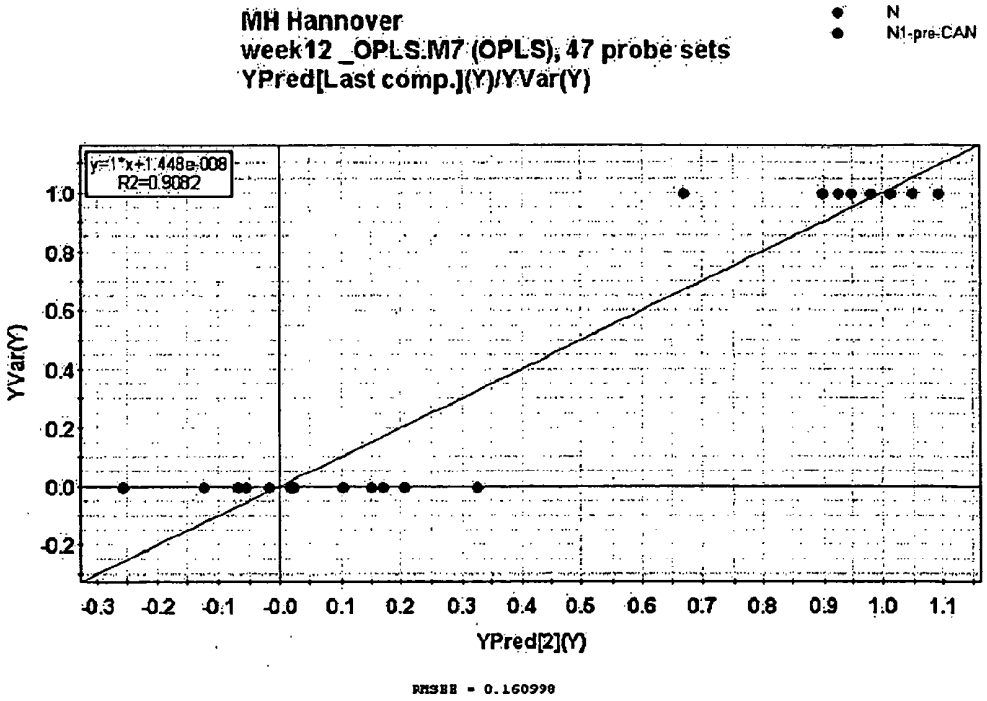

MH Hannover
week12 _OPLS:M7 (OPLS), 47 probe sets
YPred[Last comp.](Y)/YVar(Y)
N
N1-pre-CAN
y=1*x+1.448e-008
R2=0.9082
YVar(Y)
YPred[2](Y)
RMSEE = 0.160990

FIG. 8

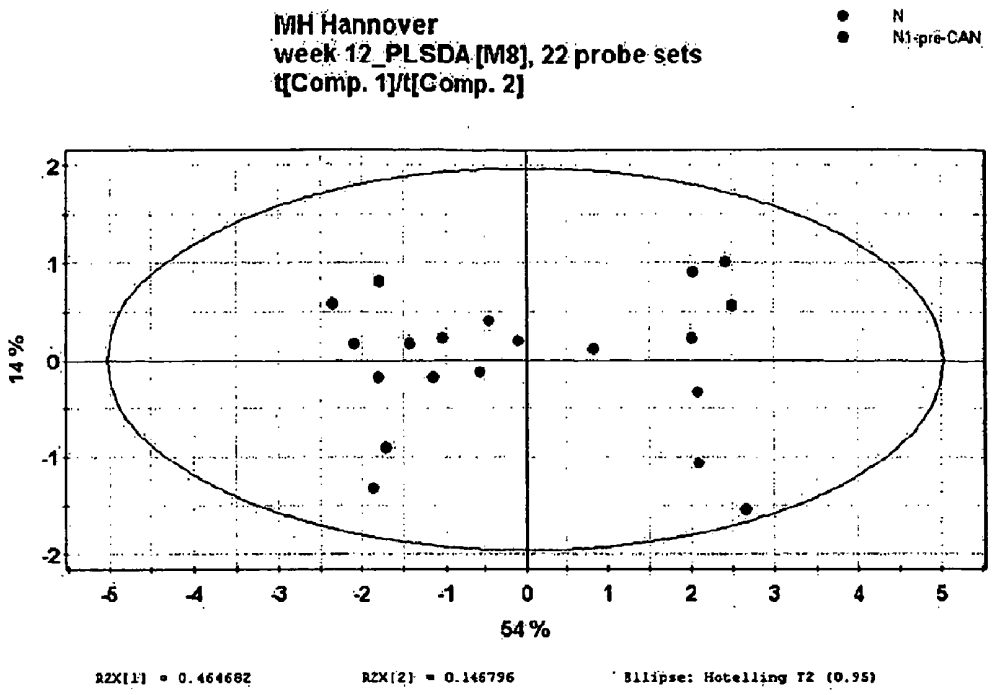

MH Hannover
week 12_PLSDA [M8], 22 probe sets
t[Comp. 1]/t[Comp. 2]
N
N1-pre-CAN
14 %
54 %
2
1
0
-1
-2
-5
-4
-3
-2
-1
0
1
2
3
4
5
R2X[1] = 0.464682
R2X[2] = 0.146796
Ellipse: Hotelling T2 (0.95)

FIG. 9

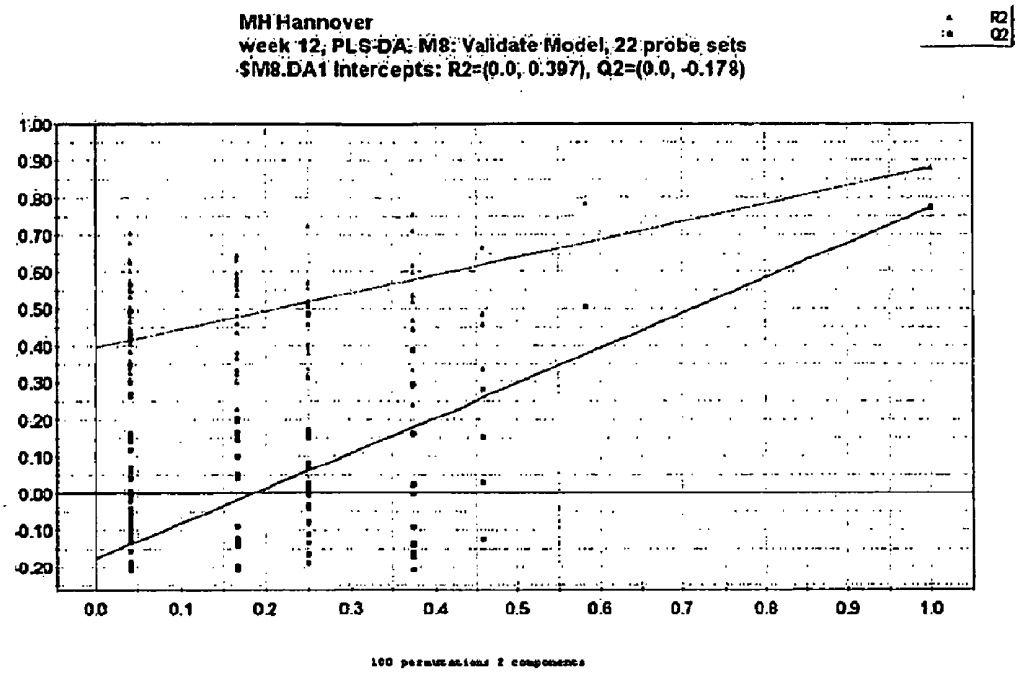

MH Hannover
week 12, PLS-DA: M8: Validate Model, 22 probe sets
$M8.DA1 Intercepts: R2=(0.0, 0.397), Q2=(0.0, -0.178)
R2
Q2
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

FIG. 10

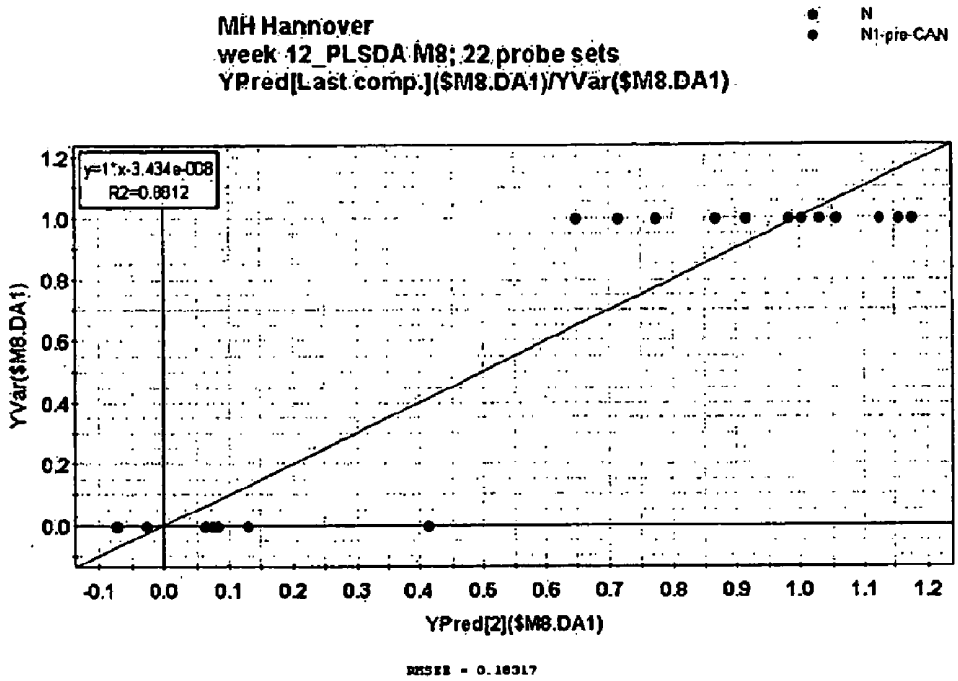

MH Hannover
week 12_PLSDA M8; 22 probe sets
YPred[Last comp.]($M8.DA1)/YVar($M8.DA1)
N
N1-pre-CAN
y=1*x-3.434e-008
R2=0.8812
YVar($M8.DA1)
YPred[2]($M8.DA1)
RMSEE = 0.16317

FIG. 11

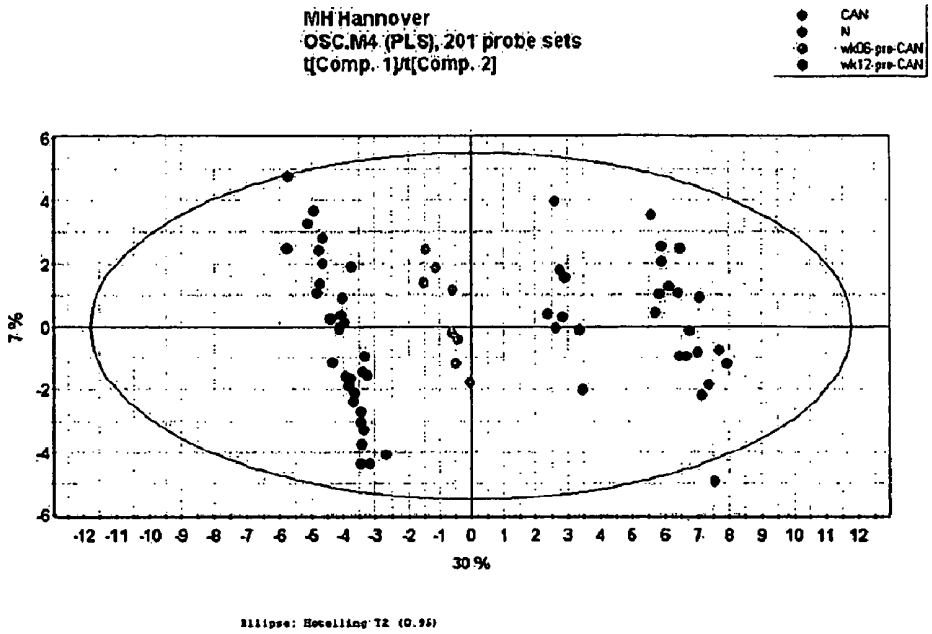

MH Hannover
OSC.M4 (PLS), 201 probe sets
t[Comp. 1]/t[Comp. 2]
CAN
N
wk06-pre-CAN
wk12-pre-CAN
7 %
30 %
-12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12
6
4
2
0
-2
-4
-6
Ellipse: Hotelling T2 (0.95)

FIG. 12

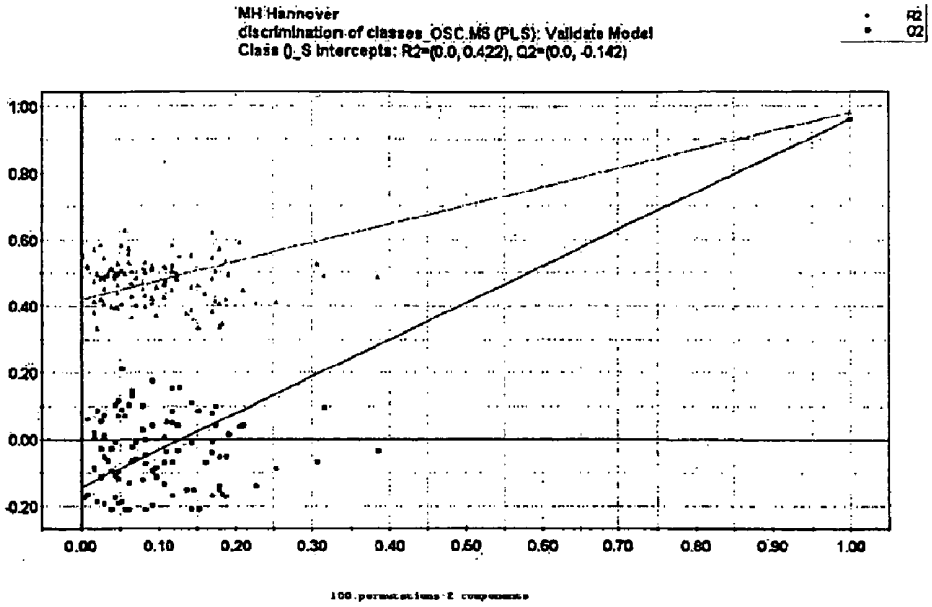

MH Hannover
discrimination of classes_OSC.M8 (PLS): Validate Model
Class 0_S intercepts: R2=(0.0, 0.422), Q2=(0.0, -0.142)
R2
Q2
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00

FIG. 13

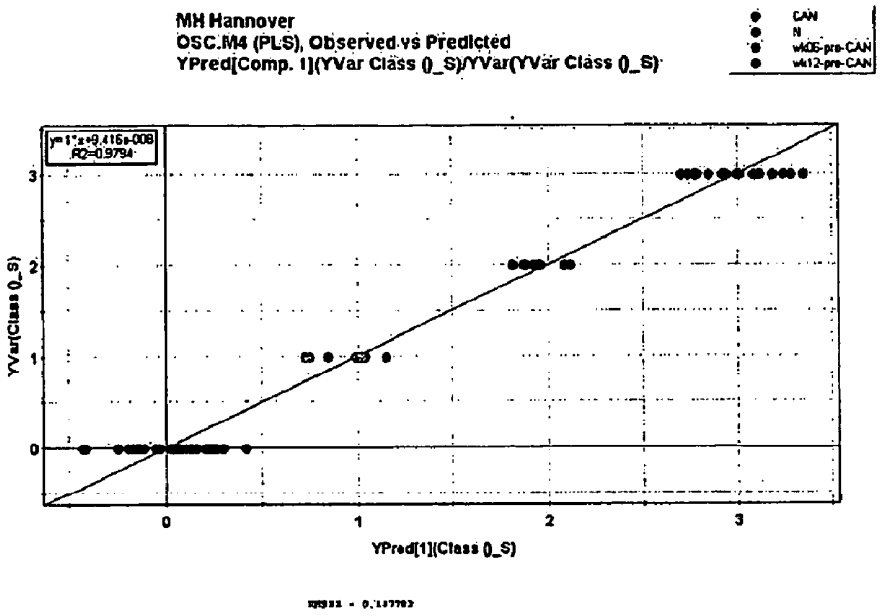

MH Hannover
OSC.M4 (PLS), Observed vs Predicted
YPred[Comp. 1](YVar Class 0_S)/YVar(YVar Class 0_S)
CAN
N
wk0-pre-CAN
wk12-pre-CAN
y=1*x+9.416e-008
R2=0.9794
YVar(Class 0_S)
YPred[1](Class 0_S)
0
1
2
3

FIG. 14

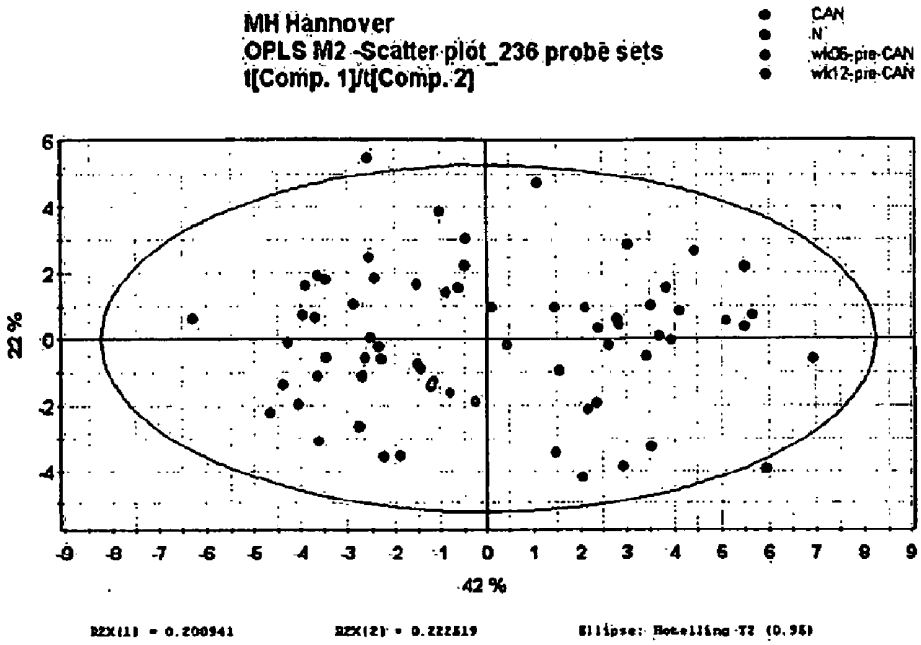

MH Hannover
OPLS M2 -Scatter plot_236 probe sets
t[Comp. 1]/t[Comp. 2]
CAN
N
wk06-pre-CAN
wk12-pre-CAN
22 %
42 %
6
4
2
0
-2
-4
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9

FIG. 15

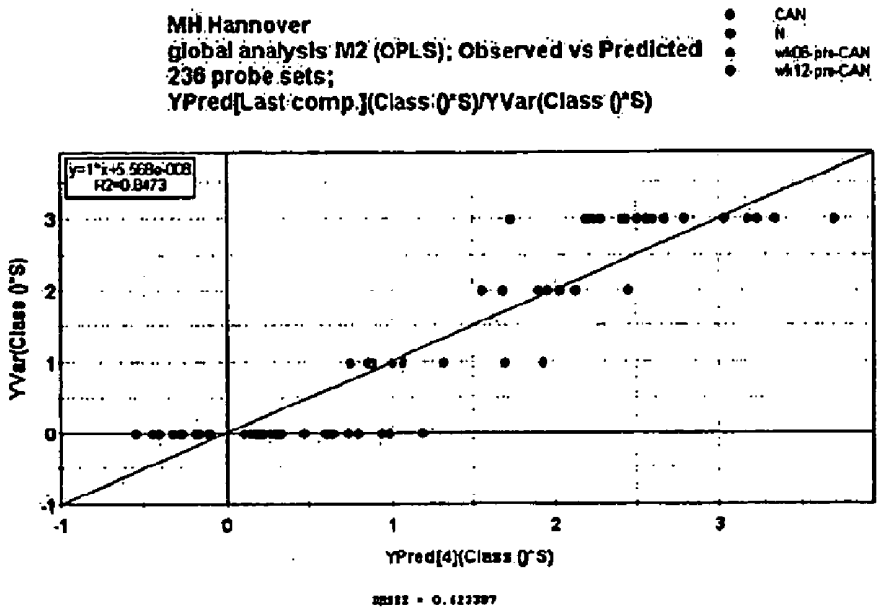

MH Hannover
global analysis M2 (OPLS); Observed vs Predicted
236 probe sets;
YPred[Last comp.](Class ()*S)/YVar(Class ()*S)
CAN
N
wk06-pre-CAN
wk12-pre-CAN
y=1*x+5.568e-008
R2=0.8473
YVar(Class ()*S)
YPred[4](Class ()*S)
3
2
1
0
-1
-1
0
1
2
3

FIG. 16

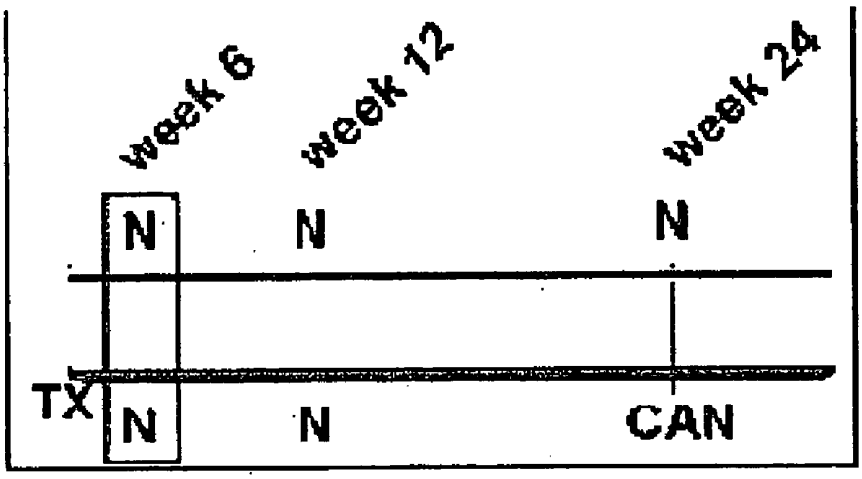


FIG. 17

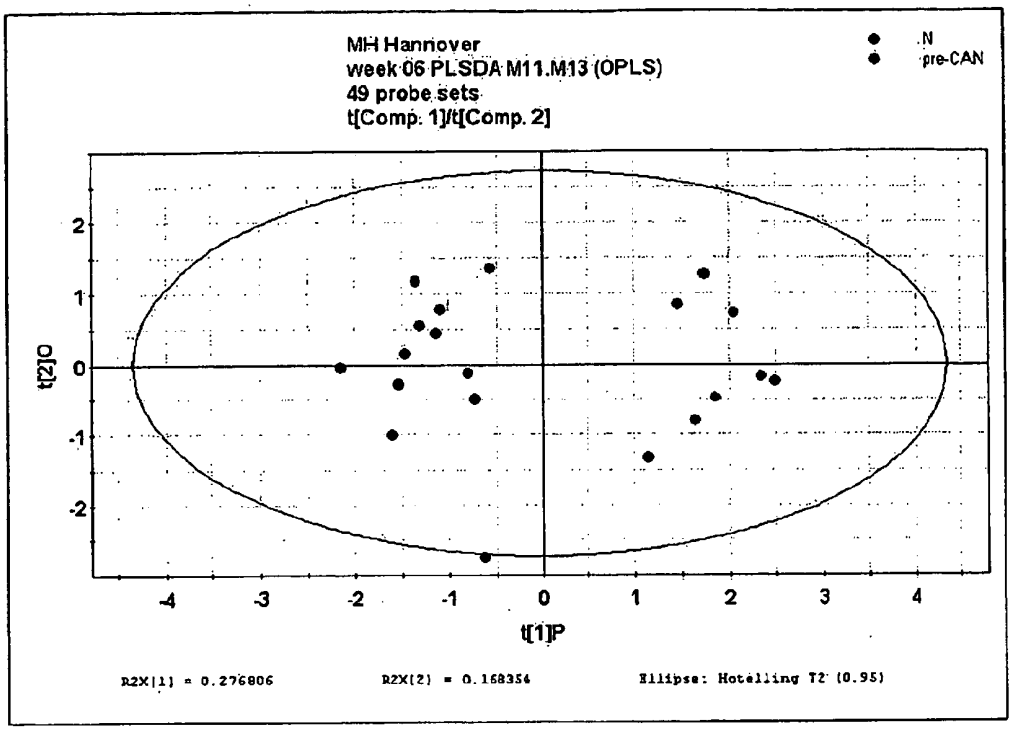

MH Hannover
week 06 PLSDA M11.M13 (OPLS)
49 probe sets
t[Comp. 1]/t[Comp. 2]
N
pre-CAN
t[2]O
t[1]P
2
1
0
-1
-2
-4
-3
-2
-1
0
1
2
3
4
R2X[1] = 0.276806
R2X[2] = 0.168356
Ellipse: Hotelling T2 (0.95)

FIG. 18

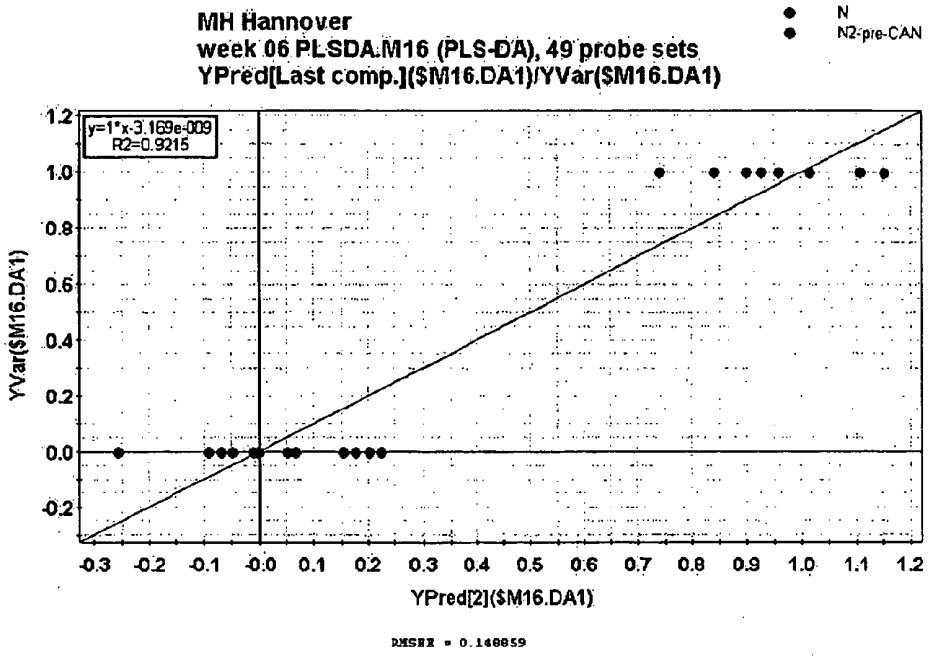

MH Hannover
week 06 PLSDA.M16 (PLS-DA), 49 probe sets
YPred[Last comp.]($M16.DA1)/YVar($M16.DA1)
N
N2-pre-CAN
y=1*x-3.169e-009
R2=0.9215
YVar($M16.DA1)
YPred[2]($M16.DA1)
RMSEE = 0.146859

FIG. 19

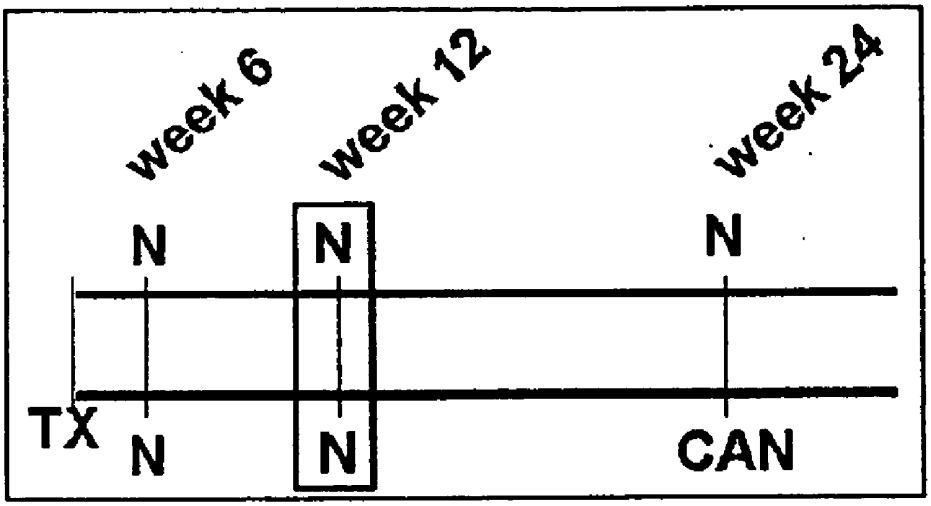


FIG. 20

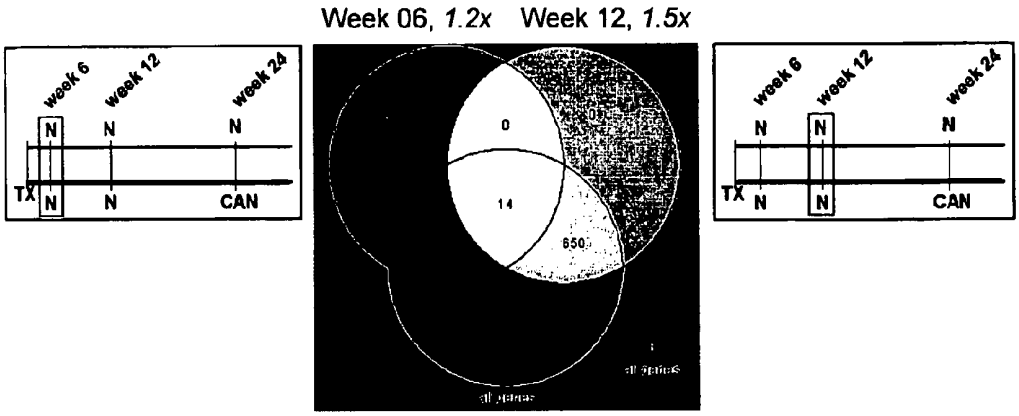
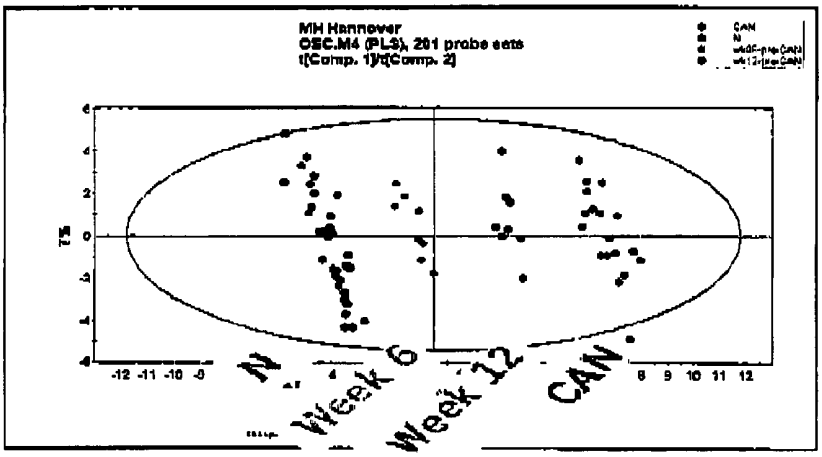
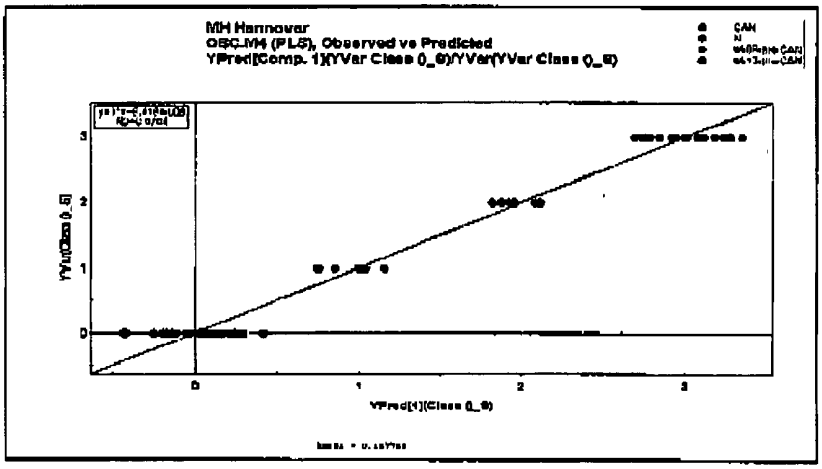

Week 06, 1.2x Week 12, 1.5x
week 6
week 12
week 24
N
N
N
TX
N
N
CAN
0
14
650
week 6
week 12
week 24
N
N
N
TX
N
N
CAN

FIG. 21

A


MH Hannover
OSC.M4 (PLS), 201 probe sets
t[Comp. 1]/t[Comp. 2]
CAN
N
wk06-pre-CAN
wk12-pre-CAN
N
Week 6
Week 12
CAN

B


MH Hannover
OSC.M4 (PLS), Observed vs Predicted
YPred[Comp. 1](YVar Class 0_6)/YVar(YVar Class 0_6)
CAN
N
wk06-pre-CAN
wk12-pre-CAN

C

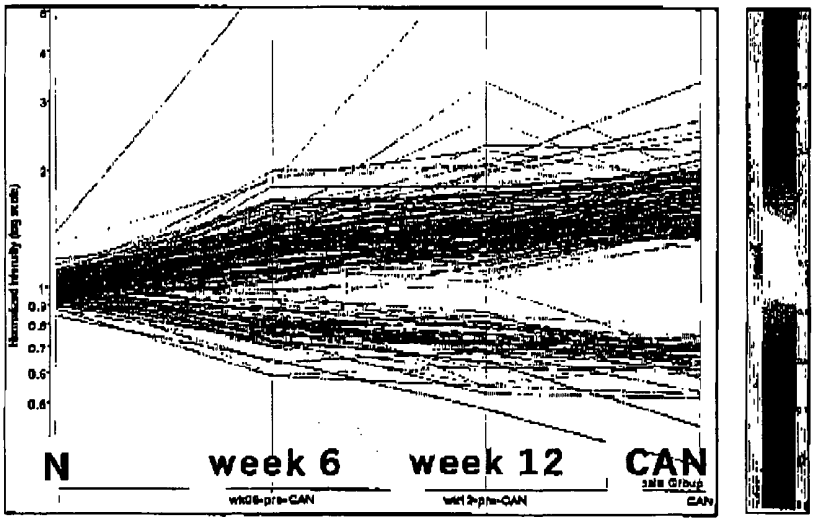

N
week 6
week 12
CAN
wk06-pre-CAN
wk12-pre-CAN
CAN

FIG. 22

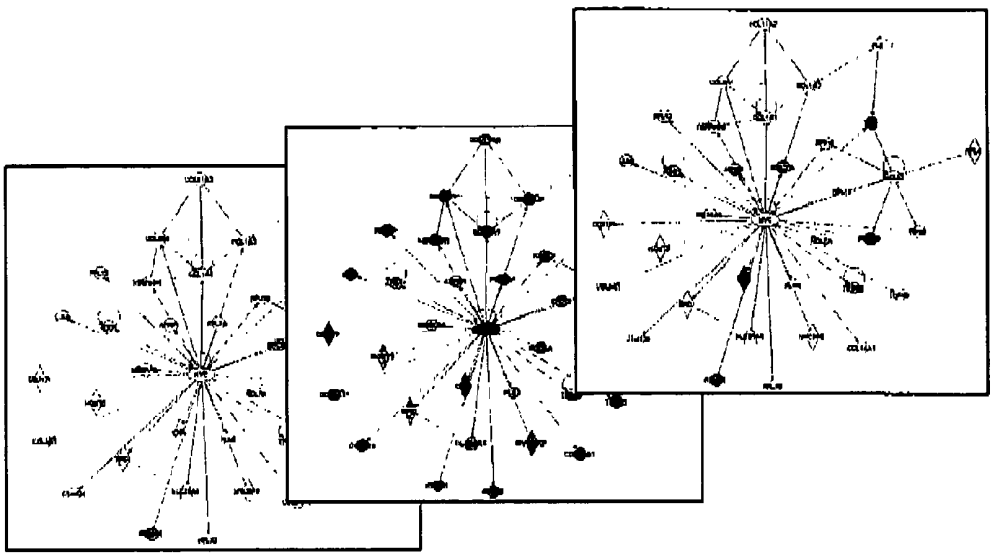
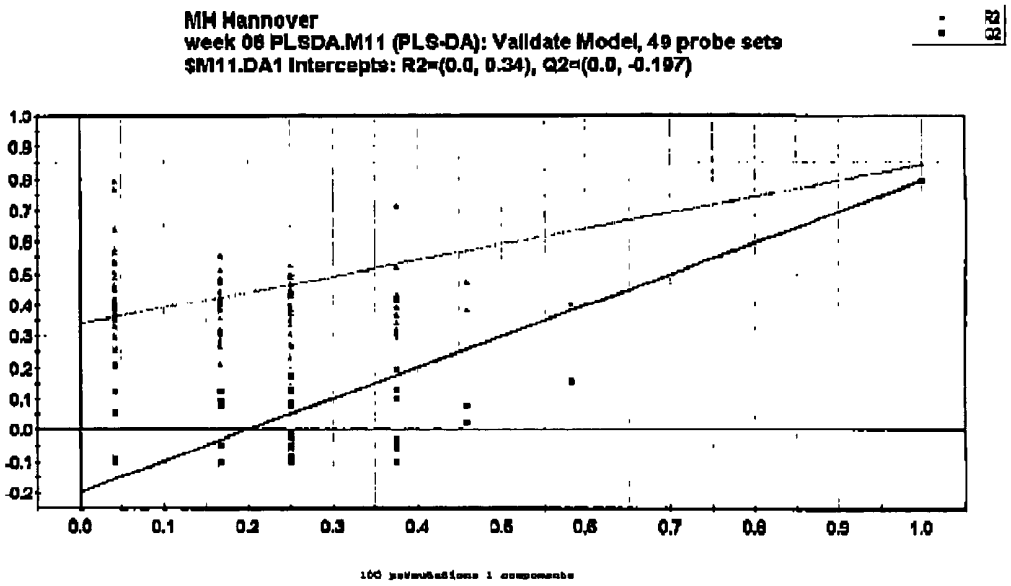


FIG. 23


MH Hannover
week 08 PLSDA.M11 (PLS-DA): Validate Model, 49 probe sets
$M11.DA1 Intercepts: R2=(0.0, 0.34), Q2=(0.0, -0.197)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

PREDICTIVE BIOMARKERS FOR CHRONIC ALLOGRAFT NEPHROPATHY

FIELD OF THE INVENTION

[0001] This invention relates generally to the analytical testing of tissue samples in vitro, and more particularly to gene- or protein-based tests useful in prediction of chronic allograft nephropathy.

BACKGROUND OF THE INVENTION

[0002] Chronic transplant dysfunction is a phenomenon in solid organ transplants displaying a gradual deterioration of graft function following transplantation, eventually leading to graft failure, and which is accompanied by characteristic histological features. Clinically, chronic transplant dysfunction in kidney grafts, e.g., chronic/sclerosing allograft nephropathy ("CAN"), manifests itself as a slowly progressive decline in glomerular filtration rate, usually in conjunction with proteinuria and arterial hypertension. Despite clinical application of potent immunoregulatory drugs and biologic agents, chronic rejection remains a common and serious post-transplantation complication. Chronic rejection is a relentlessly progressive process.

[0003] The single most common cause for early graft failure, especially within one month post-transplantation, is immunologic rejection of the allograft. The unfavorable impact of the rejection is magnified by the fact that: (a) the use of high-dose anti-rejection therapy, superimposed upon maintenance immunosuppression, is primarily responsible for the morbidity and mortality associated with transplantation, (b) the immunization against "public" HLA-specificities resulting from a rejected graft renders this patient population difficult to retransplant and (c) the return of the immunized recipient with a failed graft to the pool of patients awaiting transplantation enhances the perennial problem of organ shortage.

[0004] Histopathological evaluation of biopsy tissue is the gold standard for the diagnosis of CAN, while prediction of the onset of CAN is currently impossible. Current monitoring and diagnostic modalities are ill-suited to the diagnosis of CAN at an early stage.

SUMMARY

[0005] The invention pertains to molecular diagnostic methods using gene expression profiling further refine the BANFF 97 disease classification (Racusen L C, et al., Kidney Int. 55(2):713-23 (1999)). The invention also provides for methods for using biomarkers as predictive or early diagnostic biomarkers when applied at early time points after transplantation when graft dysfunction by other more conventional means is not yet detectable.

[0006] Accordingly, in one aspect, the invention pertains to a method for predicting the onset of a rejection of a transplanted organ in a subject, comprising the steps of: (a) obtaining a post-transplantation sample from the subject; (b) determining the level of gene expression in the post-transplantation sample of a combination of a plurality of genes selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model; (c) comparing the magnitude of gene expression of the at least one gene in the post-transplantation sample with the magnitude of gene expression of the same gene in a control sample; and (d) determining whether the expression level of at least one gene is up-regulated or down-regulated relative to the control sample, wherein up-regulation or down-regulation of at least one gene indicates that the subject is likely to experience transplant rejection, thereby predicting the onset of rejection of the transplanted organ in the subject.

[0007] The sample comprises cells obtained from the subject. The sample can be selected from the group consisting of: a graft biopsy; blood; serum; and urine. The rejection can be chronic/sclerosing allograph nephropathy. The magnitude of expression in the sample differs from the control magnitude of expression by a factor of at least about 1.5, or by a factor of at least about 2.

[0008] In another aspect, the invention pertains to a method for predicting the onset of a rejection of a transplanted organ in a subject, comprising the steps of: (a) obtaining a post-transplantation sample from the subject; (b) determining the level of gene expression in the post-transplantation sample of a combination of a plurality of genes selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model; and (c) comparing the gene expression pattern of the combination of gene in the post-transplantation sample with the pattern of gene expression of the same combination of gene in a control sample, wherein a similarity in the expression pattern of the gene expression pattern of the combination of gene in the post-transplantation sample compared to the expression pattern same combination of gene in a control sample expression profile indicates indicates that the subject is likely to experience transplant rejection, thereby predicting the onset of rejection of the transplanted organ in the subject.

[0009] In another aspect, the invention pertains to a method of monitoring transplant rejection in a subject, comprising the steps of: (a) taking as a baseline value the magnitude of gene expression of a combination of a plurality of genes in a sample obtained from a transplanted subject who is known not to develop rejection; (b) detecting a magnitude of gene expression corresponding to the combination of a plurality of genes in a sample obtained from a patient post-transplantation; and (c) comparing the first value with the second value, wherein a first value lower or higher than the second value predicts that the transplanted subject is at risk of developing rejection, wherein the plurality of genes are selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model.

[0010] In another aspect, the invention pertains to a method of monitoring transplant rejection in a subject, comprising the steps of: (a) detecting a pattern of gene expression corresponding to a combination of a plurality of genes from a sample obtained from a donor subject at the day of transplantation; (b) detecting a pattern of gene expression corresponding to the plurality of genes from a sample obtained from a recipient subject post-transplantation; and (c) comparing the first value with the second value, wherein a first value lower or higher than the second value predicts that the recipient subject is at risk of developing rejection; wherein the a plurality of genes selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model.

[0011] In another aspect, the invention pertains to a method for monitoring transplant rejection in a subject at risk thereof, comprising the steps of: (a) obtaining a pre-administration sample from a transplanted subject prior to administration of a rejection inhibiting agent; (b) detecting the magnitude of gene expression of a plurality of genes in the pre-administration sample; and (c) obtaining one or more post-administration samples from the transplanted subject; detecting the pattern of gene expression of a plurality of genes in the post-administration sample or samples, comparing the pattern of gene expression of the plurality of genes in the pre-administration sample with the pattern of gene expression in the post-administration sample or samples, and adjusting the agent accordingly, wherein the plurality of genes are selected from the group consisting of the genes of: Table 2; Table 3 and Table 4 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model.

[0012] In another aspect, the invention pertains to a method for preventing, inhibiting, reducing or treating transplant rejection in a subject in need of such treatment comprising administering to the subject a compound that modulates the synthesis, expression or activity of one or more genes or gene products encoded thereof of genes selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, so that at least one symptom of rejection is ameliorated.

[0013] In another aspect, the invention pertains to a method for identifying agents for use in the prevention, inhibition, reduction or treatment of transplant rejection comprising monitoring the level of gene expression of one or more genes or gene products selected from the group consisting of the genes of: Table 4; Table 5; Table 6; Table 7; and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model.

[0014] The transplanted subject can be a kidney transplanted subject. The pattern of gene expression can be assessed by detecting the presence of a protein encoded by the gene. The presence of the protein can be detected using a reagent which specifically binds to the protein. The pattern of gene expression can be detected by techniques selected from the group consisting of Northern blot analysis, reverse transcription PCR and real time quantitative PCR. The magnitude of gene expression of one gene or a plurality of genes can be detected.

[0015] In another aspect, the invention pertains to use of the combination of the plurality of genes or an expression products thereof as listed in Table 2, Table 3 or Table 4 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model as a biomarker for transplant rejection.

[0016] In another aspect, the invention pertains to use of a compound which modulates the synthesis, expression of activity of one or more genes as identified in Table 2, Table 3 or Table 4 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, or an expression product thereof, for the preparation of a medicament for prevention or treatment of transplant rejection in a subject.

BRIEF DESCRIPTION OF DRAWINGS

[0017] FIG. **1** is a schematic diagram detailing the time course of biopsy samples for diagnosis of stable allograft function (normal, N) and chronic allograft rejection (CAN) by histopathological evaluation;

[0018] FIG. **2** is a scatter plot derived by partial least squares discrimination analysis (PLDA) of biomarker data obtained at Biomarker week 06;

[0019] FIG. **3** is a graph derived by PLSDA of data obtained at Biomarker week 06 comparing observed versus predicted biomarker data;

[0020] FIG. **4** is a graph of biomarker data relating to the Biomarker week 06 PLSDA model: Validation by Response Permutation;

[0021] FIG. **5** is a scatter plot derived by orthogonal partial least squares analysis (OPLS) of biomarker data obtained at Biomarker week 12;

[0022] FIG. **6** is a graph of biomarker data relating to the Biomarker week 12 OPLS model: Validation by Response Permutation;

[0023] FIG. **7** is a graph derived by OPLS of data obtained at Biomarker week 12 comparing observed versus predicted biomarker data;

[0024] FIG. **8** is a scatter plot derived by PLDA of biomarker data obtained at Biomarker week 06;

[0025] FIG. **9** is a graph of biomarker data relating to the Biomarker week 12 PLSDA model: Validation by Response Permutation;

[0026] FIG. **10** is a graph derived by OPLS of data obtained at Biomarker week 12 comparing observed versus predicted biomarker data;

[0027] FIG. **11** is a scatter plot derived by orthogonal signal correction (OSC) in a global analysis of biomarker data;

[0028] FIG. **12** is a graph of biomarker data relating to Biomarker global analysis OSC model: Validation by response permutation;

[0029] FIG. **13** is a graph derived by global analysis OSC modeling of data comparing observed versus predicted biomarker data;

[0030] FIG. **14** is a scatter plot derived by OPLS in a global analysis of biomarker data; and

[0031] FIG. **15** is a graph derived by global analysis OPLS modeling of data comparing observed versus predicted biomarker data.

[0032] FIG. **16** is a chart showing week 6 post-TX timepoint, 4.5 months before clinical/histopath. evidence of CAN.

[0033] FIG. **17** is graph of biomarker identification at week 6 (4.5 months before CAN). Good separation of patient groups (PLSDA model with 49 probe sets).

[0034] FIG. **18** is graph showing cross-validation at week 6 (4.5 months before CAN). Cross-validation ("leave one group of 7 samples out"): Model provides clear separation between N and pre-CAN.

[0035] FIG. **19** is a chart showing week 6 post-TX timepoint, 3 months before clinical/histopath. evidence of CAN.

[0036] FIG. **20** is a chart showing the overlap of biomarkers identified at week 6 (t test<0.05, 1.2 FC) and week 12 (t test<0.05, 1.5 FC). Small overlap between week 06 and week 12 biological genelists may indicate the presence of different underlying biological processes/pathways at specific timepoints.

[0037] FIG. **21** is a figure the OSC model with 201 probe sets. OSC model with 201 probe sets differentiates groups by timepoint and diagnosis.

[0038] FIG. **22** is a figure showing pathway analysis and biological mechanisms. Transient activation of pathways at different timepoints.

[0039] FIG. **23** is a figure showing model validation by permutation. Model validation by Permutation analysis: 100 iterations (i.e. fit of 100 PLS models compared to fit of"real model").

DETAILED DESCRIPTION

[0040] Definitions

[0041] To further facilitate an understanding of the present invention, a number of terms and phrases are defined below:

[0042] The terms "down-regulation" or "down-regulated" are used interchangeably herein and refer to the decrease in the amount of a target gene or a target protein. The term "down-regulation" or "down-regulated" also refers to the decreases in processes or signal transduction cascades involving a target gene or a target protein.

[0043] The term "transplantation" as used herein refers to the process of taking a cell, tissue, or organ, called a "transplant" or "graft" from one subject and placing it or them into a (usually) different subject. The subject who provides the transplant is called the "donor" and the subject who received the transplant is called the "recipient". An organ, or graft, transplanted between two genetically different subjects of the same species is called an "allograft". A graft transplanted between subjects of different species is called a "xenograft".

[0044] The term "transplant rejection" as used herein is defined as functional and structural deterioration of the organ due to an active immune response expressed by the recipient, and independent of non-immunologic causes of organ dysfunction.

[0045] The term "chronic rejection" as used herein refers to rejection of the transplanted organs (e.g., kidney). The term also applies to a process leading to loss of graft function and late graft loss developing after the first 30-120 post-transplant days. In kidneys, the development of nephrosclerosis (hardening of the renal vessels), with proliferation of the vascular intima of renal vessels, and intimal fibrosis, with marked decrease in the lumen of the vessels, takes place. The result is renal ischemia, hypertension, tubular atrophy, interstitial fibrosis, and glomerular atrophy with eventual renal failure. In addition to the established influence of HLA incompatibility, the age, number of nephrons, and ischemic history of a donor kidney may contribute to ultimate progressive renal failure in transplanted patients.

[0046] The term "subject" as used herein refers to any living organism in which an immune response is elicited. The term subject includes, but is not limited to, humans, nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered.

[0047] A "gene" includes a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed and translated. Any of the polynucleotide sequences described herein may be used to identify larger fragments or full-length coding sequences of the gene with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art, some of which are described herein.

[0048] A "gene product" includes an amino acid (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

[0049] The term "magnitude of expression" as used herein refers to quantifying marker gene transcripts and comparing this quantity to the quantity of transcripts of a constitutively expressed gene. The term "magnitude of expression" means a "normalized, or standardized amount of gene expression". For example, the overall expression of all genes in cells varies (i.e., it is not constant). To accurately assess whether the detection of increased mRNA transcript is significant, it is preferable to "normalize" gene expression to accurately compare levels of expression between samples, i.e., it is a baselevel against which gene expression is compared. In one embodiment, the expressed gene is associated with a biological pathway/process selected from the group consisting of: the wnt pathway (e.g., NFAT, NE-dig, frizzled-9, hes-1), TGFbeta (e.g., NOMO, SnoN), glucose and fatty acid transport and metabolism (e.g., GLUT4), vascular smooth muscle differentiation (e.g., amnionless, ACLP, lumican), vascular sclerosis (e.g., THRA, IGFBP4), ECM (e.g., collagen), and immune response (e.g., TNF, NFAT, GM-CSF). Quantification of gene transcripts was accomplished using competitive reverse transcription polymerase chain reaction (RT-PCR) and the magnitude of gene expression was determined by calculating the ratio of the quantity of gene expression of each marker gene to the quantity of gene expression of the expressed gene.

[0050] The term "differentially expressed", as applied to a gene, includes the differential production of mRNA transcribed from a gene or a protein product encoded by the gene. A differentially expressed gene may be overexpressed or underexpressed as compared to the expression level of a normal or control cell. In one aspect, it includes a differential that is at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times or at least 10 times higher or lower than the expression level detected in a control sample. In a preferred embodiment, the expression is higher than the control sample. The term "differentially expressed" also includes nucleotide sequences in a cell or tissue which are expressed where silent in a control cell or not expressed where expressed in a control cell. In particular, this term refers to refers to a given allograft gene expression level and is defined as an amount which is substantially greater or less than the amount of the corresponding baseline expression level. Baseline is defined here as being the level of expression in healthy tissue. Healthy tissue includes a transplanted organ without pathological findings.

[0051] The term "sample" as used herein refers to cells obtained from a biopsy. The term "sample" also refers to cells obtained from a fluid sample including, but not limited to, a sample of bronchoalveolar lavage fluid, a sample of bile, pleural fluid or peritoneal fluid, or any other fluid secreted or excreted by a normally or abnormally functioning allograft, or any other fluid resulting from exudation or transudation through an allograft or in anatomic proximity to an allograft, or any fluid in fluid communication with the allograft. A fluid test sample may also be obtained from essentially any body fluid including: blood (including peripheral blood), lymphatic fluid, sweat, peritoneal fluid, pleural fluid, bronchoalveolar lavage fluid, pericardial fluid, gastrointestinal juice, bile, urine, feces, tissue fluid or swelling fluid, joint fluid, cerebrospinal fluid, or any other named or unnamed fluid gathered from the anatomic area in proximity to the allograft or gathered from a fluid conduit in fluid communication with

the allograft. A "post-transplantation fluid test sample" refers to a sample obtained from a subject after the transplantation has been performed.

[0052] Sequential samples can also be obtained from the subject and the quantification of immune activation gene biomarkers determined as described herein, and the course of rejection can be followed over a period of time. In this case, for example, the baseline magnitude of gene expression of the biomarker gene(s) is the magnitude of gene expression in a post-transplant sample taken after the transplant. For example, an initial sample or samples can be taken within the nonrejection period, for example, within one week of transplantation and the magnitude of expression of biomarker genes in these samples can be compared with the magnitude of expression of the genes in samples taken after one week. In one embodiment, the samples are taken on weeks 6, 12 and 24 post-transplantation.

[0053] The term "biopsy" as used herein refers to a specimen obtained by removing tissue from living patients for diagnostic examination. The term includes aspiration biopsies, brush biopsies, chorionic villus biopsies, endoscopic biopsies, excision biopsies, needle biopsies (specimens obtained by removal by aspiration through an appropriate needle or trocar that pierces the skin, or the external surface of an organ, and into the underlying tissue to be examined), open biopsies, punch biopsies (trephine), shave biopsies, sponge biopsies, and wedge biopsies. In one embodiment, a fine needle aspiration biopsy is used. In another embodiment, a minicore needle biopsy is used. A conventional percutaneous core needle biopsy can also be used.

[0054] The term "up-regulation" or "up-regulated" are used interchangeably herein and refer to the increase or elevation in the amount of a target gene or a target protein. The term "up-regulation" or "up-regulated" also refers to the increase or elevation of processes or signal transduction cascades involving a target gene or a target protein.

[0055] The term "gene cluster" or "cluster" as used herein refers to a group of genes related by expression pattern. In other words, a cluster of genes is a group of genes with similar regulation across different conditions, such as graft non-rejection versus graft rejection. The expression profile for each gene in a cluster should be correlated with the expression profile of at least one other gene in that cluster. Correlation may be evaluated using a variety of statistical methods. Often, but not always, members of a gene cluster have similar biological functions in addition to similar gene expression patterns.

[0056] A "probe set" as used herein refers to a group of nucleic acids that may be used to detect two or more genes. Detection may be, for example, through amplification as in PCR and RT-PCR, or through hybridization, as on a microarray, or through selective destruction and protection, as in assays based on the selective enzymatic degradation of single or double stranded nucleic acids. Probes in a probe set may be labeled with one or more fluorescent, radioactive or other detectable moieties (including enzymes). Probes may be any size so long as the probe is sufficiently large to selectively detect the desired gene. A probe set may be in solution, as would be typical for multiplex PCR, or a probe set may be adhered to a solid surface, as in an array or microarray. It is well known that compounds such as PNAs may be used instead of nucleic acids to hybridize to genes. In addition, probes may contain rare or unnatural nucleic acids such as inosine.

[0057] The terms "polynucleotide" and "oligonucleotide" are used interchangeably, and include polymeric forms of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The term also includes both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0058] A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for guanine when the polynucleotide is RNA. This, the term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be inputted into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

[0059] The term "cDNAs" includes complementary DNA, that is mRNA molecules present in a cell or organism made into cDNA with an enzyme such as reverse transcriptase. A "cDNA library" includes a collection of mRNA molecules present in a cell or organism, converted into cDNA molecules with the enzyme reverse transcriptase, then inserted into "vectors" (other DNA molecules that can continue to replicate after addition of foreign DNA). Exemplary vectors for libraries include bacteriophage, viruses that infect bacteria (e g., lambda phage). The library can then be probed for the specific cDNA (and thus mRNA) of interest.

[0060] A "primer" includes a short polynucleotide, generally with a free 3'-OH group that binds to a target or "template" present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers" or "set of primers" consisting of "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and are taught, for example, in MacPherson et al., IRL Press at Oxford University Press (1991)). All processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication". A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses (see, e.g., Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A

Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

[0061] The term "polypeptide" includes a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. As used herein the term "amino acid" includes either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly referred to as an oligopeptide. Peptide chains of greater than three or more amino acids are referred to as a polypeptide or a protein.

[0062] The term "hybridization" includes a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0063] Hybridization reactions can be performed under conditions of different "stringency". The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. Under stringent conditions, nucleic acid molecules at least 60%, 65%, 70%, 75% identical to each other remain hybridized to each other, whereas molecules with low percent identity cannot remain hybridized. A preferred, non-limiting example of highly stringent hybridization conditions are hybridization in 6x sodium chloride/sodium citrate (SSC) at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 50° C., preferably at 55° C., more preferably at 60° C., and even more preferably at 65° C.

[0064] When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. "Complementarity" or "homology" (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to hydrogen bond with each other, according to generally accepted base-pairing rules.

[0065] As used herein, the terms "marker" and "biomarker" are used interchangeably and include a polynucleotide or polypeptide molecule which is present or modulated (i.e., increased or decreased) in quantity or activity determined using a statistical model (e.g., PLSDA and OPLS), in subjects at risk for organ rejection relative to the quantity or activity in subjects that are not at risk for organ rejection. The relative change in quantity or activity of the biomarker is correlated with the incidence or risk of incidence of rejection.

[0066] As used herein, the term "panel of markers" includes a group of biomarkers determined using a statistical model (e.g., PLSDA and OPLS), the quantity or activity of each member of which is correlated with the incidence or risk of incidence of organ rejection. In certain embodiments, a panel of biomarkers may include only those biomarkers which are either increased in quantity or activity in subjects at risk for organ rejection. In other embodiments, a panel of biomarkers may include only those biomarkers which are either decreased in quantity or activity in subjects at risk for organ rejection.

[0067] Abbreviations for select terms are summarized in Table 1 below.

TABLE 1

Abbreviations:

Abbreviation	Term
AEBP/ACLP	Adipocyte enhancer binding protein/aortic carboxylase like protein
Amn	amnionless
BMD	BioMarker Development
CAN	Chronic allograft nephropathy
CP	Ceruloplasmin, ferroxidase
CSF2RB	colony stimulating factor 2 receptor, beta
CV	Coefficient of variance
Dlg3, Ne-dlg	Neuroendocrine discs large
Fzd-9	Frizzled 9
GLUT4/ SLC2A12	solute carrier family 2 (facilitated glucose transporter), member 12
Hes-1	Hairy and enhancer of split 1
HGF	hepatocyte growth factor (hepapoietin A; scatter factor)
IGFBP4	insulin-like growth factor binding protein 4
Lcn	lumican
NFAT	Nuclear factor of activated T cells
OPLS	Orthogonal projections of latent structures by means of partial least squares
PLS	Projections of latent structures by means of partial least squares
PLS-DA	Projections of latent structures by means of partial least squares-discriminant analysis
pM5/NOMO	Nodal modulator 2
Ski-l/SnoN	Ski-like (snoN)
THRA	Thyroid hormone receptor alpha

[0068] Predictive Biomarkers of Chronic Rejection

[0069] The invention is based, in part, on the discovery that select genes are modulated in CAN and these genes can be used as predictive biomarkers before the onset of overt CAN. Advances in highly parallel, automated DNA hybridization techniques combined with the growing wealth of human gene sequence information have made it feasible to simultaneously analyze expression levels for thousands of genes (see, e.g., Schena et al., 1995, Science 270:467-470; Lockhart et al., 1996, Nature Biotechnology 14:1675-1680; Blanchard et al., 1996, Nature Biotechnology 14:1649; Ashby et al., U.S. Pat. No. 5,569,588, issued Oct. 29, 1996; Perou et al., 2000, Nature 406:747-752). Methods such as the gene-by-gene quantitative RT-PCR are highly accurate but relatively labor intensive. While it is possible to analyze the expression of thousands of genes using quantitative PCR, the effort and expense would be enormous. Instead, as an example of large scale analysis, an entire population of mRNAs may be converted to cDNA and hybridized to an ordered array of probes that represent anywhere from ten to ten thousand or more genes. The relative amount of cDNA that hybridizes to each of these probes is a measure of the expression level of the corresponding gene. The data may then be statistically analyzed to reveal informative patterns of gene expression. Indeed, early diagnosis of renal allograft rejection and new

prognostic biomarkers are important minimize and personalize immunosuppression. In addition to histopathological differential diagnosis, gene expression profiling significantly improves disease classification by defining a "molecular signature."

[0070] Several previous studies have successfully applied a transcriptomic approach to distinguish different classes of kidney transplants. However, the heterogeneity of microarray platforms and various data analysis methods complicates the identification of robust signatures of CAN.

[0071] To address this issue, comparative multivariate data analyses (e.g., PLSDA; OPLS; OSC) was performed on gene expression profiles of serial renal protocol biopsies from patients with stable graft function throughout at least one year after renal transplantation and patients who had diagnosed chronic allograft nephropathy (CAN; grade 1) at the week 24 biopsy but not at biopsies of earlier time points (week 06 and week 12). As presented in Example I, these studies identify molecular signatures predictive of the onset of CAN. The molecular signature comprises a combination of algorithm and genes identified by the algorithm at various time points. That is, the present invention relates to the identification of genes, which are modulated (i.e., up-regulated or down-regulated) during rejection, in particular during early CAN. A highly statistically significant correlation has been found between the expression of one or more biomarker gene(s) and CAN, thereby providing a "molecular signature" for transplant rejection (e.g., CAN). These biomarker genes and their expression products can be used in the management, prognosis and treatment of patients at risk of transplant rejection as they are useful to identify organs that are likely to undergo rejection.

[0072] Clinical Features of CAN

[0073] Chronic transplant dysfunction is a phenomenon in solid organ transplants displaying a gradual deterioration of graft function months to years after transplantation, eventually leading to graft failure, and which is accompanied by characteristic histological features. Clinically, chronic allograft nephropathy in kidney grafts (i.e., CAN) manifests itself as a slowly progressive decline in glomerular filtration rate, usually in conjunction with proteinuria and arterial hypertension.

[0074] The cardinal histomorphologic feature of CAN in all parenchymal allografts is fibroproliferative endarteritis. The vascular lesion affects the whole length of the arteries in a patchy pattern. There is concentric myointimal proliferation resulting in fibrous thickening and the characteristic 'onion skin' appearance of the intima in small arteries. Other findings include endothelial swelling, foam cell accumulation, disruption of the internal elastic lamina, hyalinosis and medial thickening, and presence of subendothelial T-lymphocytes and macrophages (Hruban R H, et al., Am J Pathol 137(4):871-82 (1990)). In addition, a persistent focal perivascular inflammation is often seen.

[0075] In addition to vascular changes, kidneys undergoing CAN also show interstitial fibrosis, tubular atrophy, and glumerulopathy. Chronic transplant glumerolopathy—duplication of the capillary walls and mesangial matrix increase—has been identified as a highly specific feature of kidneys with CAN (Solez K, Clin Transplant.; 8(3 Pt 2):345-50 (1994)). Less specific lesions are glomerular ischemic collapse, tubular atrophy, and interstitial fibrosis. Furthermore, peritubular capillary basement splitting and laminations are associated with late decline of graft function (Monga M, et al., Ultrastruct Pathol. 14(3):201-9 (1990)). The criteria for histological diagnosis of CAN in kidney allografts are internationally standardized in the Banff 97 scheme for Renal Allograft Pathology (Racusen L C, et al., Kidney Int. 55(2):713-23 (1999)); (adopted from Kouwenhoven et al., Transpl Int. 2000;13(6):385-401. 2000). Table 2 summarizes the Banff 97 criteria for chronic/sclerosing allograft nephropathy (CAN) (Racusen L C, et al., Kidney Int. 55(2):713-23 (1999)).

TABLE 2

Grade	Histopathological Findings
I - mild	Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific changes suggesting chronic rejection
II - moderate	Moderate interstitial fibrosis and tubular atrophy (a) or (b)
III - severe	Severe interstitial fibrosis and tubular atrophy and tubular loss (a) or (b)

[0076] For Banff 97, an "adequate" specimen is defined as a biopsy with 10 or more glumeruli and at least two arteries. Two working hypotheses are proposed to understand the process of CAN (Kouwenhoven et al., Transpl Int. 2000;13(6): 385-401. 2000). The first and probably the most important set of risk factors have been lumped under the designation of "alloantigen-dependent", immunological or rejection-related factors. Among these, late onset and increased number of acute rejection episodes; younger recipient age; male-to-female sex mismatch; a primary diagnosis of autoimmune hepatitis or biliary disease; baseline immunosuppression and non-caucasian recipient race have all been associated with an increased risk of developing chronic rejection. More specifically, (a) histoincompatibility: long-term graft survival appear to be strongly correlated with their degree of histocompatibility matching between donor and recipient; (b) Acute rejections: onset, frequency, and severity of acute rejection episodes are independent risk factors of CAN. Acute rejection is the most consistently identified risk factor for the occurrence of CAN; (c) Suboptimal immunosuppression due to too low maintenance dose of cyclosporine or non-compliance; and (d) Anti-donor specific antibodies: many studies have shown that following transplantation, the majority of patients produce antibodies. The second set of risk factors are referred to as "non-alloantigen-dependent" or "non-immunological" risk factors that also contribute to the development of chronic rejection include advanced donor age, pre-existing atherosclerosis in the donor organ, and prolonged cold ischemic time. Non-alloimmune responses to disease and injury, such as ischemia, can cause or aggravate CAN. More specifically, (a) recurrence of the original disease, such as glomerulonephritis; (b) consequence of the transplantation surgical injury; (c) duration of ischemia: intimal hyperplasia correlates with duration of ischemia; (d) kidney grafts from cadavers versus those from living related and unrelated donors; (e) viral infections: CMV infection directly affects intercellular adhesion molecules such as ICAM-1; (f) hyperlipidemia; (g) hypertension; (h) age; (i) gender: the onset of transplant arterosclerosis was earlier in male than in female; (j) race; and (k) the amount of functional tissue—reduced number of nephrons and hyperfiltration.

[0077] CAN is characterized by morphological evidence of destruction of the transplanted organ. The common denominator of all parenchymal organs is the development of intimal hyperplasia. T cells and macrophages are the predominant graft-invading cell types, with an excess of $CD4^+$ over $CD8^+$

T cells. Increased expression of adhesion molecules (ICAM-1, VCAM-1) and MHC antigens are seen in allografts with CAN, and increased TGF-β is frequently found. A short description of the route through which a graft may develop CAN follows:

[0078] Endothelial Cell Activation by Ischemia, Surgical Manipulation, and Reperfusion Injury.

[0079] In consequence, the endothelial cells produce oxygen free radicals and they release increased amounts of the cytokines IL-1, IL-6, IFN-γ, TNF-α and the chemokines IL-8, macrophage chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1α and 1β (MIP-1α MIP-1 β), colony stimulating factors, and multiple growth factors such as, platelet derived growth factor (PDGF), insulin like growth factor 1 (IGF-1), transforming growth factor β (TGF-β), and pro-thrombotic molecules such as tissue factor and plasminogen activator inhibitor (PAI). These cytokines activate the migration of neutrophils, monocytes/macrophages and T-lymphocytes to the site of injury where they interact with the endothelial cells by means of adhesion molecules, including ICAM-1, VCAM-1, P- and E-selectin. The increased expression of these adhesion molecules is induced by the cytokines IL-1β, IFN-γ, and TNF-α. Extravasation of leucocytes is facilitated by activated complement and oxygen-free radicals that increase the permeability between endothelial cells.

[0080] Limitations to Current Clinical Approaches for CAN Diagnosis

[0081] The differentiation of the diagnosis of rejection, e.g., CAN, from other etiologies for graft dysfunction and institution of effective therapy is a complex process because: (a) the percutaneous core needle biopsy of grafts, the best of available current tools to diagnose rejection is performed usually after the "fact", i.e., graft dysfunction and graft damage (irreversible in some instances) are already present, (b) the morphological analysis of the graft provides modest clues with respect to the potential for reversal of a given rejection episode, and minimal clues regarding the likelihood of recurrence ("rebound"), and (c) the mechanistic basis of the rejection phenomenon, a prerequisite for the design of therapeutic strategies, is poorly defined by current diagnostic indices, including morphologic features of rejection.

[0082] The diagnosis of, for example, renal allograft rejection is made usually by the development of graft dysfunction (e.g., an increase in the concentration of serum creatinine) and morphologic evidence of graft injury in areas of the graft also manifesting mononuclear cell infiltration. Two caveats apply, however, to the use of abnormal renal function as an indicator of the rejection process: first, deterioration in renal function is not always available as a clinical clue to diagnose rejection since many of the cadaveric renal grafts suffer from acute (reversible) renal failure in the immediate post-transplantation period due to injury from harvesting and ex vivo preservation procedures. Second, even when immediately unimpaired renal function is present, graft dysfunction might develop due to a non-immunologic cause, such as immunosuppressive therapy itself.

[0083] For example, cyclosporine (CsA) nephrotoxicity, a complication that is not readily identified solely on the basis of plasma/blood concentrations of CsA, is a common complication. The clinical importance of distinguishing rejection from CsA nephrotoxicity cannot be overemphasized since the therapeutic strategies are diametrically opposite: escalation of immunosuppressants for rejection, and reduction of CsA dosage for nephrotoxicity.

[0084] The invention is based, in part, on the observation that increased or decreased expression of on or more genes and/or the encoded proteins is associated with certain graft rejection states. As a result of the data described herein, methods are now available for the rapid and reliable diagnosis of acute and chronic rejection, even in cases where allograft biopsies show only mild cellular infiltrates. Described herein is an analysis of genes that are modulated (e.g., up-regulated or down-regulated) simultaneously and which provide a molecular signature to accurately detect transplant rejection.

[0085] The invention further provides classic molecular methods and large scale methods for measuring expression of suitable biomarker genes. The methods described herein are particularly useful for detecting chronic transplant rejection and preferably early chronic transplant rejection. In one embodiment, the chronic transplant rejection is the result of CAN. Most typically, the subject (i.e., the recipient of a transplant) is a mammal, such as a human. The transplanted organ can include any transplantable organ or tissue, for example kidney, heart, lung, liver, pancreas, bone, bone marrow, bowel, nerve, stem cells (or stem cell-derived cells), tissue component and tissue composite. In a preferred embodiment, the transplant is a kidney transplant.

[0086] The methods described herein are useful to assess the efficacy of anti-rejection therapy. Such methods involve comparing the pre-administration magnitude of the transcripts of the biomarker genes to the post-administration magnitude of the transcripts of the same genes, where a post-administration magnitude of the transcripts of the genes that is less than the pre-administration magnitude of the transcripts of the same genes indicates the efficacy of the anti-rejection therapy. Any candidates for prevention and/or treatment of transplant rejection, (such as drugs, antibodies, or other forms of rejection or prevention) can be screened by comparison of magnitude of biomarker expression before and after exposure to the candidate. In addition, valuable information can be gathered in this manner to aid in the determination of future clinical management of the subject upon whose biological material the assessment is being performed. The assessment can be performed using a sample from the subject, using the methods described herein for determining the magnitude of gene expression of the biomarker genes. Analysis can further comprise detection of an infectious agent.

[0087] Biological Pathways Associated with Biomarkers of the Invention

[0088] Biomarkers of the present invention identify select biological pathways affected by CAN and, as such, these biological pathways are of relevance to solid organ allograft nephropathy. Indeed, this meta-analysis revealed robust biomarker signatures for select biological pathways which can represent gene clusters. Such biological pathways include, but are not limited to, e.g., wnt pathway (i.e., NFAT (Murphy et al., J Immunol. 69(7):3717-25 (2002)); NE-dlg (Hanada et al., Int. J. Cancer 86(4):480-8 (2000)); frizzled-9 (Karasawa et al., J. Biol. Chem. 277(40):37479-86 (2002)); Hes-1 (Deregowski et al., J Biol Chem. 281(10):6203-10 (2006); Piscione et al., Gene Expr. Patterns 4(6):707-11 (2004)), TGFbeta/Smad signaling pathway (i.e., Smad3 (Saika et al., Am. J. Pathol. 164(2):651-63 (2004); Smad2 (Ju et al., Mol. Cell Biol. 26(2):654-67 (2006); pM5/NOMO

(Hafner et al., EMBO J. Aug. 4, 2004;23(15):3041-50; SnoN (Zhu et al., Mol. Cell Biol. 25(24):10731-44 (2005); Wilkinson et al., Mol. Cell Biol. 25(3):1200-12 (2005)), glucose and fatty acid transport and metabolism (i.e., GLUT4 (Linden et al., Am J Physiol Renal Physiol. 290(1):F205-13. (2006)), vascular smooth muscle differentiation (i.e., lumican (Onda et al., 72(2): 142-9 (2002); ceruloplasmin (Chen et al., Biochem. Biophys. Res. Commun. 281(2):475-82 (2001); amnionless (Moestrup S K, Curr Opin Lipidol. 16(3):301-6 (2005); aortic carboxypeptidase-like protein (ACLP)), vascular sclerosis (THRA (Sato et al, Circ. Res. 97(6):550-7 (2005); IGFBP4; AE binding protein-1 (Layne et al., J. Biol. Chem. 273(25):15654-60 (1998); Abderrahim et al, Exp. Cell Res. 293(2):219-28 (2004)); ECM (collagen), and immune response (NFAT (Murphy et al., J Immunol. 69(7):3717-25 (2002));TNF, GM-CSF (Steinman R. M., Annu Rev. Immunol 9:271-96 (1991); Xu et al., Trends Pharmacol. Sci. 25(5): 254-8 (2004)). Jehle and coworkers have demonstrated that insulin-like growth factor binding protein 4 in serum is characteristic of chronic renal failure. Jehle et al., Kidney Int. 57(3):1209-10 (2000). Azuma and coworkers have shown that Hepatocyte growth factor (HGF) plays a renotropic role in renal regeneration and protection from acute ischemic injury and that HGF treatment greatly contribute to a reduction of susceptibility to the subsequent development of CAN in a rat model. Azuma et al. J. Am. Soc. Nephrol. 12(6):1280-92 (2001).

[0089] The advent of large scale gene expression analysis has revealed that groups of genes are often expressed together in a coordinated manner. For example, whole genome expression analysis in the yeast *Saccharomyces cerevisiae* showed coordinate regulation of metabolic genes during a change in growth conditions known as the diauxic shift (DiRisi et al., 1997, Science 278:680-686; Eisen et al., 1998, PNAS 95:14863-14868). The diauxic shift occurs when yeast cells fermenting glucose to ethanol exhaust the glucose in the media and begin to metabolize the ethanol. In the presence of glucose, genes of the glycolytic pathway are expressed and carry out the fermentation of glucose to ethanol. When the glucose is exhausted, yeast cells must metabolize the ethanol, a process that depends heavily on the Krebs cycle and respiration.

[0090] Accordingly, the expression of glycolysis genes decreases, and the expression of Krebs cycle and respiratory genes increases in a coordinate manner. Similar coordinate gene regulation has been found in various cancer cells. Genes encoding proteins involved in cell cycle progression and DNA synthesis are often coordinately overexpressed in cancerous cells (Ross et al., 2000, Nature Genet. 24:227-235; Perou et al, 1999, PNAS 96:9212-9217; Perou et al., 2000, Nature 406:747-752).

[0091] The coordinate regulation of genes is logical from a functional point of view. Most cellular processes require multiple genes, for example: glycolysis, the Krebs cycle, and cell cycle progression are all multi-gene processes. Coordinate expression of functionally related genes is therefore essential to permit cells to perform various cellular activities. Such groupings of genes can be called "gene clusters" (Eisen et al., 1998, PNAS 95:14863-68).

[0092] Clustering of gene expression is not only a functional necessity, but also a natural consequence of the mechanisms of transcriptional control. Gene expression is regulated primarily by transcriptional regulators that bind to cis-acting DNA sequences, also called regulatory elements. The pattern of expression for a particular gene is the result of the sum of the activities of the various transcriptional regulators that act on that gene. Therefore, genes that have a similar set of regulatory elements will also have a similar expression pattern and will tend to cluster together. Of course, it is also possible, and quite common, for genes that have different regulatory elements to be expressed coordinately under certain circumstances.

[0093] It is anticipated that the analysis of more than one gene cluster will be useful not only for diagnosing transplant rejection but also for determining appropriate medical interventions. For example, chronic allograft nephropathy is a general description for a disorder that has many variations and many different optimal treatment strategies. In one embodiment, the invention provides a method for simultaneously identifying graft rejection and determining an appropriate treatment. In general, the invention provides methods comprising measuring representatives of different, informative biomarker genes which can represent gene clusters, that indicate an appropriate treatment protocol.

[0094] Detecting Gene Expression

[0095] In certain aspects of the present invention, the magnitude of expression is determined for one or more biomarker genes in sample obtained from a subject. The sample can comprise cells obtained from the subject, such as from a graft biopsy. Other samples include, but are not limited to fluid samples such as blood, plasma, serum, lymph, CSF, cystic fluid, ascites, urine, stool and bile. The sample may also be obtained from bronchoalveolar lavage fluid, pleural fluid or peritoneal fluid, or any other fluid secreted or excreted by a normally or abnormally functioning allograft, or any other fluid resulting from exudation or transudation through an allograft or in anatomic proximity to an allograft, or any fluid in fluid communication with the allograft.

[0096] Many different methods are known in the art for measuring gene expression. Classical methods include quantitative RT-PCR, Northern blots and ribonuclease protection assays. Certain examples described herein use competitive reverse transcription (RT)-PCR to measure the magnitude of expression of biomarker genes. Such methods may be used to examine expression of subject genes as well as entire gene clusters. However, as the number of genes to be examined increases, the time and expense may become cumbersome.

[0097] Large scale detection methods allow faster, less expensive analysis of the expression levels of many genes simultaneously. Such methods typically involve an ordered array of probes affixed to a solid substrate. Each probe is capable of hybridizing to a different set of nucleic acids. In one method, probes are generated by amplifying or synthesizing a substantial portion of the coding regions of various genes of interest. These genes are then spotted onto a solid support. Then, mRNA samples are obtained, converted to cDNA, amplified and labeled (usually with a fluorescence label). The labeled cDNAs are then applied to the array, and cDNAs hybridize to their respective probes in a manner that is linearly related to their concentration. Detection of the label allows measurement of the amount of each cDNA adhered to the array. Many methods for performing such DNA array experiments are well known in the art. Exemplary methods are described below but are not intended to be limiting.

[0098] Microarrays are known in the art and consist of a surface to which probes that correspond in sequence to gene products (e.g., cDNAs, mRNAs, oligonucleotides) are bound at known positions. In one embodiment, the microarray is an

array (i.e., a matrix) in which each position represents a discrete binding site for a product encoded by a gene (e.g., a protein or RNA), and in which binding sites are present for products of most or almost all of the genes in the organism's genome. In a preferred embodiment, the "binding site" (hereinafter, "site") is a nucleic acid or nucleic acid derivative to which a particular cognate cDNA can specifically hybridize. The nucleic acid or derivative of the binding site can be, e.g., a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

[0099] Usually the microarray will have binding sites corresponding to at least 100 genes and more preferably, 500, 1000, 4000 or more. In certain embodiments, the most preferred arrays will have about 98-100% of the genes of a particular organism represented. In other embodiments, customized microarrays that have binding sites corresponding to fewer, specifically selected genes can be used. In certain embodiments, customized microarrays comprise binding sites for fewer than 4000, fewer than 1000, fewer than 200 or fewer than 50 genes, and comprise binding sites for at least 2, preferably at least 3, 4, 5 or more genes of any of the biomarkers of Table 4, Table 5, Table 6, Table 7, and Table 8. Preferably, the microarray has binding sites for genes relevant to testing and confirming a biological network model of interest.

[0100] The nucleic acids to be contacted with the microarray may be prepared in a variety of ways. Methods for preparing total and poly(A)+ RNA are well known and are described generally in Sambrook et al., supra. Labeled cDNA is prepared from mRNA by oligo dT-primed or random-primed reverse transcription, both of which are well known in the art (see e.g., Klug and Berger, 1987, Methods Enzymol. 152:316-325). Reverse transcription may be carried out in the presence of a dNTP conjugated to a detectable label, most preferably a fluorescently labeled dNTP. Alternatively, isolated mRNA can be converted to labeled antisense RNA synthesized by in vitro transcription of double-stranded cDNA in the presence of labeled dNTPs (Lockhart et al., 1996, Nature Biotech. 14:1675). The cDNAs or RNAs can be synthesized in the absence of detectable label and may be labeled subsequently, e.g., by incorporating biotinylated dNTPs or rNTP, or some similar means (e.g., photo-cross-linking a psoralen derivative of biotin to RNAs), followed by addition of labeled streptavidin (e.g., phycoerythrin-conjugated streptavidin) or the equivalent.

[0101] When fluorescent labels are used, many suitable fluorophores are known, including fluorescein, lissamine, phycoerythrin, rhodamine (Perkin Elmer Cetus), Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, FluorX (Amersham) and others (see, e.g., Kricka, 1992, Academic Press San Diego, Calif.).

[0102] In another embodiment, a label other than a fluorescent label is used. For example, a radioactive label, or a pair of radioactive labels with distinct emission spectra, can be used (see Zhao et al., 1995, Gene 156:207; Pietu et al., 1996, Genome Res. 6:492). However, use of radioisotopes is a less-preferred embodiment.

[0103] Nucleic acid hybridization and wash conditions are chosen so that the population of labeled nucleic acids will specifically hybridize to appropriate, complementary nucleic acids affixed to the matrix. As used herein, one polynucleotide sequence is considered complementary to another when, if the shorter of the polynucleotides is less than or equal to 25 bases, there are no mismatches using standard base-pairing rules or, if the shorter of the polynucleotides is longer than 25 bases, there is no more than a 5% mismatch.

[0104] Optimal hybridization conditions will depend on the length (e.g., oligomer versus polynucleotide greater than 200 bases) and type (e.g., RNA, DNA, PNA) of labeled nucleic acids and immobilized polynucleotide or oligonucleotide. General parameters for specific (i.e., stringent) hybridization conditions for nucleic acids are described in Sambrook et al., supra, and in Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York, which is incorporated in its entirety for all purposes. Non-specific binding of the labeled nucleic acids to the array can be decreased by treating the array with a large quantity of non-specific DNA—a so-called "blocking" step.

[0105] When fluorescently labeled probes are used, the fluorescence emissions at each site of a transcript array can be, preferably, detected by scanning confocal laser microscopy. When two fluorophores are used, a separate scan, using the appropriate excitation line, is carried out for each of the two fluorophores used. Alternatively, a laser can be used that allows simultaneous specimen illumination at wavelengths specific to the two fluorophores and emissions from the two fluorophores can be analyzed simultaneously (see Shalon et al., 1996, Genome Research 6:639-645). In a preferred embodiment, the arrays are scanned with a laser fluorescent scanner with a computer controlled X-Y stage and a microscope objective. Sequential excitation of the two fluorophores is achieved with a multi-line, mixed gas laser and the emitted light is split by wavelength and detected with two photomultiplier tubes. Fluorescence laser scanning devices are described in Schena et al., 1996, Genome Res. 6:639-645 and in other references cited herein. Alternatively, the fiber-optic bundle described by Ferguson et al., 1996, Nature Biotech. 14:1681-1684, may be used to monitor mRNA abundance levels at a large number of sites simultaneously. Fluorescent microarray scanners are commercially available from Affymetrix, Packard BioChip Technologies, BioRobotics and many other suppliers.

[0106] Signals are recorded, quantitated and analyzed using a variety of computer software. In one embodiment the scanned image is despeckled using a graphics program (e.g. Hijaak Graphics Suite) and then analyzed using an image gridding program that creates a spreadsheet of the average hybridization at each wavelength at each site. If necessary, an experimentally determined correction for "cross talk" (or overlap) between the channels for the two fluors may be made. For any particular hybridization site on the transcript array, a ratio of the emission of the two fluorophores is preferably calculated. The ratio is independent of the absolute expression level of the cognate gene, but is useful for genes whose expression is significantly modulated by drug administration, gene deletion, or any other tested event.

[0107] In one embodiment, transcript arrays reflecting the transcriptional state of a cell of interest are made by hybridizing a mixture of two differently labeled sets of cDNAs to the microarray. One cell is a cell of interest while the other is used as a standardizing control. The relative hybridization of each cell's cDNA to the microarray then reflects the relative expression of each gene in the two cells.

[0108] In preferred embodiments, expression levels of genes of a biomarker model in different samples and conditions may be compared using a variety of statistical methods. A variety of statistical methods are available to assess the degree of relatedness in expression patterns of different

genes. The statistical methods may be broken into two related portions: metrics for determining the relatedness of the expression pattern of one or more gene, and clustering methods, for organizing and classifying expression data based on a suitable metric (Sherlock, 2000, Curr. Opin. Immunol. 12:201-205; Butte et al., 2000, Pacific Symposium on Biocomputing, Hawaii, World Scientific, p. 418-29).

[0109] In one embodiment, Pearson correlation may be used as a metric. In brief, for a given gene, each data point of gene expression level defines a vector describing the deviation of the gene expression from the overall mean of gene expression level for that gene across all conditions. Each gene's expression pattern can then be viewed as a series of positive and negative vectors. A Pearson correlation coefficient can then be calculated by comparing the vectors of each gene to each other. An example of such a method is described in Eisen et al. (1998, supra). Pearson correlation coefficients account for the direction of the vectors, but not the magnitudes.

[0110] In another embodiment, Euclidean distance measurements may be used as a metric. In these methods, vectors are calculated for each gene in each condition and compared on the basis of the absolute distance in multidimensional space between the points described by the vectors for the gene. In another embodiment, both Euclidean distance and Correlation coefficient were used in the clustering.

[0111] In a further embodiment, the relatedness of gene expression patterns may be determined by entropic calculations (Butte et al. 2000, supra). Entropy is calculated for each gene's expression pattern. The calculated entropy for two genes is then compared to determine the mutual information. Mutual information is calculated by subtracting the entropy of the joint gene expression patterns from the entropy calculated for each gene individually. The more different two gene expression patterns are, the higher the joint entropy will be and the lower the calculated mutual information. Therefore, high mutual information indicates a non-random relatedness between the two expression patterns.

[0112] In another embodiment, agglomerative clustering methods may be used to identify gene clusters. In one embodiment, Pearson correlation coefficients or Euclidean metrics are determined for each gene and then used as a basis for forming a dendrogram. In one example, genes were scanned for pairs of genes with the closest correlation coefficient. These genes are then placed on two branches of a dendrogram connected by a node, with the distance between the depth of the branches proportional to the degree of correlation. This process continues, progressively adding branches to the tree. Ultimately a tree is formed in which genes connected by short branches represent clusters, while genes connected by longer branches represent genes that are not clustered together. The points in multidimensional space by Euclidean metrics may also be used to generate dendrograms.

[0113] In yet another embodiment, divisive clustering methods may be used. For example, vectors are assigned to each gene's expression pattern, and two random vectors are generated. Each gene is then assigned to one of the two random vectors on the basis of probability of matching that vector. The random vectors are iteratively recalculated to generate two centroids that split the genes into two groups. This split forms the major branch at the bottom of a dendrogram. Each group is then further split in the same manner, ultimately yielding a fully branched dendrogram.

[0114] In a further embodiment, self-organizing maps (SOM) may be used to generate clusters. In general, the gene expression patterns are plotted in n-dimensional space, using a metric such as the Euclidean metrics described above. A grid of centroids is then placed onto the n-dimensional space and the centroids are allowed to migrate towards clusters of points, representing clusters of gene expression. Finally the centroids represent a gene expression pattern that is a sort of average of a gene cluster. In certain embodiments, SOM may be used to generate centroids, and the genes clustered at each centroid may be further represented by a dendrogram. An exemplary method is described in Tamayo et al, 1999, PNAS 96:2907-12 Once centroids are formed, correlation must be evaluated by one of the methods described supra.

[0115] In another embodiment, PLSDA, OPLS and OSC multivariate analyses may be used as a means of classification. As detailed in Example I, the biomarker models of the invention (e.g., PLSDA, OPLS and OSC models and the genes identified by such models) are useful to classify tissue with latent CAN and/or early CAN.

[0116] In another aspect, the invention provides probe sets. Preferred probe sets are designed to detect expression of one or more genes and provide information about the status of a graft. Preferred probe sets of the invention comprise probes that are useful for the detection of at least two genes belonging to any of the biomarker genes of Table 4, Table 5, Table 6, Table 7, and Table 8. Probe sets of the invention comprise probes useful for the detection of no more than 10,000 gene transcripts, and preferred probe sets will comprise probes useful for the detection of fewer than 4000, fewer than 1000, fewer than 200, fewer than 100, fewer than 90, fewer than 80, fewer than 70, fewer than 60, fewer than 50, fewer than 40, fewer than 30, fewer than 20, fewer than 10 gene transcripts. The probe sets of the invention are targeted at the detection of gene transcripts that are informative about transplant status. Probe sets of the invention may also comprise a large or small number of probes that detect gene transcripts that are not informative about transplant status. In preferred embodiments, probe sets of the invention are affixed to a solid substrate to form an array of probes. It is anticipated that probe sets may also be useful for multiplex PCR. The probes of probe sets may be nucleic acids (e.g., DNA, RNA, chemically modified forms of DNA and RNA), or PNA, or any other polymeric compound capable of specifically interacting with the desired nucleic acid sequences.

[0117] Computer readable media comprising a biomarker (s) of the present invention is also provided. As used herein, "computer readable media" includes a medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. The skilled artisan will readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a biomarker of the present invention.

[0118] As used herein, "recorded" includes a process for storing information on computer readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the biomarkers of the present invention.

[0119] A variety of data processor programs and formats can be used to store the biomarker information of the present invention on computer readable medium. For example, the nucleic acid sequence corresponding to the biomarkers can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. Any number of dataprocessor structuring formats (e.g., text file or database) may be adapted in order to obtain computer readable medium having recorded thereon the biomarkers of the present invention.

[0120] By providing the biomarkers of the invention in computer readable form, one can routinely access the biomarker sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer-readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

[0121] The invention also includes an array comprising a biomarker(s) of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 4700 genes can be simultaneously assayed for expression. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

[0122] In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression per se and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

[0123] In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development and differentiation, disease progression, in vitro processes, such a cellular transformation and senescence, autonomic neural and neurological processes, such as, for example, pain and appetite, and cognitive functions, such as learning or memory.

[0124] The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells. This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

[0125] The array is also useful for ascertaining differential expression patterns of one or more genes in normal and diseased cells. This provides a battery of genes that could serve as a molecular target for diagnosis or therapeutic intervention.

[0126] Proteins

[0127] It is further anticipated that increased levels of certain proteins may also provide diagnostic information about transplants. In certain embodiments, one or more proteins encoded by genes of Table 4, Table 5, Table 6, Table 7, and Table 8 may be detected, and elevated or decreased protein levels may be used to predict graft rejection. In a preferred embodiment, protein levels are detected in a post-transplant fluid sample, and in a particularly preferred embodiment, the fluid sample is peripheral blood or urine. In another preferred embodiment, protein levels are detected in a graft biopsy.

[0128] In view of this specification, methods for detecting proteins are well known in the art. Examples of such methods include Western blotting, enzyme-linked immunosorbent assays (ELISAs), one- and two-dimensional electrophoresis, mass spectroscopy and detection of enzymatic activity. Suitable antibodies may include polyclonal, monoclonal, fragments (such as Fab fragments), single chain antibodies and other forms of specific binding molecules.

[0129] Predictive Medicine

[0130] The present invention pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenetics and monitoring clinical trials are used for prognostic (predictive) purposes to thereby diagnose and treat a subject prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining biomarker protein and/or nucleic acid expression from a sample (e.g., blood, serum, cells, tissue) to thereby determine whether a subject is likely to reject a transplant.

[0131] Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of biomarker in clinical trials as described in further detail in the following sections.

[0132] An exemplary method for detecting the presence or absence of biomarker protein or genes of the invention in a sample involves obtaining a sample from a test subject and contacting the sample with a compound or an agent capable of detecting the protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes the biomarker protein such that the presence of the biomarker protein or nucleic acid is detected in the sample. A preferred agent for detecting mRNA or genomic DNA corresponding to a biomarker gene or protein of the invention is a labeled nucleic acid probe capable of hybridizing to a mRNA or genomic DNA of the invention. Suitable probes for use in the diagnostic assays of the invention are described herein.

[0133] A preferred agent for detecting biomarker protein is an antibody capable of binding to biomarker protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (eg., Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of

the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect biomarker mRNA, protein, or genomic DNA in a sample in vitro as well as in vivo. For example, in vitro techniques for detection of biomarker mRNA include Northern hybridizations and in situ hybridizations. In vitro techniques for detection of biomarker protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of biomarker genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of biomarker protein include introducing, into a subject, a labeled anti-biomarker antibody. For example, the antibody can be labeled with a radioactive biomarker whose presence and location in a subject can be detected by standard imaging techniques.

[0134] In one embodiment, the sample contains protein molecules from the test subject. Alternatively, the sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred sample is a serum sample isolated by conventional means from a subject.

[0135] The methods further involve obtaining a control sample (e.g., biopsies from non transplanted healthy kidney or from transplanted healthy kidney showing no sign of rejection) from a control subject, contacting the control sample with a compound or agent capable of detecting biomarker protein, mRNA, or genomic DNA, such that the presence of biomarker protein, mRNA or genomic DNA is detected in the sample, and comparing the presence of biomarker protein, mRNA or genomic DNA in the control sample with the presence of biomarker protein, mRNA or genomic DNA in the test sample.

[0136] The invention also encompasses kits for detecting the presence of biomarker in a sample. For example, the kit can comprise a labeled compound or agent capable of detecting biomarker protein or mRNA in a sample; means for determining the amount of biomarker in the sample; and means for comparing the amount of biomarker in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect biomarker protein or nucleic acid.

[0137] The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant biomarker expression or activity. As used herein, the term "aberrant" includes a biomarker expression or activity which deviates from the wild type biomarker expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant biomarker expression or activity is intended to include the cases in which a mutation in the biomarker gene causes the biomarker gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional biomarker protein or a protein which does not function in a wild-type fashion, e.g., a protein which does not interact with a biomarker ligand or one which interacts with a non-biomarker protein ligand.

[0138] Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to reduce the risk of rejection, e.g., cyclospsorin. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with increased gene expression or activity of the combination of genes in Table 4, Table 5, Table 6, Table 7, and Table 8.

[0139] Monitoring the influence of agents (e.g., drugs) on the expression or activity of a genes can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase gene expression, protein levels, or up-regulate activity, can be monitored in clinical trials of subjects exhibiting by examining the molecular signature and any changes in the molecular signature during treatment with an agent.

[0140] For example, and not by way of limitation, genes and their encoded proteins that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates gene activity can be identified. In a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of genes implicated associated with rejection. The levels of gene expression (e.g., a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein. In this way, the gene expression pattern can serve as a molecular signature, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the subject with the agent.

[0141] In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a gene or combination of genes, the protein encoded by the genes, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the biomarker protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the biomarker protein, mRNA, or genomic DNA in the pre-administration sample with the a gene or combination of genes, the protein encoded by the genes, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to decrease the expression or activity of the genes to lower levels, i.e., to increase the effectiveness of the agent to protect against transplant rejection. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of biomarker to lower levels than detected, i.e., to decrease the effectiveness of the agent e.g., to avoid toxicity. According to such an embodiment, gene expression or activ-

ity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

[0142] The present invention provides for both prophylactic and therapeutic methods for preventing transplant rejection. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, includes the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a subject's genes determine his or her response to a drug (e.g., a subject's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring a subject's prophylactic or therapeutic treatment with either the biomarker molecules of the present invention or biomarker modulators according to that subject's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to subjects who will most benefit from the treatment and to avoid treatment of subjects who will experience toxic drug-related side effects.

[0143] In one aspect, the invention provides a method for preventing transplant rejection in a subject, associated with increased biomarker expression or activity, by administering to the subject a compound or agent which modulates biomarker expression. Examples of such compounds or agents are e.g., compounds or agents having immunosuppressive properties, such as those used in transplantation (e.g., a calcineurin inhibitor, cyclosporin A or FK 506); a mTOR inhibitor (e.g., rapamycin, 40-O -(2-hydroxyethyl)-rapamycin, CC1779, ABT578, AP23573, biolimus-7 or biolimus-9); an ascomycin having immuno-suppressive properties (e.g., ABT-281, ASM981, etc.); corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid or salt; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; a PKC inhibitor (e.g., as disclosed in WO 02/38561 or WO 03/82859, the compound of Example 56 or 70); a JAK3 kinase inhibitor (e.g., N-benzyl-3,4dihydroxy-benzylidene-cyanoacetamide a-cyano-3,4dihydroxy)-]N-benzylcinnamamide (Tyrphostin AG 490), prodigiosin 25-C (PNU156804), [4-(4'-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline] (WHI-P131), [4-(3'-bromo-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline] (WHI-P154), [4-(3',5'-dibromo-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline] WHI-P97, KRX-211, 3-{(3R,4R)4-methyl-3-[methyl-(7H-pyrrolo[2,3-d]pyrimidin4-yl)-amino]-piperidin-1-yl)-3-oxo-propionitrile, in free form or in a pharmaceutically acceptable salt form, e.g., mono-citrate (also called CP-690,550), or a compound as disclosed in WO 04/052359 or WO 05/066156); a S1P receptor agonist or modulator (e.g., FTY720 optionally phosphorylated or an analog thereof, e.g., 2-amino-2-[4-(3-benzyloxyphenylthio)-2-chlorophenyl]ethyl-1,3-propanediol optionally phosphorylated or 1-{4-[1-(4-cyclohexyl-3-trifluoromethyl-benzyloxyimino)-ethyl]-2-ethyl-benzyl}-azetidine-3-carboxylic acid or its pharmaceutically acceptable salts); immunosuppressive monoclonal antibodies (e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40, CD45, CD52, CD58, CD80, CD86 or their ligands); other immunomodulatory compounds (e.g., a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g., an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g., CTLA41 g (for ex. designated ATCC 68629) or a mutant thereof, e.g., LEA29Y); adhesion molecule inhibitors (e.g., LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM4 antagonists or VLA-4 antagonists). These compounds or agents may also be used in combination.

[0144] Another aspect of the invention pertains to methods of modulating biomarker protein expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a biomarker protein or agent that modulates one or more of the activities of a biomarker protein activity associated with the cell. An agent that modulates biomarker protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a biomarker protein (e.g., a biomarker protein substrate), a biomarker protein antibody, a biomarker protein agonist or antagonist, a peptidomimetic of a biomarker protein agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more biomarker protein activities. Examples of such stimulatory agents include active biomarker protein and a nucleic acid molecule encoding biomarker protein that has been introduced into the cell. In another embodiment, the agent inhibits one or more biomarker protein activities. Examples of such inhibitory agents include antisense biomarker protein nucleic acid molecules, anti-biomarker protein antibodies, and biomarker protein inhibitors. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating a subject afflicted with a disease or disorder characterized by aberrant expression or activity of a biomarker protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) biomarker protein expression or activity. In another embodiment, the method involves administering a biomarker protein or nucleic acid molecule as therapy to compensate for reduced or aberrant biomarker protein expression or activity.

[0145] Stimulation of biomarker protein activity is desirable in situations in which biomarker protein is abnormally down-regulated and/or in which increased biomarker protein activity is likely to have a beneficial effect. For example, stimulation of biomarker protein activity is desirable in situations in which a biomarker is down-regulated and/or in which increased biomarker protein activity is likely to have a beneficial effect. Likewise, inhibition of biomarker protein activity is desirable in situations in which biomarker protein is abnormally up-regulated and/or in which decreased biomarker protein activity is likely to have a beneficial effect.

[0146] The biomarker protein and nucleic acid molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on biomarker protein activity (e.g., biomarker gene expression), as identified by a screening assay described herein, can be administered to subjects to treat (prophylactically or therapeutically) biomarker-associated disorders (e.g., prostate cancer) associated with aberrant biomarker protein activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between a subject's genotype and that subject's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying

knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a biomarker molecule or biomarker modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a biomarker molecule or biomarker modulator.

[0147] One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related biomarkers (e.g., a "bi-allelic" gene biomarker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of subjects taking part in a Phase II/III drug trial to identify biomarkers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, subjects can be grouped into genetic categories depending on a particular pattern of SNPs in their subject genome. In such a manner, treatment regimens can be tailored to groups of genetically similar subjects, taking into account traits that may be common among such genetically similar subjects.

[0148] Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (e.g., a biomarker protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

[0149] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of a subject. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a biomarker molecule or biomarker modulator, such as a modulator identified by one of the exemplary screening assays described herein.

[0150] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, are incorporated herein by reference.

Examples

Example 1

Identifying Biomarkers Predictive of Chronic/Sclerosing Allograft Nephropathy

[0151] 1 Introduction and Purpose of the Studies

[0152] Histopathological evaluation of biopsy tissue is the gold standard of diagnosis of chronic renal allograft nephropathy (CAN), while prediction of the onset of CAN is currently impossible. Molecular diagnostics, like gene expression profiling, may aid to further refine the BANFF 97 disease classification (Racusen L C, et al., Kidney Int. 55(2):713-23 (1999)), and may also be employed as predictive or early diagnostic biomarkers when applied at early time points after transplantation when by other means graft dysfunction is not yet detectable. In the present study, gene expression profiling was applied to biopsy RNA extracted from serial renal protocol biopsies from patients which showed no overt deterioration of graft function within about at least one year after transplantation, and patients which had overt chronic allograft nephropathy (CAN) as diagnosed at the week 24 biopsy, but not at week 06 or week 12 biopsy (see FIG. **1**). Specifically, to identify genomic biomarkers of chronic/sclerosing allograft nephropathy which, based on mRNA expression levels derived from kidney biopsies of renal transplant patients, allows for early detection/diagnosis (prediction) of future CAN at a time point when histopathological investigations of the same kidneys fail to diagnose CAN. Three analysis approaches were followed: (1) identification of genomic biomarker for early diagnosis (prediction) at week 06 post TX (18 weeks before histopathological diagnosis of CAN); (2) identification of genomic biomarker for early diagnosis (prediction) at week 12 post TX (12 weeks before histopathological diagnosis of CAN); and (3) identification of genomic biomarker for early diagnosis (prediction) at week 06 post TX (18 weeks before histopathological diagnosis of CAN), or week 12 post TX (12 weeks before histopathological diagnosis of CAN), or the diagnosis of CAN versus N.

[0153] 1.1 Patient Stratification

[0154] Kidney biopsy samples from renal transplant patients at all three timepoints were analysed. In this study, the dataset encompassed 67 biopsy samples or subsets of these. The sample distribution across the different grades of chronic/sclerosing allograft nephropathy (CAN) is shown below in Table 3A.

TABLE 3A

Number of samples with different grade of disease recruited from two clinical centers

Grade of CAN	Patient Number from MHH
0: stable graft	33
0: Week 06: latent CAN	8
0: Week 12: latent CAN	8
I: mild	18
Total	67

[0155] The "normal" samples were stratified into the following groups as follows:

[0156] Source: patients with stable renal allograft function throughout the observation period (number of biopsy samples: 36)

[0157] Source: patients with declining renal allograft function, as diagnosed on week 24 biopsy;

[0158] Week 6 post-TX (18 weeks before histopathological evidence of CAN): 8 samples

[0159] Week 12 post-TX (12 weeks before histopathological evidence of CAN): 8 samples

[0160] The "CAN grade I" samples were obtained from patients at any time after transplantation.

TABLE 3B

Comparison of data from patients without clinical signs of rejection or nephropathy (N = 12) and patients with overt CAN at week 24 (N = 8).

CRM001-MHH # of patients	Diagnoses week 06	week 12	week 24
12	no	no	no
1	no	AR 2 = IA	no
1	no	AR 1 = I borderline	no
1	AR 1 = borderline	CAN 1 = I (mild)	no
1	AR 1 = I borderline	AR 1 = I borderline	no
8	no	no	CAN 1 = I (mild)
2	no	CAN 1 = I (mild)	CAN 1 = I (mild)
1	no	AR 1 = I borderline	CAN 1 = I (mild)
1	no	AR 1 = II borderline CR 1 = I (mild)	CAN 1 = I (mild)
1	CAN 1 = I (mild)	CAN 1 = I (mild)	CAN 1 = I (mild)
1	AR 1 = I borderline	no	CAN 1 = I (mild)
1	no	AR 1 = borderline	AR 2 = IA
1	no	CAN 1 = I (mild)	AR 2 = IA
1	no	AR 1 = I borderline	AR 3 = IB!
1	no	no	AR 2 = IA CR 1 = I (mild)
1	no	no	AR 1 = IB CR 1 = I (mild)
1	no	CAN 1 = I (mild)	AR 1 = borderline CR 1 = I (mild)
1	no	CAN 2 = II (moderate)	AR 2 = IA CR 1 = I (mild)

[0161] 2 Sample Processing

[0162] 2.1 RNA Extraction and Purification

[0163] Total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol, Invitrogen Life Technologies) from each frozen tissue section and the total RNA was then purified on an affinity resin (RNeasy, Qiagen) according to the manufacturer's instructions and quantified. Total RNA was quantified by the absorbance at λ=260 nm (A_{260nm}), and the purity was estimated by the ratio $A_{260\ nm}/A_{280nm}$. Integrity of the RNA molecules was confirmed by non-denaturing agarose gel electrophoresis. RNA was stored at approximately −80° C. until analysis.

[0164] 2.2 GeneChip Experiment

[0165] All DNA microarray experiments were conducted in the Genomics Factory EU, Basel, Switzerland, following the instructions of the manufacturer of the GeneChip system (Affymetrix, Inc., San Diego, Calif., USA) and as previously described (Lockhart D J, et al., Nat Biotechnol. 14(13):1675-80 (1996)).

[0166] Total RNA was obtained from snap frozen kidney samples by acid guanidinium isothiocyanate-phenol-chloroform extraction (Chomczynski P, et al., Anal Biochem 162 (1):156-9 (1987)) using Trizol (Invitrogen Life Technologies, San Diego, Calif., USA) and was purified on an affinity resin column (RNeasy; Qiagen, Hilden, Germany) according to the manufacturer's instructions. Human HG_133_plus2_target arrays [Affymetrix] were used, comprising more than 54,000 probe sets, analyzing over 35,000 transcripts and variants from over 28,000 well-substantiated human genes. One GeneChip was used per tissue, per animal. The resultant image files (.dat files) were processed using the Microarray Analysis Suite 5 (MAS5) software (Affymetrix). Tab-delimited files containing data regarding signal intensity (Signal) and categorical expression level measurement (Absolute Call) were obtained. Raw data were converted to expression levels using a "target intensity" of 150. The data were checked for quality prior to uploading to an electronic database.

[0167] 2.3 Data Analysis

[0168] Data analysis was performed using Silicon Genetics software package GeneSpring version 7.2 and with SIMCA-P+ (version 11) by Umetrics AB, Sweden.

[0169] 2.3.1 Filtering, Interpretation

[0170] Various filtering and clustering tools in these software packages were used to explore the datasets and identify transcript level changes that inform on altered cellular and tissue functions and that can be used to establish working hypotheses on the mode of action of the compound.

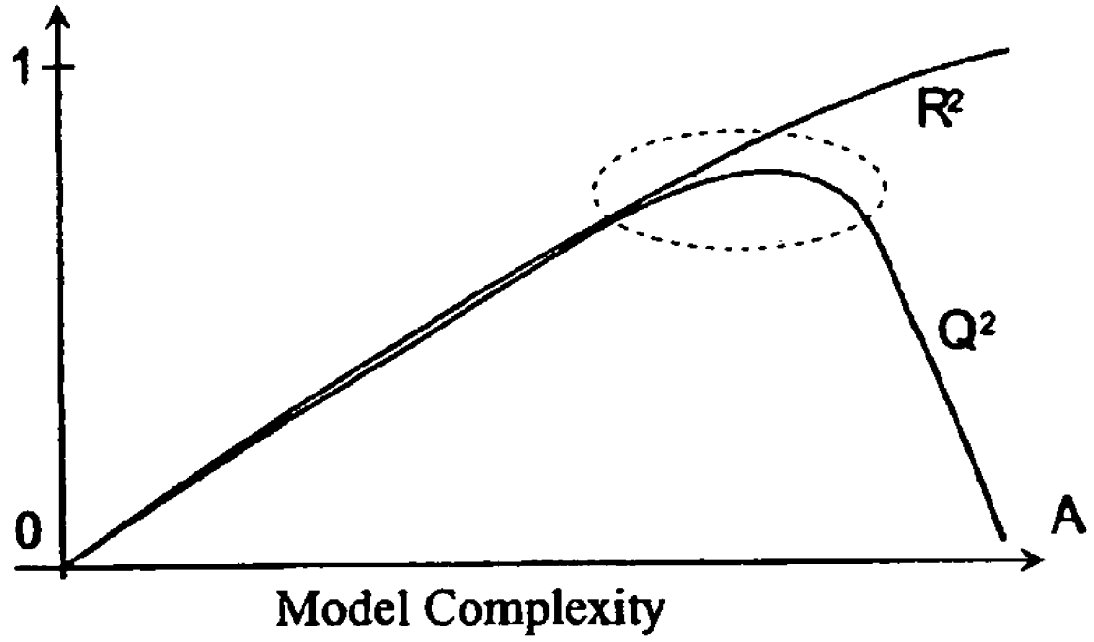
[0171] To account for experimental microarray-wide variations in intensity, all measurements on each array were normalized by dividing them by the 50th percentile of that array. Furthermore, the expression values for each gene were normalized by dividing them by the median expression value for that gene in the control group.

[0172] For the identification of the various biomarkers different filters were applied, which are described separately for each biomarker. The information content of these data, which

is a conjunction of numerical changes and biological information was evaluated by comparing the data to various databases and scientific literature. Several databases were used to explore biological relevance of the datasets, e.g., PubMed (http://www.ncbi.nlm.nih.gov), NIH David (http://david.niaid.nih.gov), Affymetrix (https://www.affymetrix.com), as well internal databases. The value of that relationship was assessed by the analyst, and any hypothesis generated from this analysis would need further validation with other analytical and experimental techniques.

[0173] 2.3.2 Predictive Modelling and Validation Techniques

[0174] The challenge of minimizing the trade off between goodness of fit (R2) and goodness of prediction (Q2) was addressed.


1
0
R²
Q²
A
Model Complexity

[0175] Normalized expression values were log-transformed and Pareto scaled. For some of the predictive models, the data underwent orthogonal signal correction. Partial Least Squares (PLS) was employed as supervised learning algorithms.

[0176] 2.3.3 Supervised Learning by Partial Least Squares

[0177] Partial Least Squares (PLS) is one of the methods of choice when the issue is the prediction of a variable and there exist a very large number of correlated predictors. It is probably one of the best statistical approaches for prediction when there is multicollineality and a much larger number of variables than observations.

[0178] The goal of PLS regression is to provide a dimension reduction strategy in a situation where we want to relate a set of response variables Y to a set of predictor variables X. We looked for orthogonal X-components $t_h=Xw_h^*$ and Y-components $u_h=Yc_h$ maximising the covariance between t_h and u_h. It was a compromise between the principal component analyses of X and Y and the canonical correlation analysis of X and Y. Note that canonical correlation analysis or multivariate regression was not directly applicable because there are many more predictors (cDNA clones) than observations; in addition, the high multicollineality observed with microarray data causes a poor performance of the multivariate regression and of canonical analysis even if a subset of expression levels were selected. The PLS methodology, in contrast, can be applied even when there are many more predictor variables than observations, as is the case with microarray data (Pérez-Encisol M, et al, Human Genetics 112(5-6):581-92 (2003)). The particular case of PLS-DA is a PLS regression where Y is a set of binary variables describing the categories of a categorical variable on X; i.e., the number dependent, or response, variables is equal to the number of categories. Alternative discrimination strategies are found in Nguyen and Rocke (Nguyen D V, et al, Bioinformatics 18:39-50 (2002)). For each response variable, y_k, a regression model on the X-components is written:

$$y_k = \sum_{h=1}^{m} (Xw_h^*)c_h + e = XW^*e + e,$$

[0179] where w_h^* is a p dimension vector containing the weights given to each original variable in the k-th component, and c_h is the regression coefficient of y_k on h-th X-component variable. We used the algorithm developed by Wold et al. (Wold et al., The multivariate calibration problem in chemistry solved by the PLS method. In: Ruhe A, Kagstrom B (eds) Proc Conf Matrix Pencils. Springer, Heidelberg, pp 286-293 (1983)) that allows for missing values. A fundamental requirement for PLS to yield meaningful answers is some preliminary variable selection. We did this by selecting the variables on the basis of the VIP for each variable. The VIP is a popular measure in the PLS literature and is defined for variable j as:

$$VIP_j = \left\{ p \sum_{h=1}^{m} \sum_{k} R^2(y_k, t_k) w_{hj}^2 \Big/ \sum_{h=1}^{w} \sum_{k} R^2(y_k, t_k) \right\}^{1/2},$$

[0180] (Eriksson L, et al., Umetrics, Umea (1999); (Tenenhaus M, La régression PLS. Editions Technip, Paris (1998)) for each j-th predictor variable J=1, p, where $R^2(a,b)$ stands for the squared correlation between items in vector a and b, and $t_h=X_{h-1}w_h$, where X_{h-1} is the residual matrix in the regression of X on components $t_1, \ldots t_{h-1}$ and w_h is a vector of norm 1 (in the PLS regression algorithm t_h is build with this normalisation constraint). Note that w_{hj} measures the contribution of each variable j to the h-th PLS component. Thus, VIP_j quantifies the influence on the response of each variable summed over all components and categorical responses (for more than two categories in Y), relative to the total sum of squares of the model; this makes the VIP an intuitively appealing measure of the global effect of each cDNA clone. The VIP has also the property of

$$\sum_{j=1}^{p} VIF\textcircled{?} = p.$$

? indicates text missing or illegible when filed

[0181] In this work, a first analysis was carried out with all variables (cDNA levels) and the VIP was assessed for each variable. The number of PLS components was selected if a new component satisfied the Q^2 criterion; i.e.,

$$Q_h^2 = 1 - \mathrm{PRESS}_h / \mathrm{RESS}_{h-1} \geqq 0.05,$$

[0182] where PRESS_h is the predicted sum of squares of a model containing h components, and RESS_{h-1} is the residual sum of squares of a model containing h−1 components. PRESS is computed by cross validation,

$$PRESS_h = \sum_{i=1}^{n} (y_{h-1,i} - \hat{y}_{h-1,-i})^2,$$

[0183] with $y_{h-1,i}$ being the residual of observation i when h−1 components are fitted, and ýh−1−1 is the predicted y_i obtained when the i-th observation is removed. Prediction of a new observation is simply obtained as

$$\hat{y}_i = \sum_{h=1}^{m} (\textcircled{?}\,\textcircled{?})c_h,$$

? indicates text missing or illegible when filed

[0184] where x_i is the vector containing the variable records for the new observation i.

[0185] Model validation was carried out via permutation. Permutation tests are part of the computer intensive procedures that have become very popular in the last years due to their flexibility and to increasing computer power (Good PI, PERMUTATION TESTS: A PRACTICAL GUIDE TO RESAMPLING METHODS FOR TESTING HYPOTHESES. Springer, New York. The principle is very simple, to test the significance of a statistic T in a given sample, the response vector (Y) N times is randomised, T_i, i=1, N is computed for each of the permutation sets, and the distribution of T under the null hypothesis is approximated by the set of T_i values; e.g., the 5% significance threshold will be the 0.05×N largest value of all T_i. In the

present example, the response vector (Y) was permuted 200 times and, redoing the analysis, the values of Q^2 and R^2 were plotted, where

$$Q_2 = 1 - \prod_{k=1}^{m} PRESS_k / RESS_{k-1}$$

[0186] and R^2 is the fraction of the total sums of squares explained by the model. Q^2 is a measurement of the predictive ability of the model, whereas R^2 is related to the model's goodness of fit. Analyses were done with SIMCA-P software (Eriksson L, et al., Umetrics, Umea (1999)).

[0187] 3 Results

[0188] 3.1 Biomarker Week 06 Post Transplantation

[0189] 3.1.1 Strategy

[0190] Gene expression profiles of renal allograft biopsy samples taken at week 06 after renal transplant ("TX") from twelve patients with stable graft function until at least 12 months post TX were compared to eight patients with declining renal graft function and histopathological diagnosis of CAN at week 24. Importantly, at time point week 06, all biopsies in this study were diagnosed as stable.

[0191] 3.1.2 Data Processing

[0192] MAS5 transformed data were normalized to the 50^{th} percentile of each microarray, then normalized on the median of all normal samples from the patients with stable graft function, according to the batch of hybridization (GeneSpring Version 7.2). The gene expression intensity per patient group was calculated as the trimmed mean (Tmean) allowing one outlier sample to the top and one to the low expression range (Windows Excel 2002). Coefficient of variance (CV) was calculated as the sixth of the difference of the 20^{th} and the 80^{th} percentile of the expression range of a group, and expressed as percentage of the Tmean of that group. Only genes with coefficient of variance (CV) smaller than 20% in the group of samples from patients with longterm stable renal allografts were included in the further analysis. These genes were then filtered by the following criteria:

[0193] (1) Tmean >100 in either of the two groups

[0194] (2) p-value of ttest (two-tailed, homoscedastic) <0.05

[0195] (3) fold change between T mean of the two groups >1.2

[0196] This filter resulted in 188 probe sets.

[0197] Normalized data were subjected to predictive modelling and validation techniques (section 2.3.2, 2.3.3) to identify the best model for this dataset.

[0198] 3.1.3 Biomarker Week 06 Post TX ("N2-pre-CAN" vs "N"), Result

[0199] In the present example, 49 probe sets were identified to be sufficient and necessary to predict the membership of each sample to the correct group.

[0200] FIG. **2** is a scatter plot of the Biomarker week 06, PLS-DA model.

[0201] A scatterplot or scatter graph is a graph used in statistics to visually display and compare two sets of related quantitative, or numerical, data by displaying only finitely many points, each having a coordinate on a horizontal and a vertical axis. In FIG. **2** each dot represents a sample of a patient. Relative distance between data points is a measure of relationship/resemblance. The separation of the "N" samples from the "pre-CAN" samples indicates the potency of the algorithm/model to discriminate between the data points with the use of 49 probe sets.

[0202] FIG. **3** is a graph comparing observed versus predicted data for the Biomarker week 06 PLSDA model.

[0203] The prediction of the Y space samples can be plotted as a scatter plot. RMSE (Root mean square error) is the standard deviation of the predicted residuals (error), and is computed as the square root of $(\Sigma\text{-}(obs\text{-}pred)^2/N)$. A small RMSE is a measure for a good fit of a model. The Y-axis of the plot represents the observed classes of the model, the X-axis the predicted classes. A match of Y- and X-values in this plot demonstrates the good fit of the model.

[0204] FIG. **4** shows the Biomarker week 06 PLSDA model: Validation by Response Permutation.

[0205] Validation by response permutation is an internal cross-validation, which creates a training set and a test set of samples. A model is fitted to explain the test set based on the training set and the values for R^2Y (explained variance) and Q^2 (predicted variance) are computed and plotted. By random permutation of the training and test sets, a number of R^2Y/Q^2 are obtained. The validate plot is then created by letting the Y-axis represent the R^2Y/Q^2-values of all models, including the "real" one, and by assigning the X-axis to the correlation coefficients between permuted and original response variables. A regression line is then fitted among the R^2Y points and another one through the Q^2 points. The intercepts of the regression lines are interpretable as measures of "background" R2Y and Q^2 obtained to fit the data. Intercepts around 0.4 and below for R2Y and around 0.05 and below for Q^2 indicate valid models. Since these criteria are met in this model it is an indication of a valid model for the present dataset.

[0206] The combination of biomarker genes that form a molecular signature 6 weeks after tissue transplantation are shown in Table 4. Stable graft should describe the group values of the group of samples from patients which will not develop CAN at any later timepoint and indicates the level of expression of the genes at the "baseline" level.

TABLE 4

Genes of the Biomarker week 06, PLSDA model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
221657_s_at	ankyrin repeat and SOCS box-containing 6	ASB6	BC001719	0.72	127
224489_at	ARF protein	LOC51326	BC006271	1.52	74
213710_s_at	calmodulin 1 (phosphorylase kinase, delta)	CALM1	AL523275	1.53	142

TABLE 4-continued

Genes of the Biomarker week 06, PLSDA model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
1558404_at	CDNA FLJ41173 fis, clone BRACE2042394		BC015390	0.78	174
201183_s_at	chromodomain helicase DNA binding protein 4	CHD4	AI613273	0.74	364
222809_x_at	chromosome 14 open reading frame 136	C14orf136	AA728758	1.38	155
222492_at	chromosome 21 open reading frame 124	C21orf124	AW262867	0.62	169
227188_at	chromosome 21 open reading frame 63	C21orf63	AI744591	1.51	243
224991_at	c-Maf-inducing protein	CMIP	AI819630	0.63	82
223495_at	coiled-coil domain containing 8	CCDC8	AI970823	0.64	351
239860_at	dihydropyrimidinase	DPYS	AI311917	0.66	143
212728_at	discs, large homolog 3 (neuroendocrine-dlg, *Drosophila*)	DLG3	T62872	0.74	113
225167_at	FERM domain containing 4	FRMD4	AW515645	0.63	254
236656_s_at	Full length insert cDNA YI37C01		AW014647	1.34	276
213645_at	gb: AF305057 /DB_XREF = gi: 11094017 /FEA = DNA_1 /CNT = 29 /TID = Hs.180433.1 /TIER = Stack /STK = 12 /UG = Hs.180433 /LL = 55556 /UG_GENE = HSRTSBETA /UG_TITLE = rTS beta protein /DEF = *Homo sapiens* RTS (RTS) gene, complete cds, alternatively spliced		AF305057	1.39	387
231951_at	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	GNAO1	AL512686	1.55	81
203394_s_at	hairy and enhancer of split 1, (*Drosophila*)	HES1	BE973687	0.72	618
241031_at	hypothetical LOC145741		BE218239	0.78	80
223542_at	hypothetical protein DKFZp761C121	DKFZp761C121	AL136560	0.71	74
215063_x_at	hypothetical protein FLJ20331	FLJ20331	AL390149	0.76	136
226485_at	hypothetical protein FLJ20674	FLJ20674	BG547864	0.71	278
230012_at	hypothetical protein FLJ34790	FLJ34790	AW574774	1.39	102
1557207_s_at	hypothetical protein LOC283177	LOC283177	AI743605	0.72	152
225033_at	hypothetical protein LOC286167	LOC286167	AV721528	1.36	160
231424_at	hypothetical protein MGC52019	MGC52019	AV700405	2.08	351
224525_s_at	hypothetical protein PTD004	PTD004	AL136546	1.63	78
209291_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	ID4	AW157094	1.53	1689
228002_at	isopentenyl-diphosphate delta isomerase 2	IDI2	AI814569	1.44	104
231850_x_at	KIAA1712	KIAA1712	AB051499	0.71	104
229095_s_at	LIM and senescent cell antigen-like domains 3		AI797263	1.83	135
229874_x_at	LOC388599 (LOC388599), mRNA		BE865517	0.70	710
213215_at	MRNA full length insert cDNA clone EUROIMAGE 42138		AI910895	1.57	246
226991_at	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2		AA489681	0.68	92

TABLE 4-continued

Genes of the Biomarker week 06, PLSDA model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
203195_s_at	nucleoporin 98 kDa	NUP98	NM_005387	0.78	109
218414_s_at	nudE nuclear distribution gene E homolog 1 (*A. nidulans*)	NDE1	NM_017668	1.89	178
206302_s_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	NUDT4	NM_019094	0.73	934
203118_at	proprotein convertase subtilisin/kexin type 7	PCSK7	NM_004716	0.77	170
203555_at	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	PTPN18	NM_014369	2.39	83
238863_x_at	ring finger protein 135	RNF135	AI524240	0.70	87
215127_s_at	RNA binding motif, single stranded interacting protein 1	RBMS1	AL517946	2.62	2152
207939_x_at	RNA binding protein S1, serine-rich domain	RNPS1	NM_006711	0.63	149
211325_x_at	RPL13-2 pseudogene	LOC283345	U72518	0.73	110
225779_at	solute carrier family 27 (fatty acid transporter), member 4	SLC27A4	AK000722	1.32	85
235579_at	splicing factor, arginine/serine-rich 2, interacting protein	SFRS2IP	AA679858	1.67	122
1316_at	thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)	THRA	X55005mRNA	2.47	115
242536_at	Transcribed sequences		AI522220	2.21	526
244018_at	Transcribed sequences		AW451618	1.44	66
244026_at	Transcribed sequences		BF063657	1.46	71
243514_at	WD repeat and FYVE domain containing 2	WDFY2	AI475902	1.75	70

[0207] In one embodiment, the preferred genes identified at 6 weeks include, but are not limited to, NFAT (Murphy et al., (2002) J. Immunol October 1;169(7):3717-25), Discs large 3, dlg3 (Hanada et al. (2000) Int. J. Cancer May 15;86(4):480-8), and thyroid hormone receptor alpha (Sato et al. Circ Res. (2005) September 16;97(6):550-7. Epub Aug. 11, 2005).

[0208] 3.2 Biomarker Week 12 Post Transplantation

[0209] 3.2.1 Strategy

[0210] Gene expression profiles of renal allograft biopsy samples taken at week 12 after renal TX from twelve patients with stable graft function until at least 12 months post TX were compared to eight patients with declining renal graft function and histopathological diagnosis of CAN at week 24. Importantly, at time point week 12, all biopsies in this study were diagnosed as stable.

[0211] 3.2.2 Data Processing

[0212] MAS5 transformed data were normalized to the 50^{th} percentile of each microarray, then normalized on the median of all normal samples from the patients with stable graft function, according to the batch of hybridization (GeneSpring Version 7.2). The gene expression intensity per patient group was calculated as the trimmed mean (T_{mean}) allowing one outlier sample to the top and one to the low expression range (Windows Excel 2002). Coefficient of variance (CV) was calculated as the sixth of the difference of the 20^{th} and the 80^{th} percentile of the expression range of a group, and expressed as percentage of the T_{mean} of that group. Only genes with coefficient of variance (CV) smaller than 20% in the group of samples from patients with longterm stable renal allografts were included in the further analysis. These genes were then filtered by the following criteria:

[0213] (1) T mean>100 in either of the two groups

[0214] (2) p-value of ttest (two-tailed, homoscedastic) <0.05

[0215] (3) fold change between T mean of the two groups >1.5

[0216] This filter resulted in 664 probe sets. Normalized data were subjected to predictive modelling and validation techniques (section 2.3.2, 2.3.3) to identify the best model for this dataset.

[0217] 3.2.3 Biomarker Week12 Post TX: OPLS Model, Result

[0218] FIG. **5** shows the Biomarker week 12 OPLS model: Scatter plot.

[0219] A scatterplot or scatter graph is a graph used in statistics to visually display and compare two sets of related quantitative, or numerical, data by displaying only finitely many points, each having a coordinate on a horizontal and a vertical axis. In FIG. **5** each dot represents a sample of a patient. Relative distance between data points is a measure of relationship/resemblance. The separation of the "N" samples from the "pre-CAN" samples indicates the potency of the algorithm /model to discriminate between the data points with the use of these probe sets.

[0220] FIG. **6** shows the Biomarker week 12 OPLS model: Validation by Response Permutation.

[0221] Validation by response permutation is an internal cross-validation, which creates a training set and a test set of samples. A model is fitted to explain the test set based on the training set and the values for R^2Y (explained variance) and

Q^2 (predicted variance) are computed and plotted. By random permutation of the training and test sets, a number of R^2Y/Q^2 are obtained. The validate plot is then created by letting the Y-axis represent the R^2Y/Q^2-values of all models, including the "real" one, and by assigning the X-axis to the correlation coefficients between permuted and original response variables. A regression line is then fitted among the R^2Y points and another one through the Q^2 points. The intercepts of the regression lines are interpretable as measures of "background" R2Y and Q^2 obtained to fit the data. Intercepts around 0.4 and below for R2Y and around 0.05 and below for Q^2 indicate valid models. Since these criteria are met in this model it is an indication of a valid model for the present dataset.

[0222] FIG. **7** shows the Biomarker week 12 OPLS model: observed vs predicted.

[0223] The prediction of the Y space samples can be plotted as a scatter plot. RMSE (Root mean square error) is the standard deviation of the predicted residuals (error), and is computed as the square root of $(\Sigma(\text{obs-pred})^2/N)$. A small RMSE is a measure for a good fit of a model. The Y-axis of the plot represents the observed classes of the model, the X-axis the predicted classes. A match of Y- and X-values in this plot demonstrates the good fit of the model.

[0224] The combination of biomarker genes that form a molecular signature 12 weeks after tissue transplantation as determined by OPLS analysis are shown in Table 5.

TABLE 5

Genes of the Biomarker week 12, OPLS model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
201792_at	AE binding protein 1	AEBP1	NM_001129	2.13	212
211712_s_at	annexin A9	ANXA9	BC005830	0.47	190
207367_at	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	ATP12A	NM_001676	0.48	108
233085_s_at	AV734843 cdA *Homo sapiens* cDNA clone cdAAHD10 5', mRNA sequence.	FLJ22833	AV734843	2.13	368
227140_at	CDNA FLJ11041 fis, clone PLACE1004405		AI343467	1.95	105
232090_at	CDNA FLJ11481 fis, clone HEMBA1001803		AI761578	1.89	102
232991_at	CDNA FLJ11613 fis, clone HEMBA1004012		AK021675	1.96	101
1570198_x_at	Clone IMAGE: 5111803, mRNA		BC019872	2.23	131
229218_at	collagen, type I, alpha 2	COL1A2	AA628535	4.04	212
232458_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)		AU146808	0.50	66
201438_at	collagen, type VI, alpha 3	COL6A3	NM_004369	8.84	1146
226237_at	collagen, type VIII, alpha 1	COL8A1	AL359062	2.00	471
227336_at	deltex homolog 1 (*Drosophila*)	DTX1	AW576405	0.42	125
210165_at	deoxyribonuclease I	DNASE1	M55983	0.55	189
220625_s_at	E74-like factor 5 (ets domain transcription factor)	ELF5	AF115403	2.26	405
221870_at	EH-domain containing 2	EHD2	AI417917	1.71	55
227353_at	epidermodysplasia verruciformis 2	EVER2	BE671663	2.42	70
242974_at	frizzled homolog 9 (*Drosophila*)	FZD9	AA446657	2.49	50
211795_s_at	FYN binding protein FYB-120/130)	FYB	AF198052	0.40	89
1560782_at	*Homo sapiens* cDNA clone IMAGE: 5186324, partial cds.		BC035326	2.69	112
242372_s_at	hypothetical protein DKFZp761N1114	DKFZp761N1114	AL542291	2.52	329
222872_x_at	hypothetical protein FLJ22833	FLJ22833	AU157541	1.94	400
224489_at	hypothetical protein LOC284058	LOC51326	BC006271	0.45	94
212768_s_at	isoform 1 match: proteins: Sw: Q07081 Tr: O95362 Tr: Q9Z2Y4 Tr: O95897 Tr: O70624 Sw: Q99972 Sw: Q99784 Sw: Q62609 Tr: Q9TV76 Tr: Q9I9K5 Sw: P01813 Tr: Q9IAK4 Tr: O35429; Human DNA sequence from clone RP11-209J19 on chromosome 13 Contains ESTs, STSs and GSSs. Contains the gene for the GW112 protein with two	GW112	AL390736	2.40	143

TABLE 5-continued

Genes of the Biomarker week 12, OPLS model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
	isoforms (GW112 and KIAA4294), complete sequence.				
201744_s_at	lumican	LUM	NM_002345	2.22	1658
229554_at	lumican	LUM	AI141861	2.05	82
227438_at	lymphocyte alpha-kinase	LAK	AI760166	2.34	55
226841_at	macrophage expressed gene 1	MPEG1	BF590697	2.17	81
212999_x_at	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	AW276186	2.00	101
226210_s_at	maternally expressed 3	MEG3	AI291123	2.43	127
212012_at	Melanoma associated gene	D2S448	BF342851	0.50	428
219666_at	membrane-spanning 4-domains, subfamily A, member 6A	MS4A6A	NM_022349	3.20	157
232113_at	MRNA; cDNA DKFZp564B182 (from clone DKFZp564B182)		N90870	3.00	158
1556183_at	MRNA; cDNA DKFZp686E1246 (from clone DKFZp686E1246)		AK097649	1.93	47
228055_at	napsin B pseudogene	NAP1L	AI763426	0.52	99
229070_at	ne10a12.s1 NCI_CGAP_Co3 *Homo sapiens* cDNA clone IMAGE: 880798 3', mRNA sequence.	C6orf105	AA470369	2.43	210
214111_at	opioid binding protein/cell adhesion molecule-like	OPCML	AF070577	2.67	103
205267_at	POU domain, class 2, associating factor 1	POU2AF1	NM_006235	2.18	39
216834_at	regulator of G-protein signalling 1	RGS1	S59049	1.98	36
218870_at	Rho GTPase activating protein 15	ARHGAP15	NM_018460	2.79	56
237639_at	SRSR846		AI913600	1.92	372
209374_s_at	synonym: MU; *Homo sapiens* immunoglobulin heavy constant mu, mRNA (cDNA clone MGC: 1228 IMAGE: 3544448), complete cds.	IGHM	BC001872	2.07	84
236203_at	te62a03.x1 Soares_NFL_T_GBC_S1 *Homo sapiens* cDNA clone IMAGE: 2091244 3' similar to gb: J02931 TISSUE FACTOR PRECURSOR (HUMAN);, mRNA sequence.		AI377755	2.84	51
203083_at	thrombospondin 2	THBS2	NM_003247	0.42	403
244061_at	Transcribed sequences		AI510829	0.45	32
209960_at	unnamed protein product; HGF (AA 1-728); Human mRNA for hepatocyte growth factor (HGF).	HGF	X16323	2.46	119
202664_at	Wiskott-Aldrich syndrome protein interacting protein	WASPIP	AW058622	2.71	385

[0225] 3.2.4 Biomarker Week12 Post TX ("N1-pre-CAN vs N"): PLSDA Model, Result

[0226] FIG. **8** shows a Biomarker week 12 PLSDA model: Scatter plot. A scatterplot or scatter graph is a graph used in statistics to visually display and compare two sets of related quantitative, or numerical, data by displaying only finitely many points, each having a coordinate on a horizontal and a vertical axis. In FIG. **8** each dot represents a sample of a patient. Relative distance between data points is a measure of relationship/resemblance. The separation of the "N" samples from the "pre-CAN" samples indicates the potency of the algorithm /model to discriminate between the data points with the use of these probe sets.

[0227] FIG. **9** shows the Biomarker week 12 PLSDA model: Validation by Response Permutation.

[0228] Validation by response permutation is an internal cross-validation, which creates a training set and a test set of samples. A model is fitted to explain the test set based on the training set and the values for R^2Y (explained variance) and Q^2 (predicted variance) are computed and plotted. By random

permutation of the training and test sets, a number of R^2Y/Q^2 are obtained. The validate plot is then created by letting the Y-axis represent the R^2Y/Q^2-values of all models, including the "real" one, and by assigning the X-axis to the correlation coefficients between permuted and original response variables. A regression line is then fitted among the R^2Y points and another one through the Q^2 points. The intercepts of the regression lines are interpretable as measures of "background" R2Y and Q^2 obtained to fit the data. Intercepts around 0.4 and below for R2Y and around 0.05 and below for Q^2 indicate valid models. Since these criteria are met in this model it is an indication of a valid model for the present dataset.

[0229] FIG. **10** shows the Biomarker week 12 PLSDA model: observed vs predicted.

[0230] The prediction of the Y space samples can be plotted as a scatter plot. RMSE (Root mean square error) is the standard deviation of the predicted residuals (error), and is computed as the square root of $(\Sigma(obs\text{-}pred)^2/N)$. A small RMSE is a measure for a good fit of a model. The Y-axis of the plot represents the observed classes of the model, the X-axis the predicted classes. A match of Y- and X-values in this plot demonstrates the good fit of the model.

[0231] The combination of biomarker genes that form a molecular signature 12 weeks after tissue transplantation as determined by PLSDA analysis are shown in Table 6.

TABLE 6

Genes of the Biomarker week 12, PLSDA model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
201792_at	AE binding protein 1	AEBP1	NM_001129	8.84	212
242974_at	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	CD47	AA446657	4.04	50
227140_at	CDNA FLJ11041 fis, clone PLACE1004405		AI343467	3.20	105
232090_at	CDNA FLJ11481 fis, clone HEMBA1001803		AI761578	3.00	102
229218_at	collagen, type I, alpha 2	COL1A2	AA628535	2.67	212
232458_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	AU146808	0.47	66
227336_at	deltex homolog 1 (*Drosophila*)	DTX1	AW576405	2.84	125
210165_at	deoxyribonuclease I	DNASE1	M55983	2.42	189
227353_at	epidermodysplasia verruciformis 2	EVER2	BE671663	2.46	70
1560782_at	*Homo sapiens* cDNA clone IMAGE: 5186324, partial cds.	C22orf1; 239AB; FAM1A	BC035326	0.42	112
242372_s_at	hypothetical protein DKFZp761N1114	DKFZp761N1114	AL542291	2.79	329
222872_x_at	hypothetical protein FLJ22833	FLJ22833	AU157541	2.18	400
212768_s_at	isoform 1 match: proteins: Sw: Q07081 Tr: O95362 Tr: Q9Z2Y4 Tr: O95897 Tr: O70624 Sw: Q99972 Sw: Q99784 Sw: Q62609 Tr: Q9TV76 Tr: Q9I9K5 Sw: P01813 Tr: Q9IAK4 Tr: O35429; Human DNA sequence from clone RP11-209J19 on chromosome 13 Contains ESTs, STSs and GSSs. Contains the gene for the GW112 protein with two isoforms (GW112 and KIAA4294), complete sequence.	bA209J19.1	AL390736	2.43	143
229554_at	lumican	LUM	AI141861	2.43	82
227438_at	lymphocyte alpha-kinase	LAK	AI760166	2.52	55
226210_s_at	maternally expressed 3	MEG3	AI291123	2.34	127
205267_at	POU domain, class 2, associating factor 1	POU2AF1	NM_006235	2.23	39
218870_at	Rho GTPase activating protein 15	ARHGAP15	NM_018460	0.45	56
237639_at	SRSR846	UNQ846	AI913600	0.42	372
209374_s_at	synonym: MU; *Homo sapiens* immunoglobulin heavy constant mu, mRNA	IGHM; MU	BC001872	2.22	84

TABLE 6-continued

Genes of the Biomarker week 12, PLSDA model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change	Stable Graft: Raw Expression Value
	(cDNA clone MGC: 1228 IMAGE: 3544448), complete cds.				
236203_at	te62a03.x1 Soares_NFL_T_GBC_S1 *Homo sapiens* cDNA clone IMAGE: 2091244 3' similar to gb: J02931 TISSUE FACTOR PRECURSOR (HUMAN);, mRNA sequence.		AI377755	0.50	51
203083_at	thrombospondin 2	THBS2	NM_003247	1.89	403

[0232] In one embodiment, the preferred genes identified at 12 weeks include, but are not limited to, lumican (Onda et al. Exp. Mol. Pathol. (2002) April;72(2):142-9), Smad3 (Saika et al., Am. J. Pathol. (2004) February;164(2):651-63), AE binding protein 1 (Layne et al. J. Biol. Chem. (1998) June 19;273(25):15654-60), and frizzled-9 (Karasawa et al. (2002) J. Biol. Chem October 4;277(40):37479-86. Epub Jul. 22, 2002.).

[0233] 3.3 Biomarker "Global Analysis": Identification of Genomic Predictive Biomarker Before and at Week 24 After Renal Transplantation

[0234] 3.3.1 Strategy

[0235] Gene expression profiles of serial renal protocol biopsy samples taken at week 12 after renal TX from eight patients with declining renal graft function and histopathological diagnosis of CAN at week 24 were compared to 33 renal biopsy samples from patients with stable allograft function at least until 12 months post TX, and 18 biopsies with histological evidence of CAN grade 1. Classes of samples were defined as:

[0236] N (normal; longterm stable renal allograft): n=33

[0237] Week 06 (biopsy from a healthy patient who develops overt CAN between week 12 and week 24 post TX): n=8

[0238] Week 12 (biopsy from a healthy patient who develops overt CAN between week 12 and week 24 post TX): n=8

[0239] CAN: histopathological evidence of chronic allograft nephropathy: n=18.

[0240] 3.3.2 Data Processing

[0241] MAS5 transformed data were normalized to the 50^{th} percentile of each microarray, then normalized by time point and batch on the median of all normal samples (n=33) from the patients with stable graft function, according to the batch of hybridization (GeneSpring Version 7.2). Only probe sets with raw expression intensity of at least 100 in at least 25% of the samples (n=18) were included in the following analysis (20,549 probe sets).

[0242] These probe sets were subjected to a Fisher's Exact Test to find an association between gene expression changes and class membership. The Find Significant Parameters using an Association Test option performs an association test for each gene, over all parameters and attributes. Both numeric and non-numeric parameters and attributes can be tested.

[0243] In this analysis the groups were defined as described in section 1.1. The test resulted in a list of 578 probe sets with a correlation of <0.0001 with the class membership described in section 1.1. Normalized data were subjected to predictive modelling and validation techniques (section 2.3.2, 2.3.3) to identify the best model for this dataset.

[0244] 3.3.3 Biomarker "Global Analysis"; OSC Model, Result

[0245] FIG. **11** shows the Biomarker global analysis OSC model: Scatter plot. A scatterplot or scatter graph is a graph used in statistics to visually display and compare two sets of related quantitative, or numerical, data by displaying only finitely many points, each having a coordinate on a horizontal and a vertical axis. In FIG. **11** each dot represents a sample of a patient. Relative distance between data points is a measure of relationship/resemblance. The separation of the "N" samples from the "week 06 pre-CAN", "week 12 pre-CAN" and "CAN" samples indicates the potency of the algorithm /model to discriminate between the data points with the use of these probe sets.

[0246] FIG. **12** shows the Biomarker global analysis OSC model: Validation by response permutation. Validation by response permutation is an internal cross-validation, which creates a training set and a test set of samples. A model is fitted to explain the test set based on the training set and the values for R^2Y (explained variance) and Q^2 (predicted variance) are computed and plotted. By random permutation of the training and test sets, a number of R^2Y/Q^2 are obtained. The validate plot is then created by letting the Y-axis represent the R^2Y/Q^2-values of all models, including the "real" one, and by assigning the X-axis to the correlation coefficients between permuted and original response variables. A regression line is then fitted among the R^2Y points and another one through the Q^2 points. The intercepts of the regression lines are interpretable as measures of "background" R2Y and Q^2 obtained to fit the data. Intercepts around 0.4 and below for R2Y and around 0.05 and below for Q^2 indicate valid models. Since these criteria are met in this model it is an indication of a valid model for the present dataset.

[0247] FIG. **13** Biomarker global analysis OSC model: Observed vs. predicted. The prediction of the Y space samples can be plotted as a scatter plot. RMSE (Root mean square error) is the standard deviation of the predicted residuals (error), and is computed as the square root of $(\Sigma(\text{obs-pred})^2/N)$. A small RMSE is a measure for a good fit of a model. The Y-axis of the plot represents the observed classes of the model, the X-axis the predicted classes. A match of Y- and X-values in this plot demonstrates the good fit of the model.

[0248] The combination of biomarker genes that form a molecular signature after tissue transplantation as determined by global data analysis using OSC model are shown in Table 7.

TABLE 7

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
244567_at	602343781F1 NIH_MGC_89 *Homo sapiens* cDNA clone IMAGE: 4453556 5', mRNA sequence.		BG165613	1.51	1.21	1.71	103
244145_at	602371458F1 NIH_MGC_93 *Homo sapiens* cDNA clone IMAGE: 4479327 5', mRNA sequence.		BG260337	1.49	1.58	1.52	102
201660_at	acyl-CoA Synthetase long-chain family member 3	ACSL3	AL525798	1.94	2.28	1.91	876
232175_at	ADP-ribosylation factor 1	ARF1	AI972094	1.43	1.58	1.78	108
232865_at	ALL1 fused gene from 5q31	AF5Q31	N59653	1.55	1.51	1.97	179
236778_at	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, *S. cerevisiae*)	ATRX	AA826176	1.08	1.17	1.87	77
1563792_at	amnionless homolog (mouse)	AMN	AK092824	1.37	1.57	1.81	98
226718_at	amphoterin-induced gene	KIAA1163	AA001423	1.12	1.24	1.37	142
227260_at	ankyrin repeat domain 10	ANKRD10	AV724266	1.32	1.59	1.54	708
230972_at	ankyrin repeat domain 9	ANKRD9	AW194999	1.16	1.33	1.66	656
206993_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B)	ATP5S	NM_015684	1.27	1.53	1.52	119
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	ABCA8	NM_007168	0.81	0.65	0.65	350
233271_at	AU145563 HEMBA1 *Homo sapiens* cDNA clone HEMBA1005133 3', mRNA sequence.		AU145563	1.18	1.95	1.50	143
215204_at	AU147295 MAMMA1 *Homo sapiens* cDNA clone MAMMA1000264 3', mRNA sequence.		AU147295	1.99	2.06	3.37	90
236892_s_at	B1 for mucin	HAB1	BF590528	1.34	1.25	1.45	312
227896_at	BRCA2 and CDKN1A interacting protein	BCCIP	AI373643	1.31	1.27	2.56	223
223679_at	catenin (cadherin-associated protein), beta 1, 88 kDa	CTNNB1	AF130085	1.64	1.73	1.58	146
233019_at	CCR4-NOT transcription complex, subunit 7	CNOT7	AU145061	1.17	1.32	1.59	89
233399_x_at	CDNA clone IMAGE: 30352956, partial cds		AU145662	1.60	1.66	1.95	183
232351_at	CDNA FLJ10150 fis, clone HEMBA1003395		AK022308	1.54	1.76	1.70	152
234074_at	CDNA FLJ10946 fis, clone PLACE1000005		AU155494	1.29	1.15	1.76	99
232544_at	CDNA FLJ11572 fis, clone HEMBA1003373		AU144916	0.89	0.77	0.69	231
232991_at	CDNA FLJ11613 fis, clone HEMBA1004012		AK021675	0.91	0.81	0.79	107
232952_at	CDNA FLJ11942 fis, clone HEMBB1000652		AU146493	0.83	0.75	0.74	83
230791_at	CDNA FLJ12033 fis, clone HEMBB1001899		AU146924	1.37	1.58	1.43	241
233296_x_at	CDNA FLJ12131 fis, clone MAMMA1000254		AU147291	0.89	0.81	0.71	425
233498_at	CDNA FLJ14142 fis, clone MAMMA1002880		AK024204	0.58	0.61	0.68	282
230986_at	CDNA FLJ30065 fis, clone ADRGL2000328		AI821447	0.95	0.83	0.73	96
241941_at	CDNA FLJ31511 fis, clone NT2RI1000035		AA778747	0.94	0.84	0.67	75
1557270_at	CDNA FLJ36375 fis, clone THYMU2008226		AA632049	1.21	1.55	1.72	283
235028_at	CDNA FLJ46440 fis, clone THYMU3016022		BG288330	0.81	0.72	0.49	659
234604_at	CDNA: FLJ21228 fis, clone COL00739		AK024881	0.68	0.69	0.64	62
233824_at	CDNA: FLJ21428 fis, clone COL04203		AK025081	0.91	0.80	0.76	114

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
228143_at	ceruloplasmin (ferroxidase)	CP	AI684991	1.44	5.78	3.93	69
223191_at	chromosome 14 open reading frame 112	C14orf112	AF151037	0.68	0.73	0.58	541
218453_s_at	chromosome 6 open reading frame 35	C6orf35	NM_018452	1.56	2.02	1.59	110
229012_at	chromosome 9 open reading frame 24	C9orf24	AW269443	0.77	0.58	0.41	142
1552455_at	chromosome 9 open reading frame 65	C9orf65	NM_138818	1.23	1.31	1.48	81
225377_at	chromosome 9 open reading frame 86	C9orf86	BE783949	0.81	0.80	0.76	173
239683_at	citrate lyase beta like	CLYBL	AI476268	0.98	1.01	0.67	243
215504_x_at	Clone 25061 mRNA sequence		AF131777	1.04	1.17	1.45	482
243329_at	Clone IMAGE: 121662 mRNA sequence		AI074450	1.33	1.65	1.62	195
231808_at	Clone IMAGE: 5302006, mRNA		AY007106	1.04	1.54	1.44	213
225288_at	collagen, type XXVII, alpha 1	COL27A1	AI949136	1.13	1.37	1.47	304
211025_x_at	cytochrome c oxidase subunit Vb	COX5B	BC006229	1.28	1.14	1.49	1299
1556820_a_at	deleted in lymphocytic leukemia, 2	DLEU2	H48516	1.36	1.37	1.78	67
1556821_x_at	deletcd in lymphocytic leukemia, 2	DLEU2	H48516	1.31	1.33	1.55	100
210165_at	deoxyribonuclease 1	DNASE1	M55983	1.22	1.16	1.55	149
218650_at	DiGeorge syndrome critical region gene 8	DGCR8	NM_022775	1.41	1.56	1.64	167
223763_at	dystrobrevin binding protein 1	DTNBP1	AL136637	1.10	1.16	1.44	82
227353_at	epidermodysplasia verruciformis 2	EVER2	BE671663	1.41	1.59	2.19	85
236520_at	EST384471 MAGE resequences, MAGL *Homo sapiens* cDNA, mRNA sequence.		AW972380	1.25	1.24	1.66	128
214805_at	eukaryotic translation initiation factor 4A, isoform 1	EIF4A1	U79273	1.24	1.25	1.61	153
242029_at	FAD104	FAD104	N32832	0.87	0.75	0.76	96
243649_at	F-box only protein 7	FBXO7	AI678692	0.91	0.75	0.74	71
230389_at	formin binding protein 1	FNBP1	BE046511	0.90	0.85	0.72	188
227163_at	glutathione S-transferase omega 2	GSTO2	AL162742	0.71	0.72	0.67	361
215203_at	golgi autoantigen, golgin subfamily a, 4	GOLGA4	AW438464	1.25	1.44	1.36	109
229255_x_at	golgi SNAP receptor complex member 2	GOSR2	BF593917	0.81	0.77	0.75	142
227085_at	H2A histone family, member V	H2AV	AI823792	0.77	0.69	0.64	234
240405_at	H326	H326	AA707411	0.87	1.16	1.40	61
203394_s_at	hairy and enhancer of split 1, (*Drosophila*)	HES1	BE973687	0.78	0.80	0.70	703
209960_at	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	X16323	1.31	1.54	1.55	118
213359_at	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37 kDa)	HNRPD	W74620	1.47	1.66	1.96	207
215553_x_at	*Homo sapiens* cDNA FLJ14253 fis, clone OVARC1001376.		AK024315	1.03	1.34	1.69	262
233813_at	*Homo sapiens* cDNA: FLJ23247 fis, clone COL03425.		AK026900	1.13	1.20	1.57	76
227298_at	Hypothetical gene supported by AK095117 (LOC401264), mRNA		AI806330	1.63	2.06	1.45	167
237108_x_at	hypothetical protein DKFZp761G0122	DKFZp761G0122	AW611845	0.83	0.82	0.70	276
219074_at	hypothetical protein FLJ10846	FLJ10846	NM_018241	1.41	1.52	1.64	418

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
1557828_a_at	hypothetical protein FLJ21657	FLJ21657	BE675061	0.81	0.69	0.72	148
222872_x_at	hypothetical protein FLJ22833	FLJ22833	AU157541	1.17	1.48	1.40	456
233085_s_at	hypothetical protein FLJ22833	FLJ22833	AV734843	1.21	1.37	1.44	415
229145_at	hypothetical protein LOC119504	LOC119504	AA541762	1.19	1.25	1.39	659
227550_at	hypothetical protein LOC143381	LOC143381	AW242720	1.01	1.07	1.36	222
227415_at	hypothetical protein LOC283508	LOC283508	BF109303	1.59	1.37	1.99	350
232288_at	hypothetical protein LOC283970	LOC283970	AK026209	4.60	6.51	13.54	77
226901_at	hypothetical protein LOC284018	LOC284018	AI214996	0.81	0.86	0.65	342
235482_at	hypothetical protein LOC285002	LOC285002	BE886868	0.82	0.82	0.73	132
227466_at	hypothetical protein LOC285550	LOC285550	BF108695	0.86	0.77	0.74	589
228040_at	hypothetical protein LOC286286	LOC286286	AW294192	1.19	1.40	1.49	468
1569189_at	hypothetical protein MGC29649	MGC29649	AF289605	0.77	0.76	0.67	75
225065_x_at	hypothetical protein MGC40157	MGC40157	AI826279	0.80	0.76	0.75	237
229444_at	hypothetical protein MGC4614	MGC4614	AI051046	0.82	0.73	0.77	198
218750_at	hypothetical protein MGC5306	MGC5306	NM_024116	1.26	1.99	1.55	239
223797_at	hypothetical protein PRO2852	PRO2852	AF130079	0.81	0.74	0.14	169
235756_at	IL2-UM0076-240300-056-G02 UM0076 *Homo sapiens* cDNA, mRNA sequence.		AW802645	1.81	1.97	1.66	75
239842_x_at	IMAGE: 20075 Soares infant brain 1NIB *Homo sapiens* cDNA clone IMAGE: 20075, mRNA sequence.		W18186	0.89	0.80	0.75	190
209374_s_at	immunoglobulin heavy constant mu	IGHM	BC001872	0.83	0.79	0.73	123
242903_at	interferon gamma receptor 1		AI458949	1.56	1.82	2.00	90
229310_at	kelch repeat and BTB (POZ) domain containing 9	KBTBD9	BE465475	0.86	0.84	0.76	175
236368_at	KIAA0368		BF059292	1.40	3.18	1.82	142
216000_at	KIAA0484 protein	KIAA0484	AA732995	1.20	1.26	1.45	74
231956_at	KIAA1618	KIAA1618	AA976354	1.62	2.80	1.80	111
238087_at	kinesin family member 2C	KIF2C	AI587389	0.82	0.83	0.74	92
1555929_s_at	laa10f11.x1 8 5 week embryo anterior tongue 8 5 EAT *Homo sapiens* cDNA 3', mRNA sequence.		BM873997	1.23	1.78	1.84	230
1557360_at	leucine-rich PPR-motif containing	LRPPRC	CA430402	1.33	1.26	1.48	103
1569003_at	likely ortholog of rat vacuole membrane protein 1	VMP1	AL541655	0.85	0.82	0.73	213
223223_at	likely ortholog of yeast ARV1	ARV1	AF321442	1.23	1.37	1.58	520
227438_at	lymphocyte alpha-kinase	LAK	AI760166	0.84	0.76	0.65	63
226841_at	macrophage expressed gene 1	MPEG1	BF590697	1.06	1.62	1.76	87
214048_at	methyl-CpG binding domain protein 4	MBD4	AI913365	1.03	0.96	0.65	89
239001_at	microsomal glutathione S-transferase 1	MGST1	AV705233	1.19	1.33	1.40	62
217980_s_at	mitochondrial ribosomal protein L16	MRPL16	NM_017840	0.82	0.84	0.65	609
231274_s_at	mitochondrial solute carrier protein	MSCP	R92925	0.79	0.81	0.69	193
1558732_at	mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4	AK074900	0.82	0.87	0.70	128

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
223218_s_at	molecule possessing ankyrin repeats induced by lipopolysaccharide (MAIL), homolog of mouse	MAIL	AB037925	0.84	0.75	0.71	708
1563469_at	MRNA; cDNA DKFZp313M0417 (from clone DKFZp313M0417)		AL832681	1.35	1.30	1.38	74
234224_at	MRNA; cDNA DKFZp434O0919 (from clone DKFZp434O0919)		AL137541	0.93	0.79	0.80	79
227576_at	MRNA; cDNA DKFZp686K1098 (from clone DKFZp686K1098)		AW003140	0.99	0.77	0.69	452
228217_s_at	MRNA; cDNA DKFZp686P09209 (from clone DKFZp686P09209)		BF973374	1.02	1.41	1.77	365
210210_at	myelin protein zero-like 1	MPZL1	AF181660	1.24	1.41	1.78	105
233539_at	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D	NAPE-PLD	AK000801	1.15	1.37	1.69	135
202000_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14 kDa	NDUFA6	BC002772	1.20	1.48	1.45	693
218320_s_at	neuronal protein 17.3	P17.3	NM_019056	0.87	0.67	0.68	993
233626_at	neuropilin 1	NRP1	AK024580	1.38	1.39	1.43	53
235985_at	nj45a06.x5 NCI_CGAP_Pr9 *Homo sapiens* cDNA clone IMAGE: 995410 similar to contains Alu repetitive element; contains element TAR1 repetitive element;, mRNA sequence.		AI821477	0.96	0.80	0.73	115
226991_at	nuclear factor of activated T-cells, cytoplsamic, calcineurin-dependent 2		AA489681	1.38	1.73	1.87	88
206302_s_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	NUDT4	NM_019094	1.29	1.35	1.52	955
238408_at	oxidation resistance 1	OXR1	AW086258	1.27	1.28	1.46	84
205336_at	parvalbumin	PVALB	NM_002854	0.87	0.71	0.74	319
204300_at	PET112-like (yeast)	PET112L	NM_004564	1.21	1.39	1.55	205
209504_s_at	pleckstrin homology domain containing, family B (evectins) member 1	PLEKHB1	AF081583	1.34	1.59	1.55	144
242922_at	pM5 protein	PM5	AU151198	1.21	1.23	1.49	60
236407_at	potassium voltage-gated channel, Isk-relatad family, member 1	KCNE1	R73518	1.28	1.47	1.52	127
1568706_s_at	Pp12719 mRNA, complete cds		AF318328	1.38	1.42	2.03	96
1558017_s_at	PRKC, apoptosis, WT1, regulator	PAWR	BG109597	1.24	1.37	1.47	179
200979_at	pyruvate dehydrogenase (lipoamide) alpha 1	PDHA1	BF739979	1.29	1.49	1.69	650
223802_s_at	retinoblastoma binding protein 6	RBBP6	AF063596	1.43	1.69	1.97	249
225171_at	Rho GTPase activating protein 18	ARHGAP18	BE644830	1.16	1.28	1.47	1407
221989_at	ribosomal protein L10	RPL10	AW057781	1.11	1.35	1.69	212
1555878_at	ribosomal protein S24	RPS24	AK094613	1.63	1.79	1.66	138
212030_at	RNA-binding region (RNP1, RRM) containing 7	RNPC7	BG251218	1.11	1.42	1.74	293
241996_at	RUN and FYVE domain containing 2	RUFY2	AI669591	1.52	1.92	1.44	194
215028_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	SEMA6A	AB002438	1.05	1.43	1.30	63

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
1559263_s_at	Similar to hypothetical protein D730019B10 (LOC340152), mRNA		BG397809	1.34	1.37	1.54	96
222145_at	Similar to PI-3-kinase-related kinase SMG-1 isoform 1; lambda/iota protein kinase C-interacting protein; phosphatidylinositol 3-kinase-related protein kinase (LOC390682), mRNA		AK027225	1.16	1.15	1.34	64
202781_s_at	skeletal muscle and kidney enriched inositol phosphatase	SKIP	AI806031	0.79	0.79	0.64	101
217591_at	SKI-like	SKIL	BF725121	1.21	1.07	1.63	114
1559351_at	solute carrier family 16 (monocarboxylic acid transporters), member 9	SLC16A9	BI668873	1.67	1.36	1.80	138
244353_s_at	solute carrier family 2 (facilitated glucose transporter), member 12	SLC2A12	AI675682	1.09	1.21	1.74	125
231437_at	solute carrier family 35, member D2	SLC35D2	AA693722	1.81	1.71	1.87	120
233123_at	solute carrier family 40 (iron-regulated transporter), member 1	SLC40A1	AU156956	1.43	1.85	2.09	120
232392_at	splicing factor, arginine/serine-rich 3	SFRS3	BE927772	1.39	1.64	1.60	565
204690_at	syntaxin 8	STX8	NM_004853	1.00	1.19	1.48	622
221617_at	TAF9-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31 kDa		AF077053	1.22	1.37	1.75	80
221938_x_at	thyroid hormone receptor associated protein 5	THRAP5	AW262690	1.18	1.11	1.73	168
228793_at	thyroid hormone receptor interactor 8	TRIP8	BF002296	1.43	1.60	1.92	395
210886_x_at	TP53 activated protein 1	TP53AP1	AB007457	1.33	1.37	2.04	182
228971_at	Transcribed sequence with moderate similarity to protein ref: NP_055301.1 (*H. sapiens*) neuronal thread protein [*Homo sapiens*]		AI357655	1.07	1.19	1.46	704
233518_at	Transcribed sequence with moderate similarity to protein ref: NP_071431.1 (*H. sapiens*) cytokine receptor-like factor 2; cytokine receptor CRL2 precusor [*Homo sapiens*]		AU144449	0.97	1.20	1.57	74
241798_at	Transcribed sequence with moderate similarity to protein sp: P39195 (*H. sapiens*) ALU8_HUMAN Alu subfamily SX sequence contamination warning entry		AI339930	0.77	0.64	0.73	69
243256_at	Transcribed sequence with weak similarity to protein ref: NP_060265.1 (*H. sapiens*) hypothetical protein FLJ20378 [*Homo sapiens*]		AW796364	1.31	1.47	1.54	157
239735_at	Transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]		N67106	1.33	1.27	1.56	150
242191_at	Transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]		AI701905	0.68	0.50	0.49	174

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
242490_at	Transcribed sequence with weak similarity to protein ref: NP_062553.1 (*H. sapiens*) hypothetical protein FLJ11267 [*Homo sapiens*]		AA564255	1.16	1.23	1.55	165
241897_at	Transcribed sequence with weak similarity to protein ref: NP_071431.1 (*H. sapiens*) cytokine receptor-like factor 2; cytokine receptor CRL2 precusor [*Homo sapiens*]		AA491949	1.32	1.49	1.93	492
230590_at	Transcribed sequences		BE675486	0.88	0.81	0.67	107
230733_at	Transcribed sequences		H98113	0.67	0.63	0.61	127
230773_at	Transcribed sequences		AA628511	1.09	1.26	1.60	131
237317_at	Transcribed sequences		AW136338	1.02	0.75	0.70	79
239238_at	Transcribed sequences		AI208857	1.35	2.25	2.19	113
240128_at	Transcribed sequences		H94876	1.18	1.34	1.62	54
241837_at	Transcribed sequences		AI289774	1.64	1.71	1.73	59
241936_x_at	Transcribed sequences		AI654130	1.07	1.17	1.51	175
241940_at	Transcribed sequences		BF477544	1.22	1.25	1.66	63
242299_at	Transcribed sequences		AW274468	0.80	0.77	0.70	82
242536_at	Transcribed sequences		AI522220	1.25	1.28	1.97	533
242579_at	Transcribed sequences		AA935461	1.35	1.16	1.73	270
242673_at	Transcribed sequences		AA931284	1.36	1.55	1.62	99
243591_at	Transcribed sequences		AI887749	1.30	1.72	2.12	106
243675_at	Transcribed sequences		BF512500	1.12	1.42	1.89	81
243933_at	Transcribed sequences		AI096634	1.15	1.24	1.48	142
244414_at	Transcribed sequences		AI148006	1.31	1.62	1.54	439
244674_at	Transcribed sequences		AA936428	1.19	1.11	1.54	131
244797_at	Transcribed sequences		AI269245	1.37	1.23	1.57	168
224566_at	trophoblast-derived noncoding RNA	TncRNA	AI042152	1.27	1.42	1.95	1769
202510_s_at	tumor necrosis factor, alpha-induced protein 2	TNFAIP2	NM_006291	1.46	1.63	1.71	211
232141_at	U2(RNU2) small nuclear RNA auxiliary factor 1	U2AF1	AU144161	1.03	1.24	1.32	109
228142_at	ubiquinol-cytochrome c reductase complex (7.2 kD)	HSPC051	BE208777	1.34	1.39	1.42	177
1557409_at	UI-CF-FN0-aex-p-22-0-UI.s1 UI-CF-FN0 *Homo sapiens* cDNA clone UI-CF-FN0-aex-p-22-0-UI 3', mRNA sequence.		CA313226	1.19	1.56	1.64	124
1558801_at	unnamed protein product; *Homo sapiens* cDNA FLJ31207 fis, clone KIDNE2003357.		AK055769	1.14	1.40	1.55	169
225198_at	VAMP (vesicle-aaaociated membrane protein)-associated protein A, 33 kDa	VAPA	AL571942	1.78	1.90	2.34	658
222303_at	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2	AV700891	1.32	1.86	2.52	177
235850_at	WD repeat domain 5B	WDR5B	BF434228	1.13	1.21	1.56	289
229647_at	wh65e08.x1 NCI_CGAP_Kid11 *Homo sapiens* cDNA clone IMAGE: 2385638 3' similar to contains Alu repetitive element; contains element MER22 repetitive element;, mRNA sequence.		AI762401	2.01	2.01	2.22	793
242406_at	wl47a04.x1 NCI_CGAP_Ut1 *Homo sapiens* cDNA clone IMAGE: 2428014 3', mRNA sequence.		AI870547	0.73	0.58	0.70	126
224590_at	X (inactive)-specific transcript	XIST	BE644917	1.26	1.44	1.54	261

TABLE 7-continued

Genes of Biomarker Global Analysis, OSC Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
238913_at	xm54d01.x1 NCI_CGAP_GC6 *Homo sapiens* cDNA clone IMAGE: 2688001 3' similar to contains Alu repetitive element; contains element MER28 MER28 repetitive element;, mRNA sequence.		AW235215	1.25	1.60	1.64	111
222281_s_at	xs86h03.x1 NCI_CGAP_Ut2 *Homo sapiens* cDNA clone IMAGE: 2776565 3' similar to contains Alu repetitive element; contains element MER38 repetitive element;, mRNA sequence.		AW517716	1.47	1.56	1.78	350
234033_at	yd35c06.s1 Soares fetal liver spleen 1NFLS *Homo sapiens* cDNA clone IMAGE: 110218 3', mRNA sequence.		T71269	1.15	1.19	1.61	130
239654_at	ye62h04.s1 Soares fetal liver spleen 1NFLS *Homo sapiens* cDNA clone IMAGE: 122359 3', mRNA sequence.		T98846	1.07	1.32	1.62	139
242241_x_at	yi33f06.s1 Soares placenta Nb2HP *Homo sapiens* cDNA clone IMAGE: 141059 3' similar to contains Alu repetitive element; contains L1 repetitive element;, mRNA sequence.		R66713	114	1.36	1.63	73
1565566_a_at	yn76g07.s1 Soares adult brain N2b5HB55Y *Homo sapiens* cDNA clone IMAGE: 174396 3' similar to contains Alu repetitive element;, mRNA sequence.		H21394	0.96	1.26	1.35	84
217586_x_at	yy28g05.s1 Soares melanocyte 2NbHM *Homo sapiens* cDNA clone IMAGE: 272600 3' similar to contains Alu repetitive element;, mRNA sequence.		N35922	1.44	1.53	1.58	370
226163_at	zinc finger and BTB domain containing 9	ZBTB9	AW291499	1.27	1.15	1.56	159
1569312_at	zinc finger protein 146	ZNF146	BE383308	1.08	1.24	1.53	85
231848_x_at	zinc finger protein 207	ZNF207	AW192569	0.94	0.56	0.66	344
239937_at	zinc finger protein 207	ZNF207	AI860558	1.02	1.17	1.52	128
215012_at	zinc finger protein 451	ZNF451	AU144775	1.35	2.08	2.60	153
219741_x_at	zinc finger protein 552	ZNF552	NM_024762	1.20	1.31	1.66	184
230503_at	zo02d03.s1 Stratagene colon (#937204) *Homo sapiens* cDNA clone IMAGE: 566501 3', mRNA sequence.		AA151917	0.69	0.68	0.68	159

[0249] 3.3.4 Biomarker Global Analysis; OPLS Model, Result

[0250] FIG. **14** shows the Biomarker global analysis OPLS model: Scatter plot. A scatterplot or scatter graph is a graph used in statistics to visually display and compare two sets of related quantitative, or numerical, data by displaying only finitely many points, each having a coordinate on a horizontal and a vertical axis. In FIG. **2** each dot represents a sample of a patient. Relative distance between data points is a measure of relationship/resemblance. The separation of the "N" samples from the "week 06 pre-CAN", "week 12 pre-CAN", "CAN" samples indicates the potency of the algorithm /model to discriminate between the data points with the use of these probe sets.

[0251] FIG. **15** shows the Biomarker global analysis OPLS model: observed vs prediction.

[0252] Validation by response permutation is an internal cross-validation, which creates a training set and a test set of samples. A model is fitted to explain the test set based on the training set and the values for R^2Y (explained variance) and Q^2 (predicted variance) are computed and plotted. By random permutation of the training and test sets, a number of R^2Y/Q^2

are obtained. The validate plot is then created by letting the Y-axis represent the R^2Y/Q^2-values of all models, including the "real" one, and by assigning the X-axis to the correlation coefficients between permuted and original response variables. A regression line is then fitted among the R^2Y points and another one through the Q^2 points. The intercepts of the regression lines are interpretable as measures of "background" R2Y and Q^2 obtained to fit the data. Intercepts around 0.4 and below for R2Y and around 0.05 and below for Q^2 indicate valid models. Since these criteria are met in this model it is an indication of a valid model for the present dataset.

[0253] FIG. **16** shows the Biomarker global analysis OPLS model: observed vs predicted.

[0254] The prediction of the Y space samples can be plotted as a scatter plot. RMSE (Root mean square error) is the standard deviation of the predicted residuals (error), and is computed as the square root of $(\Sigma(\text{obs-pred})^2/N)$. A small RMSE is a measure for a good fit of a model.

[0255] The Y-axis of the plot represents the observed classes of the model, the X-axis the predicted classes. A match of Y- and X-values in this plot demonstrates the good fit of the model.

[0256] The combination of biomarker genes that form a molecular signature after tissue transplantation as determined by global data analysis using OPLS model are shown in Table 11.

TABLE 11

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
244567_at	602343781F1 NIH_MGC_89 *Homo sapiens* cDNA clone IMAGE: 4453556 5', mRNA sequence.		BG165613	1.5	1.2	1.7	103
244145_at	602371458F1 NIH_MGC_93 *Homo sapiens* cDNA clone IMAGE: 4479327 5', mRNA sequence.		BG260337	1.2	2.0	1.7	102
232175_at	ADP-ribosylation factor 1	ARF1	AI972094	1.5	1.6	1.5	108
238996_x_at	aldolase A, fructose-bisphosphate	ALDOA	AI921586	1.9	2.3	1.9	413
232865_at	ALL1 fused gene from 5q31	AF5Q31	N59653	1.4	1.6	1.8	179
236778_at	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, *S. cerevisiae*)	ATRX	AA826176	1.6	1.5	2.0	77
1563792_at	amnionless homolog (mouse)	AMN	AK092824	1.1	1.2	1.9	98
226718_at	amphoterin-induced gene	KIAA1163	AA001423	1.4	1.6	1.8	142
229903_x_at	amylase, alpha 2B; pancreatic	AMY2B	AI632212	1.1	1.2	1.4	350
219962_at	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	ACE2	NM_021804	1.3	1.6	1.5	378
227260_at	ankyrin repeat domain 10	ANKRD10	AV724266	1.2	1.3	1.7	708
230972_at	ankyrin repeat domain 9	ANKRD9	AW194999	1.3	1.5	1.5	656
224489_at	ARF protein	LOC51326	BC006271	0.8	0.6	0.6	86
206993_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B)	ATP5S	NM_015684	1.2	2.0	1.5	119
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	ABCA8	NM_007168	2.0	2.1	3.4	350
233271_at	AU145563 HEMBA1 *Homo sapiens* cDNA clone HEMBA1005133 3', mRNA sequence.		AU145563	0.8	0.5	0.7	143
215204_at	AU147295 MAMMA1 *Homo sapiens* cDNA clone MAMMA1000264 3', mRNA sequence.		AU147295	1.3	1.2	1.5	90
236892_s_at	B1 for mucin	HAB1	BF590528	1.3	1.3	1.6	312
239791_at	B1 for mucin	HAB1	AI125255	1.0	1.0	0.7	94
227896_at	BRCA2 and CDKN1A interacting protein	BCCIP	AI373643	1.6	1.7	1.6	223
223679_at	catenin (cadherin-associated protein), beta 1, 88 kDa	CTNNB1	AF130085	1.2	1.3	1.6	146
233019_at	CCR4-NOT transcription complex, subunit 7	CNOT7	AU145061	1.6	1.7	2.0	89
204510_at	CDC7 cell division cycle 7 (*S. cerevisiae*)	CDC7	NM_003503	1.5	1.8	1.7	104
233399_x_at	CDNA clone IMAGE: 30352956, partial cds		AU145662	1.3	1.1	1.8	183
232351_at	CDNA FLJ10150 fis, clone HEMBA1003395		AK022308	0.9	0.8	0.7	152

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
234074_at	CDNA FLJ10946 fis, clone PLACE1000005		AU155494	1.4	1.3	1.4	99
227140_at	CDNA FLJ11041 fis, clone PLACE1004405		AI343467	0.8	0.7	0.7	108
232544_at	CDNA FLJ11572 fis, clone HEMBA1003373		AU144916	1.4	1.6	1.4	231
232991_at	CDNA FLJ11613 fis, clone HEMBA1004012		AK021675	0.9	0.8	0.7	107
232952_at	CDNA FLJ11942 fis, clone HEMBB1000652		AU146493	0.6	0.6	0.7	83
230791_at	CDNA FLJ12033 fis, clone HEMBB1001899		AU146924	1.0	0.9	0.7	241
233498_at	CDNA FLJ14142 fis, clone MAMMA1002880		AK024204	0.9	0.8	0.7	282
230986_at	CDNA FLJ30065 fis, clone ADRGL2000328		AI821447	1.1	1.4	1.3	96
241941_at	CDNA FLJ31511 fis, clone NT2RI1000035		AA778747	0.9	0.8	0.7	75
1557270_at	CDNA FLJ36375 fis, clone THYMU2008226		AA632049	1.2	1.5	1.7	283
235028_at	CDNA FLJ46440 fis, clone THYMU3016022		BG288330	1.5	2.1	1.5	659
234604_at	CDNA: FLJ21228 fis, clone COL00739		AK024881	1.6	2.0	1.6	62
233824_at	CDNA: FLJ21428 fis, clone COL04203		AK025081	0.8	0.7	0.5	114
216782_at	CDNA: FLJ23026 fis, clone LNG01738		AK026679	0.7	0.7	0.6	488
214196_s_at	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)	CLN2	AA602532	1.6	1.3	1.9	84
228143_at	ceruloplasmin (ferroxidase)	CP	AI684991	0.9	0.8	0.8	69
223191_at	chromosome 14 open reading frame 112	C14orf112	AF151037	0.7	0.7	0.7	541
218796_at	chromosome 20 open reading frame 42	C20orf42	NM_017671	1.4	5.8	3.9	107
218453_s_at	chromosome 6 open reading frame 35	C6orf35	NM_018452	0.7	0.7	0.6	110
229012_at	chromosome 9 open reading frame 24	C9orf24	AW269443	1.2	1.6	1.4	142
1552455_at	chromosome 9 open reading frame 65	C9orf65	NM_138818	1.6	2.0	1.6	81
225377_at	chromosome 9 open reading frame 86	C9orf86	BE783949	0.8	0.6	0.4	173
239683_at	citrate lyase beta like	CLYBL	AI476268	1.2	1.3	1.5	243
215504_x_at	Clone 25061 mRNA sequence		AF131777	0.7	0.6	0.7	482
243329_at	Clone IMAGE: 121662 mRNA sequence		AI074450	1.0	1.0	0.7	195
231808_at	Clone IMAGE: 5302006, mRNA		AY007106	1.0	1.2	1.4	213
205229_s_at	coagulation factor C homolog, cochlin (*Limulus polyphemus*)	COCH	AA669336	0.8	1.5	1.5	86
225288_at	collagen, type XXVII, alpha 1	COL27A1	AI949136	1.3	1.7	1.6	304
205159_at	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	CSF2RB	AV756141	1.0	1.5	1.4	106
211025_x_at	cytochrome c oxidase subunit Vb	COX5B	BC006229	1.4	1.5	1.4	1299
225503_at	dehydrogenase/reductase (SDR family) X-linked	DHRSX	AL547782	1.1	1.4	1.5	178
1556820_a_at	deleted in lymphocytic leukemia, 2	DLEU2	H48516	1.3	1.1	1.5	67
1556821_x_at	deleted in lymphocytic leukemia, 2	DLEU2	H48516	1.4	1.4	1.8	100
210165_at	deoxyribonuclease 1	DNASE1	M55983	1.3	1.3	1.6	149

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
218650_at	DiGeorge syndrome critical region gene 8	DGCR8	NM_022775	1.2	1.2	1.6	167
223763_at	dystrobrevin binding protein 1	DTNBP1	AL136637	1.4	1.6	1.6	82
227353_at	epidermodysplasia verruciformis 2	EVER2	BE671663	1.1	1.2	1.4	85
236520_at	EST384471 MAGE resequences, MAGL *Homo sapiens* cDNA, mRNA sequence.		AW972380	1.4	1.6	2.2	128
214805_at	eukaryotic translation initiation factor 4A, isoform 1	EIF4A1	U79273	1.5	1.4	1.7	153
230389_at	formin binding protein 1	FNBP1	BE046511	1.2	1.2	1.7	188
244509_at	G protein-coupled receptor 155	GPR155	AW449728	1.2	1.2	1.6	69
210358_x_at	GATA binding protein 2	GATA2	BC002557	0.9	07	0.8	111
227163_at	glutathione S-transferase omega 2	GSTO2	AL162742	0.9	0.7	0.7	361
215203_at	golgi autoantigen, golgin subfamily a, 4	GOLGA4	AW438464	0.9	0.8	0.7	109
229255_x_at	golgi SNAP receptor complex member 2	GOSR2	BF593917	0.7	0.7	0.7	142
240405_at	H326	H326	AA707411	1.3	1.4	1.4	61
203394_s_at	hairy and enhancer of split 1, (*Drosophila*)	HES1	BE973687	1.9	2.2	1.8	703
209960_at	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	X16323	0.8	0.8	0.7	118
213359_at	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37 kDa)	HNRPD	W74620	0.8	0.7	0.6	207
1560782_at	*Homo sapiens* cDNA clone IMAGE: 5186324, partial cds.		BC035326	1.7	1.6	1.9	101
215553_x_at	*Homo sapiens* cDNA FLJ14253 fis, clone OVARC1001376.		AK024315	1.5	1.6	2.0	262
233813_at	*Homo sapiens* cDNA: FLJ23247 fis, clone COL03425.		AK026900	1.7	1.4	1.9	76
231886_at	*Homo sapiens* mRNA; cDNA DKFZp434B2016 (from clone DKFZp434B2016).		AL137655	0.8	0.8	0.7	73
228564_at	hypothetical gene supported by BC013438		AI569804	1.3	1.5	1.5	439
241031_at	hypothetical LOC145741		BE218239	1.5	1.7	2.0	68
237108_x_at	hypothetical protein DKFZp761G0122	DKFZp761G0122	AW611845	1.0	1.3	1.7	276
219074_at	hypothetical protein FLJ10846	FLJ10846	NM_018241	1.1	1.2	1.6	418
222788_s_at	hypothetical protein FLJ11220	FLJ11220	BE888593	0.9	0.8	0.6	106
226967_at	hypothetical protein FLJ14768	FLJ14768	BG231981	1.6	2.1	1.4	156
1557828_a_at	hypothetical protein FLJ21657	FLJ21657	BE675061	0.8	0.8	0.7	148
222872_x_at	hypothetical protein FLJ22833	FLJ22833	AU157541	1.4	1.5	1.6	456
233085_s_at	hypothetical protein FLJ22833	FLJ22833	AV734843	0.8	0.7	0.7	415
229145_at	hypothetical protein LOC119504	LOC119504	AA541762	1.2	1.5	1.4	659
227550_at	hypothetical protein LOC143381	LOC143381	AW242720	1.2	1.4	1.4	222
227415_at	hypothetical protein LOC283508	LOC283508	BF109303	1.2	1.3	1.4	350
232288_at	hypothetical protein LOC283970	LOC283970	AK026209	1.6	1.5	1.6	77
226901_at	hypothetical protein LOC284018	LOC284018	AI214996	1.9	2.1	1.5	342

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
235482_at	hypothetical protein LOC285002	LOC285002	BE886868	1.6	1.4	2.0	132
228040_at	hypothetical protein LOC286286	LOC286286	AW294192	4.6	6.5	13.5	468
1569189_at	hypothetical protein MGC29649	MGC29649	AF289605	0.8	0.9	0.6	75
225065_x_at	hypothetical protein MGC40157	MGC40157	AI826279	0.8	0.8	0.7	237
218750_at	hypothetical protein MGC5306	MGC5306	NM_024116	0.9	0.8	0.7	239
223797_at	hypothetical protein PRO2852	PRO2852	AF130079	1.2	1.4	1.5	169
235756_at	IL2-UM0076-240300-056-G02 UM0076 *Homo sapiens* cDNA, mRNA sequence.		AW802645	0.8	0.8	0.7	75
239842_x_at	IMAGE: 20075 Soares infant brain 1NIB *Homo sapiens* cDNA clone IMAGE: 20075, mRNA sequence.		W18186	0.9	0.9	0.6	190
209374_s_at	immunoglobulin heavy constant mu	IGHM	BC001872	0.8	0.8	0.8	123
212827_at	immunoglobulin heavy constant mu	IGHM	X17115	0.9	0.7	0.6	95
209031_at	immunoglobulin superfamily, member 4	IGSF4	AL519710	1.3	1.8	1.6	921
201508_at	insulin-like growth factor binding protein 4	IGFBP4	NM_001552	0.8	0.7	0.8	238
226535_at	integrin, beta 6	ITGB6	AK026736	1.3	2.0	1.6	1574
242903_at	interferon gamma receptor 1		AI458949	0.8	0.7	0.7	90
224361_s_at	interleukin 17 receptor B	IL17RB	AF250309	0.9	0.7	0.7	394
229310_at	kelch repeat and BTB (POZ) domain containing 9	KBTBD9	BE465475	1.8	2.0	1.7	175
236368_at	KIAA0368		BF059292	1.7	2.5	1.5	142
216000_at	KIAA0484 protein	KIAA0484	AA732995	0.8	0.8	0.7	74
231956_at	KIAA1618	KIAA1618	AA976354	1.6	1.8	2.0	111
238087_at	kinesin family member 2C	KIF2C	AI587389	1.4	3.2	1.8	92
1555929_s_at	laa10f11.x1 8 5 week embryo anterior tongue 8 5 EAT *Homo sapiens* cDNA 3', mRNA sequence.		BM873997	1.2	1.3	1.5	230
1557360_at	leucine-rich PPR-motif containing	LRPPRC	CA430402	1.6	2.8	1.8	103
1569003_at	likely ortholog of rat vacuole membrane protein 1	VMP1	AL541655	1.2	1.8	1.8	213
223223_at	likely ortholog of yeast ARV1	ARV1	AF321442	1.3	1.3	1.5	520
229554_at	lumican	LUM	AI141861	0.8	0.8	0.7	95
227438_at	lymphocyte alpha-kinase	LAK	AI760166	1.2	1.4	1.6	63
226841_at	macrophage expressed gene 1	MPEG1	BF590697	0.8	0.8	0.7	87
214048_at	methyl-CpG binding domain protein 4	MBD4	AI913365	1.1	1.6	1.8	89
239001_at	microsomal glutathione S-transferase I	MGST1	AV705233	1.0	1.0	0.6	62
217980_s_at	mitochondrial ribosomal protein L16	MRPL16	NM_017840	0.8	0.8	0.7	609
231274_s_at	mitochondrial solute carrier protein	MSCP	R92925	1.2	1.3	1.4	193
1558732_at	mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4	AK074900	0.8	0.8	0.6	128
223218_s_at	molecule possessing ankyrin repeats induced by lipopolysaccharide (MAIL), homolog of mouse	MAIL	AB037925	0.8	0.8	0.7	708
243683_at	mortality factor 4 like 2	MORF4L2	H43976	0.8	0.9	0.7	65
1563469_at	MRNA; cDNA DKFZp313M0417 (from clone DKFZp313M0417)		AL832681	0.6	0.8	0.6	74
234224_at	MRNA; cDNA DKFZp434O0919 (from clone DKFZp434O0919)		AL137541	0.8	0.7	0.7	79

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
227576_at	MRNA; cDNA DKFZp686K1098 (from clone DKFZp686K1098)		AW003140	0.8	0.7	0.8	452
228217_s_at	MRNA; cDNA DKFZp686P09209 (from clone DKFZp686P09209)		BF973374	1.4	1.3	1.4	365
210210_at	myelin protein zero-like 1	MPZL1	AF181660	1.8	2.4	1.4	105
233539_at	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D	NAPE-PLD	AK000801	1.0	0.8	0.7	135
202000_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14 kDa	NDUFA6	BC002772	0.8	0.9	0.7	693
218320_s_at	neuronal protein 17.3	P17.3	NM_019056	1.0	1.4	1.8	993
233626_at	neuropilin 1	NRP1	AK024580	1.2	1.4	1.8	53
235985_at	nj45a06.x5 NCI_CGAP_Pr9 *Homo sapiens* cDNA clone IMAGE: 995410 similar to contains Alu repetitive element; contains element TAR1 repetitive element;, mRNA sequence.		AI821477	1.3	1.4	1.6	115
226991_at	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2		AA489681	1.1	1.4	1.7	88
209505_at	nuclear receptor subfamily 2, group F, member 1	NR2F1	AI951185	1.2	1.5	1.4	499
206302_s_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	NUDT4	NM_019094	0.9	0.7	0.7	955
244450_at	oc86a09.s1 NCI_CGAP_GCB1 *Homo sapiens* cDNA clone IMAGE: 1356568 3' similar to gb: M81181 SODIUM/POTASSIUM-TRANSPORTING ATPASE BETA-2 (HUMAN); contains element PTR5 repetitive element;, mRNA sequence.		AA741300	1.4	1.4	1.4	65
238408_at	oxidation resistance 1	OXR1	AW086258	1.0	0.8	0.7	84
205336_at	parvalbumin	PVALB	NM_002854	1.4	1.7	1.9	319
220303_at	PDZ domain containing 2	PDZK2	NM_024791	1.3	1.4	1.5	95
204300_at	PET112-like (yeast)	PET112L	NM_004564	1.3	1.3	1.5	205
209504_s_at	pleckstrin homology domain containing, family B (evectins) member 1	PLEKHB1	AF081583	0.9	0.7	0.7	144
242922_at	pM5 protein (nomo)	PM5	AU151198	1.2	1.4	1.5	60
236407_at	potassium voltage-gated channel, Isk-related family, member 1	KCNE1	R73518	1.3	1.5	1.5	127
1568706_s_at	Pp12719 mRNA, complete cds		AF318328	1.3	1.6	1.5	96
1558017_s_at	PRKC, apoptosis, WTI, regulator	PAWR	BG109597	1.1	1.1	1.6	179
229158_at	protein kinase, lysine deficient 4	PRKWNK4	AW082836	1.2	1.2	1.5	859
200979_at	pyruvate dehydrogenase (lipoamide) alpha 1	PDHA1	BF739979	1.3	1.5	1.5	650
225171_at	Rho GTPase activating protein 18	ARHGAP18	BE644830	1.3	1.2	1.5	1407
221989_at	ribosomal protein L10	RPL10	AW057781	1.4	1.4	2.0	212
1555878_at	ribosomal protein S24	RPS24	AK094613	1.2	1.4	1.5	138
212030_at	RNA-binding region (RNP1, RRM) containing 7	RNPC7	BG251218	1.3	1.5	1.7	293
241996_at	RUN and FYVE domain containing 2	RUFY2	AI669591	1.4	1.7	2.0	194

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
215028_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	SEMA6A	AB002438	1.2	1.8	1.5	63
226492_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	SEMA6D	AL036088	1.2	1.3	1.5	793
1559263_s_at	Similar to hypothetical protein D730019B10(LOC340152), mRNA		BG397809	1.1	1.3	1.7	96
222145_at	Similar to P1-3-kinase-related kinase SMG-1 isoform 1; lambda/iota protein kinase C-interacting protein; phosphatidylinositol 3-kinase-related protein kinase (LOC390682), mRNA		AK027225	1.6	1.8	1.7	64
202781_s_at	skeletal muscle and kidney enriched inositol phosphatase	SKIP	AI806031	1.1	1.4	1.7	101
217591_at	SKI-like	SKIL	BF725121	1.5	1.9	1.4	114
220503_at	solute carrier family 13 (sodium/sulfate symporters), member 1	SLC13A1	AF260824	1.3	1.4	1.5	501
1559351_at	solute carrier family 16 (monocarboxylic acid transporters), member 9	SLC16A9	BI668873	0.8	0.8	0.6	138
206872_at	solute carrier family 17 (sodium phosphate), member 1	SLC17A1	NM_005074	1.2	1.1	1.6	592
244353_s_at	solute carrier family 2 (facilitated glucose transporter), member 12	SLC2A12	AI675682	1.7	1.4	1.8	125
231437_at	solute carrier family 35, member D2	SLC35D2	AA693722	1.1	1.2	1.7	120
232597_x_at	splicing factor, arginine/serine-rich 2, interacting protein	SFRS2IP	AK025132	1.8	1.7	1.9	499
232392_at	splicing factor, arginine/serine-rich 3	SFRS3	BE927772	1.4	1.8	2.1	565
237639_at	SRSR846		AI913600	1.4	1.6	1.6	318
204690_at	syntaxin 8	STX8	NM_004853	1.0	1.2	1.5	622
242512_at	te33f12.x1 Soares_NhHMPu_S1 *Homo sapiens* cDNA clone IMAGE: 2088527 3' similar to contains L1 t3 L1 repetitive element;, mRNA sequence.		AI382029	1.2	1.4	1.8	92
1555392_at	Testin-related protein TRG mRNA, complete cds		AY143171	1.2	1.1	1.7	74
221938_x_at	thyroid hormone receptor associated protein 5	THRAP5	AW262690	1.4	1.6	1.9	168
228793_at	thyroid hormone receptor interactor 8	TRIP8	BF002296	1.3	1.4	2.0	395
232017_at	tight junction protein 2 (zona occludens 2)	TJP2	AK025185	1.1	1.2	1.5	118
228971_at	Transcribed sequence with moderate similarity to protein ref: NP_055301.1 (*H. sapiens*) neuronal thread protein [*Homo sapiens*]		AI357655	1.0	1.2	1.6	704
233518_at	Transcribed sequence with moderate similarity to protein ref: NP_071431.1 (*H. sapiens*) cytokine receptor-like factor 2; cytokine receptor CRL2 precusor [*Homo sapiens*]		AU144449	0.8	0.6	0.7	74

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
241798_at	Transcribed sequence with moderate similarity to protein sp: P39195 (*H. sapiens*) ALU8_HUMAN Alu subfamily SX sequence contamination warning entry		AI339930	1.3	1.5	1.5	69
243256_at	Transcribed sequence with weak similarity to protein ref: NP_060265.1 (*H. sapiens*) hypothetical protein FLJ20378 [*Homo sapiens*]		AW796364	1.3	1.3	1.6	157
239735_at	Transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]		N67106	0.7	0.5	0.5	150
242191_at	Transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]		AI701905	1.3	1.4	1.7	174
242490_at	Transcribed sequence with weak similarity to protein ref: NP_062553.1 (*H. sapiens*) hypothetical protein FLJ11267 [*Homo sapiens*]		AA564255	1.2	1.2	1.6	165
241897_at	Transcribed sequence with weak similarity to protein ref: NP_071431.1 (*H. sapiens*) cytokine receptor-like factor 2; cytokine receptor CRL2 precusor [*Homo sapiens*]		AA491949	1.3	1.5	1.9	492
230590_at	Transcribed sequences		BE675486	0.9	0.8	0.7	107
230733_at	Transcribed sequences		H98113	0.7	0.6	0.6	127
230773_at	Transcribed sequences		AA628511	1.1	1.3	1.6	131
236432_at	Transcribed sequences		AA682425	0.7	0.6	0.6	70
237317_at	Transcribed sequences		AW136338	1.0	0.7	0.7	79
238875_at	Transcribed sequences		BE644953	1.4	2.3	2.2	75
239238_at	Transcribed sequences		AI208857	1.4	1.2	1.4	113
240128_at	Transcribed sequences		H94876	1.2	1.3	1.6	54
241837_at	Transcribed sequences		AI289774	1.2	0.9	0.6	59
241936_x_at	Transcribed sequences		AI654130	1.6	1.7	1.7	175
241940_at	Transcribed sequences		BF477544	1.1	1.2	1.5	63
242299_at	Transcribed sequences		AW274468	1.2	1.2	1.7	82
242536_at	Transcribed sequences		AI522220	0.8	0.8	0.7	533
242579_at	Transcribed sequences		AA935461	1.2	1.3	2.0	270
242673_at	Transcribed sequences		AA931284	0.6	0.5	0.7	99
243591_at	Transcribed sequences		AI887749	1.3	1.2	1.7	106
243675_at	Transcribed sequences		BF512500	1.4	1.5	1.6	81
243933_at	Transcribed sequences		AI096634	1.3	1.7	2.1	142
244414_at	Transcribed sequences		AI148006	1.1	1.4	1.9	439
244674_at	Transcribed sequences		AA936428	2.8	2.5	1.7	131
244797_at	Transcribed sequences		AI269245	1.2	1.2	1.5	168
224566_at	trophoblast-derived noncoding RNA	TncRNA	AI042152	1.3	1.6	1.5	1769
204141_at	tubulin, beta polypeptide	TUBB	NM_001069	1.2	1.1	1.5	1453
202510_s_at	tumor necrosis factor, alpha-induced protein 2	TNFAIP2	NM_006291	1.4	1.2	1.6	211
232141_at	U2(RNU2) small nuclear RNA auxiliary factor I	U2AFI	AU144161	1.3	1.4	1.9	109
228142_at	ubiquinol-cytochrome c reductase complex (7.2 kD)	HSPC051	BE208777	1.5	1.6	1.7	177
1557409_at	UI-CF-FN0-sex-p-22-0-UI.s1 UI-CF-FN0 *Homo sapiens* cDNA clone UI-CF-FN0-aex-p-22-0-UI 3', mRNA sequence.		CA313226	1.0	1.2	1.3	124

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
1558801_at	unnamed protein product; *Homo sapiens* cDNA FLJ31207 fis, clone KIDNE2003357.		AK055769	1.2	1.6	1.6	169
225198_at	VAMP (vesicle-associated membrane protein)-associated protein A, 33 kDa	VAPA	AL571942	1.1	1.4	1.6	658
222303_at	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2	AV700891	1.8	1.9	2.3	177
235850_at	WD repeat domain 5B	WDR5B	BF434228	1.3	1.9	2.5	289
229647_at	wh65e08.x1 NCI_CGAP_Kid11 *Homo sapiens* cDNA clone IMAGE: 2385638 3' similar to contains Alu repetitive element; contains element MER22 repetitive element;, mRNA sequence.		AI762401	1.1	1.2	1.6	793
242406_at	w147a04.x1 NCI_CGAP_Ut1 *Homo sapiens* cDNA clone IMAGE: 2428014 3', mRNA sequence.		AI870547	1.0	1.0	1.6	126
224590_at	X (inactive)-specific transcript	XIST	BE644917	2.0	2.0	2.2	261
1565454_at	XAGE-4 protein	XAGE-4	AJ318895	0.7	0.6	0.7	119
230554_at	xenobiotic/medium-chain fatty acid:CoA ligase	LOC348158	AV696234	1.3	1.4	1.5	5210
238913_at	xm54d01.x1 NCI_CGAP_GC6 *Homo sapiens* cDNA clone IMAGE: 2688001 3' similar to contains Alu repetitive element; contains element MER28 MER28 repetitive element;, mRNA sequence.		AW235215	1.3	1.6	1.6	111
222281_s_at	xs86h03.x1 NCI_CGAP_Ut2 *Homo sapiens* cDNA clone IMAGE: 2776565 3' similar to contains Alu repetitive element; contains element MER38 repetitive element;, mRNA sequence.		AW517716	1.5	1.6	1.8	350
234033_at	yd35c06.s1 Soares fetal liver spleen INFLS *Homo sapiens* cDNA clone IMAGE: 110218 3', mRNA sequence.		T71269	1.1	1.2	1.6	130
239654_at	ye62h04.s1 Soares fetal liver spleen INFLS *Homo sapiens* cDNA clone IMAGE: 122359 3', mRNA sequence.		T98846	1.1	1.3	1.6	139
242241_x_at	yi33f06.s1 Soares placenta Nb2HP *Homo sapiens* cDNA clone IMAGE: 141059 3' similar to contains Alu repetitive element; contains L1 repetitive element;, mRNA sequence.		R66713	1.2	1.4	1.6	73
232216_at	YME1-like 1 (*S. cerevisiae*)	YME1L1	AA828049	1.4	1.5	1.6	70
1565566_s_at	yn76g07.s1 Soares adult brain N2b5HB55Y *Homo sapiens* cDNA clone IMAGE: 174396 3' similar to contains Alu repetitive element;, mRNA sequence.		H21394	1.8	1.8	1.5	84

TABLE 11-continued

Genes of the Biomarker Global Analysis, OPLS Model

Affymetrix Probe Set ID	Description	Common	Genbank	Fold change wk06-pre-CAN	Fold change wk12-pre-CAN	Fold change CAN	Stable Graft: Raw Expression Value
217586_x_at	yy28g05.s1 Soares melanocyte 2NbHM *Homo sapiens* cDNA clone IMAGE: 272600 3' similar to contains Alu repetitive element;, mRNA sequence.		N35922	1.3	1.2	1.6	370
226163_at	zinc finger and BTB domain containing 9	ZBTB9	AW291499	1.1	1.2	1.5	159
1569312_at	zinc finger protein 146	ZNF146	BE383308	0.9	0.6	0.7	85
231848_x_at	zinc finger protein 207	ZNF207	AW192569	1.0	1.2	1.5	344
239937_at	zinc finger protein 207	ZNF207	AI860558	1.1	1.9	1.6	128
229279_at	zinc finger protein 432	ZNF432	AW235102	1.4	1.4	1.6	93
215012_at	zinc finger protein 451	ZNF451	AU144775	1.3	2.1	2.6	153
219741_x_at	zinc finger protein 552	ZNF552	NM_024762	1.2	1.3	1.7	184
230503_at	zo02d03.s1 Stratagene colon (#937204) *Homo sapiens* cDNA clone IMAGE: 566501 3', mRNA sequence.		AA151917	0.7	0.7	0.7	159

[0257] In one embodiment, the preferred genes identified using the global analysis include, but are not limited to, ceruloplasmin (Chen et al., Biochem, Biophys Res Commun. (2001);282; 475-82), pM5/NOMO (Ju et al., Mol. Cell. Biol. (2006), 26; 654-67), colonly stimulating factor 2 receptor (Steinman et al. Annu Rev. Immunol. (1991), 9; 271-96), Hairy and enhancer of split-1 (Hes-1) (Deregowski et al. J. Biol. Chem (2006)), insulin growth factor binding protein 4 (Jehle et al, Kidney Int. (2000) 57; 1209-10), hepatocyte growth factor (hepapoietin A) (Azuma et al., J. Am. Soc. Nephrol (2001), 12; 1280-92),solute carrier family 2 (Linden et al, Am. J. Physiol Renal. Physiol. (2006) January;290(1): F205-13. Epub Aug. 9, 2005), ski-like (snoN) (Zhu et al. Mol. Cell. Biol. (2005) December;25(24):1073144).

[0258] 4 Discussion

[0259] Gene expression profiling of serial renal allograft protocol biopsies was performed with the goal to identify genomic biomarkers for prediction/early diagnosis of CAN. The biomarkers are useful as molecular tools to diagnose latent CAN grade I 18 weeks and/or 12 weeks before CAN is manifest by histological parameters.

[0260] Statistical analysis of gene expression data from serial renal protocol biopsies allowed the identification of predictive/early diagnostic biomarkers of CAN I

[0261] Individual biomarker models were generated for

[0262] 4.5 months before clinical/histopathol. evidence of CAN

[0263] 3 months before clinical/histopathol. evidence of CAN

[0264] across timepoints and diagnosis

[0265] Biomarker variables (i.e. probe sets) are quite different at individual timepoints, here: 4.5 months and 3 months before histopathological diagnosis of CAN I

[0266] The validity of the biomarkers has to be proven by validation on new datasets.

[0267] To reveal biological processes on molecular level which are involved in the development of CAN, the analysis will focus on

[0268] temporarily expressed genes and networks, and

[0269] genes present at CAN, tracking back there expression and pathways to earlier timepoints

[0270] Equivalents

[0271] The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

TABLE 12

Biomarker Identification: week 12 (3 months before CAN)*

Affymetrix Probe Set ID	Description	Common	Fold change
201792_at	AE binding protein 1	AEBP1	1.89
211712_s_at	annexin A9	ANXA9	0.55
207367_at	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	ATP12A	0.50
229218_at	collagen, type I, alpha 2	COL1A2	2.43
232458_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)		2.79
201438_at	collagen, type VI, alpha 3	COL6A3	2.13
226237_at	collagen, type VIII, alpha 1	COL8A1	2.17
227336_at	deltex homolog 1 (*Drosophila*)	DTX1	0.50
210165_at	deoxyribonuclease I	DNASE1	0.42
220625_s_at	E74-like factor 5 (ets domain transcription factor)	ELF5	0.45
221870_at	EH-domain containing 2	EHD2	2.40

TABLE 12-continued

Biomarker Identification: week 12 (3 months before CAN)*

Affymetrix Probe Set ID	Description	Common	Fold change
227353_at	epidermodysplasia verruciformis 2	EVER2	3.20
242974_at	frizzled homolog 9 (*Drosophila*)	FZD9	2.46
211795_s_at	FYN binding protein (FYB-120/130)	FYB	2.26
201744_s_at	lumican	LUM	1.95
229554_at	lumican	LUM	2.67
227438_at	lymphocyte alpha-kinase	LAK	3.00
226841_at	macrophage expressed gene 1	MPEG1	2.00
212999_x_at	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	2.49
226210_s_at	maternally expressed 3	MEG3	2.34
212012_at	Melanoma associated gene	D2S448	1.71
219666_at	membrane-spanning 4-domains, subfamily A, member 6A	MS4A6A	1.94
228055_at	napsin B pseudogene	NAP1L	1.93
214111_at	opioid binding protein/cell adhesion molecule-like	OPCML	0.40
205267_at	POU domain, class 2, associating factor 1	POU2AF1	4.04
216834_at	regulator of G-protein signalling 1	RGS1	2.69
218870_at	Rho GTPase activating protein 15	ARHGAP15	2.52
209374_s_at	immunoglobulin heavy constant mu	IGHM	8.84
203083_at	thrombospondin 2	THBS2	2.23
209960_at	hepatocyte growth factor (HGF).	HGF	2.00
202664_at	Wiskott-Aldrich syndrome protein interacting protein	WASPIP	1.96

*Probe sets of biomarker without functionally non-annotated probe sets omitted

TABLE 13

Week 12 (3 months prior to histological diagnosis of CAN):
Large overrepresentation of immune related genes

Affymetrix ID	Gene Name	FC	T test
213539_at	CD3D antigen, delta polypeptide (TiT3 complex)	2.5	1.3E−02
210031_at	CD3Z antigen, zeta polypeptide (TiT3 complex)	2.1	1.4E−02
212063_at	CD44 antigen (homing function and Indian blood group system)	2.1	8.2E−02
204118_at	CD48 antigen (B-cell membrane protein)	1.7	6.1E−02
213958_at	CD6 antigen	2.3	2.8E−02
206978_at	chemokine (C-C motif) receptor 2	2.3	6.4E−03
206337_at	chemokine (C-C motif) receptor 7	2.0	7.4E−02
205898_at	chemokine (C—X3—C motif) receptor 1	2.0	4.4E−03
217028_at	chemokine (C—X—C motif) receptor 4	2.1	1.1E−01
224733_at	chemokine-like factor super family 3	1.4	2.9E−01
224998_at	chemokine-like factor super family 4	0.6	4.3E−03
211339_s_at	IL2-inducible T-cell kinase	2.1	2.6E−02
232024_at	immunity associated protein 2	1.7	9.6E−02
211430_s_at	immunoglobulin heavy constant gamma 3 (G3m marker)	8.5	2.9E−02
209374_s_at	immunoglobulin heavy constant mu	25.6	1.2E−02
212827_at	immunoglobulin heavy constant mu	1.6	1.2E−01
212592_at	immunoglobulin J polypeptide, linker protein for immunoglobulin al⍰	9.9	1.9E−02
214677_x_at	immunoglobulin lambda joining 3	10.7	3.5E−02
215121_x_at	immunoglobulin lambda locus	14.8	5.4E−02
209031_at	immunoglobulin superfamily, member 4	0.5	1.2E−02

TABLE 13-continued

Week 12 (3 months prior to histological diagnosis of CAN):
Large overrepresentation of immune related genes

Affymetrix ID	Gene Name	FC	T test
226818_at	macrophage expressed gene 1	2.4	2.8E−02
226841_at	macrophage expressed gene 1	2.7	1.1E−04
211654_x_at	major histocompatibility complex, class II, DQ beta 1	1.8	5.9E−02
212999_x_at	major histocompatibility complex, class II, DQ beta 1	2.9	6.3E−03
209312_x_at	major histocompatibility complex, class II, DR beta 3	1.7	1.0E−01
204670_x_at	major histocompatibility complex, class II, DR beta 4	1.6	5.0E−03
208306_x_at	major histocompatibility complex, class II, DR beta 4	1.6	9.6E−03
202687_s_at	tumor necrosis factor (ligand) superfamily, member 10	1.7	2.0E−01
214329_x_at	tumor necrosis factor (ligand) superfamily, member 10	1.5	1.5E−01
204781_s_at	tumor necrosis factor receptor superfamily, member 6	1.7	2.7E−02
202510_s_at	tumor necrosis factor, alpha-induced protein 2	1.6	8.4E−02
202644_s_at	tumor necrosis factor, alpha-induced protein 3	1.6	8.2E−02
206026_s_at	tumor necrosis factor, alpha-induced protein 6	2.8	4.4E−02
210260_s_at	tumor necrosis factor, alpha-induced protein 8	1.7	3.7E−03

⍰ indicates text missing or illegible when filed

TABLE 14

Week 12 (3 months prior to histological diagnosis of CAN):
Large overrepresentation of ECM related genes

Affymetrix ID	Gene Name	FC	T test
1556499_s_at	collagen, type I, alpha 1	1.5	8.0E−02
202403_s_at	collagen, type I, alpha 2	1.7	3.2E−02
202404_s_at	collagen, type I, alpha 2	2.0	3.1E−02
229218_at	collagen, type I, alpha 2	2.3	6.4E−03
201852_x_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autos⍰	2.1	1.7E−02
215076_s_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV autos⍰	1.7	1.4E−02
212488_at	collagen, type V, alpha 1	2.0	1.5E−02
212489_at	collagen, type V, alpha 1	1.6	5.2E−02
209156_s_at	collagen, type VI, alpha 2	2.2	6.9E−02
201438_at	collagen, type VI, alpha 3	2.3	2.6E−03
226237_at	collagen, type VIII, alpha 1	2.8	2.1E−02
212865_s_at	collagen, type XIV, alpha 1 (undulin)	1.7	9.9E−03
204345_at	collagen, type XVI, alpha 1	1.6	1.1E−02
201893_x_at	decorin	1.6	4.2E−02
209335_at	decorin	1.5	3.8E−02
211813_x_at	decorin	1.5	2.1E−01
211896_s_at	decorin	1.7	3.0E−02
210495_x_at	fibronectin 1	1.5	2.1E−01
211719_x_at	fibronectin 1	1.5	2.4E−01
212464_s_at	fibronectin 1	1.5	2.2E−01
218255_s_at	fibrosin 1	0.6	1.2E−03
202995_s_at	fibulin 1	2.0	1.6E−02
202994_s_at	fibulin 1	1.6	8.8E−02
201744_s_at	lumican	1.9	2.2E−02
229554_at	lumican	2.9	8.4E−04
204259_at	matrix metalloproteinase 7 (matrilysin, uterine)	1.8	2.3E−01

⍰ indicates text missing or illegible when filed

TABLE 15

Overview for "Global Analysis".

Intention:	Identification of biomarker model across timepoints and diagnosis
Samples:	33 N samples from non-progressors ("N") 8 pre-CAN, week 6 ("week 06 pre-CAN") 8 pre-CAN, week12 ("week 12 pre-CAN") 18 CAN grd. I (week 6, 12 and 24) ("CAN") total: 67 samples
Normalization:	each group to median of N samples, by batch
Filter:	Coefficient of Variation: small (<20% in group N) Raw expression values >100 in >25% of all samples)
Analysis:	SIMCA (OSC, i.e partial least square with orthogonal signal correction)

(d) determining whether the magnitude of gene expression of the combination of the plurality of genes is up-regulated or down-regulated relative to the control sample, wherein up-regulation or down-regulation of the magnitude of expression of the combination of the plurality of genes indicates that the subject is likely to experience transplant rejection, thereby predicting the onset of rejection of the transplanted organ in the subject.

2. The method of claim **1**, wherein the post-translation sample comprises cells obtained from the subject.

3. The method of claim **1**, wherein the post-translation sample is selected from the group consisting of: a graft biopsy; blood; serum; and urine.

4. The method of claim **1**, wherein the rejection is chronic/sclerosing allograph nephropathy.

TABLE 16

Excerpt of genes from the global analysis

Affymetrix ID	Gene Name	Role	Fold change trend
223679_at	catenin (cadherin-associated protein), beta 1, 88 kDa	Wnt pathway; EMT	↑
228143_at	ceruloplasmin (ferroxidase)	copper carrier; elevated in serum in nephrotic syndrome	↑
225288_at	collagen, type XXVII, alpha 1	ECM	↑
1556820_a_at	deleted in lymphocytic leukemia, 2		↑
210165_at	deoxyribonuclease I	tubular damage	↓
227353_at	epidermodysplasia verruciformis 2		↑
203394_s_at	hairy and enhancer of split 1, (*Drosophila*)	Notch signaling; T cell; regulation of prostaglandin synthase	↓
209960_at	HGF (AA 1-728)	antagonizes TGFbeta; ameliorates interstitial inflammation; inhibits EMT	↑
212827_at	IgM heavy chain complete sequence.	immune response	↑
242903_at	interferon gamma receptor 1		↑
227438_at	lymphocyte alpha-kinase	maintenance of epithelial polarity	↑
226841_at	macrophage expressed gene 1		↑
226991_at	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	potential metabolic sensor for the arterial smooth muscle response to high glucose; immune response	↑
206302_s_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	pyrophosphate hydrolase	↓
1558017_s_at	Prostate apoptosis response-4 protein	interacts with WT1; apoptosis	↑
217591_at	SKI-like	TGFbeta pathway; interacts with Smad3	↑
221938_x_at	thyroid hormone receptor associated protein 5		↓
228793_at	thyroid hormone receptor interactor 8		↑
224566_at	trophoblast MHC class II suppressor	non-coding RNA; suppresses MHC class expression	↑
202510_s_at	tumor necrosis factor, alpha-induced protein 2		↑

1. A method for predicting the onset of a rejection of a transplanted organ in a subject, comprising the steps of:

(a) obtaining a post-transplantation sample from the subject;

(b) determining the magnitude of gene expression in the post-transplantation sample of a combination of a plurality of genes selected from the group consisting of the genes set forth in Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model;

(c) comparing the magnitude of gene expression of the combination of the plurality of genes in the post-transplantation sample with the magnitude of gene expression of the same combination of the plurality of genes in a control sample; and

5. The method of claim **1**, wherein the magnitude of expression in the post-transplantation sample differs from the magnitude of expression in the control sample by a factor of at least about 1.5.

6. The method of claim **1**, wherein the magnitude of expression in the post-transplantation sample differs from the magnitude of expression in the control sample by a factor of at least about 2.

7. A method for predicting the onset of a rejection of a transplanted organ in a subject, comprising the steps of:

(a) obtaining a post-transplantation sample from the subject;

(b) determining the gene expression pattern in the post-transplantation sample of a combination of a plurality of genes selected from the group consisting of the genes set

forth in Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model; and

(c) comparing the gene expression pattern of the combination of the plurality of genes in the post-transplantation sample with the gene expression pattern of the same combination of the plurality of genes in a control sample, wherein a similarity in the gene expression pattern of the combination of the plurality of genes in the post-transplantation sample compared to the gene expression pattern of the same combination of the plurality of genes in a control sample gene expression pattern indicates indicates that the subject is likely to experience transplant rejection, thereby predicting the onset of rejection of the transplanted organ in the subject.

8. The method of claim **7**, wherein the post-transplantation sample comprises cells obtained from the subject.

9. The method of claim **7**, wherein the post-transplantation sample is selected from the group consisting of: a graft biopsy; blood; serum; and urine.

10. The method of claim **7**, wherein the rejection is chronic/sclerosing allograft nephropathy.

11. A method of monitoring transplant rejection in a subject, comprising the steps of:

(a) taking as a first baseline value the magnitude of gene expression of a combination of a plurality of genes in a sample obtained from a transplanted subject who is known not to develop rejection;

(b) taking as a second value the magnitude of gene expression of the same combination of a the plurality of genes in a sample obtained from a the transplanted subject post-transplantation; and

(c) comparing the first baseline value with the second value, wherein a first baseline value lower or higher than the second value predicts that the transplanted subject is at risk of developing rejection, wherein the combination of the plurality of genes are selected from the group consisting of the genes set forth in Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, thereby monitoring transplant rejection in the subject.

12. A method of monitoring transplant rejection in a subject, comprising the steps of:

(a) taking as a first value a pattern of gene expression corresponding to a combination of a plurality of genes from a sample obtained from a donor subject at the day of transplantation;

(b) taking as a second value a pattern of gene expression corresponding to the combination of the plurality of genes from a sample obtained from a recipient subject post-transplantation; and

(c) comparing the first value with the second value, wherein a first value lower or higher than the second value predicts that the recipient subject is at risk of developing rejection; wherein the a combination of the plurality of genes selected from the group consisting of the genes set forth in Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, thereby monitoring transplant rejection in the subject.

13. A method for monitoring modifying transplant rejection treatment in a subject at risk thereof, comprising the steps of:

(a) obtaining a pre-administration sample or samples from a transplanted subject prior to administration of a rejection inhibiting agent;

(b) detecting the pattern of gene expression of a plurality of genes in the pre-administration sample or samples; and

(c) obtaining a post-administration sample or samples from the transplanted subject;

(d) detecting the pattern of gene expression of a the plurality of genes in the post-administration sample or samples;

(e) comparing the pattern of gene expression of the plurality of genes in the pre-administration sample with the pattern of gene expression in the post-administration sample or samples; and

(f) adjusting the agent accordingly, wherein the plurality of genes are selected from the group consisting of the genes Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, thereby modifying transplant refection treatment.

14. A method for preventing, inhibiting, reducing or treating transplant rejection in a subject in need of such treatment comprising administering to the subject a compound that modulates the synthesis, expression or activity of one or more genes or gene products encoded thereby, said genes being selected from the group consisting of the genes set forth in Table 4, Table 5, Table 6, Table 7, and Table 8 in combination with a predictive model selected from the group consisting of a PLDSA model and an OPLS model, such that at least one symptom of rejection is ameliorated.

15. (canceled)

16. The method according to claim **1**, wherein the transplanted subject is a kidney transplanted subject.

17. The method according to of claim **1**, wherein the magnitude of gene expression is assessed by detecting the presence of a protein encoded by the combination of the plurality of genes.

18. The method of claim **17**, wherein the presence of the protein is detected using a reagent which specifically binds to the protein.

19. The method of claim **12**, wherein the pattern of gene expression is detected by techniques selected from the group consisting of Northern blot analysis, reverse transcription PCR and real time quantitative PCR.

20-24. (canceled)

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专利名称(译)	慢性同种异体肾病的预测性生物标志物		
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[标]申请(专利权)人(译)	SCHERER ANDREAS		
申请(专利权)人(译)	SCHERER ANDREAS		
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摘要(译)

本发明涉及在移植排斥中调节的基因的分析和鉴定。这种基因表达的改变提供了准确检测移植排斥的分子特征。