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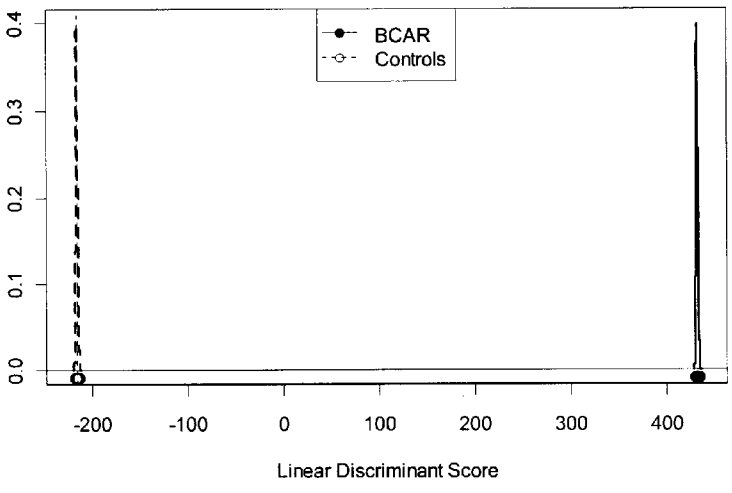
[Continued on next page]

(54) Title: METHODS OF DIAGNOSING REJECTION OF A KIDNEY ALLOGRAFT USING GENOMIC OR PROTEOMIC EXPRESSION PROFILING

Figure 1A



(57) Abstract: A method of determining the acute allograft rejection status of a subject, the method comprising the steps of: determining the nucleic acid expression profile of one or more than one nucleic acid markers, or one or more than one proteomic markers in a biological sample from the subject; comparing the expression profile of the one or more than one nucleic acid markers to a control profile; and determining whether the expression level of the one or more than one nucleic acid markers is increased relative to the control profile, wherein the increase of the one or more than one nucleic acid markers is indicative of the acute rejection status of the subject.

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METHODS OF DIAGNOSING REJECTION OF A KIDNEY ALLOGRAFT USING GENOMIC OR PROTEOMIC EXPRESSION PROFILING

This application claims priority benefit of U.S. Provisional application 61/129,022, filed May 30, 2008, the contents of which is herein incorporated by reference.

[0001] FIELD OF INVENTION

[0002] The present invention relates to methods of diagnosing rejection of a kidney allograft using genomic expression profiling or proteomic expression profiling.

BACKGROUND OF THE INVENTION

[0003] Transplantation is considered the primary therapy for patients with end-stage vital organ failure. While the availability of immunosuppressants such as cyclosporine and tacrolimus has improved allograft recipient survival and wellbeing, identification of rejection of the allograft as early and as accurately as possible, and effective monitoring and adjusting immunosuppressive medication doses is still of primary importance to the continuing survival of the allograft recipient.

[0004] Rejection of an allograft results from a recipient's immune response to nonself antigens expressed by the donor tissues, and may occur with hours or days of receiving the allograft, or months to years later. Renal allograft rejection is characterized by features comprising oliguria, rapid deterioration of renal function and mild proteinuria. Renal allograft rejection can lead to nephropathy and kidney failure.

[0005] At present, invasive biopsies (e.g. endomyocardial, liver core, and renal fine-needle aspiration) are regarded as the gold standard for the surveillance and diagnosis of allograft rejections, but are invasive procedures which carry risks of their own (e.g. Mehra MR, et al. Curr. Opin. Cardiol. 2002 Mar;17(2):131-136.). Biopsy results may also be subject to reproducibility and interpretation issues due to sampling errors and inter-observer variabilities, despite the availability of international guidelines such as the Banff schema for grading kidney and liver allograft rejection (Solez et al 2008 Am J Transplant 8: 753; Table 1) An allograft recipient may be exposed to the biopsy procedure multiple times in the first year following the transplant. Noninvasive surveillance techniques are currently used (the increase in blood creatinine levels), however serum creatinine levels are non-specifically reflective of kidney

injury. The kidney injury can be from rejection, infection, or even recurrence of the original disease, thus, the test is not specific for rejection.

[0006] Indicators of allograft rejection may include a heightened and localized immune response as indicated by one or more of localized or systemic inflammation, tissue injury, allograft infiltration of immune cells, inflammatory cells which recognize donor-specific antigens on the graft, allospecific antibodies, cytotoxic T-cell activation, altered composition and concentration of tissue- and blood- derived proteins, differential oxygenation of allograft tissue, edema, infection, necrosis of the allograft and/or surrounding tissue, and the like.

[0007] Allograft rejection may be described as 'acute' or 'chronic'. Acute rejection (also known as acute antibody-mediated rejection, AMR or active rejection) is generally considered to be rejection of a tissue or organ allograft within ~6-12 months of the subject receiving the allograft. Rejection or acute rejection may be characterized by cellular and humoral insults on the donor tissue, leading to rapid graft dysfunction and failure of the tissue or organ. Rejection of a tissue or organ allograft beyond 6-12 months is generally considered to be chronic rejection, and may occur several years after receiving the allograft. Such late or chronic rejection may be the result of sub-clinical or not fully resolved acute rejection episodes. Later-onset or chronic rejection may be characterized by progressive tissue remodeling triggered by the alloimmune response may lead to gradual neointimal formation within arteries, contributing to obliterative vasculopathy, parenchymal fibrosis and consequently, failure and loss of the graft. Depending on the nature and severity of the rejection, there may be overlap in the indicators or clinical variables observed in a subject undergoing, or suspected of undergoing, allograft rejection – either chronic or acute.

[0008] The scientific and patent literature is blessed with reports of this marker or that being important for identification/diagnosis/prediction/treatment of every medical condition that can be named. Even within the field of allograft rejection, a myriad of markers are recited (frequently singly), and conflicting results may be presented. This conflict in the literature, added to the complexity of the genome (estimates range upwards of 30,000 transcriptional units), the variety of cell types (estimates range upwards of 200), organs and tissues, and expressed proteins or polypeptides (estimates range upwards of 80,000) in the human body, renders the number of possible nucleic acid sequences, genes, proteins, metabolites or combinations thereof useful for diagnosing acute organ rejection is staggering. Variation between individuals presents

additional obstacles, as well as the dynamic range of protein concentration in plasma (ranging from 10^{-6} to 10^{3} µg/ mL) with many of the proteins of potential interest existing at very low concentrations) and the overwhelming quantities of the few, most abundant plasma proteins (constituting ~ 99% of the total protein mass.

[0009] PCT Publication WO 2006/125301 discloses nucleic acids that are differentially expressed in transplanted tissue, and methods and materials for detecting kidney tissue rejection.

[0010] US 7235258 discloses methods of diagnosing or monitoring transplant rejection, including kidney transplant rejection in a subject, by detecting the expression level of one or more genes in the subject. Oligonucleotides useful in these methods are also described.

[0011] Flechner et al. (Am J Transplant 2004: 4 (9) 1475-1489) identifies several publications that employed DNA or microarrays to identify differential expression of various genes in subjects receiving kidney transplants, and also describes use of microarray analysis and RT-PCR to examine gene expression profile of peripheral blood lymphocytes and kidney biopsy samples from kidney transplant subjects, and identified over 60 genes that were differentially expressed.

[0012] Alakulppi et al, 2007 (Transplantation 83:791-798) discloses the diagnosis of acute renal allograft rejection using RT-PCT for eight nucleic acid markers. Further investigations by Alakulppi et al. (2008, Transplantation 86:1222-8) were unable to identify a robust whole blood gene expression nucleic acid marker for subclinical rejection.

[0013] Sarwal et al. 2003 (N. Engl. J. Med 349:125) reported that genes associated with apoptosis were increased in renal biopsies during acute rejection and found transcript groups indicating lymphocyte infiltration and activation driven by NF-kappaB and IFNγ.

[0014] Mueller et al., 2007. Am J. Transplant 7:2712 identified transcripts in the kidney tissue associated with cytotoxic T-lymphocytes, IFNγ signaling, and epithelial cell injury in both mouse and human.

[0015] Mehra et al., 2008 suggests that pathways regulating T-cell homeostatis and corticosteroid sensitivity may be associated with future acute rejection of cardiac transplants, but offers no comment with respect to kidney transplantation. Expression of ITGAX is one of the 33 genes addressed.

[0016] A review by Fildes et al 2008 (Transplant Immunology 19:1-11) discusses the role of cell types in immune processes following lung transplantation, and discloses that AICL (CLEC2B) interaction with NK cell proteins may have a role in acute and chronic rejection.

[0017] Integration of multiple platforms (proteomics, genomics) has been suggested for diagnosis and monitoring of various cancers, however discordance between protein and mRNA expression is identified in the field (Chen et al., 2002.Mol Cell Proteomics1:304-313; Nishizuka et al., 2003 Cancer Research 63:5243-5250). Previous studies have reported low correlations between genomic and proteomic data (Gygi SP et al. 1999. *Mol Cell Biol.*19:1720-1730; Huber et al., 2004 Mol Cell Proteomics 3:43-55).

[0018] Several studies have been done looking at the urine proteome of kidney transplant recipients (reviewed in Schaub et al., 2008. Contrib. Nephrol 160:65-75.

[0019] Bottelli et al., 2008 (J. Am Soc Nephrol 19:1904-18) teaches that macrophage stimulating protein (MSP) is upregulated during regeneration of injured tubule cells, and suggests that it may aid recovery from acute kidney injury. Gorgi et al. (2009 Transplantation Proceedings 41:660-662) investigated the association between acute kidney transplant rejection, and a polymorphism of the MBL gene, and concluded that the polymorphism could be involved in susceptibility to acute allograft rejection in the study population. Fiane et al., 2005 (Eur Heart J 26:1660-5) disclosed that a low MBL level was related to the development of acute rejection in cardiac transplant recipients. Fildes 2008 (J. Heart Lung Transplant 27:1353-1356) teaches that heart transplant recipients with MBL deficiency had fewer rejection episodes. Neither Fiane nor Fildes offers comment with respect to kidney transplants.

[0020] Berger et al., 2005 (Am J. Transplant 5:1361-1366) teaches that higher MBL (Mannose-binding lectin) may be associated with a more severe form of rejection in kidney transplant recipients, and suggests that pre-transplantation MBL levels may be useful for risk stratification prior to kidney transplantation.

[0021] Methods of assessing or diagnosing allograft rejection that are less invasive, repeatable and more robust (less susceptible to sampling and interpretation errors) are greatly desirable.

SUMMARY OF THE INVENTION

[0022] The present invention relates to methods of diagnosing rejection of a kidney allograft using genomic expression profiling or proteomic expression profiling of one or more biological samples obtained from a subject.

[0023] The biological sample may be a blood or a plasma sample; use of such samples in the methods described herein provides an advantage over biopsy-based assessment and/or monitoring of kidney allograft rejection (including acute rejection) as such samples may be obtained in a minimally invasive manner (a peripheral blood sample, for example), with no requirement for biopsy of the allograft. Use of a blood or plasma sample provides a further advantage, in that it may reduce sampling error, and detection of proteomic or nucleic acid markers may be less subject to interpretation – the marker is present or it is not, or it is increased or decreased relative to a baseline, control or the like as described herein.

[0024] Some current surveillance techinques that do employ blood sampling (e.g. serum creatine levels) may not be specific for rejection; the nucleic acid or proteomic markers described herein, when obtained from a blood or plasma sample are specific for acute kidney allograft rejection, thus provide a further advantage of specificity.

[0025] The complex pathobiology of acute kidney allograft rejection is reflected in the heterogeneity of markers identified herein. Markers identified herein distribute over a range of biological processes: immune signal transduction, cytoskeletal reorganization, apoptosis, T-cell activation and proliferation, cellular and humoral immune responses, acute phase inflammatory pathways, and the like.

[0026] In accordance with another aspect of the invention, there is provided a method of determining the acute allograft rejection status of a subject, the method comprising the steps of: a) determining the nucleic acid expression profile of one or more than one nucleic acid markers in a biological sample from the subject, the nucleic acid markers selected from the group comprising TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX; b) comparing the expression profile of the one or more than one nucleic acid markers to a control profile; and c) determining whether the expression level of the one or more than one nucleic acid markers is increased relative to the

control profile; wherein the increase of the one or more than one nucleic acid markers is indicative of the acute rejection status of the subject.

[0027] In some aspects the biological sample is blood or plasma.

[0028] In some aspects, the group of nucleic acid markers further comprises one or more than one of SFRS16, NFYC, NCOA3, PGS1, NEDD9, LIMK2, NASP, 240057_at, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6.

[0029] In some aspects, the control profile is obtained from a non-rejecting, allograft recipient subject or a non-allograft recipient subject.

[0030] In some aspects, the method further comprises obtaining a value for one or more clinical variables.

[0031] In some aspects, the method further comprises at step a) determining the expression profile of one or more than one of the nucleic acid markers selected from Table 2.

[0032] In some aspects, the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by detecting an RNA sequence corresponding to one or more than one markers.

[0033] In some aspects, the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by PCR.

[0034] In some aspects, the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by hybridization. The hybridization may be to an oligonucleotide.

[0035] In some aspects the control is an autologous control.

[0036] In accordance with another aspect of the invention, there is provided a method of determining acute allograft rejection status of a subject, the method comprising the steps of: a) determining a proteomic expression profile of proteomic markers in a biological sample from the subject, the proteomic markers including a polypeptide encoded by one or more than one of KNG1, AFM, TTN, MSTP9/MST1, PI16, C2, MBL2, SERPINA10, F9 and UBR4; b) comparing the expression profile of the proteomic markers to a control profile; and c) determining whether the expression level of the one or more than one proteomics markers is

increased or decreased relative to the control profile; wherein the increase or decrease of the five or more proteomic markers is indicative of the acute rejection status of the subject.

[0037] In some aspects the biological sample is blood or plasma.

[0038] In some aspects,the level of polypeptides encoded by one or more than one of KNG1 and AFM are decreased relative to a control, and the level of polypeptides encoded by one or more than one of TTN, MSTP9, MST1, PI16, C2, MBL2, SERPINA10, F9 and UBR4 are increased relative to a control profile.

[0039] In some aspects the control profile is obtained from a non rejecting, allograft recipient subject or a non-allograft recipient subject.

[0040] In some aspects, the method further comprises obtaining a value for one or more clinical variables.

[0041] In some aspects, the proteomic expression profile is determined by an immunologic assay.

[0042] In some aspects, the proteomic expression profile is determined by ELISA.

[0043] In some aspects the proteomic expression profile is determined by mass spectrometry.

[0044] In some aspects the proteomic expression profile is determined by an isobaric or isotope tagging method.

[0045] In some aspects the proteomic markers further include a polypeptide encoded by one or more than one of LBP, VASN, ARNTL2, PI16, SERPINA5, CFD, USH1C, C9, LCAT, B2M, SHBG and C1S.

[0046] In some aspects the control is an autologous control.

[0047] In accordance with another aspect of the invention, there is provided a method of determining acute allograft rejection status of a subject, the method comprising the steps of: a. determining a proteomic expression profile of proteomic markers in a biological sample from the subject, the proteomic markers including a polypeptide included in one or more than one of protein group codes 111, 224, 23, 18, 100, 116, 38, 135, 125; b. comparing the expression profile of the proteomic markers to a control profile; and c. determining whether the expression

level of the one or more than one proteomics markers is increased or decreased relative to the control profile; wherein the increase or decrease of the five or more proteomic markers is indicative of the acute rejection status of the subject.

[0048] In some aspects the protein group codes further includes one or more than one of groups 18, 108, 222, 97, 104, 26, 230, 103, 69 or 29.

[0049] In some aspects the biological sample is blood or plasma.

[0050] In some aspects,the level of polypeptides encoded by one or more than one of KNG1 and AFM are decreased relative to a control, and the level of polypeptides encoded by one or more than one of TTN, MSTP9, MST1, PI16, C2, MBL2, SERPINA10, F9 andUBR4 are increased relative to a control profile.

[0051] In some aspects the control profile is obtained from a non rejecting, allograft recipient subject or a non-allograft recipient subject.

[0052] In some aspects, the method further comprises obtaining a value for one or more clinical variables.

[0053] In some aspects, the proteomic expression profile is determined by an immunologic assay.

[0054] In some aspects, the proteomic expression profile is determined by ELISA.

[0055] In some aspects the proteomic expression profile is determined by mass spectrometry.

[0056] In some aspects the proteomic expression profile is determined by an isobaric or isotope tagging method.

[0057] In some aspects the proteomic markers further include a polypeptide encoded by one or more than one of LBP, VASN, ARNTL2, PI16, SERPINA5, CFD, USH1C, C9, LCAT, B2M, SHBG and C1S.

[0058] In some aspects the control is an autologous control.

[0059] In accordance with another aspect of the invention, there is provided an array comprising one or more probe sets for one or more than one of the nucleic acid markers TncRNA, FKSG49,

ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073, ITGAX.

[0060] In some aspects, the array further comprises one or more additional probe sets for one or more than one of the nucleic acid markesrs , SFRS16, NFYC, NCOA3, PGS1, NEDD9, LIMK2, NASP, 240057_at, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6.

[0061] In some aspects, the array further comprises one or more additional probe sets for the nucleic acid markers of Table2.

[0062] In accordance with another aspect of the invention, there is provided an array comprising one or more detection reagents for one or more than one of the proteomic markers KNG1, AFM, TTN, MSTP9, MST1, PI16, C2, MBL2, SERPINA10, F9 and UBR4.

[0063] In some aspects, the array further comprises one or more additional detection reagents for one or more than one of LBP, VASN, ARNTL2, PI16, SERPINA5, CFD, USH1C, C9, LCAT, B2M, SHBG and C1S.

[0064] In accordance with another aspect of the invention, there is provided a method of assessing, monitoring or diagnosing kidney allograft rejection in a subject, the method comprising: a) determining the expression profile of at least one or more nucleic acid markers presented in Table 2 in a biological sample from the subject; b) comparing the expression profile of the at least one or more markers to a non-rejector profile; and c) determining whether the expression level of the at least one or more markers is up-regulated (increased) or down-regulated (decreased) relative to the control profile, wherein up-regulation or down-regulation of the at least one or more markers is indicative of the rejection status.

[0065] In some embodiments, the method further comprises obtaining a value for one or more clinical variables and comparing the one or more clinical variables to a control. The control is a non-rejection, allograft recipient subject or a non-allograft recipient subject. In some embodiments, the rejection is acute rejection. In some embodiments, the one or more nucleic acid markers includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleic acid markers selected from those presented in Table 2. In some embodiments, the nucleic acid markers may include one or more than one of the nucleic acid markers presented in Table 5.

[0066] In accordance with another aspect of the invention, there is provided a kit for assessing or diagnosing kidney allograft rejection in a subject, the kit comprising reagents for specific and quantitative detection of at least one or more markers presented in Table 2, along with instructions for the use of such reagents and methods for analyzing the resulting data. The kit may further comprise one or more oligonucleotides for selective hybridization to one or more of a gene, transcript or sequence unit representing one or more of the markers. Instructions or other information useful to combine the kit results with those of other assays to provide a non-rejection cutoff index or control for the diagnosis of a subject's rejection status may also be provided in the kit.

[0067] In some embodiments, the kit may further comprise instructions or materials for obtaining a value for one or more clinical variables and comparing the one or more clinical variables to a control. The control is a non-rejection, allograft recipient subject or a non-allograft recipient subject. In some embodiments, the rejection is acute rejection. In some embodiments, the one or more nucleic acid markers includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleic acid markers selected from those presented in Table 2. In some embodiments, the nucleic acid markers may include one or more than one of the nucleic acid markers presented in Table 5.

[0068] This summary of the invention does not necessarily describe all features of the invention. Other aspects, features and advantages of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0069] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0070] **Figure 1** shows the results of a subject classification using a panel of 24 nucleic acid biomarkers (presented in Table 5). Subjects were determined to have . A) 24-probe-set classifier; B) Zoomed-in view of A) to more clearly illustrate the Gaussian peaks and samples below. For A and B, acute rejection – solid circle; no rejection – open circle. C) The same dataset as in A and B, displaying the data in the same format as for Figure 2. Acute rejection (solid diamond) or no rejection (solid circle)

[0071] **Figure 2** shows the result of a subject classification using only clinical parameters (serum creatinine, GFR, BUN). Subjects were determined to have acute rejection (solid diamond) or no rejection (solid circle). .

[0072] **Figure 3:** Differential expression of probe-sets between subjects with and without BCAR detected by micro-array analysis. Points in grey indicate the probe-sets identified by LIMMA alone, while those in black indicate the 183 probe-sets identified by the intersection of LIMMA, robust LIMMA and SAM. Circles indicate the 24 probe-sets included in the primary classifier.

[0073] **Figure 4:** Principal component analysis showing separation of same subject groups, demonstrating that the centroids of all groups are clearly separated. AR – acute rejector; NR – non-rejector; N – normal control (20 non-recipient subjects).The percentage variance as explained by the principal components are provide on the X axis (56%) and Y axis (12%).

[0074] **Figure 5**. Gene ontologies and network analysis of 183 probe sets differentially expressed in BCAR. The x-axis shows –log10 (p-values). **A** Most significantly enriched Gene ontology categories ("Biological processes"), sorted by increasing p-value. **B** Most significantly enriched Gene ontology categories (GeneGO MetaCore Biological Categories), sorted by increasing p-value.

[0075] **Figure 6.** Performance of classifier. **(A)** Incremental classification accuracy demonstrating step-wise inclusion of 11 common most highly predictive probe-sets. Y-axis – classification accuracy; X-axis, biomarkers. **(B)** Linear discriminant analysis showing performance of 11 probe-set classifiers in distinguishing cases with (•, solid line) and without (°, stippled line) BCAR (biopsy-confirmed acute rejection). **(C)** Change in classifier score post-transplant relative to individual pre-transplant (baseline) value. The difference between cohorts is significant only at the time of rejection (week 1) (p=0.0001). Y axis – change from baseline (mean +/- 2 se); X axis BL- baseline; W1-W12, week 1 – week 12. . "START" indicates the beginning of the tsep-wise analysis where there are no probe-set classifiers.

[0076] **Figure 7**:Volcano plot showing all 144 protein group codes that were found in at least two thirds of the BCAR positive samples and two thirds of the BCAR negative samples. Circled points indicate the 18 protein groups whose plasma concentration differed significantly ($p<0.05$) between subjects with or without BCAR.

[0077] **Figure 8**: Linear discriminant analysis showing separation of patients with or without BCAR based upon plasma protein biomarkers. Solid line/ "X" – BCAR subjects; stippled line/ " | " – control (non-rejector) subjects.

[0078] **Figure 9**: Estimated classification accuracy demonstrating step-wise inclusion of protein groups as chosen by forward-selection stepwise discriminant analysis (SDA). Y Axis – classification accuracy; X axis – PGC codes. "START" is as for Figure 6.

[0079] **Figure 10** shows target sequences of (SEQ ID NO: 1-183) of nucleic acid markers useful for diagnosis of acute kidney allograft rejection, listed in Table 2.

DETAILED DESCRIPTION

[0080] The present invention provides for methods of diagnosing rejection in a subject that has received a tissue or organ allograft, specifically a kidney allograft.

[0081] The present invention provides genomic and proteomic expression profiles related to the assessment, prediction or diagnosis of allograft rejection in a subject. While several of the elements in the genomic or proteomic expression profiles may be individually known in the existing art, the specific combination of the altered expression levels (increased or decreased relative to a control) of specific sets of genomic, T-cell, proteomic or metabolite markers comprise a novel combination useful for assessmentf or diagnosis or allograft rejection in a subject.

[0082] An allograft is an organ or tissue transplanted between two genetically different subjects of the same species. The subject receiving the allograft is the 'recipient', while the subject providing the allograft is the 'donor'. A tissue or organ allograft may alternately be referred to as a 'transplant', a 'graft', an 'allograft', a 'donor tissue' or 'donor organ', or similar terms. A transplant between two subjects of different species is a xenograft.

[0083] Subjects may present with a variety of symptoms or clinical variables well-known in the literature as an aid for monitoring allograft rejection. A myriad of clinical variables may be used in assessing a subject having, or suspected of having, allograft rejection, in addition to biopsy of the allograft. The information from these clinical variables is then used by a clinician, physician, veterinarian or other practitioner in a clinical field in attempts to determine if rejection is

occurring, and how rapidly it progresses, to allow for modification of the immunosuppressive drug therapy of the subject. Examples of clinical variables are presented in Table1.

[0084] Clinical variables (optionally accompanied by biopsy), while currently the only practical tools available to a clinician in mainstream medical practice, are not always able to cleanly differentiate between rejecting and a non-rejecting subject, as is illustrated in Figure 2. While the extreme left and right subjects are correctly classified as rejecting or non-rejecting, the bulk of the subjects are represented in the middle range and their status is unclear. This does not negate the value of the clinical variables in the assessment of allograft rejection, but instead indicates their limitation when used in the absence of other methods.

[0085] Table 1: Clinical variables for possible use in assessment of allograft rejection.

Clinical Variable Name	Renal/Heart / Liver/ All	Variable Explanation
Primary Diagnosis	All	Diagnosis leading to transplant
Secondary Diagnosis	All	Diagnosis leading to transplant
"Transplant Procedure - Living related, Living unrelated, or cadaveric"		
Blood Type	All	Blood Type
Blood Rh	All	Blood Rh
Height (cm)	All	Height (cm)
Weight (kg)	All	Weight (kg)
BMI	All	Calculation: Weight / (Height)2
Liver Ascites	All	
HLA A1	All	
HLA A2	All	
HLA B1	All	
HLA B2	All	
HLA DR1	All	
HLA DR2	All	
CMV	All	Viral Status
CMV Date	All	Date of viral status
HIV	All	Viral Status
HBV	All	Viral Status
HBV Date	All	Date of viral status
HbsAb	All	Viral Status

HbcAb (Total)	All	Viral Status
HBvDNA	All	Viral Status
HCV	All	Viral Status
HCV Genotype	All	Hepatitis C genotype
HCV Genotype Sub	All	"Hepatitis C genotype, subtype"
EBV	All	Viral Status
Zoster	All	Viral Status
Dialysis Start Date	All	Dialysis Start Date
Dialysis Type	All	Dialysis Type
Cytoxicity Current Level	All	
Cytoxicity Current Date	All	
Cytoxicity Peak Level	All	
Cytoxicity Peak Date	All	
Flush Soln	All	Type of Flush Solution used at transplant
Cold Time 1	All	
Cold Time 2	All	
Re-Warm Time 1	All	
Re-Warm Time 2	All	
HTLV 1	All	
HTLV 2	All	
HCV RNA	All	
24hr Urine	All	24 Hour urine output
Systolic Blood Pressure	All	Blood Pressure reading
Diastolic Blood Pressure	All	Blood Pressure reading
24 Hr Urine	All	24 hour urine
Sodium	All	Blood test
Potassium	All	Blood test
Chloride	All	Blood test
Total CO2	All	Blood test
Albumin	All	Blood test
Protein	All	Blood test
Calcium	All	Blood test
Inorganic Phosphate	All	Blood test
Magnesium	All	Blood test
Uric Acid	All	Blood test
Glucose	All	Blood test
Hemoglobin A1C	All	Blood test
CPK	All	Blood test
Parathyroid Hormone	All	Blood test

Homocysteine	All	Blood test
Urine Protein	All	Urine test
Creatinine	All	Blood test
BUN	All	Blood test
Hemoglobin	All	Blood test
Platelet Count	All	Blood test
WBC Count	All	Blood test
Prothrombin Time	All	Blood test
Partial Thromboplastin Time	All	Blood test
INR	All	Blood test
Gamma GT	All	Blood test
AST	All	Blood test
Alkaline Phosphatase	All	Blood test
Amylase	All	Blood test
Total Bilirubin	All	Blood test
Direct Bilirubin	All	Blood test
LDH	All	Blood test
ALT	All	Blood test
Triglycerides	All	Blood test
Cholesterol	All	Blood test
HDL Cholesterol	All	Blood test
LDL Cholesterol	All	Blood test
FEV1	All	Lung function test
FVC	All	Lung function test
Total Ferritin	All	Blood test
TIBC	All	Blood test
Transferrin Saturated	All	Blood test
Ferritin	All	Blood test
Angiography	Heart	Heart function test
Intravascular ultrasound	Heart	Heart function test
Dobutamine Stress Echocardiography	Heart	Heart function test
Cyclosporine WB	All	Immunosuppressive levels
Cyclosporine 2 hr	All	Immunosuppressive levels
Tacrolimus WB	All	Immunosuppressive levels
Sirolimus WB	All	Immunosuppressive total daily dose
Solumedrol	All	Immunosuppressive total daily dose
Prednisone	All	Immunosuppressive total daily dose
Prednisone ALT	All	Immunosuppressive total daily dose
Tacrolimus	All	Immunosuppressive total daily dose

Cyclosporine	All	Immunosuppressive total daily dose
Imuran	All	Immunosuppressive total daily dose
Mycophonelate Mofetil	All	Immunosuppressive total daily dose
Sirolimus	All	Immunosuppressive total daily dose
OKT3	All	Immunosuppressive total daily dose
ATG	All	Immunosuppressive total daily dose
ALG	All	Immunosuppressive total daily dose
Basiliximab	All	Immunosuppressive total daily dose
Daclizumab	All	Immunosuppressive total daily dose
Ganciclovir	All	Anti-viral total daily dose
Lamivudine	All	Anti-viral total daily dose
Riboviron	All	Anti-viral total daily dose
Interferon	All	Anti-viral total daily dose
Hepatitis C Virus RNA	All	test for presence of HCV values ()
CMV Antigenemia	All	Antiviral and Virus
Valganciclovir	All	Anti-viral total daily dose
Neutrophil Number	All	Blood test
C Peptide	All	Blood test
Peg Interferon	All	Anti-viral total daily dose
GFR	All	Glomerular Filtration Rate
Complication Events	All	Complication Type
Biopsy Scores	Renal	Borderline 1A, 1B, 2A, 2B, 3 Hyperacute
Biopsy Scores	Liver	Portal inflammation, Bile duct inflammation damage, Venous endothelial inflammation, each scored from 1-2
Donor Blood Type	All	Donor Blood Type
Donor Blood Rh	All	Donor Rh
Donor HLA A1	All	Donor HLA A1
Donor HLA A2	All	Donor HLA A2
Donor HLA B1	All	Donor HLA B1
Donor HLA B2	All	Donor HLA B2
Donor HLA DR1	All	Donor HLA DR1
Donor HLA DR2	All	Donor HLA DR2
Donor CMV	All	Donor CMV
Donor HIV	All	Donor HIV
Donor HBV	All	Donor HBV
Donor HbsAb	All	Donor HbsAb
Donor HbcAb (total)	All	Donor HbcAb (total)

Donor Hbdna	All	Donor Hbdna
Donor HCV	All	Donor HCV
Donor EBV	All	Donor EBV

[0086] The multifactorial nature of allograft rejection prediction, diagnosis and assessment is considered in the art to exclude the possibility of a single biomarker that meets even one of the needs of prediction, diagnosis or assessment of allograft rejection. Strategies involving a plurality of markers may take into account this multifactorial nature. Alternately, a plurality of markers may be assessed in combination with clinical variables that are less invasive (e.g. a biopsy not required) to tailor the prediction, diagnosis and/or assessment of allograft rejection in a subject.

[0087] Regardless of the methods used for prediction, diagnosis and assessment of allograft rejection, earlier is better – from the viewpoint of preserving organ or tissue function and preventing more systemic detrimental effects. There is no 'cure' for allograft rejection, only maintenance of the subject at a suitably immunosuppressed state, or in some cases, replacement of the organ if rejection has progressed too rapidly or is too severe to correct with immunosuppressive drug intervention therapy.

[0088] Applying a plurality of mathematical and/or statistical analytical methods to a protein or polypeptide dataset, metabolite concentration data set, or nucleic acid expression dataset may indicate varying subsets of significant markers, leading to uncertainty as to which method is 'best' or 'more accurate'. Regardless of the mathematics, the underlying biology is the same in a dataset. By applying a plurality of mathematical and/or statistical methods to a microarray dataset and assessing the statistically significant subsets of each for common markers, uncertainty may be reduced, and clinically relevant core group of markers may be identified.

[0089] "Markers", "biological markers" or "biomarkers" may be used interchangeably and refer generally to detectable (and in some cases quantifiable) molecules or compounds in a biological sample. A marker may be down-regulated (decreased), up-regulated (increased) or effectively unchanged in a subject following transplantation of an allograft. Markers may include nucleic acids (DNA or RNA), a gene, or a transcript, or a portion or fragment of a transcript in reference to 'genomic' markers (alternately referred to as "nucleic acid markers"); polypeptides, peptides, proteins, isoforms, or fragments or portions thereof for 'proteomic' markers, or selected

molecules, their precursors, intermediates or breakdown products (e.g. fatty acid, amino acid, sugars, hormones, or fragments or subunits thereof). In some usages, these terms may reference the level or quantity of a particular protein, peptide, nucleic acid or polynucleotide, or metabolite (in absolute terms or relative to another sample or standard value) or the ratio between the levels of two proteins, polynucleotides, peptides or metabolites, in a subject's biological sample. The level may be expressed as a concentration, for example micrograms per milliliter; as a colorimetric intensity, for example 0.0 being transparent and 1.0 being opaque at a particular wavelength of light, with the experimental sample ranked accordingly and receiving a numerical score based on transmission or absorption of light at a particular wavelength; or as relevant for other means for quantifying a marker, such as are known in the art. In some examples, a ratio may be expressed as a unitless value. A "marker" may also reference to a ratio, or a net value following subtraction of a baseline value. A marker may also be represented as a 'fold-change', with or without an indicator of directionality (increase or decrease/ up or down). The increase or decrease in expression of a marker may also be referred to as 'down-regulation' or 'up-regulation', or similar indicators of an increase or decrease in response to a stimulus, physiological event, or condition of the subject. A marker may be present in a first biological sample, and absent in a second biological sample; alternately the marker may be present in both, with a statistically significant difference between the two. Expression of the presence, absence or relative levels of a marker in a biological sample may be dependent on the nature of the assay used to quantify or assess the marker, and the manner of such expression will be familiar to those skilled in the art.

[0090] A marker may be described as being differentially expressed when the level of expression in a subject who is rejecting an allograft is significantly different from that of a subject or sample taken from a non-rejecting subject. A differentially expressed marker may be overexpressed or underexpressed as compared to the expression level of a normal or control sample.

[0091] A "profile" is a set of one or more markers and their presence, absence, relative level or abundance (relative to one or more controls). For example, a metabolite profile is a dataset of the presence, absence, relative level or abundance of metabolic markers. A proteomic profile is a dataset of the presence, absence, relative level or abundance of proteomic markers. A genomic or nucleic acid profile a dataset of the presence, absence, relative level or abundance of expressed nucleic acids (e.g. transcripts, mRNA, EST or the like). A profile may alternately be referred to as an expression profile.

[0092] The increase or decrease, or quantification of the markers in the biological sample may be determined by any of several methods known in the art for measuring the presence and/or relative abundance of a gene product or transcript, or a nucleic acid molecule comprising a particular sequence, polypeptide or protein, metabolite or the like. The level of the markers may be determined as an absolute value, or relative to a baseline value, and the level of the subject's markers compared to a cutoff index (e.g. a non-rejection cutoff index). Alternately, the relative abundance of the marker may be determined relative to a control. The control may be a clinically normal subject (e.g. one who has not received an allograft) or may be an allograft recipient that has not or is not demonstrating rejection.

[0093] In some embodiments, the control may be an autologous control, for example a sample or profile obtained from the subject before undergoing allograft transplantation. In some embodiments, the profile obtained at one time point (before, after or before and after transplantation) may be compared to one or more than one profiles obtained previously from the same subject. By repeatedly sampling the same biological sample from the same subject over time, a composite profile, illustrating marker level or expression over time may be provided. Sequential samples can also be obtained from the subject and a profile obtained for each, to allow the course of increase or decrease in one or more markers to be followed over time For example, an initial sample or samples may be taken before the transplantation, with subsequent samples being taken weekly, biweekly, monthly, bimonthly or at another suitable, regular interval and compared with profiles from samples taken previously. Samples may also be taken before, during and after administration of a course of a drug, for example an immunosuppressive drug.

[0094] Techniques, methods, tools, algorithms, reagents and other necessary aspects of assays that may be employed to detect and/or quantify a particular marker or set of markers are varied. Of significance is not so much the particular method used to detect the marker or set of markers, but what markers to detect. As is reflected in the literature, tremendous variation is possible. Once the marker or set of markers to be detected or quantified is identified, any of several techniques may be well suited, with the provision of appropriate reagents. One of skill in the art, when provided with the set of markers to be identified, will be capable of selecting the appropriate assay (for example, a PCR based or a microarray based assay for nucleic acid markers, an ELISA, protein or antibody microarray or similar immunologic assay, or in some examples, use of an iTRAQ, iCAT or SELDI proteomic mass spectrometric based method) for performing the methods disclosed herein.

[0095] The present invention provides nucleic acid expression profiles and proteomic expression profiles related to the assessment or diagnosis of allograft rejection in a subject. While several of the elements in the genomic or T-cell expression profiles or proteomic expression profiles may be individually known in the existing art, the specific combination of the altered expression levels (increased or decreased relative to a control) of specific sets of genomic or proteomic markers comprise a novel combination useful for assessment or diagnosis of allograft rejection in a subject.

[0096] 183 probe sets were found to specifically detect (by hybridization and detection of a label) and allow for quantitation of the expression level of the expressed nucleic acids. Of this set of 183 (listed in Table 2), representing 183 individual expressed transcripts or nucleic acids, a subset of 24 probe sets (Table 5) were detected, quantified and found to demonstrate a statistically significant fold change in the AR samples relative to non-rejecting transplant (NR). Figure 10 provides nucleic acid sequence information of a portion of the nucleic acid identified by the probe sets listed in Tables 2 and 5. Sequences in Figure 10 (SEQ ID NO: 1-183) may be useful as probes for specific hybridization to the indicated gene (e.g. in a microarray, blot, or other hybridization based assay), or for the design of a primer or primers for specific amplification of the indicated gene (e.g. by PCR, RT-PCR or other amplification-based assay).

[0097] 18 significant protein group codes were found to have differential relative levels (relative to a reference sample) in AR and NR subjects, using a multiplexed iTRAQ methodology (Table 7). These protein group codes included proteomic markers encoded by one or more than one of TTN, KNG1, LBP, VASN, ARNTL2, AFM, MSTP9, MST1, PI16, SERPINA5, CFD, USH1C, C2, MBL2, SERPINA10, C9, LCAT, B2M, SHBG, C1S, UBR4 and F9. As described below, accession numbers providing specific reference to the nucleic acid sequences encoding these polypeptides, and the amino acid sequences of these polypeptides are provided herein. Unique identifiers (International Protein Index accession numbers) for each member of the indicated protein group codes are found in Table 7. Polypeptides comprising a portion of one or more of these sequences may be useful for the preparation of antibodies that specifically detect one or more of the proteomic markers, alternately, the sequences may be used to identify one or more proteomic markers in a sample subjected to tryptic digest and analysis by mass spectroscopy by comparison of the peptide fragments generated to the sequences, or to a database comprising such sequences.

[0098] Detection or determination, and in some cases quantification, of a nucleic acid may be accomplished by any one of a number methods or assays employing recombinant DNA technologies known in the art, including but not limited to, sequence-specific hybridization, polymerase chain reaction (PCR), RT-PCR, microarrays and the like. Such assays may include sequence-specific hybridization, primer extension, or invasive cleavage. Furthermore, there are numerous methods for analyzing/detecting the products of each type of reaction (for example, fluorescence, luminescence, mass measurement, electrophoresis, etc.). Furthermore, reactions can occur in solution or on a solid support such as a glass slide, a chip, a bead, or the like.

[0099] Methods of designing and selecting probes for use in microarrays or biochips, or for selecting or designing primers for use in PCR-based assays are known in the art. Once the marker or markers are identified and the sequence of the nucleic acid determined by, for example, querying a database comprising such sequences, or by having an appropriate sequence provided (for example, a sequence listing as provided herein), one of skill in the art will be able to use such information to select appropriate probes or primers and perform the selected assay.

[00100] Standard reference works setting forth the general principles of recombinant DNA technologies known to those of skill in the art include, for example: Ausubel et al, Current Protocols In Molecular Biology, John Wiley & Sons, New York (1998 and Supplements to 2001); Sambrook et al, Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Plainview, New York (1989); Kaufman et al , Eds., Handbook Of Molecular And Cellular Methods In Biology And Medicine, CRC Press, Boca Raton (1995); McPherson, Ed., Directed Mutagenesis: A Practical Approach, IRL Press, Oxford (1991).

[00101] Proteins, protein complexes or proteomic markers may be specifically identified and/or quantified by a variety of methods known in the art and may be used alone or in combination. Immunologic- or antibody-based techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), western blotting, immunofluorescence, microarrays, some chromatographic techniques (i.e. immunoaffinity chromatography), flow cytometry, immunoprecipitation and the like. Such methods are based on the specificity of an antibody or antibodies for a particular epitope or combination of epitopes associated with the protein or protein complex of interest. Non-immunologic methods include those based on physical characteristics of the protein or protein complex itself. Examples of such methods include electrophoresis, some chromatographic techniques (e.g. high performance liquid chromatography

(HPLC), fast protein liquid chromatography (FPLC), affinity chromatography, ion exchange chromatography, size exclusion chromatography and the like), mass spectrometry, sequencing, protease digests, and the like. Such methods are based on the mass, charge, hydrophobicity or hydrophilicity, which is derived from the amino acid complement of the protein or protein complex, and the specific sequence of the amino acids. Exemplary methods include those described in, for example, PCT Publication WO 2004/019000, WO 2000/00208, US 6670194. Immunologic and non-immunologic methods may be combined to identify or characterize a protein or protein complex. Furthermore, there are numerous methods for analyzing/detecting the products of each type of reaction (for example, fluorescence, luminescence, mass measurement, electrophoresis, etc.). Furthermore, reactions can occur in solution or on a solid support such as a glass slide, a chip, a bead, or the like.

[00102] Methods of producing antibodies for use in protein or antibody arrays, or other immunology based assays are known in the art. Once the marker or markers are identified and the amino acid sequence of the protein or polypeptide is identified, either by querying of a database or by having an appropriate sequence provided (for example, a sequence listing as provide herein), one of skill in the art will be able to use such information to prepare one or more appropriate antibodies and perform the selected assay.

[00103] For preparation of monoclonal antibodies directed towards a biomarker, any technique that provides for the production of antibody molecules may be used. Such techniques include, but are not limited to, hybridomas or triomas (e.g. Kohler and Milstein 1975, Nature 256:495-497; Gustafsson et al., 1991, Hum. Antibodies Hybridomas 2:26-32), human B-cell hybridoma or EBV hybridomas e.g. (Kozbor et al., 1983, Immunology Today 4:72;;Cole et al., 1985, In: Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Human, or humanized antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026- 2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, In: Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Techniques developed for the production of "chimeric antibodies" (Morrison et al, 1984, Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger et al, 1984, Nature 312:604-608; Takeda et al, 1985, Nature 314:452-454) by splicing a sequence encoding a mouse antibody molecule specific for a particular biomarker together with a sequence encoding a human antibody molecule of appropriate biological activity may be used; such antibodies are within the scope of this invention. Techniques described for the production of single chain antibodies (U.S.

Patent 4,946,778) may be adapted to produce a biomarker -specific antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al, 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for a biomarker proteins. Non-human antibodies can be "humanized" by known methods (e.g., U.S. Patent No. 5,225,539).

[00104] Antibody fragments that contain an idiotype of a biomarker can be generated by techniques known in the art. For example, such fragments include, but are not limited to, the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragment that can be generated by reducing the disulfide bridges of the F(ab')2 fragment; the Fab fragment that can be generated by treating the antibody molecular with papain and a reducing agent; and Fv fragments. Synthetic antibodies, e.g., antibodies produced by chemical synthesis, may also be useful in the present invention.

[00105] Standard reference works described herein and known to those skilled in the relevant art describe both immunologic and non-immunologic techniques, their suitability for particular sample types, antibodies, proteins or analyses. Standard reference works setting forth the general principles of immunology and assays employing immunologic methods known to those of skill in the art include, for example: Harlow and Lane, Antibodies: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. (1999); Harlow and Lane, Using Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York; Coligan et al. eds. Current Protocols in Immunology, John Wiley & Sons, New York, NY (1992-2006); and Roitt et al., Immunology, 3d Ed., Mosby-Year Book Europe Limited, London (1993). Standard reference works setting forth the general principles of peptide synthesis technology and methods known to those of skill in the art include, for example: Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2000; Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994).

[00106] A subject's rejection status may be described as "rejector" (R or "acute rejector" or AR) or as a "non-rejector" (NR) and is determined by comparison of the concentration of the

markers to that of a non-rejector cutoff index. A "non-rejector cutoff index" is a numerical value or score, beyond or outside of which a subject is categorized as having rejector status. The non-rejector cutoff index may be alternately referred to as a 'control value', a 'control index', or simply as a 'control'. A non-rejector cutoff-index may be the concentration of individual markers in a control subject population and considered separately for each marker measured; alternately the non-rejector cutoff index may be a combination of the concentration of the markers, and compared to a combination of the concentration of the markers in the subject's sample provided for diagnosing. The control subject population may be a normal or healthy control population, or may be an allograft recipient population that has not, or is not, rejecting the allograft. A control, or pool of controls, may be constant e.g. represented by a static value, or may be cumulative, in that the sample population used to obtain it may change from site to site, or over time and incorporate additional data points. For example, a central data repository, such as a centralized healthcare information system, may receive and store data obtained at various sites (hospitals, clinical laboratories or the like) and provide this cumulative data set for use with the methods of the invention at a single hospital, community clinic, for access by an end user (i.e. an individual medical practitioner, medical clinic or center, or the like). In some embodiments the cutoff index may be further characterized as being a genomic cutoff index (for genomic expression profiling of subjects), a proteomic cutoff index (for proteomic profiling of subjects), or the like.

[00107] A "biological sample" refers generally to body fluid or tissue or organ sample from a subject. For example, the biological sample may be a body fluid such as blood, serum, plasma, lymph fluid, urine or saliva. A tissue or organ sample, such as a non-liquid tissue sample may be digested, extracted or otherwise rendered to a liquid form – examples of such tissues or organs include cultured cells, blood cells, skin, liver, heart, kidney, pancreas, islets of Langerhans, bone marrow, blood, blood vessels, heart valve, lung, intestine, bowel, spleen, bladder, penis, face, hand, bone, muscle, fat, cornea or the like. A plurality of biological samples may be collected at any one time. A biological sample or samples may be taken from a subject at any time, including before allograft transplantation, at the time of transplantation or at anytime following transplantation. A biological sample may comprise "nucleic acid", such as 'deoxyribonucleic acid' (also 'DNA') or 'ribonucleic acid' (also 'RNA' or 'mRNA'), or a combination thereof, in either single or double-stranded form. A nucleic acid may also be referred to as a 'transcript'.

[00108] The methods described herein may be employed before a subject receives an allograft, or at any time following receipt of an allograft to determine whether or not the allograft is being rejected. For example, a sample obtained from a subject at any time following the receipt of the allogaft may be assessed for the presence of altered levels (increased or decreased) of one or more than one nucleic acid marker or proteomic marker listed in Tables 2 or 7. In some cases, a sample can be obtained from the subject 1, 2, 3, 4, 5, 6, 7, 8, or more hours after the allograft is received. In some cases, a sample can be obtained from the subject one or more days (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or more days) after the allograft is received. In some examples, a sample can be obtained from 2 to 7 days (e.g., 5 to 7 days) after receipt of the allograft and assessed for the presence of nucleic acid markers or proteomic markers listed in Tables 2 or 7.

[00109] The term "subject" or "patient" generally refers to mammals and other animals including humans and other primates, companion animals, zoo, and farm animals, including, but not limited to, cats, dogs, rodents, rats, mice, hamsters, rabbits, horses, cows, sheep, pigs, goats, poultry, etc. A subject includes one who is to be tested, or has been tested for prediction, assessment or diagnosis of allograft rejection. The subject may have been previously assessed or diagnosed using other methods, such as those described herein or those in current clinical practice, or may be selected as part of a general population (a control subject).

[00110] A fold-change of a marker in a subject, relative to a control may be at least 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, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 or more, or any amount there between. The fold change may represent a decrease, or an increase, compared to the control value. One or more than one includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more.

[00111] "Down-regulation" or 'down-regulated' may be used interchangeably and refer to a decrease in the level of a marker, such as a gene, nucleic acid, metabolite, transcript, protein or polypeptide. "Up-regulation" or "up-regulated" may be used interchangeably and refer to an increase in the level of a marker, such as a gene, nucleic acid, metabolite, transcript, protein or polypeptide. Also, a pathway, such as a signal transduction or metabolic pathway may be up- or down-regulated.

[00112] Once a subject is identified as an acute rejector, or at risk for becoming an acute rejector by any method (genomic or proteomic, or a combination thereof), therapeutic measures may be implemented to alter the subject's immune response to the allograft. The subject may undergo additional monitoring of clinical values more frequently, or using more sensitive monitoring methods. Additionally the subject may be administered immunosuppressive medicaments to decrease or increase the subject's immune response. Even though a subject's immune response needs to be suppressed to prevent rejection of the allograft, a suitable level of immune function is also needed to protect against opportunistic infection. Various medicaments that may be administered to a subject are known; see for example, Goodman and Gilman's *The Pharmacological Basis of Therapeutics 11th edition*. Ch 52, pp 1405-1431 and references therein; LL Brunton, JS Lazo, KL Parker editors. Standard reference works setting forth the general principles of medical physiology and pharmacology known to those of skill in the art include: Fauci et al. , Eds., Harrison 's Principles Of Internal Medicine, 14th Ed., McGraw-Hill Companies, Inc. (1998). Other preventative and therapeutic strategies are reviewed in the medical literature– see, for example Djamali et al., 2006. Clin J Am Soc Nephrol 1:623-630.

[00113] **Genomic nucleic acid expression profiling**

[00114] A method of diagnosing acute allograft rejection in a subject as provided by the present invention comprises 1) determining the expression profile of at least one or more markers in a biological sample from the subject, the markers selected from the group presented in Table 2; 2) comparing the expression profile of the at least one or more markers to a non-rejector profile; and 3) determining whether the expression level of the at least one or more markers is up-regulated (increased) or down-regulated (decreased) relative to the control profile, wherein up-regulation or down-regulation of the at least one or more markers is indicative of the rejection status.

[00115] The invention also provides for a method of predicting, assessing or diagnosing kidney allograft rejection in a subject as provided by the present invention comprising 1) measuring the increase or decrease of at least one or more markers selected from the group presented in Table 2; and 2) determining the 'rejection status' of the subject, wherein the determination of 'rejection status' of the subject is based on comparison of the subject's marker expression profile to a control marker expression profile.

[00116] The phrase "gene expression data", "gene expression profile" or "marker expression profile" as used herein refers to information regarding the relative or absolute level of expression of a gene or set of genes in a biological sample. The level of expression of a gene may be determined based on the level of RNA, such as mRNA, encoded by the gene. Alternatively, the level of expression may be determined based on the level of a polypeptide or fragment thereof encoded by the gene.

[00117] A 'polynucleotide', 'oligonucleotide', 'nucleic acid' or 'nucleotide polymer' as used herein may include synthetic or mixed polymers of nucleic acids, including RNA, DNA or both RNA and DNA, both sense and antisense strands, and may be chemically or biochemically modified or may contain non- natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.), charged linkages (e. g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), and modified linkages (e.g., alpha anomeric polynucleotides, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions.

[00118] An oligonucleotide includes variable length nucleic acids, which may be useful as probes, primers and in the manufacture of microarrays (arrays) for the detection and/or amplification of specific nucleic acids. Oligonucleotides may comprise DNA, RNA, PNA or other polynucleotide moieties as described in, for example, US 5,948,902. Such DNA or RNA strands may be synthesized by the sequential addition (5'-3' or 3'-5') of activated monomers to a growing chain which may be linked to an insoluble support. Numerous methods are known in the art for synthesizing oligonucleotides for subsequent individual use or as a part of the insoluble support, for example in arrays (Lashkari DA. *et al.* PNAS (1995) 92(17):7912-5; McGall G. *et al.* PNAS (1996) 93(24):13555-60; Albert TJ. *et al.* Nucleic Acid Res.(2003) 31(7):e35; Gao X. *et al.* Biopolymers (2004) 73(5):579-96; and Moorcroft MJ. *et al.* Nucleic Acid Res.(2005) 33(8):e75 and references therein). In general, oligonucleotides are synthesized through the stepwise addition of activated and protected monomers under a variety of conditions depending on the method being used. Subsequently, specific protecting groups may be removed to allow for further elongation and subsequently and once synthesis is complete all the protecting

groups may be removed and the oligonucleotides removed from their solid supports for purification of the complete chains if so desired.

[00119] A "gene" is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product and may include untranslated and untranscribed sequences in proximity to the coding regions (5' and 3' to the coding sequence), as well as exons and/or introns. Such non-coding sequences may contain regulatory sequences needed for transcription and translation of the sequence or splicing of introns, for example, or may as yet to have any function attributed to them beyond the occurrence of the mutation of interest. A gene may also include one or more promoters, enhancers, transcription factor binding sites, termination signals or other regulatory elements.

[00120] The term "microarray," "array," or "chip" refers to a plurality of defined nucleic acid probes coupled to the surface of a substrate in defined locations. The substrate may be a solid substrate. Microarrays have been generally described in the art in, for example, U.S. Patent Nos. 5,143,854 (Pirrung); 5,424,186, 5,445,934, 5,744,305 and 5,800,992 to Fodor, 5,677,195 and 6,040,193 to Winkler, and Fodor et al. 1991(Science, 251:767-777). Each of these references is incorporated by reference herein in their entirety.

[00121] "Hybridization" includes a reaction in which one or more polynucleotides and/or oligonucleotides interact in an ordered manner (sequence-specific) to form a complex that is stabilized by hydrogen bonding - also referred to as 'Watson-Crick' base pairing. Variant base-pairing may also occur through non-canonical hydrogen bonding includes Hoogsteen base pairing. Under some thermodynamic, ionic or pH conditions, triple helices may occur, particularly with ribonucleic acids. These and other variant hydrogen bonding or base-pairing are known in the art, and may be found in, for example, Lehninger – Principles of Biochemistry, 3rd edition (Nelson and Cox, eds. Worth Publishers, New York.), herein incorporated by reference.

[00122] Hybridization reactions can be performed under conditions of different "stringency". The stringency of a hybridization reaction can determine the ease or difficulty with which any two nucleic acid molecules will hybridize to one another. Stringency may be increased, for example, by increasing the temperature at which hybridization occurs, by decreasing the ionic (salt) concentration at which hybridization occurs, or a combination thereof. Under stringent conditions, nucleic acid molecules at least 60%, 65%, 70%, 75% or more identical to each other remain hybridized to each other, whereas molecules with low percent

identity generally do not remain hybridized. An example of stringent hybridization conditions are hybridization in 6x sodium chloride/sodium citrate (SSC) at about 44-45°C, followed by one or more washes in 0.2xSSC, 0.1% SDS at 50°C, 55°C, 60°C, 65°C, or at a temperature there between.

[00123] Hybridization between two nucleic acids may occur in an antiparallel configuration – this is referred to as 'annealing', and the paired nucleic acids are described as complementary. A double-stranded polynucleotide may be "complementary", if hybridization can occur between one of the strands of the first polynucleotide and the second. The degree of which one polynucleotide is complementary with another is referred to as homology, and 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.

[00124] In general, sequence-specific hybridization involves a hybridization probe, which is capable of specifically hybridizing to a defined sequence. Such probes may be designed to differentiate between sequences varying in only one or a few nucleotides, thus providing a high degree of specificity. A strategy which couples detection and sequence discrimination is the use of a "molecular beacon", whereby the hybridization probe (molecular beacon) has 3' and/ or 5' reporter and quencher molecules and 3' and 5' sequences which are complementary such that absent an adequate binding target for the intervening sequence the probe will form a hairpin loop. The hairpin loop keeps the reporter and quencher in close proximity resulting in quenching of the fluorophor (reporter) which reduces fluorescence emissions. However, when the molecular beacon hybridizes to the target the fluorophor and the quencher are sufficiently separated to allow fluorescence to be emitted from the fluorophor.

[00125] Probes used in hybridization may include double-stranded DNA, single-stranded DNA and RNA oligonucleotides, and peptide nucleic acids. Hybridization conditions and methods for identifying markers that hybridize to a specific probe are described in the art - see, for example, Brown, T. "Hybridization Analysis of DNA Blots" in Current Protocols in Molecular Biology. FM Ausubel et al, editors. Wiley & Sons, 2003. doi: 10.1002/0471142727.mb0210s21. Suitable hybridization probes for use in accordance with the invention include oligonucleotides, polynucleotides or modified nucleic acids from about 10 to about 400 nucleotides, alternatively from about 20 to about 200 nucleotides, or from about 30 to about 100 nucleotides in length.

[00126] Specific sequences may be identified by hybridization with a primer or a probe, and this hybridization subsequently detected.

[00127] 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 Beverly, SM. Enzymatic Amplification of RNA by PCR (RT-PCR) in Current Protocols in Molecular Biology. FM Ausubel et al, editors. Wiley & Sons, 2003. doi: 10.1002/0471142727.mb1505s56. Synthesis of the replicate copies may include incorporation of a nucleotide having a label or tag, for example, a fluorescent molecule, biotin, or a radioactive molecule. The replicate copies may subsequently be detected via these tags, using conventional methods.

[00128] A primer may 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).

[00129] A "probe set" (or sometimes 'primer set') as used herein refers to a group of oligonucleotides that may be used to detect the presence of a nucleic acid molecule (a nucleic acid marker) in a sample; the detection may be quantitative, or semi-quantitative. 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 – generally a size range from about 15 to about 25, or to about 30 nucleotides is of sufficient size. A probe set may be in solution, e.g. for use in multiplex PCR. Alternately, a probe set may be adhered to a solid surface, as in an array or microarray. A probe set may detect the expression level of a full-length gene, a splice-variant of a full-length gene, a transcriptional

unit, or a fragment of a gene or transcriptional unit. A probe set identifies a nucleic acid marker that is present in the sample.

[00130] In some embodiments of the invention, a probe set for detection of nucleic acids expressed by a set of nucleic acid markers comprising one or more than one of TncRNA, FKSG49, ZNF438, SFRS16, 1558448_a_at, CAMKK2, NFYC, NCOA3, LMAN2, PGS1, NEDD9, 237442_at, FKSG49/LOC730444, LIMK2, UNB, NASP, PRO1073, 240057_at, ITGAX, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6 is provided. Such a probe set may be useful for determining the rejection status of a subject. The probe set may comprise one or more pairs of primers for specific amplification (e.g. PCR, or RT-PCR) of nucleic acid sequences corresponding to one or more than one of TncRNA, FKSG49, ZNF438, SFRS16, 1558448_a_at, CAMKK2, NFYC, NCOA3, LMAN2, PGS1, NEDD9, 237442_at, FKSG49/LOC730444, LIMK2, UNB, NASP, PRO1073, 240057_at, ITGAX, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6. In another embodiment of the invention, the probe set is part of a microarray. In another embodiment of the invention, the nucleic acid markers include one or more than one of TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX. The markers are described in further detail below.

[00131] It will be appreciated that numerous other methods for sequence discrimination and detection are known in the art and some of which are described in further detail below. It will also be appreciated that reactions such as arrayed primer extension mini sequencing, tag microarrays and sequence-specific extension could be performed on a microarray. One such array based genotyping platform is the microsphere based tag-it high throughput array (Bortolin S. et al. 2004 Clinical Chemistry 50: 2028-36). This method amplifies genomic DNA by PCR followed by sequence-specific primer extension with universally tagged primers. The products are then sorted on a Tag-It array and detected using the Luminex xMAP system.

[00132] It will be appreciated by a person of skill in the art that any numerical designations of nucleotides within a sequence are relative to the specific sequence. Also, the same positions may be assigned different numerical designations depending on the way in which the sequence is numbered and the sequence chosen. Furthermore, sequence variations such as insertions or deletions, may change the relative position and subsequently the numerical designations of particular nucleotides at and around a mutational site. For example, the sequences represented by

accession numbers e.g. AC124566, AF211864, AI035495, AI326085, AK089167, AK131133, AK155816, AK170432, BC042840 and BC057200 all represent human *ITGAX* nucleotide sequences, but may have some sequence differences, and numbering differences between them. As another example, the sequences represented by accession numbers NP_115925, NP_444509, P20702, NP_776169, NP_000878, NP_001706, NP_04223, AAA59180, AAA51620 all represent human ITGAX polypeptide sequences, but may have some sequence differences, and numbering differences between them. Other nucleic acid markers may demonstrate variants, and are described below.

[00133] Selection and/or design of probes, primers or probe sets for specific detection of expression of any gene of interest, including any of the above genes is within the ability of one of skill in the relevant art, when provided with one or more nucleic acid sequences of the gene of interest. Further, any of several probes, primers or probe sets, or a plurality of probes, primers or probe sets may be used to detect a gene of interest, for example, an array may include multiple probes for a single gene transcript – the aspects of the invention as described herein are not limited to any specific probes exemplified.

[00134] Sequence identity or sequence similarity may be determined using a nucleotide sequence comparison program (for DNA or RNA sequences, or fragments or portions thereof) or an amino acid sequence comparison program (for protein, polypeptide or peptide sequences, or fragments or portions thereof), such as that provided within DNASIS (for example, but not limited to, using the following parameters: GAP penalty 5, #of top diagonals 5, fixed GAP penalty 10, k-tuple 2, floating gap 10, and window size 5). However, other methods of alignment of sequences for comparison are well-known in the art for example the algorithms of Smith & Waterman (1981, Adv. Appl. Math. 2:482), Needleman & Wunsch (J. Mol. Biol. 48:443, 1970), Pearson & Lipman (1988, Proc. Nat'l. Acad. Sci. USA 85:2444), and by computerized implementations of these algorithms (e.g. GAP, BESTFIT, FASTA, and BLAST), or by manual alignment and visual inspection.

[00135] If a nucleic acid or gene, polypeptide or sequence of interest is identified and a portion or fragment of the sequence (or sequence of the gene polypeptide or the like) is provided, other sequences that are similar, or substantially similar may be identified using the programs exemplified above. For example, when constructing a microarray or probe sequences, the sequence and location are known, such that if a microarray experiment identifies a 'hit' (the

probe at a particular location hybridizes with one or more nucleic acids in a sample, the sequence of the probe will be known (either by the manufacturer or producer of the microarray, or from a database provided by the manufacturer – for example the NetAffx databases of Affymetrix, the manufacturer of the Human Genome U133 Plus 2.0 Array). If the identity of the sequence source is not provided, it may be determined by using the sequence of the probe in a sequence-based search of one or more databases. For peptide or peptide fragments identified by proteomics assays, for example iTRAQ, the sequence of the peptide or fragment may be used to query databases of amino acid sequences as described above. Examples of such a database include those maintained by the National Centre for Biotechnology Information, or those maintained by the Swiss Institute of Bioinformatics, the Sanger Centre, or the European Bioinformatics Institute, such as the International Protein Index (IPI).

[00136] A protein or polypeptide, nucleic acid or fragment or portion thereof may be considered to be specifically identified when its sequence may be differentiated from others found in the same phylogenetic Species, Genus, Family or Order. Such differentiation may be identified by comparison of sequences. Comparisons of a sequence or sequences may be done using a BLAST algorithm (Altschul et al. 1009. J. Mol Biol 215:403-410). A BLAST search allows for comparison of a query sequence with a specific sequence or group of sequences, or with a larger library or database (e.g. GenBank or GenPept) of sequences, and identify not only sequences that exhibit 100% identity, but also those with lesser degrees of identity. For example, regarding a protein with multiple isoforms (either resulting from, for example, separate genes or variant splicing of the nucleic acid transcript from the gene, or post translational processing), an isoform may be specifically identified when it is differentiated from other isoforms from the same or a different species, by specific detection of a structure, sequence or motif that is present on one isoform and is absent, or not detectable on one or more other isoforms.

[00137] Access to the methods of the invention may be provided to an end user by, for example, a clinical laboratory or other testing facility performing the individual marker tests – the biological samples are provided to the facility where the individual tests and analyses are performed and the predictive method applied; alternately, a medical practitioner may receive the marker values from a clinical laboratory and use a local implementation or an internet-based implementation to access the predictive methods of the invention.

[00138] Determination of statistical parameters such as multiples of the median, standard error, standard deviation and the like, as well as other statistical analyses as described herein are known and within the skill of one versed in the relevant art. Use of a particular coefficient, value or index is exemplary only and is not intended to constrain the limits of the various aspects of the invention as disclosed herein.

[00139] Interpretation of the large body of gene expression data obtained from, for example, microarray experiments, or complex RT-PCR experiments may be a formidable task, but is greatly facilitated through use of algorithms and statistical tools designed to organize the data in a way that highlights systematic features. Visualization tools are also of value to represent differential expression by, for example, varying intensity and hue of colour (Eisen et al. 1998. Proc Natl Acad Sci 95:14863-14868). The algorithm and statistical tools available have increased in sophistication with the increase in complexity of arrays and the resulting datasets, and with the increase in processing speed, computer memory, and the relative decrease in cost of these.

[00140] Mathematical and statistical analysis of nucleic acid or protein expression profiles may accomplish several things – identification of groups of genes that demonstrate coordinate regulation in a pathway or a domain of a biological system, identification of similarities and differences between two or more biological samples, identification of features of a gene expression profile that differentiate between specific events or processes in a subject, or the like. This may include assessing the efficacy of a therapeutic regimen or a change in a therapeutic regimen, monitoring or detecting the development of a particular pathology, differentiating between two otherwise clinically similar (or almost identical) pathologies, or the like.

[00141] Clustering methods are known and have been applied to microarray datasets, for example, hierarchical clustering, self-organizing maps, k-means or deterministic annealing. (Eisen et al, 1998 Proc Natl Acad Sci USA 95:14863- 14868; Tamayo, P., et al. 1999. Proc Natl Acad Sci USA 96:2907-2912; Tavazoie, S., et al. 1999. Nat Genet 22:281-285; Alon, U., et al. 1999. Proc Natl Acad Sci USA 96:6745-6750). Such methods may be useful to identify groups of genes in a gene expression profile that demonstrate coordinate regulation, and also useful for the identification of novel genes of otherwise unknown function that are likely to participate in the same pathway or system as the others demonstrating coordinate regulation.

[00142] The pattern of nucleic acid or proteomic expression in a biological sample may also provide a distinctive and accessible molecular picture of its functional state and identity. Two different samples that have related gene expression patterns are may be biologically and functionally similar to one another; conversely two samples that demonstrate significant differences in the pattern of nucleic acid or proteomic expression may not only be differentiated by the complex expression pattern displayed, but may indicate a diagnostic subset of gene products or transcripts that are indicative of a specific pathological state or other physiological condition, such as allograft rejection.

[00143] Applying a plurality of mathematical and/or statistical analytical methods to a microarray dataset may indicate varying subsets of significant markers, leading to uncertainty as to which method is 'best' or 'more accurate'. Regardless of the mathematics, the underlying biology is the same in a dataset. By applying a plurality of mathematical and/or statistical methods to a microarray dataset and assessing the statistically significant subsets of each for common markers to all, the uncertainty is reduced, and clinically relevant core group of markers is identified.

[00144] **Genomic expression profiling markers**

[00145] The present invention provides for a core group of nucleic acid markers useful for the assessment or diagnosis of allograft rejection, including acute kidney allograft rejection, comprising one or more than one of the nucleic acid markers presented in Table 2, and may include one or more than one of TncRNA, FKSG49, ZNF438, SFRS16, 1558448_a_at, CAMKK2, NFYC, NCOA3, LMAN2, PGS1, NEDD9, 237442_at, FKSG49/LOC730444, LIMK2, UNB, NASP, PRO1073, 240057_at, ITGAX, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6.

[00146] 183 probe sets were detected, quantified and found to demonstrate a statistically significant discrimination, with a false discovery rate (FDR) below 1%, comparing the rejection (AR) samples and non-rejecting transplant (NR) controls in all of the three moderated t-tests applied, and may represent an increase/up-regulation or decrease/down-regulation of the gene or transcript in question. These probe sets specifically detect (by hybridization and detection of a label) and allow for quantitation of the expression level of the expressed nucleic acids. Of this set of 183 (listed in Table 2), representing 183 individual expressed transcripts or nucleic acids, a subset of 24 probe sets (Table 5) were detected, quantified and found to demonstrate a

statistically significant fold change in the AR samples relative to non-rejecting transplant (NR) controls in all of the three moderated t-tests applied, and may represent an increase/up-regulation or decrease/down-regulation of the gene or transcript in question. Of these 24 probe sets, at least 18 detect specific genes (known, or known but not described) genes or transcripts. Figure 10 provides nucleic acid sequence information of a portion of the nucleic acid identified by the probe sets listed in Tables 2 and 5.

[00147] In some embodiments, the present invention provides a method for the assessment, monitoring, prediction or diagnosis of allograft rejection, including acute kidney allograft rejection, comprising measuring the expression level of at least one or more of the markers or probe sets selected from the group listed in Table 2, and referred to by the indicated gene symbol. These probe sets are associated with and may specifically measure the expression level individual and unique genes or gene fragments referenced by the gene symbol.

[00148] The genes or markers indicated in Tables 2 or 5 may have a biological role in the allograft rejection process, and represent a therapeutic target.

[00149] In another embodiment, the present invention provides for a group of nucleic acid markers, useful for the assessment or diagnosis of acute allograft rejection, including kidney allograft rejection, comprising one or more than one of TncRNA, FKSG49, ZNF438, SFRS16, 1558448_a_at, CAMKK2, NFYC, NCOA3, LMAN2, PGS1, NEDD9, 237442_at, FKSG49/LOC730444, LIMK2, UNB, NASP, PRO1073, 240057_at, ITGAX, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6.

[00150] In another embodiment, the present invention provides for a subset of markers selected from the group of 24, that may be useful for the assessment, monitoring, prediction or diagnosis of allograft rejection, including acute kidney allograft rejection, comprising one or more than one ofTncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX.

[00151] In another embodiment, the present invention provides for a subset of markers selected from the group of 24, that may be useful for the assessment, monitoring, prediction or diagnosis of allograft rejection, including acute kidney allograft rejection, comprising TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX and one or more than one of SFRS16, NFYC, NCOA3, PGS1,

NEDD9, LIMK2, NASP, 240057_at, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6. One or more than one includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or more.

[00152] The results of Examples 1-3 illustrate the above embodiments - a 24 nucleic acid classifer set (TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073, ITGAX;SFRS16, NFYC, NCOA3, PGS1, NEDD9, LIMK2, NASP, 240057_at, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6) are useful for discerning acute rejecting subjects from non-rejecting subjects. Any combination of one or more than one of the set of 24 may also be useful for discerning acute rejecting subjects from non-rejecting subjects. The intersecting set of 11 nucleic acid markers (TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX) may also be useful for discerning acute rejecting subjects from non-rejecting subjects.

[00153] Table 2: Differentially expressed probe sets, exhibiting about a 1.39 to 1.4-fold difference (or greater) between AR and NR subject. The target sequence is the portion of the consensus or exemplar sequencer from which the probe sequences were selected (Affymetrix™ NetAffx™ Annotation database Update Release 25, March 2008) . The consensus or exemplary sequence is the sequence used at the time of design of the array to represent the transcript that the GeneChip U133 2.0 probe set measures. A consensus sequence results from base-calling algorithms that align and combine sequence data into groups. An exemplar sequence is a representative cDNA sequence for each gene.

Affymetrix Probe Set ID	Gene Symbol	Gene Title	RefSeq Accession No.	log2 (Fold Change)	Fold Change	Direction (AR vs NR)	FDR value	Representative sequence (SEQ ID NO:)
1552264_a_at	MAPK1	mitogen-activated protein kinase 1	NM_002745 NM_138957	0.73	1.66	up	0.00749	1
1552542_s_at	TAGAP	T-cell activation GTPase activating protein	NM_054114 NM_138810 NM_152133	0.95	1.93	up	0.00996	2
1553186_x_at	RASEF	RAS and EF-hand domain containing	NM_152573	1.12	2.18	up	0.00760	3
1553297_a_at	CSF3R	colony stimulating factor 3 receptor (granulocyte)	NM_000760 NM_156038 NM_156039 NM_172313	0.81	1.76	up	0.00574	4
1554691_a_at	PACSIN2	protein kinase C and casein kinase substrate in neurons 2	NM_007229	0.74	1.67	up	0.00574	5
1555420_a_at	KLF7	Kruppel-like factor 7 (ubiquitous)	NM_003709	1.08	2.11	up	0.00638	6
1555467_a_at	CUGBP1	CUG triplet repeat, RNA binding protein 1	NM_001025596 NM_006560 NM_198700	0.79	1.73	up	0.00363	7

Affymetrix Probe Set ID	Gene Symbol	Gene Title	RefSeq Accession No.	log2 (Fold Change)	Fold Change	Direction (AR vs NR)	FDR value	Representative sequence (SEQ ID NO:)
1555797_a_at	ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa	NM_005717	0.76	1.70	up	0.00711	8
1555852_at	1555852_at	---	---	0.90	1.87	up	0.00721	9
1555950_a_at	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	NM_000574	0.85	1.80	up	0.00703	10
1557924_s_at	ALPL	alkaline phosphatase, liver/bone/kidney	NM_000478	0.77	1.71	up	0.00758	11
1558448_a_at	1558448_a_at	CDNA FLJ35687 fis, clone SPLEN2019349	---	0.79	1.73	up	0.00677	12
1563509_at	1563509_at	MRNA; cDNA DKFZp313O229 (from clone DKFZp313O229)	---	0.84	1.79	up	0.00219	13
1565484_x_at	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	NM_005228 NM_201282 NM_201283 NM_201284	-1.23	2.35	down	0.00562	14
1565599_at	1565599_at	Clone 23712 mRNA sequence	---	0.49	1.40	up	0.00760	15
1565717_s_at	FUS	fusion (involved in t(12;16) in malignant liposarcoma)	NM_001010850 NM_004960	1.04	2.06	up	0.00375	16
1568609_s_at	FAM91A2/FLJ39739/LOC727820	family with sequence similarity 91, member A2 hypothetical FLJ39739 hypothetical protein LOC727820	NM_207400 XM_001125827 XM_934503	0.87	1.83	up	0.00703	17
1569003_at	TMEM49	transmembrane protein 49	NM_030938	0.95	1.94	up	0.00769	18

Affymetrix Probe Set ID	Gene Symbol	Gene Title	RefSeq Accession No.	log2 (Fold Change)	Fold Change	Direction (AR vs NR)	FDR value	Representative sequence (SEQ ID NO:)
200709_at	FKBP1A	FK506 binding protein 1A, 12kDa	NM_000801 NM_054014	0.59	1.50	up	0.00775	19
200739_s_at	SUMO3	SMT3 suppressor of mif two 3 homolog 3 (S. cerevisiae)	NM_006936	0.59	1.50	up	0.00711	20
200796_s_at	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	NM_021960 NM_182763	1.32	2.50	up	0.00269	21
200797_s_at	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	NM_021960 NM_182763	0.60	1.52	up	0.00872	22
200805_at	LMAN2	lectin, mannose-binding 2	NM_006816	0.72	1.64	up	0.00265	23
200852_x_at	GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	NM_005273	0.53	1.44	up	0.00872	24
200904_at	HLA-E	major histocompatibility complex, class I, E	NM_005516	0.66	1.58	up	0.00760	25
200959_at	FUS	fusion (involved in t(12;16) in malignant liposarcoma)	NM_001010850 NM_004960	0.74	1.67	up	0.00254	26
201043_s_at	ANP32A	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	NM_006305	0.81	1.75	up	0.00087	27
201090_x_at	TUBA1B	tubulin, alpha 1b	NM_006082	0.63	1.55	up	0.00468	28
201440_at	DDX23	DEAD (Asp-Glu-Ala-Asp) box polypeptide 23	NM_004818	0.54	1.46	up	0.00826	29
201473_at	JUNB	jun B proto-oncogene	NM_002229	0.76	1.69	up	0.00638	30
201531_at	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)	NM_003407	0.86	1.81	up	0.00265	31

Affymetrix Probe Set ID	Gene Symbol	Gene Title	RefSeq Accession No.	log2 (Fold Change)	Fold Change	Direction (AR vs NR)	FDR value	Representative sequence (SEQ ID NO:)
201651_s_at	PACSIN2	protein kinase C and casein kinase substrate in neurons 2	NM_007229	0.68	1.60	up	0.00891	32
201729_s_at	KIAA0100	KIAA0100	NM_014680	0.59	1.51	up	0.00711	33
201861_s_at	LRRFIP1	leucine rich repeat (in FLII) interacting protein 1	NM_004735	1.11	2.16	up	0.00222	34
201950_x_at	CAPZB	capping protein (actin filament) muscle Z-line, beta	NM_004930	0.70	1.62	up	0.00872	35
201954_at	ARPC1B/LOC653888	actin related protein 2/3 complex, subunit 1B, 41kDa similar to Actin-related protein 2/3 complex subunit 1B (ARP2/3 complex 41 kDa subunit) (p41-ARC)	NM_005720 XM_936251	0.68	1.60	up	0.00219	36
201970_s_at	NASP	nuclear autoantigenic sperm protein (histone-binding)	NM_002482 NM_152298 NM_172164	0.52	1.43	up	0.00917	37
202150_s_at	NEDD9	neural precursor cell expressed, developmentally down-regulated 9	NM_006403 NM_182966	0.49	1.40	up	0.00879	38
202180_s_at	MVP	major vault protein	NM_005115 NM_017458	0.82	1.77	up	0.00703	39
202216_x_at	NFYC	nuclear transcription factor Y, gamma	NM_014223	0.55	1.46	up	0.00775	40
202423_at	MYST3	MYST histone acetyltransferase (monocytic leukemia) 3	NM_001099412 NM_001099413 NM_006766	0.52	1.43	up	0.00798	41
202510_s_at	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	NM_006291	0.86	1.81	up	0.00465	42

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202531_at	IRF1	interferon regulatory factor 1	NM_002198	0.85	1.81	up	0.00998	43
202897_at	SIRPA	signal-regulatory protein alpha	NM_001040022 NM_001040023 NM_080792	0.99	1.99	up	0.00219	44
202910_s_at	CD97	CD97 molecule	NM_001025160 NM_001784 NM_078481	0.93	1.90	up	0.00760	45
202951_at	STK38	serine/threonine kinase 38	NM_007271	0.78	1.71	up	0.00879	46
203233_at	IL4R	interleukin 4 receptor	NM_000418 NM_001008699	0.92	1.90	up	0.00912	47
203239_s_at	CNOT3	CCR4-NOT transcription complex, subunit 3	NM_014516	0.69	1.61	up	0.00857	48
203254_s_at	TLN1	talin 1	NM_006289	0.83	1.78	up	0.00434	49
203471_s_at	PLEK	pleckstrin	NM_002664	0.80	1.74	up	0.00879	50
203509_at	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing	NM_003105	0.68	1.60	up	0.00652	51
203591_s_at	CSF3R	colony stimulating factor 3 receptor (granulocyte)	NM_000760 NM_156038 NM_156039 NM_172313	0.88	1.85	up	0.00638	52
203624_at	SFRS17A	splicing factor, arginine/serine-rich 17A	NM_005088	0.72	1.65	up	0.00972	53
203748_x_at	RBMS1	RNA binding motif, single stranded interacting protein 1	NM_002897 NM_016836 NM_016839	0.80	1.74	up	0.00219	54
204166_at	SBNO2	strawberry notch homolog 2 (Drosophila)	NM_001100122 NM_014963	0.80	1.74	up	0.00314	55
204978_at	SFRS16	splicing factor, arginine/serine-rich 16	NM_007056	0.76	1.70	up	0.00552	56

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205220_at	GPR109B	G protein-coupled receptor 109B	NM_006018 XM_001134375	1.15	2.22	up	0.00552	57
205285_s_at	FYB	FYN binding protein (FYB-120/130)	NM_001465 NM_199335	0.62	1.53	up	0.00872	58
205539_at	AVIL	advillin	NM_006576	0.92	1.89	up	0.00087	59
205921_s_at	SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	NM_003043	0.65	1.57	up	0.00756	60
206130_s_at	ASGR2	asialoglycoprotein receptor 2	NM_001181 NM_080912 NM_080913 NM_080914	0.83	1.78	up	0.00419	61
206323_x_at	OPHN1	oligophrenin 1	NM_002547	0.57	1.48	up	0.00922	62
207127_s_at	HNRPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	NM_012207 NM_021644	0.64	1.56	up	0.00749	63
207266_x_at	RBMS1	RNA binding motif, single stranded interacting protein 1	NM_002897 NM_016836 NM_016839	0.92	1.89	up	0.00222	64
207446_at	TLR6	toll-like receptor 6	NM_006068	0.78	1.72	up	0.00470	65
207643_s_at	TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	NM_001065	0.77	1.71	up	0.00288	66
207782_s_at	PSEN1	presenilin 1 (Alzheimer disease 3)	NM_000021	0.70	1.63	up	0.00971	67
208018_s_at	HCK	hemopoietic cell kinase	NM_002110	0.87	1.83	up	0.00930	68
208120_x_at	FKSG49/LOC730444	FKSG49 hypothetical protein LOC730444	XM_001125803	0.56	1.47	up	0.00356	69
208488_s_at	CR1	complement component (3b/4b) receptor 1 (Knops blood group)	NM_000573 NM_000651 XM_001126036	0.78	1.72	up	0.00749	70
208702_x_at	APLP2	amyloid beta (A4) precursor-like protein 2	NM_001642	0.76	1.69	up	0.00870	71

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208772_at	ANKHD1/MASK-BP3	ankyrin repeat and KH domain containing 1 MASK-4E-BP3 alternate reading frame gene	NM_017747 NM_017978 NM_020690 NM_024668	0.85	1.81	up	0.00775	72
208811_s_at	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	NM_005494 NM_058246	0.82	1.77	up	0.00777	73
208885_at	LCP1	lymphocyte cytosolic protein 1 (L-plastin)	NM_002298	0.77	1.70	up	0.00912	74
208919_s_at	NADK	NAD kinase	NM_023018	0.81	1.76	up	0.00872	75
208922_s_at	NXF1	nuclear RNA export factor 1	NM_001081491 NM_006362	0.64	1.56	up	0.00703	76
209060_x_at	NCOA3	nuclear receptor coactivator 3	NM_006534 NM_181659	0.83	1.77	up	0.00652	77
209083_at	CORO1A	coronin, actin binding protein, 1A	NM_007074	0.82	1.77	up	0.00760	78
209286_at	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	NM_006449	0.91	1.88	up	0.00222	79
209868_s_at	RBMS1	RNA binding motif, single stranded interacting protein 1	NM_002897 NM_016836 NM_016839	0.88	1.84	up	0.00337	80
210184_at	ITGAX	integrin, alpha X (complement component 3 receptor 4 subunit)	NM_000887 XM_001127869	0.65	1.57	up	0.00392	81
210190_at	STX11	syntaxin 11	NM_003764	1.00	1.99	up	0.00684	82
210191_s_at	PHTF1	putative homeodomain transcription factor 1	NM_006608	0.90	1.87	up	0.00972	83
210483_at	MGC31957	hypothetical protein MGC31957	---	0.59	1.51	up	0.00652	84

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210484_s_at	MGC31957/TNFRSF10C	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain hypothetical protein MGC31957	NM_003841	0.89	1.85	up	0.00262	85
210514_x_at	HLA-G	HLA-G histocompatibility antigen, class I, G	NM_002127	0.58	1.50	up	0.00875	86
210563_x_at	CFLAR	CASP8 and FADD-like apoptosis regulator	NM_003879	0.83	1.78	up	0.00722	87
210569_s_at	SIGLEC9	sialic acid binding Ig-like lectin 9	NM_014441	0.67	1.59	up	0.00087	88
210686_x_at	SLC25A16	solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	NM_152707	0.74	1.67	up	0.00179	89
210754_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	NM_002350	0.71	1.64	up	0.00758	90
210787_s_at	CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta	NM_006549 NM_153499 NM_153500 NM_172214 NM_172215 NM_172216 NM_172226	0.62	1.54	up	0.00560	91
210992_x_at	FCGR2C	Fc fragment of IgG, low affinity IIc, receptor for (CD32)	NM_001005410 NM_001005411 NM_001005412 NM_201563	0.83	1.77	up	0.00522	92
211058_x_at	TUBA1B	tubulin, alpha 1b	NM_006082	0.57	1.48	up	0.00761	93

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211072_x_at	TUBA1B	tubulin, alpha 1b	NM_006082	0.60	1.51	up	0.00409	94
211251_x_at	NFYC	nuclear transcription factor Y, gamma	NM_014223	0.49	1.40	up	0.00888	95
211395_x_at	FCGR2C	Fc fragment of IgG, low affinity IIc, receptor for (CD32)	NM_001005410 NM_001005411 NM_001005412 NM_201563	0.84	1.79	up	0.00562	96
211454_x_at	FKSG49	FKSG49	---	0.75	1.69	up	0.00087	97
211521_s_at	PSCD4	pleckstrin homology, Sec7 and coiled-coil domains 4	NM_013385	0.69	1.61	up	0.00219	98
211571_s_at	VCAN	versican	NM_004385	1.47	2.77	up	0.00470	99
211750_x_at	TUBA1C	tubulin, alpha 1c	NM_032704	0.61	1.52	up	0.00840	100
211787_s_at	EIF4A1	eukaryotic translation initiation factor 4A, isoform 1	NM_001416	0.58	1.50	up	0.00715	101
211794_at	FYB	FYN binding protein (FYB-120/130)	NM_001465 NM_199335	0.79	1.73	up	0.00711	102
211795_s_at	FYB	FYN binding protein (FYB-120/130)	NM_001465 NM_199335	1.02	2.03	up	0.00756	103
211797_s_at	NFYC	nuclear transcription factor Y, gamma	NM_014223	0.81	1.76	up	0.00760	104
211823_s_at	PXN	paxillin	NM_002859 XM_001132665	0.65	1.57	up	0.00356	105
211974_x_at	RBPJ	recombination signal binding protein for immunoglobulin kappa J region	NM_005349 NM_015874 NM_203283 NM_203284	0.70	1.63	up	0.00179	106

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211996_s_at	DKFZp547E087/LOC23117/LOC440345/LOC613037/LOC728888	KIAA0220-like protein hypothetical gene LOC283846 hypothetical protein LOC440345 nuclear pore complex interacting protein pseudogene similar to Protein KIAA0220	NR_002555 NR_002603 XM_496136 XM_931802 XM_931808 XM_931814 XM_931818 XM_931827 XM_931837 XM_931840 XM_933834 XM_933869 XR_015786 XR_015889	0.88	1.84	up	0.00711	107
212036_s_at	PNN	pinin, desmosome associated protein	NM_002687	1.34	2.53	up	0.00688	108
212550_at	STAT5B	signal transducer and activator of transcription 5B	NM_012448	0.66	1.58	up	0.00760	109
212639_x_at	TUBA1B	tubulin, alpha 1b	NM_006082	0.65	1.56	up	0.00369	110
212680_x_at	PPP1R14B	protein phosphatase 1, regulatory (inhibitor) subunit 14B	NM_138689	0.77	1.71	up	0.00519	111
212708_at	MSL-1	male-specific lethal-1 homolog	NM_001012241 XM_932082 XM_932097 XM_932107 XM_943695 XM_943702	0.80	1.74	up	0.00826	112
212974_at	DENND3	DENN/MADD domain containing 3	NM_014957	0.91	1.87	up	0.00564	113
213505_s_at	SFRS14	splicing factor, arginine/serine-rich 14	NM_001017392 NM_014884	0.55	1.46	up	0.00998	114

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213596_at	CASP4	caspase 4, apoptosis-related cysteine peptidase	NM_001225 NM_033306 NM_033307	1.00	2.00	up	0.00996	115
213646_x_at	TUBA1B	tubulin, alpha 1b	NM_006082	0.63	1.55	up	0.00468	116
214369_s_at	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	NM_001098670 NM_001098671 NM_005825 NM_153819	0.67	1.59	up	0.00703	117
215210_s_at	DLST/DLSTP	dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex) dihydrolipoamide S-succinyltransferase pseudogene (E2 component of 2-oxo-glutarate complex)	NM_001933	0.48	1.39	up	0.00826	118
215236_s_at	PICALM	phosphatidylinositol binding clathrin assembly protein	NM_001008660 NM_007166	1.21	2.31	up	0.00869	119
215415_s_at	LYST	lysosomal trafficking regulator	NM_000081 NM_001005736	0.74	1.67	up	0.00369	120
215646_s_at	VCAN	versican	NM_004385	1.39	2.61	up	0.00761	121
215760_s_at	SBNO2	strawberry notch homolog 2 (Drosophila)	NM_001100122 NM_014963	0.80	1.74	up	0.00339	122
215832_x_at	PICALM	phosphatidylinositol binding clathrin assembly protein	NM_001008660 NM_007166	0.71	1.63	up	0.00879	123
215990_s_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	NM_001706 NM_138931	1.64	3.13	up	0.00439	124

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216236_s_at	SLC2A14/SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3 solute carrier family 2 (facilitated glucose transporter), member 14	NM_006931 NM_153449	0.90	1.86	up	0.00959	125
216950_s_at	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	NM_000566	1.44	2.70	up	0.00653	126
216985_s_at	STX3	syntaxin 3	NM_004177	0.92	1.89	up	0.00996	127
217436_x_at	LOC730399/LOC731974	hypothetical protein LOC730399 hypothetical protein LOC731974	XR_015561 XR_015670	0.60	1.51	up	0.00777	128
217475_s_at	LIMK2	LIM domain kinase 2	NM_001031801 NM_005569 NM_016733	0.78	1.71	up	0.00468	129
217507_at	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	NM_000578	1.09	2.13	up	0.00864	130
217728_at	S100A6	S100 calcium binding protein A6	NM_014624	0.84	1.79	up	0.00745	131
217992_s_at	EFHD2	EF-hand domain family, member D2	NM_024329	0.77	1.70	up	0.00879	132
218157_x_at	CDC42SE1	CDC42 small effector 1	NM_001038707 NM_020239	0.72	1.65	up	0.00591	133
218380_at	NLRP1	NLR family, pyrin domain containing 1	NM_001033053 NM_014922 NM_033004 NM_033006 NM_033007	0.75	1.68	up	0.00333	134

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219100_at	OBFC1	oligonucleotide/oligosaccharide-binding fold containing 1	NM_024928	0.69	1.62	up	0.00998	135
219183_s_at	PSCD4	pleckstrin homology, Sec7 and coiled-coil domains 4	NM_013385	0.80	1.74	up	0.00574	136
219394_at	PGS1	phosphatidylglycerophosphate synthase 1	NM_024419	1.02	2.03	up	0.00293	137
220046_s_at	CCNL1	cyclin L1	NM_020307	1.05	2.08	up	0.00919	138
220305_at	MGC3260	hypothetical protein MGC3260	NM_024030	0.97	1.96	up	0.00219	139
220326_s_at	FLJ10357	hypothetical protein FLJ10357	NM_018071	0.91	1.88	up	0.00909	140
221432_s_at	SLC25A28	solute carrier family 25, member 28	NM_031212	0.56	1.47	up	0.00671	141
221695_s_at	MAP3K2	mitogen-activated protein kinase kinase kinase 2	NM_006609 XM_001128799	0.89	1.85	up	0.00995	142
222244_s_at	TUG1	taurine upregulated gene 1	NR_002323	0.57	1.48	up	0.00711	143
222435_s_at	UBE2J1	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	NM_016021	0.97	1.96	up	0.00658	144
222955_s_at	FAM45A/FAM45B/LOC731832	family with sequence similarity 45, member B family with sequence similarity 45, member A similar to family with sequence similarity 45, member A	NM_018472 NM_207009 XM_001130983	0.66	1.58	up	0.00814	145
223009_at	C11orf59	chromosome 11 open reading frame 59	NM_017907	0.63	1.55	up	0.00760	146

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223578_x_at	PRO1073	PRO1073 protein	---	0.84	1.79	up	0.00991	147
223591_at	RNF135	ring finger protein 135	NM_032322 NM_197939	0.72	1.64	up	0.00879	148
224254_x_at	224254_x_at	---	---	0.88	1.84	up	0.00499	149
224566_at	TncRNA	trophoblast-derived noncoding RNA	NR_002802	1.33	2.52	up	0.00536	150
224807_at	GRAMD1A	GRAM domain containing 1A	NM_020895	0.74	1.67	up	0.00219	151
224909_s_at	PREX1	phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1	NM_020820	0.94	1.91	up	0.00468	152
225673_at	MYADM	myeloid-associated differentiation marker	NM_001020818 NM_001020819 NM_001020820 NM_001020821 NM_138373	0.88	1.84	up	0.00749	153
226266_at	PGS1	phosphatidylglycerophosphate synthase 1	NM_024419	0.91	1.88	up	0.00552	154
226334_s_at	AHSA2	AHA1, activator of heat shock 90kDa protein ATPase homolog 2 (yeast)	NM_152392	1.01	2.01	up	0.00468	155
226872_at	RFX2	regulatory factor X, 2 (influences HLA class II expression)	NM_000635 NM_134433	0.81	1.76	up	0.00879	156
227396_at	PTPRJ	protein tyrosine phosphatase, receptor type, J	NM_001098503 NM_002843	0.86	1.82	up	0.00814	157
227490_at	WDFY2	WD repeat and FYVE domain containing 2	NM_052950	0.51	1.43	up	0.00342	158
227510_x_at	PRO1073	PRO1073 protein	---	1.16	2.24	up	0.00265	159
227697_at	SOCS3	suppressor of cytokine signaling 3	NM_003955	1.12	2.18	up	0.00470	160

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228216_at	228216_at	Transcribed locus	---	0.91	1.88	up	0.00982	161
228582_x_at	228582_x_at	Transcribed locus	---	1.20	2.30	up	0.00591	162
228793_at	JMJD1C	jumonji domain containing 1C	NM_004241 NM_032776	1.31	2.48	up	0.00870	163
229120_s_at	CDC42SE1	CDC42 small effector 1	NM_001038707 NM_020239	0.92	1.89	up	0.00362	164
230735_at	230735_at	Transcribed locus	---	0.93	1.91	up	0.00652	165
232555_at	232555_at	CDNA FLJ11431 fis, clone HEMBA1001094	---	0.89	1.86	up	0.00362	166
233303_at	233303_at	Homo sapiens, clone IMAGE:4295366, mRNA	---	1.20	2.30	up	0.00872	167
234640_x_at	234640_x_at	CDNA: FLJ22614 fis, clone HSI05089	---	1.86	3.64	up	0.00425	168
235167_at	DKFZp547E087	hypothetical gene LOC283846	XM_496136 XM_931802 XM_931808 XM_931814 XM_931818 XM_931827 XM_931837 XM_931840	1.56	2.94	up	0.00326	169
236155_at	ZCCHC6	Zinc finger, CCHC domain containing 6	NM_024617	1.07	2.10	up	0.00823	170
236528_at	236528_at	Transcribed locus	---	1.14	2.20	up	0.00677	171
237442_at	237442_at	---	---	1.03	2.05	up	0.00562	172
237544_at	237544_at	Transcribed locus	---	1.07	2.10	up	0.00711	173
238320_at	TncRNA	trophoblast-derived noncoding RNA	NR_002802	1.34	2.54	up	0.00023	174
238712_at	238712_at	Transcribed locus	---	0.78	1.71	up	0.00917	175

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239021_at	239021_at	Transcribed locus, moderately similar to XP_530714.1 hypothetical protein XP_530714 [Pan troglodytes]	---	1.19	2.28	up	0.00891	176
240057_at	240057_at	Transcribed locus	---	0.52	1.43	up	0.00703	177
241774_at	241774_at	Transcribed locus	---	1.20	2.29	up	0.00891	178
242907_at	242907_at	---	---	1.16	2.23	up	0.00494	179
244356_at	244356_at	Transcribed locus	---	1.17	2.25	up	0.00254	180
244556_at	LCP2	Lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)	NM_005565	0.87	1.83	up	0.00777	181
244752_at	ZNF438	zinc finger protein 438	NM_182755	0.67	1.59	up	0.00468	182
37028_at	PPP1R15A	protein phosphatase 1, regulatory (inhibitor) subunit 15A	NM_014330	0.52	1.43	up	0.00470	183

The Representative sequence indicated in Table 6 refers to the target sequences for the corresponding probe set. The target sequence comprises a portion of the expressed nucleic acid marker found to be differentially expressed in the AR and NR subject samples. A target sequence may be used to obtain a sequence of the full gene or expressed nucleic acid marker by, for example, use of a BLAST search at a suitable database, such as is described herein.

[00154] **Biological pathways associated with genomic biomarkers of the invention**

[00155] Large scale gene expression analysis methods, such as microarrays have indicated that groups of genes that have an interaction (often with two or more degrees of separation) are expressed together and may have common regulatory elements. Other examples of such coordinate regulation are known in the art, see, for example, the diauxic shift of yeast (DiRisi et al 1997 Science 278:680-686; Eisen et al. 1998. Proc Natl Acad Sci 95:14863-14868).

[00156] Microarray analysis using peripheral blood samples may be used to document the biological processes invoked during graft rejection; identification of nucleic acid markers of BCAR has also been demonstrated in the preceding examples. These markers have been demonstrated to correctly classify samples with high cross-validation specificity. The biological functions of the genes differentially expressed during rejection (Table 2) encompass three major biological categories of processes related to immune signal transduction, cytoskeletal reorganization, and apoptosis, and emphasize the participation of the cytokine-activated Jak-Stat pathway, interferon signaling, and lymphocyte activation, proliferation, chemotaxis and adhesion.

[00157] Upregulation of 4 mammalian Jak family kinases was identified in the rejecting subjects, as well as STAT3, STAT5 and STAT6 in patients with BCAR - the Jak tyrosine kinase-Stat transcription factor pathway is known to be involved in immune cell development, proliferation and function While acute rejection may be classically ascribed to cytotoxic T cell mediated events, these data demonstrate that Th2/STAT6 processes are also important. Genes involved in interferon (IFN) signaling are also upregulated in BCAR, including interferon-inducible guanylate-binding protein (GBP), the interferon-response factor 1 (IRF1) and STAT1. Two MHC class I genes, HLA-E and HLA-G are known to have immunomodulatory functions and are increased in AR subjects.

[00158] T cell activation and proliferation are known to involve actin remodeling. On MHC-peptide/TCR engagement, the actin cytoskeleton is bundled at the site of engagement and is essential to forming the immune synapse; this bundling is known to be mediated by structural proteins like SLP-76, and ADAP, CDC42EP, and the actin bundling protein LCP-2. The actin cytoskeleton is remodeled to link to the integrin-receptor complex through proteins like talin and paxillin. The genes encoding these proteins are upregulated in AR subjects. AVIL (Advillin) was

one of the most highly differentially expressed genes, and codes for known to be a Ca^{2+} regulated actin-binding protein and a member of the gelsolin/villin family of actin regulatory proteins.

Apoptotic cell death, another central theme detected in this dataset, was represented by caspase 4, presenilin1, NACHT leucine rich repeat and PYD containing 1 (NLRP1), and tumor necrosis factor receptor 1 (TNF-R1). ANP32A (Acidic nuclearphosphoprotein 32 family, member a), was a highly differentially expressed nucleic acid marker and this gene encodes a protein known to have pro-apoptotic function and as illustrated in this dataset, is linked to acute rejection in AR subjects. The apoptotic signature detected in peripheral blood samples of AR subjects may thus represent a combination of T cell activation (TNF-R1 is a T cell co-receptor) and activation induced cell death (AICD) of cells which have transited from the organ. Interestingly, SIGLEC-9 (Sialic-acid binding Ig-like lectin 9), another of the most highly differentially-expressed genes, encodes a cell-adhesion molecule expressed on blood leukocytes which is upregulated during inflammation and is known to negatively regulate T cell and other leukocytes through induction of apoptosis.

[00159] A product of the CAMKK2 (calcium/calmodulin-dependent protein kinase kinase 2, beta) gene encodes a protein which belongs to the Serine/Threonine protein kinase family, and plays a role in calcium-mediated signaling. Seven transcript variants encoding six distinct isoforms have been identified for this gene. CAMKK2 beta is ubiquitously expressed and known to regulate activation of the transcription factor NfkappaB. Additional splice variants have been described but their full-length nature has not been determined. The identified isoforms undergo autophosphorylation and also phosphorylate other kinases. Nucleotide sequences of human CAMKK2 are known (e.g. GenBank Accession No. AB018081, CH473973).

[00160] A product of the FKBP1A (FK506 binding protein 1A, 12kDa) gene encodes a protein which is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. Nucleotide sequences of human FKBP1A are known (e.g. AB241120, AB241121, AB241122, AF483488, AF483489, AI847849, AK002777, AK010693, AK019362, AK085599, AK141261, AK145400, AK145986, AK151047, AK154751, AK168333, AK169186, AK169242, AL928719, BC004671, BG074872, BY065108, CH466551, U65098, U65099, U65100, X60203).

[00161] A product of the HLA-G (HLA-G histocompatibility antigen, class I, G) gene encodes a protein which belongs to the HLA class I heavy chain paralogues and is a heterodimer consisting of a heavy chain and a light chain. Nucleotide sequences of human HLA-G are known (e.g. AB088083, AB103589).

[00162] A product of the ITGAX (integrin, alpha X (complement component 3 receptor 4 subunit) gene encodes a heterodimeric integral membrane protein composed of an alpha chain and a beta chain. Nucleotide sequences of human ITGAX are known (e.g. AC124566, AF211864, AI035495, AI326085, AK089167, AK131133, AK155816, AK170432, BC042840, BC057200).

[00163] A product of the JUNB (jun B proto-oncogene) gene encodes a . Nucleotide sequences of human JUNB are known (e.g. BC053234, BX548032, EC268690).

[00164] A product of the LIMK2 (LIM domain kinase 2) gene encodes a protein which belongs to the LIM-domain containing family of proteins. LIMK2 is involved in regulation of actin cytoskeleton. Nucleotide sequences of human LIMK2 are known (e.g. NC_000022.9 NT_011520.11).

[00165] A product of the LMAN2 (lectin, mannose-binding 2) gene encodes an intracellular lectin which is known to function as a chaperone protein and transmembrane cargo receptor in the endoplasmic reticulum and golgi apparatus. Nucleotide sequences of human LMAN2 are known (e.g. X76392).

[00166] A product of the NASP (nuclear autoantigenic sperm protein (histone-binding)) gene encodes a protein which is involved in transporting histones into the nucleus of dividing cells. Multiple isoforms are encoded by transcript variants of this genes. The nucleotide sequence of the human NASP are known (e.g. BC081913, CH474008).

[00167] A product of the NCOA3 (nuclear receptor coactivator 3) gene encodes a nuclear receptor coactivator that interacts with nuclear hormone receptors to enhance their transcriptional activator functions. Nucleotide sequences of the human NCOA3 are known (e.g. AF322224, BC088343, CH474005)

[00168] A product of the NEDD9 (neural precursor cell expressed, developmentally down-regulated 9) gene encodes a docking protein which plays a central coordinating role for tyrosine-

kinase-based signaling related to cell adhesion. Nucleotide sequences of the human NEDD9 are known (e.g. AC167669, AF009366, AK030985, AK033729, AK046357, AK054179, AK083374, BB458177, BC004696, BC053713, CH466546, CT025639, D10919).

[00169] A product of the NFYC (nuclear transcription factor Y, gamma) gene encodes one subunit of a trimeric complex, forming a highly conserved transcription factor that binds with high specificity to CCAAT motifs in the promoter regions in a variety of genes. Nucleotide sequences of human MFYC are known (e.g. BC045364, BC065645, BC155102, CR388024, CT027763).

[00170] A product of the PGS1 (phosphatidylglycerophosphate synthase 1) gene encodes a protein which is a phosphatidyltransferase and participates in metabolic pathways. Nucleotide sequences of human PGS1 are known (e.g. AC061992, AK024529, AK225030, AL359590, BC008903, BC015570, BC025951, BC035662, BC108732, CH471099, CR594011, CR749720, DQ892813, DQ896059).

[00171] A product of the RBMS1 (RNA binding motif, single stranded interacting protein 1) gene encodes a protein which is a member of a small family of proteins which bind single stranded DNA/RNA. Nucleotide sequences of human RBMS1 are known (e.g. AB009975).

[00172] A product of the SFRS16 (splicing factor, arginine/serine-rich 16) gene encodes a protein which may participate in processes such as mRNA processing or RNA splicing.. Nucleotide sequences for human SFRS16 are known (e.g. AC011489, AF042800, AF042802, AF042803, AF042804, AF042805, AF042806, AF042807, AF042808, AF042809, AF042810, AK074590, AK094681, AL080189, AY358944, BC013178, BC080554, BC131496, CH471126, CR604154).

[00173] A product of the SLC6A6 (solute carrier family 8 (neurotransmitter transporter, taurine) member 6) gene encodes a protein which may have a role in amino acid transport or neurotransmitter transport. Nucleotide sequences of human SLC6A6 are known (e.g. NC_006602, NW_876271).

[00174] A short noncoding RNA, designated TncRNA (trophoblast-derived ncRNA), originates from the 3-prime end of NEAT1 and is expressed exclusively in trophoblasts. TncRNA is known to suppress MHC class II expression in mice through inhibition of CIITApIII activity, and may be a target for TP53 (p53), suggesting involvement in apoptosis or cell cycle

control Nucleotide sequences of human TncRNA are known (e.g. AF001892, AF001893, AF080092, AF508303, AK027191, AP000769, AP000944, CR611820, CR618687, U60873).

[00175] A product of the ZNF438 (zinc finger protein 438) gene encodes a protein which belongs to the family of zinc-finger motif containing proteins and may play a role in regulation of DNA-dependent transcription of immunoglobulins. Nucleotide sequences of human ZNF438 are known (e.g. AF428258, AF440405, AK057323, AK131357, AK292730, AL359532, AL591707, AL596113, AL833056, BC101622, BC104757, CH471072, DQ356011, DQ356012).

[00176] A product of the PRO1073 gene (MALAT1, metastasis associated lung adenocarcinoma transcript 1) encodes a protein which may be involved in cell cycle progression. Nucleotide sequences of human PRO1073 are known (e.g. AE017126, NP_875465).

[00177] Probe set 1558448_a_at is unannotated in the Affymetrix™ NetAffx™ Annotation database, but the target sequence is part of the IMAGE clone 5215251, according to NCBI Blast. IMAGE clone 5215251 is uncharacterized. A nucleotide sequence of IMAGE clone 5215251 is known (e.g. GenBank Accession No. BC0324515.1).

[00178] Probe set 208120_x_at is unannotated in the Affymetrix™ NetAffx™ Annotation database, but the target sequence is part of the gene FKSG63, according to NCBI Blast. FKSG63 is uncharacterized. A nucleotide sequence of FKSG63 is known (e.g. GenBank Accession No. AF338192).

[00179] Probe set 237442_is unannotated in the Affymetrix™ NetAffx™ Annotation database identifies a nucleic acid marker that includes sequences on chromosome 10 and may be part of the gene APBB1IP (amyloid beta (A4) precursor protein-binding family B member 1 interacting protein).Nucleotide sequence of APBB1IP is known (e.g. GenBank Accession No. A160287.18).

[00180] Probe set 240057_at is unannotated in the Affymetrix™ NetAffx™ Annotation database, and is part of an EST, according to NCBI Blast. Nucleotide sequence of the human EST is known (e.g. GenBank Accession No. AP000763.5).

[00181] Probe set 217436_x_at is annotated as coding for a "hypothetical protein" in the Affymetrix™ NetAffx™ Annotation database, but was found to be part of Homo sapiens major histocompatibility complex, class I, G, mRNA (cDNA clone IMAGE:4694038), partial cds in

NCBI Blast. Nucleotide sequences of human HLA-I, G, are known (e.g. GenBank Accession No. BC020891.1)

[00182] FKSG49 is unannotated in the Affymetrix™ NetAffx™ Annotation database. Nucleotide sequence of the human FKSG49 is known (e.g. GenBank Accession No. AC113404.3).

[00183] While the specific biological roles of FKSG49, FKSG49/LOC730444, and 1558448_a_at are as yet unknown, their identification and upregulation in AR samples is indicative of their suitability as nucleic acid markers of acute rejection.

[00184] **Proteomic profiling for diagnosing allograft rejection**

[00185] Proteomic profiling may also be used for diagnosing allograft rejection. Proteomic profiling may be used alone, or in combination with genomic expression profiling or metabolite profiling.

[00186] In some embodiments, the invention provides for a method of assessing or diagnosing allograft rejection, including acute kidney allograft rejection in a subject comprising 1) determining the expression profile of one or more than one proteomic markers in a biological sample from the subject, the proteomic markers selected from the group comprising a polypeptide encoded by TTN, KNG1, LBP, VASN, ARNTL2, AFM, MSTP9, MST1, PI16, SERPINA5, CFD, USH1C, C2, MBL2, SERPINA10, C9, LCAT, B2M, SHBG, C1S, UBR4 and F9; 2) comparing the expression profile of the one or more than one proteomic markers to a non-rejector profile; and 3) determining whether the expression level of the one or more than one proteomic markers is increased or decreased relative to the control profile, wherein increase or decrease of the one or more than one proteomic markers is indicative of the acute rejection status. These markers are described in further detail below.

[00187] The invention also provides for a method of assessing or diagnosing allograft rejection, including acute kidney allograft rejection, in a subject as provided by the present invention comprises 1) measuring the increase or decrease of one or more than one proteomic markers selected from the group comprising a polypeptide encoded by TTN, KNG1, LBP, VASN, ARNTL2, AFM, MSTP9, MST1, PI16, SERPINA5, CFD, USH1C, C2, MBL2, SERPINA10, C9, LCAT, B2M, SHBG, C1S, UBR4, and F9; and 2) determining the 'rejection status' of the subject, wherein the determination of 'rejection status' of the subject is based on

comparison of the subject's proteomic marker expression profile to a control proteomic marker expression profile.

[00188] In some embodiments, the one or more than one proteomic markers are KNG1, AFM, TTN, MSTP9/MST1, PI16, C2, MBL2, SERPINA10 and UBR4.

[00189] A myriad of methods for protein identification and quantitation are currently available, such as glycopeptide capture (Zhang et al., 2005. Mol Cell Proteomics 4:144-155), multidimensional protein identification technology (Mud-PIT)Washburn et al., 2001 Nature Biotechnology (19:242-247), and surface-enhanced laser desorption ionization (SELDI-TOF) (Hutches et al., 1993. Rapid Commun Mass Spec 7:576-580). In addition, several isotope labelling methods which allow quantification of multiple protein samples, such as isobaric tags for relative and absolute protein quantification (iTRAQ) (Ross et al, 2004 Mol Cell Proteomics 3:1154-1169); isotope coded affinity tags (ICAT) (Gygi et al., 1999 Nature Biotechnology 17:994-999), isotope coded protein labelling (ICPL) (Schmidt et al., 2004. Proteomics 5:4-15), and N-terminal isotope tagging (NIT) (Fedjaev et al., 2007 Rapid Commun Mass Spectrom 21:2671-2679; Nam et al., 2005. *J Chromatogr B Analyt Technol Biomed Life Sci.* 826:91-107), provide a format suitable for high-throughput performance, a trait particularly useful in biomarker screening/identification studies.

[00190] A multiplexed iTRAQ methodology was employed for identification of plasma proteomic markers in allograft recipients. iTRAQ was first described by Ross et al, 2004 (Mol Cell Proteomics 3:1154-1169). Briefly, subject plasma samples (control and allograft recipient) were depleted of the 14 most abundant proteins and quantitatively analyzed by iTRAQ-MALDI-TOF/TOF, resulting in the identification of 460 protein group codes in at least one BCAR positive and BCAR negative sample. 144 protein group codes were detected in at least 8 out of 11 BCAR positive samples, and in at least 14 of 21 controls. Table 7 presents the 18 significant protein group codes identified.

[00191] Thus, while a single candidate biomarkers may not clearly differentiate AR and NR subjects, *together*, a set of proteomic markers comprising KNG1, AFM, TTN, MSTP9/MST1, PI16, C2, MBL2, SERPINA10 and UBR4 achieved a satisfactory classification (63% sensitivity and 86% specificity). As described below and in the accompanying examples, amino acids sequences of the isoforms of the proteomic markers identified as members of the

protein group codes are known, and may be specifically identified by the accession numbers described herein (e.g. GenBank, GenPept, IPI or the like).

[00192] While iTRAQ was one exemplary method used to detect the peptides, other methods described herein, for example immunological based methods such as ELISA may also be useful. Alternately, specific antibodies may be raised against the one or more proteins, isoforms, precursors, polypeptides, peptides, or portions or fragments thereof, and the specific antibody used to detect the presence of the one or more proteomic marker in the sample. Methods of selecting suitable peptides, immunizing animals (e.g. mice, rabbits or the like) for the production of antisera and/or production and screening of hybridomas for production of monoclonal antibodies are known in the art, and described in the references disclosed herein.

[00193] **Proteomic expression profiling markers ("proteomic markers")**

[00194] One or more precursors, splice variants, isoforms may be encoded by a single gene Examples of genes and the isoforms, precursors and variants encoded are provided in Table 7, under the respective Protein Group Code (PGC).

[00195] A polypeptide encoded by TTN (Titin, Connectin,TMD, CMH9, CMD1G, CMPD4, EOMFC, HMERF, LGMD2J, FLJ26020, FLJ26409, FLJ32040, FLJ34413, FLJ39564, FLJ43066, DKFZp451N061) is a muscle protein expressed in regions of cardiac and skeletal muscle. Nucleotide sequences encoding TTN are known (e.g. GenBank Accession Nos. AC009948.3, AF321609.2, NM_133437.2, NM_133432.2, NM_003319.3, NM_133378.3, NM133379.2,). Amino acid sequences for TTN are known (e.g. GenPept Accession Nos. NP_597676.2, NP_596870.2, NP_597681.2NP_003310.3, NP_596869.3, Q4ZG20, Q8WZ50, Q6ZP81, Q8WZ42.2).

[00196] A polypeptide encoded by KNG1 (Kininogen 1, BDK) may have a role in assembly of plasma kallikrein, and has high and low molecular weight isoforms, generated by alternate splicing. Nucleotide sequences encoding KNG1 are known (e.g. GenBank Accession Nos. NM_000893.2, NM001102416.1, AC109780.7, AI133186.1, BC060039.1,). Amino acid sequences for KNG1 are known (e.g. GenPept Accession Nos. NP_000884.1, NP_001095886.1, AAH600396.1, P01042.2, Q05CF8).

[00197] A polypeptide encoded by LBP(lipopolysaccharide binding protein) may have a role in an acute-phase immunologic response to a bacterial infection. Nucleotide sequences

encoding LBP are known (e.g. GenBank Accession Nos. NM_004139.2, AF013512.1, AF106067/1, M35533.1, DQ891394.2). Amino acid sequences for LBP are known (e.g. GenPept Accession Nos. NP_004130.2, AAC39547.1, AAD21962.1, AAA59493.1, ABM85360.1, P18428.3, Q8TCF0).

[00198] A polypeptide encoded by VASN (vasorin) is a TGF-beta binding protein found in vascular smooth muscle cells. Nucleotide sequences encoding VASN are known (e.g. GenBank Accession Nos. NM_138440.2, CH471112.2, AY166584.1). Amino acid sequences for VASN are known (e.g. GenPept Accession Nos. NP_612449.2, EAW85311.1, Q6EMK4.1, AAO27704.1).

[00199] A polypeptide encoded by ARNTL2 (aryl hydrocarbon receptor nuclear translocator-like-2, BMAL2, MOP9) is a member of the basic helix-loop-helix family of transcription factors, which may have roles in various physiological processes including circadian rhythms. Nucleotide sequences encoding ARNTL2 are known (e.g. GenBank Accession Nos. NM_020183.3, AC068794.25, AB03992.1). Amino acid sequences for ARNTL2 are known (e.g. GenPept Accession Nos. NP_064568.3, Q8WYA1.2, BAB01485.4).

[00200] A polypeptide encoded by AFM (afamin, ALB2, ALBA, ALF, MGC125338, MGC125339, AFM) is a serum transport protein of the albumin gene family. Nucleotide sequences encoding AFM are known (e.g. GenBank Accession Nos. NM_001133.2, AC108157.3, AK290556.1). Amino acid sequences for AFM are known (e.g. GenPept Accession Nos. NP_001124.1, BAF83245.1, P43652.1, Q4W5C5).

[00201] A polypeptide encoded by MSTP9 is a putative macrophage-stimulating protein (brain rescue factor 1), and a homolog of hepatocyte growth factor-like protein. Nucleotide sequences encoding MSTP9 are known (e.g. GenBank Accession Nos. AF083416.1, AF116647.1, AY192149.1, U28055.1). Amino acid sequences for MSTP9 are known (e.g. GenPept Accession Nos. Q2TV78.2, AAP20103.12, AAC35412.1).

[00202] A polypeptide encoded by MST1 (macrophage stimulating 1, MSP, HGFL, NF15S2, D3F15S2) may have a role in inflammatory bowel disease. Nucleotide sequences encoding MST1 are known (e.g. GenBank Accession Nos. NM020998.3, AC099668.2, AK222893.1, M74178.1). Amino acid sequences for MST1 are known (e.g. GenPept Accession Nos. NP_066278.3, P26928.2, Q13208, Q49A61, Q53GN8, BAD96613.1, AAA50165.1).

[00203] A polypeptide encoded by PI16 (Peptidase inhibitor 16, PSPBP, CRISP9, MSMBBP, MGC45378, DKFZp586B1817) is a blood protein that may interact with prostate secretory proteins. Nucleotide sequences encoding PI16 are known (e.g. GenBank Accession Nos. NM_153370.2, AL122034.29, AK075470.1, AK124589.1, AK302193.1, AK312785.1, BC022399.1). Amino acid sequences for PI16 are known (e.g. GenPept Accession Nos. NP_699201.2, Q6UXB8.1, BAC11640.1, BAG35648.1, AAH22399.2).

[00204] A polypeptide encoded by SERPINA5 (serpin peptidase inhibitor, clade A member 5, PAI3, PCI, PROCI, protein C inhibitor) is a plasma protein inhibitor of activated protein C. Nucleotide sequences encoding SERPINA5 are known (e.g. GenBank Accession Nos. NM_000624.4, AF361796.1, AK096131.1, BC018915.2, U35464.1). Amino acid sequences for SERPINA5 are known (e.g. GenPept Accession Nos.NP_000615.3, P05154.2AAB60386.1, AAH08915.1, BAG53218.1).

[00205] A polypeptide encoded by CFD (complement factor D, adipsin) is a member of the trypsin factor of peptidases. Nucleotide sequences encoding CFD are known (e.g. GenBank Accession Nos. NM_001928.2, AC112706.2,AJ313463.1, BC034529.1, BC057807.1, M84526.1). Amino acid sequences for CFD are known (e.g. GenPept Accession Nos. NP_001919.2, P00746.5, Q6FHW3, AAA35527.1, AAH570807.1, CAC48304.1).

[00206] A polypeptide encoded by USH1C is a scaffold protein that functions in the assembly of Usher protein complexes. Nucleotide sequences encoding USH1C are known (e.g. GenBank Accession Nos. NM_005709.3, NM_153676.3, kAC124799.5, AB006955.1, AF039699.1, AK000936.1, BK000147.1). Amino acid sequences for USH1C are known (e.g. GenPept Accession Nos. NP_005700.2, NP_710142.1,AAC18049.1, BAG62565.1, DAA00086.1, Q7RTU8, Q9H758, Q9Y6N9.3).

[00207] A polypeptide encoded by C2 (complement component 2, CO2, DKFZp779M0311) is a serum glycoprotein having a role in the classical complement pathway. Nucleotide sequences encoding C2 are known (e.g. GenBank Accession Nos. NM_000063.4, NM_001145903.1, AF019413.1, AK096258.1, BC029781.1, BX537504.1, M26301.1, X04481.1). Amino acid sequences for C2 are known (e.g. GenPept Accession Nos. NP_000054.2, NP_001139375.1, AAA35604.1, CAA28169.1, CAD97767.1).

[00208] A polypeptide encoded by MBL2 (mannose binding lectin 2, MBL, MBP, MBP1, COLEC1, HSMBPC, MGC116832, MGC116833) is a soluble mannose-binding lectin found in serum. Nucleotide sequences encoding MBL2 are known (e.g. GenBank Accession Nos. NM_000242.2, AB025350.1, AF360991.1, BC096181.2). Amino acid sequences for MBL2 are known (e.g. GenPept Accession Nos. NP_000233.1, BAB17020.1, AAK52907.1, AAH96182.3, P11226.2, Q5SQS3, Q9HCS8).

[00209] A polypeptide encoded by SERPINA10 (serpin peptidase inhibitor clade A ember 10, ZPI, PDI) is a serpin that inhibits the activated coagulation factors X and XI. Nucleotide sequences encoding SERPINA10 are known (e.g. GenBank Accession Nos. NM_001100607.1, NM_016186.2, CH471061.1, AF181467.1, BC022261.1, CR606434.1). Amino acid sequences for SERPINA10 are known (e.g. GenPept Accession Nos. NP_001094077.1, NP_057270.1, EAW81564.1, AAD53962.1, CAD62339.1, Q9UK55.1).

[00210] A polypeptide encoded by LCAT (lecithin-cholesterol acetyltransferase) is an extracellular cholesterol esterifying enzyme, affecting cholesterol transport. Nucleotide sequences encoding LCAT are known (e.g. GenBank Accession Nos. NM_000229.1, AC040162.5, BC014781.1, X06537.1). Amino acid sequences for LCAT are known (e.g. GenPept Accession Nos. NP_000299.1, P04180.1, Q53XQ3, Q9Y5N3, AAH14781.1, CAB56610.1).

[00211] A polypeptide encoded by B2M (Beta-2-Microglobulin) is a serum protein found in association with the major histocompatibility complex (MHC) class 1 heavy chain on the surface of most nucleated cells. Nucleotide sequences encoding B2M are known (e.g. GenBank Accession No. NM_004048, BU658737.1, BC032589.1 and AI686916.1). Amino acid sequences for B2M are known (e.g. GenPept Accession No. P61769, AAA51811, CAA23830).

[00212] A polypeptide encoded by SHBG (Sex-hormone binding globulin, androgen-binding protein, ABP, testosterone-binding beta-globulin, TEBG) is a plasma glycoprotein that binds sex steroids. Nucleotide sequences encoding SHBG are known (e.g. GenBank Accession No. AK302603.1, NM_001040.2). Amino acid sequences for SHBG are known (e.g. GenPept Accession No. P04728.2, CAA34400.1, NP001031.2).

[00213] A polypeptide encoded by C1S (complement component 1, S subcomponent) is a serine protease and a component of the human complement C1. Nucleotide sequences encoding

C1S are known (e.g. GenBank Accession Nos. NM_001734.3, NM_201442.2, AB009076.1, AK025309.1, J04080.1, M18767.1). Amino acid sequences for C1S are known (e.g. GenPept Accession Nos. NP_001725.1, NP_958850.1, BAA86864.1, AAA51852.1, AAA51853.1).

[00214] A polypeptide encoded by UBR4 (ubiquitin protein ligase D3 component n-recognin 4, p600; ZUBR1; RBAF600; FLJ41863; KIAA0462; KIAA1307; RP5-1126H10.1) may have a role in regulation of anchorage-independent growth associated with some oncogenic viruses. Nucleotide sequences encoding UBR4 are known (e.g. GenBank Accession Nos. NM_020765.2, AL137127.7, AA748129.1, AB007931.1, BC096758.1). Amino acid sequences for UBR4 are known (e.g. GenPept Accession Nos. NP_065816.2, CAI19268.1, BAA32307.1, AAH96758.1, Q5T4S7.1, Q6ZUC7, Q96HY5).

[00215] A polypeptide encoded by F9 (coagulation factor XI) is a vitamin K-dependent coagulation factor found in the blood as an active zymogen. Nucleotide sequences encoding F9 are known (e.g. GenBank Accession Nos. NM_000133.3, A01819.1, AB186358.1, A13997.1, M11390.1). Amino acid sequences for F9 are known (e.g. GenPept Accession Nos. NP_1000124.1, CAA00205.1, BAD89383.1, P00740.2, Q14316, CAA01140.1, AAA52023.1).

[00216] Table 7 and the IPI accession numbers provided therein further indicate database records where the amino acid sequence information of specific isoforms of the indicated protein group code members may be obtained.

[00217] Interpretation of the large body of expression data obtained from, for example, iTRAQ protein or proteomic experiments, but is greatly facilitated through use of algorithms and statistical tools designed to organize the data in a way that highlights systematic features. Visualization tools are also of value to represent differential expression by, for example, varying intensity and hue of colour. The algorithm and statistical tools available have increased in sophistication with the increase in complexity of arrays and the resulting datasets, and with the increase in processing speed, computer memory, and the relative decrease in cost of these.

[00218] Mathematical and statistical analysis of protein or polypeptide expression profiles may accomplish several things – identification of groups of genes that demonstrate coordinate regulation in a pathway or a domain of a biological system, identification of similarities and differences between two or more biological samples, identification of features of a gene expression profile that differentiate between specific events or processes in a subject, or the like.

This may include assessing the efficacy of a therapeutic regimen or a change in a therapeutic regimen, monitoring or detecting the development of a particular pathology, differentiating between two otherwise clinically similar (or almost identical) pathologies, or the like.

[00219] Methods for selecting and manufacturing such antibodies, as well as their inclusion on a 'chip' or an array, or in an assay, and methods of using such chips, arrays or assays are referenced or described herein.

[00220] **Other embodiments**

[00221] Nucleic acid profiling may also be used in combination with metabolite ("metabolomics") or proteomic profiling. Minor alterations in a subject's genome, such as a single nucleotide change or polymorphism, or expression of the genome (e.g. differential gene expression) may result in rapid response in the subject's small molecule metabolite profile. Small molecule metabolites may also be rapidly responsive to environmental alterations, with significant metabolite changes becoming evident within seconds to minutes of the environmental alteration – in contrast, protein or gene expression alterations may take hours or days to become evident. The list of clinical variables includes, for example, cholesterol, homocysteine, glucose, uric acid, malondialdehyde and ketone bodies. Other non-limiting examples of small molecule metabolites are listed in Table 3.

[00222] Table 3: Metabolites identified and quantified in NMR spectra of serum samples obtained from subject population.

Compound Name	
Glucose	Lactate
Glutamine	Alanine
Glycine	Proline
Glycerol	Valine
Taurine	Lysine
Citrate	Serine
Leucine	Ornithine
Creatinine	Tyrosine
Phenylalanine	Pyruvate
Histidine	Carnitine
Glutamate	Acetate
Isoleucine	Asparagine
Betaine	3-Hydroxybutyrate
Creatine	Propylene glycol
2-Hydroxybutyrate	Formate

Methionine	Choline
Acetone	

[00223] Various techniques and methods may be used for obtaining a metabolite profile of a subject. The particulars of sample preparation may vary with the method used, and also on the metabolites of interest – for example, to obtain a metabolite profile of amino acids and small, generally water soluble molecules in the sample may involve filtration of the sample with a low molecular weight cutoff of 2-10 kDa, while obtaining a metabolite profile of lipids, fatty acids and other generally poorly-water soluble molecules may involve one or more steps of extraction with an organic solvent and/or drying and resolubilization of the residues. While some exemplary methods of detecting and/or quantifying markers have been indicated herein, others will be known to those skilled in the art and readily usable in the methods and uses described in this application.

[00224] Some examples of techniques and methods that may be used (either singly or in combination) to obtain a metabolite profile of a subject include, but are not limited to, nuclear magnetic resonance (NMR), gas chromatography (GC), gas chromatography in combination with mass spectroscopy (GC-MS), mass spectroscopy, Fourier transform MS (FT-MS), high performance liquid chromatography or the like. Exemplary methods for sample preparation and techniques for obtaining a metabolite profile may be found at, for example, the Human Metabolome Project website (Wishart DS et al., 2007. Nucleic Acids Research 35:D521-6).

[00225] Standard reference works setting forth the general principles of such methods useful in metabolite profiling as would be known to those of skill in the art include, for example, *Handbook of Pharmaceutical Biotechnology*, (ed. SC Gad) John Wiley & Sons, Inc., Hoboken, NJ, (2007), *Chromatographic Methods in Clinical Chemistry and Toxicology* (R Bertholf and R. Winecker, eds.) John Wiley & Sons, Inc., Hoboken, NJ, (2007), *Basic One- and Two-Dimensional NMR Spectroscopy* by H., Friebolin. Wiley-VCH 4th Edition (2005).

[00226] Access to the methods of the invention may be provided to an end user by, for example, a clinical laboratory or other testing facility performing the individual marker tests – the biological samples are provided to the facility where the individual tests and analyses are performed and the predictive method applied; alternately, a medical practitioner may receive the marker values from a clinical laboratory and use a local implementation or an internet-based implementation to access the predictive methods of the invention.

[00227] **Kits**

[00228] The invention also provides for a kit for use in assessing or diagnosing a subject's rejection status. The kit may comprise reagents for specific and quantitative detection of one or more nucleic acid markers, selected from the group comprising TncRNA, FKSG49, ZNF438, SFRS16, 1558448_a_at, CAMKK2, NFYC, NCOA3, LMAN2, PGS1, NEDD9, 237442_at, FKSG49/LOC730444, LIMK2, UNB, NASP, PRO1073, 240057_at, ITGAX, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6, along with instructions for the use of such reagents and methods for analyzing the resulting data. In some embodiments, the nucleic acid markers are TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX. The kit may be used alone for predicting or diagnosing a subject's rejection status, or it may be used in conjunction with other methods for determining clinical variables, or other assays that may be deemed appropriate. The kit may include, for example, a labelled oligonucleotide capable of selectively hybridizing to the marker. The kit may further include, for example, an oligonucleotide operable to amplify a region of the marker (e.g. by PCR). Instructions or other information useful to combine the kit results with those of other assays to provide a non-rejection cutoff index for the prediction or diagnosis of a subject's rejection status may also be provided.

[00229] The invention also provides for a nucleic acid array. The array may be a two-dimensional array, and may contain at least 10 different nucleic acid molecules (e.g., at least 20, at least 30, at least 50, at least 100, or at least 200 different nucleic acid molecules). Each nucleic acid molecule may have any length sufficient to specifically identify a nucleic acid marker by hybridization. For example, each nucleic acid molecule may be between 10 and 250 nucleotides (e.g., between 12 and 200, 14 and 175, 15 and 150, 16 and 125, 18 and 100, 20 and 75, or 25 and 50 nucleotides, or any amount therebetween) in length. For example, the nucleic acid molecules of the arrays provided herein may comprise sequences that hybridize with and specifically identify one or more than one of the nucleic acid markers presented in Table 2. Exaples of such sequences include SEQ ID NO: 1-183.

[00230] The invention also provides for a kit for use in assessing or diagnosing a subject's rejection status. The kit may comprise reagents for specific and quantitative detection of one or more than one proteomic markers selected from the group comprising TTN, KNG1, LBP, VASN, ARNTL2, AFM, MSTP9, MST1, PI16, SERPINA5, CFD, USH1C, C2, MBL2,

SERPINA10, C9, LCAT, B2M, SHBG, C1S, UBR4 and F9, along with instructions for the use of such reagents and methods for analyzing the resulting data. In some embodiments, the one or more than one proteomic markers are KNG1, AFM, TTN, MSTP9, MST1, PI16, C2, MBL2, SERPINA10, F9 and UBR4. For example, the kit may comprise antibodies or fragments thereof, specific for the proteomic markers (primary antibodies), along with one or more secondary antibodies that may incorporate a detectable label; such antibodies may be used in an assay such as an ELISA. Alternately, the antibodies or fragments thereof may be fixed to a solid surface, e.g. an antibody array. The kit may be used alone for predicting or diagnosing a subject's rejection status, or it may be used in conjunction with other methods for determining clinical variables, or other assays that may be deemed appropriate. Instructions or other information useful to combine the kit results with those of other assays to provide a non-rejection cutoff index for the prediction or diagnosis of a subject's rejection status may also be provided.

[00231] The invention also provides for computer-readable storage medium configured with instructions for causing a programmable processor to determine whether an allograft is being rejected. Methods for determining whether an allograft is being rejected (rejection status of the subject) are described herein, and the processor comprises instructions to receive a signal (e.g. light emission, a change in intensity or frequence of fluorescence, or the like, representative of the relative quantity of the nucleic acid or proteomic marker present in the sample) and assess the level of a nucleic acid or proteomic marker relative to a control and determine if the level is increased or decreased. The processor may be further provided with instructions to interpret the pattern of increase and/or decrease of the indicated nucleic acid or proteomic marker, and provide information to a user (for example a physician) on the rejection status of the subject. Instruction and information for removal of baseline noise or other aberrant signals from the detected signals may also be included. The instructions may be provided on a computer-readable storage medium and may be implemented in a high level procedural or object oriented programming language to communicate with a computer system. Alternatively, such instructions can be implemented in assembly or machine language. The language further can be compiled or interpreted language.

[00232] The nucleic acid detection signals can be obtained using an apparatus (e.g., a chip or an array reader) and a determination of tissue rejection can be generated using a separate processor (e.g., a computer). Alternatively, a single apparatus having a programmable processor may combine these and/or other functions and obtain the detection signals and process the

signals to generate a determination of the rejection status of the subject. The processing step may be performed simultaneously with the step of collecting the detection signals (e.g., "real- time").

[00233] Methods for selecting and manufacturing such antibodies, as well as their inclusion on a 'chip' or an array, or in an assay, and methods of using such chips, arrays or assays are referenced or described herein.

[00234] **Methods**

Subjects and Specimens

[00235] All subjects in this study received a renal transplant between 2005 and 2007 at St. Paul's Hospital or Vancouver General Hospital, Vancouver, UBC, Canada, and appropriate consent was obtained. Immunosuppression was mainly based on Mycophenolate Mofetil (MMF) in combination with Tacrolimus and/or Prednisolone. Age, gender, ethnicity and primary disease of the subjects are summarized in Table 4, below. Whole blood was drawn using PAXgene^{TM} tubes pre-transplant (baseline) and post-transplant at 0.5, 1, 2, 3, 4, 8, 12, and 26 weeks, every 6 months through year 3, and at the time of suspected rejection. Urine samples were obtained for the same time points. PAXgene^{TM} whole blood samples were also taken from a cohort of control subjects with no disease using representative ages and sexes from the transplant patients. All samples were stored at -80°C until selection for analysis. 33 subjects were included in the genomic marker study, and 32 of these 33 were included in the proteomic marker study. .

[00236] Table 4: Kidney transplant subject demographics.

	Subjects with AR (n=11)	Subjects without AR (n=22)
Mean Age (standard deviation)	41.85 (11.98)	48.97 (10.57)
Gender (n, % male)	8 (72.73%)	14 (63.64%)
Ethnicity (n,%)		
Caucasian	9 (81.82%)	15 (68.18%)
North American Indian	1 (9.09%)	2 (9.09%)
Asian	0 (0%)	2 (9.09%)
Indian Sub-continent	1 (9.09%)	2 (9.09%)
Other	0 (0%)	1 (4.55%)
Primary Disease (n, %)		
Chronic renal failure, aetiology uncertain	4 (36.36%)	2 (9.09%)
Cortical or tubular necrosis	1 (9.09%)	1 (4.55%)
Diabetic nephropathy associated with Type II	1 (9.09%)	1 (4.55%)
Focal glomerulosclerosis - adults	3 (27.27%)	4 (18.18%)

Polycystic kidneys, adult type (dominant)	0 (0%)	5 (22.73%)
IgA Nephropathy (proven by immunofluorescence)	2 (18.18%)	1 (4.55%)
Other	0 (0%)	8 36.36%)
Donor		
Living	3 (27.3%)	8 (38.1%)
Deceased	8 (72.7%)	13 (61-9%)

[00237] All kidney transplant subject clinical data was reviewed. Samples were selected from subjects with acute rejection, borderline rejection or no rejection who had no significant co-morbidities (infections, disease recurrence, or other co-morbid events). To ensure homogeneous phenotypes and to minimize biological variability for this analysis, patients were considered eligible if they were less than 75 years of age; were not receiving immunosuppression prior to transplantation; had not received pre-transplant immunological desensitization; had received a kidney transplant from a deceased or non-HLA-identical living donor; had a negative AHG-CDC anti-donor T-cell cross-match; had not received depleting antibody induction therapy with ATG or OKT3; were able to receive oral medication, had immediate graft function, and had no clinical or laboratory evidence of infections, disease recurrence, and other major co-morbid events. Biopsies were diagnosed and recorded using the Banff criteria (Solez et al 2008 Am J Transplant 8: 753; Table 1). The cohort for this study consisted of 11 acute rejection (AR) subjects within the first week, and 22 non-rejection (NR) subjects within the first week (biopsy-confirmed acute rejection, BCAR). For all NR subjects data was available at weeks 1, 2, 3, 4 and baseline (BL). One AR subject did not have a baseline sample, and three subjects did not have a week 1, week 2 and week 4 sample, respectively. Several subjects had data for additional time points at weeks 8 and 12. Two AR patients had their rejection at day 3. For the analysis, these rejections were considered in the week 1 group. 20 normal samples from 20 healthy individuals are included to calculate results relative-to-normal. Thus, the analysis includes samples from 53 individuals, 33 of which were patients who provided samples at different time points during the 3-month post-transplant period

[00238] The study employed a closed cohort case-control design to compare differential gene expression in subjects with or without BCAR during the first 3 months post-transplant. Patients with BCAR (cases) diagnosed during the first 12 weeks post-transplant were matched 1:2 with those who did not have evidence of clinical or BCAR (controls) during the same period of observation. All rejection episodes were diagnosed by conventional clinical and laboratory parameters, were confirmed by biopsy, and graded according to the Banff criteria for working

classification of renal allograft pathology. Banff categories 2 and 4 (antibody-mediated or acute/active cellular rejection) were considered significant. Subjects with borderline changes (Category 3) were analyzed separately. All baseline demographic and follow-up data were recorded in the transplant program electronic database and there was no loss to follow-up during the period of study.

[00239] **Immunosuppression**: Immunosuppression consisted of basiliximab at 20 mg i.v. on days 0 and 4, with tacrolimus 0.075 mg/kg b.i.d and mycophenolate 1000 mg b.i.d. Drug concentrations were measured by tandem mass spectrometry; the tacrolimus dose was adjusted to achieve 12-hour trough levels of 8-12 ng/mL for the first month post-transplant, 6-9 ng/ml for the second month, then 4-8 ng/ml thereafter. First graft and non-sensitized subjects received methylprednisolone 125 mg iv on the day of transplantation, and oral prednisone of 1 mg/kg on day 1, declining to zero by day 3 post-transplant. For recipients of a second or subsequent graft, the prednisone dose was reduced slowly and in a stepwise fashion to a maintenance dose of 10 mg on alternate days after three months. Rejection episodes were treated with methylprednisolone 500 mg i.v. daily for 3-5 days. Steroid resistant rejections were treated with OKT3 5 mg i.v. or ALG 15mg/kg i.v daily for 7-10 days.

[00240] **Plasma collection and depletion:** Whole blood samples from transplant recipients, taken at the scheduled time-points and at the time of suspected rejection, and similar blood samples from normal disease-free controls of comparable ages and sexes, were drawn into EDTA tubes, stored on ice before processing. Plasma was separated and stored at -80 ^{o}C within 2 hours then transferred to liquid nitrogen until selected for analysis. Plasma samples were then thawed to room temperature, diluted 5 times with 10 mM phosphate buffered saline (PBS) at pH 7.6, and filtered with spin-X centrifuge tube filters. Diluted plasma was injected via a 325 µL sample loop onto a 5 mL avian antibody affinity column (Genway Biotech; San Diego, CA) capable of removing the 14 most abundant plasma proteins: HAS, IgG, fibrinogen, transferring, IgA, IgM, haptoglobin, α2-macroglobulin, α1-acid glycoprotein, α1-antitrypsin, Apoliprotein-I, Apoliprotein-II, Complement C3 and low density lipoproteins (mainly Apoliprotein B). Flow-through fractions were collected and precipitated by adding TCA to a final concentration of 10% and incubated at 4 ^{o}C for 16-18 hours. The protein precipitate was recovered by centrifugation at 3200g at 4 ^{o}C for 1 hour, washed three times with ice cold acetone (EMD; Gibbstown, NJ) and re-hydrated with 200-300 µL iTRAQ buffer consisting of 45:45:10 saturated urea (J.T. Baker;

Phillipsburg, NJ), 0.05 M TEAB buffer (Sigma-Aldrich; St Louis, MO), and 0.2% SDS (Sigma-Aldrich; St Louis, MO). Each sample was then stored at -80 °C.

RNA Extraction and Microarray Analysis

[00241] RNA extraction was performed on thawed samples using the PAXgene™ Blood RNA Kit [Cat #762134] to isolate total RNA. Between 4 and 10 µg of RNA was routinely isolated from 2.5 ml whole blood and the RNA quality confirmed using the Agilent BioAnalyzer. Samples with 1.5 µg of RNA, an RIN (RNA integrity number) >5, and A240/A280 >1.9 were packaged on dry ice and shipped by overnight courier to the Microarray Core (MAC) Laboratory, Children's Hospital, Los Angeles, CA for Affymetrix microarray analysis. The microarray analysis was performed by a single technician at the CAP/CLIA accredited MAC laboratory. Nascent RNA was used for double stranded cDNA synthesis. The cDNA was then labeled using the Affymetrix cDNA Synthesis Kit (Affymetrix Inc., Santa Clara, CA), fragmented, mixed with hybridization cocktail and hybridized onto GeneChip Human Genome U133 Plus 2.0 Arrays. The arrays were scanned with the Affymetrix System in batches of 48 with an internal RNA control made from pooled normal whole blood. Microarrays were checked for quality issues using Affymetrix version 1.16.0 and affyPLM version 1.14.0 BioConductor packages (Bolstad, B., *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. 2004, University of California, Berkeley; Irizarry et al. 2003. Biostatistics 4(2): 249-64). The arrays with lower quality were repeated with a different RNA aliquot from the same time point. The Affymetrix™ NetAffx™ Annotation database Update Release 25 (March 2008) was used for identification and analysis of microarray results.

Gene expression analysis

[00242] The microarray analysis produced one Cel file per sample with 54,000 probe sets that analyzes over 47,000 transcripts and variants from over 38,500 well-substantiated human genes. All Cel files were pre-processed before the final analysis. The pre-processing steps were: (1) quality control of gene chip results, (2) adjustment of background intensities, (3) normalization of all data together, (4) summarization of probe-level data into probe-set intensity values, and (5) filtering of probe-sets to removed probe-sets that did not show a high enough intensity across samples.

[00243] Quality control was performed using issues using Affy version 1.16.0 and affyPLM version 1.14.0 BioConductor packages. Samples with low quality were repeated. Cel

files were RMA normalized (Bolstad, et al. Bioinformatics, 2003. **19**(2): p. 185–93) and log2-transformed with the Affy BioConductor package version 1.16.0 (Bolstad, 2004, supra). A raw expression filter left 21,771 probe sets with a signal intensity of 2^6=64 in at least 3 of 416 samples. The filtering step was then used to include probe-sets with a log2-expression value of at least 6 in at least 3 samples over all 416 samples that were used in the normalization. The overall number of samples included in the pre-processing steps was 416; 33 of these were from transplant subject samples were used in the final analysis.

[00244] **Trypsin Digest and iTRAQ labeling**: Total protein concentration was determined using the bicinchoninic acid assay (BCA) (Sigma-Aldrich, St Louis, MO USA) were used to obtain 100 µg of total protein from each sample. Each sample was then precipitated by the addition of 10 volumes of HPLC grade acetone at -20 °C (Sigma-Aldrich, Seelze, Germany) and incubated for 16-18 hours at -20 °C. The protein precipitate was recovered by centrifugation at 16,110g for 10 min and dissolved in 50 mM TEAB buffer (Sigma-Aldrich; St Louis, MO) and 0.2% electrophoresis grade SDS (Fisher Scientific; Fair Lawn, NJ). Proteins in each sample were reduced with TCEP (Sigma-Aldrich; St Louis, MO) at 3.3 mM and incubated at 60 °C for 60 min. Cysteines were blocked with methyl methane thiosulfonate at a final concentration of 6.7 mM and incubated at room temperature for 10 min.

[00245] Reduced and blocked samples were then digested with sequencing grade modified trypsin (Promega; Madison, WI) and incubated at 37 °C for 16-18 hours. Trypsin digested peptide samples were then dried in a speed vacuum (Thermo Savant; Holbrook, NY) and labeled with iTRAQ reagent according to the manufacturer's protocol (Applied Biosystems; Foster City, CA). Labeled samples were pooled and acidified to pH 2.5-3.0 with concentrated phosphoric acid (ACP Chemicals Inc; Montreal, QC, Canada).

[00246] **2D-LC Chromatography:** iTRAQ labeled peptides were separated by strong cation exchange chromatography (SCX) using a 4.6 mm internal diameter (ID) and 100 mm in length polysulphoethyl A column packed with 5 µm beads with 300 Å pores (PolyLC Inc., Columbia, MD USA) on a VISION workstation (Applied Biosystems; Foster City, CA). Mobile phases used were Buffer A composed of 10 mM monobasic potassium phosphate (Sigma-Aldrich; St Louis, MO) and 25% acetonitrile (EMD Chemicals; Gibbstown, NJ) pH 2.7, and Buffer B that was the same as A except for the addition of 0.5 M potassium chloride (Sigma-Aldrich St Louis, MO, USA). Fractions of 500 µL were collected over an 80 minute gradient divided into two

linear profiles: 1) 0-30 min with 5% to 35% of Buffer B, and 2) 30-80 min with 35% to 100% of Buffer B. The 20 to 30 fractions with the most peptides detected by UV trace were selected and their volumes were reduced to 150µL in preparation for nano reverse phase chromatography. Peptides were desalted by loading fractions onto a C18 PepMap guard column (300 µm ID x 5 mm, 5 µm, 100 Å, LC Packings, Amsterdam) and washing for 15 min at 50 µL/min with mobile phase A consisting of water/acetonitrile/TFA 98:2:0.1 (v/v). The trapping column was then switched into the nano flow stream at 200 nL/min where peptides were loaded onto a Magic C18 nano LC column (15 cm, 5 µm pore size, 100 Å, Michrom Bioresources Inc., Auburn CA, USA) for high resolution chromatography. Peptides were eluted by the following gradient: 0-45 min with 5% to 15% B (acetonitrile/water/TFA 98:2:0.1, v/v); 45-100 min with 15% to 40% B, and 100-105 min with 40% to 75% B. The eluent was spotted directly onto MALDI ABI 4800 plates using a Probot microfraction collector (LC Packings, Amsterdam, Netherlands). Matrix solution, 3 mg/mL α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich, St Louis, MO USA) in 50% ACN, 0.1% TFA, was then added at 0.75 µL per spot.

[00247] **Proteomic Methodology:** Proteomic analysis was performed using iTRAQ-MALDI-TOF/TOF methodology. The multiplexing capability of iTRAQ technology allows simultaneous processing of four samples per experimental run. To ensure interpretable results across different experimental runs, a reference sample was processed together with 3 patient samples in all iTRAQ runs. The reference sample consisted of a pool of plasma from 16 healthy individuals and was consistently labeled with iTRAQ reagent 114. Patient samples were randomly labeled between reagents 115, 116 and 117. Each iTRAQ run enabled the identification and quantitation of proteins of 3 patient samples relative to the reference sample.

[00248] **Mass Spectrometry and Data Processing:** For each experiment, peptides spotted on MALDI plates and analyzed using the 4800 MALDI TOF/TOF analyzer (Applied Biosystems; Foster City, CA) controlled using 4000 series Explorer version 3.5 software. The mass spectrometer was set in the positive ion mode with an MS/MS collision energy of 1 keV. A maximum of 1400 shots/spectrum were collected for each MS/MS run causing the total mass time to range from 35 to 40 hours. Peptide identification and quantitation was carried out by ProteinPilot™ Software v2.0 (Applied Biosystems/MDS Sciex, Foster City, CA USA) with the integrated Paragon™ Search Algorithm (Applied Biosystems) and Pro Group™ Algorithm. Database searching was performed against the International protein index (IPI HUMAN v3.39) (Kersey et al., 2004. Proteomics 4:1985-8) to identify the polypeptides present in the samples.

The precursor tolerance was set to 150 ppm and the iTRAQ fragment tolerance was set to 0.2 Da. Identification parameters were set for trypsin cleavages, cysteine alkylation by MMTS, with special factors set at urea denaturation and an ID focus on biological modifications. The detected protein threshold was set at the 85% confidence interval.

[00249] Pro Group™ Algorithm (Applied Biosystems) assembled the peptide evidence from the Paragon™ Algorithm into a comprehensive summary of proteins in the sample. The set of identified proteins from each iTRAQ run were organized into protein groups to avoid redundancies. Relative protein levels (levels of labels 115, 116 and 117 relative to 114, respectively) were estimated for each protein group by Protein Pilot based on a weighted average of the log ratios of the individual peptides for each protein. The weight of each log ratio is the inverse of the Error Factor, an estimate of the error in the quantitation, calculated by Pro Group Algorithm. These weighted averages were then converted back into the linear space and corrected for experimental bias using the Auto Bias correction option in Pro Group Algorithm. Peptide ratios coming from the following cases are excluded from the calculation of the corresponding average protein ratios: shared peptides (i.e., the same peptide sequence is claimed by more than one protein), peptides with a precursor overlap (i.e., the spectrum yielding the identified peptide is also claimed by a different protein but with an unrelated peptide sequence), peptides with a low confidence (i.e., peptide ID confidence < 1.0%), peptides that do not have an iTRAQ modification, peptides with only one member of the reagent pair identified, and peptide ratios where the sum of the signal-to-noise ratio for all of the peak pairs is less than 9. When all (non-blank) peptide ratios are 0 or 9999 (indicating that only one member of the reagent pair was identified), the average ratio for the corresponding protein is shown as 0 or 9999. Further information on these and other quantitative measures assigned to each protein and on the bias correction are given in ProteinPilot Software documentation.

[00250] Although each protein group in an iTRAQ experiment may consist of more than one identified protein, a single set of three iTRAQ ratios was assigned for the entire group based on its corresponding list of identified peptides. An in-house algorithm, called the Protein Group Code Algorithm (PGCA) was employed to link protein groups across all iTRAQ experiments. PGCA assigns an identification code to all the protein groups within each iTRAQ run and a common code to similar protein groups across runs. The latter code, also referred to as the protein group code (PGC), was then used to match proteins across different iTRAQ runs. This

process ensures common identifier nomenclature for related proteins and protein families across all experimental runs.

[00251] **Statistical Analysis**

[00252] The statistical analysis for the microarray experiments was performed using SAS version 9.1, R version 2.6.1 and BioConductor version 2.1 (Gentleman, R., et al., Genome Biology, 2004. **5**: p. R80). Robust Multi-array Average (RMA) (Bolstad, 2003, supra) technique was used for background correction, normalization and summarization as available in the Affy BioConductor package. A noise minimization was then performed; probe sets with expression values consistently lower than 50 across at least 3 samples were considered as noise and eliminated from further analysis. The remaining probe sets were analyzed using three different moderated T-tests. Two of the methods are available in the Linear Models for Microarray data (limma) BioConductor package - robust fit combined with eBayes and least square fit combined with eBayes. The third statistical analysis method, Statistical Analysis of Microarrays (SAM), is available in the same BioConductor package. A gene was considered statistically significant if it had a false discovery rate (FDR) <0.01 in all three methods (Smyth, G., *Limma: linear models for microarray data*, in *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, R. Gentleman, et al., Editors. 2005, Springer: New York). The fold-change and maximum FDR value [the highest FDR from the 3 methods] are presented in Table 2.

[00253] The nucleic acid markers were identified by applying Stepwise Discriminant Analysis (SDA) with forward selection on the statistically significant probe sets. Linear Discriminant Analysis (LDA) was used to train and test the biomarker panel as a 'classifier marker' to generate a minimal or small subset of markers with optimal diagnostic qualities. An 11-fold cross-validation of the entire process of classifier construction was used to evaluate the performance of the principal classifier based on the biomarker panel. Samples were randomly divided into 11 disjoint sets, each consisting of one sample from subjects with and two without BCAR, mirroring the one-to-two distribution in the overall study cohort. For each of the 11 disjoint sets, a new classifier was constructed in the same manner as the principal classifier: identification of a list of differentially expressed probe sets based on 3 moderated t-tests, followed by forward selection discriminant analysis. The classification accuracy (sensitivity and specificity) of each of the 11 classifiers was then determined based on the 3 samples left out at

each fold. Sensitivity and specificity for the principal classifier were estimated by averaging the performance across the 11-fold cross-validation samples.

[00254] **Statistical analysis for proteomics:** A one-protein at a time evaluation of differential relative levels was performed using a robust moderated t-test (empirical Bayes, eBayes; Smyth et al., 2004 Stat Appl Genet Mol Biol 3: Article 3) on a set of proteins that have been detected, using the assigned protein group code by PGCA, in at least two thirds within each analyzed group). Using the eBayes approach decreases the number of false positives caused by artificially low sample variance estimates when the sample size in the study is small. In addition, its robust version assigns less analytical weight to protein levels that are statistical outliers. This makes the procedure less sensitive to observations deviating from the bulk of the data than classical, non-robust tests. Protein group codes with mean relative concentrations (relative to pool control's level) differing significantly between BCAR positive and negative (i.e., with p-value < 0.05) were identified as potential markers.

[00255] The proteomic biomarker panel proteins were then determined using a forward selection stepwise discriminant analysis (SDA) based on the identified list of potential markers. The SDA algorithm incorporates one protein group code at a time from the list of potential markers. In the first step it identifies the protein group code that best classifies samples based on leave-one-out cross validation. In the second step it identifies the second protein group code that, together with the previously identified code, best classify samples in a leave-one-out cross validation. This procedure is repeated until all protein group codes are sequentially incorporated or until (n-2) steps are performed, where n is the number of available samples. The proteomic biomarker panel is defined by the first k protein group codes selected by the SDA algorithm, where $k=k_0+k_m$ is the step at which the maximum cross-validation accuracy is reached for the first time (k_0) and maintained for k_m additional steps. In each cross-validation, sample classification is performed using a linear discriminant analysis (LDA) with prior probabilities for each group set to 0.5. In LDA, the relative concentration for each protein undetected in patient sample(s) and/or pooled control was imputed using the average relative concentration calculated from remaining training samples in each group (BCAR positive and negative).

[00256] **Internal validation (proteomics data):** Statistical validation was performed by a leave-one-out cross-validation of the entire process of biomarker panel selection. More specifically, at each step of the leave-one-out cross-validation one sample is left out for

classification (test set) and the remaining samples are used to build a classifier (training set). The entire biomarker selection process is then performed on the training set, i.e., from the selection of protein group codes detected in at least 2/3 of the samples in each group through the biomarker panel selection by SDA. A classifier based on the resulting proteomic biomarker panel is built using LDA and tested on the test set (priors and missing values have been treated as explained above). This process is repeated until all samples are used as test set once. The overall specificity and sensitivity are estimated based on the classification accuracy of each run. All statistical analyses were implemented using R version 2.7.0 (The R Project for Statistical Computing).

[00257] **Technical validation:** 2 proteins from a panel of 9 proteomic biomarkers were selected for validation by Enzyme-Linked ImmunoSorbent Assay (ELISA) using commercially available kits, following manufacturer's directions: Hepatocyte growth factor-like protein homolog (R&D DHG00) and E3 ubiquitin-protein ligase UBR4 (DiaPharma - DPGR032A).

[00258] The present invention will be further illustrated in the following examples. However it is to be understood that these examples are for illustrative purposes only, and should not be used to limit the scope of the present invention in any manner.

[00259] **Example 1:Comparison of biomarkers with clinical diagnosis**

[00260] A total of 33 subjects were included in the study, comprising 11 patients with an acute rejection within the first week of transplantation, and 22 patients who were free of rejection for at least 6 months following transplantation. The 33 transplanted patients were clinically stable 3 months following renal transplantation. A total of 183 probe sets representing 160 genes were found to be statistically significantly and consistently differentially expressed between AR and NR subjects (Table 2). The sequences that the probe sets represent are presented in Figure 10. Samples from subjects with acute rejection within the first week after transplantation clustered together, separately from samples from non-rejection patients.

[00261] Classifying the test subjects using the panel of nucleic acid markers listed in Table 5 divided the subjects into rejectors (AR) or non-rejectors (NR) (Figure 1A-C).

[00262] As a comparison, an independent classification of a set of subjects using only clinical parameters did allow for separation of AR and NR subject, however the boundary between the two groups was not as clear as demonstrated for the set of subjects illustrated in Figures 1A-C, as some overlap of AR subjects and NR subjects was observed (Figure 2).

[00263] Table 5: Primary classifier (24 nucleic acid markers) associated with acute graph rejection.

Affymetrix Probe Set ID	Gene Symbol	Gene Title	log2 (Fold Change)	Fold Change	Direction (AR versus NR)	SEQ ID NO:
238320_at	++TncRNA	trophoblast-derived noncoding RNA	1.34	2.54	up	150
211454_x_at	++FKSG49	FKSG49	0.75	1.69	up	69
244752_at	++ZNF438	zinc finger protein 438	0.67	1.59	up	182
204978_at	SFRS16	splicing factor, arginine/serine-rich 16	0.76	1.70	up	56
1558448_a_at	++1558448_a_at	CDNA FLJ35687 fis, clone SPLEN2019349	0.79	1.73	up	12
210787_s_at	++CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta	0.62	1.54	up	91
211251_x_at	NFYC	nuclear transcription factor Y, gamma	0.49	1.40	up	40
209060_x_at	NCOA3	nuclear receptor coactivator 3	0.83	1.77	up	77
200805_at	++LMAN2	lectin, mannose-binding 2	0.72	1.64	up	23
226266_at	PGS1	phosphatidylglycerophosphate synthase 1	0.91	1.88	up	137
202150_s_at	NEDD9	neural precursor cell expressed, developmentally down-regulated 9	0.49	1.40	up	38
237442_at	++237442_at	---	1.03	2.05	up	172
208120_x_at	++FKSG49/LOC730444	FKSG49 hypothetical protein LOC730444	0.56	1.47	up	69
217475_s_at	LIMK2	LIM domain kinase 2	0.78	1.71	up	129
201473_at	++JUNB	jun B proto-oncogene	0.76	1.69	up	30
201970_s_at	NASP	nuclear autoantigenic sperm protein (histone-binding)	0.52	1.43	up	37
227510_x_at	++PRO1073	PRO1073 protein	1.16	2.24	up	147
240057_at	240057_at	Transcribed locus	0.52	1.43	up	177
210184_at	++ITGAX	integrin, alpha X (complement component 3 receptor 4 subunit)	0.65	1.57	up	81
217436_x_at	LOC730399/LOC731974	hypothetical protein LOC730399 hypothetical protein LOC731974	0.60	1.51	up	128
200709_at	FKBP1A	FK506 binding protein 1A, 12kDa	0.59	1.50	up	19

210514_x_at	HLA-G	HLA-G histocompatibility antigen, class I, G	0.58	1.50	up	86
203748_x_at	RBMS1	RNA binding motif, single stranded interacting protein 1	0.80	1.74	up	54
205921_s_at	SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	0.65	1.57	up	60

++ intersection of the 11 probe sets identified in the cross-validation process to estimate out-of-sample performance

[00264] **Example 2**:

[00265] **Subjects:** Of the 305 subjects who received a renal transplant during the period of observation, 27 (8.9%) developed BCAR with a Banff grade of ≥ 1a during the first 3 months post-transplant, while a further 24 (7.9%) had only borderline changes. A total of 11/27 (40.74%) subjects with grade ≥ 1a rejection on biopsy (range: 3-10 days, mean: 6 days) fulfilled the case selection criteria with immediate graft function, and absence of infection or other confounding co-morbid events, as did 5/24 (20.83%) subjects with borderline changes on biopsy (range: 5-7 days, mean: 6 days). A further 22 subjects who had immediate graft function, with no clinical or BCAR for at least 6 months following transplantation, and no confounding clinical co-morbid events, were selected as matched controls, and 20 normal control subjects served as a comparator group. Demographic details are shown in Table 4. Graft function was significantly inferior in cases with BCAR at the first week post-transplant (27±10 vs. 42±13 ml/min/1.73M^2, P = 0.004), but was comparable in both cases and controls by month 3 (48±11 vs. 51±8 ml/min/1.73M^2, P = 0.359) and remained clinically stable with good allograft function throughout the 12 months period of observation (54±13 vs. 53±15 ml/min/1.73M^2 at month 12, P = 0.859).

[00266] **Micro-array expression**: Peripheral blood samples were selected from each of the cases with BCAR at the time of biopsy for acute rejection, and from the respective controls without BCAR at a time-point identical to the respective case, and were compared with samples from normal comparators. Microarray analysis of the samples from patients with or without BCAR at an FDR < 0.01 identified a total of 239 probe-sets that were differentially expressed using LIMMA, 575 probe-sets with robust LIMMA and 2677 probe-sets using SAM. The intersection of the three methods found a more restricted set of 183 probe sets which were differentially expressed between cases (BCAR) and controls (no BCAR) for all three analytical methods. Of the 183 significantly differentially expressed probe sets, 182 were over-expressed in subjects

with BCAR while one (1565484_x_at coding for the epidermal-growth factor receptor; EGFR) was under-expressed (Figure 3).

[00267] Unsupervised two-way hierarchical clustering and principal component analysis based upon these probe-sets showed discrete separation between normal subjects, patients with BCAR and those without BCAR. A principle component analysis (Figure 4) illustrates the separation of the subject groups (AR, NR and N), demonstraing that the centroids of all groups are clearly separated. When samples from subjects with borderline changes were introduced, they were distributed heterogeneously among the cases and controls with and without BCAR . The biological processes encompassed by the 183 differentially expressed probe sets, representing approximately 160 genes, are shown in Figure 5. Combination of overlapping networks in which probe-sets were shared identified three major biological categories implying involvement of processes related to immune responses, signal transduction, and cytoskeletal reorganization. Analysis of gene-gene and protein-protein networks (Ekins et al., 2007. Methods Mol Biol 356:319-50) revealed that the cytokine-activated Jak-Stat pathway, interferon signaling, lymphocyte activation, proliferation, chemotaxis, and apoptosis were prominently represented among the 183 differentially expressed probe-sets.

[00268] **Classifier selection**: Although many genes were highly associated with BCAR, co-linearity implied that not all were necessary to develop a classifier for this event. Forward selection discriminant analysis was therefore employed to identify a linear discriminant function consisting of a more parsimonious classifier from among the 183 differentially expressed probe-sets initially documented. The principal 24 probe-sets identified within this classifier, and their respective genes, are shown in Table 5.

[00269] **Example 3: Cross validation of nucleic acid biomarkers**

[00270] Cross-validation of the entire gene set using the same reductive process was employed to enhance the robustness of this classifier and to estimate the out-of-sample performance. An 11 nucleic acid marker set lists produced by this process contained a mean of 103 probe-sets, and the six most significantly differentially expressed of the original 183 probe-sets (TncRNA, FKSG49, AVIL, SIGLEC9, ANP32A, SLC25A16) were present in each list. Forward selection discriminant analysis identified a group of 11 classifiers with a union of 87 probe-sets. Eleven of these probe-sets, depicted in Table 5 , were contained within the original 24 probe-set classifier.

Cross-validation yielded an overall mean sensitivity of 73% and specificity of 91% for the identification of samples with or without BCAR.

[00271] Performance of the final 11 probe-set (nucleic acid marker) classifier is shown in Figure 6. The set of 11 nucleic acid markers included TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX.

[00272] Diagnostic accuracy improved rapidly with addition of sequential probe-sets (Figure 6A), and the linear discriminant scores for the full 11 probe-set classifier showed clear separation of the samples with and without BCAR (Figure 6B). Finally, longitudinal monitoring over the first 3 months post-transplant showed a significant increase in classifier score at the time of BCAR ($p=0.001$), with a subsequent return to the baseline value following treatment and resolution of the rejection episode. No comparable increase occurred in subjects who did not experience BCAR and there was no significant difference between these curves at any other time post-transplant (Figure 6C).

[00273] An 11 cross-validation analysis demonstrated an average prediction accuracy of 72.7% (sensitivity) for AR and 90.9% (specificity) for NR (Table 6) and is an estimate of the prediction accuracy of the panel of 24 biomarkers listed presented in Table 5. The "++" designation in Table 5 indicates the nucleic acid markers in the intersecting set of the 11 probe sets identified in the cross-validation process to estimate out-of-sample performance.

[00274] Table 6: Sensitivity and specificity outcome of cross-validation analysis of nucleic acid markers.

	Sensitivity	Specificity
Fold 1	100%	100%
Fold 2	0%	100%
Fold 3	100%	100%
Fold 4	100%	100%
Fold 5	100%	100%
Fold 6	100%	100%
Fold 7	100%	100%
Fold 8	100%	100%
Fold 9	0%	100%

Fold 10	100%	50%
Fold 11	0%	50%

[00275] **Example 4: Proteomics biomarker identification and validation**

[00276] A total of 305 subjects received a renal transplant during the period of observation, of whom 27 (8.8%) developed BCAR ≥ 1a during the first 3 months post-transplant. Eleven of these fulfilled the case selection criteria, with immediate graft function, BCAR ≥ 1a within the first 4 weeks post transplant (range: 3-10 days, mean: 6 days), and no infection or other confounding co-morbid events. A further 21 subjects who had immediate graft function, with no clinical or BCAR for at least 6 months following transplantation, and no confounding clinical co-morbid events, were selected as controls; for a total of 32 transplanted subjects. Except for the incidence of BCAR, all patients were otherwise clinically stable, with good allograft function throughout the 12-month period of observation. Six additional BCAR negative samples were selected for an internal validation, one each from three patients without BCAR included in the discovery study, and three from new patients.

[00277] After depletion of the 14 most abundant proteins (albumin, fibrinogen, transferin, IgG, IgA, IgM, haptoglobin, α2-macroglobulin, α1-acid glycoprotein, α1-antitrypsin, Apoliprotein-I, Apoliprotein-II, complement C3 and Apoliprotein B) by immuno-affinity chromatography (Genway Biotech; San Diego, CA), less than 5% of the total protein mass remained. The remaining protein was trypsin digested with sequencing grade modified trypsin (Promega; Madison, WI) and labelled with iTRAQ reagents according to manufacturer's (Applied Biosystems; Foster City, CA) protocol and was examined to identify plasma proteomic markers of renal acute rejection. A total of 460 protein group codes were identified in at least one BCAR positive sample and one BCAR negative sample, among which 144 protein group codes were detected in at least 8 out of 11 BCAR positive samples and in at least 14 out of 21 controls, passing the two-thirds selection criteria per group. Analysis of the 144 protein group codes with the robust eBayes identified a total of 18 protein group codes whose concentrations differed significantly ($p<0.05$) between the two groups (Figure 7). The results for the 18 significant protein group codes are shown in Table 7.

[00278] Forward selection stepwise discriminant analysis (SDA) identified a subset of 9 protein group codes that constitutes the proteomic biomarker panel (blue bold font in Table 7).

Seven of the biomarker panel PGCs were up-regulated (TTN, MSTP9, PI16, C2, MBL2, SERPINA10, UBR4) and two were down-regulated (KNG1 and AFM) in patients with compared to those without BCAR. Figure 8 illustrates the marginal classification performance achieved by protein group codes at each step of the forward selection. The x-axis shows the protein group code selected at each step to join the protein group codes selected in previous steps. The y-axis shows the classification accuracy achieved by each successively larger panel. The marginal gain in prediction accuracy quickly stabilized as protein group codes were added into the panel, and even three proteins were sufficient to achieve maximum accuracy (Figure 8).

[00279] Table 7: Plasma proteins with differential relative concentrations at p-value<0.05. Protein group identified in the column AR vs NR constitute the plasma proteomic biomarker panel. The column "PGC" contains the code assigned by the PGCA. Accession numbers and protein names of all proteins in each group, corresponding genes, p-values calculated by the robust-eBayes test, fold changes and their directions (up- or down-regulated) in BCAR positive relative to negative are given in the remaining columns.

Accession #	PGC	Gene Symbol	Protein Name	P-Value	Adj. P-Value	Fold Change	AR vs NR
IPI00759754.1		TTN	Isoform 1 of Titin				
IPI00749039.2		TTN	titin isoform N2-A				
IPI00179357.2		TTN	Isoform 7 of Titin				
IPI00023283.3		TTN	Isoform 2 of Titin				
IPI00759542.1		TTN	Isoform 8 of Titin				
IPI00759637.1		TTN	Isoform 4 of Titin				
IPI00759613.1		TTN	Isoform 5 of Titin				
IPI00375499.2	**111	TTN	titin isoform novex-2	0.00003	0.0045	1.21	up*
IPI00375498.2		TTN	titin isoform novex-1				
IPI00455173.4		TTN	Isoform 3 of Titin				
IPI00412307.8		TTN	2268 kDa protein				
IPI00436021.3		TTN	Titin (Fragment)				
IPI00884109.1		-	Cellular titin isoform PEVK variant 3 (Fragment)				
IPI00789376.1		KNG1	KNG1 protein				
IPI00797833.3		KNG1	Kininogen 1				
IPI00032328.2	**18	KNG1	Isoform HMW of Kininogen-1 precursor	0.00149	0.1108	1.18	down
IPI00215894.1		KNG1	Isoform LMW of Kininogen-1 precursor				
IPI00032311.4	108	LBP	Lipopolysaccharide-binding protein precursor	0.00641	0.2024	1.22	up

IPI00395488.2		VASN	Vasorin precursor				
IPI00827866.1		ARNTL2	Isoform 7 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00142781.3		ARNTL2	Isoform 1 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00163662.3		ARNTL2	Isoform 2 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00465306.3	222	ARNTL2	Isoform 5 of Aryl hydrocarbon receptor nuclear translocator-like protein 2	0.00666	0.2024	1.14	up
IPI00788724.2		ARNTL2	Isoform 6 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00789255.2		ARNTL2	Isoform 3 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00795339.2		ARNTL2	Isoform 4 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00827897.1		ARNTL2	Isoform 8 of Aryl hydrocarbon receptor nuclear translocator-like protein 2				
IPI00019943.1	**23	AFM	Afamin precursor	0.00679	0.2024	1.29	down
IPI00873854.1	**224	MSTP9	64 kDa protein	0.00863	0.2143	1.09	up
IPI00292218.4		MST1	Hepatocyte growth factor-like protein precursor				
IPI00384647.1		MST1	Hepatocyte growth factor-like protein homolog				
IPI00718805.1		MSTP9	Brain-rescue-factor-1				
IPI00816378.1		-	21 kDa protein				
IPI00847702.2		MST1	14 kDa protein				
IPI00301143.5	**135	PI16	Isoform 1 of Peptidase inhibitor 16 precursor	0.01286	0.2738	1.25	up
IPI00845506.1		PI16	Isoform 2 of Peptidase inhibitor 16 precursor				
IPI00007221.1	97	SERPIN A5	Plasma serine protease inhibitor precursor	0.01925	0.2870	1.22	down

IPI00165972.3	104	CFD	Complement factor D preproprotein Harmonin (Usher syndrome type-1C protein) (Autoimmune enteropathy- related antigen AIE-75) (Antigen NY-CO-38/NY-CO-37) (PDZ-73 protein) (Renal carcinoma antigen NY-REN-3). Isoform 3	0.02044	0.2870	1.43	up
IPI00218195.3		USH1C	harmonin isoform b3				
IPI00412105.2		USH1C	Isoform 4 of Harmonin				
IPI00478105.4		USH1C	Isoform 3 of Harmonin				
IPI00478519.3		USH1C	29 kDa protein				
IPI00790818.1		USH1C	60 kDa protein				
IPI00872537.1							
IPI00303963.1	**38	C2	Complement C2 precursor (Fragment)	0.01939	0.2870	1.09	up
IPI00643506.3		C2	Complement component 2				
IPI00004373.1	**116	MBL2	Mannose-binding protein C precursor	0.02119	0.2870	1.3678	up
IPI00007199.4	**125	SERPINA10	Protein Z-dependent protease inhibitor precursor	0.02335	0.2899	1.2317	up
IPI00022395.1	26	C9	Complement component C9 precursor	0.02917	0.2962	1.1321	up
IPI00022331.1	230	LCAT	Phosphatidylcholine-sterol acyltransferase precursor	0.03031	0.2962	1.1822	down
IPI00868938.1	103	-	Beta-2-microglobulin	0.03179	0.2962	1.2735	up
IPI00796379.1		B2M	B2M protein				
IPI00004656.2		B2M	Beta-2-microglobulin				
IPI00219583.1	69	SHBG	Isoform 2 of Sex hormone-binding globulin precursor	0.03180	0.2962	1.1924	down
IPI00023019.1		SHBG	Isoform 1 of Sex hormone-binding globulin precursor				
IPI00749179.2	29	C1S	Uncharacterized protein C1S	0.04030	0.3532	1.0817	up
IPI00017696.1		C1S	Complement C1s subcomponent precursor				
IPI00385294.2		C1S	Putative uncharacterized protein DKFZp686M10257				
IPI00791987.1		C1S	17 kDa protein				
IPI00877989.1		C1S	Protein				
IPI00878772.1		C1S	19 kDa protein				

IPI00843999.2		UBR4	Isoform 1 of E3 ubiquitin-protein ligase UBR4				
IPI00640981.3		UBR4	Isoform 4 of E3 ubiquitin-protein ligase UBR4				
IPI00296176.2		F9	Coagulation factor IX precursor				
IPI00180305.7	**100	UBR4	Isoform 5 of E3 ubiquitin-protein ligase UBR4	0.04317	0.3574	1.0943	up
IPI00646605.3		UBR4	Isoform 3 of E3 ubiquitin-protein ligase UBR4				
IPI00746934.2		UBR4	Isoform 2 of E3 ubiquitin-protein ligase UBR4				
IPI00816532.1		F9	Coagulation factor IX (Fragment)				

[00280] *"Up" with respect to "AR vs NR" indicates that one or more members of the specified protein group code are increased in the AR subjects, relative to the NR subjects. "Down" with respect to "AR vs NR" indicates that one or more members of the specified protein group code are decreased in the AR subjects, relative to the NR subjects.

[00281] ** Indicates the protein group codes selected by SDA. One or more of the members of the indicated protein group code are increased or decreased (as indicated in the right-most column) in the AR subject, relative to the NR subject.

[00282] The Accession # is the International Protein Index (IPI) accession number; the amino acid sequence of the corresponding polypeptide is available from the IPI database as indicated in the methods section.

[00283] In an internal validation, two approaches were taken to estimate the ability of the proteomic biomarker panel to classify new samples. First, a leave-one-out cross-validation using LDA estimated a sensitivity of 63% and a specificity of 86% associated with the outlined discovery strategy. Second, a classifier based on the 9 protein group codes in the biomarker panel was built using LDA and was tested on 6 new NR samples. Four out of these 6 samples were correctly classified.

[00284] All citations are herein incorporated by reference, as if each individual publication was specifically and individually indicated to be incorporated by reference herein and as though it were fully set forth herein. Citation of references herein is not to be construed nor considered as an admission that such references are prior art to the present invention.

[00285] One or more currently preferred embodiments of the invention have been described by way of example. The invention includes all embodiments, modifications and variations substantially as hereinbefore described and with reference to the examples and figures. It will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims. Examples of such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way.

WHAT IS CLAIMED IS:

1. A method of determining the acute allograft rejection status of a subject, the method comprising the steps of:

   a. determining the nucleic acid expression profile of one or more than one nucleic acid markers in a biological sample from the subject, the nucleic acid markers selected from the group comprising TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, FKSG49/LOC730444, JUNB, PRO1073 and ITGAX;

   b. comparing the expression profile of the one or more than one nucleic acid markers to a control profile; and

   c. determining whether the expression level of the one or more than one nucleic acid markers is increased relative to the control profile;

   wherein the increase of the one or more than one nucleic acid markers is indicative of the acute rejection status of the subject.

2. The method of claim 1 wherein the group of nucleic acid markers further comprises one or more than one of SFRS16, NFYC, NCOA3, PGS1, NEDD9, LIMK2, NASP, 240057_at, LOC730399/LOC731974, FKBP1A, HLA-G, RBMS1 and SLC6A6.

3. The method of claim 1 wherein the control profile is obtained from a non-rejecting, allograft recipient subject or a non-allograft recipient subject.

4. The method of claim 1, further comprising obtaining a value for one or more clinical variables.

5. The method of claim 1, further comprising at step a) determining the expression profile of one or more than one of the nucleic acid markers selected from Table 2.

6. The method of claim 1, wherein the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by detecting an RNA sequence corresponding to one or more than one markers.

7. The method of claim 1, wherein the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by PCR.

8. The method of claim 1, wherein the nucleic acid expression profile of the one or more than one nucleic acid markers is determined by hybridization.

9. The method of claim 9, wherein the hybridization is to an oligonucleotide.

10. A method of determining acute allograft rejection status of a subject, the method comprising the steps of:

a. determining a proteomic expression profile of proteomic markers in a biological sample from the subject, the proteomic markers including a polypeptide encoded by KNG1, AFM, TTN, MSTP9/MST1, PI16, C2, MBL2, SERPINA10, F9 and UBR4;

b. comparing the expression profile of the proteomic markers to a control profile; and

c. determining whether the expression level of the one or more than one proteomics markers is increased or decreased relative to the control profile;

wherein the increase or decrease of the five or more proteomic markers is indicative of the acute rejection status of the subject.

11. The method of claim 10 wherein the level of polypeptides encoded by KNG1 or AFM are decreased relative to a control, and the level of polypeptides encoded by TTN, MSTP9/MST1, PI16, C2, MBL2, SERPINA10, F9, or UBR4 are increased relative to a control profile.

12. The method of claim 10 wherein the control profile is obtained from a non rejecting, allograft recipient subject or a non-allograft recipient subject.

13. The method of claim 10 further comprising obtaining a value for one or more clinical variables.

14. The method of claim 10, wherein the proteomic expression profile is determined by an immunologic assay.

15. The method of claim 10, wherein the proteomic expression profile is determined by ELISA.

16. The method of claim 10, wherein the proteomic expression profile is determined by mass spectrometry.

17. The method of claim 10, wherein the proteomic expression profile is determined by an isobaric or isotope tagging method.

18. The method of claim 10 wherein the proteomic markers further include a polypeptide encoded by one or more than one of LBP, VASN, ARNTL2, PI16, SERPINA5, CFD, USH1C, C9, LCAT, B2M, SHBG and C1S.

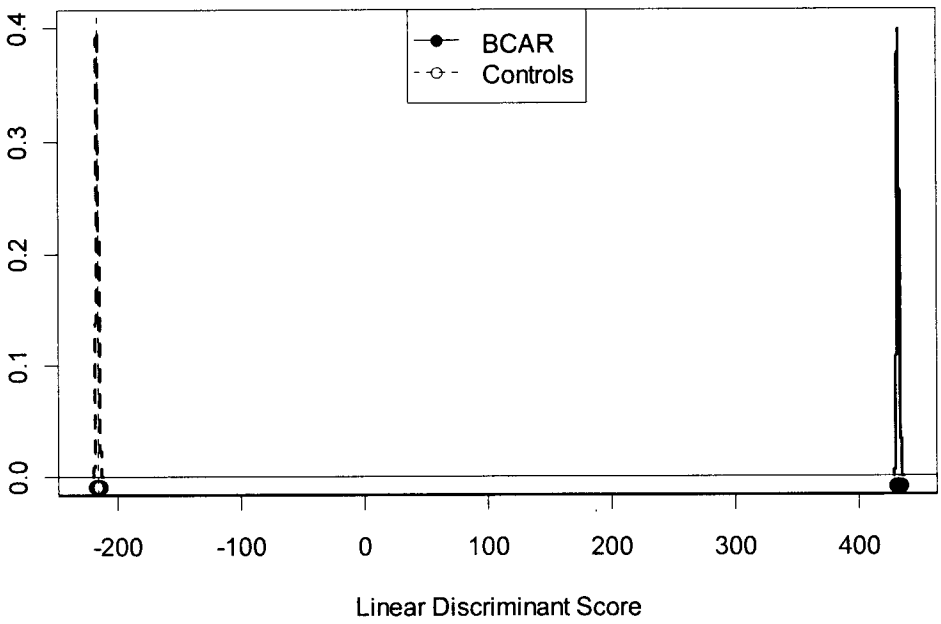
19. The method of claim 1 wherein the control is an autologous control.

20. The method of claim 10 wherein the control is an autologous control.

21. The method of claim 1 wherein the biological sample is blood or plasma.

22. The method of claim 10 wherein the biological sample is blood or plasma.

Figure 1A



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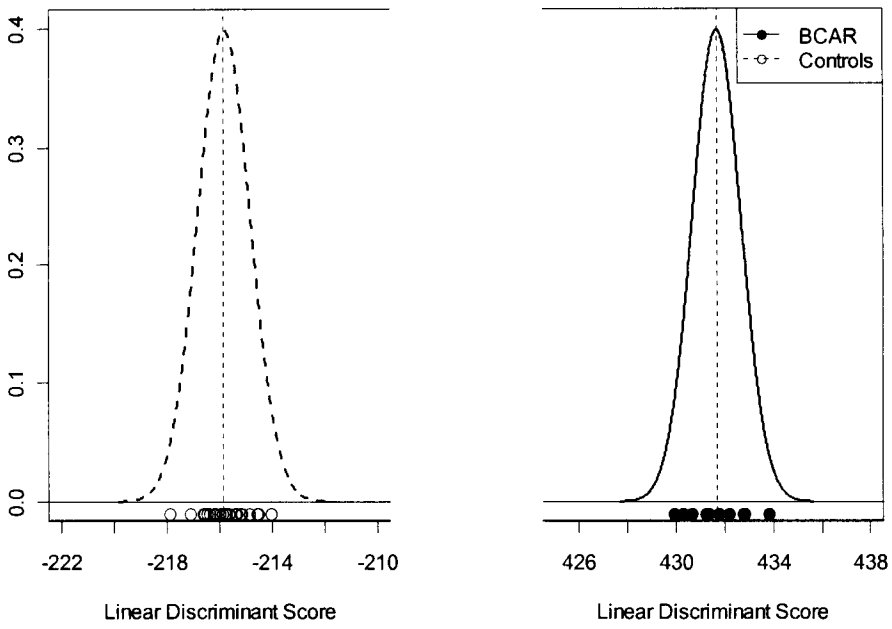
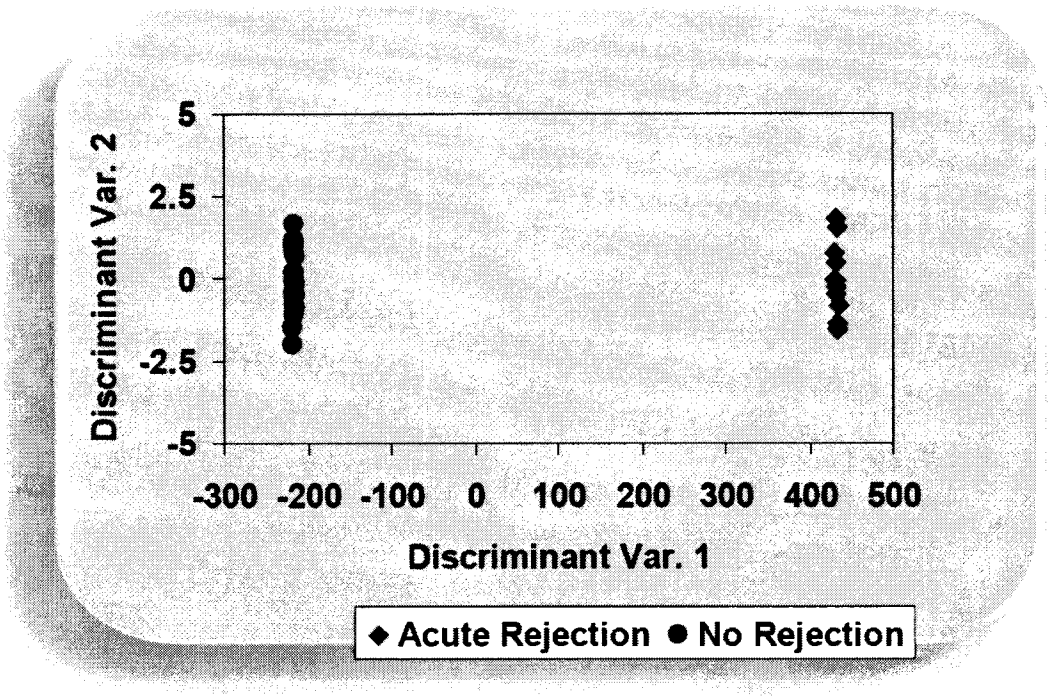


Figure 1C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-5
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Discriminant Var. 1
◆ Acute Rejection ● No Rejection

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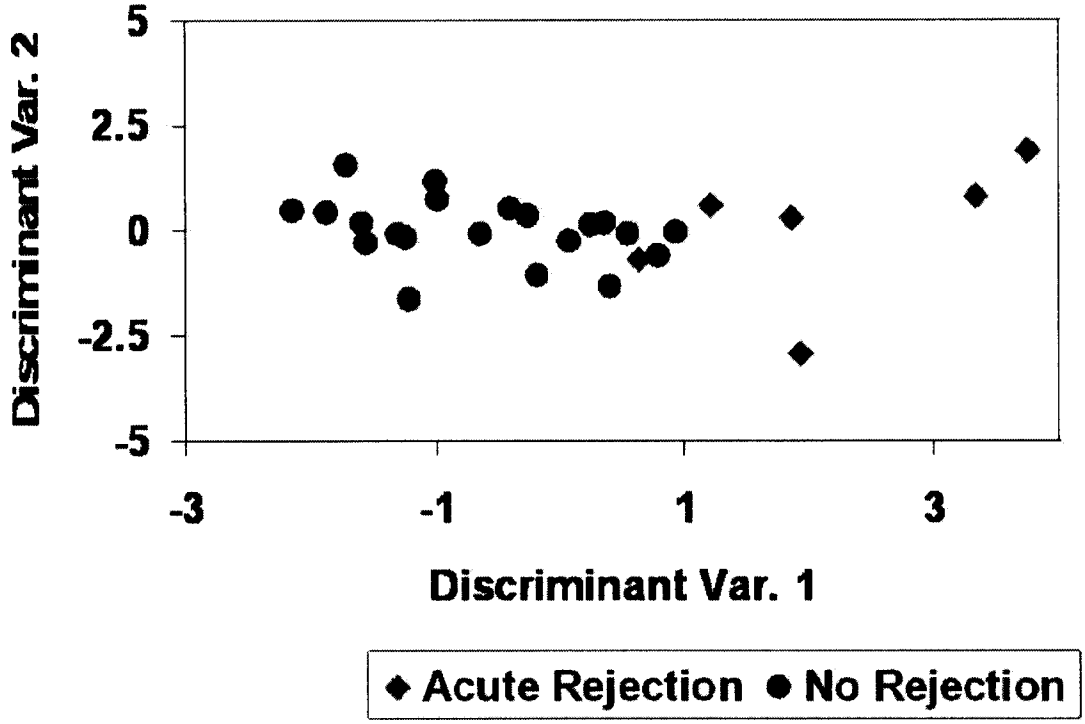
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Acute Rejection
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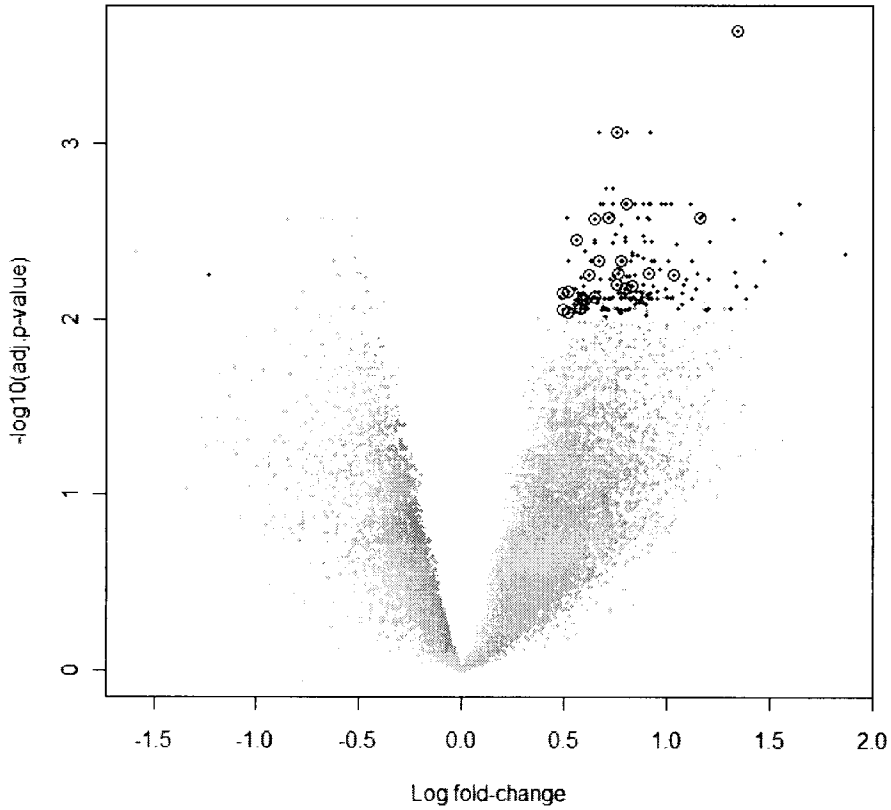

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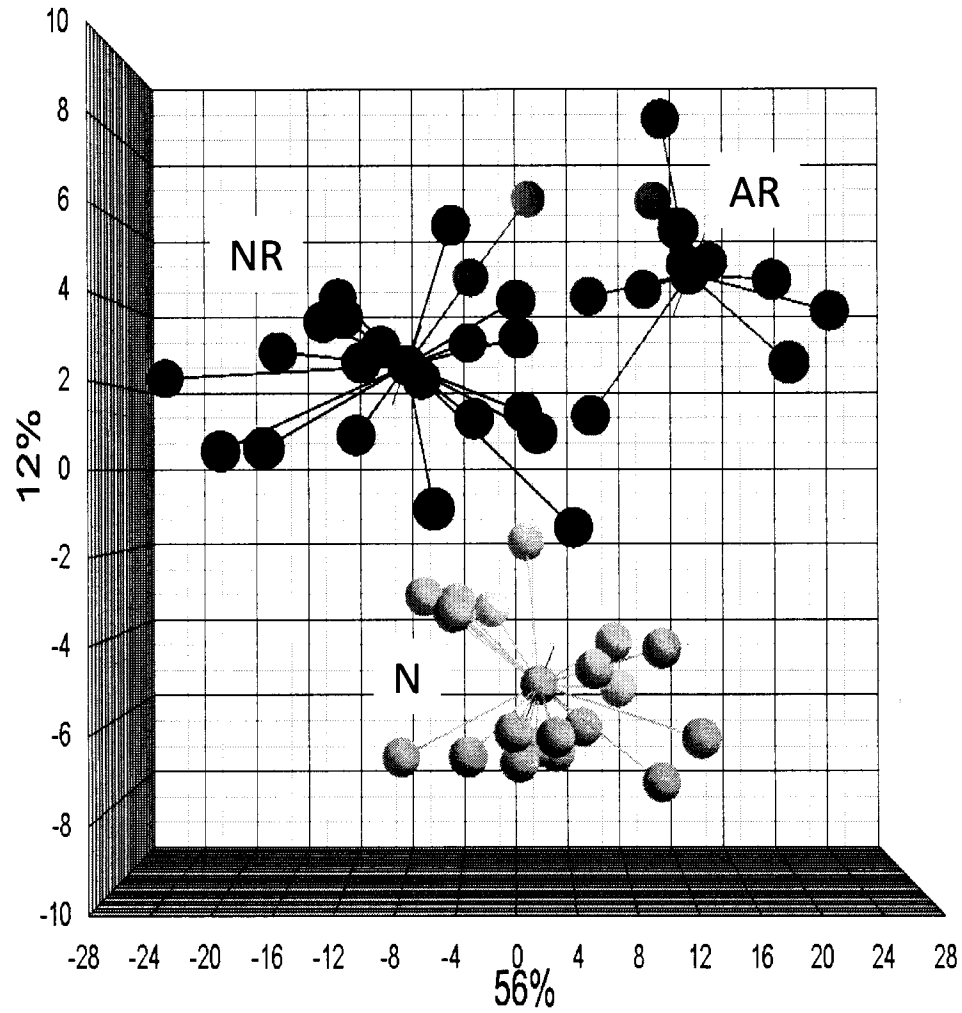
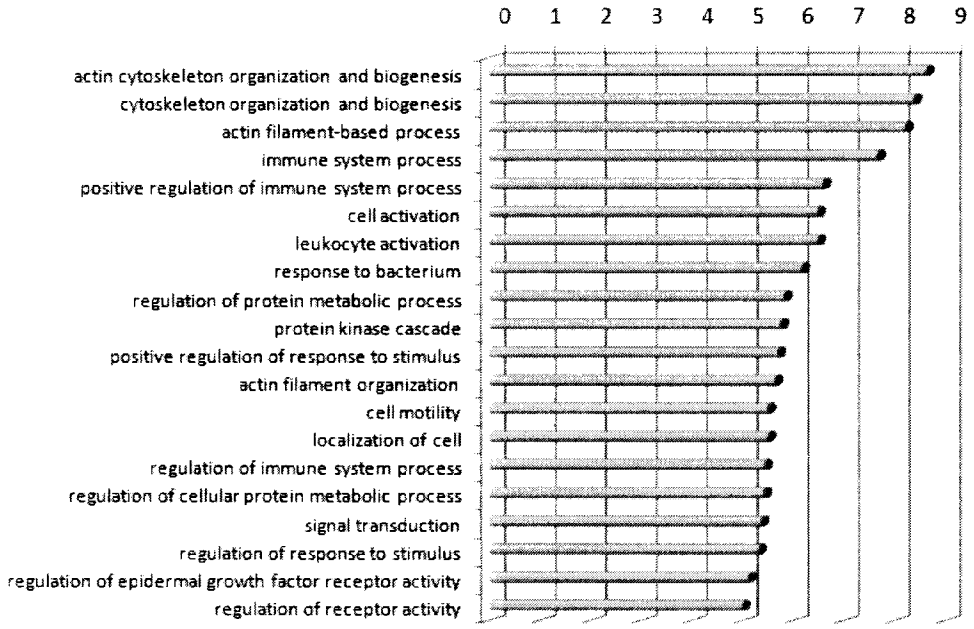


Figure 5A



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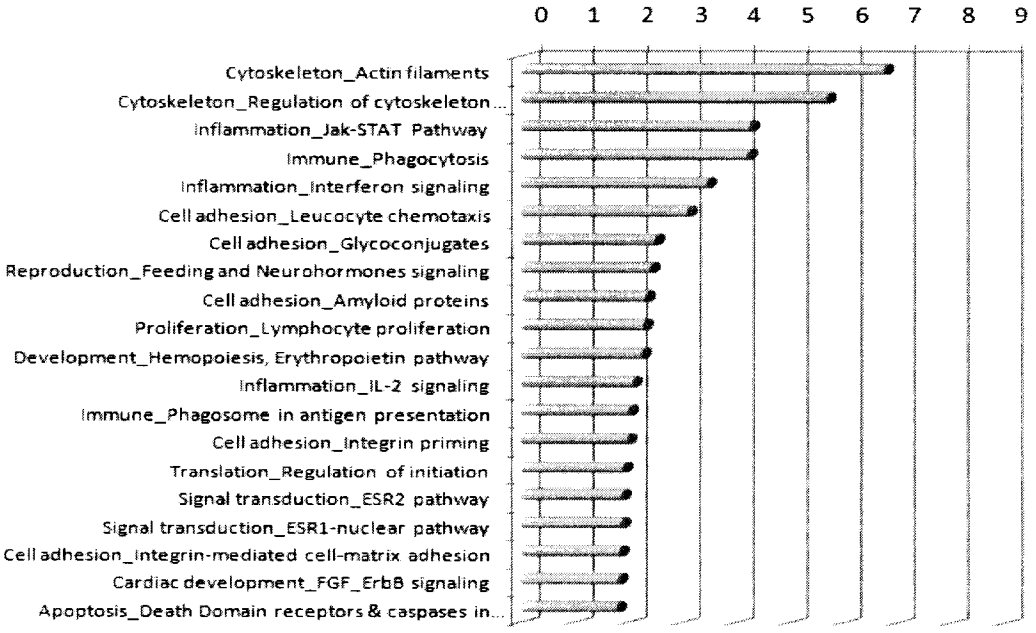

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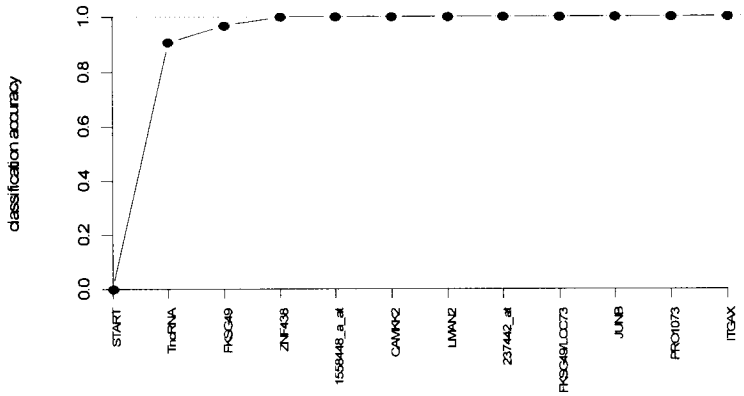


Figure 6B

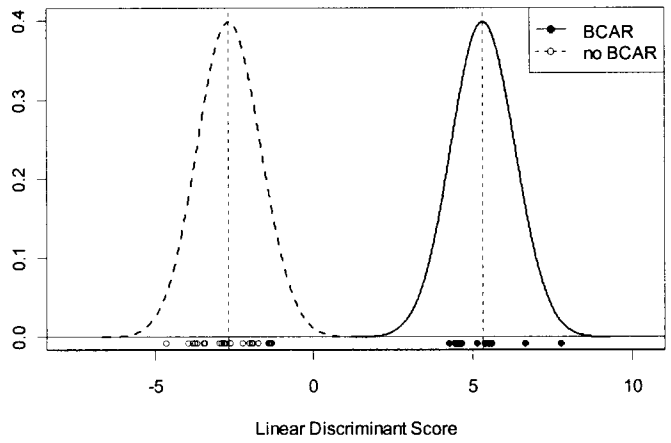
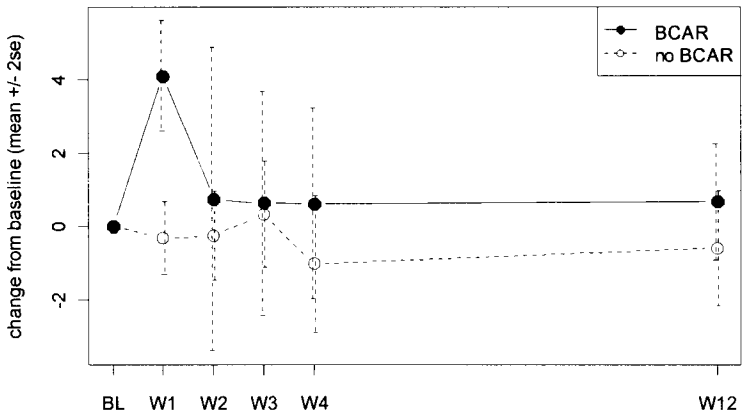


Figure 6C



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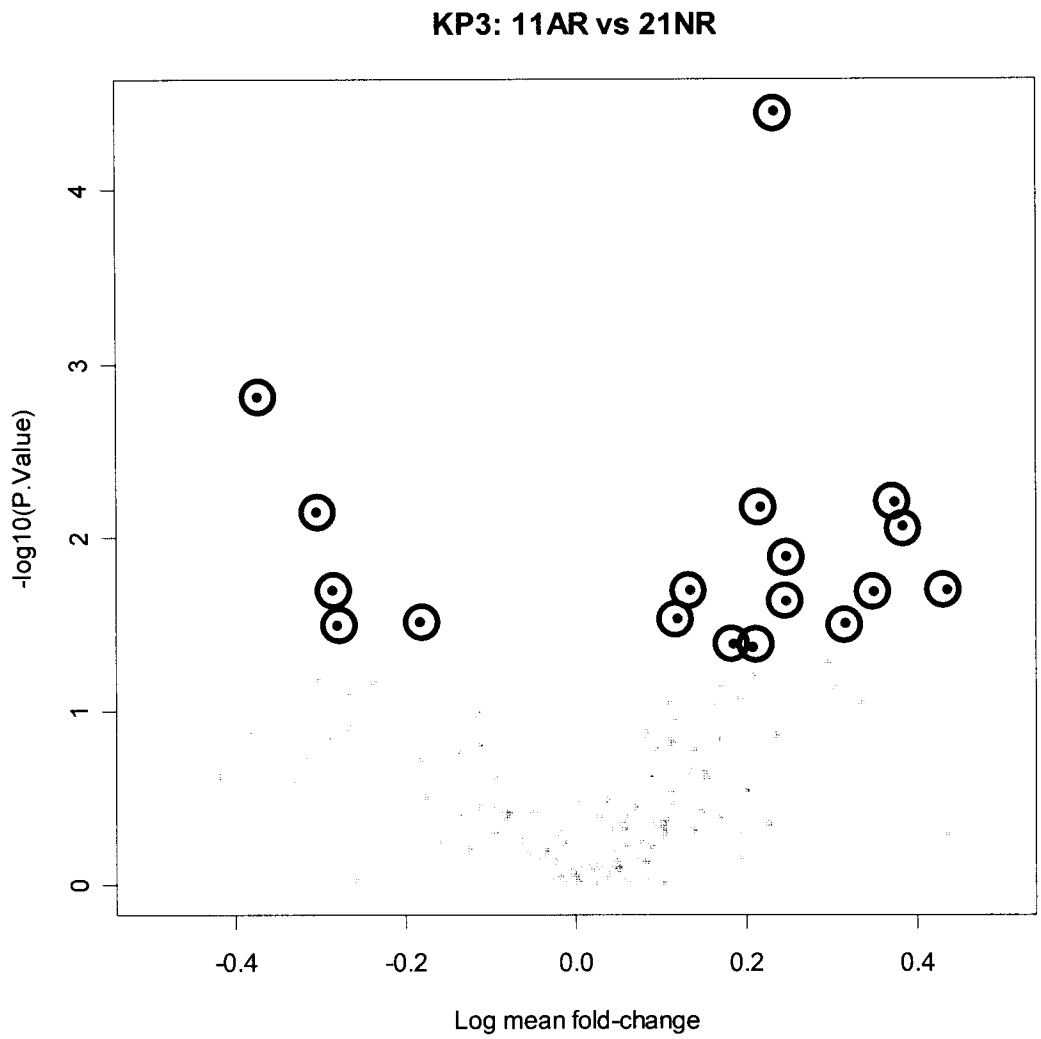
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2
3
4
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Log mean fold-change

Figure 8

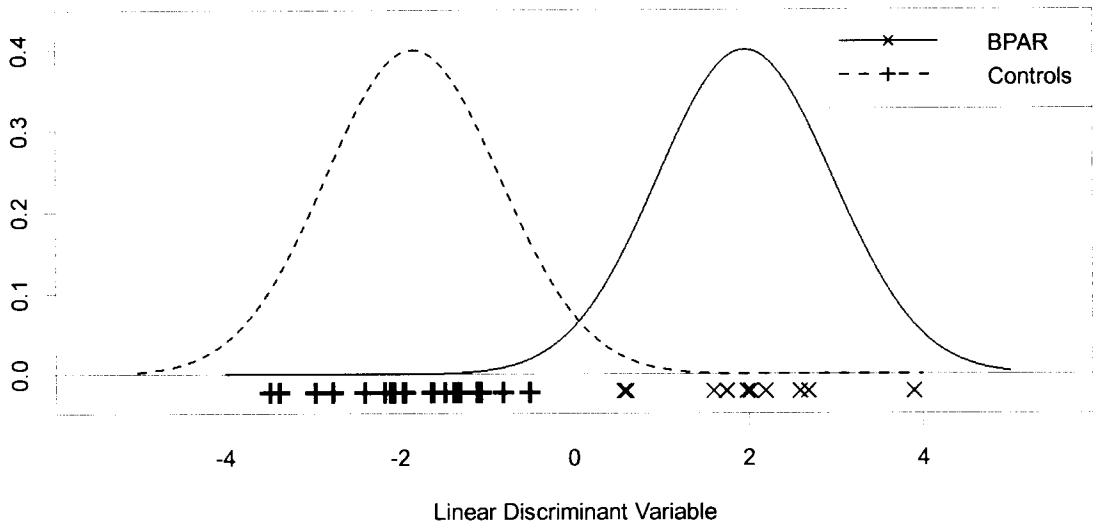

Figure 9

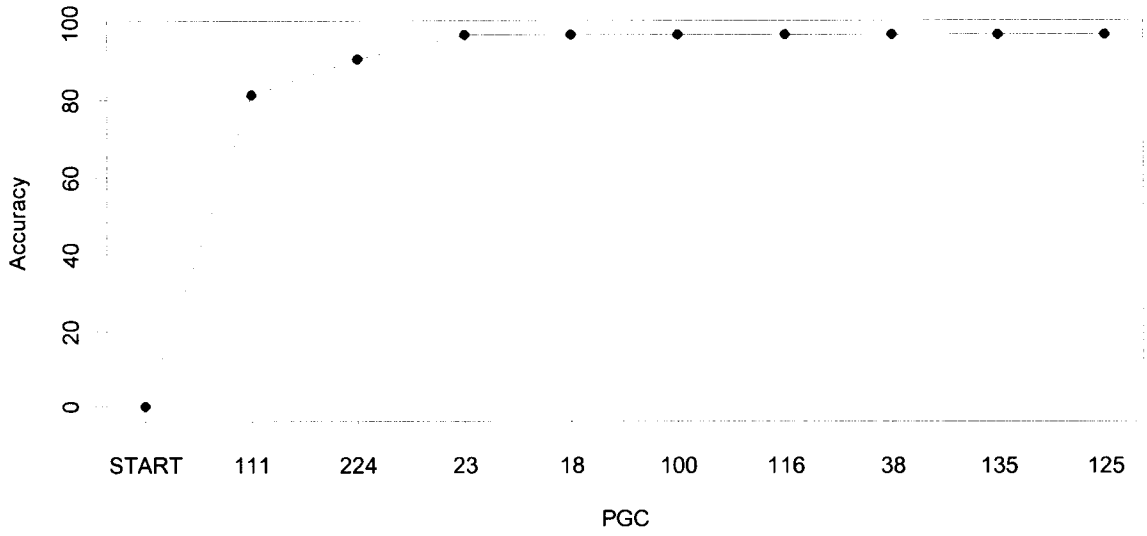

Figure 10

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31	201531_at	atcaagtaatccccttttccagaatgcattaacccactcccctgacctcacgctggggcaggtccccaagtgtgca agctcagtattcatgatggtgggggatggagtgtcttccgaggttcttgggggaaaaaaaattgtagcatatttaag ggaggcaatgaaccctctccccccacctcttccctgcccaaatctgtctcctagaatcttatgtgctgtgaataatagg ccttcactgcccctccagtttttatagacctgaggttccagtgtctcctggtaactggaacctctcctgaggggggaat cctggtgctcaaattaccctccaaaagcaagtagccaaagccgttgccaaaccccacccataaatcaatgggccc tttatttatgacgactttatttattctaatatgattttatagtatttatatatattgggtcgtctgcttcccctt
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34	201861_s_at	gcagttctctccctgaaaacacagtacaggttgagtcaaatgaggtcatgggtgcaccagatgacaggaccagaa ctccccttgagccatccaactgttggagtgacttagatggtgggaaccacacagagaatgtgggagaggcagca gtgactcaggttgaagagcaggcaggcacagtggcctcgtgtcctttagggcatagtgatgacacagtttatcatg atgacaaatgtatggtagaggtcccccaagagttagagacaagcacagggcatagtttagagaaagaattcacca accaggaagcagctgagcccaaggaggttccagcgcacagtacagaagtaggtagggatcacaacgaagaag agggtgaagaaacaggattaagggacgagaaaccaatcaagacagaagttcctggttctccagcaggaactga gggcaactgtcaggaagcgacaggtccaagtacagtagacactcaaaatgaacccttag
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Figure 10 (cont'd)

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39	202180_s_at	tgagcatggccgtggagagcaccgggactgccaaggcggaggccgagtcccgtgcggaggcagcccggatt gagggagaagggtccgtgctgcaggccaagctaaaagcacaggccttggccattgaaacggaggctgagctc cagagggtccagaaggtccgagagctggaactggtctatgcccgggcccagctggagctggaggtgagcaag gctcagcagctggctgaggtggaggtgaagaagttcaagcagatgacagaggccataggccccagcaccatca gggaccttgctgtggctgggcctgagatgcaggtaaaactgctccagtccctgggcctgaaatcaaccctcatca ccgatggctccactcccatcaacctcttcaacacagcctttgggctgctggggatggggcccgagggtcagccc ctgggcagaagggtgccagtggcccagccctggggagggatatccccccagtctgctcaggcccctcaagct cctggagacaaccacgtggtgcctgtactgcgctaactcctgattaata
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Figure 10 (cont'd)

SEQ ID NO:	Probe Set ID	Target Sequence
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46	202951_at	aaagcatcatttgcacctatgtgggaactttgcctgttgcaaagtattgtggccgagctgcagctgggagcctgcttt ctgccagtcttgaggttctgaagatcagctttgaaaggaaagtatgtcctagcttagccattcagaagagaaaaatg gaatatcagagttacagttgtcagtgaaactactttggattttaacctcttagaggaagaaaaaaggttagggaagtg tcaactctggatgaaggtgatgtgtttgcctctcagtctttcattcatagcctgctagtgaaaaggaagtaaatgagat tcttttgtgtgactttgtagtctctttgtattaccaaatagttggggtgttgactcctgtgtgttttgcaagaatgtgtggta agcctgggtaaagagaaggaactgcggtgttgggagagtctttgtgttggggagtggcaggggatgatttgtt
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50	203471_s_at	aaggactcagagccacacagaacttctgagaggggctgttagcattgcgcagcatcttcagttctccagtaaatga tattgcgttcgtgcctcagctttaagcacaagtagcagcagctcctgcttgagttctgagggcatcatggccctatga ttaaccagagtgatctaacctagactaaaattgggaacttatttgcaatttttgaccctgaccactaactagtgattctt ctccaaaattgagaaagacagcacccattgaaacagatatgtgtgtgaaagtatatttttcaattccagatttttaatttt aaggctccaggaaagaaaggagagtagaacatttttcctcattttatcaaatcctctcttgccctccctcaattcccct gtaacattcctgaagctgttcccactcccagatggttttatcaatagcctagaggtaaagaactgtctttttctctgattc tttaataaattatctttatagaatatgcacaagtttttctacactcagtgt
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52	203591_s_at	ctatgtgctccaggggacccaagagcagtttccacccagccccaatcccagtctggcaccagcgatcaggtcc tttatgggcagctgctgggcagccccacaagcccagggccagggcactatctccgctgtgactccactcagccc ctcttggcgggcctcacccccagccccaagtcctatgagaacctctggttccaggccagccccttggggaccctg gtaaccccagccccaagccaggaggacgactgtgtctttgggccactgctcaacttcccccctcctgcaggggatc cgggtccatgggatggaggcgctggggagcttctagggcttcctggggttcccttcttgggcctgcctcttaaagg cctgagctagctggagaagaggggagggtccataagcccatgactaaaaactaccccagcccaggctctcacc atctccagtcaccagcatctccctctcctcccaatctccataggctgggcctcccaggcgatctgcatacttaagg accagatcatgctccat

Figure 10 (cont'd)

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54	203748_x_at	caagtatcggggctctgctatcaaggtgcaaagtccttcgtggatgcaacctcaaccatatattctacagcaccctg gtgccgtgttaactccctcaatggagcacaccatgtcactacagcccgcatcaatgatcagccctctggcccagca gatgagtcatctgtcactaggcagcaccggaacatacatgcctgcaacgtcagctatgcaaggagcctacttgcc acagtatgcacatatgcagacgacagcggttcctgttgaggaggcaagtggtcaacagcaggtggctgtcgaga cgtctaatgaccattctccatataccttcaacctaataagtaactgtgagatgtacagaaaggtgttcttacatgaag aagggtgtgaaggctgaacaatcatggatttttctgatcaattgtgctttaggaaattattgacagttttgcacaggttc ttgaaaacgttatttataatgaaatcaactaaaactatttttgctataagttctataaggtgcataaaaccctttaaattcat ctagtagctgttccccc
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58	205285_s_at	gccaaagattcggaacaccagccagcttgaccaccagagaccccgaggcgaaagtgggatttctgaaacctgta ggccccaagcccatcaacttgcccaaagaagattccaaacctacatttccctggcctcctggaaacaagccatctc ttcacagtgtaaaccaagaccatgacttaaagccactaggcccgaaatctgggcctactcctccaacctcagaaaa tgaacagaagcaagcgtttcccaaattgactggggttaaagggaaatttatgtcagcatcacaagatcttgaaccc aagcccctcttccccaaacccgcctttggccagaagccgcccctaagtaccgagaactcccatgaagacgaaag ccccatgaagaatgtgtcttcatcaaaagggtccccagctcccctgggagtcaggtccaaaagcggccctttaaa accagcaagggaagactcagaaaataaagaccatgcaggggagatttcaagtttgccctttcctggagtggttttg a
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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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93	211058_x_at	atgtcaatgctgccattgccaccatcaaaaccaagcgcagcatccagtttgtggattggtgccccactggcttcaa ggttggcatcaactaccagcctcccactgtggtgcctggtggagacctggccaaggtacagagagctgtgtgcat gctgagcaacaccacagccattgctgaggcctgggctcgcctggaccacaagtttgacctgatgtatgccaagc gtgcctttgttcactggtacgtgggtgaggggatggaggaaggcgagttttcagaggcccgtgaagatatggctg cccttgagaaggattatgaggaggttggtgtggattctgttgaaggagagggtgaggaagaaggagaggaatac taattatccattccttttggccctgcagcatgtcatgctcccagaatttcagcttcagcttaactgacagacgttaaagc tttctggttagattgttttcacttggtgatcatgtctttccatgtgtacctgtaat
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Figure 10 (cont'd)

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Figure 10 (cont'd)

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108	212036_s_at	gcagtagcagttctagtagcagttcaaccagtagcagcagtggaagtagttccagcagtggaagtagtagcagtc gcagtagttccagtagcagctccagtacaagtggcagcagcagagatagtagcagnnnnnntagcactag tagtagtagtgagagtagaagtcggagtaggggccggggnacataatagagatagaaagcacagaaggagcg tggatcggaagagaagggatacttcaggactagaaagaagtcacaaatcttcaaaaggtggtagtagtagagata caaaaggatcaaaggataagaattcccggtccgacagaaagaggtctatatcagagagtagtcgatcaggcaaa agatcttcaagaagtgaaagagaccgaaaatcagacaggaaagacaaaaggcgttaatggaagaagccaggct ttcttagccattctttgcagc
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112	212708_at	gtgctcaatgtatttgcctgcttacaacactgggagatgtgtttgccagtaagttgctcatcacaagagcaccagact tgggggtgtaatctccggcaacttgcatgccctctgaaagaagggttttctgtgctgtgaaatgcatagaactatact ttgccatgcacgactgttcctgcaattgatattgtgtgaaatctgggagggtggtctttgggtgttctcaggggccaa tggtaatttttgggttggggagccagcttggggtggggaattttcacctgggcctccgctctttaactatataaacatt tatctgtatatctatgtccctgtctgggggggcaggaggaatctgccaaagaccaacagtcttacttttatcttactatact tcacaaaggttctaaaatgtgaagagtttacttggattgcagtagcccattggttgtt
113	212974_at	gtaacgaaatgcccatgacgcttccggagacaaccctggaaacactgaagcataaaatcaacccctcggcggg ggaggcgttcccacaagcggtggacgtgctgctctacactccagggcatcttgacccagccgaaaaagttgaag atgctcaccccaagttatggtgtgctctgagcgaaggcaaggtgaccgtgttcaatgcttcttcatggaccatccac cagcactcctttaaagtgggcactgcaaaagtgaactgcatggtgatggccgaccagaaccaggtgtgggttgg ctcggaagactccgtcatctacatcatcaacgtccacagcatgtcctgcaacaagcagctcacagcccactgctcc agtgtcacggatttgattgtgcaggacggacaggaggcacccagcaacgtgtactcgtgca
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Figure 10 (cont'd)

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118	215210_s_at	gagcagattctagcacatcatggcagtgaccaagcgtggtcccgagaagggccagagcctggtagagactagg gaagggaggtctcctctagactgactcacattgccttgagcttttcagttaagttgctgtaagcacctgggctgagg aggctgtttttgttccttcctgcgttatagcggggccttgtctcttcctctgcaggacacagatctggaggacgtgga ctggggtaggaaaccacctgagggtgttagtacctagtggtgaaatggatgaggtcatttctaaggtgtgttgccc gtggatctgggcacaatcattggaattccttggagccactgggattcatggctttgtatccaactgcatccaggcct gaggctgctgacgtttgacaccaggccagtagagagtgccctttgtatcttaagccaagaagtgaggcctgggg gtgggggaggggggaaggggtgggagccaatactgagtgcctgca
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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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Figure 10 (cont'd)

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159	227510_x_at	aagaccatcccaaaatgcttcaagtaaaaaataacaagtttaaggggttaagcacttttaaagtctgattaaggggg tgggggggaaaaaagagtaactaccagccatttctccaatggacatctcttccacagacctcaacgtgagaactgct ctagtttctataaactgtaaacctgtggtggtctgattatcctgatattggattttcttgttttctgttacaccttgagtcattt gcctttaggattctagacagacctaagggaaaaagaactgaaaacatattttgccccccaccccacaaaaaaaaat actgaaaactcccccccgcctcagttacacatccaaactctacatttacaaaacgaattcagggtgaggaagtaaa aacaggtcatctattcacaaaactgaaatacttcattaccccaactaa
160	227697_at	ctgccatagcactgatcagtgacaatttacaggaatgtagcagcgatggaattacctggaacagtttttgttttgtttt ttgttttgttttgtgggggggggcaactaaacaaacacaaagtattctgtgtcaggtattgggctggacagggcag ttgtgtgttggggtggttttttctctatttttttgtttgtttcttgttttttaataatgtttacaatctgcctcaatcactctgtctt ttataaagattccacctccagtcctctctcctccccctactcaggcccttgaggctattaggagatgcttgaagaac tcaacaaaatcccaatccaagtcaaactttgcacatatttatattta
161	228216_at	actacctaagattattttcacagctaccaatttgtcagaaactttggtggtgttttgngggaggctgagatttttaaatt aaaaatcaagtgaaaacacaaagactctaagacacgatcctgatttgtgtcatttctaagtatcagatttgttctccctt catttgacaggtgttctcagtctctcctcctgtaagattctgtccttcccctgatgacaagccaatagttcttggtgggg ttgcatgtctcctagagccccaggaccccctgtcgtgtcggggaaggggggagggcaggagggtgggcanaga ctcgggttgggggagggagttcaaagaaagagtgaaaatatgggtttatataaacatatttaaagcagtttagcaaa agctttctcgttgaacagctttaagaacaatgtgaatgaaatcttagcaacttggttagtaatc
162	228582_x_at	aacatttccacttgccagtttaatttcttgaagactgttgcttgtttggaatgtttcttgtcactgattttaaggttgcatctg gaaaagactaaaggcttcagtcccctcccaccaccagaaatgaacaaaaagcattttacctaaaaatacaccagc aaaatgtactcagcttcaatcacaaatacgactgcttaaaactgcagaaatttcctcaacactcagcctttatcactca gctggatttttttccttcaacaatcactactccaagcattggggaacacaacttttaatcatactccagtcgtttcacaat gcattctaatagcagcgggatcagaacagtactgcatttacttgccaacagaacagacagacctgaagtcaagac aactgcattctctgtga
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166	232555_at	actgcagcaaactttctagcatctgatattggataagnatagcttgtgctanangntggagatnaatcnggtctgct gnctngcancnttagagnctgnatctnatggttggtgtnaggatgttgttgacagttctgaaagttagccatcaatt cctgtgcagggtggagtcagacccagtgacttccttttcaatgtcagcaagagttttctcatgcctgctttggtcacttt ctcttggaaacttcacgcatttgacttgcagcttcttgacccgaggaatcaactgagctcccagtgctggctaccttg ggcaaataaaatctgtggttgagagagttctctttgctgtgccacagtccctgtgatgtgccaaatggcaccagcatt tgcagcaaagctcgttaaatctttttggaatgggcaaatatttttttcaacttgagccctgctgagc

Figure 10 (cont'd)

SEQ ID NO:	Probe Set ID	Target Sequence
167	233303_at	cttacctattgcctctgatatttacttgcttaaatttttttttattggaaatccagaaaaagtggatttagagaacaacact aactcccacctaatctatgacagagatnnnnnanagagtanctgtgaaaaatgtgaaagtatctgaaaaatgtaa cctttggcagcctgagcatagtcaaccagaaaaactatctgaattaaaataattggtccataggtactattttatttggt ccataaggattatttttttcaacttttttnnaagtgtattattatgtcatttcccacgtaggttactgatacctgaagacttttt tccacctttaaccttactcgttgaggagctttg
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Figure 10 (cont'd)

SEQ ID NO:	Probe Set ID	Target Sequence
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181	244556_at	ctctcaagtagcttttctcatggacttaagtccccatcggtgacacggagggaaaagaacttcagaaccaactgttg gccctgaaaagacctcaaagagggtgcaatccagttgatttttacattgaaaggtcacagttcccccctctgagagtn tgacggnttgtacaancccctncccccaggaancctgtatacacagtcanccancccatgaagcagtctcaggct aagaactntattatggaccgtagcccaatctgttcatttttacagatgaggcaactgagtccacaggagagcagaac gtgacntgcgtagaacacaaagttagttagaggcagacctaggacccaggtaacca
182	244752_at	gacctcacagctctttcagggaaagcacactttgtaagcaagataacatctagtaaaccttctgctgttgccagtga aaaatttaaagaacaagttgatcttgcaaaaaccatgaccaatttatcaccaaccattcttggcaatgcagttcagttg atctcttcagtcccaaagggaaactgccaatcccaccctactcaagaatgaagacaatggaggtttacaaaatca aatcagatgctaacattgcaggtttttctttaccaggacctaaggccgactgtgataagataccctccaccacagaa ggctttaatgcagccaccaaggtggcaagcaggctacctgttccacaagtgtcacagcagagtgcctgtgaaagt gccttt
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000744

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12Q 1/68*** (2006.01) , ***C40B 30/00*** (2006.01) , ***G01N 33/483*** (2006.01) , ***G01N 33/53*** (2006.01) , ***G01N 33/543*** (2006.01) , ***G01N 33/68*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q 1/68 (2006.01) , ***C40B 30/00*** (2006.01) , ***G01N 33/483*** (2006.01) , ***G01N 33/53*** (2006.01) , ***G01N 33/543*** (2006.01) , ***G01N 33/68*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Delphion, Scopus, NCBI PubMed database, Google Patents. Key Words: Allograft, Rejection, Genomic, Proteomic, expression profiling, Biomarkers, TncRNA, FKSG49, ZNF438, 1558448_a_at, CAMKK2, LMAN2, 237442_at, JUNB, etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	COLOMBO, G. et al. Treatment with alpha-melanocyte stimulating hormone preserves calcium regulatory proteins in rat heart allografts. Brain, Behavior, and Immunity. August 2008; Volume 22(6), pages 817-823. ISSN: 0889-1591. Abstract.	1-9
X	SARKAR, S. et al. Induction of indoleamine 2,3-dioxygenase by interferon-γ in human islets. Diabetes. January 2007; Volume 56(1), pages 72-79. ISSN: 0012-1797. Whole document.	1-9
X	CA2600168 A1 (KIENLE, S. et al.) 5 March 2008. Whole document.	10-22
A	US5939270 (HAUNSØ, S. et. al.) 17 August 1999.	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2009 (10-08-2009)	24 August 2009 (24-08-2009)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Adnan Ali 819- 934-7930

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000744

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

   a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [X] on paper

      [X] in electronic form

   c. time of filing/furnishing

      [ ] contained in the international application as filed.

      [ ] filed together with the international application in electronic form

      [X] furnished subsequently to this Authority for the purposes of search.

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments :

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FAMULSKI, K. et al. Changes in the transcriptome in allograft rejection: IFN-γ-induced transcripts in mouse kidney allografts. American Journal of Transplantation. June 2006; Volume 6(6), pages 1342-1354. ISSN:1600-6135.	
A	SARWAL, M. et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. The New England Jounral of Medicine. 10 July 2003; Volume 349(2), pages 125-138. ISSN: 0028-4793.	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2009/000744

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA 2600168 A1	05-03-2008	EP 1905846 A2	02-04-2008
		EP 1905846 A3	09-07-2008
		GB 0617429D0	18-10-2006
		US 2008153092A1	26-06-2008
US 5939270	17-08-1999	AU 702368 B2	18-02-1999
		AU 1414695 A	10-07-1995
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		DK 145393D0	23-12-1993
		EP 0736042 A1	09-10-1996
		JP 10504097 T	14-04-1998
		WO 9517425 A2	29-06-1995
		WO 9517425 A3	05-10-1995