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(54) **METHOD FOR DIAGNOSING SUBCLINICAL AND CLINICAL ACUTE REJECTION BY ANALYSIS OF PREDICTIVE GENE SETS**

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(57) **ABSTRACT**

Disclosed herein are methods for diagnosing acute cellular rejection (ACR) of an allograft by analysis of predictive gene sets and kits for practicing these methods.

Specification includes a Sequence Listing.

METHOD FOR DIAGNOSING SUBCLINICAL AND CLINICAL ACUTE REJECTION BY ANALYSIS OF PREDICTIVE GENE SETS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of co-pending U.S. application Ser. No. 15/321,885, filed Dec. 23, 2016, which is a U.S. National Stage application, and claims priority of International Application No. PCT/US15/38171, filed Jun. 26, 2015, which claims priority of U.S. Provisional Application Ser. No. 62/017,784, filed Jun. 26, 2014. The contents of all of the prior applications are incorporated herein by reference in their entirety.

GOVERNMENT GRANT CLAUSE

[0002] This invention was made with government support under grant no. 1U01A1070107-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing that has been submitted electronically as an XML file named "[27527-0135002SEQ.XML." The XML file, created on Feb. 6, 2023, is 88,902 bytes in size. The material in the XML file is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[0004] This invention relates to methods for diagnosing acute cellular rejection (ACR) of an allograft by analysis of predictive gene sets and kits for practicing these methods. The methods comprise analyzing the blood of kidney allografts recipients by determining the expression level of a gene signature set containing at least 7 preselected genes in order to identify and treat such patients. An altered expression level of one or more genes in the blood of the allograft recipient compared to the same genes in a control indicates the patient is at risk for allograft rejection. The greater the level of alteration, the greater the risk of rejection. A logistic regression fitting model can be applied to normalized expression values (e.g. read counts of genes from next generation sequencing technology) to derive a tatistical model from which a probability score for risk of acute cellular rejection can be calculated for each patient.

BACKGROUND OF THE INVENTION

[0005] The existing tests for renal allograft rejection are cumbersome and expensive. Such tests usually require obtaining a biopsy specimen from the patient. Often by the time rejection is recognized it is too late to do anything. An increase in serum creatinine or an increase of protein in the urine may be warnings of rejection but are not entirely predictive. There has been a need in the field for an improved assay that is easy to conduct, eliminates the need for a biopsy and is more predictive of the risk of renal allograft rejection or fibrosis.

[0006] The present expression profile test addresses this need and provides a blood based assay that is easily administered repetitively to transplant patients. Renal transplant patients are examined by their physician very frequently post transplantation—in most instances twice per week for the first month moving to weekly and then every other week getting to monthly after 4 to 5 months, with time intervals between visits gradually increasing thereafter. During this time, the patients' renal function and the immunosuppression levels are monitored. Steroids are tapered to 5 mg by 3 months post-surgery and the tacrolimus (a drug that suppresses the immune system and is used to prevent rejection of transplanted organs) levels are gradually reduced to a steady level by 6-12 months if the post-transplant course has no complications and the patient is not high immunological risk. The gene expression profiles described below can be employed as a standard test to be performed at the time of a clinical visit. A positive test result (i.e. the patient is expressing these genes at an altered level compared to a control) indicates that the patient is at risk of rejecting the transplanted organ, and would be treated by increasing the dose of immunosuppressive drugs and by discontinuing the customary taper of immunosuppressive drugs. Repeat testing (which can be done economically since the assay is preferably a blood based test) will guide the reinitiation of immunosuppression tapering. Thus, for example if 2 subsequent tests were negative the prednisone dose may decrease by 2.5 or 5 mg, or the target level for tacrolimus would be lowered by 0.5 mg/dl. If the profile test is positive in the presence of an increase in creatinine this would indicate a clinical acute rejection as evidenced by renal injury. In this instance, the patient would be treated with either high dose steroids or anti-lymphocyte agents depending on the overall immunological risk of the individual.

SUMMARY OF THE INVENTION

[0007] In one aspect, a method is provided for identifying a kidney allograft recipient at risk of allograft rejection or loss by obtaining a biological specimen from the renal allograft recipient, measuring the expression level of a preselected gene signature set in the specimen, comparing the expression level of the preselected gene signature set in the specimen with the expression level of the preselected gene signature set in a control, determining that the allograft recipient is at increased risk for rejection of the allograft if the expression level of the gene signature set in the specimen is altered compared to the expression level in the control.

[0008] In another aspect, a method is provided for identifying a kidney allograft recipient at risk of allograft rejection or loss by obtaining a biological specimen from the renal allograft recipient, measuring the expression level of a preselected gene signature set in the specimen, comparing the expression level of the preselected gene signature set in the specimen with the expression level of the preselected gene signature set in a control, determining that the allograft recipient is at increased risk for rejection of the allograft if the expression level of one or more genes in the gene signature set in the specimen is altered compared to the expression level of one or more genes in the gene signature set in the control.

[0009] In another aspect, a method is provided for treating a kidney allograft recipient at risk of allograft rejection or loss by obtaining a biological specimen from the renal allograft recipient, measuring the expression level of a preselected gene signature set in the specimen, comparing the expression level of the preselected gene signature set in the specimen with the expression level of the preselected gene signature set in a control sample, determining that the allograft recipient is at increased risk for rejection of the

allograft if the expression level of the gene signature set in the specimen is altered compared to the expression level of the preselected gene signature set in the control and treating a recipient determined to be at risk of rejection.

[0010] In another aspect, a method is provided for treating a kidney allograft recipient at risk of allograft rejection or loss by obtaining a biological specimen from the renal allograft recipient, measuring the expression level of a preselected gene signature set in the specimen, comparing the expression level of the preselected gene signature set in the specimen with the expression level of a control sample, determining that the allograft recipient is at increased risk for rejection of the allograft if the expression level of one or more genes in the gene signature set in the specimen is altered compared to the expression level of one or more genes in the gene signature set in the control and treating a recipient determined to be at risk of rejection

[0011] In another embodiment, a method is provided for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a selected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of a control patient, determining that the recipient is at risk for rejection—of the allograft if the expression level of the gene set in the specimen is altered compared to the expression level of the gene set in the control, and treating a recipient determined to be at risk of rejection.

[0012] In another embodiment, a method is provided for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a selected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of a control, determining that the recipient is at risk for—rejection—of the allograft if the expression level of one or more members of the gene set in the specimen is altered compared to the expression level of one or more members of the gene signature set in the control, and treating a recipient determined to be at risk of rejection.

[0013] Another embodiment provides a method for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a selected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of a control, determining that the patient is at risk of rejection of the allograft if the expression level of the gene set in the specimen is altered relative to the expression level of the gene set in the control, and treating the recipient determined to be at risk of rejection by administering immunosuppressive drugs to the recipient or by administering high dose steroids or antilymphocyte agents.

[0014] Another embodiment provides a method for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a selected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of the gene set in a control, determining that the patient is at risk of rejection of the allograft if the expression level of one or more genes of the gene set in the specimen is altered relative to the expression level of one or more members of the gene set in the control, and treating the recipient determined to be at risk of rejection by administering immunosuppressive drugs to the recipient or by administering high dose steroids or antilymphocyte agents.

[0015] In another aspect a kit for use in determining if an allograft recipient is at risk of ACR is provided. The kit comprises an assay for a preselected gene signature set, primers for a preselected gene signature set, buffers and positive and negative controls and instructions for use.

[0016] In one aspect, a sequencing panel is provided comprising at least 7 genes from among the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1

[0017] In another aspect, a sequencing panel is provided comprising the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7

[0018] In another aspect, a sequencing panel is provided comprising the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7.

[0019] In another aspect, a sequencing panel is provided comprising the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1.

[0020] In another aspect, a sequencing panel is provided comprising the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

[0021] Yet another aspect-is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7, buffers, positive and negative controls and instructions for use.

[0022] Yet another aspect is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7, buffers, positive and negative controls and instructions for use.

[0023] Yet another aspect is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1, buffers, positive and negative controls and instructions for use.

[0024] Another aspect is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP and C1GALT1C1, buffers, positive and negative controls and instructions for use.

[0025] Another aspect of the invention is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising genes selected from the

group consisting of SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1, buffers, positive and negative controls and instructions for use.

[0026] A further aspect-provides a method for identifying kidney allograft recipients suffering from subclinical and clinical acute rejection and at risk for graft loss comprising the steps of providing a blood specimen from a renal allograft recipient, isolating mRNA from the blood specimen, synthesizing cDNA from the mRNA, and measuring the expression levels of a gene panel comprising a preselected gene signature set with MiSEQ sequence system (Illumina, Inc. San Diego Calif.), Nanostring (nCounter® miRNA Expression Assay-Nanostring Technologies, Inc. Seattle Wash.) or qPCR. The results of the gene set analysis are compared to a control. The gene expression result can be used to predict the onset of an allograft rejection response, to diagnose an allograft rejection response, and/or to characterize an allograft rejection response in a transplant patient. If the patient is expressing the gene signature set at an altered level relative to the expression level in the control, the patient is at risk for rejection. The greater the patients expression level of the signature gene set is altered compared to the control, the greater the risk of rejection.

[0027] In another aspect, the results of the assay are applied to a penalized logistic regression fitting model (log (p(x))/(1−p(x))=β*0+β*1g1+β*igi+ . . . +β*ngn (where (p(x) is the probability of ACR, β*i is penalized coefficiency and gi is the read count of gene i) that can be used to compute a probability score for acute rejection for each patient.

[0028] In another aspect the invention provides a method for assessing the likelihood of renal graft rejection in a patient by determining the level of expression of a preselected gene set containing at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7, in a sample from the patient and comparing the expression level of the preselected gene set genes in the sample with the expression level of the preselected gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0029] In another aspect the invention provides a method for assessing the likelihood of renal graft rejection in a patient by determining the level of expression of a preselected gene set containing at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7 in a sample from the patient and comparing the expression level of the preselected gene set genes in the sample with the expression level of the preselected gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0030] In another aspect the invention provides a method for assessing the likelihood of renal graft rejection in a patient by determining the level of expression of a preselected gene set containing at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1 in a sample from the patient and comparing the expression level of the preselected gene set genes in the sample with the expression level of the gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0031] In another aspect the invention provides a method for assessing the likelihood of renal graft rejection in a patient by determining the level of expression of a preselected gene set containing at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1 in a sample from the patient and comparing the expression level of the preselected gene set genes in the sample with the expression level of the preselected gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0032] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a gene set comprising at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7 from a sample from the patient who has received the allograft and comparing the gene set expression level in the sample with the expression level of the gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0033] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a gene set comprising at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7 from a sample from the patient who has received the allograft and comparing the gene set expression level in the sample with the expression level of the gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0034] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a gene set comprising at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1 from a sample from the patient who has received the allograft and comparing the gene set expression level in the sample with the expression level of the gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0035] In another aspect the invention provides a method for assessing the likelihood of renal graft rejection in a patient by determining the level of expression of a preselected gene set containing at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1 in a sample from the patient and comparing the expression level of the preselected gene set genes in the sample with the expression level of the gene set genes in a control to assess the likelihood of renal graft rejection in the patient.

[0036] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a gene set comprising at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, C1GALT1C1, SENP6, CLK1 and SENP7 from a sample from the patient who has received the allograft.

[0037] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of an eleven member gene set comprising at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1,

CLK1, and C1GALT1C1 from a sample from the patient who has received the allograft.

[0038] In another aspect the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a 17 member gene set comprising at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1 from a sample from the patient who has received the allograft.

[0039] In another aspect the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for the preselected gene set, positive and negative controls, buffers and instructions for use.

[0040] In another aspect the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for a 17 member gene set, positive and negative controls, buffers and instructions for use.

[0041] In another aspect the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for an 11 member gene set, positive and negative controls, buffers and instructions for use.

[0042] In another aspect the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for a 9 member gene set, positive and negative controls, buffers and instructions for use.

[0043] In another aspect the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for a 7 member gene set, positive and negative controls, buffers and instructions for use.

[0044] In another aspect the invention provides a method for selecting a renal allograft recipient for treatment to reduce the risk of renal allograft rejection which comprises

[0045] (a) providing a blood specimen from the renal allograft recipient;

[0046] (b) determining the expression levels of a preselected gene set in the specimen;

[0047] (c) comparing the expression levels of the preselected gene set genes in the specimen with the expression levels of the preselected gene set genes in a control, and

[0048] (d) selecting the recipient for treatment for allograft rejection if the expression level of one or more genes in the gene set in the specimen is altered compared to the expression level of one or more of the gene set genes in the control.

[0049] In another aspect the invention provides a method of selecting a renal allograft patient for treatment to reduce the risk of renal allograft rejection or allograft loss which comprises comparing the expression level of a preselected gene set obtained from the patient with the expression level of the preselected gene set in a control sample obtained from an allograft recipient that did not suffer allograft rejection, and selecting the patient for treatment for allograft rejection or loss if the expression level of one or more genes in the preselected gene set from the patient is altered compared to the expression level of one or more of the preselected gene set genes in the control.

[0050] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0051] As used herein, the term "about" or "approximately" usually means within an acceptable error range for the type of value and method of measurement. For example, it can mean within 20%, more preferably within 10%, and most preferably still within 5% of a given value or range. Alternatively, especially in biological systems, the term "about" means within about a log (i.e., an order of magnitude) preferably within a factor of two of a given value.

[0052] As used herein "ACR" means acute cellular rejection.

[0053] In accordance with the present invention, there may be employed conventional molecular biology, microbiology, recombinant DNA, immunology, cell biology and other related techniques within the skill of the art. See, e.g., Sambrook et al., (2001) Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.; Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.; Ausubel et al., eds. (2005) Current Protocols in Molecular Biology. John Wiley and Sons, Inc.: Hoboken, N.J.; Bonifacino et al., eds. (2005) Current Protocols in Cell Biology. John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al., eds. (2005) Current Protocols in Immunology, John Wiley and Sons, Inc.: Hoboken, N.J.; Coico et al., eds. (2005) Current Protocols in Microbiology, John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al., eds. (2005) Current Protocols in Protein Science, John Wiley and Sons, Inc.: Hoboken, N.J.; Enna et al., eds. (2005) Current Protocols in Pharmacology John Wiley and Sons, Inc.: Hoboken, N.J.; Hames et al., eds. (1999) Protein Expression: A Practical Approach. Oxford University Press: Oxford; Freshney (2000) Culture of Animal Cells: A Manual of Basic Technique. 4th ed. Wiley-Liss; among others. The Current Protocols listed above are updated several times every year

[0054] The terms "decrease", "decreased", "reduced", "reduction" or "down-regulated" are all used herein generally to mean a decrease by a statistically significant amount. However, for avoidance of doubt, "reduced", "reduction", "down-regulated" "decreased" or "decrease" means a decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. absent level as compared to a reference sample), or any decrease between 10-100% as compared to a reference level, or at least about a 0.5-fold, or at least about a 1.0-fold, or at least about a 1.2-fold, or at least about a

1.5-fold, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold decrease, or any decrease between 1.0-fold and 10-fold or greater as compared to a reference level.

[0055] The terms "increased", "increase" or "up-regulated" are all used herein to generally mean an increase by a statistically significant amount; for the avoidance of any doubt, the terms "increased" or "increase" means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 0.5-fold, or at least about a 1.0-fold, or at least about a 1.2-fold, or at least about a 1.5-fold, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 1.0-fold and 10-fold or greater as compared to a reference level.

[0056] As used herein, "determining the level of expression," "determining the expression level" or "detecting the level of express", as in, for example, "determining the expression level of a gene refers to quantifying the amount of mRNA present in a sample. Detecting expression of the specific mRNAs, can be achieved using any method known in the art or described herein. Typically, mRNA detection methods involve sequence specific detection, such as by RT-PCR. mRNA-specific primers and probes can be designed using nucleic acid sequences, which are known in the art.

[0057] Throughout the application and in the appended claims, it should be understood and is intended to be understood that use of the terms "drug", "medication", "agent" and "therapeutic agent" are interchangeable expressions defining the same or similar entities. A "drug" refers generally to a chemical compound, small molecule, or other biologic composition, such as an antisense compound, antibody, protease inhibitor, hormone, chemokine or cytokine, capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

[0058] As used herein, "treating" or "treatment" of a state, disorder or condition includes: (1) preventing or delaying the appearance of clinical or sub-clinical symptoms of the state, disorder or condition developing in a mammal that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition (e.g., fibrosis of a renal allograft and/or allograft loss); and/or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or sub-clinical symptom thereof; and/or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms; and/or (4) causing a decrease in the severity of one or more symptoms of the disease. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0059] As used herein, the term "inhibiting" of disease or condition (e.g. ACR and/or allograft loss) means for example, to stop the development of one or more symptoms of a disease in a subject before they occur or are detectable, e.g., by the patient or the patient's doctor. Preferably, the disease or condition does not develop at all, i.e., no symptoms of the disease are detectable. However, it can also result in delaying or slowing of the development of one or more symptoms of the disease.

[0060] As used herein, a "altered" level of expression of a mRNA compared to reference level or control level is an at least 0.5-fold (e.g., at least: 1-2-; 3-; 4-; 5-; 6-; 7-; 8-; 9-; 10-; 15-; 20-; 30-; 40-; 50-; 75-; 100-; 200-; 500-; 1,000-; 2000-; 5,000-; or 10,000-fold) altered level of expression of the mRNA. It is understood that the alteration can be an increase or a decrease. Alternatively, altered expression level is defined as an increase in the acute rejection probability score using parameters in the logistic regression model established from a training patient group, comparing the probability score to the cutoff derived from the training set.

[0061] "Control" is defined as a sample obtained from a patient that received an allograft transplant that is not suffering from acute cellular rejection.

[0062] It has now been determined that enhanced transcription of a preselected set of genes can be used to predict the likelihood that a renal allograft recipient will reject a transplanted kidney before the patient manifests any overt symptoms. The assay can also be employed to diagnose whether a kidney transplant patient is experiencing acute rejection. Over expression of the gene sets can be used to identify patients at risk for acute rejection.

[0063] If the patient is expressing the preselected genes at an altered level relative to control, the patient is at risk for rejection. The greater the alteration in the expression level of the preselected genes compared to the control, the greater the risk of rejection. The information obtained with the assay can also be applied to a penalized logistic regression fitting model ($\log(p(x))/(1-p(x))=\beta*0+\beta*1g1+\beta*igi+\ldots+\beta*9g9$ (where $(p(x)$ is the probability of ACR, $\beta*i$ is penalized coefficiency and gi is the read count of gene i), that can be used to compute a probability score for acute rejection for each patient. In one embodiment, a higher score above indicates that the patient is at high risk of rejecting the transplanted organ.

[0064] Currently, the diagnosis of acute rejection requires a renal allograft biopsy triggered by an elevation of creatinine in the presence of renal injury. The collection and assaying of patient biopsy samples is time consuming and expensive. The present assay technique is a biological tissue assay, preferably a blood based assay that avoids the need for biopsy specimens. The assay can be used to predict the likelihood that a renal allograft recipient will reject a transplanted kidney before the patient manifests any overt symptoms. The assay can also be employed to diagnose whether a kidney transplant patient is experiencing acute rejection. The assay is inexpensive, highly accurate, reproducible and non-invasive.

[0065] Peripheral blood signatures using gene signature sets comprising at least 7 and up to 17 preselected genes have been identified. These preselected gene sets can be used to accurately diagnose subclinical rejection of kidney allografts and identify kidney allografts at risk for subsequent histological and functional decline as well as the risk of graft rejection.

[0066] The gene signature sets useful in practicing the methods disclosed herein are selected from the following genes: SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82,

NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

[0067] The seven gene peripheral blood signature set of the present invention is: ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7.

[0068] The nine gene peripheral blood signature of the present invention is: TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7.

[0069] The 11-gene peripheral blood signature of the present invention is: CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1.

[0070] The 17-gene peripheral blood signature of the present invention is: SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

[0071] Each of these blood signature sets can be used to practice the present invention. However, the 11-gene blood signature set is a preferred embodiment.

[0072] The present invention provides a method for identifying kidney allograft recipients suffering from subclinical and clinical acute rejection and at risk for graft loss comprising the steps of providing a blood specimen from a renal allograft recipient, isolating mRNA from the blood specimen, synthesizing cDNA from the mRNA, and measuring the expression levels of a gene panel comprising a selected gene signature set with MiSEQ sequence system (Illumina, Inc. San Diego Calif.), Nanostring (nCounter® miRNA Expression Assay-Nanostring Technologies, Inc. Seattle Wash.) or qPCR. The results of the gene set analysis are compared to a control. The gene expression result can be used to predict the onset of an allograft rejection response, to diagnose an allograft rejection response, and/or to characterize an allograft rejection response in a transplant patient. If the patient is expressing the gene signature set at an altered level relative to the expression level of the gene signature set genes in the control, the patient is at risk for rejection. The greater the patient's expression level is altered compared to the control, the greater the risk of rejection.

[0073] In one aspect, a method is provided for treating a kidney allograft recipient by obtaining a biological specimen from the renal allograft recipient, measuring the expression level of a preselected gene signature set in the specimen, comparing the expression level of the preselected gene signature set in the specimen with the expression level of the gene signature genes a control sample, and determining that the allograft recipient is at increased risk for acute T-cell mediated rejection of the allograft if the expression level of the gene signature set in the specimen is altered relative the expression level in the gene signature set in the control.

[0074] In another embodiment, a method is provided for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a selected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of a control patient, determining that the recipient is at risk for rejection of the allograft if the expression level of the gene set in the specimen is altered relative to the expression level of the gene set in the control, and treating a patient determined to be at risk of rejection to prevent rejection.

[0075] Another embodiment provides a method for treating a kidney allograft recipient by obtaining a biological specimen from the allograft recipient, measuring the expression level of a preselected gene set in the specimen, comparing the expression level of the gene set in the specimen with the expression level of a control, determining that the patient is at risk of rejection if the expression level of the gene set in the specimen is altered relative to the expression level of the gene set in the control, and treating the recipient determined to be at risk of rejection by administering immunosuppressive drugs to the recipient or by administering high dose steroids or antilymphocyte agents

[0076] In yet another embodiment, an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss is provided comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7, buffers, positive and negative controls and instructions for use.

[0077] In yet another embodiment, an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss is provided comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7, buffers, positive and negative controls and instructions for use.

[0078] Yet another aspect is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes CCDC82, F13A1, TUBB1, TSC22D1, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1, buffers, positive and negative controls and instructions for use.

[0079] Yet another aspect is an assay kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers: an assay for a gene signature set comprising at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1, buffers, positive and negative controls and instructions for use.

[0080] In a further embodiment, the present invention provides a method for identifying kidney allograft recipients suffering from subclinical and clinical acute rejection and at risk for graft loss comprising the steps of providing a blood specimen from a renal allograft recipient, isolating mRNA from the blood specimen, synthesizing cDNA from the mRNA, and measuring the expression levels of a gene panel comprising a selected nine gene signature set with MiSEQ sequence system (Illumina, Inc. San Diego Calif.), Nanostring (nCounter® miRNA Expression Assay-Nanostring Technologies, Inc. Seattle Wash.) or qPCR. The results of the gene set analysis are compared to the expression level of the signature set genes in a control. The gene expression result can be used to predict the onset of an allograft rejection response, to diagnose an allograft rejection response, and/or to characterize an allograft rejection response in a transplant patient. If the patient is expressing the gene signature set at an altered level relative to the control, the patient is at risk for reject of the signature set

genes ion. The greater the alteration (increase and/or decrease) in the patient's expression level of the gene signature genes compared to the control, the greater the risk of rejection.

[0081] In another embodiment, the results of the assay are applied to a penalized logistic regression fitting model (log (p(x))/(1−p(x))=β*0+β*1g1+β*igi+ . . . +β*ngn (where (p(x) is the probability of ACR, β*i is penalized coefficiency and gi is the read count of gene i) that can be used to compute a probability score for acute rejection for each patient. If the probability score of the patient is higher than the probability score of the control then the patient is at risk for acute cellular rejection.

[0082] In another embodiment the invention provides a method of determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft by evaluating the expression level of a 17 member gene set comprising at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1 from a sample from the patient who has received the allograft.

[0083] In some methods herein, it is desirable to detect and quantify mRNAs present in a sample. Detection and quantification of RNA expression can be achieved by any one of a number of methods well known in the art. Using the known sequences for RNA family members, specific probes and primers can be designed for use in the detection methods described below as appropriate.

[0084] In some cases, detection and quantification of RNA expression requires isolation of nucleic acid from a sample, such as a cell or tissue sample. Nucleic acids, including RNA and specifically mRNA, can be isolated using any suitable technique known in the art. For example, phenol-based extraction is a common method for isolation of RNA. Phenol-based reagents contain a combination of denaturants and RNase inhibitors for cell and tissue disruption and subsequent separation of RNA from contaminants. Phenol-based isolation procedures can recover RNA species in the 10-200-nucleotide range (e.g., precursor and mature miRNAs, 5S and 5.8S ribosomal RNA (rRNA), and U1 small nuclear RNA (snRNA)). In addition, extraction procedures such as those using TRIZOL™ or TRI REAGENT™, will purify all RNAs, large and small, and are efficient methods for isolating total RNA from biological samples that contain miRNAs and small interfering RNAs (siRNAs). Extraction procedures such as those using QIAGEN-ALLprep kit are also contemplated.

[0085] In some embodiments, use of quantitative RT-PCR is desirable. Quantitative RT-PCR (qRT-PCR) is a modification of polymerase chain reaction used to rapidly measure the quantity of a product of polymerase chain reaction. qRT-PCR is commonly used for the purpose of determining whether a genetic sequence is present in a sample, and if it is present, the number of copies in the sample. Any method of PCR that can determine the expression of a nucleic acid molecule, including a mRNA, falls within the scope of the present disclosure. There are several variations of the qRT-PCR method known in the art, three of which are described below.

[0086] In another embodiment the invention provides a kit for determining whether a patient that has received an allograft is undergoing an acute rejection of the allograft comprising in one or more containers primer pairs for the 17 member gene set, positive and negative controls, buffers and instructions for use.

[0087] In a typical embodiment, a clinical lab will obtain the expression value using the patient's sample and send it to the patient's doctor. The doctor will then communicate this value to his web based service provider. The service provider will enter that value in the bioinformatics system which already has the penalized co-efficiency for each gene of the preselected gene set and the cutoff from the logistic regression model from the training set. The bioinformatics system will use this information to calculate the probability score for the patient. The calculated score will reflect the patient's ACR status.

[0088] The overall procedure of application of the gene signature in ACR diagnosis is described below using the nine-gene signature as a non-limiting example.

[0089] 1) Selecting training group: A group of kidney transplant patients with balanced ACR and noACR (control) cases (total number N=100) will be carefully selected. The training group will have well-characterized demographics and clinical indications which have been reviewed by at least two pathologists.

[0090] 2) Measuring expression of 9 genes: Expression levels of 9 genes from the blood sample post-transplant of each patient in the training group can be measured using any technique, and preferably by MiSEQ, RT-PCR or Nanostring technology. Use of these techniques is described in Examples 1-3 below.

[0091] 3) Establishing a regression model and cut off: A penalized logistic regression fitting model using the logistf R package (a statistical package available from r-project. org) will be then applied on expression values of the 9 genes to derive the statistical model from which the β* value will be derived for each gene and the probability score of acute rejection for each patient will be calculated from the following equation:

$$(\log (p(x))/(1-p(x))=(\beta*0+(\beta*1g1+\beta*igi+ \ldots +(\beta*9g9$$

(where (p(x) is the probability of ACR, β*i is penalized coefficiency and gi is the read count of gene i)

[0092] Based on the probability score, the prediction statistics such as prediction AUC (area under the curve) of ROC (Receive operating characteristic) curve of the true positive rate versus the false positive, sensitivity/specificity, the positive values (PPV) and the negative predictive values (NPV) will be determined. At a given specificity (90%), a probability score cut off will be established which best detects the presence of acute rejection. It is expected that there will be a clear cut off into two groups in that if a patient is in the top group they have a high likelihood of having acute rejection and the test is determined to be positive but if they are in the bottom they have a very low likelihood of having acute rejection and the test is determined to be negative.

[0093] The alternative is that patients will be broken in to tertiles based on their probability score determined as above. In this case if the patient is in (1) the top tertile they have a high likelihood of having acute rejection and the test is determined to be positive; (2) the second tertile or intermediate group their risk cannot be accurately determined; and (3) the bottom tertile they have a very low likelihood of having acute rejection and the test is determined to be negative.

[0094] The coefficiency (β* value) and the cutoff derived from the training group will be entered and stored into a web-based bioinformatics computer system which can be accessed from clinical lab/doctor office via the internet.

[0095] 4) Diagnosis of a new case: For a new patient, the expression levels of the 9 gene set will be measured by the same technology used for the training set in the clinical lab. By using a web-based bioinformatics system, the probability score will be calculated by summarizing the expression value (gi) of the 9 genes multiplied by their β* values which are derived from the training set. The probability score will be compared to the cutoff to determine the ACR status. An increase in the probability score in the patient relative to the probability score in a control indicates that the patient is at an increased risk for allograft rejection. A clinical lab will send the testing results to the doctor.

[0096] The methods disclosed herein accurately diagnose subclinical and clinical rejection and accurately identify allografts at risk for subsequent histological and functional decline and allograft recipients at risk for graft loss.

[0097] When such allografts recipients are identified, the present invention includes methods for treating such patients. The methods include, without limitation, increased administration of immunosuppressive drugs, i.e. a calcineurin inhibitor (CNI), such as cyclosporine or tacrolimus, or a less fibrogenic immunosuppressive drug such as mycophenolate mofetil (MMF) or sirolimus. The main class of immunosuppressants are the calcineurin inhibitors (CNIs), which includes tacrolimus (Prograf® and Advagraf®/Astagraf XL (Astellas Pharma Inc.) and generics of Prograf®) and cyclosporine (Neoral® and Sandimmune® (Novartis AG) and generics). Steroids such as prednisone may also be administered to treat patients at risk for graft loss or functional decline. Antiproliferative agents such as Mycophenolate Mofetil, Mycophenolate Sodium and Azathioprine are also useful in such treatments. Immunosuppression can be achieved with many different drugs, including steroids, targeted antibodies and CNIs like tacrolimus. Of these, tacrolimus is one of the more potent in terms of suppressing the immune system or administration of high dose steroids or antilymphcytic agents depending on the presence or absence of an elevated creatinine. The currently preferred treatment regimen for clinical rejection is either high does steroids or antilymphocytic agents. Another preferred agent is Nulojix® (belatacept, Bristol-Myers Squibb), an infusional biologic agent.

[0098] Kits

[0099] In certain embodiments, kits are provided for determining a renal allograft recipient's risk for allograft loss.

[0100] The kits will contain primers for the 17 member gene signature sea as set forth in Example 5 below (for Nanostring assays), primers for 2 housekeeping genes, beta actin (ACTB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and a control probe, 18S ribosomal RNA (for qPCR assays).

[0101] A kit can further contain one or more mRNA extraction reagents and/or reagents for cDNA synthesis. In other embodiments, the kit can comprise, one or more containers into which the biological agents are placed and, preferably, suitably aliquotted. The components of the kits may be packaged either in aqueous media or in lyophilized form. The kits can also comprise one or more pharmaceutically acceptable excipients, diluents, and/or carriers. Non-limiting examples of pharmaceutically acceptable excipients, diluents, and/or carriers include RNAse-free water, distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, reaction buffers, labeling buffers, washing buffers, and hybridization buffers.

[0102] The kits of the invention can take on a variety of forms. Typically, a kit will include reagents suitable for determining gene set expression levels (e.g., those disclosed herein) in a sample. Optionally, the kits may contain one or more control samples. Also, the kits, in some cases, will include written information (indicia) providing a reference (e.g., predetermined values), wherein a comparison between the gene expression levels in the subject and the reference (predetermined values) is indicative of a clinical status.

[0103] In some cases, the kits comprise software useful for comparing gene set expression levels or occurrences with a reference (e.g., a prediction model). Usually the software will be provided in a computer readable format such as a compact disc, but it also may be available for downloading via the internet. However, the kits are not so limited and other variations with will be apparent to one of ordinary skill in the art.

[0104] The present methods can also be used for selecting a treatment and/or determining a treatment plan for a subject, based on the expression levels of a gene set (e.g., those disclosed herein).

[0105] Expression levels and/or reference expression levels may be stored in a suitable data storage medium (e.g., a database) and are, thus, also available for future diagnoses. This also allows efficiently diagnosing prevalence for a disease because suitable reference results can be identified in the database once it has been confirmed (in the future) that the subject from which the corresponding reference sample was obtained did develop fibrosis of the allograft and/or experience allograft rejection.

[0106] As used herein a "database" comprises data collected (e.g., analyte and/or reference level information and/or patient information) on a suitable storage medium. Moreover, the database, may further comprise a database management system. The database management system is, preferably, a network-based, hierarchical or object-oriented database management system. More preferably, the database will be implemented as a distributed (federal) system, e.g. as a Client-Server-System. More preferably, the database is structured as to allow a search algorithm to compare a test data set with the data sets comprised by the data collection. Specifically, by using such an algorithm, the database can be searched for similar or identical data sets being indicative of renal allograft rejection risk. Thus, if an identical or similar data set can be identified in the data collection, the test data set will be associated with renal allograft rejection risk. Consequently, the information obtained from the data collection can be used to diagnose an allograft recipient's risk for allograft loss or based on a test data set obtained from a subject. More preferably, the data collection comprises characteristic values of all analytes comprised by any one of the groups recited above.

[0107] The invention further provides for the communication of assay results or diagnoses or both to technicians, physicians or patients, for example. In certain embodiments, computers will be used to communicate assay results or diagnoses or both to interested parties, e.g., physicians and their patients.

[0108] In some embodiments, the method disclosed herein further comprise modifying the recipient's clinical record to

identify the recipient as being at risk for developing ACR and/or allograft loss. The clinical record may be stored in any suitable data storage medium (e.g., a computer readable medium).

[0109] In some embodiments of the invention, a diagnosis based on the methods provided herein is communicated to the allograft recipient as soon as possible after the diagnosis is obtained. The diagnosis may be communicated to the recipient by the recipient's treating physician. Alternatively, the diagnosis may be sent to a recipient by email or communicated to the subject by phone. The diagnosis may be sent to a recipient by in the form of a report. A computer may be used to communicate the diagnosis by email or phone. In certain embodiments, the message containing results of a diagnostic test may be generated and delivered automatically to the recipient using a combination of computer hardware and software which will be familiar to artisans skilled in telecommunications.

[0110] Aspects of the present invention include computer program products for identifying a subject who has undergone a renal allograft and is at risk for acute rejection, wherein the computer program product, when loaded onto a computer, is configured to employ a gene expression result from a sample derived from the subject to determining whether a subject who has undergone a renal allograft is at risk for allograft rejection wherein the gene expression result comprises expression data for at least one gene signature set.

[0111] Also provided are reference expression profiles for a phenotype that is one of: (a) low risk for acute rejection; or (b) high risk; wherein the expression profile is recorded on a computer readable medium that is accessible by a user, e.g., in a user readable format. In certain embodiments, the expression profile includes. In certain embodiments, the expression profile is a profile for a phenotype that is low risk. In certain embodiments, the expression profile is a profile for a phenotype that is high risk.

[0112] The expression profiles and databases thereof may be provided in a variety of media to facilitate their use. "Media" refers to a manufacture that contains the expression profile information of the present invention. The databases of the present invention can be recorded on computer readable media, e.g. any medium that can be read and accessed directly by a user employing a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. One of skill in the art can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising a recording of the present database information.

[0113] "Recorded" refers to a process for storing information on computer readable medium, using any such methods a known in the art. Any convenient data storage structure may be chosen, based on the means used to access the stored information. A variety of data processor programs and formats can be used for storage, e.g. word processing text file, database format, etc. Thus, the subject expression profile databases are accessible by a user, i.e., the database files are saved in a user-readable format (e.g., a computer readable format, where a user controls the computer).

[0114] As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the information of the present invention. The minimum hardware of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention. The data storage means may comprise any manufacture comprising a recording of the present information as described above, or a memory access means that can access such a manufacture.

[0115] A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention, e.g., to and from a user. One format for an output means ranks expression profiles possessing varying degrees of similarity to a reference expression profile. Such presentation provides a skilled artisan with a ranking of similarities and identifies the degree of similarity contained in the test expression profile.

[0116] The present invention is described below in examples which as intended to further describe the invention without limiting the scope thereof.

[0117] As described in the Examples, below, a molecular signature to predict development/progression of renal allograft acute cellular rejection was discovered. The data demonstrated the use of peripheral mRNA profiling for surveillance and to stratify patients at risk for fibrosis and graft loss, obviating the need for allograft biopsy, and identifying those who may benefit from early interventions to prevent chronic allograft loss.

[0118] In the examples below, the following materials and methods were used:

Example 1: MiSEQ Assay

[0119] 1) Custom Assay (barcoded probesets for 9 gene panel including a housekeeping gene panel)

[0120] 2) Illumina® TruSeq® RNA Sample Preparation Kit v 2

[0121] 3) QIAGEN RNeasy® Kit for extraction of high quality total RNA

[0122] MiSEQ Experiments

[0123] The total RNA will be extracted using QIAGEN RNeasy® Kit. The sequencing library will be generated using the Illumina® TruSeq® RNA Sample Preparation Kit v 2 by following the manufacturer's protocol: briefly, polyA-containing mRNA will be first purified and fragmented from the total RNA. The first-strand cDNAs synthesis will be performed using random hexamer primers and reverse transcriptase and followed by the second strand cDNA synthesis. After the endrepair process which converts the overhangs into blunt ends of cDNAs, multiple indexing adapters will be added to the end of the double stranded cDNA and PCR will be performed to enrich the targets using the primer pairs specific for the gene panel and housekeeping genes. Finally the indexed libraries will validated, normalized and pooled for sequencing on the MiSEQ sequencer.

[0124] MiSEQ Data Processing

[0125] The raw RNAseq data generated by the MiSEQ sequencer will be processed by the following procedure: The reads with good quality will be first aligned to several human reference databases including hg19 human genome, exon, splicing junction and contamination database including ribosome and mitochondria RNA sequences using the BWA[1] alignment algorithm. After filtering reads that mapped to the

contamination database, the reads that are uniquely aligned with a maximal 2 mis-matches to the desired amplicon (i.e. PCR product from the paired primers) regions will be then counted as expression level for the corresponding gene and further subjected to quantile normalization across samples after log 2 transformation using R statistical programs.

Example 2: Nanostring Assay

[0126] 1) Custom CodeSet (barcoded probesets for the 9 gene panel including 3 housekeeping genes and negative controls provided by Nanostring).

[0127] 2) nCounter® Master Kit including nCounter Cartridge, nCounter Plate Pack and nCounter Prep Pack.

[0128] 3) QIAGEN RNeasy® Kit for extraction of high quality total RNA

[0129] Nanostring Experiments:

[0130] Total RNA will be extracted using QIAGEN RNeasy® Kit by following the manufacturer's protocol; Barcode probes will be annealed to the total RNA in solution at 65° C. with the master kit. The capture probe will capture the target to be immobilized for data. After hybridation, the sample will be transferred to the nCounter Pre Station and probe/target will be immobilized on the nCounter Cartridge and the probes are then counted by nCounter Digital Analyzer.

[0131] mRNA Transcriptomic Data Analysis

[0132] The raw count data from Nanostring analyzer will be processed using the following procedure: the raw count data will be first normalized to the count of the housekeeping genes and the mRNAs with counts lower than the median plus 3 standard deviation of the counts of negative controls will be filtered out. Due to data variation arising from the reagent lot, the count for each mRNA from different reagent lots will be calibrated by multiplying a factor of the ratio of the averaged counts of the samples on different reagent lots. The calibrated counts from different experimental batches will be further adjusted the ComBat package.

Example 3: qPCR Assay

[0133] 1) Primer container (16 tubes with one qPCR assay per tube for 12 genes including the 9 gene-panel and 2 housekeeping genes (ACTB and GAPDH) and the control probe 18S ribosomal RNA). The assays will be ordered from LifeTech.

[0134] 2) TaqMan® Universal Master Mix II: reagents for qPCR reactions

[0135] 3) TaqMan® ARRAY 96-WELL PLATE 6×16

[0136] 4) Agilent AffinityScript QPCR cDNA Synthesis Kit: for the highest efficiency conversion of RNA to cDNA and fully optimized for real-time quantitative PCR (QPCR) applications.

[0137] Total RNA will be extracted from the allograft biopsy samples using the Allprep kit (QIAGEN-ALLprep kit, Valencia, Calif. USA). cDNA will be synthesized using the AffinityScript RT kit with oligo dt primers (Agilent Inc. Santa Clara, Calif.). TaqMan qPCR assays for the 9 gene set, 2 housekeeping genes (ACTB, GAPDH) and 18S will be purchased from ABI Life Technology (Grand Island, N.Y.). qPCR experiments will be performed on cDNAs using the TAQMAN universal mix and PCR reactions will be monitored and acquired using an ABI7900HT system. Samples will be measured in triplicate. Cycle Times (CT) values for the prediction gene set as well as the 2 housing genes will be generated. The ΔCT value of each gene will be computed by subtracting the average CT value for the housekeeping genes from the CT value of each gene.

Example 4: Performance of the Assay on Transplant Patients

[0138] Study Population

[0139] Eighty adult renal transplant patients were studied using gene expression profiling to determine the presence of subclinical acute rejection on their biopsy. The recipients were predominantly male (80%) with a mean age of 49.7, ranging from 19 to 75. Recipient race was 55% white, 22.5% African American, 11.25% Asian, and 11.25% Hispanic. Donors were 40% living donor and 60% deceased donor. The average donor age was 41.5 years with a range from 3 to 75 years. Thirty percent of the patients received anti-IL-2 receptor blocker for induction, 27.5% received Thymoglobulin, 27.5% received no induction and 0.5% received Campath-1H. All patients received prednisone, prograf and mycophenolate mofetil.

[0140] Methods

[0141] 10 cc's of peripheral blood was taken from 80 renal transplant recipients post-transplant and stored in a Paxgene tube. Blood is stored in a 4° C. refrigerator; if the assay cannot be performed immediately then the assay can be performed the following day. If the blood is to be stored longer it should be stored at −80° C.

[0142] The MiSEQ assay was performed as described in Example 1 above.

[0143] Prediction Analysis

[0144] The expression data obtained from the MiSEQ assay is then entered in to the computer program product for monitoring a subject who has received an allograft for an acute rejection (AR) response that accompanies the kit. This computer program product, when loaded onto a computer, is configured to employ a gene expression result from the sample derived from each of the subjects to determine a score and provide this ACR score to the user in a user-readable format. The ACR score is based on probability scores derived from reference experiments from which the diagnostic reference ranges have been validated.

[0145] Negative for ACR: ACR probability score below 0.16.

[0146] Positive for ACR: ACR probability score above 0.5.

[0147] The results are shown in Tables 1 and 2 attached hereto.

[0148] The following results are based on quantitation of 7 of 9 genes by MiSEQ assay. The 7 genes are: ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7.

[0149] As shown in the Tables 1, of the 80 patients 28 patients have a score of 0.16 or less and 18 have a score of 0.5 or above. Thirty-two patients are in the intermediate range and therefore categorized as indeterminate.

[0150] Comparison with conventional diagnostic methods, specifically pathology shows that on biopsy only one of the 28 patients diagnosed as no ACR (Group 1) by peripheral blood analysis has any evidence of acute rejection on biopsy (Table). However, this patient has BK nephropathy which causes inflammation on the biopsy from other causes. Patients with this diagnosis are over immunosuppressed and have a different peripheral blood profile than ACR. This data shows that BK inflammation can be differentiated from ACR inflammation on peripheral blood using the claimed assay.

Four of the 18 ACR patients were found to have no ACR on biopsy; however, 2 of these patients went on to have ACR, thus the assay correctly identified them as high risk.

[0151] Of the 32 patients that are intermediate (Group 2) 11 patient are found to have biopsies that are suspicious (n=7) or ACR (n=4).

[0152] Of the 18 patients diagnosed with ACR (Group 3) 4 were diagnosed as having no ACR but 2 of these patients went on to have ACR at a later time point.

[0153] Using a reference range based on these results the assay has a sensitivity and specificity of 1 and 0.875 respectively with an NPV and a PPV of 1 and 0.78, respectively.

[0154] Management of Patients

1. Patients group 1 (ACR score negative), continue with the standard immunosuppression tapering protocol whereby patients are reduced to prednisone 5 mg if patients are still taking it, the prograf target level is reduced to 5-7 mg/dl and mycophenolate mofetil.

2. Patient group 2 (ACR indeterminate), the approach will be determined by the treating physician. Potential approaches include:

[0155] a. Immunsuppression will not be tapered further and patients will have ongoing monitoring and if they test becomes positive they will be treated.

[0156] b. A biopsy will be performed to confirm or rule out the presence of ACR.

3. Patient group 3 (ACR score positive), patients will receive a short course of high dose steroids e.g. 500 mg Prednisone for 3 days. The assay will be repeated one week after the completion of the steroids to determine if the ACR profile is now normal. If it is not a biopsy may be warranted.

Example 5: Primers

[0157] The primer pairs for the 17 genes are as follows:

ANXA5	CAATTTAGAGCAACTACTCCTTGCTGT (SEQ ID NO: 1)	TATTCGAAGTATACCTGCCTACCTTGC (SEQ ID NO: 2)
ANXA5	ATGAAGCTCAAGTTGAACAAGATGCTC (SEQ ID NO: 3)	AGAACTTAAATGGGGGACAGATGAAGA (SEQ ID NO: 4)
AP1M1	GTTCGAGCTCATGTCCTACCGTCTCAA (SEQ ID NO: 5)	CCTTTGATATGGATCGAGTCGGTGATC (SEQ ID NO: 6)
AP1M1	CCACAGCCGCATCGAGTACATGATCAA (SEQ ID NO: 7)	CAAAAGCCAGTTCAAGCGGCGGTCAA (SEQ ID NO: 8)
C1GALT1C1	GCATGTGATGATGTATGGGGTATACCG (SEQ ID N0:9)	GGGCATTTGGGCATATTTTCAATGATG (SEQ ID NO: 10)
C1GALT1C1	CCTGAAATATGCTGGAGTATTTGCAGA (SEQ ID NO: 11)	TGCAGAAGATGCTGATGGAAAAGATG (SEQ ID NO: 12)
CCDC82	CAATGACAGAAGAAGTTGAAGATGAAC (SEQ ID NO: 13)	AGAAACAGTGGAAAGAATTTTCAGGCG (SEQ ID NO: 14)
CCDC82	TTCTCTTTCAAATAGATTTCAGGCCTC (SEQ ID NO: 15)	TCAACCCTCACATTCAGGAATAATTTT (SEQ ID NO: 16)
CLK1	GGACCTCTACCAAAACATATGATACAG (SEQ ID NO: 17)	ATTTTCACCACGATCGATTAGACTGGG (SEQ ID NO: 18)
CLK1	TCTGACTACACAGAGGCGTATAATCCC (SEQ ID NO: 19)	TGATGAACGCACCTTAATAAATCCAGA (SEQ ID NO: 20)
EFTUD2	AATTCATGATCAAAACCCGCCGTAGG (SEQ ID NO: 21)	GAGCATCAGCAAATTCTTCGATGATCC (SEQ ID NO: 22)
EFTUD2	AACCATAACCGAACCCCGAGGCAATGA (SEQ ID NO: 23)	GACCCTTGAAGTTCAATACCACATCTG (SEQ ID NO: 24)
ETAA1	TGGGAAAACTTACTAGGTAGTGAACCT (SEQ ID NO: 25)	AAATATCGACATGCCTGAACTCTTTCC (SEQ ID NO: 26)
ETAA1	AGTAACCCAAATCAGACTAGTGCATCA (SEQ ID NO: 27)	TCTTTGATGATTGGAATGATCCCTCAT (SEQ ID NO: 28)
F13A1	CCAATTTGATGCACCTTTTGTTTTTGC (SEQ ID NO: 29)	GCGACCTCATTTACATTACAGCTAAGA (SEQ ID NO: 30)
F13A1	TGGAGTAACAAGACCAATGAAGAAGAT G SEQ ID NO: 31)	AACTCCACCGTGCAGTGGGAAGAAGT (SEQ ID NO: 32)
MAP1A	GAAAAAGACAAGGCCCTGGAACAGAA (SEQ ID NO: 33)	AAGATTCCAGAAGAGAAAGACAAAGCC (SEQ ID NO: 34)
MAP1A	CTGAAGGCAGAGAAGCGAAAGCTGAT (SEQ ID NO: 35)	CAAGGTAGGGAAAAAGCACCTTAAAGA (SEQ ID NO: 36)

-continued

NFYB	CCTCTGAAATTATACCTTCAGAAATTCAG (SEQ ID NO: 37)	CTATGAAAGGAGAAAAGGGAATTGGTGG (SEQ ID NO: 38)
NFYB	TGTTATGGTTTACACAACATCATATCAAC (SEQ ID NO: 39)	TCTGGTGTTCAGCAAATTCAGTTTTCA (SEQ ID NO: 40)
SENP6	GGCATTTAAAGCCTACTATCTGTAAAC (SEQ ID NO: 41)	TATCCTACTTATGGACTCACTCCGAGG (SEQ ID NO: 42)
SENP6	TGAGAAGGATTTTATTTTTGTACCCCT (SEQ ID NO: 43)	CACTGGTTTTTGGCTGTTGTTTGTTTC (SEQ ID NO: 44)
SENP7	GACACTGTCTTTGAGTGCAGAGGATT (SEQ ID NO: 45)	GTACCGAGTCGAATATGTCAGTACCAA (SEQ ID NO: 46)
SENP7	ATTAGAACACTCTGTATTAAGCCAGCA (SEQ ID NO: 47)	TCATTTTCCTTGAACTACACAATCCTG (SEQ ID NO: 48)
SPCS3	TTATTCCATTTGTCTTAGGAAGGCCCA (SEQ ID NO: 49)	TTACATGTGACTAGCAACTTTCTCCAC (SEQ ID NO: 50)
SPCS3	GTCAAAGCTTAAAAATCAGGTGTGTCC (SEQ ID NO: 51)	GATAAGCCTGCAGTCTTAACCAGACCT (SEQ ID NO: 52)
TSC22D1	ACTGTGCCTCTTTCTTCTCAAACAATG (SEQ ID NO: 53)	TGAGAGTGACAAAATGGTGACAGGTAG (SEQ ID NO: 54)
TSC22D1	TCACCCATTTCATTGCTCGCTGCGAAA (SEQ ID NO: 55)	GTGAGACTGACATATGCCATTATCTCT (SEQ ID NO: 56)
TUBB1	GTTCTGTCTATCCACCAGCTGATTGAG (SEQ ID NO: 57)	ATGCCTGTTTCTGCATTGACAATGAGG (SEQ ID NO: 58)
TUBB1	AATACCTGGTCCAAACAAGAAAAACAA (SEQ ID NO: 59)	GACAAGCAAAACTAAAGAACTGCAGTC (SEQ ID NO: 60)
ZMAT1	ACCTAGTCATTCAAAGTAGAAACCCAC (SEQ ID NO: 61)	TGCTTTCAGCTTTTATCCTGAGAGTGG (SEQ ID NO: 62)
ZMAT1	CACATGCAAGGAAGTGAACATCAAATT (SEQ ID NO: 63)	ATCTAGTGAAGAATTCAAGGAAGACAC (SEQ ID NO: 64)
ZNF493	AGCCTTTAGTATTTTCTCAACCCCTAC (SEQ ID NO: 65)	TCACACTGAAGAGAAATCCCACAGATG (SEQ ID NO: 66)
ZNF493	CAGTCCTCAACTCCTAGTAAACATAAT (SEQ ID NO: 67)	AAACCATACAACTGTGAAGAATGTGGC (SEQ ID NO: 68)

[0158] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims

[0159] It is further to be understood that all values are approximate, and are provided for description. Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

TABLE 1

7 genes mapped			
Array ID	prob	AR_3m_time_point	ANXA5
P16637	0.017452	0	TSC22D 1
P6610	0.024695	0	APlMl

TABLE 1-continued

7 genes mapped			
Array ID	prob	AR_3m_time_point	ANXA5
P41684	0.035203	0	CLKl
P12351	0.036215	0	EFTUD2
P36378	0.039165	0	SENP6
P6485	0.041505	0	SENP7
P29934	0.049993	0	
P12683	0.051149	0	
P20741	0.052609	0	
P18463	0.062466	0	
P12673	0.067403	0	
P29561	0.068859	0	
P15313	0.0694	0	
P17064	0.077704	0	
P6646	0.084929	0	
P21093	0.108601	0	
P19792	0.116445	0	
P30609	0.119677	0	
P44056	0.120967	0	
P15762	0.121857	0	
P22987	0.123687	0	

TABLE 1-continued

7 genes mapped				
Array ID	prob	AR_3m_time_point		ANXA5
P27071	0.12519	0		
P7764	0.128861	0		
P24797	0.130973	0		
P17145	0.146718	0		
P8751	0.14781	0		
P24369	0.160478	0	Specificity	0.875
P20891	0.168029	0	NPV	1
P18496	0.170695	0		
P39362	0.175342	1		
P14029	0.17648	1		
P6491	0.181622	1		
P8403	0.193804	0		
P16828	0.199011	0		
P19996	0.203301	0		
P42810	0.206721	0		
P36244	0.208747	1		
P14212	0.220862	0		
P14307	0.229719	0		
P14768	0.232455	1		
P12616	0.237291	0		
P35866	0.249239	0		
P16865	0.265036	0		
P41082	0.300904	1		
P33638	0.302312	1		
P6650	0.308944	0		
P15063	0.323717	0		
P27250	0.331253	0		
P28901	0.351753	0		
P44124	0.353624	0		
P36051	0.419688	0		

TABLE 1-continued

7 genes mapped				
Array ID	prob	AR_3m_time_point		ANXA5
P44629	0.424203	0		
P21504	0.439369	0		
P21990	0.449395	1		
P39437	0.455526	1		
P22526	0.457334	0		
P43535	0.462036	1		
P27398	0.463876	1		
P20787	0.464346	1		
P12599	0.474293	0		
P35241	0.496918	0		
P7145	0499596	0		
P21279	0.524475	1	Sensitivity	1
P26670	0.591051	0	PPV	0.78
P20593	0.613555	1		
P43146	0.615636	1		
P41240	0.630852	0		
P21968	0.63729	1		
P14608	0.656409	1		
P7149	0.686441	1		
P34400	0.741234	0		
P30944	0.766445	1		
P21713	0.772967	1		
P32937	0.78113	1		
P6607	0.78321	1		
P41626	0.797169	0		
P43144	0.882298	1		
P28942	0.920221	1		
P44035	0.941066	1		
P33263	0.945597	1		

TABLE 2

Participants ID	Probability Score	ACR at M3	BAFF ACR Type	Age	Gender	Race
16637	0.017451688	No	None	41	Male	Hispanic
6610	0.024694693	No	None	28	Male	White/Caucasian
41684	0.035203116	No	None	32	Male	White/Caucasian
12351	0.036214623	No	None	29	Male	Pacific Islander
36378	0.039165316	No	None	62	Male	Asian
6485	0.041505122	No	None	68	Female	Hispanic
29934	0.049993206	No	None	47	Male	Black or African American
12683	0.051148938	No	None	69	Male	White/Caucasian
20741	0.052609243	No	None	58	Female	Hispanic
18463	0.062455375	No	None	37	Female	Hispanic
12673	0.067403192	No	None	68	Male	White/Caucasian
29561	0.068859041	No	None	70	Female	White/Caucasian
15313	0.069400299	No	None	41	Female	Black or African American
17064	0.077704169	No	None	72	Female	Unknown or Not Reported
6646	0.084929087	No	None	41	Female	White/Caucasian
21093	0.108600537	No	None	28	Male	Hispanic
19792	0.116445383	No	None	40	Female	White/Caucasian
30609	0.119676671	No	None	42	Female	White/Caucasian
44056	0.120967164	No	None	66	Male	Asian
15762	0.9121856593	No	None	61	Male	Black or African American
22987	0.123686939	No	None	61	Male	White/Caucasian
27071	0.125189794	No	None	21	Female	White/Caucasian
7764	0.128860617	No	None	63	Male	Black or African American
24797	0.130973269	No	None	54	Male	White/Caucasian
17145	0.146717843	No	None	66	Female	Black or African American
8751	0.147809602	No	None	65	Male	Black or African American
24369	0.160477933	No	None	29.8	Female	White/Caucasian
20891	0.168028954	No	None	43	Male	Hispanic

TABLE 2-continued

18496	0.170695322	Yes	Suspicious	31	Female	Black or African American
39362	0175341677	Yes	2A	41	Male	Black or African American
14029	0.176479641	Yes	1B	41	Male	White/Caucasian
6491	0.181621512	No	None	44	Male	White/Caucasian
8403	0.193804199	No	None	71	Male	Hispanic
16828	0.199010826	No	None	72	Male	Black or African American
19996	0.203301439	No	None	66	Female	Black or African American
42810	0.206720744	No	None	28	Female	White/Caucasian
36244	0.208764518	Yes	Suspicious	60	Male	White/Caucasian
14212	0.220862475	No	None	34	Male	Asian
14307	0.229719188	No	None	60	Female	White/Caucasian
14768	0.232454702	Yes	1A	66	Male	White/Caucasian
12616	0.237291459	No	None	67	Female	Hispanic
35866	0.249239272	No	None	36	Male	White/Caucasian
16865	0.265036479	No	None	49	Male	White/Caucasian
41082	0.300903823	Yes	Suspicious	39	Female	Pacific Islander
33638	0.302311711	Yes	Suspicious	58	Male	Black or African American
6650	0.30894397	No	None	19	Male	White/Caucasian
15063	0.323717452	No	None	38	Male	Black or African American
27250	0.331253148	No	None	52	Female	Black or African American
28901	0.35175294	No	None	43	Male	White/Caucasian
44124	0.353624404	No	None	64	Male	Pacific/Islander
36051	0.419687549	No	None	65	Male	White/Caucasian
44629	0.424202859	No	None	47	Male	White/Caucasian
21504	0.439368688	No	None	59	Female	White/Caucasian
21990	0.449395338	Yes	1A	58	Male	White/Caucasian
39437	0.455525639	Yes	Suspicious	61	Male	Asian
22526	0.457334127	No	None	41	Male	Black or African American
43535	0.462035743	Yes	Suspicious	29	Female	Asian
27398	0.463875597	Yes	Suspicious	37	Female	White/Caucasian
20787	0.464346411	Yes	1B	67	Female	Black or African American
12599	0.474293044	No	None	63	Male	Black or African American
35241	0.496918307	No	None	65	Male	White/Caucasian
7145	0.499595501	No	None	50	Male	Pacific/Islander
21279	0.524475479	Yes	Suspicious	42	Male	Black or African American
26670	0.591050623	No	None	48	Male	White/Caucasian
20593	0.613554651	Yes	2A	41	Female	White/Caucasian
43146	0.615635695	Yes	1A	42	Female	Asian
41240	0.630852103	No	None	61	Male	White/Caucasian
21968	0.637290262	Yes	Suspicious	24	Female	Hispanic
14608	0.656408931	Yes	Suspicious	37	Male	White/Caucasian
7149	0.686440543	Yes	Suspicious	35	Female	White/Caucasian
34400	0.741234395	No	None	66	Male	White/Caucasian
30944	0.766444547	Yes	Suspicious	43.4	Male	Asian
21713	0.772966899	Yes	2A	68	Male	Asian
32937	0.7311295	Yes	Suspicious	53	Female	Black or African American
6607	0.783210207	Yes	Suspicious	42	Male	Asian
41626	0.797168871	No	None	42	Male	White/Caucasian
43144	0.882297725	Yes	Suspicious	67	Male	White/Caucasian
28942	0.920220945	Yes	Suspicious	42	Female	White/Caucasian
44035	0.941065679	Yes	Suspicious	64	Female	White/Caucasian
33263	0.945596684	Yes	Suspicious	32	Male	White/Caucasian

Donor Age	Donor Gender	Donor Race	Deceased/ Living	Immunosuppression
43	Male	Hispanic	LURD	None
52	Male	White/Caucasian	LRD	None
33	Male	White/Caucasian	CadavericS	Basiliximab
27	Female	White.Caucasian	LURD	None
63	Female	Other	CadavericS	Thymoglobulin
20	Male	White/Caucasian	CadavericS	Basiliximab
49	Male	White/Caucasian	CadavericS	Basiliximab
54	Male	Hispanic	CadavericS	None
43	Female	Hispanic	LRD	Thymoglobulin

TABLE 2-continued

35	Male	Hispanic	LURD	None
54	Male	Hispanic	CadavericS	None
48	Female	White/Caucasian	LRD	None
20	Male	Black or African/	LRD	Basiliximab
69	Male	White/Caucasian	CadavericS	Thymoglobulin
26	Female	White/Caucasian	CadavericS	None
12	Male	Hispanic	CadavericS	Campath-1
42	Female	White/Caucasian	LRD	None
20	Female	White/Caucasian	LURD	Thymoglobulin
45	Male	White/Caucasian	CadavericS	Thymoglobulin
29	Male	White/Caucasian	CadavericS	Thymoglobulin
38	Male	Black or African/	CadavericS	Campath-1
16	Female	White/Caucasian	CadavericS	Thymoglobulin
44	Female	White/Caucasian	CadavericS	None
24	Male	White/Caucasian	CadavericS	Campath-1
66	Male	Black or African/	LURD	Thymoglobulin
68	Female	White/Caucasian	CadavericS	Thymoglobulin
37	Female	White/Caucasian	CadavericS	Basiliximab
35	Male	Hispanic	LRD	None
27	Male	Black or African/	CadavericS	Daclizumab
20	Male	White/Caucasian	CadavericS	Thymoglobulin
24	Female	White/Caucasian	CadavericS	Basiliximab
17	Male	White/Caucasian	CadavericS	Daclizumab
16	Female	White/Caucasian	CadavericS	None
59	Male	White/Caucasian	CadavericS	Campath-1
36	Female	White/Caucasian	CadavericS	Thymoglobulin
16	Female	White/Caucasian	CadavericS	Basiliximab
47	Female	White/Caucasian	LRD	Basiliximab
67	Male	Asian	LRD	Basiliximab
19	Male	White/Caucasian	CadavericS	None
65	Female	White/Caucasian	LURD	Basiliximab
55	Female	White/Caucasian	CadavericS	Thymo/IVG
22	Female	White/Caucasian	CadavericS	None
39	Female	White/Caucasian	CadavericS	Campath-1
53	Female	White/Caucasian	CadavericS	Basiliximab
58	Male	White/Caucasian	CadavericS	Basiliximab
51	Female	White/Caucasian	LRD	None
3	Female	White/Caucasian	CadavericS	Thymoglobulin
41	Male	White/Caucasian	CadavericS	Thymoglobulin32
32	Female	White/Caucasian	CadavericS	Basiliximab
63	Male	White/Caucasian	CadavericS	Basiliximab
56	Male	White/Caucasian	LURD	None
38	Female	White/Caucasian	LRD	Basiliximab
66	Male	White/Caucasian	LURD	Basiliximab
57	Female	White/Caucasian	LURD	Basiliximab
64	Female	Asian	LURD	Basiliximab
16	Male	Hispanic	CadavericS	Campath-1
17	Male	White/Caucasian	CadavericS	Basiliximab
30	Male	White/Caucasian	LURD	Basiliximab
55	Male	White/Caucasian	CadavericS	Thymoglobulin
55	Female	White/Caucasian	CadavericS	Thymoglobulin
57	Female	White/Caucasian	CadavericS	None
39	Female	White/Caucasian	CadavericS	None
36	Male	Black or African	LRD	Thymo/IVG
9	Male	White/Caucasian	CadavericS	None
47	Male	Black or African	CadavericS	Thymo/IVG
30	Male	White/Caucasian	CadavericS	Basiliximab
57	Male	White/Caucasian	CadavericS	Basiliximab
48	Female	Hispanic	CadavericS	Thymoglobulin
34	Female	White/Caucasian	CadavericS	Basiliximab
64	Male	White/Caucasian	CadavericS	Basiliximab
63	Male	White/Caucasian	LRD	Thymoglobulin
44	Female	Asian	LURD	None
58	Female	White/Caucasian	CadavericS	None
50	Male	Black or African	CadavericS	Thymoglobulin
75	Female	White/Caucasian	LURS	None
42	Female	White/Caucasian	LURD	Basiliximab
65	Female	White/Caucasian	LRD	Basiliximab
30	Female	White/Caucasian	LRD	Basiliximab
52	Male	White/Caucasian	CadavericS	Basiliximab
26	Female	White/Caucasian	LURD	Thymoglobulin

SEQUENCE LISTING

Sequence total quantity: 68

SEQ ID NO: 1 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 1
caatttagag caactactcc ttgctgt 27

SEQ ID NO: 2 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 2
tattcgaagt atacctgcct accttgc 27

SEQ ID NO: 3 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 3
atgaagctca agttgaacaa gatgctc 27

SEQ ID NO: 4 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 4
agaacttaaa tgggggacag atgaaga 27

SEQ ID NO: 5 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 5
gttcgagctc atgtcctacc gtctcaa 27

SEQ ID NO: 6 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 6
cctttgatat ggatcgagtc ggtgatc 27

SEQ ID NO: 7 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 7
ccacagccgc atcgagtaca tgatcaa 27

SEQ ID NO: 8 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides

-continued

source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 8
caaaagccag ttcaagcggc ggtcaa 26

SEQ ID NO: 9 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 9
gcatgtgatg atgtatgggg tataccg 27

SEQ ID NO: 10 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 10
gggcatttgg gcatattttc aatgatg 27

SEQ ID NO: 11 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 11
cctgaaatat gctggagtat ttgcaga 27

SEQ ID NO: 12 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 12
tgcagaagat gctgatggaa aagatg 26

SEQ ID NO: 13 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 13
caatgacaga agaagttgaa gatgaac 27

SEQ ID NO: 14 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 14
agaaacagtg gaaagaattt tcaggcg 27

SEQ ID NO: 15 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 15
ttctctttca aatagatttc aggcctc 27

-continued

SEQ ID NO: 16 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 16
tcaaccctca cattcaggaa taatttt 27

SEQ ID NO: 17 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 17
ggacctctac caaaacatat gatacag 27

SEQ ID NO: 18 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 18
attttcacca cgatcgatta gactggg 27

SEQ ID NO: 19 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 19
tctgactaca cagaggcgta taatccc 27

SEQ ID NO: 20 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 20
tgatgaacgc accttaataa atccaga 27

SEQ ID NO: 21 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 21
aattcatgat caaaacccgc cgtagg 26

SEQ ID NO: 22 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 22
gagcatcagc aaattcttcg atgatcc 27

SEQ ID NO: 23 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA

-continued

	organism = synthetic construct
SEQUENCE: 23	
aaccataacc gaaccccgag gcaatga	27
SEQ ID NO: 24	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 24	
gacccttgaa gttcaatacc acatctg	27
SEQ ID NO: 25	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 25	
tgggaaaact tactaggtag tgaacct	27
SEQ ID NO: 26	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 26	
aaatatcgac atgcctgaac tctttcc	27
SEQ ID NO: 27	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 27	
agtaacccaa atcagactag tgcatca	27
SEQ ID NO: 28	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 28	
tctttgatga ttggaatgat ccctcat	27
SEQ ID NO: 29	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 29	
ccaatttgat gcacctttg ttttgc	27
SEQ ID NO: 30	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 30	
gcgacctcat ttacattaca gctaaga	27
SEQ ID NO: 31	moltype = DNA length = 28
FEATURE	Location/Qualifiers

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misc_feature 1..28
note = synthetically generated oligonucleotides
source 1..28
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 31
tggagtaaca agaccaatga agaagatg 28

SEQ ID NO: 32 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 32
aactccaccg tgcagtggga agaagt 26

SEQ ID NO: 33 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 33
gaaaaagaca aggccctgga acagaa 26

SEQ ID NO: 34 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 34
aagattccag aagagaaaga caaagcc 27

SEQ ID NO: 35 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 35
ctgaaggcag agaagcgaaa gctgat 26

SEQ ID NO: 36 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 36
caaggtaggg aaaaagcacc ttaaaga 27

SEQ ID NO: 37 moltype = DNA length = 29
FEATURE Location/Qualifiers
misc_feature 1..29
note = synthetically generated oligonucleotides
source 1..29
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 37
cctctgaaat tataccttca gaaattcag 29

SEQ ID NO: 38 moltype = DNA length = 28
FEATURE Location/Qualifiers
misc_feature 1..28
note = synthetically generated oligonucleotides
source 1..28
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 38

-continued

ctatgaaagg agaaaaggga attggtgg 28

SEQ ID NO: 39 moltype = DNA length = 29
FEATURE Location/Qualifiers
misc_feature 1..29
note = synthetically generated oligonucleotides
source 1..29
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 39
tgttatggtt tacacaacat catatcaac 29

SEQ ID NO: 40 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 40
tctggtgttc agcaaattca gttttca 27

SEQ ID NO: 41 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 41
ggcatttaaa gcctactatc tgtaaac 27

SEQ ID NO: 42 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 42
tatcctactt atggactcac tccgagg 27

SEQ ID NO: 43 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 43
tgagaaggat tttatttttg tacccct 27

SEQ ID NO: 44 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 44
cactggtttt tggctgttgt ttgtttc 27

SEQ ID NO: 45 moltype = DNA length = 26
FEATURE Location/Qualifiers
misc_feature 1..26
note = synthetically generated oligonucleotides
source 1..26
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 45
gacactgtct ttgagtgcag aggatt 26

SEQ ID NO: 46 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides

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source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 46
gtaccgagtc gaatatgtca gtaccaa 27

SEQ ID NO: 47 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 47
attagaacac tctgtattaa gccagca 27

SEQ ID NO: 48 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 48
tcattttcct tgaactacac aatcctg 27

SEQ ID NO: 49 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 49
ttattccatt tgtcttagga aggccca 27

SEQ ID NO: 50 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 50
ttacatgtga ctagcaactt tctccac 27

SEQ ID NO: 51 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 51
gtcaaagctt aaaaatcagg tgtgtcc 27

SEQ ID NO: 52 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 52
gataagcctg cagtcttaac cagacct 27

SEQ ID NO: 53 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 53
actgtgcctc tttcttctca aacaatg 27

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SEQ ID NO: 54 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 54
tgagagtgac aaaatggtga caggtag 27

SEQ ID NO: 55 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 55
tcacccattt cattgctcgc tgcgaaa 27

SEQ ID NO: 56 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 56
gtgagactga catatgccat tatctct 27

SEQ ID NO: 57 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 57
gttctgtcta tccaccagct gattgag 27

SEQ ID NO: 58 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 58
atgcctgttt ctgcattgac aatgagg 27

SEQ ID NO: 59 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 59
aatacctggt ccaaacaaga aaaacaa 27

SEQ ID NO: 60 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 60
gacaagcaaa actaaagaac tgcagtc 27

SEQ ID NO: 61 moltype = DNA length = 27
FEATURE Location/Qualifiers
misc_feature 1..27
note = synthetically generated oligonucleotides
source 1..27
mol_type = other DNA

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	organism = synthetic construct
SEQUENCE: 61	
acctagtcat tcaaagtaga aacccac	27
SEQ ID NO: 62	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 62	
tgctttcagc ttttatcctg agagtgg	27
SEQ ID NO: 63	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 63	
cacatgcaag gaagtgaaca tcaaatt	27
SEQ ID NO: 64	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 64	
atctagtgaa gaattcaagg aagacac	27
SEQ ID NO: 65	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 65	
agcctttagt attttctcaa cccctac	27
SEQ ID NO: 66	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 66	
tcacactgaa gagaaatccc acagatg	27
SEQ ID NO: 67	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 67	
cagtcctcaa ctcctagtaa acataat	27
SEQ ID NO: 68	moltype = DNA length = 27
FEATURE	Location/Qualifiers
misc_feature	1..27
	note = synthetically generated oligonucleotides
source	1..27
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 68	
aaaccataca actgtgaaga atgtggc	27

What is claimed is:

1. A method for identifying a renal allograft recipient at risk of allograft rejection comprising the steps of:

(a) providing a blood specimen from the renal allograft recipient;

(b) determining the expression levels of a preselected gene set in the blood of the recipient;

(c) comparing the expression levels of the preselected genes with the expression levels the preselected genes in a control; and

(d) determining the recipient is at risk for allograft rejection if the expression level of one or more genes in the gene set in the specimen is altered from the expression level of the same one or more gene set genes in the control.

2. The method of claim **1** wherein the alteration comprises an increase and/or decrease in the expression level of one or more of the gene set genes in the specimen compared to the same one or more gene set genes in the control.

3. The method of claim **1** wherein the preselected gene set comprises at least 7 genes selected from the group consisting of SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

4. The method of claim **1** wherein the determining step comprises applying the expression levels determined in the patient's sample to a penalized logistic regression fitting model (log (p(x))/(1−p(x))=β*0+β*1g1+β*igi+ . . . +β*9g9 (where (p(x) is the probability of rejection β*i is penalized coefficiency and gi is the read count of gene i) to determine the probability of allograft rejection.

5. A method for treating a renal allograft recipient at risk of rejection of the allograft comprising the steps of:

(a) obtaining a blood specimen from the renal allograft recipient;

(b) isolating mRNA from the blood specimen;

(c) synthesizing cDNA from the miRNA;

(d) determining the expression levels of a signature gene set in said recipient's blood;

(e) diagnosing the allograft recipient as being at high risk for allograft rejection and allograft loss if the expression level of one or more genes in the gene signature set in the allograft recipient's blood specimen is altered compared to the expression level of the same one or more genes in the control blood specimen and treating the recipient identified as being at high risk to prevent allograft rejection or allograft loss.

6. The method of claim **5** comprising administering an anti-rejection drug or a high dose steroid to the recipient diagnosed as being high risk for ACR or graft loss.

7. The method of claim **2** wherein the gene signature set comprises at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7.

8. The method of claim **2** wherein the gene signature set comprises at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7.

9. The method of claim **2** wherein the gene signature set comprises at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1.

10. The method of claim **2** wherein the gene signature set comprises at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

11. The method of claim **5**, wherein the anti-rejection drug is an immunosuppressive or anti-proliferative agent.

12. The method of claim **11**, wherein the immunosuppressive agent is a member selected from the group consisting of a calcineurin inhibitor (CNI) (e.g., cyclosporine or tacrolimus), mycophenolate mofetil (MMF), sirolimus, prednisone, Mycophenolate Mofetil, Mycophenolate Sodium and Azathioprine.

13. The method of claim **1** wherein the expression levels are determined by a method selected from the group consisting of Nanostring, MiSEQ and quantitative polymerase chain reaction (qPCR).

14. A kit for identifying renal allograft recipients suffering from subclinical and clinical acute rejection and at risk for allograft loss comprising in one or more separate containers primer pairs for the preselected signature set, SEQ ID NOs: 1-34, buffers, positive and negative controls and instructions for use.

15. The kit of claim **14** wherein said signature set comprises at least the genes ANXA5, TSC22D1, AP1M1, CLK1, EFTUD2, SENP6, and SENP7.

16. The kit of claim **14** wherein the gene signature set comprises at least the genes TSC22D1, ANKA5, EFTUD2, AP1M1, MAP1A, C1GALT1C1, SENP6, CLK1 and SENP7.

17. The kit of claim **14** wherein the gene signature set comprises at least the genes CCDC82, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, and C1GALT1C1.

18. The kit of claim **14** wherein the gene signature set comprises at least the genes SPCS3, ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, F13A1, TUBB1, TSC22D1, SENP6, ANXA5, EFTUD2, SENP7, AP1M1, CLK1, MAP1A and C1GALT1C1.

19. The kit of claim **14** further comprising housekeeping genes and primers for the housekeeping genes.

20. The method of claim **5** further comprising calculating the probability score of acute rejection for said patient using the equation:

$$\log (p(x))/(1-p(x))=\beta*0+\beta*1g1+\beta*igi+ \ldots +\beta*4g4$$

wherein (p(x) is the probability of developing fibrosis, β*i is the penalized coefficiency and gi is the expression value of gene i.

21. The method of claim **20** wherein the probability score is determined using a computer based system.

22. The method of claim **20** wherein the probability score is used to determine the cut off value.

23. The method for selecting a renal allograft recipient for treatment to reduce the risk of renal allograft rejection which comprises:

(a) providing a blood specimen from the renal allograft recipient;

(b) determining the expression levels of a preselected gene set in the specimen;

(c) comparing the expression levels of the preselected gene set genes in the specimen with the expression levels of the preselected gene set genes in a control, and

(d) selecting the recipient for treatment for allograft rejection if the expression level of one or more genes in

the gene set in the specimen is altered compared to the expression level of one or more of the gene set genes in the control.

24. The method of selecting a renal allograft patient for treatment to reduce the risk of renal allograft rejection or allograft loss which comprises:

comparing the expression level of a preselected gene set obtained from the patient with the expression level of the preselected gene set in a control sample obtained from an allograft recipient that did not suffer allograft rejection, and selecting the patient for treatment for allograft rejection or loss if the expression level of one or more genes in the preselected gene set from the patient is altered compared to the expression level of one or more of the preselected gene set genes in the control.

* * * * *