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(54) **METHODS AND COMPOSITIONS FOR EVALUATING GRAFT SURVIVAL IN A SOLID ORGAN TRANSPLANT RECIPIENT**

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(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Methods are provided for evaluating a subject for graft survival, e.g., in terms of predicting graft survival, identifying the presence of a deleterious graft condition, such as CAN and DT, identifying the severity and class of acute rejection, etc, in a subject are provided. In practicing the subject methods, the expression of at least one gene in a sample from the subject, e.g., a blood or biopsy sample, is assayed, e.g., at the nucleic acid and/or protein level, to evaluate the subject. Also provided are compositions, systems and kits that find use in practicing the subject methods. The methods and compositions find use in a variety of applications.

14 Claims, 4 Drawing Sheets

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FIG. 1

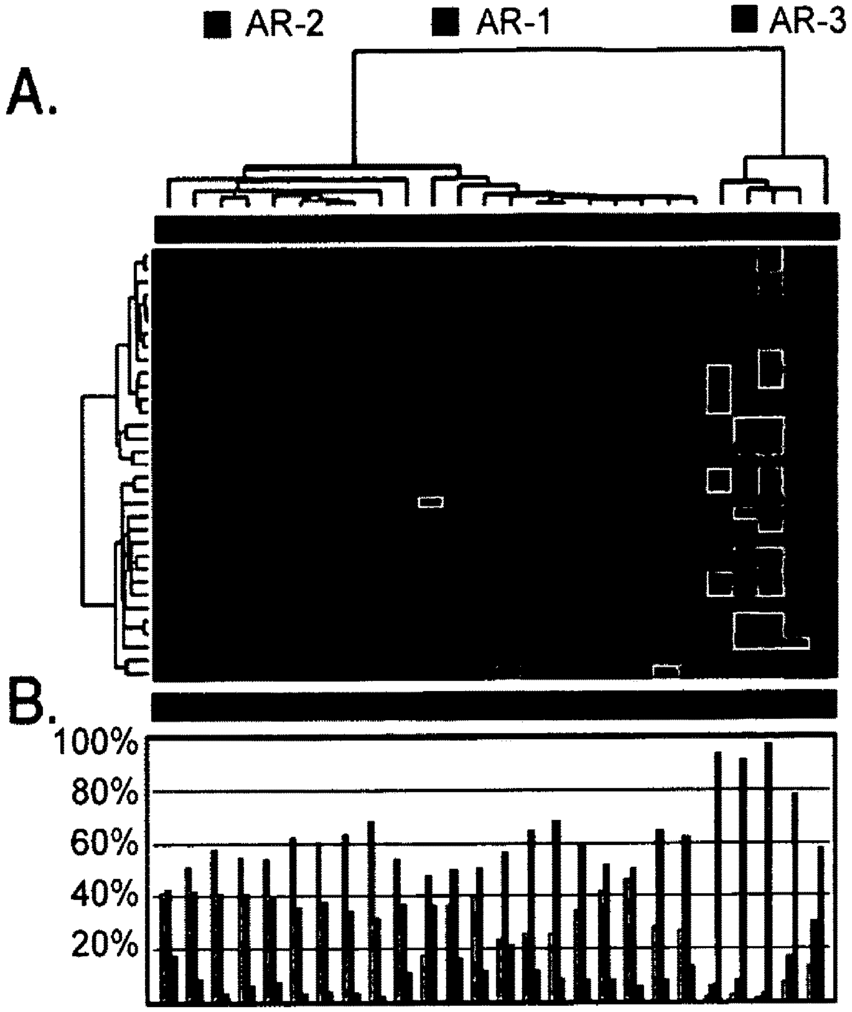
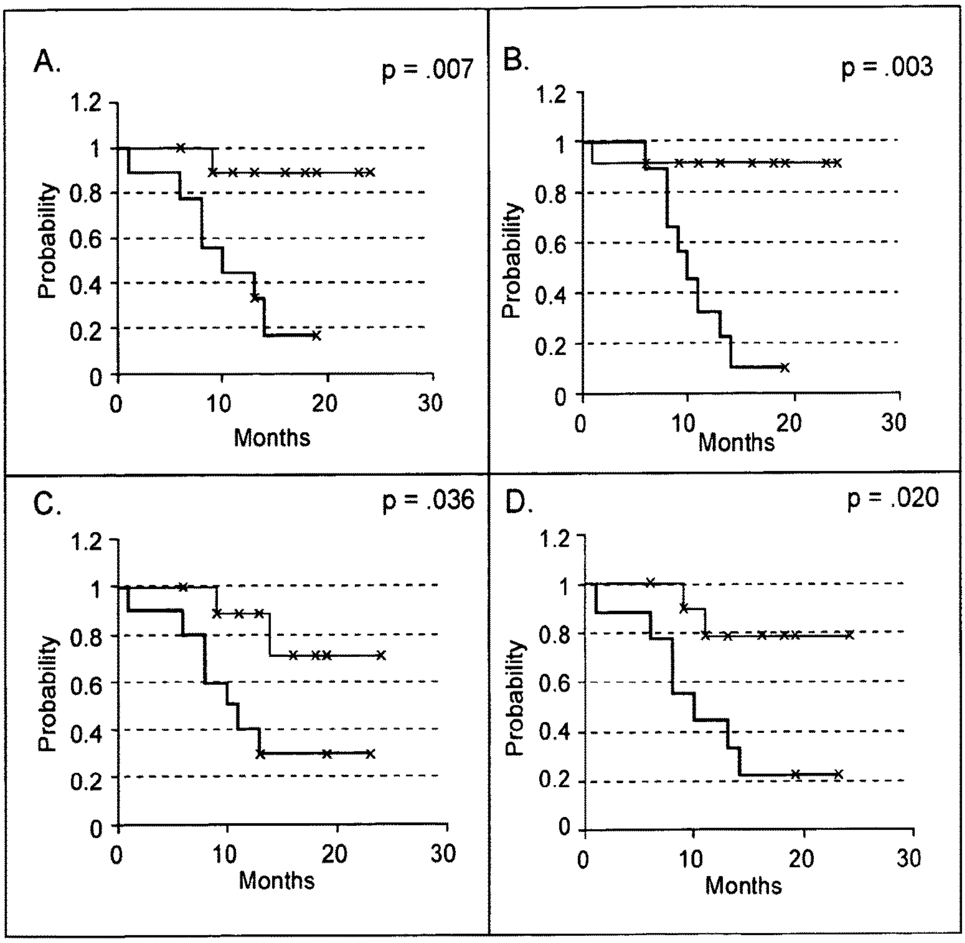


FIG. 2


A.
p = .007
Probability
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
Months
B.
p = .003
Probability
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
Months
C.
p = .036
Probability
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
Months
D.
p = .020
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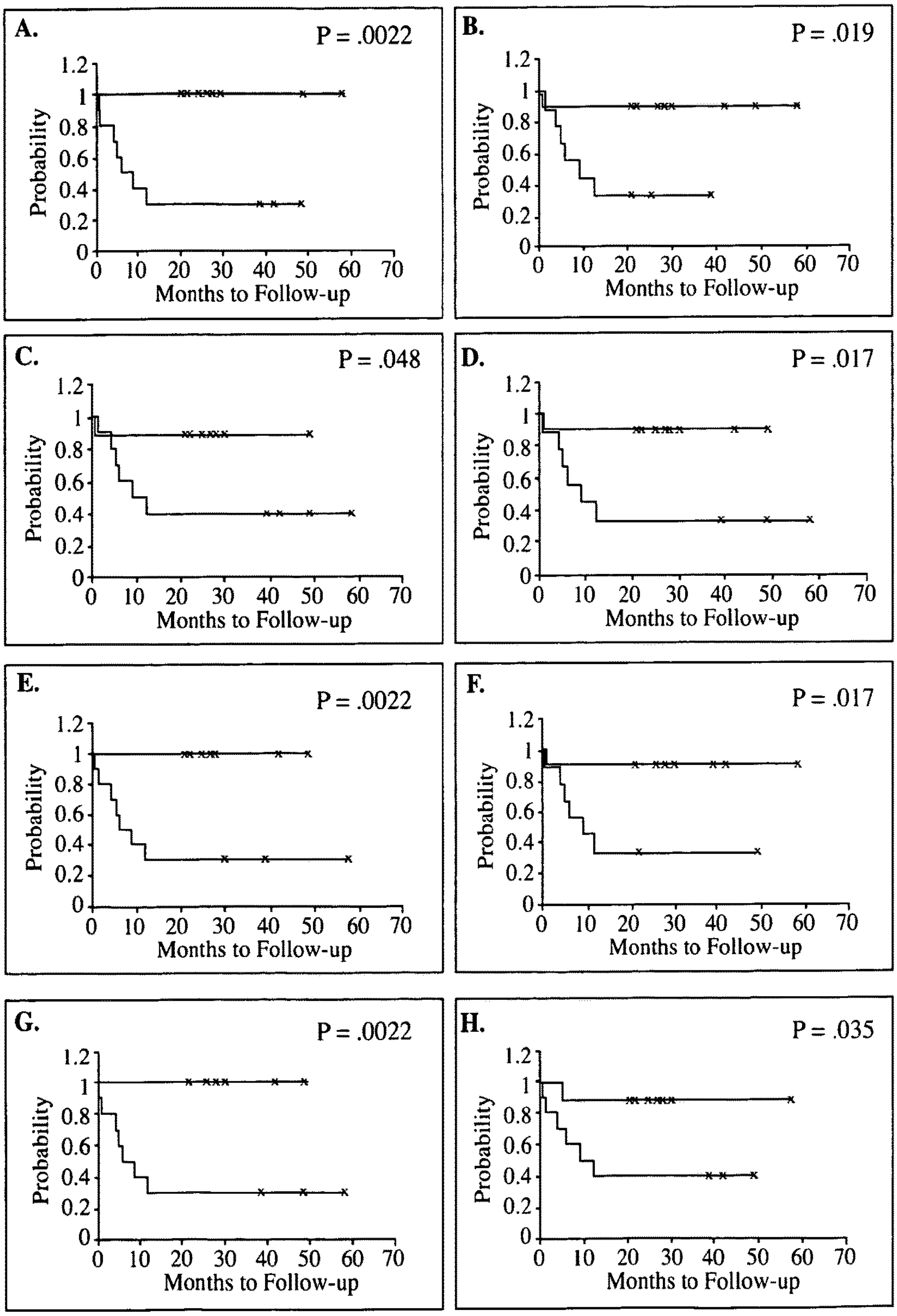
20
30
Months

FIG. 3


A.
P = .0022
Probability
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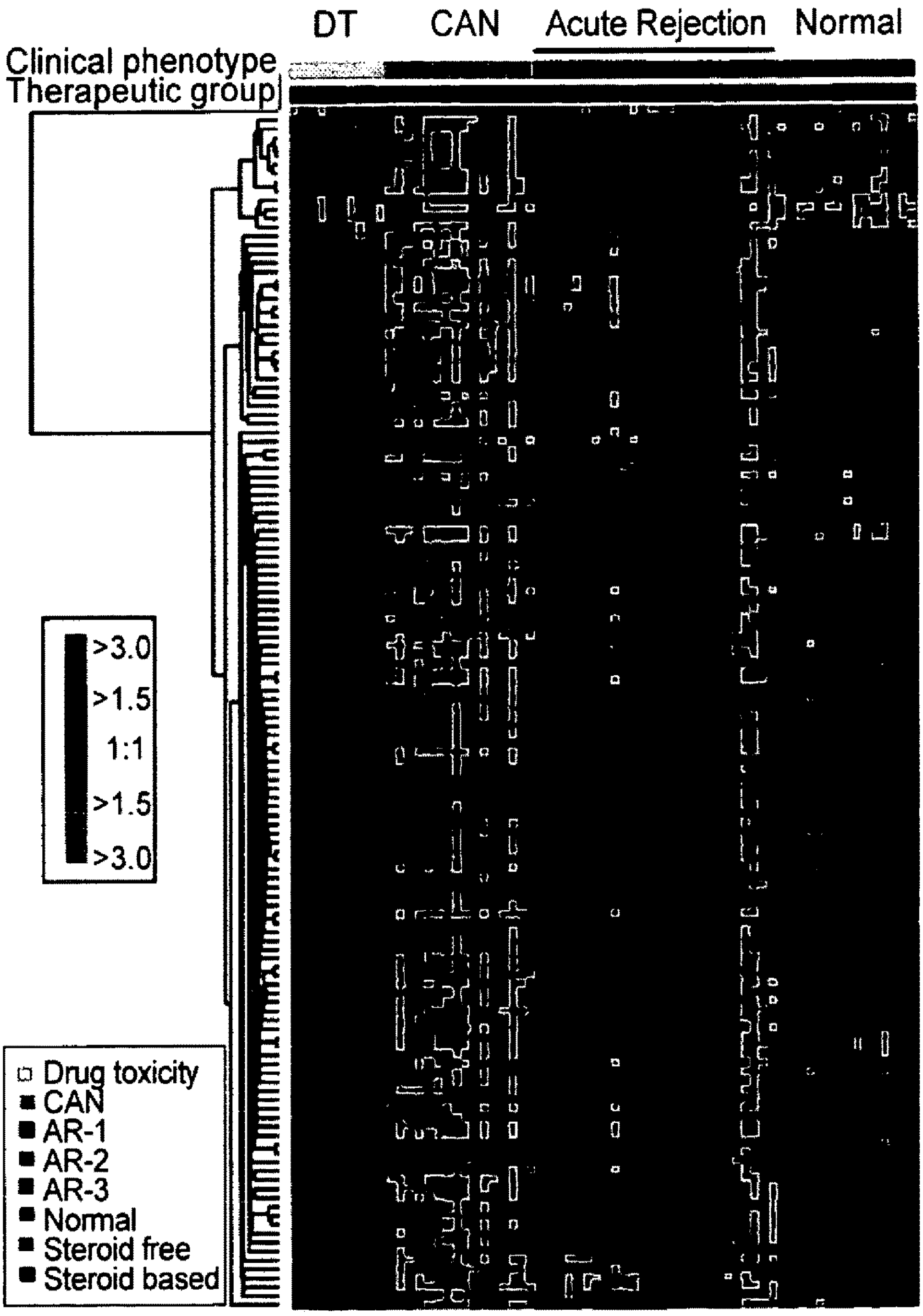
1
0.8
0.6
0.4
0.2
0
0 10 20 30 40 50 60 70
Months to Follow-up
B.
P = .019
C.
P = .048
D.
P = .017
E.
P = .0022
F.
P = .017
G.
P = .0022
H.
P = .035

FIG. 4


DT
CAN
Acute Rejection
Normal
Clinical phenotype
Therapeutic group
>3.0
>1.5
1:1
>1.5
>3.0
▫ Drug toxicity
■ CAN
■ AR-1
■ AR-2
■ AR-3
■ Normal
■ Steroid free
■ Steroid based

METHODS AND COMPOSITIONS FOR EVALUATING GRAFT SURVIVAL IN A SOLID ORGAN TRANSPLANT RECIPIENT

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS-REFERENCE TO RELATED APPLICATIONS

Notice: More than one reissue application has been filed for the reissue of Pat. No. 7,741,038. The reissue applications are application Ser. No. 13/943,626 (the present application) and Ser. No. 13/529,768 (a reissue application), filed Jun. 21, 2012.

[Pursuant to 35 U.S.C. § 119 (e), this application] *This application is a continuation reissue of U.S. patent application Ser. No. 13/529,768, filed on Jun. 21, 2012, which application is a reissue application of U.S. Pat. No. 7,741,038, which patent* claims priority to [the filing date of] U.S. Provisional Patent Application Ser. No. 60/662,083 filed on Mar. 14, 2005[; the disclosure]. *The disclosures* of [which application is] *all of the above applications are* herein incorporated by reference.

BACKGROUND

Transplantation of a graft organ or tissue from a donor to a host patient is a feature of certain medical procedures and treatment protocols. Despite efforts to avoid graft rejection through host-donor tissue type matching, in transplantation procedures where a donor organ is introduced into a host, immunosuppressive therapy is generally required to the maintain viability of the donor organ in the host.

After an organ has been transplanted into the patient, the patient's immune system is suppressed to prevent rejection of the new organ. Despite the wide use of immunosuppressive therapy, organ transplant rejection can occur.

Organ transplant rejection comprises three separate categories: hyperacute, acute and chronic. Hyperacute rejection is characterized by rapid thrombotic occlusion of the graft vasculature within minutes to hours after organ transplantation. Hyperacute rejection is mediated in large part by pre-existing antibodies that bind to the epithelium and activate the complement cascade. Complement activation results in endothelial cell damage and subsequent exposure of the basement membrane, resulting in the activation of platelets, leading to thrombosis and vascular occlusion. As the field of transplantation has matured, hyperacute rejection has become less common due to blood antigen and MHC molecule matching between the donor organ and the recipient.

Acute rejection is sub-classified into acute vascular rejection and acute cellular rejection. Acute vascular rejection is characterized by necrosis of individual cells in the graft blood vessels. The process is similar to that of hyperacute rejection, but onset is often slower, within one week of rejection, and a T cell component may be involved. Acute vascular rejection is initiated by a response to alloantigens present on the vascular endothelial cells of the donor organ, resulting in the release of a cytokine cascade, inflammation, and eventual necrosis. Acute cellular rejection is often characterized by necrosis of the essential or parenchymal cells of the transplanted organ caused by the infiltration of host T lymphocytes and macrophages. The lymphocytes involved are usually cytotoxic T lymphocytes (CTL) and macrophages, both resulting in lysis of targeted cells. The CTLs are usually specific for graft alloantigens displayed in the context of MHC class I molecules.

Chronic rejection is the major cause of allograft loss and is characterized by fibrosis and loss of normal organ structures. Fibrosis may be the result of wound healing following the cellular necrosis of acute rejection, or may occur independently and without prior acute rejection. In addition, chronic rejection may lead to vascular occlusions thought to stem from a delayed type hypersensitivity response to alloantigens present on the transplanted organ. These alloantigens stimulate lymphocytes to secrete cytokines which attract macrophages and other effector cells eventually leading to an arteriosclerosis-like blockage.

In many cases, chronic graft injury or rejection (CR) is largely due to calcineurin-inhibitor drug nephrotoxicity (DT) and chronic allograft nephropathy (CAN), two conditions which may result in loss of graft function and early graft loss, premature to the life expectancy of the recipient. The incidence of chronic graft loss has remained unchanged over the last decade.

A biopsy is the only current gold standard for CAN and DT diagnosis. As both conditions are progressive post-transplantation, multiple graft protocol biopsies are required. However, the invasiveness of biopsy procedures is a limitation to this form of monitoring. In addition, variability of biopsy sampling and pathology analysis (2) adds a confounder to the differential diagnosis of these 2 conditions—the result of either too much drug (DT) vs. too little/inappropriate drugs (CAN)—with a common outcome of chronic fibrotic injury from differing mechanisms (non-immune vs. immune).

There is currently no method available to detect or to monitor future graft loss at the time of transplantation or acute rejection (AR) episodes. AR is a risk factor both for eventual graft loss, delayed recovery of graft function and even chronic rejection. Non-invasive monitoring methods for AR stratification, CR, DT and developing or established tolerance is currently not available, but would be very valuable, as the transplant biopsy, though the current gold standard, fails to stratify or prognosticate AR, differentiate CR clearly from DT or diagnose tolerance.

Accordingly, of interest would be the ability to evaluate likelihood of graft survival in a transplant recipient, e.g., following an AR episode, such that treatment protocols for transplant patients may be customized.

SUMMARY OF THE INVENTION

Methods are provided for evaluating a subject for graft survival, e.g., in terms of predicting graft survival, identifying the presence of a deleterious graft condition, such as CAN and DT, identifying the severity and class of acute rejection, etc, in a subject are provided. In practicing the subject methods, the expression of at least one gene in a sample from the subject, e.g., a blood or biopsy sample, is assayed, e.g., at the nucleic acid and/or protein level, to evaluate the subject. Also provided are compositions, systems and kits that find use in practicing the subject methods.

DEFINITIONS

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

"Acute rejection or AR" is the rejection by the immune system of a tissue transplant recipient when the transplanted tissue is immunologically foreign. Acute rejection is characterized by infiltration of the transplanted tissue by immune cells of the recipient, which carry out their effector function and destroy the transplanted tissue. The onset of acute rejection is rapid and generally occurs in humans within a few weeks after transplant surgery. Generally, acute rejection can be inhibited or suppressed with immunosuppressive drugs such as rapamycin, cyclosporin A, anti-CD40L monoclonal antibody and the like.

"Chronic transplant rejection or CR" generally occurs in humans within several months to years after engraftment, even in the presence of successful immunosuppression of acute rejection. Fibrosis is a common factor in chronic rejection of all types of organ transplants. Chronic rejection can typically be described by a range of specific disorders that are characteristic of the particular organ. For example, in lung transplants, such disorders include fibroproliferative destruction of the airway (bronchiolitis obliterans); in heart transplants or transplants of cardiac tissue, such as valve replacements, such disorders include fibrotic atherosclerosis; in kidney transplants, such disorders include, obstructive nephropathy, nephrosclerorsis, tubulointerstitial nephropathy; and in liver transplants, such disorders include disappearing bile duct syndrome. Chronic rejection can also be characterized by ischemic insult, denervation of the transplanted tissue, hyperlipidemia and hypertension associated with immunosuppressive drugs.

The term "transplant rejection" encompasses both acute and chronic transplant rejection.

The term "stringent assay conditions" as used herein refers to conditions that are compatible to produce binding pairs of nucleic acids, e.g., surface bound and solution phase nucleic acids, of sufficient complementarity to provide for the desired level of specificity in the assay while being less compatible to the formation of binding pairs between binding members of insufficient complementarity to provide for the desired specificity. Stringent assay conditions are the summation or combination (totality) of both hybridization and wash conditions.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization (e.g., as in array, Southern or Northern hybridizations) are sequence dependent, and are different under different experimental parameters. Stringent hybridization conditions that can be used to identify nucleic acids within the scope of the invention can include, e.g., hybridization in a buffer comprising 50% formamide, 5×SSC, and 1% SDS at 42° C., or hybridization in a buffer comprising 5×SSC and 1% SDS at 65° C., both with a wash of 0.2×SSC and 0.1% SDS at 65° C. Exemplary stringent hybridization conditions can also include hybridization in a buffer of 40% formamide, 1 M NaCl, and 1% SDS at 37° C., and a wash in 1×SSC at 45° C. Alternatively, hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C., and washing in 0.1×SSC/0.1% SDS at 68° C. can be employed. Yet additional stringent hybridization conditions include hybridization at 60° C. or higher and 3×SSC (450 mM sodium chloride/45 mM sodium citrate) or incubation at 42° C. in a solution containing 30% formamide, 1M NaCl, 0.5% sodium sarcosine, 50 mM MES, pH 6.5. Those of ordinary skill will readily recognize that alternative but comparable hybridization and wash conditions can be utilized to provide conditions of similar stringency.

In certain embodiments, the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is specifically hybridized to a surface bound nucleic acid. Wash conditions used to identify nucleic acids may include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50° C. or about 55° C. to about 60° C.; or, a salt concentration of about 0.15 M NaCl at 72° C. for about 15 minutes; or, a salt concentration of about 0.2×SSC at a temperature of at least about 50° C. or about 55° C. to about 60° C. for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2×SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1×SSC containing 0.1% SDS at 68° C. for 15 minutes; or, equivalent conditions. Stringent conditions for washing can also be, e.g., 0.2×SSC/0.1% SDS at 42° C.

A specific example of stringent assay conditions is rotating hybridization at 65° C. in a salt based hybridization buffer with a total monovalent cation concentration of 1.5 M (e.g., as described in U.S. patent application Ser. No. 09/655,482 filed on Sep. 5, 2000, the disclosure of which is herein incorporated by reference) followed by washes of 0.5×SSC and 0.1×SSC at room temperature.

Stringent assay conditions are hybridization conditions that are at least as stringent as the above representative conditions, where a given set of conditions are considered to be at least as stringent if substantially no additional binding complexes that lack sufficient complementarity to provide for the desired specificity are produced in the given set of conditions as compared to the above specific conditions, where by "substantially no more" is meant less than about 5-fold more, typically less than about 3-fold more. Other stringent hybridization conditions are known in the art and may also be employed, as appropriate.

As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid comprising an open reading frame encoding a polypeptide, including exon and (optionally) intron sequences. The term "intron" refers to a DNA sequence present in a given gene that is not translated into protein and is generally found between exons in a DNA molecule. In addition, a gene may optionally include its natural promoter (i.e., the promoter with which the exons and introns of the gene are operably linked in a non-recombinant cell, i.e., a naturally occurring cell), and associated regulatory sequences, and may or may not have sequences upstream of the AUG start site, and may or may not include untranslated leader sequences, signal sequences, downstream untranslated sequences, transcriptional start and stop sequences, polyadenylation signals, translational start and stop sequences, ribosome binding sites, and the like.

A "protein coding sequence" or a sequence that "encodes" a particular polypeptide or peptide, is a nucleic acid sequence that is transcribed (in the case of DNA) and is translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from viral, procaryotic or eukaryotic mRNA, genomic DNA sequences from viral, procaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

The terms "reference" and "control" are used interchangeably to refer to a known value or set of known values against which an observed value may be compared. As used herein, known means that the value represents an understood parameter, e.g., a level of expression of a marker gene in a graft survival or loss phenotype.

The term "nucleic acid" includes DNA, RNA (double-stranded or single stranded), analogs (e.g., PNA or LNA molecules) and derivatives thereof. The terms "ribonucleic acid" and "RNA" as used herein mean a polymer composed of ribonucleotides. The terms "deoxyribonucleic acid" and "DNA" as used herein mean a polymer composed of deoxyribonucleotides. The term "mRNA" means messenger RNA. An "oligonucleotide" generally refers to a nucleotide multimer of about 10 to 100 nucleotides in length, while a "polynucleotide" includes a nucleotide multimer having any number of nucleotides.

The terms "protein" and "polypeptide" used in this application are interchangeable. "Polypeptide" refers to a polymer of amino acids (amino acid sequence) and does not refer to a specific length of the molecule. Thus peptides and oligopeptides are included within the definition of polypeptide. This term does also refer to or include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylation and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

The term "assessing" and "evaluating" are used interchangeably to refer to any form of measurement, and includes determining if an element is present or not. The terms "determining," "measuring," "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations. Assessing may be relative or absolute. "Assessing the presence of" includes determining the amount of something present, as well as determining whether it is present or absent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. Predictive Analysis of Microarrays (PAM) using a set of 3,170 differentially expressed genes identifies the 33 classifiers with similar power (FIG. **1**A). The PAM classification scores grouped the samples with 100% concordance to assigned classes and reported scores are aligned with the clustered samples (FIG. **1**B).

FIG. **2**. Kaplan-Meier survival analysis for graft loss (red) and no-loss (blue). The genes include ICAM5 (FIG. **2**A; p=0.007), IL6R (FIG. **2**B; p=0.003), STAT1 (FIG. **2**C; p=0.036), and STAT6 (FIG. **2**D (p=0.020).

FIG. **3**. Kaplan-Meier survival curves for 8 genes from whole blood samples that are predictive of graft loss. Genes include AHSA2 (FIG. **3**A), IGHG1 (FIG. **3**B), IFNAR2 (FIG. **3**C), IGKC (FIG. **3**D), HIST1H2BC (FIG. **3**E), IL1R2 (FIG. **3**F), MAPK1 (FIG. **3**G), and MAPK9 (FIG. **3**H).

FIG. **4**. Demonstrates that gene expression is generally uniform/consistent across the full clinical groups analyzed as the gene expression levels segregate well within patient groups.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods are provided for evaluating a subject for graft function, e.g., in terms of predicting graft survival, identifying the presence of a deleterious graft condition, such as CAN and DT, identifying the severity and class of acute rejection, etc, in a subject are provided. In practicing the subject methods, the expression of at least one gene in a sample from the subject, e.g., a blood or biopsy sample, is assayed, e.g., at the nucleic acid and/or protein level, to evaluate the subject. Also provided are compositions, systems and kits that find use in practicing the subject methods. The methods and compositions find use in a variety of applications.

Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, representative illustrative methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

As summarized above, the subject invention is directed to methods of evaluating graft function in a subject, as well as reagents and kits for use in practicing the subject methods. In further describing the invention, the subject methods are described first, followed by a review of the reagents and kits for use in practicing the subject methods.

Methods of Evaluating Graft Function

As reviewed above, the subject invention provides methods for evaluating a subject for graft survival. The methods provide for evaluating a subject for graft survival in terms of a number of different factors. In certain embodiments, the factor evaluated is a basic prediction of graft survival. In certain embodiments, the factor evaluated is the presence of a deleterious graft condition, such as CAN and DT. In certain embodiments, the factor identified is the severity and/or class of acute rejection, where these embodiments are distinguished from methods that just identify the presence of acute rejection, since one is further determining the severity and/or class of acute rejection, and therefore an aspect of graft survival

As such, certain embodiments of the invention provide methods of evaluating, e.g., in terms of predicting, graft survival in a subject comprising a graft. As such, the subject invention provides methods of evaluating whether a graft in a transplant patient or subject will survive or be lost. In certain embodiments, the methods may be viewed as methods of determining whether a transplant subject has a graft survival phenotype, i.e., a phenotype in which the graft will survive. A graft survival phenotype is a phenotype characterized by the presence of long-term graft survival. By "long-term" graft survival is meant graft survival for at least about 5 years beyond current sampling, despite the occurrence of one or more prior episodes of AR. In certain embodiments, graft survival is determined for patients in which at least one episode of acute rejection (AR) has occurred. As such, these embodiments are methods of determining or predicting graft survival following AR. Graft survival is determined or predicted in certain embodiments in the context of transplant therapy, e.g., immunosuppressive therapy, where immunosuppressive therapies are known in the art. In yet other embodiments, methods of distinguishing being organ rejection disease conditions, such as CAN and DT, are provided. In yet other embodiments, methods of determining the class and/or severity of acute rejection (and not just the presence thereof are provided.

As in known in the transplantation field, the graft organ, tissue or cell(s) may be allogeneic or xenogeneic, such that the grafts may be allografts or xenografts. Organs and tissues of interest include, but are not limited to: skin, heart, kidney, liver, bone marrow, and other organs.

In practicing the subject methods, a subject or patient sample, e.g., cells or collections thereof, e.g., tissues, is assayed to evaluate graft survival in the host, e.g., whether the graft will survive in the host from which the assayed sample was obtained. Accordingly, the first step of the subject methods is to obtain a suitable sample from the subject or patient of interest, i.e., a patient having at least one graft, e.g., allograft.

The sample is derived from any initial suitable source, where sample sources of interest include, but are not limited to, many different physiological sources, e.g., CSF, urine, saliva, tears, tissue derived samples, e.g., homogenates (such as biopsy samples of the transplanted tissue or organ (including, but not limited to kidney, heart, lung biopsies), and blood or derivatives thereof.

In certain embodiments, a suitable initial source for the patient sample is blood. As such, the sample employed in the subject assays of these embodiments is generally a blood-derived sample. The blood derived sample may be derived from whole blood or a fraction thereof, e.g., serum, plasma, etc., where in certain embodiments the sample is derived from blood cells harvested from whole blood. Of particular interest as a sample source are peripheral blood lymphocytes (PBL). Any convenient protocol for obtaining such samples may be employed, where suitable protocols are well known in the art and a representative protocol is reported in the Experimental Section, below.

In practicing the subject methods, the sample is assayed to obtain an expression evaluation, e.g., expression profile, for one or more genes, where the term expression profile is used broadly to include a genomic expression profile, e.g., an expression profile of nucleic acid transcripts, e.g., mRNAs, of the one or more genes of interest, or a proteomic expression profile, e.g., an expression profile of one or more different proteins, where the proteins/polypeptides are expression products of the one or more genes of interest. As such, in certain embodiments the expression of only one gene is evaluated. In yet other embodiments, the expression of two or more, e.g., about 5 or more, about 10 or more, about 15 or more, about 25 or more, about 50 or more, about 100 or more, about 200 or more, etc., genes is evaluated. Accordingly, in the subject methods, the expression of at least one gene in a sample is evaluated. In certain embodiments, the evaluation that is made may be viewed as an evaluation of the transcriptosome, as that term is employed in the art. See e.g., Gomes et al., Blood (2001 Jul. 1) 98(1): 93-9.

In generating the expression profile, in certain embodiments a sample is assayed to generate an expression profile that includes expression data for at least one gene/protein, usually a plurality of genes/proteins, where by plurality is meant at least two different genes/proteins, and often at least about 5, typically at least about 10 and more usually at least about 20 different genes/proteins or more, such as 50 or more, 100 or more, etc.

In the broadest sense, the expression evaluation may be qualitative or quantitative. As such, where detection is qualitative, the methods provide a reading or evaluation, e.g., assessment, of whether or not the target analyte, e.g., nucleic acid or expression product, is present in the sample being assayed. In yet other embodiments, the methods provide a quantitative detection of whether the target analyte is present in the sample being assayed, i.e., an evaluation or assessment of the actual amount or relative abundance of the target analyte, e.g., nucleic acid in the sample being assayed. In such embodiments, the quantitative detection may be absolute or, if the method is a method of detecting two or more different analytes, e.g., target nucleic acids, in a sample, relative. As such, the term "quantifying" when used in the context of quantifying a target analyte, e.g., nucleic acid(s), in a sample can refer to absolute or to relative quantification. Absolute quantification may be accomplished by inclusion of known concentration(s) of one or more control analytes and referencing the detected level of the target analyte with the known control analytes (e.g., through generation of a standard curve). Alternatively, relative quantification can be accomplished by comparison of detected levels or amounts between two or more different target analytes to provide a relative quantification of each of the two or more different analytes, e.g., relative to each other.

Genes/proteins of interest are graft survival/loss indicative genes, i.e., genes/proteins that are differentially expressed or present at different levels in graft survival and graft loss individuals (more specifically, individuals in which graft loss will occur vs. individuals in which a graft will survive). Representative genes/proteins of interest in certain embodiments include, but are not limited to, the genes/proteins provided in Tables 1 and 2. (Note that for Tables 1 and 2, the exact sequence of the clone identified in the table can be determined through the NCBI Entrez nucleotide database located at the website produced by placing "http://www." before: "ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&db=nucleotide" in the navigation window of a web browser (e.g., Netscape); the sequence for a specific clone is then obtained by entering the clone ID in quotes as the search term).

TABLE 1

Genes of known function in whole blood predictive of graft loss following acute rejection

Rank	Clone	Symbol	Gene	UnigeneID
1	IMAGE: 214006	HI5T1H2BC	Histone 1, H2bc	Hs.356901
2	IMAGE: 826131	IGHG3	Ig heavy constant gamma 3	Hs.413826
3	IMAGE: 626318	UBN1	Ubinuclein 1	Hs.21479
4	IMAGE: 511387	GLG1	Golgi apparatus protein 1	Hs.78979
5	IMAGE: 810057	CSDA	Cold shock domain protein A	Hs.221889
6	IMAGE: 283919	HIST1H2AC	Histone 1, H2ac	Hs.28777
7	IMAGE: 453710	PLEK2	Pleckstrin 2	Hs.170473
8	IMAGE: 840821	SSR4	Signal sequence receptor, delta	Hs.409223
9	IMAGE: 70201	MSCP	Mitochondrial solute carrier	Hs.283716
10	IMAGE: 66686	RPL10	Ribosomal protein L10	Hs.77091
11	IMAGE: 1306420	AHSA2	Activator of heat shock ATPase	Hs.122440
12	IMAGE: 2578221	UBB	Ubiquitin B	Hs.356190
13	IMAGE: 811062	CGI-69	CGI-69 protein	Hs.237924
14	IMAGE: 1272566	TNFRSF10D	TNF receptor superfamily 10d	Hs.129844
15	IMAGE: 1240649	RPL10	Ribosomal protein L10	Hs.77091
16	IMAGE: 85224	RBM25	RNA binding motif protein 25	Hs.197184
17	IMAGE: 2114004	HIST1H3D	Histone 1, H3d	Hs.239458
18	IMAGE: 789091	HIST1H2AC	Histone 1, H2ac	Hs.28777
19	IMAGE: 591025	JMJD3	Jumonji domain containing 3	Hs.103915
20	IMAGE: 1354406	SSR4	Signal sequence receptor, delta	Hs.409223
21	IMAGE: 812276	SNCA	Synuclein	Hs.76930
22	IMAGE: 344720	GYPC	Glycophorin C	Hs.81994
23	IMAGE: 683899	JMJD3	Jumonji domain containing 3	Hs.103915
24	IMAGE: 825006	CYorf15A	Chromosome Y ORF	Hs.171857
25	IMAGE: 1492412	UBA52	Ubiquitin A-52 fusion product 1	Hs.5308
26	IMAGE: 854079	ACTN1	Actinin, alpha 1	Hs.119000
27	IMAGE: 366884	IFNAR2	Interferon (a- B- and o) receptor 2	Hs.86958
28	IMAGE: 812967	TM4SF9	Transmembrane 4 superfamily	Hs.8037
29	IMAGE: 207794	NFE2	Erythroid nuclear factor	Hs.75643
30	IMAGE: 359835	SAT	Spermidine Ni-acetyltransferase	Hs.28491
31	IMAGE: 565849	KLHL12	Kelch-like 12 (*Drosophila*)	Hs.3826
32	IMAGE: 256260	RFC3	Replication factor C activator	Hs.115474
33	IMAGE: 191826	MSCP	Mitochondrial solute carrier protein	Hs.283716
34	IMAGE: 202242	MIF	Macrophage migration inhibitor	Hs.407995
35	IMAGE: 323506	MAPK1	Mitogen-activated protein kinase 1	Hs.324473
36	IMAGE: 1286850	MME	Membrane metallo-endopeptidase	Hs.259047
37	IMAGE: 129725	RBPSUH	Recombining binding protein	Hs.347340
38	IMAGE: 882522	ASS	Argininosuccinate synthetase	Hs.160786
39	IMAGE: 2129439	UBE2B	Ubiquitin-conjugating enzyme E2B	Hs.385986
40	IMAGE: 1687138	HIST1H2AM	Histone 1, H2am	Hs.134999
41	IMAGE: 209655	TGFBR3	TGFb receptor III	Hs.342874
42	IMAGE: 75254	CSRP2	Cysteine and glycine-rich protein 2	Hs.10526
43	IMAGE: 1715851	HBG2	Hemoglobin, gamma G	Hs.302145
44	IMAGE: 155467	SLC9A3R2	Solute carrier family 9	Hs.440896
45	IMAGE: 561743	PPP1R1A	Protein phosphatase 1	Hs.435238
46	IMAGE: 565075	STC1	Stanniocalcin 1	Hs.25590
47	IMAGE: 1541958	P0U2AF1	POU domain associating factor	Hs.2407
48	IMAGE: 324122	ESM1	Endothelial cell-specific molecule 1	Hs.129944
49	IMAGE: 80338	SELENBP1	Selenium binding protein 1	Hs.334841
50	IMAGE: 1472754	COX6B1	Cytochrome c oxidase (ubiquitous)	Hs.431668
51	IMAGE: 233583	IL1R2	Interleukin 1 receptor, type II	Hs.25333
52	IMAGE: 490060	RNF159	Ring finger protein (C3HC4 type)	Hs.246914
53	IMAGE: 1185475	ABCC5	ATP-binding cassette C	Hs.22010
54	IMAGE: 120551	LPIN2	Lipin 2	Hs.437425
55	IMAGE: 162772	EGR1	Early growth response 1	Hs.326035
56	IMAGE: 322029	MAPK9	Mitogen-activated protein kinase 9	Hs.348446
57	IMAGE: 1305158	KIAA1219	KIAA1219 protein	Hs.348929
58	IMAGE: 2505604	SCYE1	Endothelial monocyte-activating)	Hs.105656
59	IMAGE: 1240813	IGKC	Immunoglobulin kappa constant	Hs.377975
60	IMAGE: 257637	RRBP1	Ribosome binding protein 1 homolog	Hs.98614
61	IMAGE: 381522	PP1057	Hypothetical protein PP1057	Hs.108557
62	IMAGE: 455123	MTSS1	Metastasis suppressor 1	Hs.77694

TABLE 2

Genes of known function in renal biopsies whole blood predictive of graft loss following acute rejection.

Rank	Clone	Symbol	Gene	Unigene ID
1	IMAGE: 2134209	ZNF41	Zinc finger protein 41	Hs.143700
2	IMAGE: 1241524	TCL1A	T-cell leukemia/lymphoma 1A	Hs.2484
3	IMAGE: 704915	TAP1	Transporter 1 (MDR/TAP)	Hs.352018
4	IMAGE: 267600	STAT6	Interleukin-4 induced STAT6	Hs.437475
5	IMAGE: 26599	STAT1	Interleukin-4 induced STAT1	Hs.21486
6	IMAGE: 210405	PSME2	Proteasome activator	Hs.434081
7	IMAGE: 1240661	PSMB9	Proteasome beta type, 9	Hs.381081
8	IMAGE: 705046	PML	Promyelocytic leukemia	Hs.89633
9	IMAGE: 824340	NCF1	Neutrophil cytosolic factor 1	Hs.1583
10	IMAGE: 753313	LAPTM5	Lysosomal-associated protein-5	Hs.436200
11	IMAGE: 1351990	ISG20	Interferon stimulated gene 20 kDa	Hs.105434
12	IMAGE: 1672498	IGLV@	Ig lambda variable group	Hs.449601
13	IMAGE: 1240590	IGLC2	Ig lambda constant 2	Hs.405944
14	IMAGE: 1240813	IGKC	Ig kappa constant	Hs.377975
15	IMAGE: 1604703	HLA-F	MHC complex, class I, F	Hs.411958
16	IMAGE: 2448698	HLA-DRB6	MHC, class II, DR beta 6 (pseudogene)	Hs.534338
17	IMAGE: 461769	HLA-DRB5	MHC complex, class II, DR beta 5	Hs.308026
18	IMAGE: 1241341	HLA-DRB3	MHC complex, class II, DR beta 3	Hs.520049
19	IMAGE: 1241211	HLA-DPB1	MHC complex, class II, DP beta 1	Hs.368409
20	IMAGE: 203527	HLA-A	MHC complex, class IA	Hs.181244
21	IMAGE: 853906	HCG4P6	HLA complex group 4 pseudogene 6	Hs.512759
22	IMAGE: 841008	GBP1	Guanylate binding 1, interferon-inducible	Hs.62661
23	IMAGE: 277522	DAF	Decay accelerating factor complement (CD55)	Hs.408864
24	IMAGE: 269295	CD83	CD83 antigen (Activated B lymphocytes)	Hs.444310
25	IMAGE: 276727	CD69	CD69 antigen (early T-cell activation antigen)	Hs.82401
26	IMAGE: 200720	CD38	CD38 antigen (p45)	Hs.174944
27	IMAGE: 2000918	CAS1	O-acetyltransferase	Hs.324725
28	IMAGE: 67042	APOM	Apolipoprotein M	Hs.247323
29	IMAGE: 488143	IGHM	Immunoglobulin heavy locus	Hs.103995
30	IMAGE: 207718		TASS Ig light chain variable region	Hs.449578

In certain embodiments, at least one of the genes/proteins in the prepared expression profile is a graft survival/rejection indicative gene from Tables 1 and/or 2, where the expression profile may include expression data for 5, 10, 20, 50, 75 or more of, including all of, the genes/proteins listed in Tables 1 and/or 2. The number of different genes/proteins whose expression and/or quantity data, i.e., presence or absence of expression, as well as expression/quantity level, that are included in the expression profile that is generated may vary, but may be at least 2, and in certain embodiments ranges from 2 to about 100 or more, sometimes from 3 to about 75 or more, including from about 4 to about 70 or more.

In certain embodiments, additional genes beyond those listed in Tables 1 and/or 2, may be assayed, such as genes whose expression pattern can be used to evaluate additional transplant characteristics, including but not limited to: acute rejection (e.g., the genes identified as AR in Table 3, below); chronic allograft injury (chronic rejection) in blood (e.g., the genes identified as CR in Table 3, below); immunosuppressive drug toxicity or adverse side effects including drug-induced hypertension (e.g., the genes identified as DT in Table 3, below); age or body mass index associated genes that correlate with renal pathology or account for differences in recipient age-related graft acceptance (e.g., the genes identified as BMI in Table 3, below); immune tolerance markers in whole blood (e.g., the genes identified as TOL in Table 3, below); genes found in literature surveys with immune modulatory roles that may play a role in transplant outcomes (e.g., the genes identified as Lit. in Table 3, below); as well as other array assay function related genes, e.g., for assessing sample quality (3'- to 5'-bias in probe location), sampling error in biopsy-based studies, cell surface markers, and normalizing genes for calibrating hybridization results (see e.g., the genes identified as Contr. in Table 3, below); and the like.

A representative collection of genes that includes not only graft survival/rejection genes of Tables 1 and 2 above, but also additional graft characterizing genes (e.g., specific for DT, CAN, and immune tolerance) is in Table 3.

TABLE 3

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
ACOX1	Acyl-Coenzyme A oxidase 1, palmitoyl	NM_004035	Blood	AR
ADD3	Adducin 3 (gamma)	NM_016824	Blood	AR
ADM	Adrenomedullin	NM_001124	Blood	AR
AHR	Aryl hydrocarbon receptor	NM_001621	Blood	AR
ATP1A1	ATPase, Na+/K+ transporting, alpha 1	NM_000701	Blood	AR
BUB1B	BUB1 budding uninhibited by benzimidazoles	NM_001211	Blood	AR
CASP8	Caspase 8, apoptosis-related cysteine protease	NM_001228	Blood	AR
CASP8AP2	CASP8 associated protein 2	NM_012115	Blood	AR

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
CCNC	Cyclin C	NM_005190	Blood	AR
CD21	CD21 B-cell receptor for complement C3d0	Y00649	Blood	AR
CD69	CD69 antigen (early T-cell activation antigen)	NM_001781	Blood	AR
CD8A	CD8 antigen, alpha polypeptide (p32)	NM_001768	Blood	AR
CDIPT	Phosphatidylinositol synthase	NM_145752	Blood	AR
COX6C	Cytochrome c oxidase subunit VIc	NM_004374	Blood	AR
CSNK1A1	Casein kinase 1, alpha 1	NM_001892	Blood	AR
DUSP1	Dual specificity phosphatase 1	NM_004417	Blood	AR
DUSP3	Dual specificity phosphatase 3	NM_004090	Blood	AR
EIF1A	Eukaryotic translation initiation factor 1A	NM_001412	Blood	AR
EIF2S3	Eukaryotic translation initiation factor 2	NM_001415	Blood	AR
GNLY	Granulysin	NM_006433	Blood	AR
GOLGIN-67	Golgin-67	XM_496064	Blood	AR
AHSA2	Activator of heat shock ATPase	NM_152392	Blood	AR
HIST1H2BC	Histone 1, H2bc	NM_003526	Blood	AR
IFNAR2	Interferon (alpha, beta and omega) receptor 2	NM_000874	Blood	AR
IGHG1	Ig heavy constant gamma 1 (G1m marker)	AB067073	Blood	AR
IL1R2	Interleukin 1 receptor, type II	NM_004633	Blood	AR
MAPK1	Mitogen-activated protein kinase 1	NM_002745	Blood	AR
MIF	Macrophage migration inhibitory factor	NM_002415	Blood	AR
SCYE1	Endothelial monocyte-activating	NM_004757	Blood	AR
TGFBR3	TGFb receptor III (betaglycan)	NM_003243	Blood	AR
TM4SF9	Transmembrane 4 superfamily member 9	NM_005723	Blood	AR
IGHM	Immunoglobulin heavy constant mu	X58529	Blood	AR
ISG20	Interferon stimulated gene 20 kDa	NM_002201	Blood	AR
KIAA1014	FNBP4 formin binding protein 4	AB023231	Blood	AR
LIV-1	SLC39A6 metal ion transporter	NM_015359	Blood	AR
MAPKAPK5	Mitogen-activated protein kinase	NM_003668	Blood	AR
MDM4	p53 binding protein	NM_002393	Blood	AR
MYT1	Myelin transcription factor 1	NM_004535	Blood	AR
NAB1	EGR1 binding protein 1	NM_005966	Blood	AR
NFKB1	NFkB enhancer in B-cells 1 (p105)	NM_003998	Blood	AR
PC4	RNA polymerase II transcription cofactor 4	NM_006713	Blood	AR
PKM2	Pyruvate kinase, muscle	NM_002654	Blood	AR
PTP4A1	Protein tyrosine phosphatase	NM_003463	Blood	AR
RBL2	Retinoblastoma-like 2 (p130)	NM_005611	Blood	AR
RBM3	RNA binding motif 3 (RNP1, RAM)	NM_006743	Blood	AR
REL	V-rel viral oncogene homolog	NM_002908	Blood	AR
RPL22	Ribosomal protein L22	NM_000983	Blood	AR
RPS24	Ribosomal protein S24	NM_033022	Blood	AR
RPS27	Ribosomal protein S27	NM_001030	Blood	AR
RPS4Y	RPS4Y ribosomal protein S4	NM_001008	Blood	AR
SATB1	Special AT-rich sequence binding protein	NM_002971	Blood	AR
SDS3	Likely ortholog of mouse Sds3	NM_022491	Blood	AR
SSBP1	Single-stranded DNA binding protein 1	NM_003143	Blood	AR
SSI-3	SOCS3 suppressor of cytokine signaling 3	NM_003955	Blood	AR
STK4	Serine/threonine kinase 4	NM_006282	Blood	AR
TBRG1	Transforming growth factor beta regulator 1	NM_032811	Blood	AR
TCF7	Transcription factor 7 (T-cell specific)	NM_201633	Blood	AR
TOP2B	Topoisomerase (DNA) II beta 180 kDa	NM_001068	Blood	AR
TRIM	T-cell receptor interacting molecule	NM_016388	Blood	AR
TRRAP	Transcription domain-associated protein	NM_003496	Blood	AR
UBA52	Ubiquitin A-52-ribosomal protein fusion	NM_003333	Blood	AR
UBB	Ubiquitin B	NM_018955	Blood	AR
UBE2B	Ubiquitin-conjugating enzyme E2B	NM_003337	Blood	AR
UBN1	Ubinuclein 1	NM_016936	Blood	AR
USP25	Ubiquitin specific protease 25	NM_013396	Blood	AR
AIM1	Absent in melanoma 1	XM_166300	Biopsy	AR
CD38	CD38 antigen (p45)	NM_001775	Biopsy	AR
CDS1	CDP-diacylglycerol synthase	NM_001263	Biopsy	AR
CSF1R	Feline sarcoma viral (v-fms) homolog	NM_005211	Biopsy	AR
DR1	Down-regulator of transcription 1	NM_001938	Biopsy	AR
FGL2	Fibrinogen-like 2	NM_006682	Biopsy	AR
FLJ13612	Calcium binding protein	AI635773	Biopsy	AR
HLA-A	MHC class I, A	NM_002116	Biopsy	AR
HLA-B	MHC class I, B	NM_005514	Biopsy	AR
HLA-C	MHC class I, C	NM_002117	Biopsy	AR
HLA-DPA1	MHC class II, DP alpha 1	NM_033554	Biopsy	AR
HLA-DRA	MHC class II, DR alpha	NM_019111	Biopsy	AR
IGKC	Ig kappa constant	AB064140	Blood	AR
TNFSF10	TNF superfamily, member 10	NM_003810	Blood	AR
IGLJ3	IGLa Immunoglobulin lambda	AI146764	Biopsy	AR
MYH10	Myosin, heavy polypeptide 10	NM_005964	Biopsy	AR
NKTR	Natural killer-tumor recognition sequence	NM_005385	Biopsy	AR
PAX8	Paired box gene 8	NM_013951	Biopsy	AR

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
POLR2B	Polymerase (RNA) II polypeptide B	NM_000938	Biopsy	AR
RGN	Regucalcin (senescence marker protein-30)	NM_004683	Biopsy	AR
SCNN1A	Sodium channel, nonvoltage-gated 1 alpha	NM_001038	Biopsy	AR
SIM2	Single-minded homolog 2	NM_009586	Biopsy	AR
TACSTD2	Calcium signal transducer 2	NM_002353	Biopsy	AR
VCAM1	Vascular cell adhesion molecule 1	NM_001078	Biopsy	AR
YARS	Tyrosyl-tRNA synthetase	NM_003680	Biopsy	AR
ZFP36L1	Zinc finger protein 36	NM_004926	Biopsy	AR
HLA-DPB1	MHC, class II, DP beta 1	NM_002121	Biopsy	AR
HLA-DRB3	MHC, class II, DR beta 4	NM_022555	Biopsy	AR
ACK1	Cdc42-associated kinase 1	NM_005781	Biopsy	AR
HLA-F	MHC, class I, F	NM_018950	Biopsy	AR
ICAM5	Intercellular adhesion molecule 5	NM_003259	Biopsy	AR
REG1A	Regenerating islet-derived 1 alpha	NM_002909	Biopsy	AR
GSTA2	Glutathione 5-transferase A2	NM_000846	Biopsy	AR
HLA-DRB5	MHC class II, DR beta 4	NM_002125	Biopsy	AR
HLA-DQA1	MHC class II, DQ alpha 1	NM_002122	Biopsy	AR
HLA-DQB1	MHC class II, DQ beta 1	NM_002123	Biopsy	AR
RFXANK	Regulatory factor X-associated ankyrin	NM_003721	Biopsy	AR
STAT6	Interleukin-4 induced STAT6	NM_003153	Biopsy	AR
TAP1	Transporter 1 (MDR/TAP)	NM_000593	Biopsy	AR
DAF	Decay accelerating factor (CD55)	NM_000574	Biopsy	AR
CD83	CD83 antigen (activated B lymphocytes)	NM_004233	Biopsy	AR
STAT1	Interleukin-4 induced STAT1	NM_007315	Biopsy	AR
LTBR	Lymphotoxin beta receptor	NM_002342	Biopsy	AR
KCNJ1	Potassium inwardly-rectifying channel	NM_000220	Biopsy	AR
SLPI	Secretory leukocyte protease inhibitor	NM_003064	Biopsy	AR
CD34	CD34 antigen	NM_001773	Biopsy	AR
HOXB5	Homeo box B5	NM_002147	Biopsy	AR
IL6R	Interleukin 6 receptor	NM_181359	Biopsy	AR
DAPK1	Death-associated protein kinase 1	NM_004938	Biopsy	AR
HOXD9	Homeo box D9	NM_014213	Biopsy	AR
TCF21	Transcription factor 21	NM_003206	Biopsy	AR
MAL	T-cell differentiation protein	NM_022438	Biopsy	AR
MAF	V-maf fibrosarcoma homolog	NM_005360	Blood	AR
NCOR2	Nuclear receptor co-repressor 2	NM_006312	Blood	CR
ZFP106	Zinc finger protein 106 homolog	NM_022473	Blood	CR
RPL23	Ribosomal protein L23	NM_000978	Blood	CR
CPVL	Carboxypeptidase, vitellogenic-like	NM_019029	Blood	CR
ENO2	Enolase 2 (gamma, neuronal)	NM_001975	Blood	CR
CAPN2	Calpain 2, (m/II) large subunit	NM_001748	Blood	CR
FGFR4	Fibroblast growth factor receptor 4	NM_002011	Blood	CR
CD68	CD68 antigen	NM_001251	Blood	CR
HK3	Hexokinase 3 (white cell)	NM_002115	Blood	CR
DUSP6	Dual specificity phosphatase 6	NM_001946	Blood	CR
IL6ST	Interleukin 6 signal transducer	NM_002184	Blood	CR
LATS2	LATS, large tumor suppressor 2	NM_014572	Blood	CR
MIC2	CD99 antigen	NM_002414	Blood	CR
MMP23B	Matrix metalloproteinase 23B	NM_006983	Blood	CR
ZNF511	Zinc finger protein 511	NM_145806	Blood	CR
ANXA5	Annexin A5	NM_001154	Blood	CR
ID2	Inhibitor of DNA binding 2	NM_002166	Blood	CR
PRKRIR	RNA dependent p58 repressor	NM_004705	Blood	CR
SGK	Serum/glucocorticoid regulated kinase	NM_005627	Blood	CR
S100A10	S100 calcium binding protein A10	NM_002966	Blood	CR
CYP51	Cytochrome P450, family 51A	NM_000786	Blood	CR
ITGA4	Integrin, alpha 4 (antigen CD49D)	NM_000885	Blood	CR
ADAM10	A disintegrin and metalloproteinase10	NM_001110	Blood	CR
HNRPK	Nuclear ribonucleoprotein K	NM_031262	Blood	CR
ITGAV	Integrin, alpha V (CD51)	NM_002210	Blood	CR
JUN	V-jun sarcoma virus 17 homolog	NM_002228	Blood	CR
PRKAR2B	Protein kinase regulator	NM_002736	Blood	CR
TIE	Tyrosine kinase with Ig and EGF domains	NM_005424	Blood	CR
IQGAP2	GTPase activating protein 2	NM_006633	Blood	CR
MAP4K1	Mitogen-activated protein kinase 1	NM_007181	Blood	CR
ILF3	Interleukin enhancer binding factor 3	NM_012218	Blood	CR
SGKL	Serum/glucocorticoid regulated kinase-like	NM_013257	Blood	CR
GLS	Glutaminase	NM_014905	Blood	CR
DPYD	Dihydropyrimidine dehydrogenase	NM_000110	Blood	CR
ACADM	Acyl-Coenzyme A dehydrogenase	NM_000016	Biopsy	DT
AUTS2	Autism susceptibility candidate 2	NM_015570	Biopsy	DT
CA2	Carbonic anhydrase II	NM_000067	Biopsy	DT
CTNNA1	Catenin (cadherin-associated protein)	NM_001903	Biopsy	DT
CXCL12	Stromal cell-derived factor 1	NM_000609	Biopsy	DT
DDR1	Discoidin domain receptor family, member 1	NM_013994	Biopsy	DT

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
DECR1	2,4-dienoyl CoA reductase 1, mitochondrial	NM_001359	Biopsy	DT
DEDD	Death effector domain containing	NM_032998	Biopsy	DT
DPP4	Dipeptidylpeptidase 4 (CD26)	NM_001935	Biopsy	DT
ITM2B	Integral membrane protein 2B	NM_021999	Biopsy	DT
KIAA0436	L-type neutral amino acid transporter	AB007896	Biopsy	DT
LDHB	Lactate dehydrogenase B	NM_002300	Biopsy	DT
LEPR	Leptin receptor	NM_002303	Biopsy	DT
LRBA	LPS-responsive vesicle trafficking	NM_006726	Biopsy	DT
MUT	Methylmalonyl Coenzyme A mutase	NM_000255	Biopsy	DT
NAT1	N-acetyltransferase 1	NM_000662	Biopsy	DT
NAT2	N-acetyltransferase 2	NM_000015	Biopsy	DT
NUP50	Nucleoporin 50 kDa	NM_153645	Biopsy	DT
PAFAH1B1	Platelet-activating factor	NM_000430	Biopsy	DT
PDZK3	PDZ domain containing 3	NM_178140	Biopsy	DT
PLCL2	Phospholipase C-like 2	NM_015184	Biopsy	DT
PPP2CB	Protein phosphatase 2	NM_004156	Biopsy	DT
PRKCM	Protein kinase C, mu	NM_002742	Biopsy	DT
PTPN3	Protein tyrosine phosphatase	NM_002829	Biopsy	DT
REST	RE1-silencing transcription factor	NM_005612	Biopsy	DT
SGCB	Sarcoglycan, beta	NM_000232	Biopsy	DT
SHB	Src homology 2 domain containing	NM_003028	Biopsy	DT
SORL1	Sortilin-related receptor, L	NM_003105	Biopsy	DT
SULT1E1	Sulfotransferase family 1E	NM_005420	Biopsy	DT
CBL	Cas-Br-Transforming sequence	NM_005188	Biopsy	DT
CXCL1	Chemokine (C—X—C motif) ligand 1	NM_001511	Biopsy	DT
FGF2	Fibroblast growth factor 2 (basic)	NM_002006	Biopsy	DT
GPRK5	G protein-coupled receptor kinase 5	NM_005308	Biopsy	DT
ITSN2	Intersectin 2	NM_006277	Biopsy	DT
BCL2L13	BCL2-like 13 (apoptosis facilitator)	AA279535	Biopsy	BMI
BDKRB2	Bradykinin receptor B2	NM_000623	Biopsy	BMI
DDX3	DEAD/H (Asp-Glu-Ala-Asp/His) box 3	NM_001356	Biopsy	BMI
FOXM1	Forkhead box M1	NM_021953	Biopsy	BMI
HMOX2	Heme oxygenase (decycling) 2	NM_002134	Biopsy	BMI
IFNGR1	Interferon gamma receptor 1	NM_000416	Biopsy	BMI
IGFBP1	Insulin-like growth factor binding protein 1	NM_000596	Biopsy	BMI
IGFBP5	Insulin-like growth factor binding protein 5	NM_000599	Biopsy	BMI
LRP2	Low density lipoprotein-related protein 2	NM_004525	Biopsy	BMI
MCM7	Minichromosome maintenance deficient 7	NM_182776	Biopsy	BMI
NPPB	Natriuretic peptide precursor B	NM_002521	Biopsy	BMI
NPR1	Natriuretic peptide receptor A	NM_000906	Biopsy	BMI
PAXIP1L	PAX transcription activation interacting	NM_007349	Biopsy	BMI
PDCD5	Programmed cell death 5	NM_004708	Biopsy	BMI
RBX1	Ring-box 1	NM_014248	Biopsy	BMI
RPL27	Ribosomal protein L27	NM_000988	Biopsy	BMI
SBA2	WD repeat and SOCS box containing protein	AA043793	Biopsy	BMI
SERPINB6	Proteinase inhibitor, clade B (ovalbumin)	NM_004568	Biopsy	BMI
SLC22A5	Solute carrier family 22	NM_003060	Biopsy	BMI
SLC38A2	Solute carrier family 38, member 2	NM_018976	Biopsy	BMI
SMT3H2	Suppressor of MIF	NM_006937	Biopsy	BMI
TJP4	Tight junction protein 4 (peripheral)	NM_080604	Biopsy	BMI
TP53INP1	p53 inducible nuclear protein 1	NM_033285	Biopsy	BMI
BHLHB2	Basic helix-loop-helix domain containing	NM_003670	Biopsy	BMI
CSPG2	Chondroitin sulfate proteoglycan 2	NM_004385	Biopsy	BMI
GPD1	Glycerol-3-phosphate dehydrogenase 1	NM_005276	Biopsy	BMI
GTPBP4	GTP binding 4; Chronic renal failure gene	NM_012341	Biopsy	BMI
HIF1A	Hypoxia-inducible factor 1, alpha	NM_001530	Biopsy	BMI
MMP7	Matrix metalloproteinase 7	NM_002423	Biopsy	BMI
SLC2A3	Facilitated glucose transporter	NM_006931	Biopsy	BMI
THBS1	Thrombospondin 1	NM_003246	Biopsy	BMI
TNC	Tenascin C (hexabrachion)	NM_002160	Biopsy	BMI
HLA-G	HLA-G histocompatibility antigen, class I, G	NM_002127	Blood	TOL
IGHG3	Ig heavy constant gamma 3	AK097306	Blood	TOL
BUR1	Budding uninhibited (cell cycle regulator)	NM_004336	Blood	TOL
CCNB2	Cyclin B2	NM_004701	Blood	TOL
TACSTD1	Tumor-associated calcium signaling	NM_002354	Blood	TOL
DHRS2	Dehydrogenase/reductase (SDR family)	AK092834	Blood	TOL
BMP7	Bone morphogenetic protein 7	NM_001719	Blood	TOL
AKR1C1	Aldo-keto reductase family 1C1	NM_001353	Blood	TOL
B4GALT2	UDP-Gal 1,4-galactosyltransferase	NM_003780	Blood	TOL
TCEB3	Transcription elongation factor B (SIII)	NM_003198	Blood	TOL
MLPH	Melanophilin	NM_024101	Blood	TOL
SERPINH2	Heat shock protein 47 (proteinase inhibitor)	NM_001235	Blood	TOL
RRM2	Ribonucleotide reductase M2 polypeptide	NM_001034	Blood	TOL
SERPINA3	Alpha-1 antiproteinase, antitrypsin	NM_001085	Blood	TOL
SERPINA5	Alpha-1 antiproteinase, antitrypsin	NM_000624	Blood	TOL

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
CTNNAL1	Catenin (cadherin-associated protein)	NM_003798	Blood	TOL
SPARC	Secreted protein, cysteine-rich (osteonectin)	NM_003118	Blood	TOL
C1S	C1S complement component 1	NM_001734	Blood	TOL
SRPUL	SRPUL sushi-repeat protein	NM_006307	Blood	TOL
MMP2	Matrix metalloproteinase 2	NM_004530	Blood	TOL
SLC7A7	Cationic amino acid transporter	NM_003982	Blood	TOL
EPOR	Erythropoietin receptor	NM_000121	Blood	TOL
PRAME	Preferentially expressed antigen in melanoma	NM_006115	Blood	TOL
AFP	Alpha-fetoprotein	NM_001134	Blood	TOL
MAPK9	Mitogen-activated protein kinase 9	NM_002752	Blood	TOL
NR2F2	Nuclear receptor subfamily 2F2	NM_021005	Blood	TOL
PFN2	Profilin 2	NM_053024	Blood	TOL
SLC38A6	Solute carrier family 38, member 6	BC050349	Blood	TOL
MYOM2	Myomesin (M-protein) 2, 165 kDa	NM_003970	Blood	TOL
RBP1	Retinol binding protein 1, cellular	NM_002899	Blood	TOL
TK1	Thymidine kinase 1, soluble	NM_003258	Blood	TOL
IFITM3	Interferon induced transmembrane protein 3	NM_021034	Blood	TOL
APOH	Apolipoprotein H (beta-2-glycoprotein I)	NM_000042	Blood	TOL
EVI2A	Ecotropic viral integration site 2A	NM_014210	Blood	TOL
CD9	CD9 antigen (p24)	NM_001769	Blood	TOL
NKG7	Natural killer cell group 7 sequence	NM_005601	Blood	TOL
CDKN3	Cyclin-dependent kinase inhibitor 3	NM_005192	Blood	TOL
TCL1A	T-cell leukemia/lymphoma 1A	NM_021966	Blood	TOL
PYCR1	Pyrroline-5-carboxylate reductase 1	NM_153824	Blood	TOL
TM4SF5	Transmembrane 4 superfamily member 5	NM_003963	Blood	TOL
GAGEB1	G antigen, family B, 1 (prostate associated)	NM_003785	Blood	TOL
PCP4	Purkinje cell protein 4	NM_006198	Blood	TOL
LGMN	Legumain	NM_005606	Blood	TOL
PIR	Pirin (iron-binding nuclear protein)	NM_178238	Blood	TOL
PAICS	Phosphoribosylaminoimidazole carboxylase	NM_006452	Blood	TOL
IGFBP3	Insulin-like growth factor binding protein 3	NM_000598	Blood	TOL
PSMB9	Proteasome subunit	NM_002800	Blood	TOL
N33	Putative prostate cancer tumor suppressor	NM_006765	Blood	TOL
DP1	Polyposis locus protein 1 (DP1)	NM_005669	Blood	TOL
TFDP1	Transcription factor Dp-1	NM_007111	Blood	TOL
OSF-2	OSF-2 osteoblast specific factor 2	NM_000358	Blood	TOL
COL3A1	Collagen, type III, alpha 1	NM_000090	Blood	TOL
TIMP3	TIMP3 tissue inhibitor of metalloproteinase 3	NM_000362	Blood	TOL
SPP1	Osteopontin, early T-lymphocyte activation 1	NM_000582	Blood	TOL
NQO1	NQO1 NAD(P)H dehydrogenase	NM_000903	Blood	TOL
TOP2A	Topoisomerase (DNA) II alpha 170 kDa	NM_001067	Blood	TOL
CCND2	Cyclin D2	NM_001759	Blood	TOL
CNN3	CNN3 calponin 3, acidic AI969128	NM_001839	Blood	TOL
COL6A1	Collagen, type VI, alpha 1	NM_001848	Blood	TOL
CTGF	Connective tissue growth factor	NM_001901	Blood	TOL
EGR1	Early growth response 1 (EGR1)	NM_001964	Blood	TOL
SDC2	Syndecan 2	NM_002998	Blood	TOL
TCF3	Transcription factor 3	NM_003200	Blood	TOL
TFAP2C	Transcription factor AP-2 gamma	NM_003222	Blood	TOL
NRP1	Neuropilin 1	NM_003873	Blood	TOL
GITR	TNF receptor superfamily18 (TNFRSF18)	NM_004195	Blood	TOL
COL6A3	Collagen, type VI, alpha 3	NM_004369	Blood	TOL
EPHA2	EPHA2 EphA2	NM_004431	Blood	TOL
PDE1A	ARHE ras homolog gene family	NM_005168	Blood	TOL
FAT	Tumor suppressor homolog 1	NM_005245	Blood	TOL
KIFC3	Kinesin family member C3	NM_005550	Blood	TOL
NR2F1	Nuclear receptor subfamily 2F1	NM_005654	Blood	TOL
CAP2	CAP, adenylate cyclase-associated 2	NM_006366	Blood	TOL
BACE2	Beta-site APP-cleaving enzyme 2	NM_012105	Blood	TOL
FADS1	Fatty acid desaturase 1	NM_013402	Blood	TOL
MELK	Maternal embryonic leucine zipper kinase	NM_014791	Blood	TOL
DKK3	Dickkopf homolog 3 (*Xenopus laevis*)	NM_015881	Blood	TOL
CCNB1	Cyclin B1	NM_031966	Blood	TOL
CALD1	Caldesmon 1	NM_033138	Blood	TOL
CASP1	Caspase 1, (interleukin 1b convertase)	NM_033292	Blood	TOL
KNSL5	Kinesin-like 5 (mitotic kinesin-like protein 1)	NM_138555	Blood	TOL
STK6	Serine/threonine kinase 6	NM_198433	Blood	TOL
CD59	CD59 antigen p18-20	NM_203330	Blood	TOL
FN1	Fibronectin 1	NM_212482	Blood	TOL
SERPINE2	Serine proteinase inhibitor	NM_006216	Blood	TOL
CDH2	Cadherin 2, type 1, N-cadherin	NM_001792	Blood	TOL
CCNE1	Cyclin E1	NM_001238	Blood	TOL
SEMA3F	Ig short basic domain, secreted	NM_004186	Blood	TOL
MAD2L1	MAD2 mitotic arrest deficient-like 1	NM_002358	Blood	TOL
CYR61	Cysteine-rich, angiogenic inducer, 61	NM_001554	Blood	TOL

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
TNFRSF7	CD27 TNF receptor superfamily 7	NM_001242	Blood	TOL
FOXP3	Forkhead box P3 (FOXP3), mRNA	NM_014009	Blood	TOL
ABCA4	ATP-binding cassette, sub-family A (ABC1)	NM_000350	Biopsy	Control
HNK-1	HNK-1 sulfotransferase	AF033827	Biopsy	Control
UCP2	Uncoupling protein 2	NM_003355	Biopsy	Control
DAB2	Mitogen-responsive phosphoprotein	NM_001343	Biopsy	Control
AQP3	Aquaporin 3	NM_004925	Biopsy	Control
CRABP1	Cellular retinoic acid binding protein 1	NM_004378	Biopsy	Control
KCNAB2	Potassium voltage-gated channel	NM_003636	Biopsy	Control
TNNT2	Troponin T2, cardiac	NM_000364	Biopsy	Control
APP	Amyloid beta (A4) precursor protein	NM_000484	Biopsy	Control
FABP3	Fatty acid binding protein 3	NM_004102	Biopsy	Control
PODXL	Podocalyxin-like	NM_005397	Biopsy	Control
ALPI	Alkaline phosphatase, intestinal	NM_001631	Biopsy	Control
MAPT	Microtubule-associated protein tau	NM_005910	Biopsy	Control
KHK	Ketohexokinase (fructokinase)	NM_000221	Biopsy	Control
18S	18s ribosomal RNA	M10098	All	Control
ACTB	Actin, beta	NM_001101	All	Control
GAPD	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	All	Control
GSUSB	Glucuronidase, beta	NM_000181	All	Control
HPRT1	Hypoxanthine phosphoribosyltransferase 1	NM_000194	All	Control
SCYA3	Chemokine (C—C motif) ligand 3	NM_002983	All	Control
LMO2	LIM domain only 2 (LMO2)	NM_005574	All	Control
BCL6	B-cell CLL/lymphoma 6	NM_001706	All	Control
IkB2	NFkB enhancer in B-cells inhibitor	NM_020529	All	Control
APC	Adenomatosis polyposis coli	NM_000038	All	Control
BAG2	BCL2-associated athanogene 2 (BAG2)	NM_004282	All	Control
CREBBP	CREB binding protein	NM_004380	All	Control
KLRB1	Killer cell lectin-like receptor B1	NM_002258	All	Control
TRADD	TNFRSF1A-associated via death domain	NM_003789	All	Control
CXCL14	Chemokine (C—X—C motif) ligand 14	NM_004887	All	Control
IL1A	Interleukin 1, alpha	NM_000575	All	Control
MMP1	Matrix metalloproteinase 1	NM_002421	All	Control
MMP9	Matrix metalloproteinase 9	NM_004994	All	Control
VEGFC	Vascular endothelial growth factor C	NM_005429	All	Control
CD8A	CD8 antigen, alpha polypeptide (p32)	NM_171827	Blood	Control
CD3G	CD3G antigen, gamma (TiT3 complex)	NM_000073	Blood	Control
CD44	CD44 antigen	NM_000610	Blood	Control
CD4	CD4 antigen (p55)	NM_000616	Blood	Control
CD3D	CD3D antigen, delta (TiT3 complex)	NM_000732	Blood	Control
CD3E	CD3E antigen, epsilon (TiT3 complex)	NM_000733	Blood	Control
CD3Z	CD3Z antigen, zeta (TiT3 complex)	NM_000734	Blood	Control
CD19	CD19 antigen	NM_001770	Blood	Control
B220	Protein tyrosine phosphatase receptor	NM_002838	Blood	Control
CD138	CD138 syndecan 1 (SDC1)	NM_002997	Blood	Control
CD43	Sialophorin (CD43)	NM_003123	Blood	Control
CD8B1	CD8 antigen, beta polypeptide 1 (p37)	NM_004931	Blood	Control
API5	Apoptosis inhibitor 5	NM_006595	All	Lit.
Axin1	Axin 1	NM_003502	All	Lit.
Axin2	Axin 2 (conductin, axil)	NM_004655	All	Lit.
BAD	BCL2-antagonist of cell death	NM_032989	All	Lit.
BIK	BCL2-interacting killer (apoptosis-inducing)	NM_001197	All	Lit.
BMP4	Bone morphogenetic protein 4	NM_001202	All	Lit.
BTG1	B-cell translocation gene 1	NM_001731	All	Lit.
CASP10	Caspase 10, apoptosis-related cysteine protease	NM_001230	All	Lit.
CASP3	Caspase 3, apoptosis-related cysteine protease	NM_004346	All	Lit.
CASP4	Caspase 4, apoptosis-related cysteine protease	NM_001225	All	Lit.
CASP7	Caspase 7, apoptosis-related cysteine protease	NM_001227	All	Lit.
CASP9	Caspase 9, apoptosis-related cysteine protease	NM_001229	All	Lit.
CCL18	Chemokine (C—C motif) ligand 18	NM_002988	All	Lit.
CD161	Killer cell lectin-like receptor B1	BCO27885	All	Lit.
CD20	Membrane-spanning 4A1	NM_152866	All	Lit.
CD22	CD22 antigen	NM_001771	All	Lit.
CD48	CD48 antigen (B-cell membrane protein)	NM_001778	All	Lit.
CD80	CD80 antigen (B7-1 antigen)	NM_005191	All	Lit.
CDA08	T-cell immunomodulatory protein	NM_030790	All	Lit.
CDC2	Cell division cycle 2, G1 to S and G2 to M	NM_001786	All	Lit.
CDw108	Semaphorin Ig and GPI membrane anchor 7A,	NM_003612	All	Lit.
CDW52	CDW52 antigen (CAMPATH-1 antigen)	NM_001803	All	Lit.
CIS4	STAT induced STAT inhibitor-4	NM_004232	All	Lit.
CTLA4	Cytotoxic T-lymphocyte-associated protein 4	NM_005214	All	Lit.
DAD1	Defender against cell death 1	NM_001344	All	Lit.
DAP3	Death associated protein 3	NM_033657	All	Lit.
DAPK2	Death-associated protein kinase 2	NM_014326	All	Lit.
DAPK3	Death-associated protein kinase 3	NM_001348	All	Lit.

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
DAXX	Death-associated protein 6	NM_001350	All	Lit.
EBF	Early B-cell factor	NM_024007	All	Lit.
FCGR1A	Fc fragment of IgG (receptor for CD64)	NM_000566	All	Lit.
GADD45B	Growth arrest and DNA-damage-inducible	NM_015675	All	Lit.
GSR	Glutathione reductase	NM_000637	All	Lit.
GZMA	Granzyme A	NM_006144	All	Lit.
GZMB	Granzyme B	NM_004131	All	Lit.
Gzmc	Granzyme C	M18459	All	Lit.
GZMK	Granzyme K	NM_002104	All	Lit.
HLA-E	MHC class I, E	NM_005516	All	Lit.
ICAM1	Intercellular adhesion molecule 1 (CD54)	NM_000201	All	Lit.
ICAM3	Intercellular adhesion molecule 3	NM_002162	All	Lit.
IFI16	Interferon, gamma-inducible protein 16	NM_005531	All	Lit.
IFI35	Interferon-induced protein 35	NM_005533	All	Lit.
IFNG	Interferon, gamma	NM_000619	All	Lit.
IGBP1	Ig (CD79A) binding protein 1	NM_001551	All	Lit.
IGJ	Ig J polypeptide, linker protein	NM_144646	All	Lit.
IK	IK cytokine, down-regulator of HLA II	NM_006083	All	Lit.
IL2RA	Interleukin 2 receptor, alpha	NM_000417	All	Lit.
IL4R	Interleukin 4 receptor	NM_000418	All	Lit.
IL6	Interleukin 6 (interferon, beta 2)	NM_000600	All	Lit.
IL7R	Interleukin 7 receptor	NM_002185	All	Lit.
IL8RB	Interleukin 8 receptor, beta	NM_001557	All	Lit.
IRF1	Interferon regulatory factor 1	NM_002198	All	Lit.
ITGAE	Integrin, alpha E (CD103)	NM_002208	All	Lit.
JAK1	Janus kinase 1	NM_002227	All	Lit.
JAK2	Janus kinase 2	NM_004972	All	Lit.
MADH2	SMAD, mothers against DPP	NM_005901	All	Lit.
MAPK3	Mitogen-activated protein kinase 3	NM_002746	All	Lit.
MDM2	p53 binding protein	NM_002392	All	Lit.
MHC2TA	MHC class II transactivator	NM_000246	All	Lit.
NK4	Natural killer cell transcript 4	NM_004221	All	Lit.
NMI	N-myc (and STAT) interactor	NM_004688	All	Lit.
PCNA	Proliferating cell nuclear antigen	NM_002592	All	Lit.
PDCD2	Programmed cell death 2	NM_002598	All	Lit.
PDCD7	Programmed cell death 7	NM_005707	All	Lit.
PDCD8	Programmed cell death 8	NM_004208	All	Lit.
PDGFRB	Platelet-derived growth factor receptor	NM_002609	All	Lit.
RhoA	Ras homolog gene family, member A	NM_001664	All	Lit.
SIMRP7	Multidrug resistance-associated protein 7	NM_033450	All	Lit.
SOD2	Superoxide dismutase 2, mitochondrial	NM_000636	All	Lit.
SSI-1	suppressor of cytokine signaling 1	NM_003745	All	Lit.
STAT2	Signal transducer2, 113 kDa	NM_005419	All	Lit.
STAT3	Signal transducer 3 (acute-phase response factor)	NM_139276	All	Lit.
STAT4	Signal transducer 4	NM_003151	All	Lit.
STAT5A	Signal transducer 5A	NM_003152	All	Lit.
STAT5B	Signal transducer a5B	NM_012448	All	Lit.
STK21	Rho-interacting	NM_007174	All	Lit.
TA-LRRP	TNF receptor-associated factor 6	NM_145803	All	Lit.
TCRA	T-cell receptor active alpha-chain	M12423	All	Lit.
TCRB	T cell receptor beta locus	X60096	All	Lit.
TCRD	T-cell receptor delta chain (VJC-region)	M21624	All	Lit.
TCRG	T cell receptor gamma locus	X06774	All	Lit.
TFRC	Transferrin receptor (p90, CD71)	NM_003234	All	Lit.
TGFA	Transforming growth factor, alpha	NM_003236	All	Lit.
TGFB2	Transforming growth factor, beta 2	NM_003238	All	Lit.
THBS2	Thrombospondin 2	NM_003247	All	Lit.
TIA1	Cytotoxic granule-associated RNA binding	NM_022173	All	Lit.
TIEG2	TGFB inducible early growth response 2	NM_003597	All	Lit.
TLR5	Toll-like receptor 5	NM_003268	All	Lit.
TNFRSF1A	TNF receptor superfamily, member 1A	NM_001065	All	Lit.
TNFRSF1B	TNF receptor superfamily, member 1B	NM_001066	All	Lit.
TNFSF7	TNF (ligand) superfamily, member 7	NM_001252	All	Lit.
TP53BP1	Tumor protein p53 binding protein, 1	NM_005657	All	Lit.
TP53BP2	Tumor protein p53 binding protein, 2	NM_005426	All	Lit.
TRAF1	TNF receptor-associated factor 1	NM_005658	All	Lit.
TRAF2	TNF receptor-associated factor 2	NM_021138	All	Lit.
TRAF3	TNF receptor-associated factor 3	NM_003300	All	Lit.
TRAF4	TNF receptor-associated factor 4	NM_004295	All	Lit.
TRAP1	TNF receptor-associated protein 1	NM_004257	All	Lit.
TTK	TTK protein kinase	NM_003318	All	Lit.
UBE1L	Ubiquitin-activating enzyme E1-like	NM_003335	All	Lit.
VPREB3	Pre-B lymphocyte gene 3	NM_013378	All	Lit.
WNT1	MMTV integration site (WNT1)	NM_005430	All	Lit.
ACE1	Ig receptor (PIGR) IgA nephritis	NM_002644	All	Lit.

TABLE 3-continued

Genes of known function of prognostic value compiled for a custom transplantation chip (TxChip VI).

Symbol	Name	mRNA	Tissue	Study
BAX	BCL2-associated X protein	NM_138763	All	Lit.
BCL2	B-cell CLL/lymphoma 2	NM_000633	All	Lit.
C3	Complement component 3	NM_000064	All	Lit.
CD28	CD28 antigen (Tp44)	NM_006139	All	Lit.
CD86	CD86 antigen (B7-2 antigen)	NM_006889	All	Lit.
ICOS	Inducible T-cell co-stimulator	NM_012092	All	Lit.
IL10	Interleukin 10	NM_000572	All	Lit.
IL15	Interleukin 15	NM_000585	All	Lit.
IL2	Interleukin 2	NM_000586	All	Lit.
IL4	Interleukin 4	NM_000589	All	Lit.
IL7	Interleukin 7	NM_000880	All	Lit.
IL8	Interleukin 8	NM_000584	All	Lit.
PRF1	Perforin 1 (pore forming protein)	NM_005041	All	Lit.
RANTES	Chemokine (C—C motif) ligand 5 (CCL5)	NM_002985	All	Lit.
TBET	Th1-specific T-box transcription factor	NM_013351	All	Lit.
TGFB1	TGF beta 1	NM_000660	All	Lit.
TNF	TNF superfamily, member 2	NM_000594	All	Lit.
TNFB	Lymphotoxin alpha (TNF1 or LTA)	NM_000595	All	Lit.
TNFRSF5	CD40 TNF receptor superfamily 5	NM_001250	All	Lit.
TNFRSF6	CD95 = Fos TNF receptor superfamily 6	NM_000043	All	Lit.
VEGF	Vascular endothelial growth factor	NM_003376	All	Lit.

In certain embodiments, a collection of genes from Table 3 is assayed, where in these embodiments the number of genes from Table 3 may be at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90% or more, including all of the genes from Table 3.

In certain embodiments, the expression profile obtained is a genomic or nucleic acid expression profile, where the amount or level of one or more nucleic acids in the sample is determined, e.g., the nucleic acid transcript of the gene of interest. In these embodiments, the sample that is assayed to generate the expression profile employed in the diagnostic methods is one that is a nucleic acid sample. The nucleic acid sample includes a plurality or population of distinct nucleic acids that includes the expression information of the phenotype determinative genes of interest of the cell or tissue being diagnosed. The nucleic acid may include RNA or DNA nucleic acids, e.g., mRNA, cRNA, cDNA etc., so long as the sample retains the expression information of the host cell or tissue from which it is obtained. The sample may be prepared in a number of different ways, as is known in the art, e.g., by mRNA isolation from a cell, where the isolated mRNA is used as is, amplified, employed to prepare cDNA, cRNA, etc., as is known in the differential expression art. In certain embodiments, the sample is prepared from a cell or tissue harvested from a subject to be diagnosed, e.g., via biopsy of tissue, using standard protocols, where cell types or tissues from which such nucleic acids may be generated include any tissue in which the expression pattern of the to be determined phenotype exists, including, but not limited to, peripheral blood lymphocyte cells, etc, as reviewed above.

The expression profile may be generated from the initial nucleic acid sample using any convenient protocol. While a variety of different manners of generating expression profiles are known, such as those employed in the field of differential gene expression analysis, one representative and convenient type of protocol for generating expression profiles is array-based gene expression profile generation protocols. In certain embodiments, such applications are hybridization assays in which a nucleic acid array that displays "probe" nucleic acids for each of the genes to be assayed/profiled in the profile to be generated is employed. In these assays, a sample of target nucleic acids is first prepared from the initial nucleic acid sample being assayed, where preparation may include labeling of the target nucleic acids with a label, e.g., a member of signal producing system. Following target nucleic acid sample preparation, the sample is contacted with the array under hybridization conditions, whereby complexes are formed between target nucleic acids that are complementary to probe sequences attached to the array surface. The presence of hybridized complexes is then detected, either qualitatively or quantitatively. Specific hybridization technology which may be practiced to generate the expression profiles employed in the subject methods includes the technology described in U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. In these methods, an array of "probe" nucleic acids that includes a probe for each of the phenotype determinative genes whose expression is being assayed is contacted with target nucleic acids as described above. Contact is carried out under hybridization conditions, e.g., stringent hybridization conditions, and unbound nucleic acid is then removed.

The resultant pattern of hybridized nucleic acid provides information regarding expression for each of the genes that have been probed, where the expression information is in terms of whether or not the gene is expressed and, typically, at what level, where the expression data, i.e., expression profile (e.g., in the form of a transcriptosome), may be both qualitative and quantitative.

Alternatively, non-array based methods for quantitating the levels of one or more nucleic acids in a sample may be employed, including quantitative PCR, and the like.

Where the expression profile is a protein expression profile, any convenient protein quantitation protocol may be employed, where the levels of one or more proteins in the assayed sample are determined. Representative methods include, but are not limited to: proteomic arrays, flow cytometry, standard immunoassays (e.g., ELISA assays), protein activity assays, including multiplex protein activity assays, etc.

Following obtainment of the expression profile from the sample being assayed, the expression profile is compared with a reference or control profile to determine the particular graft tolerant/intolerant phenotype of the cell or tissue, and therefore host, from which the sample was obtained/derived. The terms "reference" and "control" as used herein mean a standardized pattern of gene expression or levels of expression of certain genes to be used to interpret the expression signature of a given patient and assign a graft tolerant/intolerant phenotype thereto. The reference or control profile may be a profile that is obtained from a cell/tissue known to have the desired phenotype, e.g., tolerant phenotype, and therefore may be a positive reference or control profile. In addition, the reference/control profile may be from a cell/tissue known to not have the desired phenotype, e.g., an intolerant phenotype, and therefore be a negative reference/control profile.

In certain embodiments, the obtained expression profile is compared to a single reference/control profile to obtain information regarding the phenotype of the cell/tissue being assayed. In yet other embodiments, the obtained expression profile is compared to two or more different reference/control profiles to obtain more in depth information regarding the phenotype of the assayed cell/tissue. For example, the obtained expression profile may be compared to a positive and negative reference profile to obtain confirmed information regarding whether the cell/tissue has the phenotype of interest.

The comparison of the obtained expression profile and the one or more reference/control profiles may be performed using any convenient methodology, where a variety of methodologies are known to those of skill in the array art, e.g., by comparing digital images of the expression profiles, by comparing databases of expression data, etc. Patents describing ways of comparing expression profiles include, but are not limited to, U.S. Pat. Nos. 6,308,170 and 6,228,575, the disclosures of which are herein incorporated by reference. Methods of comparing expression profiles are also described above.

The comparison step results in information regarding how similar or dissimilar the obtained expression profile is to the control/reference profile(s), which similarity/dissimilarity information is employed to determine the phenotype of the cell/tissue being assayed and thereby evaluate graft survival in the subject. For example, similarity with a positive control indicates that the assayed cell/tissue has a graft survival phenotype. Likewise, similarity with a negative control indicates that the assayed cell/tissue has a graft loss phenotype.

Depending on the type and nature of the reference/control profile(s) to which the obtained expression profile is compared, the above comparison step yields a variety of different types of information regarding the cell/tissue that is assayed. As such, the above comparison step can yield a positive/negative determination of a graft survival phenotype of an assayed cell/tissue. In many embodiments, the above-obtained information about the cell/tissue being assayed is employed to diagnose a host, subject or patient with respect to graft survival, as described above. In certain embodiments, the determination/prediction of graft survival and loss can be coupled with a determination of additional characteristics of the graft and function thereof. For example, in certain embodiments one can predict not only whether graft loss will occur, but the mechanism of graft loss, e.g., via CAN or DT. The first 9 genes in the cluster illustrated in FIG. **4** are highly-differentially expressed between CAN and DT. As such, evaluating one or more of these genes permits these two overlapping conditions to be readily distinguished, such that one can readily determine the presence of CAN or DT.

The subject methods further find use in pharmacogenomic applications. In these applications, a subject/host/patient is first diagnosed for graft function according to the subject invention, and then treated using a protocol determined, at least in part, on the results of the diagnosis. For example, a host may be evaluated for the presence of absence of the graft survival phenotype using a protocol such as the diagnostic protocol described in the preceding section. The subject may then be treated using a protocol whose suitability is determined using the results of the diagnosis step. In embodiments, where the host is evaluated for the presence or absence of CAN or DT, treatment protocols may correspondingly be adjusted based on the obtained results. For example, where the subject methods are employed to determine the presence of CAN, immunosuppressive therapy can be modulated, e.g., increased or drugs changed, as is known in the art for the treatment of CAN. Likewise, where the subject methods are employed and detect the presence of DT, the immunosuppressive therapy can be reduced in order to treat the DT. In practicing the subject methods, a subject is typically screened for the presence of a graft survival or loss phenotype following receipt of a graft or transplant. The subject may be screened once or serially following transplant receipt, e.g., weekly, monthly, bimonthly, half-yearly, yearly, etc. In certain embodiments, the subject is screened following occurrence of acute rejection (AR). In such embodiments, the methods are employed to evaluate, e.g., predict, ultimate graft loss or survival in the subject following AR.

The subject methods may be employed with a variety of different types of transplant subjects. In many embodiments, the subjects are within the class mammalian, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), lagomorpha (e.g. rabbits) and primates (e.g., humans, chimpanzees, and monkeys). In certain embodiments, the animals or hosts, i.e., subjects (also referred to herein as patients) will be humans.

The methods may be used to evaluate survival of a variety of different types of grafts. Grafts of interest include, but are not limited to: transplanted heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, bone, bone marrow, muscle, bladder or parts thereof.

Databases of Expression Profiles of Phenotype Determinative Genes

Also provided are databases of expression profiles of graft survival and/or graft loss phenotype determinative genes. Such databases will typically comprise expression profiles of various cells/tissues having graft tolerant phenotypes, negative expression profiles, etc., where such profiles are further described below.

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 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. "Recorded" refers to a process for storing information on computer readable medium, using any such methods as 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.

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.

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. 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.

Reagents, Systems and Kits

Also provided are reagents, systems and kits thereof for practicing one or more of the above-described methods. The subject reagents, systems and kits thereof may vary greatly. Reagents of interest include reagents specifically designed for use in production of the above-described expression profiles of phenotype determinative genes, i.e., a gene expression evaluation element made up of one or more reagents. The term system refers to a collection of reagents, however compiled, e.g., by purchasing the collection of reagents from the same or different sources. The term kit refers to a collection of reagents provided, e.g., sold, together.

One type of such reagent is an array of probe nucleic acids in which the phenotype determinative genes of interest are represented. A variety of different array formats are known in the art, with a wide variety of different probe structures, substrate compositions and attachment technologies. Representative array structures of interest include those described in U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280.

In certain embodiments, the arrays include probes for at least 1 of the genes listed in Tables 1 and/or 2. In certain embodiments, the number of genes that are from Tables 1 and/or 2 that is represented on the array is at least 5, at least 10, at least 25, at least 50, at least 75 or more, including all of the genes listed in Tables 1 and/or 2. The subject arrays may include only those genes that are listed in Tables 1 and/or 2, or they may include additional genes that are not listed in Tables 1 and/or 2, such as probes for genes whose expression pattern can be used to evaluate additional transplant characteristics, including but not limited to: acute rejection; chronic allograft injury (chronic rejection) in blood; immunosuppressive drug toxicity or adverse side effects including drug-induced hypertension; age or body mass index associated genes that correlate with renal pathology or account for differences in recipient age-related graft acceptance; immune tolerance markers in whole blood; genes found in literature surveys with immune modulatory roles that may play a role in transplant outcomes (see e.g., Table 3 for a list of representative additional genes); as well as other array assay function related genes, e.g., for assessing sample quality (3'- to 5'-bias in probe location), sampling error in biopsy-based studies, cell surface markers, and normalizing genes for calibrating hybridization results; and the like. Where the subject arrays include probes for such additional genes, in certain embodiments the number % of additional genes that are represented and are not directly or indirectly related to transplantation does not exceed about 50%, usually does not exceed about 25%. In certain embodiments where additional genes are included, a great majority of genes in the collection are transplant characterization genes, where by great majority is meant at least about 75%, usually at least about 80% and sometimes at least about 85, 90, 95% or higher, including embodiments where 100% of the genes in the collection are phenotype determinative genes. Transplant characterization genes are genes whose expression can be employed to characterize transplant function in some manner, e.g., presence of rejection, etc.

Another type of reagent that is specifically tailored for generating expression profiles of phenotype determinative genes is a collection of gene specific primers that is designed to selectively amplify such genes. Gene specific primers and methods for using the same are described in U.S. Pat. No. 5,994,076, the disclosure of which is herein incorporated by reference. Of particular interest are collections of gene specific primers that have primers for at least 1 of the genes listed in one Tables 1 and/or 2, often a plurality of these genes, e.g., at least 2, 5, 10, 15 or more. In certain embodiments, the number of genes that are from Tables 1 and/or 2 that have primers in the collection is at least 5, at least 10, at least 25, at least 50, at least 75 or more, including all of the genes listed in Tables 1 and/or 2. The subject gene specific primer collections may include only those genes that are listed in Tables 1 and/or 2, or they may include primers for additional genes that are not listed in Tables 1 and/or 2, such as probes for genes whose expression pattern can be used to evaluate additional transplant characteristics, including but not limited to: acute rejection; chronic allograft injury (chronic rejection) in blood; immunosuppressive drug toxicity or adverse side effects including drug-induced hypertension; age or body mass index associated genes that correlate with renal pathology or account for differences in recipient age-related graft acceptance; immune tolerance markers in whole blood; genes found in literature surveys with immune modulatory roles that may play a role in transplant outcomes (see e.g., Table 3 for a list of representative additional genes); as well as other array assay function related genes, e.g., for assessing sample quality (3'- to 5'-bias in probe location), sampling error in biopsy-based studies, cell surface markers, and normalizing genes for calibrating hybridization results; and the like. Where the subject arrays include probes for such additional genes, in certain embodiments the number % of additional genes that are represented and are not directly or indirectly related to transplantation does not exceed about 50%, usually does not exceed about 25%. In certain embodiments where additional genes are included, a great majority of genes in the collection are transplant characterization genes, where by great majority is meant at least about 75%, usually at least about 80% and sometimes at least about 85, 90, 95% or higher, including embodiments where 100% of the genes in the collection are phenotype determinative genes.

The systems and kits of the subject invention may include the above-described arrays and/or gene specific primer collections. The systems and kits may further include one or more additional reagents employed in the various methods, such as primers for generating target nucleic acids, dNTPs and/or rNTPs, which may be either premixed or separate, one or more uniquely labeled dNTPs and/or rNTPs, such as biotinylated or Cy3 or Cy5 tagged dNTPs, gold or silver particles with different scattering spectra, or other post synthesis labeling reagent, such as chemically active derivatives of fluorescent dyes, enzymes, such as reverse transcriptases, DNA polymerases, RNA polymerases, and the like, various buffer mediums, e.g. hybridization and washing buffers, prefabricated probe arrays, labeled probe purification reagents and components, like spin columns, etc., signal generation and detection reagents, e.g. streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like.

The subject systems and kits may also include a phenotype determination element, which element is, in many embodiments, a reference or control expression profile that can be employed, e.g., by a suitable computing means, to make a phenotype determination based on an "input" expression profile, e.g., that has been determined with the above described gene expression evaluation element. Representative phenotype determination elements include databases of expression profiles, e.g., reference or control profiles, as described above.

In addition to the above components, the subject kits will further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, CD, etc., on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

I. Introduction

The objective of this study was to determine whether gene expression markers could be identified in RNA extracted from peripheral blood leukocytes (PBL) or renal biopsies predictive of future graft loss following AR.

II. Array Experiments

Each microarray contained approximately 32,000 DNA spots representing approximately 12,440 human genes. Total RNA was isolated (Tri Reagent; MRC Inc., Cincinnati, Ohio) from buffy coats isolated from whole blood samples. A common reference RNA pool (Perou et al., Nature (2000) 406: 747-52) was used as an internal standard. Sample or reference RNA were subjected to two successive rounds of amplification before hybridization to microarrays using an improved protocol based on the method described by Wang et al (please provide entire cite). Array data for 62 renal biopsy samples and 56 whole blood samples were stored in the Stanford Microarray database (Sherlock et al., Nuc. Acids Res. (2001) 29:152-55) and gene lists filtered at retrieval to provide expression markers with high fidelity. The two groups of samples were analyzed in two separate studies. All PBL were used for initial unsupervised hierarchical clustering (Eisen et al., Proc. Nat'l Acad. Sci. USA (1998) 95:14863-8), for subsequent supervised analyses between groups (Significance Analysis of Microarrays; SAM (Tusher et al., Proc. Nat'l Acad. Sci. USA (2001) 98:5116-21).

III. Customizing a Minimal Gene-Set for AR Class Prediction and Risk Assessment

We used Predictive Analysis of Microarrays (PAM) (Tusher et al., supra) to identify only 97 genes within the renal biopsy dataset, all having >5-fold difference in expression level, which classify our learning set of 26 AR samples with 100% concordance to assigned phenotype. Another analysis using a larger set of 3,170 differentially expressed genes identifies the 33 classifiers with similar power (FIGS. **1**A and **1**B). Reproducibility of the diagnostic signature, in particular within the majority of the AR-1 samples, is evident by the short branches in the cluster dendogram. AR expression overlaps with the innate immune response to infection, as evidenced by cluster analysis and by differential expression of several TGF-β-modulated genes including RANTES, MIC-1, several cytokines, chemokines, and cell-adhesion molecules. AR-1 is the most severe class with the highest rate of graft loss and highest expression of B-cell specific genes. AR-2 resembles a drug-toxicity signature and also co-clusters with patients with active viral infections. The most striking feature of AR-3 is the expression of genes involved in cellular proliferation and cell cycling suggesting active tissue repair and regeneration. The presence of proliferating-cell nuclear antigen (PCNA), a marker of cell proliferation, was confirmed in all AR-3 samples tested (Sarwal et al. New Engl. J. Med. 2003 349(2):125-38).

The PAM classification scores grouped the samples with 100% concordance to assigned classes and reported scores are aligned with the clustered samples (FIG. **1**B). In addition, all 33 genes selected by PAM have Significance Analysis of Microarrays significance scores of 0.09% or lower suggesting that they would be highly significant biomarkers for a customized array list.

A. PAM Class Prediction—

PAM class prediction has also proven to be a powerful approach to identify putative biomarkers for graft recovery and graft loss. We have used both Cox-regression and PAM to correlate expression differences with graft outcome with good success. Both methods yield significant results in Kaplan-Meier survival analysis although at the initial 2-year follow-up genes identified by PAM also yield greater significance. (FIG. **2**—Kaplan-Meier survival analysis for graft loss (red) and no-loss (blue. The genes include ICAM5-FIG. **2**A; (p=0.007), IL6R; FIG. **2**B; (p=0.003), STAT1; FIG. **2**C; (p=0.036), and STAT6; FIG. **2**D; (p=0.020)).

The gene signature is dominated by increased expression of cell adhesion genes, selected cytokines, B-cell genes, representatives in the STAT signaling pathway and several immune response genes including multiple representatives of both class I and class II HLA genes.

Representative genes include those from HLA class I (HLA-F, HLA-G), HLA class II (HLA-DRB1, HLA-DRB5, HLA-DRB4), signal transducers (STAT1, STAT6), immunoglobulin genes (IGKC, IGHG3), and 2 interferon gamma induced genes (ICAM5, IL6R).

A similar approach was used to identify graft-loss markers in whole blood samples. The list of the most highly-predictive significant genes in blood is summarized in Table 4, including the Kaplan-Meier survival significance score.

TABLE 4

Symbol	Gene	Unigene ID	Fold Loss/No-loss	p-value
HIST1H2BC	Histone 1, H2bc	Hs.356901	−3.46	0.00018
IGHG3	Ig heavy constant gamma 3 (G3m marker)	Hs.413826	4.14	0.00134
AHSA2	Activator of heat shock ATPase	Hs.122440	2.91	0.00041
TNFRSF10D	TNF receptor superfamily 10b	Hs.129844	−2.55	0.00010
MAPK9	Mitogen-activated protein kinase 9	Hs.348446	8.14	0.00444
IFNAR2	Interferon (alpha, beta and omega) receptor 2	Hs.86958	−2.37	0.01760
TM4SF9	Transmembrane 4 superfamily member 9	Hs.8037	−15.29	0.00580
MIF	Macrophage migration inhibitory factor	Hs.407995	−2.31	0.00674
SCYE1	Small inducible cytokine (Monocyte-activating)	Hs.105656	2.51	0.00154
MAPK1	Mitogen-activated protein kinase 1	Hs.324473	−2.32	0.00019
TGFBR3	TGFb receptor III (betaglycan)	Hs.342874	−2.94	0.00318
IGKC	Immunoglobulin kappa constant	Hs.377975	2.35	0.00290
IL1R2	Interleukin 1 receptor, type II	Hs.25333	−4.06	0.01762
IGL	Immunoglobulin lambda light chain		3.04	0.02093

The Kaplan-Meier survival curves for 8 of these genes are illustrated in FIG. **3**. The genes in FIG. **3** include A) AHSA2, B) IGHG1, C) IFNAR2, D) IGKC, E) HIST1H2BC, F) IL1R2, G) MAPK1, and H) MAPK9.

The functional composition of genes associated with acute rejection, predicted by analysis of Gene Ontology annotations, is summarized in Table 5.

TABLE 5

Gene Category	Genes	Genes on Array	EASE Score	Fisher Exact
defense response	105	747	7.15E−12	3.35E−12
response to stimulus/ acute phase response	152	1482	0.00000108	7.24E−07
apoptosis	50	361	0.00000772	3.63E−06
cell cycle	71	597	0.0000174	9.84E−06
cell proliferation	96	899	0.0000403	0.0000256
protein metabolism	176	1941	0.000228	0.000172
antigen presentation	9	29	0.000707	0.000123
cell growth and/or maintenance	244	2887	0.000766	0.000623
phosphorylation	53	512	0.00539	0.00353
protein modification	84	902	0.00775	0.00545
hemopoiesis	10	53	0.0116	0.00374
DNA replication	17	122	0.0125	0.00571
B-cell activation	6	22	0.0171	0.00356

The full list of known genes (in ranked order) in whole blood that are predictive of graft loss following acute rejection is summarized in Table 1. Of the 81 cDNA clones identified to have the highest predictive power, 62 are of known function or assigned unique Unigene Cluster IDs. Similarly, the list of known genes identified in renal biopsies predictive of graft loss following acute rejection is summarized in Table 2 (including 30 unique genes of known function from the 50 cDNA associated clones).

IV. Generation of a Transplant Custom Expression Chip

TxChip

We have compiled the gene lists described in this document for AR and graft loss along with other expression-based markers identified to be associated with clinical outcomes and severity of:

1. Acute rejection—including markers associated with graft loss and/or rate of recovery of renal function following AR (Table 3);
2. Chronic allograft injury (chronic rejection) in blood (Table 3);
3. Immunosuppressive drug toxicity or adverse side effects including drug-induced hypertension (Table 3);
4. Age or body mass index associated genes that correlate with renal pathology or account for differences in recipient age-related graft acceptance (Table 3);
5. Immune tolerance markers in whole blood (Table 3);
6. Control genes for assessing sample quality (3'- to 5'-bias in probe location), sampling error in biopsy-based studies, cell surface markers, and normalizing genes for calibrating hybridization results;
7. Genes found in literature surveys with immune modulatory roles that may play a role in transplant outcomes (Table 3) to produce the list for a representative array having probes to genes listed in Table 3.

A. Test of Expression Uniformity Across a Pilot Study of Renal Biopsies.

In the identification of the gene markers described in this invention disclosure, we compared the expression across a set of 67 renal biopsies described in detail by our laboratory. A subset of the biopsy-generated gene expression markers was used clustered to compare expression profiles in patients with confirmed cases of DT, CAN, AR and no significant abnormality (Normal). These patients were on two very different immunosuppressant regimes, either steroid-based or steroid-free (clinical regiment previously described in (Sarwal et al., Transplantation (2001) 72:13-21) and Sarwal et al., Transplantation (2003) 76:1331-9).

FIG. **4** illustrates that the gene expression is generally uniform/consistent across the full clinical groups analyzed as the gene expression levels segregate well within patient groups. Further, within each group (DT, CAN, AR or Normal) expression levels of these marker genes are independent of immunosuppression use.

The 479 gene list of Table 3 comprises design and specification for a customized thematic Transplant Chip (TxChip V1) and full-length mRNA sequences for these genes are listed in Table 3. The gene listing is cross-indexed to the studies listed above. We observe a modest overlap in the list of informative genes. For example, expression levels of IGHM positively correlate with acute rejection risk and negatively correlate with immune tolerance. An advantage of having the full compilation of genes on a common platform is that new discoveries like this can be made in future studies.

It is evident that subject invention provides a convenient and effective way of determining whether a graft in a subject will survive, e.g., following acute rejection. As such, the subject invention provides a number of distinct benefits, including the ability to identify clinically relevant AR groups with differing therapeutic responses and prognosis, and allow for individualized treatment and monitoring. As such, the subject invention represents a significant contribution to the art.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

What is claimed is:

[1. A method of evaluating graft survival in a subject, said method comprising:

assessing expression of at least two genes in a sample from said subject to evaluate graft survival in said subject, wherein said at least two genes comprises HIST1H2B and IGHG3.**]**

[2. The method according to claim **1**, wherein said expression of at least two genes is assessed by assaying said sample for a nucleic acid transcript of said gene.**]**

[3. The method according to claim **1**, wherein said expression of at least two genes is assessed by assaying said sample for an expression product of said gene.**]**

[4. The method according to any of claim **1**, wherein said sample is a blood sample.**]**

[5. The method according to claim **4**, wherein said blood sample is a peripheral blood sample.**]**

[6. The method according to claim **1**, wherein said sample is a tissue biopsy sample.**]**

[7. A method according to claim **1**, wherein the method comprises: obtaining an expression profile for a sample from said subject.**]**

[8. The method according to claim **7**, wherein said expression profile is compared to a reference expression profile.**]**

[9. The method according to claim **8**, wherein said expression profile is a nucleic acid expression profile.**]**

[10. The method according to claim **8**, wherein said expression profile comprises expression measurements for at least 5 different genes.**]**

[11. The method according to claim **8**, wherein said expression profile is determined using a microarray.**]**

[12. The method according to claim **11**, wherein said microarray is a genomic array.**]**

[13. A method of managing post-transplantation therapy in a subject, said method comprising:

(a) evaluating graft survival in said subject by a method according to claims **1**; and

(b) determining a post-transplantation therapy protocol based on said evaluation step (a);

to manage post-transplantation therapy in said subject.**]**

[14. The method according to claim **13**, wherein said subject is a human.**]**

[15. The method according to claim **1**, wherein said at least two genes further comprises one or more genes selected from: AHSA2, TNFRSF10D, MAPK9, IFNAR2, TM4SF9, MIF, SCYE1, MAPK1, TGFBR3, IGKC, IL1R2 and IGL.**]**

16. A method of treating a transplant recipient comprising the steps of:

(a) determining that the transplant recipient has a graft survival phenotype by evaluating results previously obtained from a quantitative determination of nucleic acid expression levels of at least three genes in a sample from said transplant recipient; and

treating said transplant recipient by maintaining a current therapeutic regimen; or

(b) determining that the transplant recipient has a graft loss phenotype by evaluating results previously obtained from a quantitative determination of the nucleic acid expression levels of at least three genes in a sample from said transplant recipient; and

treating said transplant recipient by increasing or decreasing a therapeutic regimen;

wherein, said evaluating comprises comparing said results to a reference nucleic acid expression profile comprising said at least three genes, and

wherein said at least three genes are selected from the group consisting of GZMK, DUSP1, IFNGR1, MAPK9, EPOR, FOXP3, IL7R, NKTR, ACTB, GBP1, IL7, ISG20, NFE2, PSMB9, STAT3 and TNFRSF1A.

17. The method according to claim 16, wherein said at least three genes comprises GZMK, NKTR, and EPOR.

18. The method according to claim 16, wherein said sample is a blood sample.

19. The method according to claim 16, wherein said blood sample is a peripheral blood sample.

20. The method according to claim 16, wherein said sample is a tissue biopsy sample.

21. The method according to claim 16, wherein said reference nucleic acid expression profile is predictive of a graft loss or graft survival phenotype.

22. The method according to claim 16, wherein said at least three genes is at least 5 genes.

23. The method according to claim 22, wherein said quantitative determination was performed using a microarray.

24. The method according to claim 23, wherein said microarray is a genomic array.

25. The method according to claim 16, wherein said at least three genes comprises DUSP1, IFNGR1, and NKTR.

26. The method according to claim 16, wherein said at least three genes comprises EPOR, CEACAM4, and MAPK9.

27. The method according to claim 16, wherein said at least three genes are selected from the group consisting of: GZMK, NKTR, EPOR, IFNGR1, DUSP1, and MAPK9.

28. The method according to claim 16, comprising: determining that the transplant recipient has a graft loss phenotype that is calcineurin-inhibitor drug nephrotoxicity (DT); and decreasing an immunosuppressive therapy.

29. The method according to claim 16, comprising: (i) determining that the transplant recipient has a graft loss phenotype that is chronic allograft nephropathy (CAN); and (ii) increasing an immunosuppressive therapy, or changing an immunosuppressive therapy by administering a different immunosuppressive drug.

* * * * *

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摘要(译)

提供了用于评估移植物存活的受试者的方法，例如就移植物存活的预测而言，鉴定受试者中存在有害的移植物状况，例如CAN和DT，识别急性排斥的严重程度和类别等提供。在实施本发明方法时，例如在核酸和/或蛋白质水平上测定来自受试者（例如血液或活检样品）的样品中至少一种基因的表达，以评估受试者。还提供了可用于实践主题方法的组合物，系统和试剂盒。该方法和组合物可用于各种应用。

FIG. 1