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(71) Applicant (for all designated States except US): **THE GOVERNORS OF THE UNIVERSITY OF ALBERTA** [CA/CA]; TEC Edmonton, 10230 - Jasper Avenue, Suite 4000, Edmonton, Alberta T5J 4P6 (CA).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **HALLORAN, Philip F.** [CA/CA]; 163 Wolf Willow Crescent, Edmonton, Alberta T5T 1T3 (CA).

(74) Agents: **BARTOSZEWICZ, Lola, A.** et al.; Sim & McBurney, 330 University Avenue 6th Floor, Toronto, Ontario M5G 1R7 (CA).

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(54) Title: MOLECULAR DIAGNOSIS OF ANTIBODY MEDIATED REJECTION

(57) Abstract: This document relates to methods and materials involved in assessing antibody mediated rejection of tissues (e.g., transplanted organs) in mammals.



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MOLECULAR DIAGNOSIS OF ANTIBODY MEDIATED REJECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application
5 Serial No. 61/366,786, filed on July 22, 2010.

TECHNICAL FIELD

This document is related to methods and materials involved in assessing tissue
rejection (e.g., organ rejection) in mammals. For example, this document relates to
10 methods and materials involved in detecting antibody mediated tissue rejection (e.g.,
kidney rejection) in mammals.

BACKGROUND

The diagnosis of allograft rejection remains an important issue in kidney
15 transplantation. Rejection can manifest as an acute episode or as subtle loss of function,
proteinuria, scarring, and graft loss (Meier-Kriesche et al., *Am J Transplant*, 4(3):378-383
(2004)). Two mechanisms of rejection are recognized in the Banff histologic
classification (Solez et al., *Am J Transplant*, 7(3):518-526 (2007); Racusen et al., *Am J*
Transplant, 4(10):1562-1566 (2004)): T cell mediated rejection (TCMR), diagnosed by
20 scoring interstitial inflammation, tubulitis, and vasculitis; and antibody-mediated rejection
(ABMR), a hallmark of which is C4d deposition in peritubular capillaries (Racusen et al.,
Am J Transplant, 3(6):708-714 (2003)). Histologically, the diagnosis of acute/active
ABMR also requires the presence of at least one of the following lesions: microthrombi,
arterial fibrinoid necrosis, glomerulitis, capillaritis, or acute tubular necrosis. In addition,
25 active episodes of antibody mediated immune responses can be superimposed on chronic
antibody mediated allograft pathology, which is hallmarked by arterial intimal fibrosis,
interstitial fibrosis/tubular atrophy, duplication of the glomerular basement membrane
(i.e., transplant glomerulopathy), and lamination of peritubular capillary (PTC) basement
membranes. The first two of these findings are nonspecific, and an accurate
30 demonstration of PTC basement membrane lamination also requires electron microscopy,
which is not routinely performed at most centers.

ABMR was first defined as a syndrome of graft dysfunction, biopsy findings of microcirculation damage (glomerulitis, capillaritis, microthrombi), and circulating donor specific antibody (DSA) (Halloran et al., *Transplant*, 49(1):85-91 (1990); and Trpkov et al., *Transplant*, 61:1586-1592 (1996)). ABMR was later found to correlate with C4d deposition in PTC (Feucht et al., *Kidney Int*, 43:1333-1338 (1993)). This led to the realizations that ABMR is responsible for many cases of late kidney transplant rejection, and that persistent ABMR leads to transplant glomerulopathy (Issa et al., *Transplant*, 86(5):681-685 (2008); Gloor et al., *Am J Transplant*, 7(9):2124-2132 (2007); Sis et al., *Am J Transplant*, 7(7):1743-1752 (2007); and Haas et al., *Am J Transplant*, 7(3):576-585 (2007)). The Banff diagnostic criteria for ABMR requires C4d positivity (Racusen et al., *Am J Transplant*, 3(6):708-714 (2003)), although ABMR often is C4d-negative.

ABMR can be fulminant or subtle, early or late. The pathogenesis of ABMR is the result of damage incurred onto microvascular endothelium by DSA, and the effector mechanisms involved in the early cases may differ from those acting in the subtle late cases. DSA to class I HLA often is a feature of the early cases, whereas DSA to class II HLA is predominant in late cases (Lee et al., *Transplant*, 88(4):568-574 (2009)). The pathogenesis of the endothelial damage in ABMR may involve direct actions of DSA (Zhang and Reed, *Am J Transplant*, 9(11):2459-2465 (2009) or complement mediated damage, but the presence of mononuclear cells and neutrophils in the glomerular or peritubular capillaries strongly suggests that cells could mediate the endothelial injury through their Fc receptors and/or complement receptors (Sis and Halloran, *Curr Opin Organ Transplant*, 15(1):42-48 (2010)), and opens the possibility of antibody-dependent cell-mediated cytotoxicity (ADCC), which can be mediated by NK cells and to some extent monocytes and neutrophils.

SUMMARY

This document provides methods and materials involved in assessing tissue rejection (e.g., organ rejection) in mammals. For example, this document provides methods and materials involved in the detection of ABMR in transplanted tissue (e.g., human kidney transplants) through the analysis of kidney biopsies for cause (BFC). In some embodiments, methods provided herein can include detection of expression levels of Donor Specific Antibody Selective Transcripts (DSASTs) in kidney biopsies to selectively identify ABMR. The methods were developed based on the finding that DSA

status (positive or negative) can allow identification of transcripts selectively expressed in ABMR in the same population, and on the identification of molecules associated with DSA and their use in defining new diagnostic criteria for ABMR. Early diagnosis of patients rejecting transplanted tissue can allow those patients to be treated sooner, which
5 can increase graft survival rates. Further, DSAST expression levels can be used to selectively identify ABMR with improved accuracy over conventional histopathology. For example, the methods described herein can be used to identify subtle ABMR cases where histopathology error rates are high. The improved accuracy of detection may lead to more appropriate patient therapy regimes and better endpoints for clinical trials.

10 This document is based in part on the discovery of nucleic acids that are differentially expressed in DSA positive and DSA negative kidney tissues undergoing rejection. The levels of these nucleic acids and/or polypeptides encoded by these nucleic acids can be used to determine whether tissue transplanted into a mammal is undergoing ABMR. The levels of multiple nucleic acids or polypeptides can be detected
15 simultaneously using nucleic acid or polypeptide arrays.

Methods for detecting ABMR of tissue transplanted into a mammal are provided herein. The methods can comprise, or consist essentially of, determining whether or not tissue transplanted into a mammal contains cells having an ABMR signature, wherein the presence of the cells indicates that the tissue is undergoing ABMR. The mammal can be a
20 human. The tissue can be kidney tissue. The tissue can be a kidney. The methods can comprise using kidney cells obtained from a biopsy to assess the presence or absence of the ABMR signature. The determining step can comprise analyzing nucleic acids, or analyzing polypeptides.

Unless otherwise defined, all technical and scientific terms used herein have the
25 same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the
30 present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and

advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

5 Figures 1A-1C are diagrams depicting algorithms used to define transcripts associated with DSA. Figure 1A is a diagram of an algorithm for class comparison between biopsies from DSA-positive patients to biopsies from DSA-negative (DSA neg) patients, which yielded DSA-associated transcripts. Figure 1B is a diagram of an algorithm for class comparisons between biopsies from HLA class I DSA-positive (DSA I+)
10 I+) patients to biopsies from HLA class I DSA negative (DSA I neg) patients, and between biopsies from HLA class II DSA-positive (DSA II+) patients to those from HLA class II DSA negative (DSA II neg) patients. Figure 1C is a Venn diagram showing the overlap in the number of transcripts selected by each of the algorithms depicted in Figure 1A and Figure 1B.

15 Figure 2A is a graph plotting biological annotation of 132 transcripts associated with DSA generated in a class comparison across all 145 biopsies. Biological annotation was based on previous annotation in pathogenesis based transcript lists, expression in a human primary cell panel, or gene name. Figure 2B is a graph plotting biological annotation of 40 transcripts associated with DSA generated in a class comparison across
20 89 late (>1 year post-transplant) biopsies. Biological annotation was based on previous annotation in pathogenesis based transcript lists, expression in a human primary cell panel, or gene name. Figure 2C is a graph plotting transcript set scores generated for each individual biopsy using 132 DSA-associated transcripts. Biopsies were arranged according to their revised diagnosis and biopsies from DSA-positive patients are
25 indicated by striped dots, biopsies from DSA-negative patients as stippled dots, and those from PRA negative patients as gray dots. Normal nephrectomy samples were used as controls and are depicted as white dots.

 Figure 3A is a diagram of an algorithm for the identification of DSASTs. DSASTs were generated by starting with the 132 DSA-associated transcripts and
30 determining how many remained differentially expressed between DSA-positive and DSA-negative groups when compared across the 78 rejection-classified biopsies. Figures 3B-3D are graphs plotting probeset signals for the indicated genes in the indicated cell types. Figure 3B shows DSASTs with preferential expression in human NK cells. Figure

3C shows DSASTs with preferential expression in HUVECs. Figure 3D shows DSASTs with high expression in HUVECs or inducible in HUVECs and also expressed in other cell types.

Figure 4 is a graph plotting DSAST scores according to HLA antibody status.

5 DSAST set scores were generated for each individual biopsy and biopsies were arranged according to their revised diagnosis. Biopsies from DSA-positive patients are indicated by striped dots, biopsies from DSA-negative patients as stippled dots, and those from PRA negative patients as gray dots. Normal nephrectomy samples were used as controls and are depicted as white dots.

10 Figure 5A is a picture of a kidney transplant biopsy section showing peritubular capillaritis (Periodic acid-Schiff, original magnification x600). Figure 5B is a graph comparing mean numbers of intraluminal CD56+, CD68+, or CD3+ cells in five peritubular capillaries in biopsies with antibody-mediated rejection (ABMR) versus T cell-mediated rejection (TCMR). Figure 5C is a representative picture showing
15 intracapillary CD56+ NK cells (left panel), CD68+ macrophages (center panel), and CD3+ T cells (right panel; immunoperoxidase, original magnification x600).

DETAILED DESCRIPTION

This document provides methods and materials related to assessing tissue
20 rejection (e.g., organ rejection). For example, this document provides methods and materials that can be used to identify a mammal (e.g., a human) as having transplanted tissue that is being rejected via ABMR. A mammal can be identified as having transplanted tissue that is undergoing ABMR if it is determined that the transplanted tissue in the mammal contains cells having an ABMR signature, in which genes disclosed
25 herein have increased expression compared to typical expression levels in non-rejected tissue. For the purposes of this document, the term "ABMR signature" refers to a nucleic acid or polypeptide profile in a sample where one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1, or one or more of the
30 nucleic acids or polypeptides encoded by members of a subset of the nucleic acids listed in Table 1, are present at an elevated level compared to the corresponding expression levels in non-rejected tissue. In some embodiments, an ABMR signature can be a nucleic acid or polypeptide profile in a sample where one or more (e.g., one, two, three, four,

five, six, seven, eight, nine, ten, 11, 12, or 13) of the nucleic acids or polypeptides encoded by *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, and *TM4SF18* are present at an elevated level compared to corresponding expression levels in non-rejected tissue. In some cases, an

5 ABMR signature can be a nucleic acid or polypeptide profile in a sample where five or more (e.g., five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1 are present at an elevated level compared to the corresponding expression level in non-rejected tissue, provided that at least one of the five or more nucleic acids or polypeptides

10 is encoded by *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, or *TM4SF18*. In some embodiments, an ABMR signature can be a nucleic acid or polypeptide profile in a sample where at least forty percent (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1

15 are present at an elevated level compared to corresponding expression levels in non-rejected tissue.

It will be appreciated that the mean expression level of one or more of the nucleic acids or polypeptides encoded by the nucleic acids comprising the ABMR signature described herein can be used to identify mammals as having tissue that is being rejected

20 via ABMR. For example, a mammal can be identified as having transplanted tissue that is undergoing ABMR if it is determined that the transplanted tissue in the mammal contains cells having a mean ABMR signature. For purposes of this document, the term "mean ABMR signature" refers to a nucleic acid or polypeptide profile in a sample where the mean expression level of one or more (e.g., one, two, three, four, five, six, seven,

25 eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1, or the mean expression level of one or more of the nucleic acids or polypeptides encoded by a subset of the nucleic acids listed in Table 1, is elevated as compared to the corresponding level in unrejected tissue. In some embodiments, a mean ABMR signature can be a nucleic acid or

30 polypeptide profile in a sample where the mean expression level of one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, or 13) of the nucleic acids or polypeptides encoded by *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, and *TM4SF18* is elevated as compared

to corresponding levels in unrejected tissue. In some cases, a mean ABMR signature can be a nucleic acid or polypeptide profile in a sample where the mean expression level of five or more (e.g., five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1 is elevated as compared to the corresponding level in unrejected tissue, provided that at least one of the five or more nucleic acids or polypeptides is encoded by *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, or *TM4SF18*. In some embodiments, a mean ABMR signature can be a nucleic acid or polypeptide profile in a sample where the mean expression level of forty percent or more (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the nucleic acids or polypeptides encoded by the nucleic acids listed in Table 1 are elevated as compared to the corresponding level in unrejected tissue.

The methods and materials provided herein can be used to assess tissue rejection in any mammal such as a human, monkey, horse, dog, cat, cow, pig, mouse, or rat. In addition, the methods and materials provided herein can be used to detect rejection of any type of transplanted tissue including, without limitation, kidney, heart, liver, pancreas, and lung tissue. For example, the methods and materials provided herein can be used to determine whether or not a human who received a kidney transplant is rejecting that transplanted kidney.

Any appropriate sample can be used to determine whether or not transplanted tissue is being rejected in a mammal. For example, biopsy (e.g., punch biopsy, aspiration biopsy, excision biopsy, needle biopsy, or shave biopsy), tissue section, lymph fluid, and blood samples can be used. In some cases, a tissue biopsy sample can be obtained directly from the transplanted tissue. In some cases, a lymph fluid sample can be obtained from one or more lymph vessels that drain from the transplanted tissue. In some cases, a urine sample can be used.

The term "elevated level" as used herein with respect to the level of a nucleic acid or polypeptide encoded by a nucleic acid disclosed herein is any level that is greater than a reference level for that nucleic acid or polypeptide. The term "suppressed level" as used herein with respect to the level of a nucleic acid or polypeptide encoded by a nucleic acid disclosed herein is any level that is lower than a reference level for that nucleic acid or polypeptide. The term "reference level" as used herein with respect to a nucleic acid or polypeptide encoded by a nucleic acid described herein that is being used to identify a

mammal as having transplanted tissue that is being rejected can be the level of that nucleic acid or polypeptide typically expressed by cells in tissues that are free of rejection. For example, a reference level of a nucleic acid or polypeptide can be the mean expression level of that nucleic acid or polypeptide, respectively, in cells isolated from
5 kidney tissue that has not been transplanted into a mammal.

Any appropriate number of samples can be used to determine a reference level. For example, cells obtained from one or more mammals (e.g., at least 5, 10, 15, 25, 50, 75, 100, or more mammals) can be used to determine a reference level. It will be appreciated that levels from comparable samples are used when determining whether or
10 not a particular level is an elevated level. For example, levels from one type of cells are compared to reference levels from the same type of cells. In addition, levels measured by comparable techniques are used when determining whether or not a particular level is an elevated or a suppressed level.

An elevated or suppressed level of a nucleic acid or polypeptide described herein
15 can be any level provided that the level is greater or lower, respectively, than a corresponding reference level for that nucleic acid or polypeptide. For example, an elevated level of a nucleic acid or polypeptide can be 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8, 3, 3.3, 3.6, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 15, 20, or more times greater than a reference level for that nucleic acid or polypeptide, respectively. A
20 suppressed level of a nucleic acid or polypeptide can be 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8, 3, 3.3, 3.6, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 15, 20, or more times lower than a reference level for that nucleic acid or polypeptide, respectively. In addition, a reference level can be any amount. For example, a reference level can be zero. In this case, any level greater than zero would be an elevated level.

25 Any appropriate method can be used to determine the level of a nucleic acid or polypeptide disclosed herein in a sample from a mammal. For example, quantitative PCR, in situ hybridization, or microarray technology can be used to measure the level of a nucleic acid. In some cases, polypeptide detection methods, such as immunochemistry techniques, can be used to measure the level of a polypeptide encoded by a nucleic acid
30 described herein. For example, antibodies specific for a polypeptide encoded by a nucleic acid disclosed herein can be used to determine the level of the polypeptide in a sample.

Once the level of a nucleic acid or polypeptide encoded by a nucleic acid described herein is determined in a sample from a mammal, then the level can be

compared to a reference level for that nucleic acid or polypeptide and used to assess tissue rejection in the mammal. For example, a level of one or more than one nucleic acid or polypeptide encoded by a nucleic acid disclosed herein as having increased expression in transplanted tissue undergoing ABMR as compared to normal nephrectomy tissue (e.g.,
 5 a level of one or more than one nucleic acid or polypeptide encoded by a nucleic acid listed in Table 1) that is higher in a sample from a mammal than the corresponding one or more than one reference level can indicate that the mammal comprises transplanted tissue that is being rejected, or that the mammal is susceptible to tissue rejection.

In some cases, the mean (e.g., geometric mean) of the expression levels of more
 10 than one nucleic acid or polypeptide encoded by a nucleic acid comprising an ABMR signature as described herein can be used to assess tissue rejection in a mammal. For example, the mean of the expression levels of two or more (e.g., two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids disclosed herein as having increased
 15 expression in tissue undergoing ABMR as compared to normal nephrectomy tissue can be used to determine whether a transplanted tissue is undergoing ABMR. In some embodiments, the mean of the expression levels of two or more (e.g., two, three, four, five, six, seven, eight, nine, ten, 11, 12, or 13) of *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, and *TM4SF18*
 20 can be used to determine whether a transplanted tissue is undergoing ABMR. In some cases, the mean of the expression levels of five or more (e.g., five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21) of the nucleic acids or polypeptides encoded by the nucleic acids disclosed herein as having increased expression in tissue undergoing ABMR as compared to normal nephrectomy tissue can be used to determine
 25 whether a transplanted tissue is undergoing ABMR, provided that at least one of the five or more nucleic acids is selected from the group consisting of *ICAM2*, *ROBO4*, *MALL*, *COL13A1*, *PLAT*, *SOX7*, *PLA1A*, *HLA-DRB3*, *SH2D1B*, *MYBL1*, *CX3CR1*, *GNLY*, and *TM4SF18*. In some embodiments, the mean of the expression levels of forty percent or more (e.g., 40%, 45%, 55%, 65%, 75%, 85%, 95%, or 100%) of the nucleic acids or
 30 polypeptides encoded by the nucleic acids disclosed herein as having increased expression in tissue undergoing ABMR as compared to normal nephrectomy tissue can be used to determine whether a transplanted tissue is undergoing ABMR.

The methods and materials provided herein can be used at any time following a

tissue transplantation to determine whether or not the transplanted tissue will be rejected. For example, a sample obtained from transplanted tissue at any time following the tissue transplantation can be assessed for the presence of cells expressing an elevated level of one or more nucleic acids or polypeptides encoded by nucleic acids provided herein. In some cases, a sample can be obtained from transplanted tissue 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more hours after the transplanted tissue was transplanted. In some cases, a sample can be obtained from transplanted tissue one or more days (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more days) after the transplanted tissue was transplanted. For example, a sample can be obtained from transplanted tissue 2 to 7 days (e.g., 4 to 6 days) after transplantation and assessed for the presence of cells expressing an elevated level of a nucleic acid or polypeptide encoded by a nucleic acid provided herein. Typically, a biopsy can be obtained any time after transplantation if a patient experiences reduced graft function.

Methods and materials provided herein can be used to assess the effectiveness of a treatment for transplant rejection in a mammal. For example, it can be determined whether or not a mammal having transplanted tissue that is being rejected, and having received a treatment for the transplant rejection, has a mean expression level of nucleic acids or polypeptides encoded by nucleic acids disclosed herein as having increased expression in rejected tissue as compared to unrejected tissue (e.g., nucleic acids or polypeptides encoded by nucleic acids listed in Table 1) that is lower than a corresponding expression level observed prior to treatment. The presence of the lower level can indicate that the treatment is effective. The absence of the lower level can indicate that the treatment is not effective.

This document also provides methods and materials to assist medical or research professionals in determining whether or not a mammal is undergoing tissue rejection. Medical professionals can be, for example, doctors, nurses, medical laboratory technologists, and pharmacists. Research professionals can be, for example, principle investigators, research technicians, postdoctoral trainees, and graduate students. A professional can be assisted by (1) determining the level of one or more than one nucleic acid or polypeptide encoded by a nucleic acid described herein in a sample, and (2) communicating information about each level to that professional.

Any method can be used to communicate information to another person (e.g., a professional). For example, information can be given directly or indirectly to a

professional. In addition, any type of communication can be used to communicate the information. For example, mail, e-mail, telephone, and face-to-face interactions can be used. The information also can be communicated to a professional by making that information electronically available to the professional. For example, the information can
5 be communicated to a professional by placing the information on a computer database such that the professional can access the information. In addition, the information can be communicated to a hospital, clinic, or research facility serving as an agent for the professional.

This document also provides nucleic acid arrays. The arrays provided herein can
10 be two-dimensional arrays, and can contain at least two different nucleic acid molecules (e.g., at least three, at least five, at least ten, at least 20, at least 30, at least 40, at least 50, or at least 60 different nucleic acid molecules). Each nucleic acid molecule can have any length. For example, each nucleic acid molecule can be between 10 and 250 nucleotides (e.g., between 12 and 200, 14 and 175, 15 and 150, 16 and 125, 18 and 100, 20 and 75, or
15 25 and 50 nucleotides) in length. In some cases, an array can contain one or more cDNA molecules encoding, for example, partial or entire polypeptides. In addition, each nucleic acid molecule can have any sequence. For example, the nucleic acid molecules of the arrays provided herein can contain sequences that are present within nucleic acids listed in Table 1.

20 In some cases, at least 25% (e.g., at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%, at least 95%, or 100%) of the nucleic acid molecules of an array provided herein contain a sequence that is (1) at least 10 nucleotides (e.g., at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or more nucleotides) in length and (2) at least about 95 percent (e.g., at least about 96, 97, 98, 99, or 100) percent
25 identical, over that length, to a sequence present within a nucleic acid disclosed herein. For example, an array can contain 60 nucleic acid molecules located in known positions, where each of the 60 nucleic acid molecules is 100 nucleotides in length while containing a sequence that is (1) 90 nucleotides in length, and (2) 100 percent identical, over that 90 nucleotide length, to a sequence of a nucleic acid provided herein. A nucleic acid
30 molecule of an array provided herein can contain a sequence present within a nucleic acid described herein where that sequence contains one or more (e.g., one, two, three, four, or more) mismatches.

The nucleic acid arrays provided herein can contain nucleic acid molecules attached to any suitable surface (e.g., plastic, nylon, or glass). In addition, any appropriate method can be used to make a nucleic acid array. For example, spotting techniques and *in situ* synthesis techniques can be used to make nucleic acid arrays.

5 Further, the methods disclosed in U.S. Patent Nos. 5,744,305 and 5,143,854 can be used to make nucleic acid arrays.

This document also provides arrays for detecting polypeptides. The arrays provided herein can be two-dimensional arrays, and can contain at least two different polypeptides capable of detecting polypeptides, such as antibodies (e.g., at least three, at

10 least five, at least ten, at least 20, at least 30, at least 40, at least 50, or at least 60 different polypeptides capable of detecting polypeptides). The arrays provided herein also can contain multiple copies of each of many different polypeptides. In addition, the arrays for detecting polypeptides provided herein can contain polypeptides attached to any suitable surface (e.g., plastic, nylon, or glass).

15 A polypeptide capable of detecting a polypeptide can be naturally occurring, recombinant, or synthetic. The polypeptides immobilized on an array also can be antibodies. An antibody can be, without limitation, a polyclonal, monoclonal, human, humanized, chimeric, or single-chain antibody, or an antibody fragment having binding activity, such as a Fab fragment, F(ab') fragment, Fd fragment, fragment produced by a

20 Fab expression library, fragment comprising a VL or VH domain, or epitope binding fragment of any of the above. An antibody can be of any type, (e.g., IgG, IgM, IgD, IgA or IgY), class (e.g., IgG1, IgG4, or IgA2), or subclass. In addition, an antibody can be from any animal including birds and mammals. For example, an antibody can be a mouse, chicken, human, rabbit, sheep, or goat antibody. Such an antibody can be capable

25 of binding specifically to a polypeptide described herein. The polypeptides immobilized on the array can be members of a family such as a receptor family.

Antibodies can be generated and purified using any suitable methods known in the art. For example, monoclonal antibodies can be prepared using hybridoma, recombinant, or phage display technology, or a combination of such techniques. In some cases,

30 antibody fragments can be produced synthetically or recombinantly from a nucleic acid encoding the partial antibody sequence. In some cases, an antibody fragment can be enzymatically or chemically produced by fragmentation of an intact antibody. In addition, numerous antibodies are available commercially. An antibody directed against

a polypeptide encoded by a nucleic acid disclosed herein can bind the polypeptide at an affinity of at least 10^4 mol^{-1} (e.g., at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} mol^{-1}).

Any method can be used to make an array for detecting polypeptides. For example, methods disclosed in U.S. Patent No. 6,630,358 can be used to make arrays for
5 detecting polypeptides. Arrays for detecting polypeptides can also be obtained commercially, such as from Panomics, Redwood City, CA.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

10

EXAMPLES

Example 1 – Materials and Methods

Patients and Sample Collection: The patient population in this study and details of the HLA antibody results have been described (Hidalgo et al., *Am J Transplant*, 9(11):2532-2541 (2009)). All consenting renal transplant patients were undergoing a
15 transplant biopsy for clinical indication (deterioration in function, proteinuria, stable impaired function) as standard of care. Biopsies were obtained under ultrasound guidance by spring-loaded needles (ASAP Automatic Biopsy, Microvasive, Watertown, MA). Only one biopsy – the last biopsy available – for each patient (N=145) was included in the analyses described herein.

20 *Histopathology:* Paraffin sections were graded according to Banff criteria (Solez et al., *Am J Transplant*, 8(4):753-760 (2008)) by a renal pathologist. C4d staining was performed on frozen sections using a monoclonal anti-C4d antibody (Quidel, San Diego, CA, USA) by indirect immunofluorescence. Diffuse linear C4d staining (>50% of biopsy area) was interpreted as positive. The criteria for C4d-negative ABMR or C4d-negative
25 Mixed ABMR plus TCMR were based on a previous description (Einecke et al., *Am J Transplant*, 9(11):2520-2531 (2009)): microvascular lesions of inflammation (g, or ptc>0) or microvascular deterioration (cg>0) from a patient with detectable HLA antibody (PRA positive) at the time of biopsy.)

Immunohistochemistry: The number of CD3+ (T cells), CD68+ (macrophages),
30 and CD56+ (NK cells / a minor subset of CD3+8+ T cells) was assessed by immunoperoxidase staining in 16-18 biopsies (depending on the marker stained) with available tissue. Antigen retrieval of formalin-fixed and paraffin-embedded tissue sections was performed with an ethylenediaminetetraacetic acid- (EDTA-) based buffer

on a Ventana BENCHMARK[®] XT. Tissue sections were incubated with anti-human CD68 (1:50, clone PG-M1, DAKO, Carpinteria, CA) or CD56 (1:25, clone 1B6, Novocastra, Australia) or rabbit polyclonal CD3 (1:75, DAKO, Carpinteria CA) primary antibody. Absolute numbers of intraluminal positive cells in cross-sections of five peritubular capillaries with the highest number of infiltrates were counted.

HLA antibody screening: Antibody specificities were determined by FLOWPRA[®] specific class I and or II and/or FLOWPRA[®] single antigen I and II beads (One Lambda Canoga Park, CA). Manufacturer's instructions for staining and acquiring were followed. Beads were analyzed on a BD FACSCalibur[™] cytometer (Becton Dickinson Biosciences, Mississauga Ontario, Canada).

Antibody screening was performed using FLOWPRA[®] beads. These beads have HLA-A, -B, -Cw -DR -DQ and -DP antigens represented. Further testing for specificities was only done if the screen was positive ($\geq 5\%$ PRA or clear pattern of reactivity with screening beads). Single antigen beads were used to test for antibodies against HLA-A, -B, -DRB1, -DRB3, 4 and 5, -DQB1 and DP. Specificities to Cw were not tested. Donor typing for DP was not performed and therefore DSA were not attributed to DP. *De novo* DSA was defined as a new DSA detected by single antigen bead technology and/or a donor specific flow crossmatch that was negative pre-transplant and positive at the time of biopsy. Flow T and B cell crossmatches were performed as previously described (Campbell et al., *Am J Transplant*, 7(10):2311-2317 (2007)).

HLA typing: Low to medium resolution HLA Class I and II typing was performed using the One Lambda Micro SSP assay as previously shown (Campbell et al., *supra*). Manufacturer's instructions for amplification and electrophoresis were followed.

Microarray analysis: One additional 18-gauge biopsy core was collected for gene expression analysis. The tissue was placed immediately in RNALater and stored at -20°C. RNA extraction, labeling and hybridization to the HG_U133_Plus_2.0 GeneChip (Affymetrix, SantaClara, CA) were carried out according to manufacturer's protocols. Microarrays were scanned using GeneArrayScanner (Affymetrix) and processed with GeneChip Operating Software Version 1.4.0 (Affymetrix). Microarray data were preprocessed by robust multi-array analysis (RMA), and implemented in Bioconductor version 2.4.

Microarray results from DSA-positive and DSA-negative classes were compared. For each probeset, the mean of the gene expression values in the two classes was

compared using the Genespring GX 7.3.1 (Agilent Technologies, Santa Clara, CA). False discovery rates (FDR) were calculated using the Benjamini and Hochberg test for multiple testing corrections. Transcript set scores were calculated using DSA-associated transcripts (N=132) or DSASTs (N=23) as the geometric mean of fold changes across all probesets within each transcript set. Results for the fold increases for the individual DSASTs in each of the biopsies in the study are included in Table S1.

Biopsies were categorized according to the patient's DSA results:

HLA class I DSA-positive – biopsies from patients who were HLA class I DSA-positive or HLA class I + II DSA-positive at the time of biopsy (N=23).

10 *HLA class I DSA-negative* – biopsies from patients who were HLA class II DSA-positive or DSA-negative or PRA negative at the time of biopsy (N=122).

HLA class II DSA-positive – biopsies from patients who were HLA class II DSA-positive or HLA class I + II DSA-positive at the time of biopsy (N=41).

15 *HLA class II DSA-negative* – biopsies from patients who were HLA class I DSA-positive or DSA-negative or PRA negative at the time of biopsy (N=104).

Rejection classifier method and statistics were performed as described previously (Reeve et al., *Am J Transplant*, 9(8):1802-1810 (2009)).

20 *Pathogenesis-based transcript sets (PBTs)*: Transcript sets defining distinct biological processes involved in allograft rejection are detailed online at transplants.med.ualberta.ca. Abbreviations for all transcripts conform to the Gene names (World Wide Web at ncbi.nlm.nih.gov/sites/entrez).

Cell Isolations and treatments: Purified cell populations from peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of healthy volunteers as previously described (Hidalgo et al., *Am J Transplant*, 8(3):627-636 (2008)). All leukocyte cell cultures were maintained in complete RPMI (RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, β -mercaptoethanol, non-essential amino acids, sodium pyruvate, and antibiotic/antimycotic (Invitrogen Life Technologies, Burlington, ON, Canada) in 5% CO₂ at 37°C. Human umbilical vein endothelial cells (HUVECs) (ATCC, Manassas, VA) and renal proximal tubule epithelial cells (RPTECs) (Lonza, Inc., Allendale, NJ) were maintained in tissue culture media as recommended by the supplier.

Example 2 – Transcripts in the biopsy associated with circulating DSA

A cohort of 145 patients undergoing a renal transplant biopsy for clinical indications was used. The same cohort previously had been studied for histopathology lesions associated with HLA antibodies (Hidalgo et al. (2009), *supra*). These biopsies
 5 included all consenting patients presenting for a BFC for whom HLA antibody results were available, and were not selected for diagnoses.

Transcripts associated with DSA were defined by comparing transcript expression in biopsies from DSA-positive versus DSA-negative patients. 290 transcripts more identified as being more strongly expressed in biopsies from DSA-positive versus DSA-
 10 negative patients (Figure 1A) at a FDR<0.05 and 132 transcripts at FDR<0.005. To simplify biological annotation of transcripts, subsequent analysis used the 132 transcripts selected at FDR<0.005.

The relative contribution of HLA class I versus class II specificities to the DSA associated transcripts was then analyzed. Biopsies from 41 anti-class II DSA-positive
 15 patients were compared to 104 biopsies from anti-class II DSA-negative patients, and 272 transcripts were identified as being differentially expressed at a FDR<0.05 (Figure 1B). Most class II DSA associated transcripts (198/272) also were in the total DSA associated transcript set (Figure 1C). In contrast, comparison of biopsies from 23 anti-class I DSA-positive patients versus 122 biopsies from anti-class I DSA-negative patients yielded no
 20 differentially expressed transcripts (FDR<0.05).

Example 3 – Biological annotation of DSA associated transcripts

The biological processes represented by the 132 DSA-associated transcripts were examined based on their annotation as members of pathogenesis-based transcript sets
 25 (PBTs; online at transplants.med.ualberta.ca) or reports in the literature. Many transcripts (50/132) reflected IFNG effects, including 32 HLA transcripts (Figure 2A). Also associated with DSA were 11 macrophage transcripts, nine transcripts selectively associated with NK cells, and 10 transcripts with high expression in NK cells as well as CD8 positive CTL. Typical T cell transcripts (e.g., CD2, CD3D, ITK) were not found,
 30 however. Fourteen transcripts were associated with endothelial cells, while 17 showed high expression across multiple inflammatory cells including CTL, NK cells, B cells, and macrophages. Immunoglobulin transcripts (18/132) also were identified, probably reflecting plasma cells, but no previously defined B cell selective transcripts (Einecke et

al., *Am J Transplant*, 8(7):1434-1443 (2008)) were identified. Three transcripts had not been previously annotated.

Because DSA is more frequent in late biopsies, some DSA-associated transcripts may actually be features of late biopsies rather than truly DSA associated. To distinguish
 5 the true DSA association from associations with time, transcripts associated with DSA across late biopsies only (more than one year post-transplant, N=89) were defined. This yielded 40 transcripts (FDR<0.01), 30 of which were shared with the original DSA associated transcripts (Figure 2B). Although most transcripts reflected the same biological processes as the original DSA-associated transcripts, immunoglobulin
 10 transcripts were eliminated. Thus, immunoglobulin transcripts are associated with late biopsies (Einecke et al. (2008), *supra*) but are not truly associated with DSA.

The mean expression of the 132 DSA-associated transcripts was compared across all biopsies to the histological diagnoses and HLA antibody status (Figure 2C): borderline (N=20), C4d-positive ABMR (N=13), C4d-negative ABMR (N=20), TCMR (N=16),
 15 C4d-positive mixed (N=3), C4d-negative mixed (N=5), polyoma virus nephropathy (N=3), glomerulonephritis (GN) (N=23), and other (N=43), compared to control kidneys. DSA-associated transcripts were more highly expressed in biopsies with ABMR, including C4d-positive and C4d-negative ABMR and Mixed, but also highly expressed in many biopsies with TCMR.

20

Example 4 – Defining DSA-selective transcripts

It was hypothesized that DSA-associated transcripts selective for ABMR might be selectively expressed in rejecting cases with DSA, and not in rejecting cases lacking DSA. Biopsies were defined as having molecular features of rejection (ABMR or
 25 TCMR) using the previously described classifier (Reeve et al., *supra*), which indicated that 78/145 biopsies had rejection. Transcripts preferentially expressed in DSA-positive compared to DSA-negative rejection then were identified; 23 of the 132 transcripts were identified as DSA *selective* transcripts (DSASTs) (FDR<0.05) (Figure 3A).

Example 5 – DSASTs are primarily expressed either in NK cells or endothelium

30 The expression of the 23 DSASTs (for 21 unique genes) was studied in primary human cell types, either purified or cultured (Figure 3B, 3C, and 3D, and Table 1): allostimulated CD4 and CD8 CTL, NK cells, B cells, macrophages, HUVECs, and

RPTECs. Macrophages, HUVECs and RPTECs treated with human IFN- γ also were included. DSASTs were preferentially expressed in two cell types: endothelium and NK cells. In contrast, no DSASTs were selectively expressed in CTL, B cells, macrophages, or RPTECs.

5 Seven transcripts (six unique genes) were selective for NK cells (Figure 3B): fractalkine receptor (CX3CR1), myeloblastosis viral oncogene homolog (MYBL1), fibroblast growth factor binding protein 2 (FGFBP2, also known as KSP37), killer cell lectin-like receptor F1 (KLRF1, also known as NKp80), and SH2 domain containing 1B (SH2D1B, also known as EAT2). Transcripts encoding the cytotoxic molecule
 10 granulysin (GNLY) showed high expression in NK cells but were also expressed in CD4 and CD8 positive CTL as described previously (Hidalgo et al. (2008), *supra*). Thus these DSASTs are selective for NK cells, although some are shared with T cells, as expected from the close relationships between these cell types (Pingiotti et al., *Ann N Y Acad Sci*, 1107:32-41 (2007)). Nevertheless, most T cell-specific transcripts (e.g., CD2, CD3D,
 15 etc.) were not increased in biopsies from DSA-positive patients, although they are prominent in TCMR (Famulski et al., *Am J Transplant*, 10(4):810-820 (2010)). Thus the expression of NK-selective DSASTs reflects the presence of NK cells, and cannot be explained by the presence of T cells.

 Eight DSASTs were primarily expressed in endothelium. Some were previously
 20 described as increased in ABMR, including cadherin 13 (CDH13), cadherin 5 (CDH5), and MALL (mal, T-cell differentiation protein-like, BENE) (Sis et al., *Am J Transplant*, 9(10):2312-2323 (2009)). CDH5, MALL, ROBO4, and SOX7 showed the most selective expression in HUVECs (Figure 3C). Intercellular adhesion molecule 2 (ICAM2) showed high expression in HUVECs but was also expressed in some leukocytes, particularly NK
 25 cells (Figure 3D).

 Most of the remaining eight DSASTs (seven unique genes) showed high expression in HUVECs, and were expressed in other cell types. Guanine nucleotide binding protein (GNG11), tissue plasminogen activator (PLAT), and transmembrane 4L six family member 18 (TM4SF18) all showed high expression in HUVECs and were also
 30 expressed in RPTECs (Figure 3D). Neither Duffy blood group (DARC) nor phosphoglucomutase 5 (PGM5) showed high expression in HUVECs, but DARC is expressed in postcapillary venule endothelium (Peiper et al., *J Exp Med*, 181(4):1311-1317 (1995)). Phospholipase A1 member A (PLA1A) was IFNG inducible in HUVECs

and RPTECs, while HLA-DRB3 showed high expression in macrophages and B cells as expected.

Example 6 – DSASTs are selectively increased in biopsies with ABMR

5 DSAST expression was compared to the histopathology diagnosis of each biopsy that had previously been established, independent of molecular features. Of the 25 biopsies with the highest DSAST scores (Table 2), 22 (88%) were diagnosed by histology as ABMR, either C4d-positive or C4d-negative. Of interest, all three biopsies with high DSAST score that did not meet current criteria for histologic ABMR were nevertheless
10 from *de novo* DSA-positive patients, raising the possibility that they had ABMR features that are not currently recognized. Thus every one of the 25 biopsies with the highest DSAST scores was either previously diagnosed as ABMR or was from a DSA-positive patient. Nine of 13 Banff C4d-positive ABMR biopsies were in the top 25 DSAST scores.

15 DSAST scores were examined across all biopsies with regard to histological diagnosis (Figure 4). Scores were highest for biopsies diagnosed as ABMR or Mixed, with C4d-positive ABMR biopsies carrying overall higher scores than C4d-negative ABMR. One biopsy diagnosed as C4d-positive ABMR and another diagnosed as C4d-positive Mixed showed low DSAST scores in each category; these biopsies had received
20 steroid bolus treatment within two weeks prior to the biopsy. Biopsies diagnosed as Borderline, Other, or polyoma virus nephropathy showed overall low DSAST scores. The DSASTs were selective for ABMR: most TCMR biopsies show a low DSAST score compared to ABMR or Mixed biopsies. Biopsies with GN show DSAST scores similar to TCMR but those with the highest scores were primarily from DSA-positive patients,
25 suggesting that subtle forms of ABMR may be missed in some biopsies.

Example 7 – NK cells and macrophages are selectively increased in PTC in ABMR

A representative of peritubular capillaritis in a kidney transplant biopsy (Periodic acid-Schiff, original magnification x 600) is shown in Figure 5A. Cell counting was
30 performed in sections stained for CD3, CD68, and CD56 in biopsies where tissue was available (six C4d-positive ABMR, six C4d-negative ABMR, and six TCMR). Many biopsies for cause did not have adequate tissue remaining for these immunostaining studies. The average number of CD56+ NK cells ($p=0.006$) and CD68+ macrophages

($p=0.03$) in peritubular capillaries was higher in C4d-positive or C4d-negative ABMR biopsies versus those with TCMR (Figure 5B). In contrast, CD3+ T cells in peritubular capillary were not elevated in ABMR. Representative pictures of intracapillary CD3+, CD68+, or CD56+ cells are shown in Figure 5C. Therefore NK cells, as well as
5 macrophages, are found in areas of antibody-mediated microcirculation injury.

Taken together, these studies identified transcript associations in BFCs from DSA-positive patients at the time of biopsy. Comparison of biopsies from DSA-positive versus negative patients revealed 132 transcripts more strongly expressed in BFC from DSA-positive patients ($FDR < 0.005$) primarily reflecting IFNG effects, NK cells, and
10 endothelium. Thus DSA in the recipient at the time of a BFC was associated with transcripts reflecting a strong inflammatory state in the biopsy, some features of which were shared with TCMR. After removing transcripts expressed in rejecting biopsies from DSA-negative patients, 23 transcripts were identified as being selectively expressed in rejecting biopsies from DSA-positive patients – the DSASTs (Table 1). The selectivity of
15 DSASTs for ABMR is evidenced by the highest 25 DSAST scores being from BFC from HLA antibody positive patients, and 88% had already been diagnosed as ABMR or mixed based on histologic criteria. Immunostaining showed CD56+ and CD68+ cells selectively increased in biopsies with ABMR. The results associate NK cells with ABMR but not with TCMR, and are compatible with a role for NK cells in the mechanism of
20 microcirculation injury in ABMR. The fact that NK cells are found selectively in peritubular capillaries in ABMR suggests that NK markers could be developed as diagnostic aids.

Table 1: Detailed annotation of the transcripts making up the DSASTs ($N=23$).

Affymetrix probeset	Gene Title	Gene Symbol	Alias	Genbank	Biological Association
208335_s at	Duffy blood group	DARC	FY	NM_002036	Endothelium
213620_s at	intercellular adhesion molecule 2	ICAM2		AA126728	Endothelium
226028_at	roundabout homolog 4, magic roundabout (Drosophila)	ROBO4		AA156022	Endothelium
204677_at	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	VE-cadherin	NM_001795	Endothelium
204115_at	guanine nucleotide binding protein (G protein), gamma 11	GNG11		NM_004126	Endothelium
209373_at	T-cell differentiation protein-like	MALL	BENE	BC003179	Endothelium
211343_s at	collagen, type XIII, alpha 1	COL13A1		M33653	Endothelium
201860_s at	plasminogen activator, tissue	PLAT	tPA	NM_000930	Endothelium
204726_at	cadherin 13, H-cadherin (heart)	CDH13	H-cadherin	NM_001257	Endothelium
206702_at	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)	TEK		NM_000459	Endothelium
228698_at	SRY (sex determining region Y)-box 7	SOX7		A1808807	Endothelium
219584_at	phospholipase A1 member A	PLA1A	PSPLA1	NM_015900	IFN-g response
221491_x at	major histocompatibility complex, class II, DR beta 3	HLA-DRB3	HLA-DR52	AA807056	MHC
223836_at	fibroblast growth factor binding protein 2	FGFBP2	KSP37	AB021123	NK cells
1553177_at	SH2 domain-containing molecule EAT2	SH2D1B	EAT2	BC022407	NK cells
213906_at	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	MYBL1	MYBL1	AW592266	NK cells
205898_at	chemokine (C-X3-C motif) receptor 1	CX3CR1	Fractalkine receptor	U20350	NK cells
220646_s at	killer cell lectin-like receptor subfamily F, member 1	KLRF1	NKp80	NM_016523	NK cells
205495_s at	granulysin	GNLY		NM_006433	NK cells/CTL
226303_at	phosphoglucomutase 5	PGM5		AA706788	Multiple
228489_at	transmembrane 4 L six family member 19	TM4SF18		A1721164	Multiple

Table 2: Biopsy code, revised diagnosis and DSA status of the 25 biopsies with highest DSAST scores. DSA status; de novo DSA – de novo, pre-existing DSA – pre-ex, DSA-negative – NDSA

Biopsy Code	Revised Diagnosis	DSA status	DSAST score
T29	C4d neg Mixed	de novo	4.01
A10	C4d pos ABMR	de novo	3.54
M3	C4d pos Mixed	pre-ex	3.12
O36	C4d neg ABMR	NDSA	3.10
A17	C4d pos ABMR	de novo	2.81
A12	C4d pos ABMR	de novo	2.73
A14	C4d pos ABMR	de novo	2.68
O10	C4d neg ABMR	pre-ex	2.56
O32	C4d neg ABMR	de novo	2.40
A6	C4d pos ABMR	de novo	2.34
A4	C4d pos ABMR	de novo	2.30
B23	C4d neg ABMR	de novo	2.26
A15	C4d pos ABMR	de novo	2.26
M4	C4d pos Mixed	pre-ex	2.22
A9	C4d pos ABMR	de novo	2.20
A8	C4d pos ABMR	de novo	2.16
T44	C4d neg Mixed	de novo	2.08
O100	GN	de novo	2.07
B12	C4d neg ABMR	pre-ex	2.05
O103	C4d neg ABMR	de novo	2.05
O80	C4d neg ABMR	NDSA	1.90
O115	GN	de novo	1.86
O22	C4d neg ABMR	de novo	1.86
O13	C4d neg ABMR	pre-ex	1.85
T30	TCMR	de novo	1.83

5

Some complexity is anticipated: DSAST expression is unlikely to correspond to all histologic lesion-based ABMR diagnoses, because transcripts reflect disease activity, whereas lesions such as transplant glomerulopathy require sustained microcirculation

injury over time and persist when ABMR is inactive. DSAST scores probably do correlate with activity, being higher in C4d-positive ABMR than C4d-negative ABMR and perhaps reflecting greater activity in C4d-positive cases (Smith et al., *Am J Transplant*, 6(8):1790-1798 (2006)). Thus the emergence of the DSASTs, and potentially
5 other markers, offers an opportunity to reclassify ABMR on the basis of intensity and activity.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction
10 with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An *in vitro* method for detecting antibody mediated rejection (ABMR) of tissue transplanted into a mammal, said method comprising determining whether or not said tissue contains cells having an ABMR signature, wherein the presence of said cells indicates that said tissue is undergoing ABMR.
2. The method of claim 1, wherein said mammal is a human.
3. The method of claim 1, wherein said tissue is kidney tissue.
4. The method of claim 1, wherein said tissue is a kidney.
5. The method of claim 1, wherein said method comprises using kidney cells obtained from a biopsy to assess the presence or absence of said ABMR signature.
6. The method of claim 1, wherein said determining step comprises analyzing nucleic acids.
7. The method of claim 1, wherein said determining step comprises analyzing polypeptides.

Figure 1A

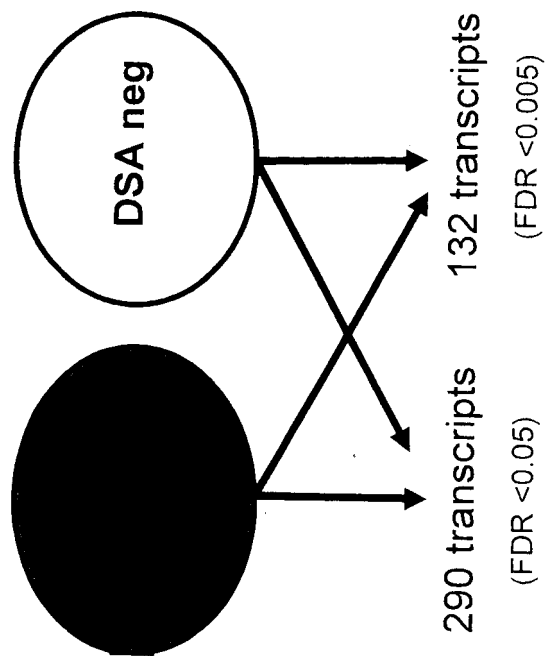


Figure 1B

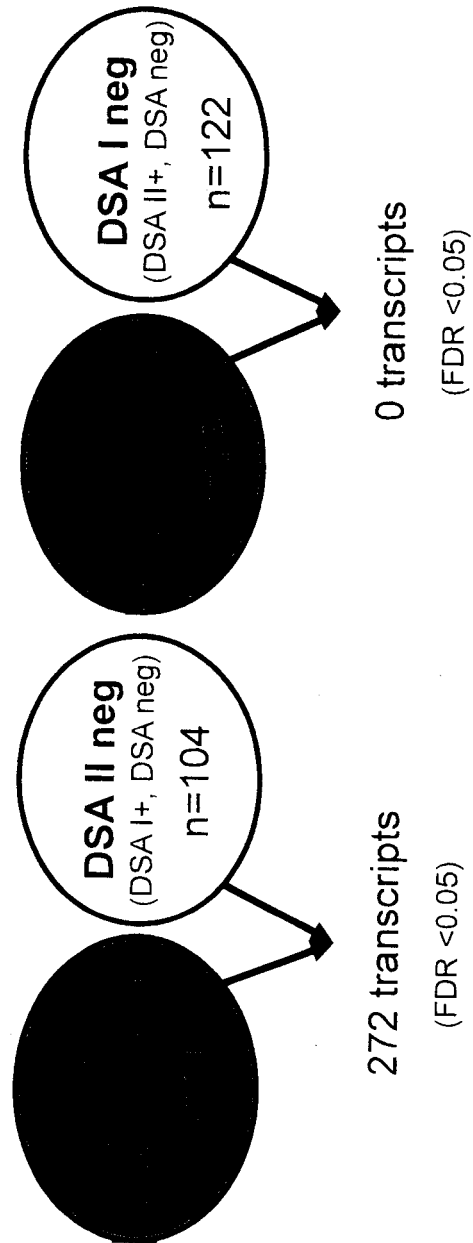


Figure 1C

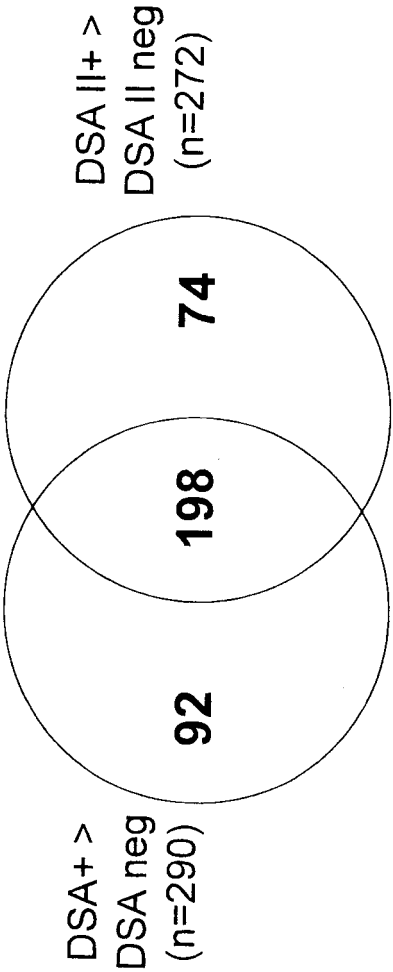


Figure 2A

Annotation of 132
DSA associated
transcripts using
all 145 biopsies:

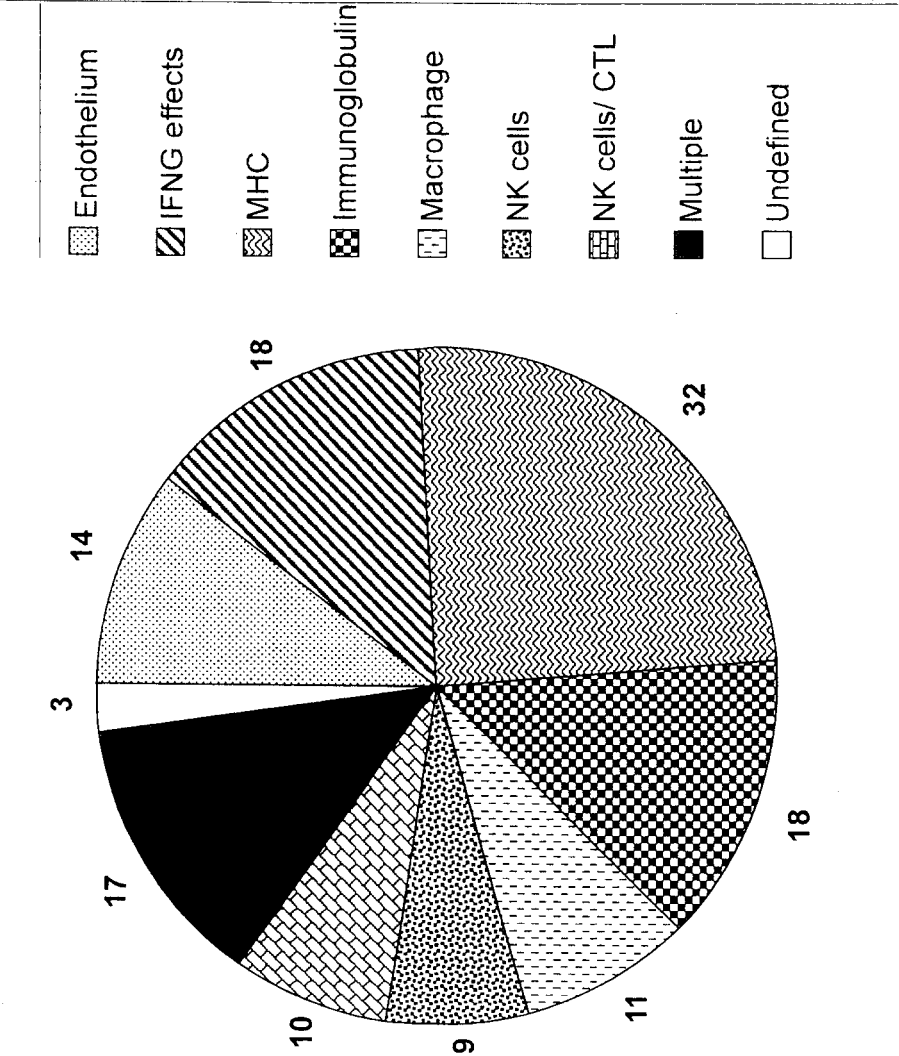
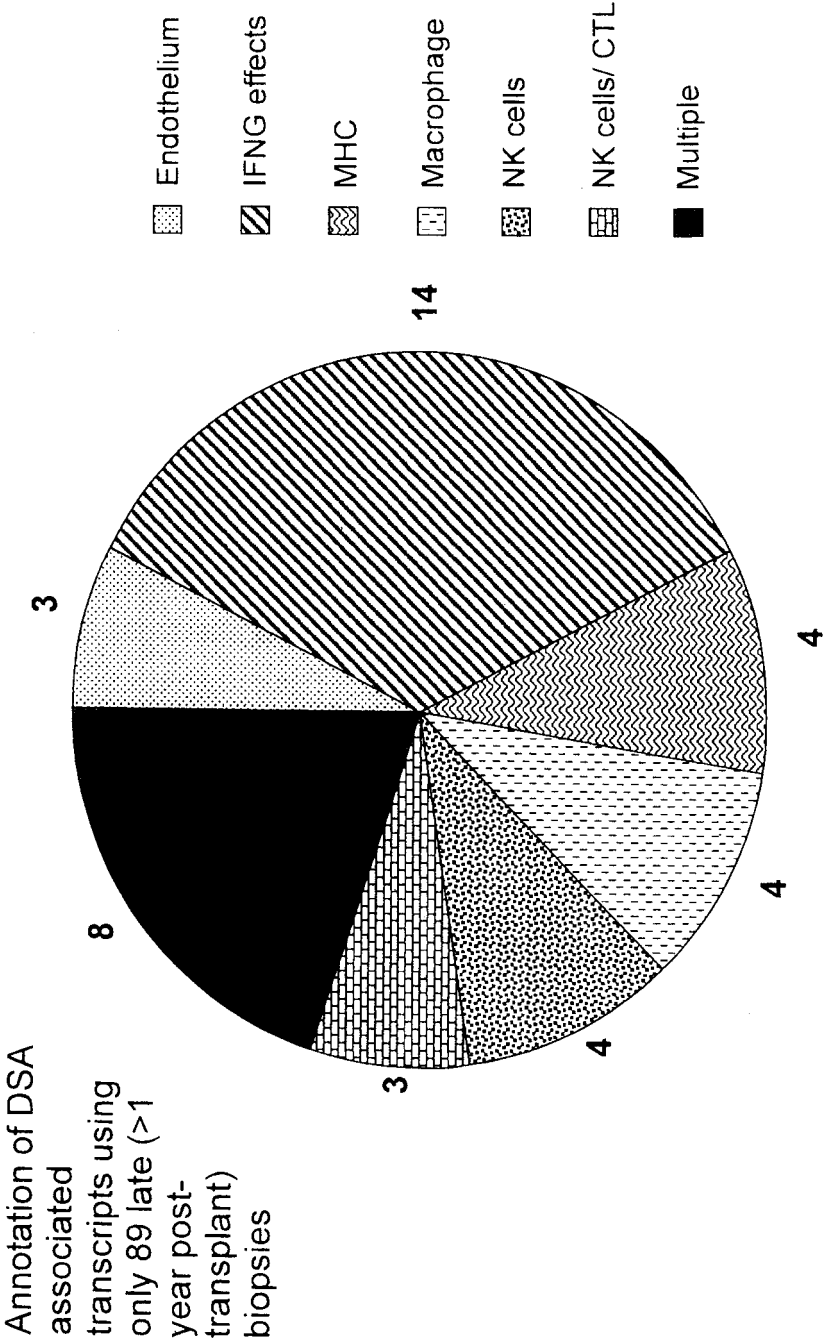


Figure 2B



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Figure 2C

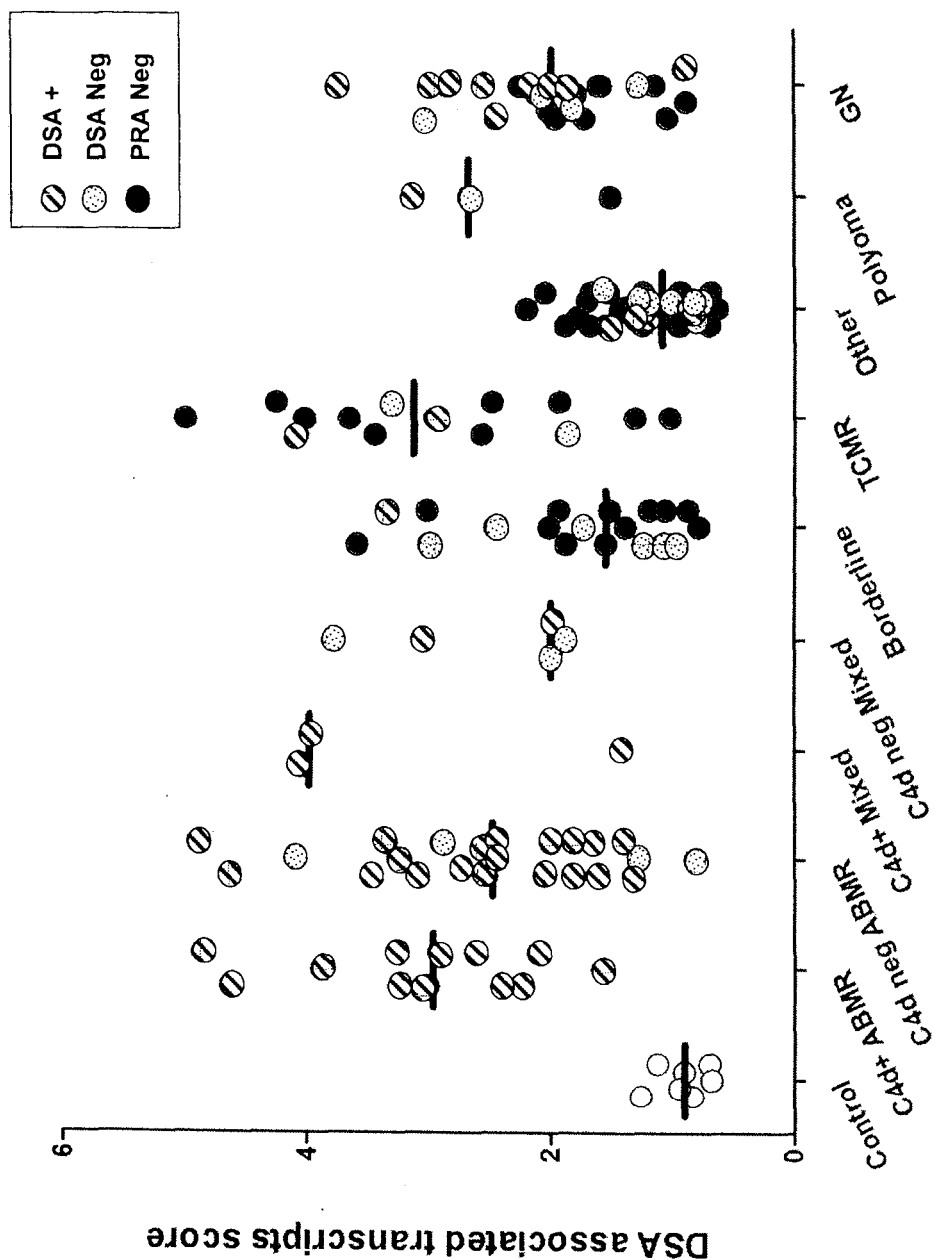


Figure 3A

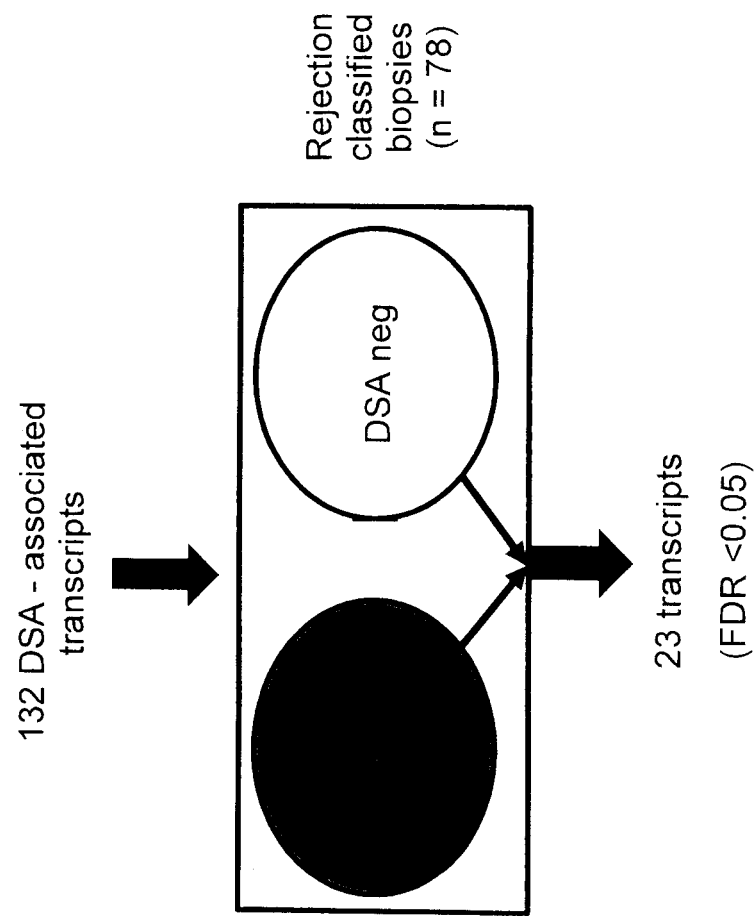
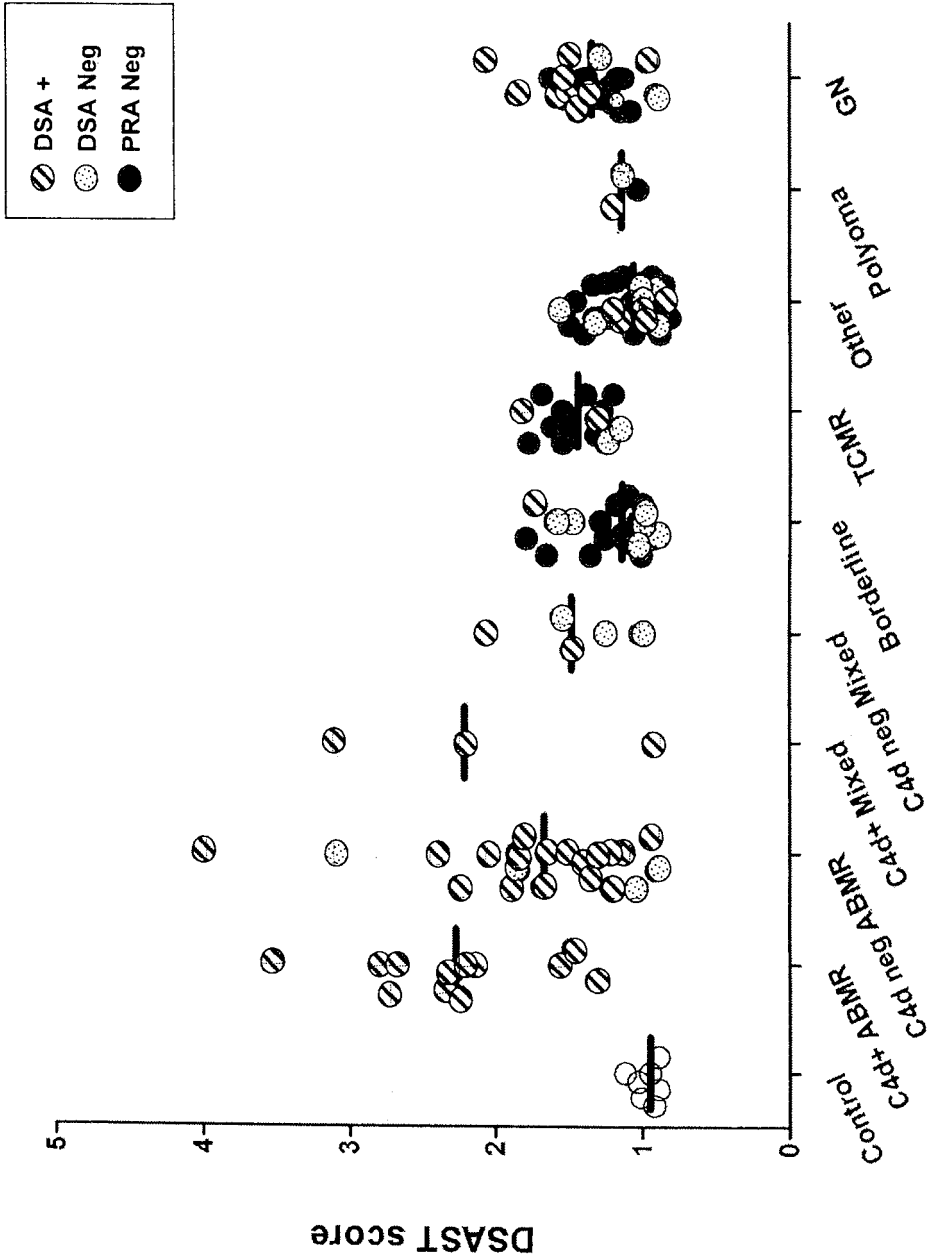


Figure 4



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Figure 5A

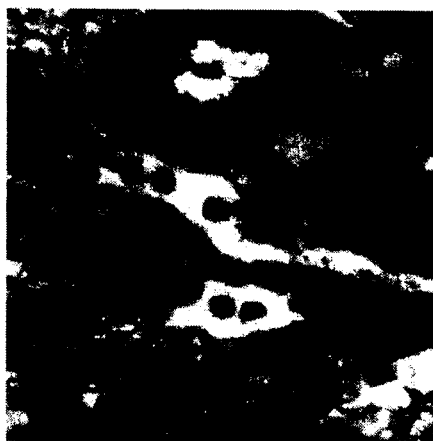


Figure 5B

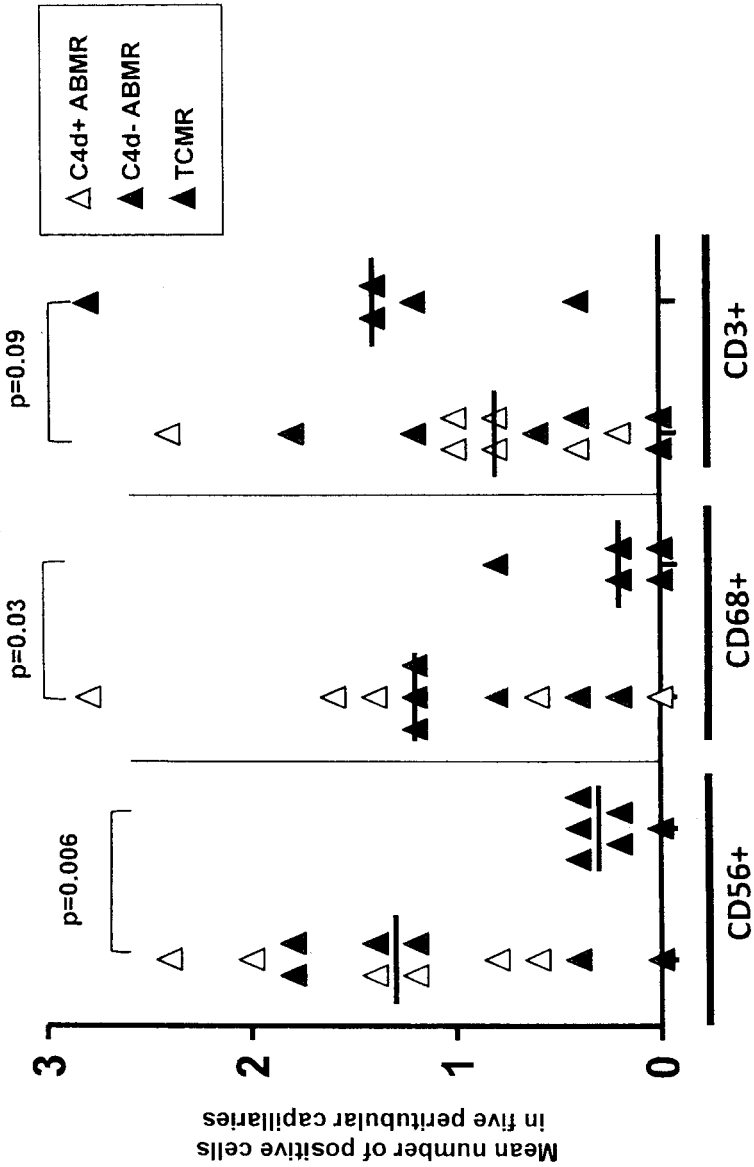
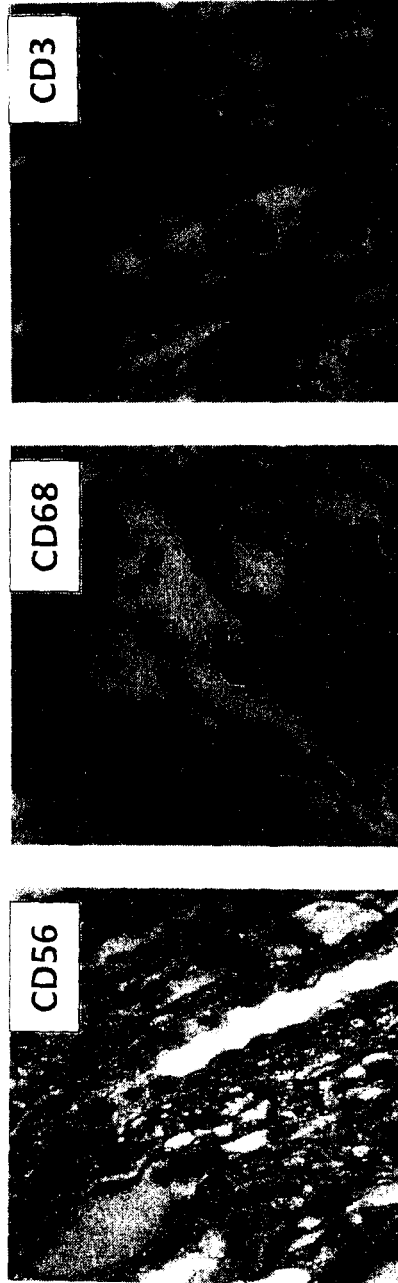


Figure 5C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/000839

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12Q 1/68* (2006.01) , *C12Q 1/02* (2006.01) , *G01N 33/48* (2006.01) , *G01N 33/68* (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: *C12Q 1/68* (2006.01) , *C12Q 1/02* (2006.01) , *G01N 33/48* (2006.01) , *G01N 33/68* (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: TotalPatent, PubMed, Scopus

Keywords: ABMR, expression, ICAM2, HLA-DRB3, HLA-DR52, SH2, ROBO4, PLA1A, PSPLA1, DRB3, EAT2, SF18, TM4, PLAT, tPA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/128333A1 (HALLORAN, P.F.) 30 October 2008 (30-10-2008) whole document	1-7
X	SIS, B. et al. "Endothelial Gene Expression in Kidney Transplants with Alloantibody Indicates Antibody-Mediated Damage Despite Lack of C4d Staining" AMERICAN JOURNAL OF TRANSPLANTATION. October 2009 (10-2009), Vol. 9, p. 2312-2323. ISSN: 1600-6143. whole document	1-7
A	HIDALGO, L.G. et al. "The transcriptome of human cytotoxic T cells: measuring the burden of CTL-associated transcripts in human kidney transplants" AMERICAN JOURNAL OF TRANSPLANTATION. March 2008 (03-2008), Vol. 8, p. 637-646. ISSN 1600-6143. whole document	1-7

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

[X] See patent family annex.

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

Date of the actual completion of the international search

17 October 2011 (17-10-2011)

Date of mailing of the international search report

21 October 2011 (21-10-2011)

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

Authorized officer

Rena Oulton (819) 997-4567

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/000839

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	HIDALGO, L.G. et al. "NK Cell Transcripts and NK Cells in Kidney Biopsies from Patients with Donor-Specific Antibodies: Evidence for NK Cell Involvement in Antibody-Mediated Rejection" AMERICAN JOURNAL OF TRANSPLANTATION. August 2010 (08-2010), Vol. 10, p. 1812-1822. ISSN: 1600-6143. whole document	1-7

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

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

1. ☐ Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. ☒ Claim Nos. : 1-7
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

See extra sheet.

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

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

- Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2011/000839

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2008/128333A1	30-10-2008	EP2148930A4	16-06-2010
		EP2148930A1	03-02-2010
		US2010190166A1	29-07-2010

Continued from Box No. II:

Claims 1-7 encompass methods that are not defined in terms of clear and/or distinguishing technical features as required under Rule 6.3(a) of the PCT. The description provides support within the meaning of Article 6 of the PCT for only a limited number of embodiments. In the present case, the claims lack clarity and/or support, and a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been established for the parts of the application which appear to be clear and supported. Specifically, the antibody mediated rejection (ABMR) signature profiles employed for the methods of the instant claims relate to a specific list of genes in Table 1 that are assessed for expression in a tissue. A profile may consist of one or more of the genes listed in Table 1 (see page 5, lines 26-32 of the instant application). Thus, the number of possible profiles for search is multitudinous, especially when considering combinations of genes within a profile. Therefore, a search of methods that employ expression profiling to detect antibody mediated rejection has been carried out, matching of single genes within the instant profile list of Table 1 with those found in the prior art.