

## Supplementary Appendix

### Supplementary Methods

#### ***Study cohorts***

We measured absolute levels of 26 mRNAs and 18S rRNA in 84 urine samples from 84 kidney transplant recipients. All recipients provided written informed consent to participate in the study and our Institutional Review Board approved the study. The clinical and research activities that we report are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'. A single pathologist (SVS), with no prior information about the urinary cell gene expression results, evaluated the biopsy specimens and categorized them using the Banff '07 update of the Banff '97 classification.<sup>1</sup>

There were 26 ACR biopsies (interstitial inflammation and tubulitis with minimal microcirculatory inflammation and absence of peritubular capillary C4d staining) from 26 patients, 26 AMR biopsies (microcirculatory inflammation and presence of C4d staining with minimal interstitial inflammation and tubulitis) from 26 patients and 32 ATI biopsies (attenuation or loss of brush border or necrosis and sloughing of tubular epithelium with or without isometric vacuolization and no interstitial inflammation, tubulitis, or microcirculatory inflammation and absence of C4d staining) from 32 patients. Among the 26 patients with AMR, 22 had results available, and were positive, for circulating anti-HLA donor specific antibodies. The remaining 4 patients did not have results available for circulating anti-HLA donor specific antibodies and hence should be categorized as suspicious for AMR based on Banff classification.<sup>1</sup>

### **Quantification of mRNAs**

We obtained approximately 50ml of urine at the time of allograft biopsy. Urine was centrifuged at 1250g for 30 minutes at room temperature within 4 hours of collection. We isolated total RNA from urinary cells using the RNeasy mini kit (Qiagen, Valencia, CA). The quantity (absorbance at 260nm) and purity (ratio of the absorbance at 260nm and 280nm) of the RNA isolated from the urine cell pellet were measured using the NanoDrop® ND-1000 spectrophotometer (Thermo Scientific). We reverse transcribed the RNA to complementary DNA using TaqMan Reverse Transcription Reagents (Applied Biosystems) at a final concentration of 1.0µg of total RNA in 100µl volume. We designed gene-specific oligonucleotide primers and fluorescent probes, using Primer Express software (Applied Biosystems, Foster City, CA), for the measurement of 26 mRNAs and a housekeeping/reference gene, 18S rRNA. This mechanistically informative panel of 26 mRNAs was designed based on our single center experience and as informed from the literature. The probes were labeled with 6-carboxy-fluorescein (FAM) at the 5' end and 6-carboxy-tetramethylrodamine (TAMRA) or dihydrocyclopyrroloindole tripeptide minor groove binder (MGB) at the 3' end. FAM functioned as the reporter dye and TAMRA or MGB as the quencher dye. We did a two-step PCR assay, a preamplification step<sup>2</sup> followed by measurement of the absolute levels of mRNAs, using our previously described standard curve method<sup>3</sup> in an ABI Prism 7500HT Fast detection system. The values of mRNAs and the 18S rRNA were expressed as copies per microgram of total RNA. The standard curve copy numbers in our PCR assays ranged from 25 to 2.5 million copies, and for data analysis, mRNA copy numbers <25 were scored as 12.5 copies per microgram of total RNA. In each of the 84 specimens, 18S rRNA value of >5X10<sup>7</sup> copies/ug total RNA and TGFβ1 mRNA value of >1X10<sup>2</sup> copies/ug total RNA

were used as a measure of transcript adequacy in that specimen.<sup>3</sup>

Three patients (2 in the AMR biopsies group and 1 in the ATI biopsy group) had BK virus replication in the urine, defined as  $\geq 10^6$  copies of BK virus VP1 mRNA per microgram of total RNA from urinary cells<sup>4</sup>, collected at the time of allograft biopsy. These three patients however did not have BK virus nephropathy as defined by negative immunohistochemistry for renal tubular epithelial nuclear SV40 large T antigen.

### ***Rationale for the mRNAs selected for inclusion in the mRNA panel***

Our choice of mRNAs for inclusion in our mRNA panel was based on our single center experience with urinary cell mRNA profiling and from the evolving literature regarding potential participants in allograft rejection. CD3 $\epsilon$  is a marker of post-thymic T-cells, the effector cell of ACR, while granzyme and perforin are attack molecules elaborated by cytotoxic T-cells.<sup>5</sup> Both FoxP3, a specification factor of regulatory T-cells and OX40, a costimulatory molecule of T cells have been associated with acute rejection.<sup>2,6</sup> CD105, CD146, and von Willebrand factor are expressed on endothelial cells, the target of antibody-mediated injury. Increased number of circulating endothelial cells has been associated with acute vascular rejection.<sup>7</sup> Immunoglobulin J has been associated with tubular injury and interstitial inflammation in transplant biopsies.<sup>8</sup> Immunoproteasome beta subunit 10 (PSMB10)<sup>9</sup> and TRIB1<sup>10</sup>, a serine-threonine kinase-like molecule, expressed in antigen presenting cells and endothelial cells has been associated with chronic antibody mediated rejection. TLR-4 is an activator of innate immunity and is associated with acute rejection.<sup>11</sup> CD14 is a marker of monocytes, a cell type that has been associated with acute rejection.<sup>12</sup> C3 and C5 are complement components while properdin, complement factor B, CD55 and CD46 are regulators of complement activity. Complement system plays an important role in acute rejection.<sup>13</sup> Vimentin is a major

intermediate filament protein expressed by mesenchymal cells. Regenerating kidney tubular cells but not healthy tubular cells express vimentin. NKCC2 is a transporter protein and E cadherin is an adhesion molecule expressed by kidney tubular cells. We have developed a urinary cell signature for kidney allograft fibrosis using levels of vimentin, NKCC2 and E cadherin.<sup>14</sup> IL-6 is a proinflammatory cytokine secreted by monocytes. In a recent study, measurement of IL-6 level in the blood differentiated acute rejection from no rejection, predominantly chronic allograft damage.<sup>15</sup> CXCL13 is a B-lymphocyte chemokine that is associated with B-cell cluster formation during acute rejection<sup>16</sup> while CD20 is a marker of B-cells.<sup>17</sup>

### ***Statistical analysis***

The levels of urinary cell transcripts were natural logarithm (ln) transformed to reduce the deviation from normality. We compared the levels of transcripts in the three diagnostic categories; ACR, AMR and ATI, using the Kruskal-Wallis test followed by Dunn's post-test. We used a two-step approach to develop our diagnostic signatures. In both the steps we first calculated the AUC for each mRNA measure to differentiate the two diagnostic categories, AR vs. ATI and ACR vs. AMR. We then used quadratic discriminant function analysis to develop a linear combination of variables that best predicted the diagnostic category.<sup>18</sup> We used 25 of the 26 mRNAs measured and 18S rRNA as independent variables. Discriminant analysis measures the distance from each point in the data to each group's multivariate mean and calculates a posterior probability of group membership. The analysis also takes into account the prior probability of group membership for calculating the posterior probability. To mimic the approximate prevalence of AR and ATI in consecutive biopsies done for acute allograft dysfunction, we assigned, for step-1, a prior probability of 0.6 for AR and 0.4 for ATI. For the same

reason, in step-2, we assigned a prior probability of 0.65 for ACR and 0.35 for AMR. We used step-wise backward estimation; all 25 mRNAs and 18SrRNA was entered in the model and were removed one at a time on the basis of their discriminating power. At  $P < 0.05$ , no further variables were removed and the existing variables were considered as the final parsimonious model. The linear combination of variables yielded a discriminant score that constituted the diagnostic signature. We tested if the signature better predicted the diagnostic outcome than individual mRNAs using the likelihood ratio test. We did 10-fold cross validation to internally validate our diagnostic signatures. The entire cohort was randomly divided into ten equal groups. Within each of the ten groups, the proportion of samples was similar to the undivided cohort. At the first run, group 1 was excluded and the signature was derived from the remaining 9 groups (90% of samples) including both variables selection and model fitting. Next, this newly derived signature was applied to samples of group 1 (10% of samples) to predict their diagnostic outcome. In the second run, group 2 was excluded and the signature was derived from the remaining 9 groups (90% of samples) including both variables selection and model fitting. This newly derived signature was applied to samples of group 2 (10% of samples) to predict their diagnostic outcome. This iteration was done for all the 10 groups. Thus, all observations are used for both deriving and validating a model and each observation is used for validation exactly once. Accordingly, the predicted probability for an individual patient was derived from a model that does not include any data from that patient. The predicted probability for each patient from the cross validation was then used to calculate discrimination statistics and in decision curve analysis, which quantifies the clinical benefit of the diagnostic signature in terms of the number of unnecessary biopsies that can be avoided in the diagnosis of AR. Decision curve analysis is a widely used method for evaluating predictions.<sup>19-22</sup> It is a weighted sum of true and false

positives, with the latter weighted by the odds at the threshold probability for biopsy, a value that can be modified to reflect different preferences about the harms of unnecessary biopsies compared to that of delayed diagnosis of acute rejection. We used JMP 10.0.2 software (SAS Institute Inc., Cary, NC) for discriminant analysis and Stata 11.2 software (StataCorp, College Station, TX) for decision curve analysis.

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**Table S1: Design of Oligonucleotide Primers and Probes**

mRNA	Accession number	Sequence	Location
CD3ε	NM_000733	Sense: 5'-AAGAAATGGGTGGTATTACACAGACA-3' Antisense: 5'-TGCCATAGTATTTCCAGATCCAGGAT-3' Probe: 5'-FAM-CCATCTCTGGAACCACAGTAATATTGACATGCC-TAMRA-3'	131-156 233-209 170-202
Granzyme B	J04071	Sense: 5'-GCGAATCTGACTTACGCCATTATT-3' Antisense: 5'-CAAGAGGGCCTCCAGAGTCC-3' Probe: 5'-FAM-CCCACGCACAACCTCAATGGTACTGTCTG-TAMRA-3'	534-557 638-619 559-585
Perforin	M28393	Sense: 5'-GGACCAGTACAGCTTACAGCACTG-3' Antisense: 5'-GCCCTCTTGAAGTCAGGGTG-3' Probe: 5'-FAM-TGCCGCTTCTACAGTTTCCATGTGGTACAC-TAMRA-3'	492-514 587-568 526-555
FoxP3	NM_014009	Sense: 5'-GAGAAGCTGAGTCCATGCA-3' Antisense: 5'-GGAGCCCTTGTCCGATGAT-3' Probe: 5'-FAM-TGCCATTTTCCCAGCCAGGTGG-TAMRA-3'	939-958 1025-1007 962-983
OX40	NM_003327.2	Sense: 5'-ACGACGTGGTCAGCTCCAA-3' Antisense: 5'-TCCGCTCACTCCACTTCTG-3' Probe: 5'-FAM- AAGCCCTGCACGTGG -MGB-3'	223-241 291-272 249-263
CD105	NM_000118.2	Sense: 5'-CAGCCTCAGCCCCACAAGT-3' Antisense: 5'-GGCCACAGGCTGAAGGT-3' Probe: 5'-FAM- TTGCAGAAACAGTCCATT-MGB-3'	464-482 522-505 484-501
CD146	NM_006500.2	Sense: 5'-CCTGGA CTTGGACACCATGAT-3' Antisense: 5'-ACTCGGAGCTCAGACACATAGTTC 3' Probe: 5'-FAM- TGCTGAGTGAACCACAGG -MGB 3'	995-1015 1049-1072 1021-1038
von Willebrand Factor	X04385.1	Sense: 5'-CCTCAAAGGCGGTGGTCAT-3' Antisense: 5'-AGCGATCTCCAATTC AATAGG-3' Probe: 5'-FAM- CCAACAGAGTGACAGTGT -MGB-3'	5474-5492 5590-5569 5549-5566
IgJ	NM_144646.3	Sense: 5'-TGGAGAGAAACATCCGAATATTG- 3' Antisense: 5'- TGGTGAGGTGGGATCAGAGATA-3' Probe: 5'- FAM- TCCTCTGAACAACAGGGA -MGB-3'	308-331 375-354 333-350
PSMB10	NM_002801.3	Sense: 5'-AGAGCTGCGAAGATCCACTT-3' Antisense: 5'-CTCCAGCCCCACAGCA-3' Probe: 5'-FAM- ATCGCCCCAAAAT -MGB-3'	331-352 388-369 354-367
TRIB1	BC063292.1	Sense: 5'-GGGCGCTGTGCATCCA-3' Antisense: 5'-AAGGCCTGATTTTGTCTGGTA-3' Probe: 5'-FAM- CGCTGCAAGGTGTTT -MGB-3'	902-917 982-961 934-948
TLR4	NM_138554.1	Sense: 5'-CATGGCCTTCTCTCCTGC-3' Antisense: 5'-GAAATTCAGCTCCATGCATTGA-3' Probe: 5'-FAM-AGGAACCCACTCCACGCAAGGCT-TAMRA-3'	209-227 302-281 269-247
CD14	NM_000591	Sense: 5'-GCTGTGTAGAAAGAGCTAAAGCACTT-3' Antisense: 5'-TGGCGTGGTCGCAGAGA-3' Probe: 5'-FAM- CTTATCGACCATGGAGCGCGGT TAMRA 3'	51-77 185-169 110-132
Complement Factor 3	NM_000064.2	Sense: 5'-CAGCACCGAAACAGAAAAGAG-3' Antisense: 5'- CCCCAGTACTGGTACAGATC-3' Probe: 5'-FAM-AAGAACAATATGATCCTTG -MGB -3'	4168-4189 4243-4143 4203-4221
Complement Factor 5	NM_001735.2	Sense: 5'-TTCCTTGGGAGGCCAGTGA-3' Antisense: 5'- AGCCAAGCCACTGCCAAA-3' Probe: 5'-FAM-ACCTCATTGTCAAGTACAGG -MGB -3'	4027-4046 4101-4084 4064-4082
Properdin	NM_002621.2	Sense: 5'-GATGTGCCGGCAACAG-3' Antisense: 5'- CACTCTGACCATGATCCTTTCAAG-3' Probe: 5'-FAM-TATCCGGCACTGCTACA -MGB -3'	1318-1334 1396-1378 1340-1356
Complement Factor B	NM_001710.5	Sense: 5'-TGCGGCCCTTGATAGT-3' Antisense: 5'- CCCAGCTGATTACACCACTTG-3' Probe: 5'-FAM-CACAAGAGAAGTCTGTTTCA -MGB -3'	2375-2392 2436-2415 2394-2312
CD55	NM_001114752.1	Sense: 5'-CACCACCTGAATGCAGAGAA-3' Antisense: 5'- GAACATTTACTGTGGTAGTTTCTGAAC-3' Probe: 5'-FAM-CTAACTTCCAAGGTCCC -MGB -3'	1130-1150 1207-1180 1156-1172
CD46	NM_002389.3	Sense: 5'-GATCGGAATCATACATGCTACCT-3' Antisense: 5'- GGCCATTTAAAGGATCCCCTATA-3' Probe: 5'-FAM-CTCAGATGACGCTGTTTATAGAGAAACATGTCCA TAMRA -3'	397-420 481-459 423-456
Vimentin	NM_003380.2	Sense: 5'-TCAGAGAGAGGAAGCCGAAAAC-3' Antisense: 5'-CCAGAGACGCATTGTCAACATC-3' Probe: 5'-FAM-CCCTGCAATCTTTCAGAC-MGB-3'	706-727 770-749 729-746
NKCC2	BC040138.2	Sense: 5'-TCAGGCAACTCGAAAAGA-3' Antisense: 5'-TCCATCACCGTTAGCAACTC-3' Probe: 5'-FAM-TGTGGCAGTCACCCCAAGTTCAGC-TAMRA-3'	588-607 658-638 609-632

E-cadherin	XM_007840	Sense: 5'-TGAGTGTCCCCGGTATCTTC-3' Antisense: 5'-CAGCCGCTTTCAGATTTTCAT-3' Probe: 5'-FAM-CCTGCCAATCCCGATGAAATTGGAAAT-TAMRA-3'	2469-2489 2549-2529 2495-2521
IL-6	NM_000600	Sense: 5'-CCAGGAGCCAGCTATGAAC-3' Antisense: 5'-CCCAGGGAGAAGGCAACTG-3' Probe: 5'-FAM-CCTTCTCCACAAGCGCCTTCGGT TAMRA-3'	49-68 112-94 70-92
CXCL13	NM_006419.2	Sense: 5'-CCCGTGGGAATGGTTGTC-3' Antisense: 5'-GGGTCCACACACAATTGACT-3' Probe: 5'-FAM-ATCATAGTCTGGAAGAAGAA-MGB-3'	242-259 314-293 271-290
CD20	NM_021950	Sense: 5'-AACTCCCCTACTACCAATACTGTT-3' Antisense: 5'-AGAAGGCAAGATCAGCATCACT-3' Probe: 5'-FAM-CAGCATACAATCTCTGTTCTTGGGCATTTG-TAMRA-3'	616-640 697-675 642-672
TGFβ1	NM_000660	Sense: 5'-GCGTGCTAATGGTGGAAACC-3' Antisense: 5'-CGGAGCTCTGATGTGTTGAAGA-3' Probe: 5'-FAM-ACAACGAAATCTATGACAAGTTCAAGCAGAGTACACA-TAMRA-3'	1170-1189 1263-1242 1191-1227
18S rRNA	K03432	Sense: 5'-GCCGGAAGCGTTTACTTTGA-3' Antisense: 5'-TCCATTATTCCTAGCTGCGGTATC-3' Probe: 5'-FAM-AAAGCAGGCCCGAGCCGCC-TAMRA-3'	929-948 1009-986 965-983

We used Primer Express software (Applied Biosystems, Foster City, CA) to design gene-specific oligonucleotide primers and TaqMan probes for measurement of urinary cell mRNA by the use of real-time PCR assay. The probes were labeled with 6-carboxy-fluorescein (FAM) at the 5' end and 6-carboxy-tetramethylrodamine (TAMRA) or dihydrocyclopyrroloindole tripeptide minor groove binder (MGB) at the 3' end. FAM functioned as the reporter dye and TAMRA or MGB as the quencher dye.

**Table S2: Receiver Operating Characteristic Curve Analysis of Urinary Cell mRNAs to Differentiate Acute Rejection (N=52) from Acute Tubular Injury (N=32)**

Urinary Cell mRNA	Area Under the Curve	95% Confidence Interval	
CD3ε	0.8786	0.8078	0.9493
FoxP3	0.8705	0.8030	0.9379
Perforin	0.8395	0.7538	0.9252
IgJ	0.8338	0.7395	0.9281
Granzyme B	0.8242	0.7356	0.9128
CD20	0.8131	0.7175	0.9086
OX40	0.7930	0.6974	0.8885
CXCL13	0.7683	0.6601	0.8765
CD105	0.7479	0.6405	0.8552
PSMB10	0.7121	0.6019	0.8223
TGFβ1	0.7112	0.5991	0.8234
Complement Factor 3	0.7046	0.5867	0.8224
Complement Factor 5	0.6989	0.5806	0.8171
Complement Factor B	0.6869	0.5669	0.8068
CD146	0.6812	0.5595	0.8028
TLR4	0.6572	0.5341	0.7801
von Willebrand Factor	0.6665	0.5439	0.7892
E-cadherin	0.6656	0.5431	0.7879
Vimentin	0.6502	0.5265	0.7739
CD46	0.6487	0.5270	0.7704
IL-6	0.6469	0.5201	0.7737
CD14	0.6352	0.5112	0.7591
NKCC2	0.6247	0.5019	0.7474
18S rRNA	0.6130	0.4860	0.7399
TRIB1	0.5980	0.4676	0.7282
CD55	0.5965	0.4653	0.7276
Properdin	0.5895	0.4605	0.7185

Receiver-operating-characteristic (ROC) curve analyses of the urinary cell mRNA measures to differentiate AR (both types) from ATI are shown. Urinary cell levels of CD3ε mRNA differentiated the two groups best. To determine if combination of mRNAs better differentiated AR from ATI, we then used stepwise quadratic discriminant analysis to develop a linear combination of variables that best predicted the diagnostic groups. A six-gene model of natural logarithm (ln)-transformed mRNA values of CD3ε, CD105, TLR4, CD14, Complement factor B, and Vimentin emerged as the parsimonious model yielding a diagnostic signature that distinguished AR from ATI. This diagnostic signature better differentiated AR from ATI than CD3ε mRNA value alone (likelihood ratio test,  $X^2=40.6$ ,  $P<0.0001$ ).

**Table S3: Receiver Operating Characteristic Curve Analysis of Urinary Cell mRNAs to Differentiate Acute T-Cell Mediated Rejection (N=26) from Acute Antibody Mediated Rejection (N=26)**

Urinary Cell mRNA	Area Under the Curve	95% Confidence Interval	
CD3ε	0.8735	0.7758	0.9711
FoxP3	0.8735	0.7741	0.9729
PSMB10	0.8462	0.7415	0.9507
CD14	0.8351	0.7256	0.9445
CXCL13	0.8299	0.7207	0.9390
Granzyme B	0.8262	0.7129	0.9394
Perforin	0.8062	0.6864	0.9259
Ox40	0.8129	0.6968	0.9289
TGFβ1	0.8047	0.6871	0.9223
CD46	0.8010	0.6782	0.9238
Vimentin	0.7966	0.6725	0.9206
Complement Factor Properdin	0.7840	0.6604	0.9075
CD105	0.7751	0.6427	0.9075
TRIB1	0.7714	0.6430	0.8999
Complement Factor 5	0.7700	0.6401	0.8998
18S rRNA	0.7663	0.6378	0.8948
CD55	0.7611	0.6319	0.8902
IL-6	0.7382	0.6033	0.8730
TLR4	0.7352	0.5997	0.8706
E-cadherin	0.7241	0.5847	0.8634
CD20	0.7204	0.5694	0.8714
Complement Factor 3	0.7145	0.5714	0.8575
NKCC2	0.7034	0.5602	0.8465
IgJ	0.6746	0.5202	0.8288
CD146	0.6686	0.5192	0.8180
von Willebrand Factor	0.6546	0.5029	0.8062
Complement Factor B	0.5673	0.4088	0.7258

After identifying acute rejections noninvasively using the six-gene diagnostic signature, we next determined if the two types of acute rejections; ACR and AMR, could be differentiated using the same assay results without the need for an invasive biopsy. The diagnostic value of individual mRNAs to differentiate ACR from AMR, ascertained using the ROC curve analysis is shown. Urinary cell levels of CD3ε mRNA differentiated the two groups best. To determine if combination of mRNAs better differentiated ACR from AMR, we then used stepwise quadratic discriminant analysis to develop a linear combination of variables that best predicted the diagnostic groups. A five-gene model of ln-transformed mRNA values of CD3ε, CD105, CD14 and CD46 as well as ln-transformed 18S rRNA emerged as the parsimonious model yielding a diagnostic signature that distinguished ACR from AMR. This diagnostic signature better differentiated ACR from AMR than CD3ε mRNA value alone (likelihood ratio test,  $X^2=30.4$ ,  $P<0.0001$ ).

**Table S4: Characteristics of Kidney Allograft Recipients Classified by Time From Transplantation to Biopsy/Urine Specimen Collection**

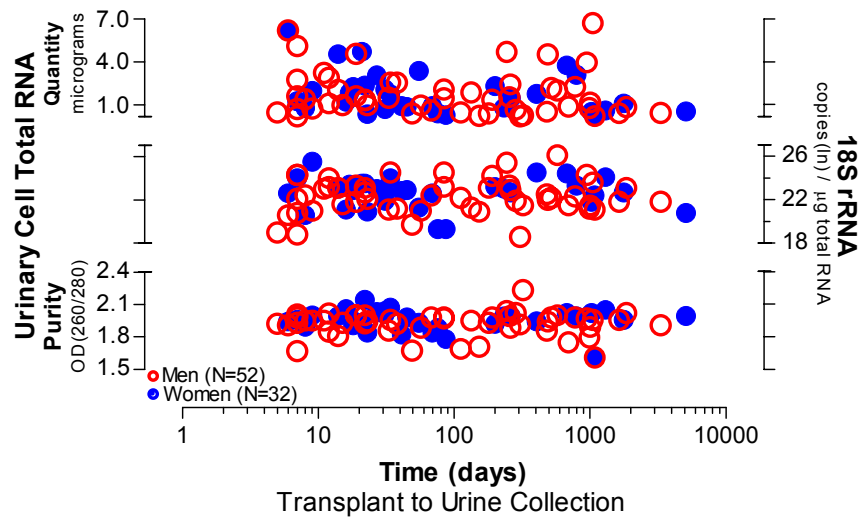
Variables	Time From Transplantation to Biopsy						P Value <sup>a</sup>		
	< 1-Month N=31			> 1-Month N=53			< 1-Month vs. > 1-Month		
	ACR N=10	AMR N=9	ATI N=12	ACR N=16	AMR N=17	ATI N=20	ACR	AMR	ATI
Age, years, <i>mean (SD)</i>	47 (8)	48 (13)	52 (12)	52 (17)	49 (14)	51 (17)	0.6	0.7	0.9
Gender, female, <i>N (%)</i>	2 (20)	5 (56)	5 (42)	6 (38)	6 (35)	8 (40)	0.4	0.3	0.9
Racial categories, black, <i>N (%)</i>	5 (50)	3 (33)	4 (33)	6 (38)	5 (29)	5 (25)	0.5	0.8	0.6
Thymoglobulin <sup>®</sup> induction, <i>N (%)</i>	6 (60)	7 (78)	10 (83)	10 (63)	9 (53)	17 <sup>b</sup> (85)	0.9	0.2	0.9
Corticosteroids maintenance, <i>N (%)</i>	7 (70)	7 (78)	3 (25)	9 (56)	10 (59)	4 (20)	0.4	0.3	0.7
History of delayed graft function, <i>N (%)</i>	4 (40)	2 (22)	6 (50)	4 (25)	3 (18)	7 (35)	0.4	0.8	0.4
History of acute rejection, <i>N (%)</i>	0 (0)	0 (0)	0 (0)	2 (13)	2 (12)	0 (0)	0.2	0.3	-
History of bacterial urinary tract infections <sup>c</sup> , <i>N (%)</i>	2 (20)	2 (22)	1 (8)	7 (44)	6 (35)	4 (20)	0.2	0.5	0.4
Serum creatinine at biopsy, mg/dl, <i>median (interquartile range)</i>	3.50 (1.90-7.23)	4.30 (3.05-5.90)	5.11 (2.96-7.91)	3.20 (1.91-4.08)	2.30 (1.79-2.83)	3.00 (2.40-3.31)	0.5	<0.01	0.01
Serum tacrolimus trough at biopsy, ng/ml, <i>median (interquartile range)</i>	6.6 (4.9-10.0)	7.7 (3.8-12.6)	9.2 (7.6-10.5)	4.4 (4.0-5.5)	5.9 (3.4-7.3)	9.3 (8.0-10.1)	0.03	0.2	0.8
Urinary cell total RNA quantity, µg, <i>median (inter quartile range)</i>	1.8 (1.3-3.4)	1.2 (0.7-3.9)	2.0 (0.9-2.9)	1.9 (0.8-2.4)	0.9 (0.5-2.2)	0.8 (0.4-1.7)	0.8	0.3	0.04
Urinary cell total RNA purity, OD260/OD280 ratio, <i>median (inter quartile range)</i>	1.98 (1.90-2.00)	1.95 (1.91-1.99)	1.95 (1.92-2.01)	1.98 (1.95-2.02)	1.96 (1.90-1.99)	1.88 (1.78-1.98)	0.4	0.9	0.1

a: P value derived by Chi-square test for categorical variables or Mann-Whitney test for continuous variables

b: Includes one patient with Alemtuzumab (Campath<sup>®</sup>) induction

c: Defined as the presence of  $\geq 10^5$  colony forming units per milliliter of urine

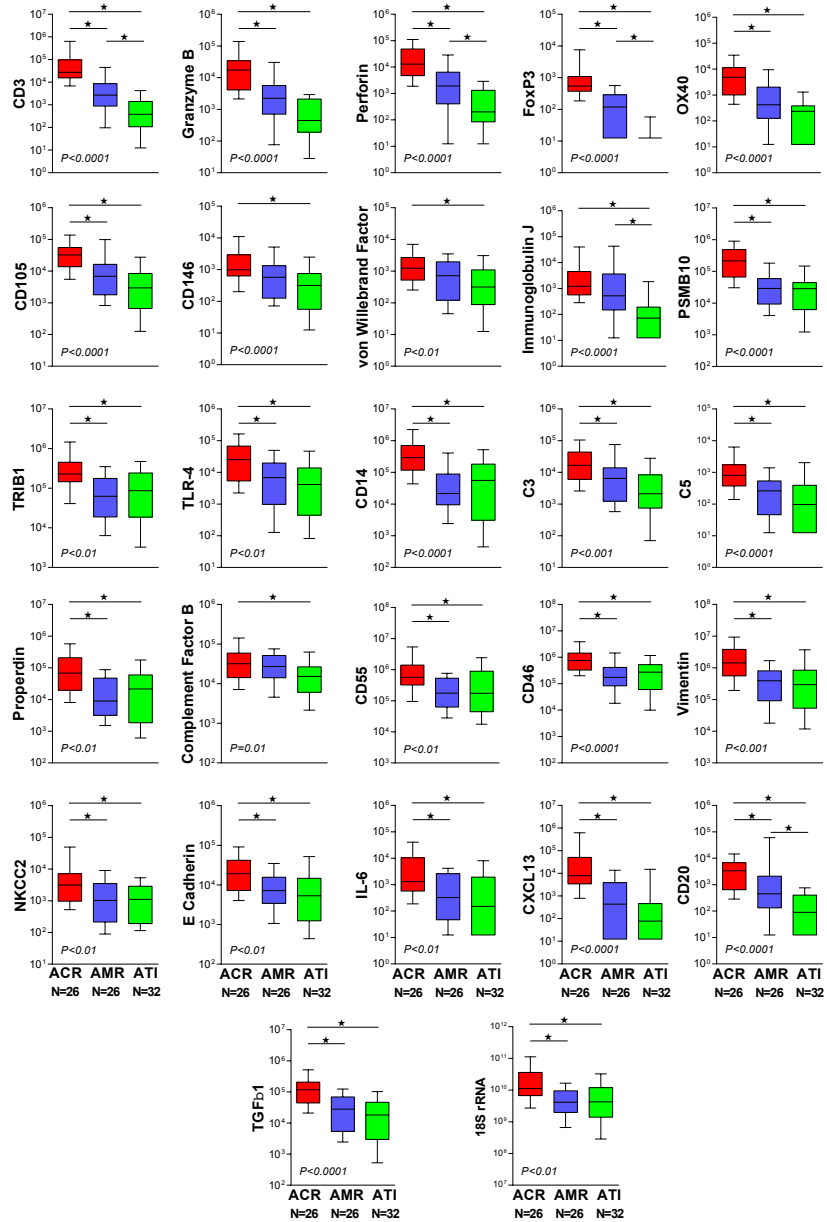
**Figure S1: RNA quantity and purity and 18S rRNA levels in urinary cells from male or female patients, as a function of time post-transplantation**



Scatter plot shows the relation between time from kidney transplantation to the collection of urine samples (X-axis) and the quantity of total RNA isolated in urinary cells (Y-axis top), the purity of RNA as assessed by the ratio of the optical density (OD) at 260 nm to the OD at 280 nm (Y-axis bottom) and the levels of endogenous control 18S ribosomal RNA. Total RNA was reverse transcribed to cDNA. 18S rRNA level in urinary cells was quantified using gene specific primers and probes by real-time PCR assay and expressed as natural log-transformed copies per one microgram of total RNA. Blue closed circles represent samples from women and red open circles represent samples from men. By Spearman rank order correlation, there was no statistically significant association ( $P > 0.05$ ) between time from transplant to urine collection and each of the variables represented on the Y-axis. The OD260/280 ratio of pure RNA is ~2.



Figure S2: Levels of mRNA in Urinary Cells



Box plots show the quantity of the 26 mRNAs and 18S rRNA measured in the urinary cells of kidney transplant recipients at the time of for-cause (diagnostic) kidney biopsies. The X-axis shows the three groups: acute T-cell mediated rejection (ACR, red, N=26), acute antibody mediated rejection (AMR, blue, N=26) and acute tubular injury (ATI, green, N=32). The 26 mRNAs and 18S rRNA were quantified using gene specific primers and probes by real-time PCR assay and expressed as copies per microgram of total RNA. The horizontal line within each box represents the median, the bottom and top of each box represent the 25th and 75th percentile values, and the whiskers represent the 10th and 90th percentile values. P values are based on the Kruskal–Wallis test. Individual groups were compared by Dunn's test and if significant ( $P < 0.05$ ) are represented by an asterisk.