MOLECULAR CORRELATES OF CHRONIC T-CELL MEDIATED REJECTION

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Meeting Report

The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology

Chronic active ABMR[2]	All three features must be present for diagnosis. As with acute/active ABMR, biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious, and it should be noted if the lesion is C4d-positive or C4d-negative, based on the criteria listed: 1 Histologic evidence of chronic tissue injury, including one or more of the following: • TG (cg >0), if no evidence of chronic thrombotic microangiopathy; includes changes evident by EM only (cg1a; Table 4) • Severe peritubular capillary basement membrane multilayering (requires EM)[3] • Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of biopsy-proven TCMR with arterial involvement but are not required 2 Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥2 alone is not sufficient and g must be ≥1 • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated 3 Serologic evidence of DSAs (HLA or other antigens): • Biopsies suspicious for ABMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing
Chronic active TCMR	Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neointima); note that such lesions may represent chronic active ABMR as well as TCMR; the latter may also be manifest in the tubulointerstitial compartment

American Journal of Transplantation 2007; 7: 2712–2722
Blackwell Munksgaard

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Microarray Analysis of Rejection in Human Kidney Transplants Using Pathogenesis-Based Transcript Sets

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Personal Viewpoint

The Molecular Phenotype of Kidney Transplants

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mediated rejection; ATN, acute tubular necrosis; PBTs, pathogenesis-based transcript sets; SLC, solute carrier.

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Table 1: Pathogenesis-based transcript sets (PBTs): a system for deconstructing complex changes into biologic mechanisms and interpreting changes in individual transcripts

PBT	PBT Name	Biological Description
QCAT	Quantitative cytotoxic T cell-associated transcripts	Burden of effector/effector-memory T cells
GRIT	Interferon-gamma- and rejection- induced transcripts	Interferon-gamma effects on the tissue and inflammatory cells
QCMAT	Quantitative constitutive macrophage-associated transcripts	Burden of macrophages
AMAT1	Alternative macrophage activation-associated transcripts	Alternatively activated macrophages
ENDAT	Endothelium-associated transcripts	Microcirculation response to injury
IRITD3	Injury- and repair-induced transcripts day 3	Active injury–repair response: 'injury-up' Increased in isografts, peaking day 3
IRITD5	Injury- and repair-induced transcripts day 5	Active injury–repair response: 'injury-up' Increased in isografts peaking day 5
KT1	Kidney transcripts—set 1	Active injury–repair response: 'injury-down' Parenchymal transcripts
KT2	Kidney transcripts—set 2	Active injury–repair response: 'injury-down' Solute carrier transcripts

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doi: 10.1111/j.1600-6143.2010.03201.x

NK Cell Transcripts and NK Cells in Kidney Biopsies from Patients with Donor-Specific Antibodies: Evidence for NK Cell Involvement in Antibody-Mediated Rejection

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antibodies; DSA, Donor-specific antibodies; NK cell, Natural killer cell; DSASTs, DSA selective transcripts.

Received 02 March 2010, revised 29 April 2010 and

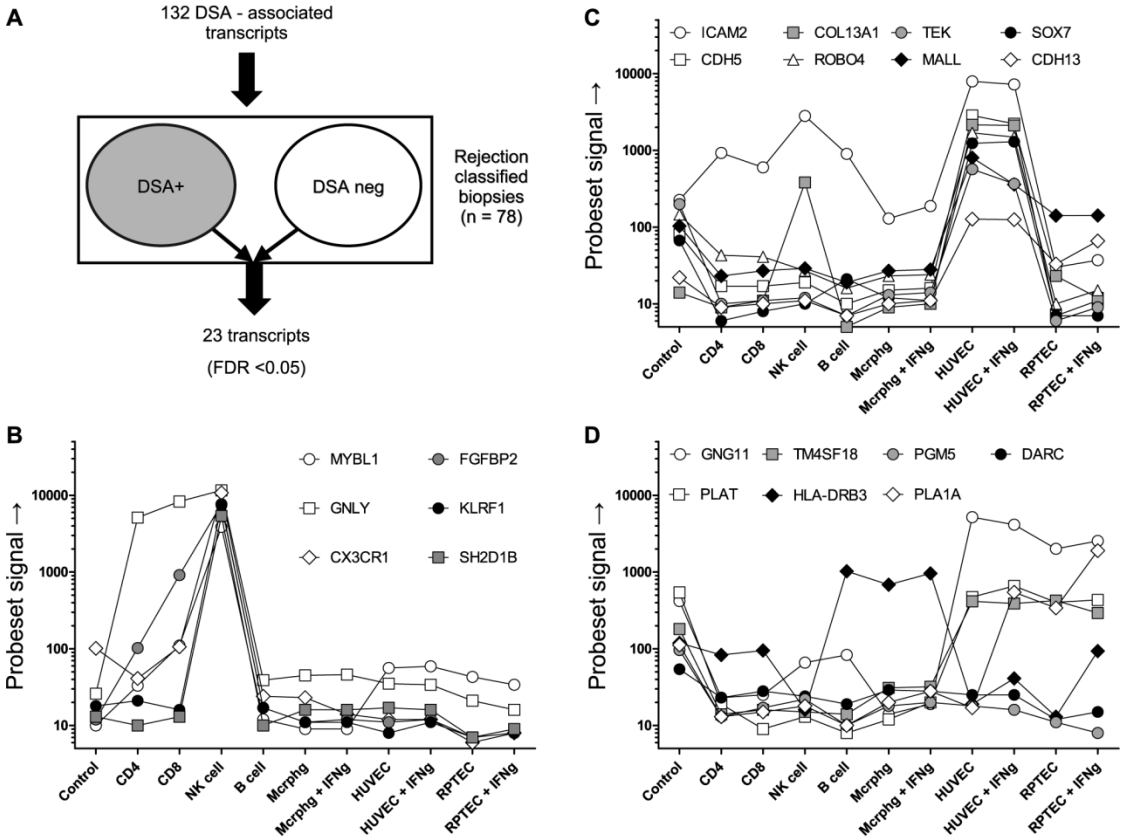

Figure 3: Algorithm for the generation of DSAST and expression of DSAST in primary human cells. (A) DSASTs were generated by starting with the 132 DSA-associated transcripts and determining how many remained differentially expressed between DSA-positive and DSA-negative groups when compared across the 78 rejection-classified biopsies. (B) DSASTs with preferential expression in human NK cells. (C) DSASTs with preferential expression in HUVECs. (D) DSASTs with high expression in HUVECs or inducible in HUVECs and also expressed in other cell types.

http://www.kidney-international.org

clinical investigation

Clinical and molecular significance of microvascular inflammation in transplant kidney biopsies

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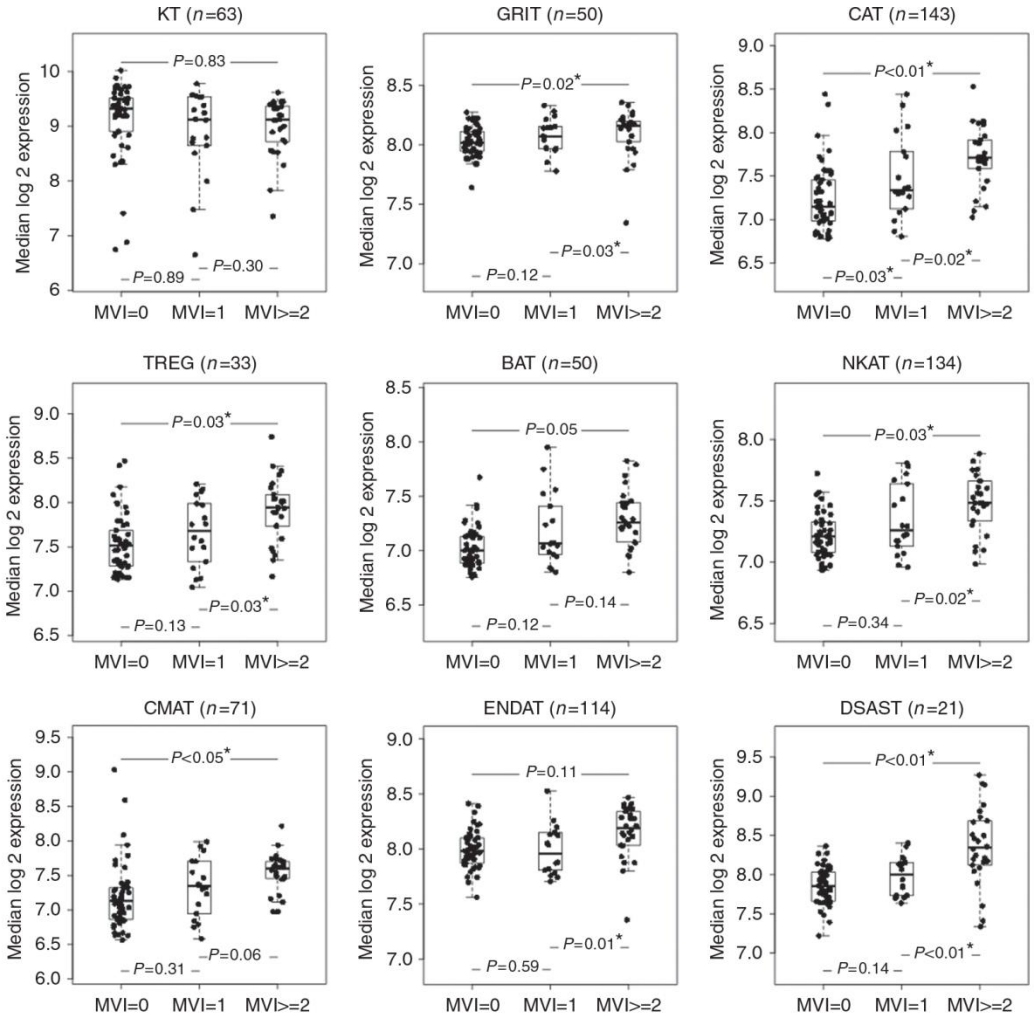

Figure 1 | Median log 2 expression levels for pathogenesis-based transcripts in biopsies from the microvascular inflammation (MVI) score = 0 ($n = 49$), MVI = 1 ($n = 18$), and MVI $\geqslant$ 2 ($n = 26$) patient groups. Associated *P*-values are taken from the *limma romer* analysis. Asterisks denote significant *P*-values, defined as $\leqslant 0.05$. BAT, B cell–associated transcripts; CAT, cytotoxic T cell–associated transcripts; CMAT, constitutive macrophage–associated transcripts; DSAST, transcripts differentially expressed between rejection-classified biopsies from DSA+ patients compared with DSA − patients; ENDAT, endothelial cell–associated transcripts; GRIT, γ-IFN and rejection-induced transcripts; KT, kidney-specific transcripts 2; NKAT, natural killer cell–selective transcripts; TREG, regulatory T cell–associated transcripts.

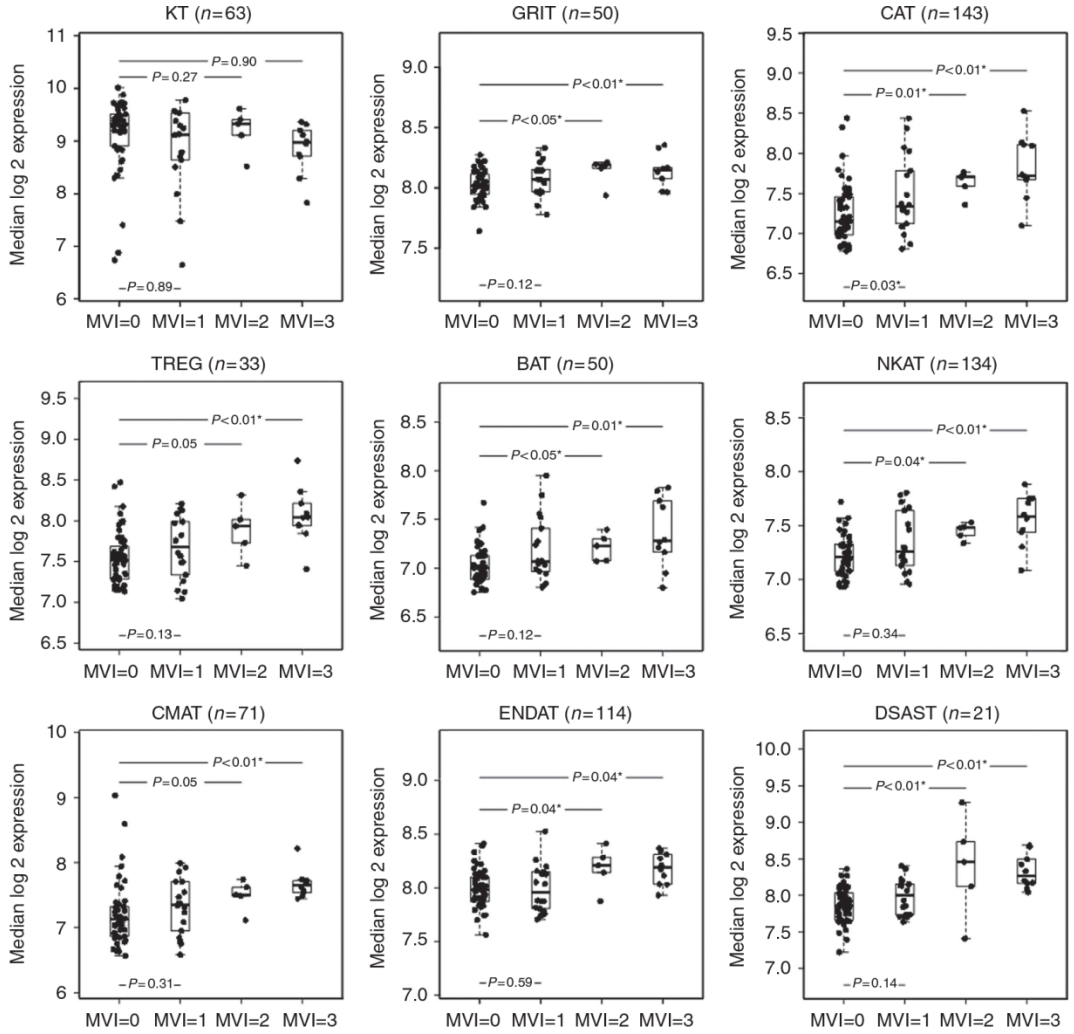

Figure 2 | Median log 2 expression levels for pathogenesis-based transcripts in the microvascular inflammation (MVI) score = 0 ($n = 49$), MVI = 1 ($n = 18$), MVI = 2 ($n = 5$), and MVI = 3 ($n = 10$) patient groups. Associated *P*-values are taken from the *limma romer* analysis. Asterisks denote significant *P*-values, defined as $\leqslant 0.05$. BAT, B cell–associated transcripts; CAT, cytotoxic T cell–associated transcripts; CMAT, constitutive macrophage–associated transcripts; DSAST, transcripts differentially expressed between rejection-classified biopsies from DSA+ patients compared with DSA − patients; ENDAT, endothelial cell–associated transcripts; GRIT, γ-IFN and rejection-induced transcripts; KT, kidney-specific transcripts 2; NKAT, natural killer cell–selective transcripts; TREG, regulatory T cell–associated transcripts.

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doi: 10.1111/ajt.12150

Molecular Diagnosis of Antibody-Mediated Rejection in Human Kidney Transplants

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status, histology, or C4d staining, and predicts future failure.

Key words: Antibody-mediated rejection, classifier, kidney transplant, microarrays, molecular diagnostics

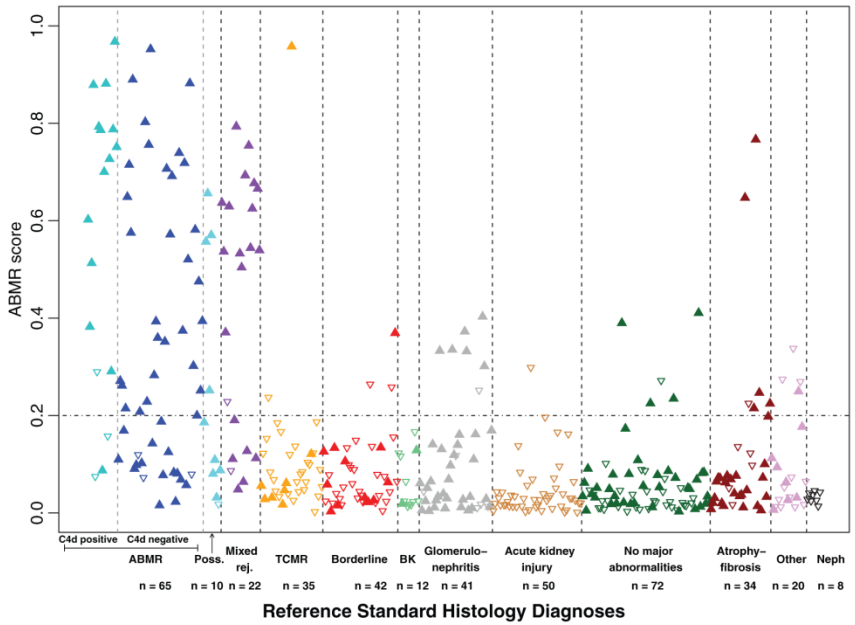

Figure 2: Relationship between the median ABMR score and the Reference Standard histology–DSA diagnoses, assigned primarily by the readings of pathologist B as outlined in the Methods section. Ordering within each diagnosis is random. The horizontal line shows our threshold of 0.2 for defining high versus low ABMR scores. The different symbols represent time posttransplantation: early (<1 year: inverted empty triangles); and late (>1 year: solid triangles). Colors cycle through the diagnostic categories as an aid to separating biopsies close to the vertical partitions between diagnostic stacks. ABMR, antibody-mediated rejection; TCMR = T cell-mediated rejection; BK = polyoma virus nephropathy; Neph. = nephrectomies: Poss. = possible ABMR.

Table 4: Agreement of the ABMR score with histology-DSA diagnosis in 221 late (>1 year) biopsies[1]

	Histology-DSA diagnosis											
				Detailed diagnosis in non–ABMR								
ABMR score	ABMR (C4d+ve and −ve)	Mixed	Non-BMR	Possible ABMR	TCMR	Borderline	BK	GN	Acute kidney injury	No major abnormalities	Atrophy-fibrosis	Other	Total
> 0.5	**26**	**13**	**6**	*3*	*1*	*0*	*0*	*0*	*0*	*0*	*2*	*0*	45
0.2–0.5	**16**	**1**	**16**	*1*	*0*	*1*	*0*	*6*	*0*	*4*	*3*	*1*	33
< 0.2	**17**	**6**	**120**	*5*	*7*	*12*	*2*	*29*	*0*	*35*	*23*	*7*	143
Total	**59**	**20**	**142**	*9*	*8*	*13*	*2*	*35*	*0*	*39*	*28*	*8*	221

[1]The data were split into four cells for a chi-square test: above versus below 0.2 ABMR score and (ABMR and mixed) versus non-ABMR

Table 6: Association between the ABMR score and DSA at the time of biopsy[1]

ABMR score	DSA positive (class II or I/II)	HLA antibody not demonstrably donor specific (NDSA)	HLA antibody negative	Not done[2]	Total
> 0.5	40 (35)	5	0	0	45
0.2 - 0.5	23 (20)	4	14	4	45
< 0.2	61 (40)	61	151	40	313
Total	124 (95)	70	165	44	403

[1]When the data were split into four cells for a chi-square test, ABMR scores above versus below 0.2 and DSA positive versus (NDSA and HLA antibody negative), the p-value was <0.001. The cases in which HLA antibody was not assessed ("not done") were excluded. ABMR = antibody-mediated rejection; DSA = donor-specific antibody; PRA = panel reactive antibody; NDSA = PRA positive but no DSA identified.
[2]In this prospective study, centers were asked to perform HLA antibody testing at their own expense but in some cases they elected not to perform testing. Those cases were significantly less likely to have high ABMR scores than those in which HLA antibody testing was done (Fisher's exact test for "not done" versus all others, one-tailed, was p = 0.015.

Table 7: Univariable and multivariable death-censored Cox regression analysis of graft failures in the 315 patients[1]

Univariable results	LL	HR	UL	p-Value
Molecular ABMR score[2]	1.50	1.76	2.06	5×10^{-12}
Histology-DSA ABMR (C4d+/-/mixed)	2.33	3.71	5.90	3×10^{-8}
Histology-DSA C4d+ ABMR(both alone and mixed)	1.2	2.4	4.7	0.01
Histology-DSA C4d- ABMR (both alone and mixed)	2.0	3.3	5.5	2×10^{-6}
Multivariable results[3]	**LL**	**HR**	**UL**	**p-value**
Molecular ABMR score[2]	1.21	1.53	1.91	2.6×10^{-4}
Histology-DSA ABMR (C4d+/-/mixed)	0.93	1.79	3.43	0.08

[1]One biopsy per patient, selected at random. HR = hazard ratio; LL, UL = lower and upper 95% confidence limits.
[2]The ABMR scores were standardized (scaled and centered) before analysis.
[3]For the multivariable analysis, only the molecular ABMR score and the histology-DSA ABMR definition with the lowest p-value (C4d+/−/mixed) were entered.

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doi: 10.1111/ajt.13572

Using Molecular Phenotyping to Guide Improvements in the Histologic Diagnosis of T Cell–Mediated Rejection

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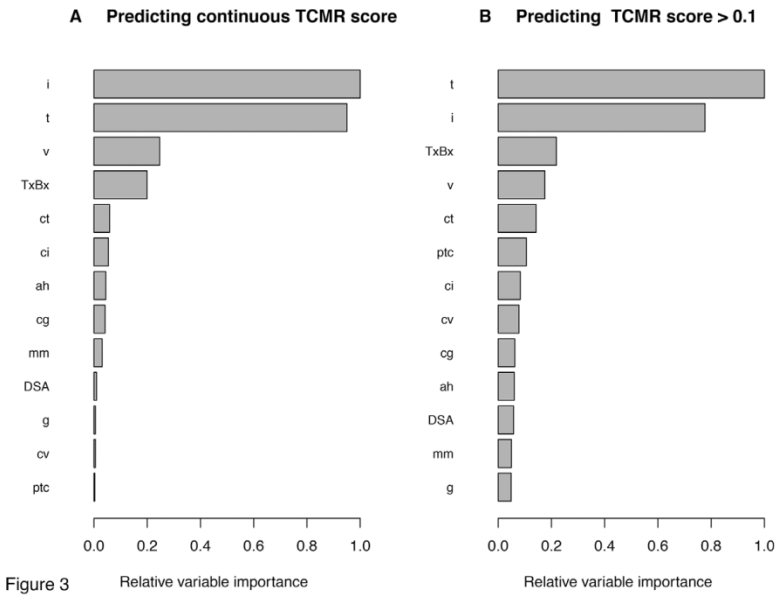


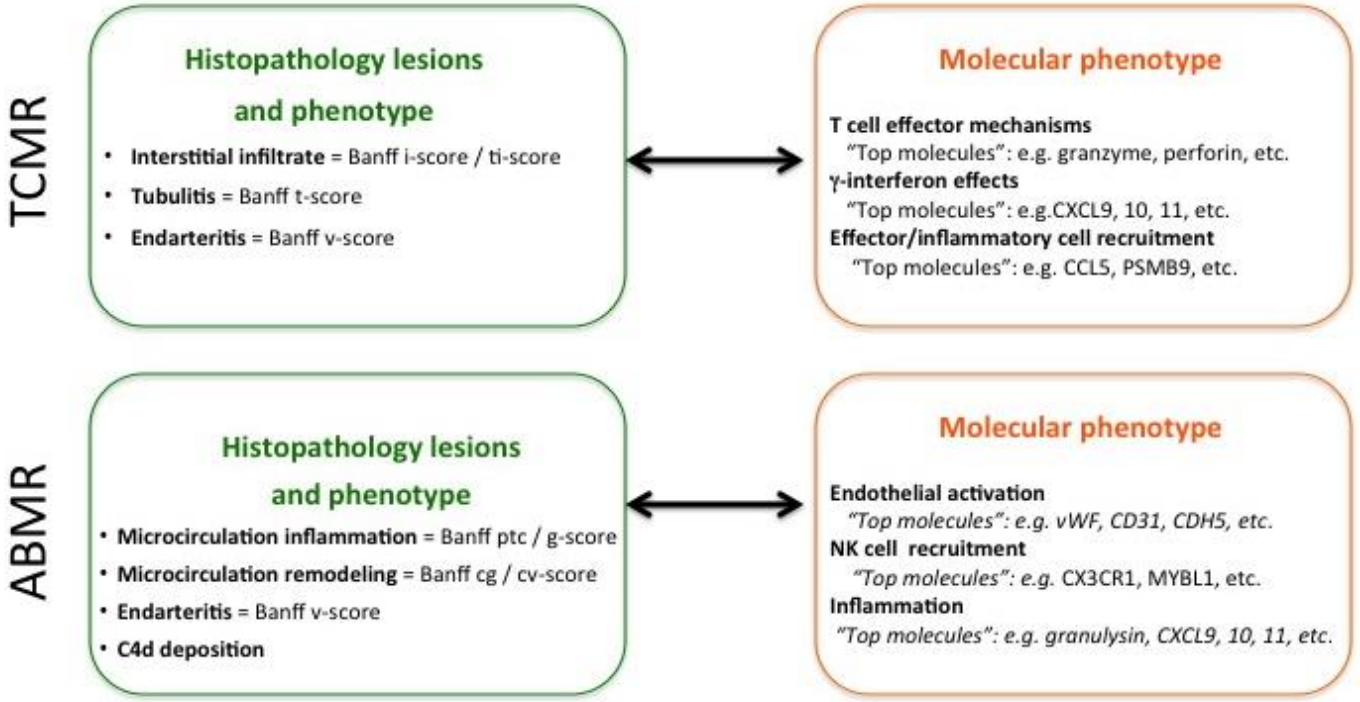
Figure 3

Figure 3: Random forest analysis of the relative importance of histology lesions, TxBx and HLA antibodies detected in predicting the molecular TCMR classifier score. DSA indicates DSA positive vs. panel reactive antibodies (PRA) negative. (A) Predicting continuous TCMR scores (0.0–1.0). (B) Predicting positive TCMR scores (>0.10). The variables' importance is standardized to that of the best predictor (1.0). ah, arteriolar hyalinosis; cg, transplant glomerulopathy; ci, fibrosis; ct, atrophy; cv, arterial fibrous intimal thickening; DSA, donor-specific antibody; g, glomerulitis; i, interstitial inflammation; mm, mesangial matrix increase; ptc, peritubular capillaritis; t, tubulitis; TCMR, T cell-mediated rejection; TxBx, time of biopsy after transplant; v, arteritis.

Table 1: Logistic regression predicting molecular TCMR score >0.1

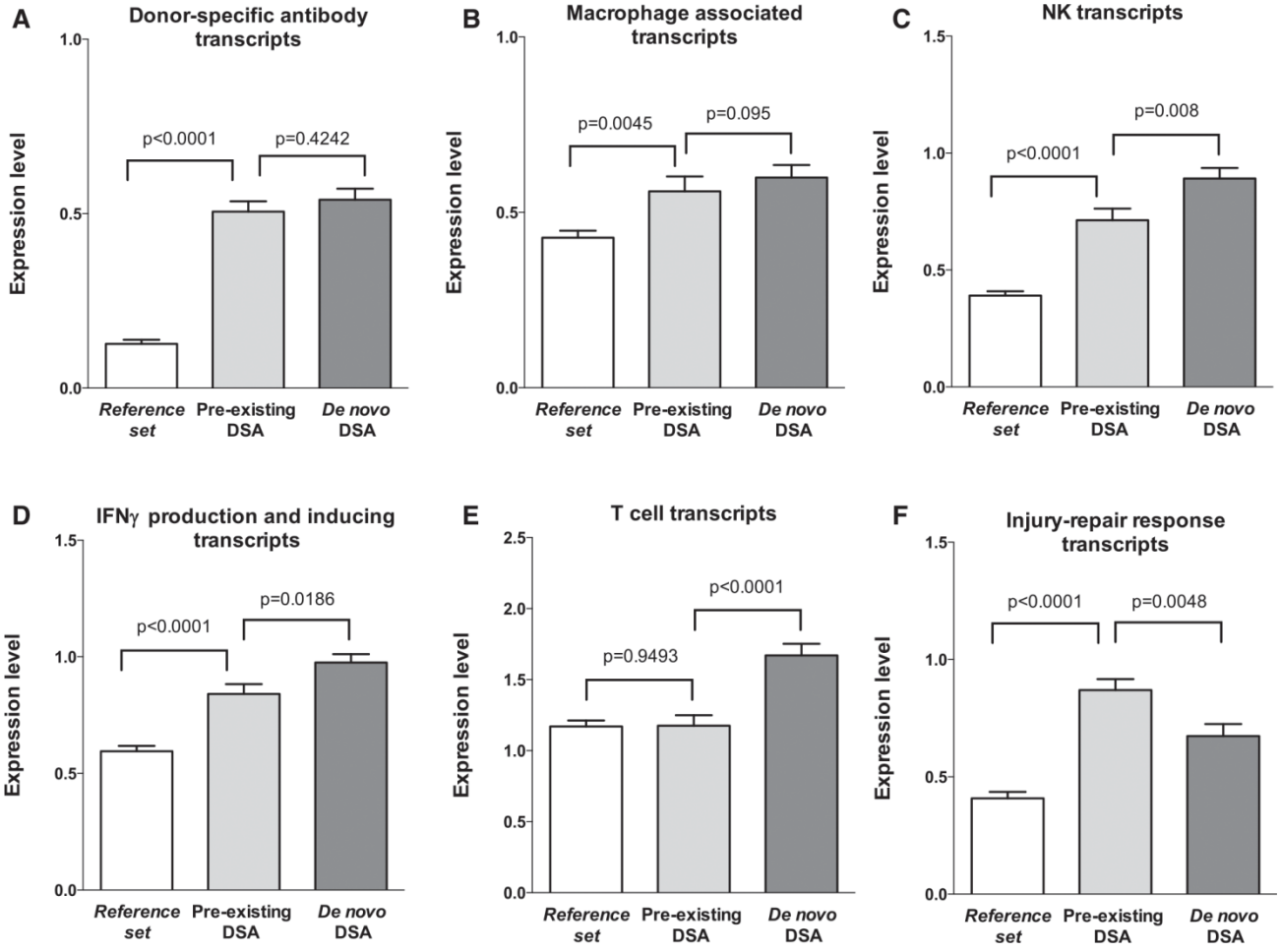
Coefficients	Estimate	p-value
(Intercept)	−5.66	<0.001
t-lesion score	0.72	<0.001
i-lesion score	0.88	<0.001
v-lesion score	0.90	0.006
TxBx	2.93	0.008
$TxBx^2$	−0.76	0.15

The Banff 2015 – 2025 Molecular Process



Antibody-Mediated Rejection Due to Preexisting versus *De Novo* Donor-Specific Antibodies in Kidney Allograft Recipients

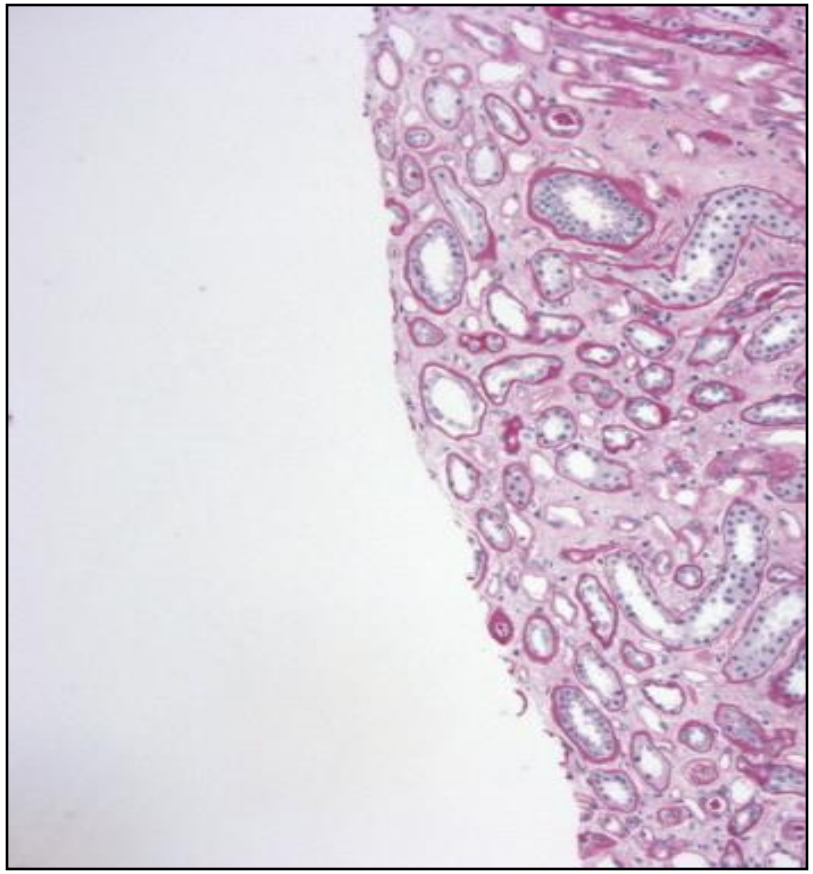
Olivier Aubert,* Alexandre Loupy,*†‡ Luis Hidalgo,§‖ Jean-Paul Duong van Huyen,¶ Sarah Higgins,** Denis Viglietti,*†† Xavier Jouven,* Denis Glotz,*†† Christophe Legendre,*†‡ Carmen Lefaucheur,*†† and Philip F. Halloran‖‡‡

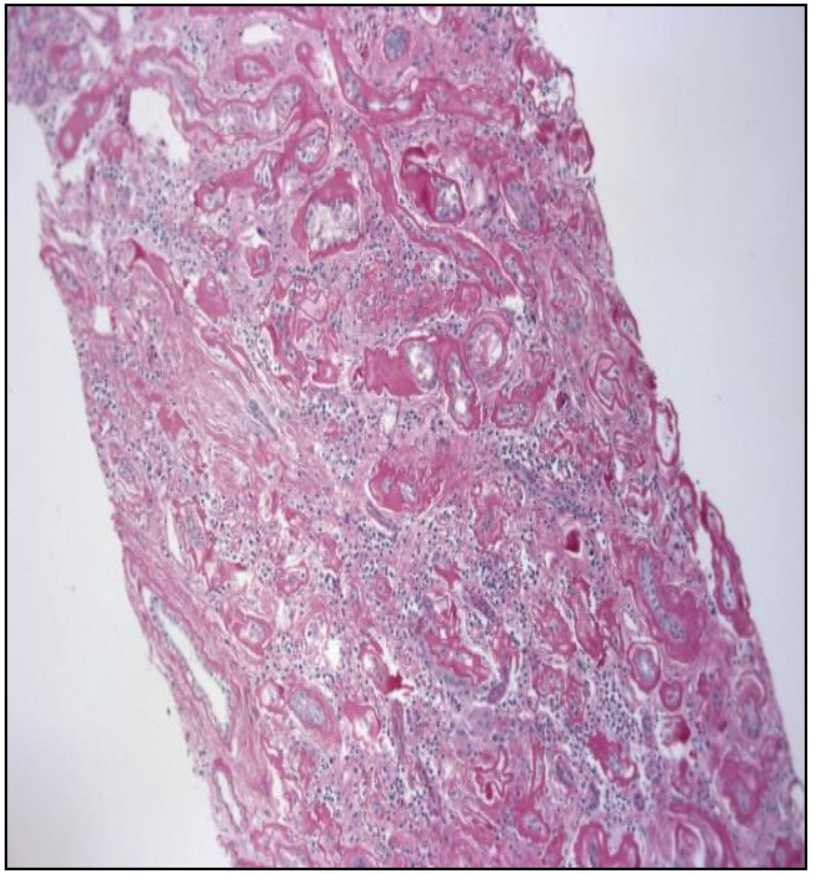


CHRONIC ACTIVE T-CELL MEDIATED REJECTION (POSSIBLE CONTENDERS)

- Interstitial fibrosis and tubular atrophy (IFTA) with interstitial inflammation (i) > 0
- DSA-/C4d- transplant glomerulopathy

INTERSTITIAL FIBROSIS WITH AND WITHOUT INFLAMMATION



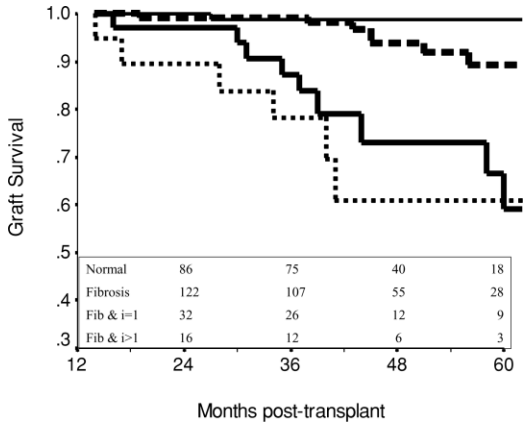


CLINICAL SIGNIFICANCE OF INFILTRATES IN FIBROTIC AREA

Cosio FG et al. AJT 2005; 5:2464

Table 2: Diagnostic classification of 1-year surveillance biopsies

Group	N (%)	I score > 0	CI score > 0	CG score >0
1. Normal	87 (30)	No	No	No
2. Inflammation	6 (2)	Yes	No	No
3. Fibrosis	131 (45)	No	Yes	No
4. Fibrosis and inflammation	53 (18)	Yes	Yes	No
5. Transplant glomerulopathy	15 (5) 15 (5)	Yes (20%)	Yes (80%)	Yes



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Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2010.03240.x

Inflammation in Areas of Tubular Atrophy in Kidney Allograft Biopsies: A Potent Predictor of Allograft Failure

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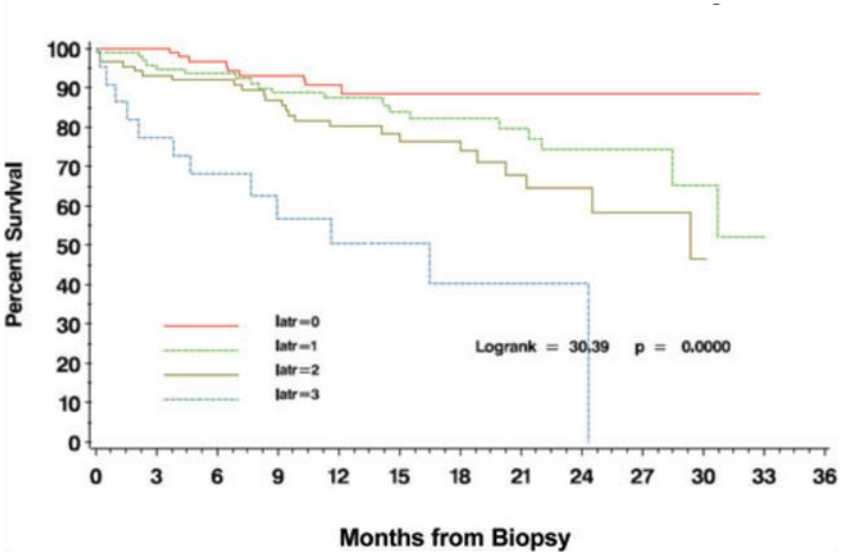
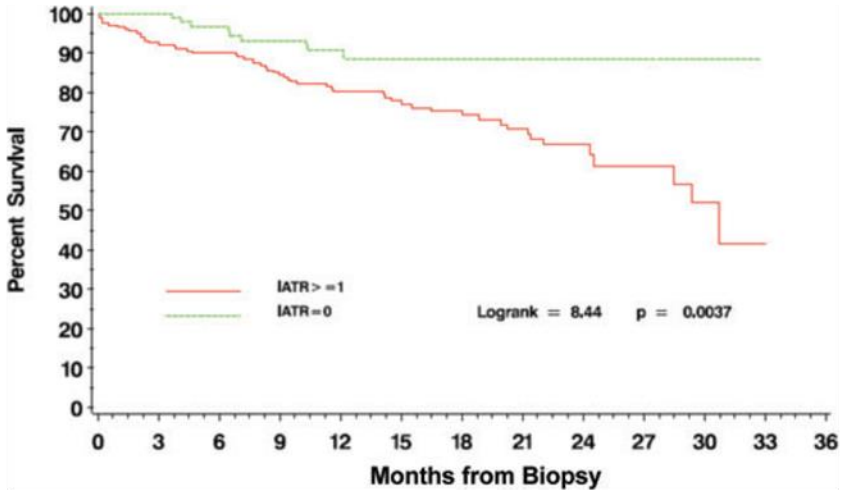
better prognostic information. Phenotypic characterization of these inflammatory cells and appropriate treatment may ameliorate late allograft failure.

Key words: Banff schema, biopsy, inflammation, fibrosis, graft failure, injury





FIBROSIS WITH INFLAMMATION AT ONE YEAR PREDICTS FUNCTIONAL DECLINE

Park et al. JASN 2010; 21 : 1987

151 living-donor recipients
86 normal histology
45 IF/TA alone
20 IF/TA with inflammation

Microarray profiles of biopsies:
Acute rejection associated genes in biopsies with IF/TA with inflam.

Table 2. Graft function and graft survival of 151 noncomplicated, HLA-mismatched, LD KTxs between 1 and 5 years after transplantation

Parameter	Normal	IF Alone	IF+i	*P*[a]
Length of follow-up after T12 surveillance biopsy (days; mean ± SD)	1515 ± 535	1677 ± 586	1416 ± 806	0.2, 0.4, 0.1
Graft survival at most recent follow-up (%)	97	91	85	0.2, 0.04, 0.5
Death-censored graft survival at most recent follow-up (%)	99	98	85	0.6, 0.003, 0.05
GFRu by C_{ioth} (ml/min; mean ± SD [*n*])				
1 month	66 ± 16 (82)	61 ± 17 (41)	61 ± 20 (17)	0.08, 0.04, 0.49
12 months	71 ± 18 (83)	67 ± 23 (43)	55 ± 17 (19)	0.20, 0.01, 0.09
24 months	68 ± 18 (76)	62 ± 16 (30)	53 ± 13 (12)	0.11, 0.01, 0.14
36 months	70 ± 23 (66)	67 ± 25 (33)	52 ± 18 (12)	0.28, 0.01, 0.10
48 months	68 ± 24 (50)	64 ± 23 (27)	44 ± 20 (10)	0.57, 0.01, 0.05

[a]*P* values based on Wilcoxon rank sum tests of normal *versus* IF alone; normal *versus* IF+i, and IF alone *versus* IF+i, respectively.

MOLECULAR FEATURES OF INFLAMATION IN SCARRED AREAS

Mengel M et al. AJT 2009; 9 : 169

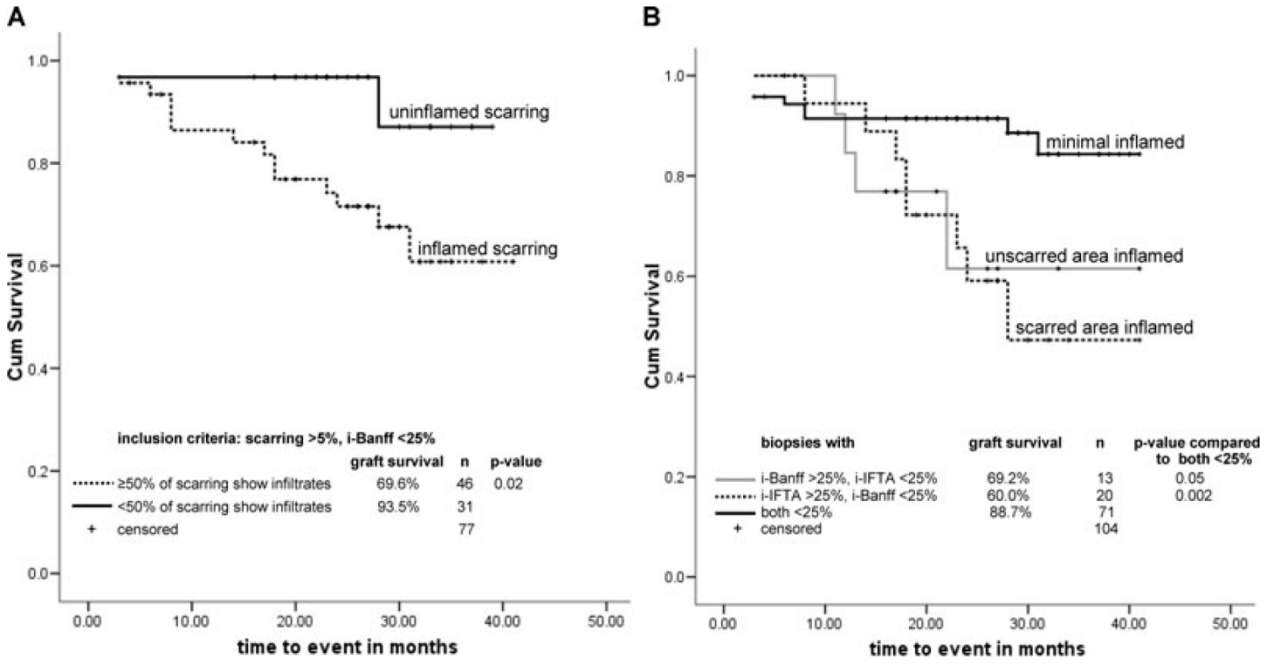


Figure 3: (A and B) IFTA and inflammation in biopsies for cause and allograft survival. Kaplan–Meier curves show that allografts with scarring lacking considerable inflammation in this compartment have better outcome than those with extensively inflamed scarring (A). Figure 3B shows that inflammation in either cortical compartment (unscarred areas and scarring) above the current Banff threshold for rejection (i.e. >25%) is associated with an inferior prognosis compared to allografts with infiltrates below this threshold. Events are defined as either allograft loss with return to dialysis or persistent (>3 months) low (<30 mL/min) eGFR. Given is the time between the last biopsy of a patient and an event.

MOLECULAR FEATURES OF INFLAMATION IN SCARRED AREAS

Mengel M et al. AJT 2009; 9 : 169

Gene expression profiles of biopsies with IF/TA and inflammation showed upregulation of genes related to CTL, IFN-γ, macrophages, B cells, immunoglobulins, injury and repair induced transcripts

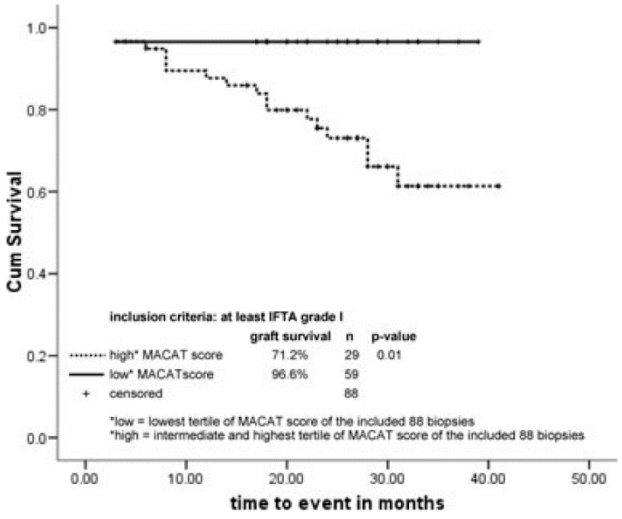
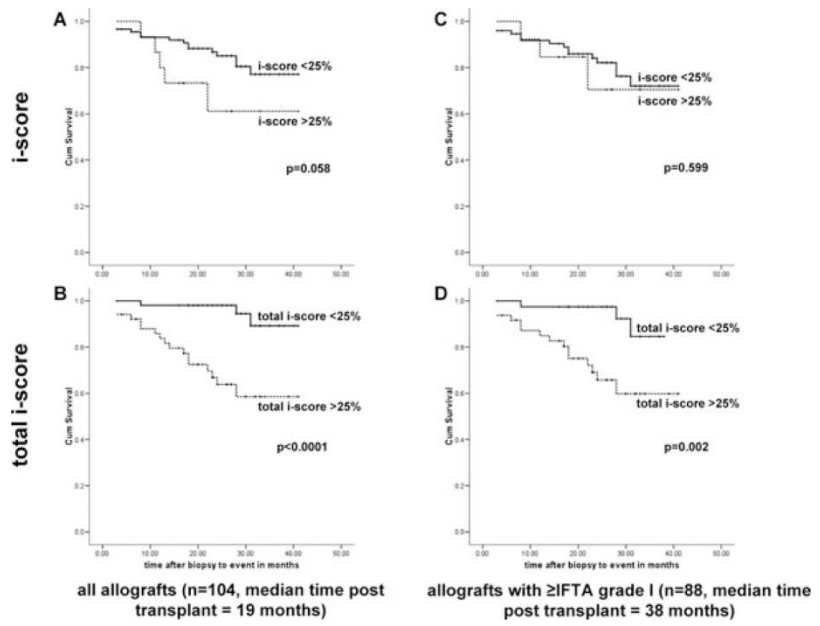
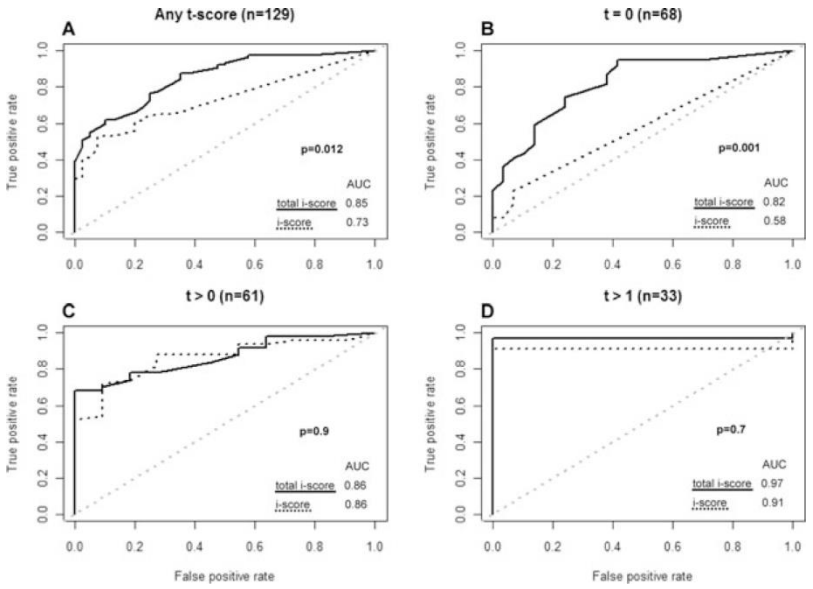


Figure 5: Mast cell PBT and allograft survival. Kaplan–Meier curves show that within allografts with scarring (at least Banff grade I) those with high expression of mast cell-associated transcripts have a worse prognosis. Events are defined as either allograft loss with return to dialysis or persistent (>3 months) low (<30 mL/min) eGFR. Given is the time between the last biopsy of a patient and an event.

TOTAL INFLAMMATION SCORE IS SUPERIOR TO i-BANFF SCORE

Mengel M et al. AJT 2009; 9:1859





(*Transplantation* 2011;91: 657–665)

Gene Expression Changes Are Associated With Loss of Kidney Graft Function and Interstitial Fibrosis and Tubular Atrophy: Diagnosis Versus Prediction

Mariano J. Scian,[1] Daniel G. Maluf,[1] Kellie J. Archer,[1,2] Jihee L. Suh,[1] David Massey,[1] Ryan C. Fassnacht,[1] Benjamin Whitehill,[1] Amit Sharma,[1] Anne King,[1] Todd Gehr,[1] Adrian Cotterell,[1] Marc P. Posner,[1] and Valeria Mas[1,3,4]

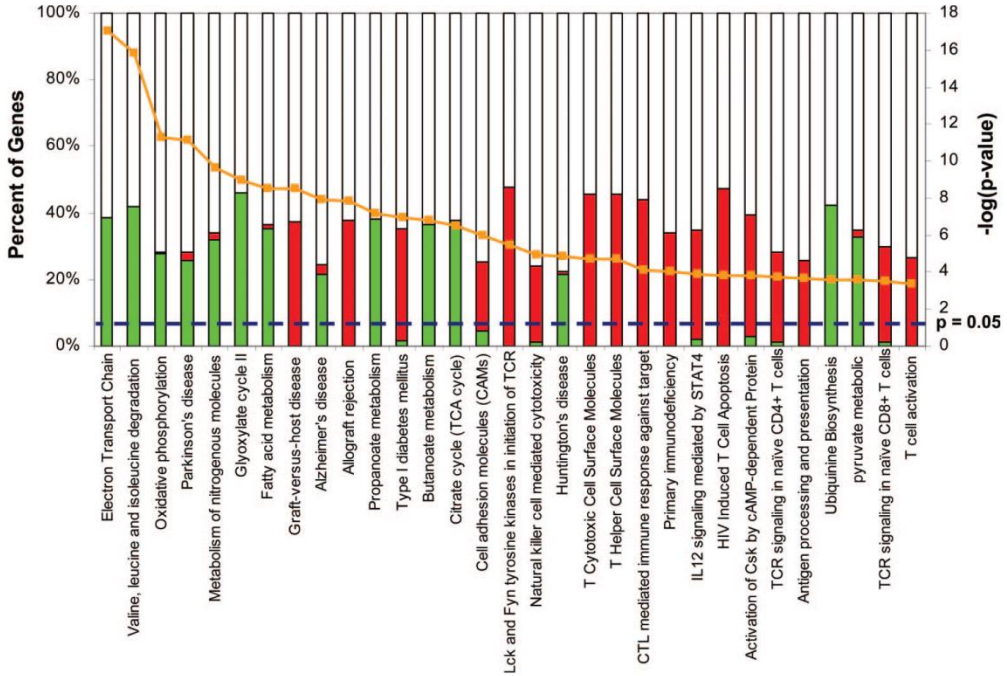


FIGURE 3. Canonical pathways identified from the differentially expressed genes. Each bar represents the percentage of up-regulated (*red*) or down-regulated (*green*) or unaffected/undetected (*white*) genes within the identified pathway. The *line* represents the −log(*P* value).

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doi: 10.1111/ajt.13728

Gene Expression in Biopsies of Acute Rejection and Interstitial Fibrosis/Tubular Atrophy Reveals Highly Shared Mechanisms That Correlate With Worse Long-Term Outcomes

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Table 2: Shared differentially expressed transcripts between IFTA subphenotypes (IFTA plus AR, IFTA with inflammation, and IFTA without inflammation) and clinical acute rejection (cAR)[1]

	All samples with IFTA (n = 78)	IFTA without inflammation (n = 40)	IFTA with inflammation (n = 10)	IFTA plus AR (n = 28)
Number of DEGs	4705	3280	1513	6229
Number (%) shared with cAR differentially expressed transcript list	3817 (81%)	2610 (80%)	1040 (69%)	4466 (72%)

AR, acute rejection; cAR, clinical acute rejection; DEGs, differentially expressed genes; IFTA, interstitial fibrosis and tubular atrophy; FC, fold-change; FDR, false discovery rate.

[1]In comparison of AR samples to patients with normal, well-functioning transplants (control; TX), there were 5345 differentially expressed transcripts (FDR*<0.05; FC*>1.2). This table shows the large number and percent of gene transcripts shared between cAR and each IFTA subphenotype.

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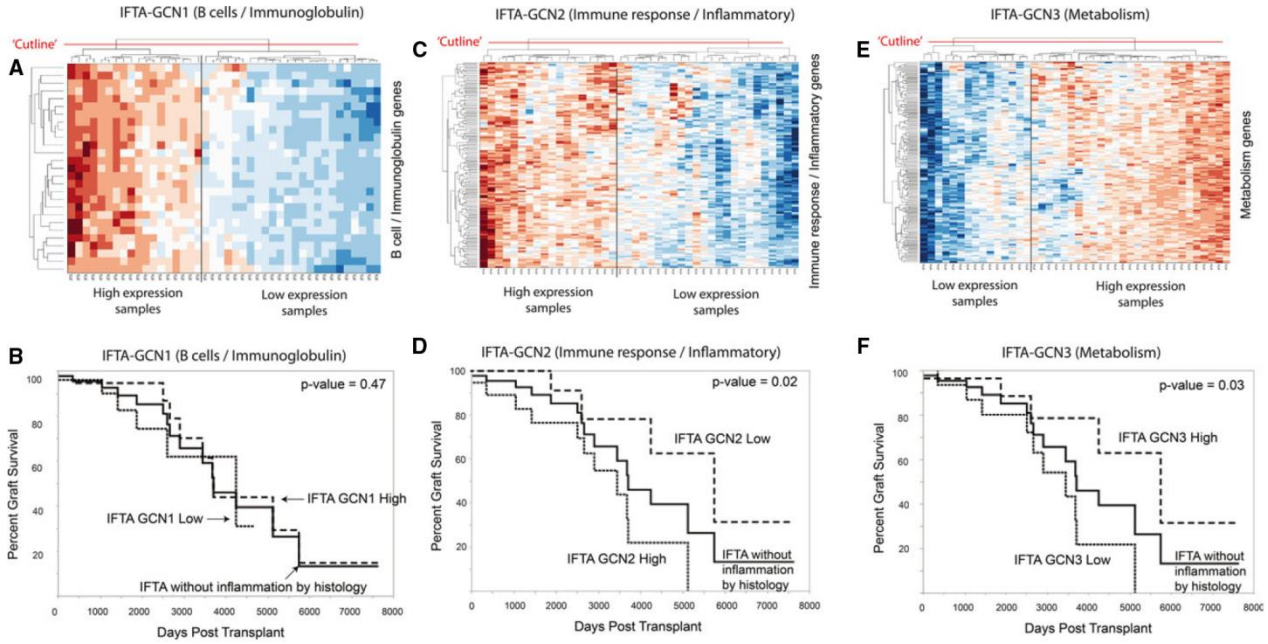


Figure 7: Graft survival of subjects with IFTA without inflammation according to expression of our three gene coexpression networks (GCNs). (A) Interstitial fibrosis and tubular atrophy (IFTA) without inflammation samples clustered into two clusters based on high versus low expression of GCN1 (B cell/immunoglobulin genes). (B) High versus low expression of GCN1 did not demonstrate a difference in graft survival ($p = 0.47$). (C and D) In contrast, when this analysis is repeated using GCN2 (immune response/inflammatory), graft survival of subjects with IFTA without inflammation correlates with relative expression of GCN2 ($p = 0.02$). (E and F) Relative expression of GCN3 (metabolism/tissue integrity) also correlates with graft survival ($p = 0.03$).

SUMMARY:
IFTA WITH INFLAMMATION

- Lower allograft survival compared to IFTA w/o inflammation
- Increased intragraft gene transcripts associated with immune activity (CTL, IFN-γ, macrophages, B cells)
- Potential for diagnosis of chronic TCMR

CHRONIC ACTIVE T-CELL MEDIATED REJECTION (POSSIBLE CONTENDERS)

- Interstitial fibrosis and tubular atrophy (IFTA) with interstitial inflammation (i) > 0
- DSA-/C4d- transplant glomerulopathy

Transplant Glomerulopathy May Occur in the Absence of Donor-Specific Antibody and C4d Staining

Enver Akalin,*† Rajani Dinavahi,*† Steven Dikman,‡ Graciela de Boccardo,*† Rex Friedlander,§ Bernd Schroppel,*† Vinita Sehgal,*† Jonathan S. Bromberg,† Peter Heeger,*† and Barbara Murphy*†

*Renal Division, †Recanati/Miller Transplantation Institute, and ‡Department of Pathology, Mount Sinai School of Medicine, and §Immunogenetics Laboratory, Rogosin Institute, New York, New York

Clin J Am Soc Nephrol 2: 1261–1267, 2007.

Table 2. Histopathologic features of patients with CAN and TGP, anti-HLA antibodies, and graft outcome

Feature	CAN (51 Patients)	TGP (36 Patients)
CAN grade per Banff 97 (*n* [%])		
I	23 (45)	9 (25)
II	21 (41)	21 (58)
III	7 (14)	6 (17)
TGP grade (*n* [%])		
I	6 (17)	
II	9 (25)	
III	13 (36)	
electron microscopy alone	8 (22)	
C4d positivity (diffuse; %)	4	11
C4d positivity (focal; %)	3	4
Nodular arteriolar hyalinosis (%)	31	31
DSA (%)[a]	33	36
anti-HLA class I	20	10
anti-HLA class II	40	50
both classes I and II	40	40
Non-DSA (%)[a]	23	29
No anti-HLA antibodies (%)	60	60
Graft loss (%)	16	28
Graft loss time after biopsy (yr; mean ± SD)	0.7 ± 0.7	1.1 ± 1.1

15% C4d

+

36% DSA+

American Journal of Transplantation 2003; 3: 1116–1120
Blackwell Munksgaard

ISSN 1600-6135

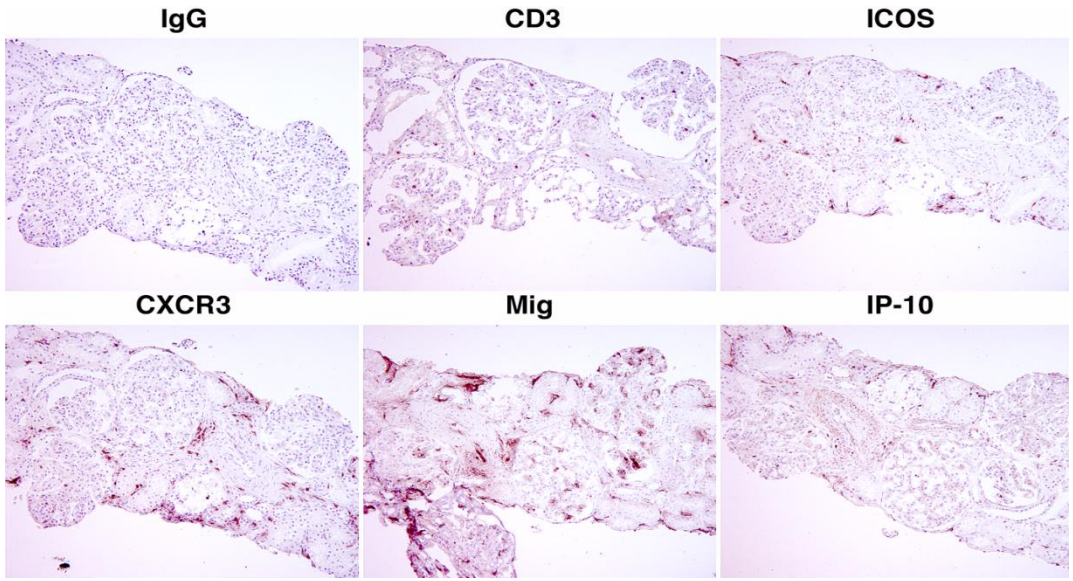
Glomerular Infiltration by CXCR3+ ICOS+ Activated T Cells in Chronic Allograft Nephropathy with Transplant Glomerulopathy

E. Akalin[a,c,*], S. Dikman[b], B. Murphy[a,c], J. S. Bromberg[c] and W. W. Hancock[d]

CAN patients. Recent studies suggest the importance of antibody-mediated immune mechanisms in the pathogenesis of CAN as indicated by C4d deposits in peritubular capillaries

Table 2: Leukocyte labeling of renal biopsies from patients with CAN

Pathology	Number	ICOS	CXCR3	Mig	IP-10	CCR2	MCP-1
CAN+ TGP+	6	6/6	6/6	6/6	4/6	0/5	0/6
		100%	100%	100%	67%	0%	0%
CAN+ TGP–	11	0/9	0/7	0/9	0/9	0/11	0/11
		0%	0%	0%	0%	0%	0%



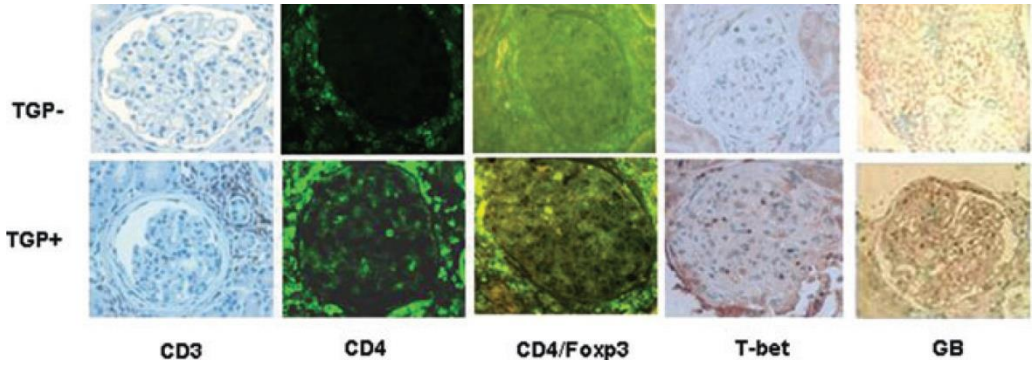
Predominant Th1 and Cytotoxic Phenotype in Biopsies from Renal Transplant Recipients with Transplant Glomerulopathy

American Journal of Transplantation 2009; 9: 1230–1236

S. Homs[a,†], H. Mansour[a,†], D. Desvaux[a], C. Diet[a], M. Hazan[c], M. Buchler[b], Y. Lebranchu[b], D. Buob[d], C. Badoual[f], M. Matignon[a], V. Audard[a], P. Lang[a] and P. Grimbert[a,*]

Key words: Chronic transplant glomerulopathy, immune function, renal transplant, renal transplant pathology

Received 29 September 2008, revised 10 February 2009 and accepted for publication 11 February 2009



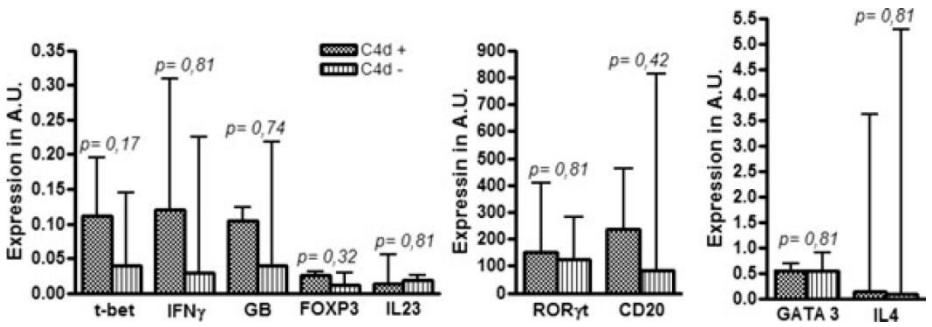


Figure 3: Expression levels of nine genes (encoding Foxp3, GB, IFNγ, T-bet, GATA3, IL-4, IL23, CD20 and RORγt) in biopsy samples from patients with TGP with (C4d+) or without (C4d–) C4d staining in peritubular capillaries.

GENE EXPRESSION PROFILES OF TRANSPLANT GLOMERULOPATHY

Elster EA…Mannon RB. J Mol Diag 2010 12 : 653

- TGP was identified in 20 biopsies of 18 patients (11%) of 963 core biopsies from 166 patients. Ten patients (56%) had DSAs
- 87 genes related to immune function and fibrosis were studied by RT-PCR
- 32 biopsies from 19 stable patients were used as control
- Cell-mediated immune response related genes upregulation, ICAM-1 (>3.15 fold), IL-10 (> 16.9 fold) and CCL3 (> 3.15 fold) and the probability of TGP is 99.67%

American Journal of Transplantation 2014; 14: 685–693
Wiley Periodicals Inc.

doi: 10.1111/ajt.12592

Brief Communication

Immune Responses to Collagen-IV and Fibronectin in Renal Transplant Recipients With Transplant Glomerulopathy

N. Angaswamy[1,†], C. Klein[2,†], V. Tiriveedhi[1,‡], J. Gaut[3], S. Anwar[2], A. Rossi[2], D. Phelan[4], J. R. Wellen[1], S. Shenoy[1], W. C. Chapman[1] and T. Mohanakumar[1,3,*]

Table 2: Development of Abs to Col-IV and FN in KTx recipients with TG

Patients	Pre/post-Tx	DSA+	HLA+	Col-IV+	FN+	Col-II
Biopsy proven TG	Pre-Tx	–	–	4/18 (22.2%)	4/18 (22.2%)	–
	Post-Tx	12/26 (46%)	4/26 (15.4%)	22/26 (84.6%)	22/26 (84.6%)	–
		I (4/12, 33%)	I (2/4, 50%)			
		II (8/12, 67%)	II (2/4, 50%)			
Biopsy proven stable	Pre-Tx	–	–	1/10 (10%)	1/10 (10%)	–
	Post-Tx	2/10 (20%)	–	2/10 (20%)	2/10 (20%)	–

Abs, antibodies; Col-II/IV, Collagen-II/IV; DSA, donor-specific antibodies; FN, fibronectin; KTx, kidney transplantation; TG, transplant glomerulopathy.

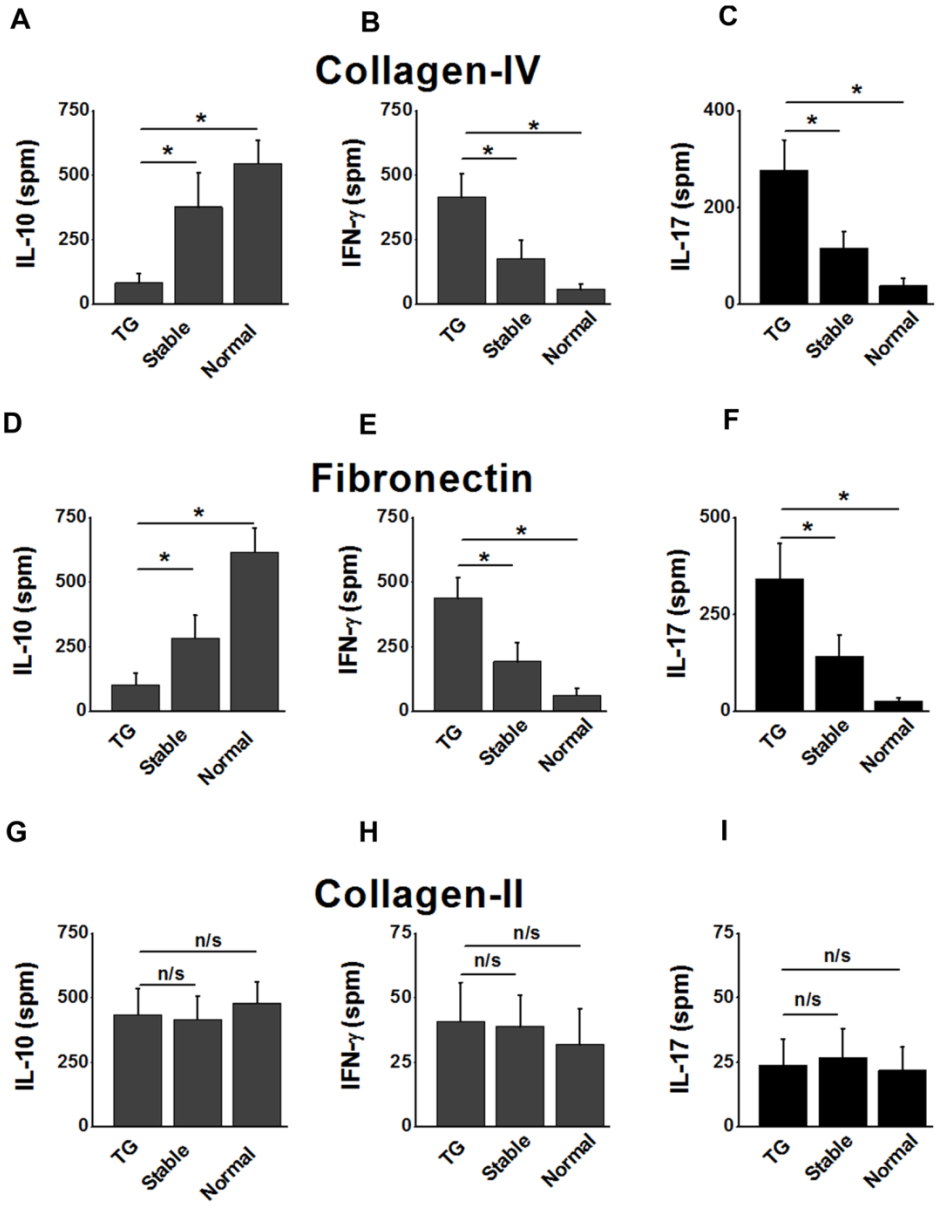


Figure 5: Increased frequency of self-Ag specific Th1 and Th17 response in patients with TG. Frequency of CD4 + T cells with specific reactivity to Col-IV (A–C), FN (D–F) and Col-II (G–I) in patients with TG (n = 26), stable (n = 10) and normal (n = 33) cohorts as determined by ELISPOT. A and D represent CD4 + T cells that induce IL-10 secretion upon specific reactivity to Col-IV and FN, respectively. B and E represent CD4 + T cells that induce IFN-γ secretion upon specific reactivity to Col-IV and FN, respectively. C and F represent CD4 + T cells that induce IL-17 secretion upon specific reactivity to Col-IV and FN, respectively. Col-II (G–I) is used as negative control in this study. Data represented as mean ± SD; statistical significance (*) ascertained if $p < 0.05$. n/s, not significant. Col-II/IV, Collagen-II/IV; FN, fibronectin; IFN-γ, interferon gamma; self-Ags, self-antigens; TG, transplant glomerulopathy.

The Clinical and Genomic Significance of Donor-Specific Antibody–Positive/C4d-Negative and Donor-Specific Antibody–Negative/C4d-Negative Transplant Glomerulopathy

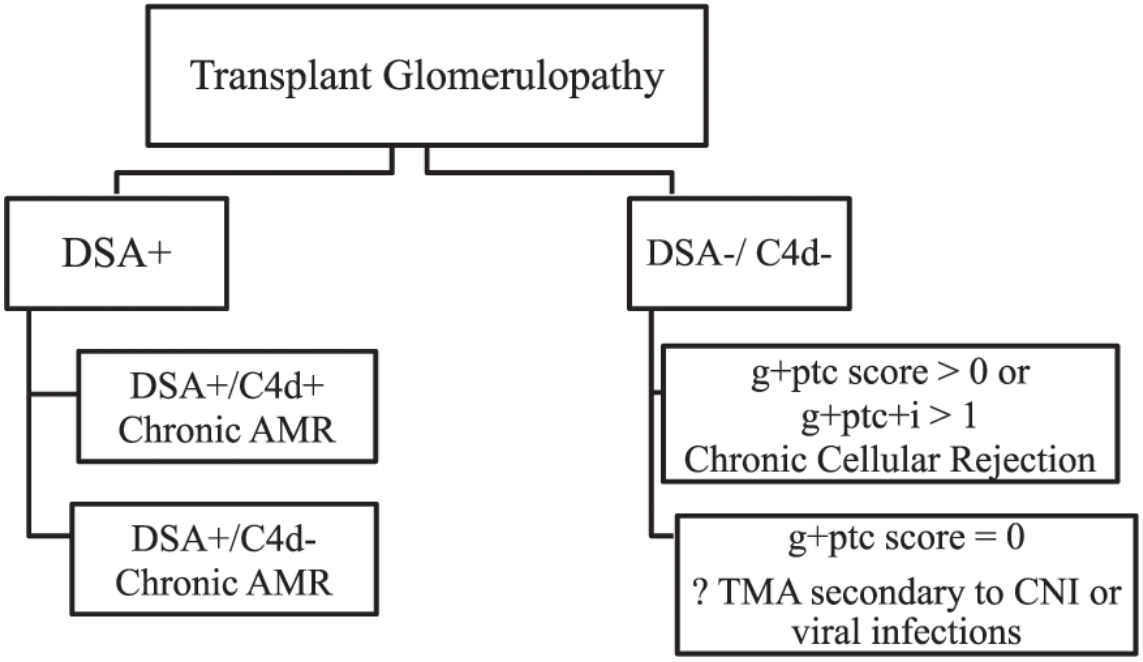
Nicole Hayde, Yi Bao,† James Pullman,‡ Bin Ye,§ R. Brent Calder,§ Monica Chung,‡ Daniel Schwartz,‡ Michelle Lubetzky,†‖ Maria Ajaimy,†‖ Graciela de Boccardo,†‖ and Enver Akalin†‖*

*Division of Pediatric Nephrology, †Montefiore–Einstein Center for Transplantation, ‡Department of Pathology, §Computational

Summary

Background This study investigated the mechanisms involved in development of donor-specific antibody (DSA) and/or C4d-negative transplant glomerulopathy (TGP) by allograft gene expression profiles using microarrays.

Clin J Am Soc Nephrol 2013; 8: 2141–21



HISTOPATHOLOGY

Banff Classification	CAMR (DSA+/C4d+) (*n*=18)	TGP (DSA+/C4d−) (*n*=14)	TGP (DSA−/C4d−) (*n*=25)	IFTA (DSA−/C4d−) (*n*=47)	*P* Value
g	0.72±0.83	0.64±0.84	0.48±0.82	0.06±0.25	0.008
ptc	1.39±1.14	1.07±1.11	0.25±0.44	0.21±0.59	<0.001
i	1.61±1.03	1.07±0.73	0.88±0.6	0.68±0.89	0.006
g + ptc	2.11	1.71	0.75	0.28	<0.001
g + ptc = 0 (%)	5 (28)	3 (21)	12 (48)	39 (83)	<0.001
g + ptc > 0 (%)	13 (72)	11 (79)	13 (52)	8 (17)	<0.001
g + ptc + i	3.72	2.79	1.63	0.96	<0.001
g + ptc + i = 0 (%)	0	1 (7)	2 (8)	23 (49)	<0.001
g + ptc + i = 1 (%)	4 (22)	0	10 (42)	13 (28)	<0.001
g + ptc + i > 1 (%)	14 (78)	13 (93)	12 (50)	11 (23)	<0.001
Tubulitis	0.56±0.78	0.29±0.47	0.04±0.2	0.11±0.31	0.15
Mesangial matrix	0.83±0.99	0.93±0.73	0.68±0.9	0.28±0.45	0.03
Interstitial fibrosis	1.67±0.97	1.21±0.70	1.48±0.77	1.62±0.85	0.51
Tubular atrophy	1.83±1.1	1.29±0.61	1.36±0.91	1.51±0.83	0.46
Intimal arteritis	0.06±0.24	0	0	0.02±0.15	0.99
Chronic vascular score	0.83±0.71	0.71±0.91	0.96±0.89	0.76±0.61	0.78
Arteriolar hyalinization	0.94±1.09	1.14±1.1	1.04±1.02	0.64±0.92	0.22

Variables shown as mean ± SD or *n* (%). *P* values represent comparisons between the four groups. CAMR, chronic antibody-mediated rejection; DSA, donor-specific antibody; TGP, transplant glomerulopathy; IFTA, interstitial fibrosis/tubular atrophy; g, glomerulitis; ptc, peritubular capillaritis; i, interstitial inflammation.

INTRAGRAFT GENE EXPRESSION PROFILES

PBT	IFTA to Normal	DSA− TGP to Normal	DSA+ TGP to Normal	CAMR To Normal	DSA− TGP to IFTA	DSA+ TGP to IFTA	CAMR to IFTA	DSA+ TGP to DSA− TGP	CAMR to DSA− TGP	CAMR to DSA+ TGP
KT	0.76	0.91	0.98	0.94	0.82	0.94	0.90	0.33	0.74	0.32
IRIT	0.63	0.44	0.98	0.31	0.18	0.20	0.10	0.34	0.23	0.31
GRIT	0.43	0.17	0.04	0.03	0.08	0.004	0.003	0.02	0.008	0.16
QCAT	0.30	0.04	0.02	0.01	0.008	<0.001	0.002	0.11	0.04	0.16
CMAT	0.15	0.06	0.04	0.006	0.12	0.06	0.004	0.11	0.006	0.03
BAT	0.27	0.09	0.12	0.12	0.08	0.11	0.07	0.72	0.29	0.22
NKAT	0.49	0.33	0.09	0.09	0.05	0.02	0.02	0.15	0.10	0.30
ENDAT	0.51	0.44	0.22	0.07	0.36	0.17	0.02	0.22	0.05	0.08
DSAST	0.33	0.12	<0.001	<0.001	0.12	<0.001	<0.001	<0.001	<0.001	0.007

P values are calculated from *t*-statistics for upregulated genes in gene set analyzed. $P<0.05$ was considered to represent statistically significant difference. PBT, pathogenesis-based transcripts; IFTA, interstitial fibrosis/tubular atrophy; DSA, donor-specific antibody; TGP, transplant glomerulopathy; CAMR, chronic antibody-mediated rejection; KT, kidney transcripts; IRIT, injury and repair-induced transcripts; GRIT, γ-IFN and rejection-induced transcripts; QCAT, quantitative cytotoxic T cell–associated transcripts; CMAT, quantitative constitutive macrophage-associated transcripts; BAT, B cell–associated transcripts; NKAT, natural killer cell–associated transcripts; ENDAT, endothelial cell–associated transcripts; DSAST, donor-specific antibody selected transcripts.

CONCLUSIONS

- Increased ptc scores were only observed in DSA+ (CAMR and DSA+/C4d- TGP) but not in DSA-/C4d- TGP biopsies and increased g scores in all TGP biopsies regardless of DSA or C4d status suggest that peritubular capillaritis is a more specific histopathologic marker of CAMR than glomerulitis.
- Both CAMR and DSA+/C4d- TGP biopsies had high microvascular inflammation (g+ptc) scores and similar gene expression profiles with activation of cytotoxic T cells, natural killer cells, macrophages, and gene transcripts associated with cellular and antibody-mediated rejection suggesting that DSA+/C4d- TGP may be classified as CAMR.
- DSA-/C4d- TGP biopsies did not demonstrate gene transcripts of CAMR, but showed increased CAT, suggesting T-cell activation as a mechanism of ongoing injury.

MOLECULAR SIGNIFICANCE OF MVI AND C4d NEGATIVE TGP

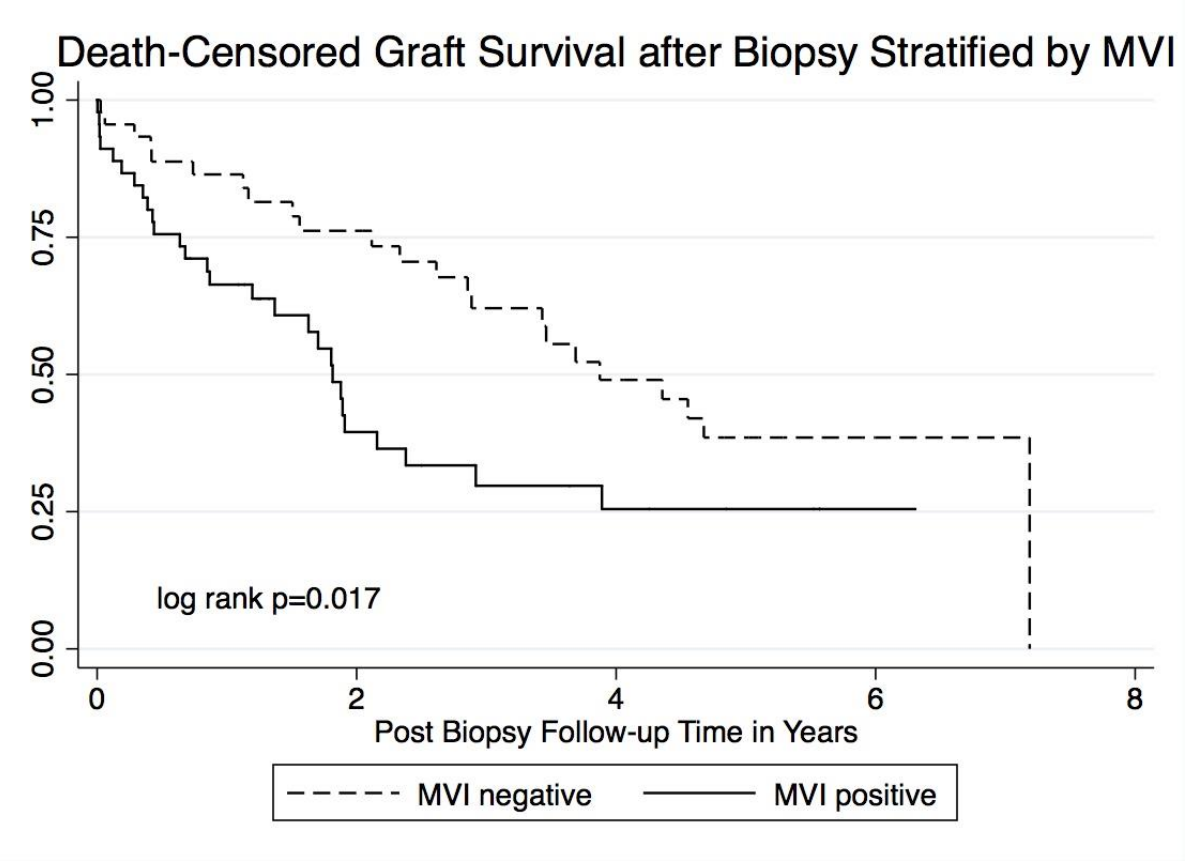
Lubetzky et al. ATC 2016, Abstract# 56

- A total of 100 patients with biopsy proven TGP
  - 49 with MVI score <2 and 51 with MVI score ≥2. Median follow up time was similar between the two groups (9.71 vs 8.3 years, p=0.21).
- A total of 44 allograft biopsies were studied using Affymetrix HuGene 1.0 ST expression arrays;
  - Group 1:12 biopsies with normal biopsy findings
  - Group 2:17 biopsies with a diagnosis of TGP, c4d positive and/or a MVI score >1
  - Group 3:15 biopsies with a diagnosis of TGP and MVI and C4d negative
    - Group 3A:10 biopsies no DSA
    - Group 3B: 5 biopsies with DSA

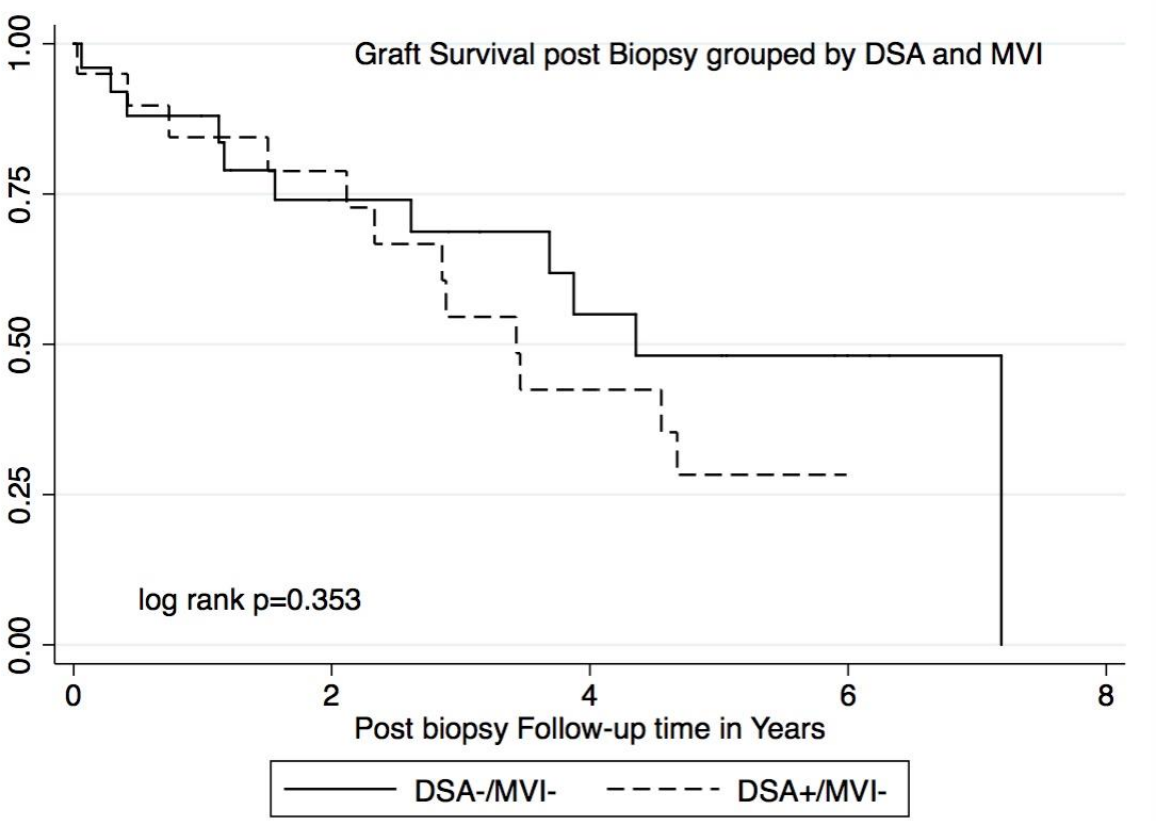
DEMOGRAPHICS

	MVI negative (n=49)	MVI positive (n=51)	P value
Age at Transplant	45.29±13.53	38.55±13.85	**0.01**
Sex (Male)	59.2%	58.8%	0.97
Race (African American)	63.3%	70.6%	0.44
Type of Transplant (Deceased Donor)	65.3%	62.7%	0.79
Cause of Kidney Disease (DM)	24.5%	15.7%	0.10
HCV negative	92.1%	100%	0.15
Induction Type (r-antithymocyte globulin)	53.1%	52.9%	0.54
DSA at time of transplant	4.1%	7.8%	0.43
Immunosupression (CNI+MMF+Pred)	75.5%	82.4%	0.35
History of prior rejection	2.0%	27.5%	**<0.001**
De novo DSA	12.2%	56.9%	**<0.001**
Class 1 PRA %	22 ± 27%	25 ± 30%	0.44
Class II PRA %	29 ± 36%	44 ± 34%	0.08
Time to biopsy	6.41 (3.27-9.35)	6.58 (3.32-10.12)	0.58
Last Creatinine	4.67±3.0	5.07±3.79	0.89
Proteinuria	3.15±3.88	2.34±2.54	0.46
Follow up time	9.71 (7.32-12.56)	8.3 (5.05-12.84)	0.21

GRAFT SURVIVAL

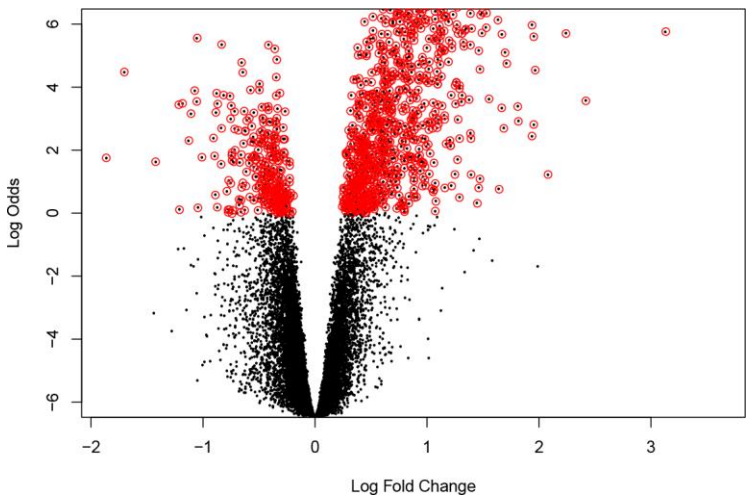


GRAFT SURVIVAL



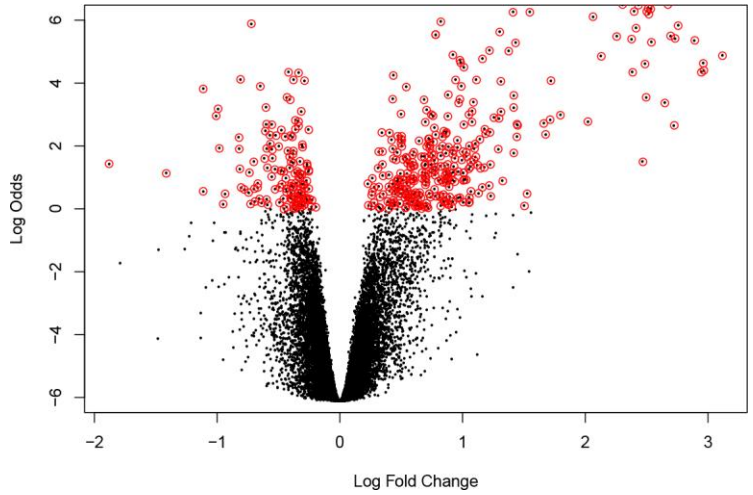
DIFFERENTIALLY EXPRESSED GENE TRANSCRIPTS

- **MVI AND/OR C4d+ TGP**



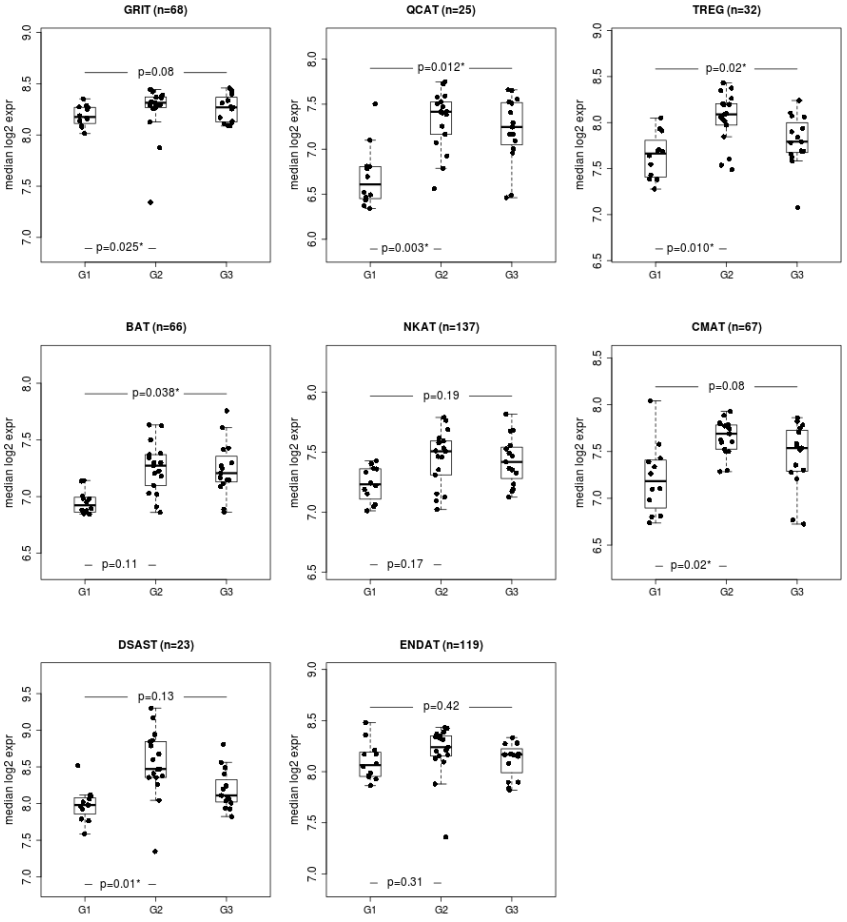
- Comparisons FDR p < 0.05 (2932) Fold Change >2 (340) Both (331)

- **MVI AND/OR C4d- TGP**



- Comparisons FDR p < 0.05 (831) Fold Change >2 (140) Both (122)

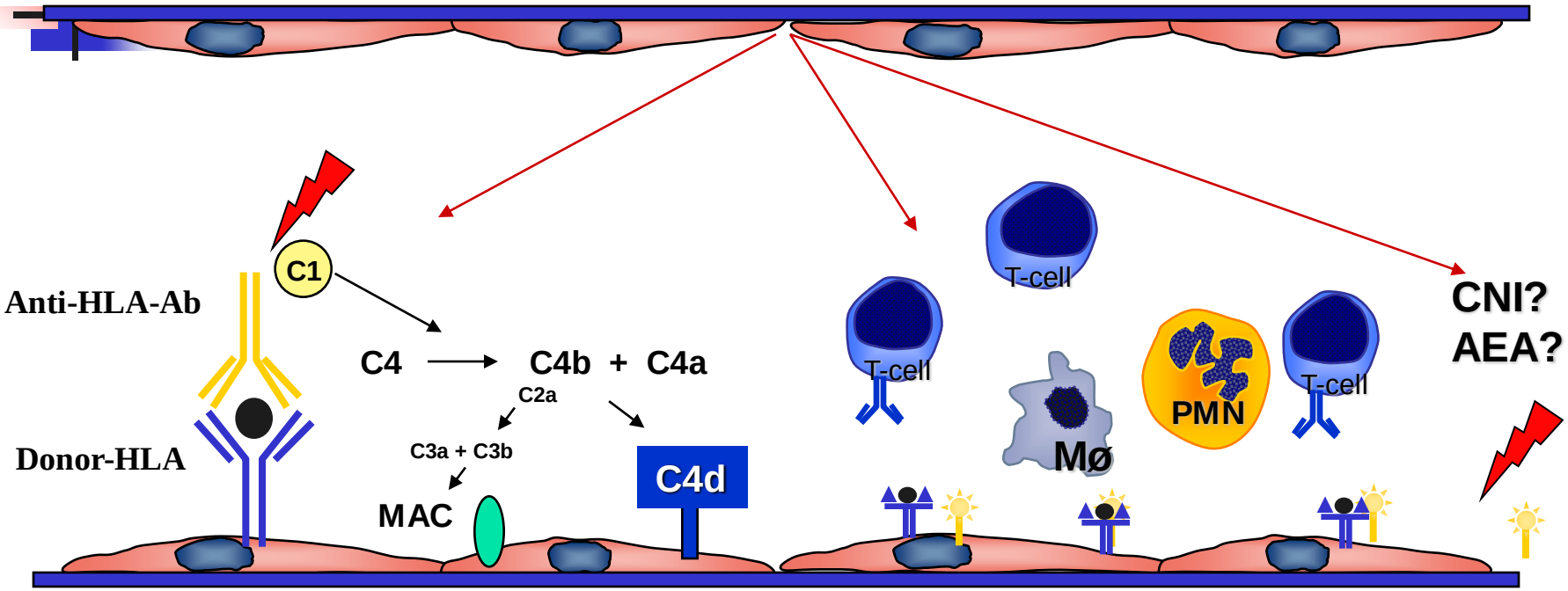
Pathogenesis base gene transcript expression in MVI positive (G2) and negative TG (G3) to normal (G1)



MECHANISMS OF TRANSPLANT GLOMERULOPATHY

Allo-antibody mediated mechanisms

Chronic cellular rejection

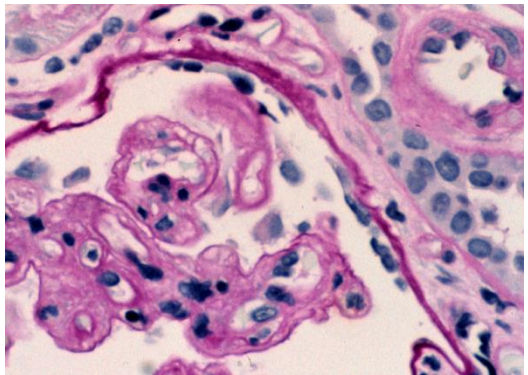


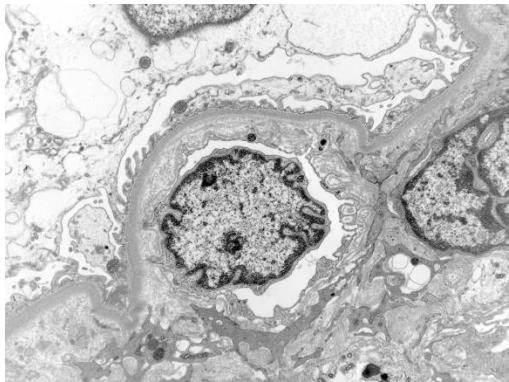
CHRONIC INJURY DUE TO:

CELLULAR IMMUNE RESPONSE

ANTIBODY-MEDIATED RESPONSE

AUTOANTIBODIES?





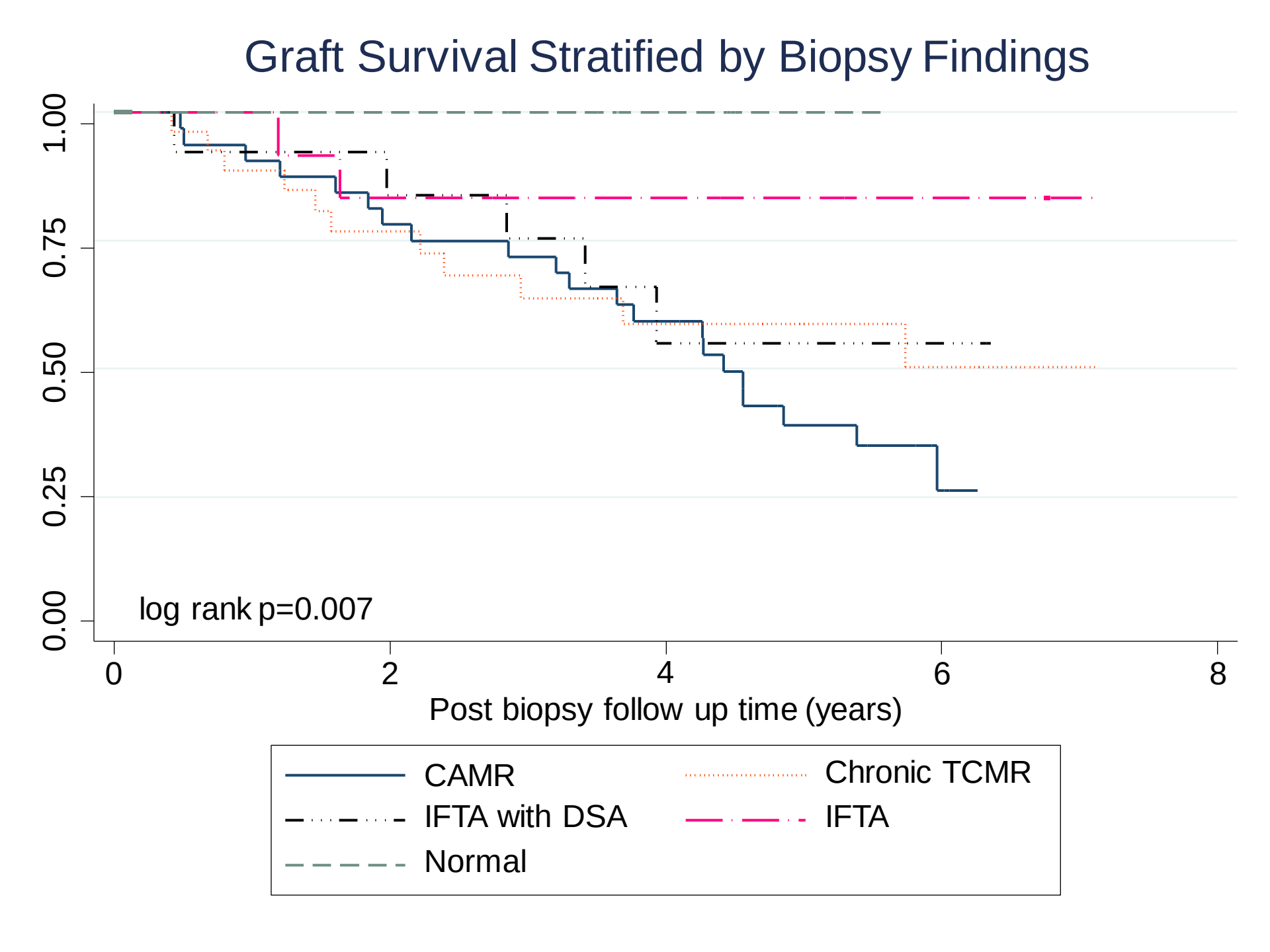
A MOLECULAR APPROACH TO CHRONIC REJECTION: CHRONIC ANTIBODY VERSUS T CELL MEDIATED REJECTION

ATC 2017

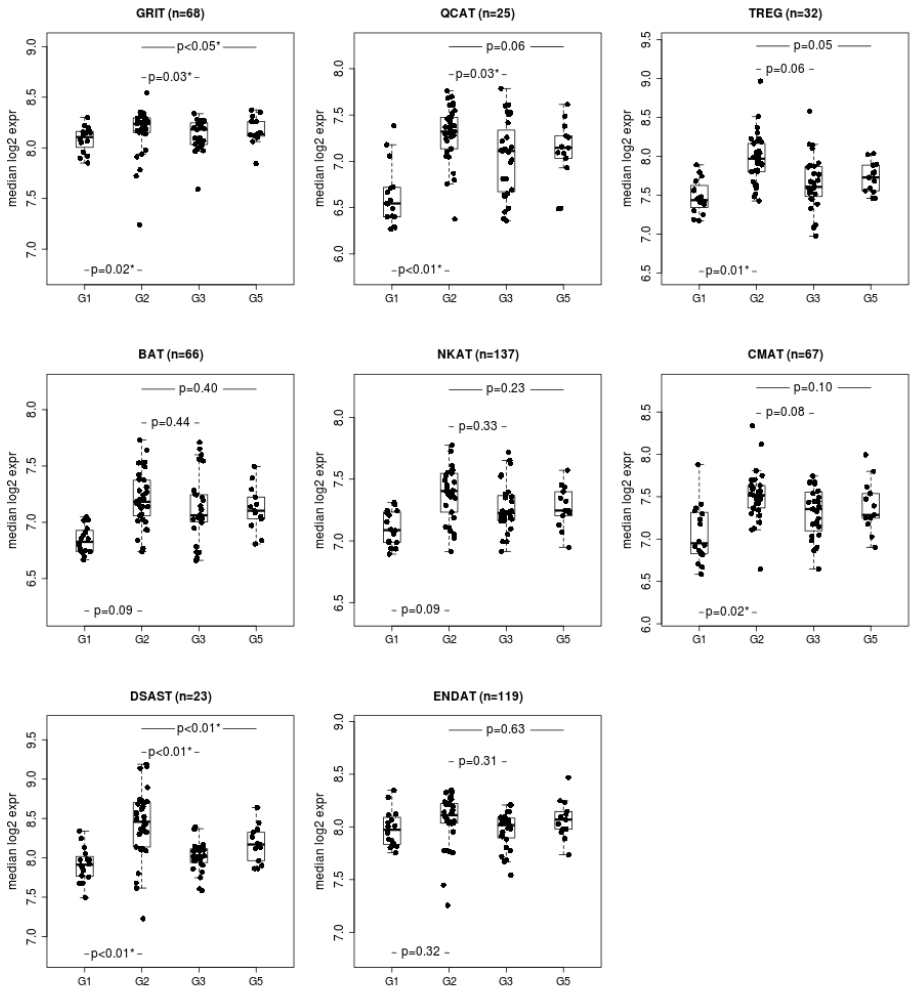
- Group 1 (G1): Normal transplant kidney biopsies (n=16)
- Group 2 (G2): Chronic AMR (n=33)
- Group 3 (G3): Chronic TCMR (n=27)
  - G3A: DSA negative IFTA with i > 0 (n=17)
  - G3B: DSA/C4d negative TGP (n=10)
- Group 4 (G4): IFTA without inflammation (n=12)
- Group 5 (G5): DSA positive IFTA with i>0 without C4d deposition or microvascular inflammation (n=13)

BANFF ALLOGRAFT INJURY SCORES

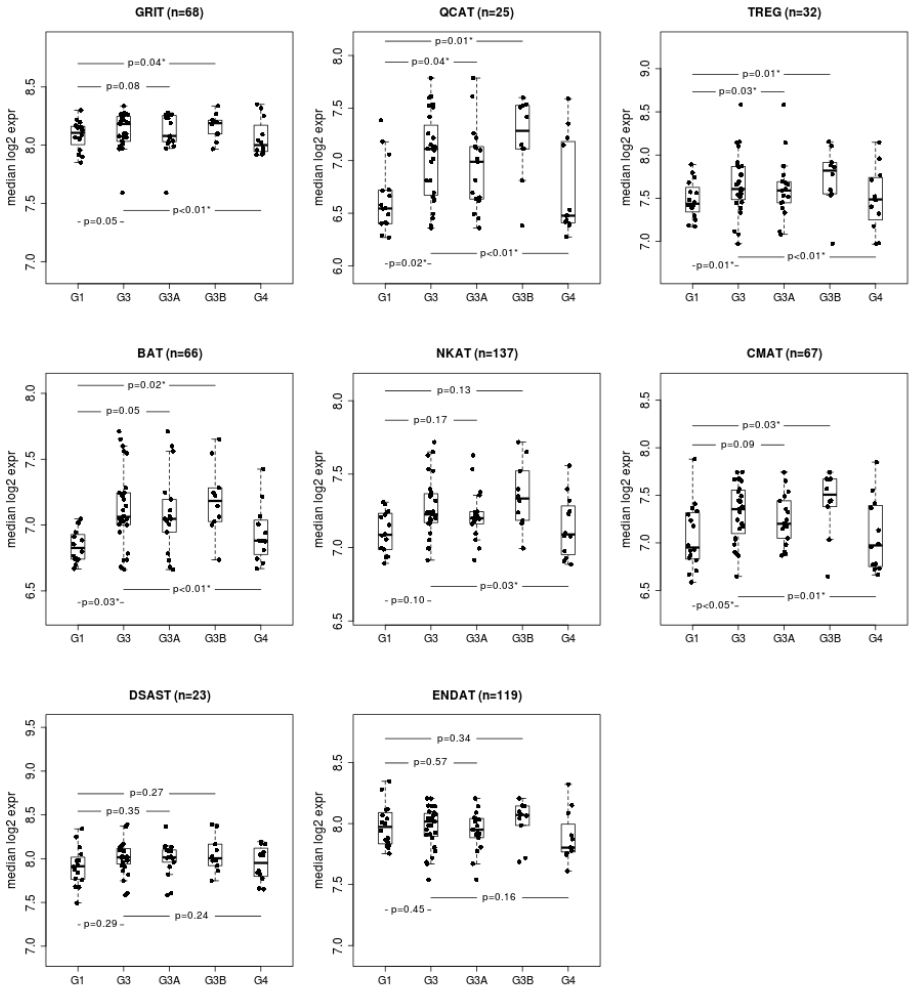
	Group 1 (Normal Biopsy)	Group 2 (CAMR)	Group 3 (CTCMR)	Group 4 (IFTA alone)	Group 5 (DSA+ i-IFTA)
g	0	0.75±0.83	0.29±0.6	0.08±0.27	0
ptc	0	1.48±1	0.44±0.75	0.16±0.37	0.23±0.42
MVI	0	2.2±1.5	0.74±1.1	0.25±0.43	0.23±0.42
i	0	1.39±0.96	1.07±0.78	0	1.1±0.7
ci	0	1.62±0.8	1.74±0.85	1.61±0.86	1.69±0.94
ct	0	1.69±0.95	1.73±0.91	1.84±0.8	1.53±0.96
cv	0	0.75±0.79	0.81±0.47	0.5±0.5	1.15±0.86
ci+ct+cv		4±2	4.25±1.95	3.75±1.63	4.3±2.2
cg	0	1.12±1.16	0.5±0.81	0	0
C4d+ (% biopsies)	0	67	0	0	0


Graft Survival Stratified by Biopsy Findings
1.00
0.75
0.50
0.25
0.00
log rank p=0.007
0
2
4
6
8
Post biopsy follow up time (years)
CAMR
Chronic TCMR
IFTA with DSA
IFTA
Normal

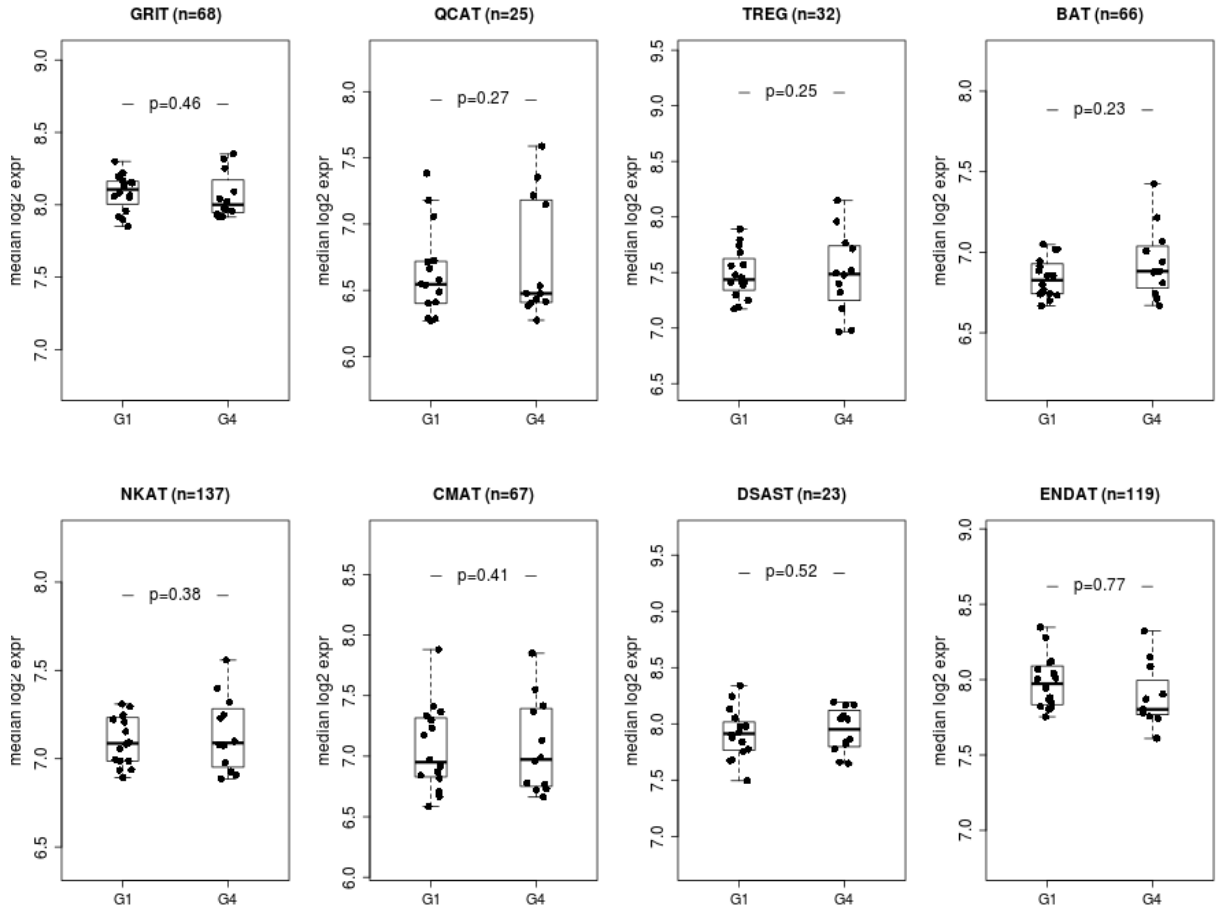
INTRAGRAFT GENE TRANSCRIPTS CHRONIC ANTIBODY-MEDIATED REJECTION



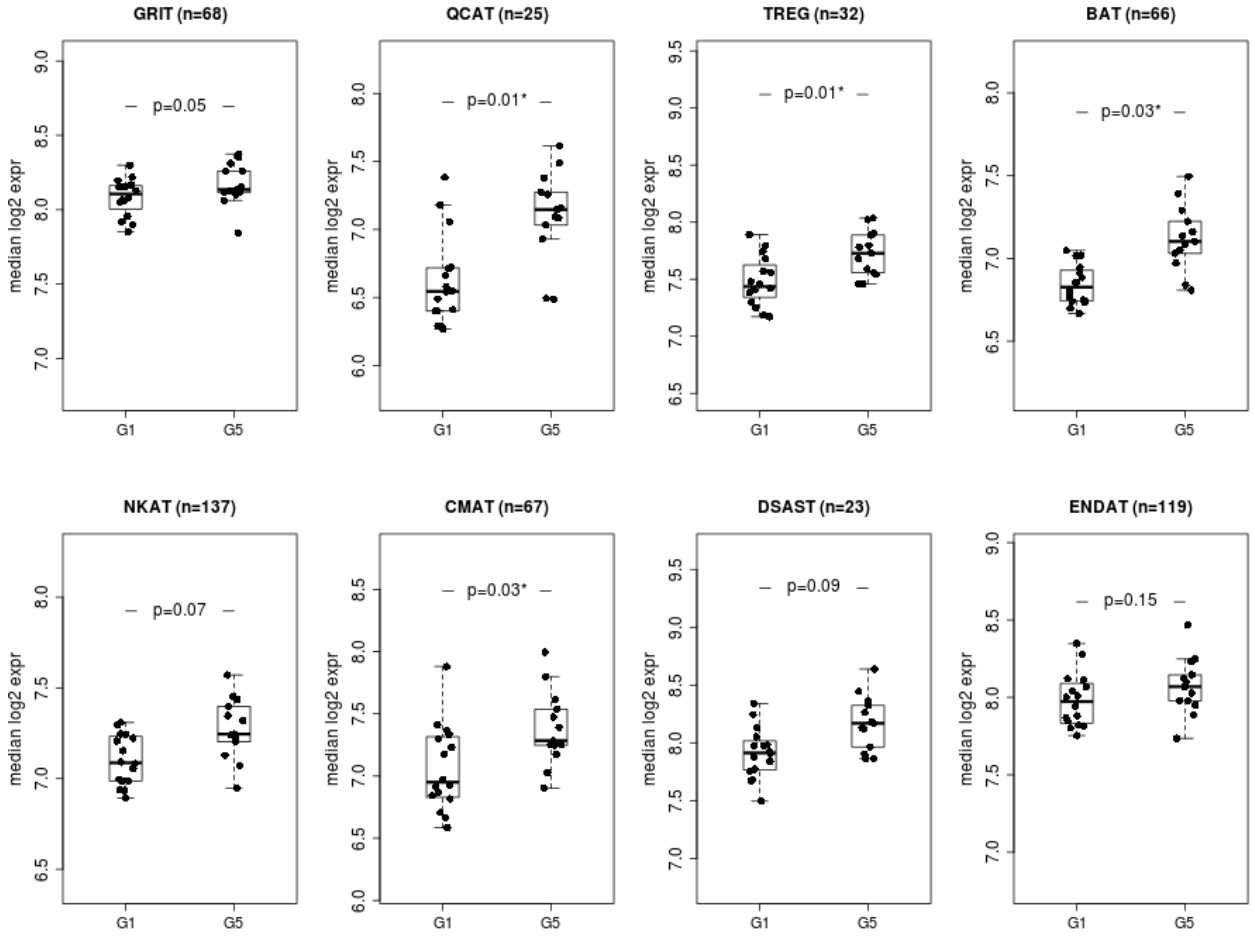
INTRAGRAFT GENE TRANSCRIPTS CHRONIC T CELL-MEDIATED REJECTION



INTRAGRAFT GENE TRANSCRIPTS IFTA ALONE



INTRAGRAFT GENE TRANSCRIPTS DSA+ IFTA WITH INFLAMMATION



SUMMARY

- Increased DSAST transcripts is a specific biomarker for molecular diagnosis of AMR
- DSA/C4d negative TGP and IFTA with inflammation biopsies (i>0) have a unique molecular signature with increased expression of CAT, TREG, and BAT but not DSAST suggesting that it could be classified as chronic TCMR
- DSA+ biopsies with IFTA with inflammation biopsies (i>0) but without C4d or MVI did not show molecular features of chronic AMR but chronic TCMR

LIMITATIONS

- A small sample size, which in the context of microarray studies, provides a statistical constraint.
- Lack of protocol biopsies at different time points after transplantation.
- Although we have good gene transcript sets for molecular diagnosis of AMR (such as DSAST), we do not have proven gene transcript sets for chronic T cell mediated rejection. How do we differentiate, acute T cell mediated rejection from chronic T cell mediated rejection by molecular microscope?
- We do not have any specific immunohistopathologic marker for chronic T cell mediated rejection

FUTURE DIRECTIONS

- BANFF working group for T cell mediated rejection was created (Volker Nickeleit and Parmjeet Randhawa are leading the working group)
- Michael Mengel is leading a working group to generate consensus in Molecular Transplant Diagnostics
- Phil Halloran is leading INTERCOM study for molecular diagnosis of transplant kidney biopsies

THANKS TO ALL

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  - Graciela de Boccardo
  - Michelle Lubetzky
  - Maria Ajaimy
  - Layla Kamal
- <u>Transplant surgeons</u>
  - Milan Kinkhabwala
  - Juan Rocca
  - Stuart Greenstein
  - Jay Graham

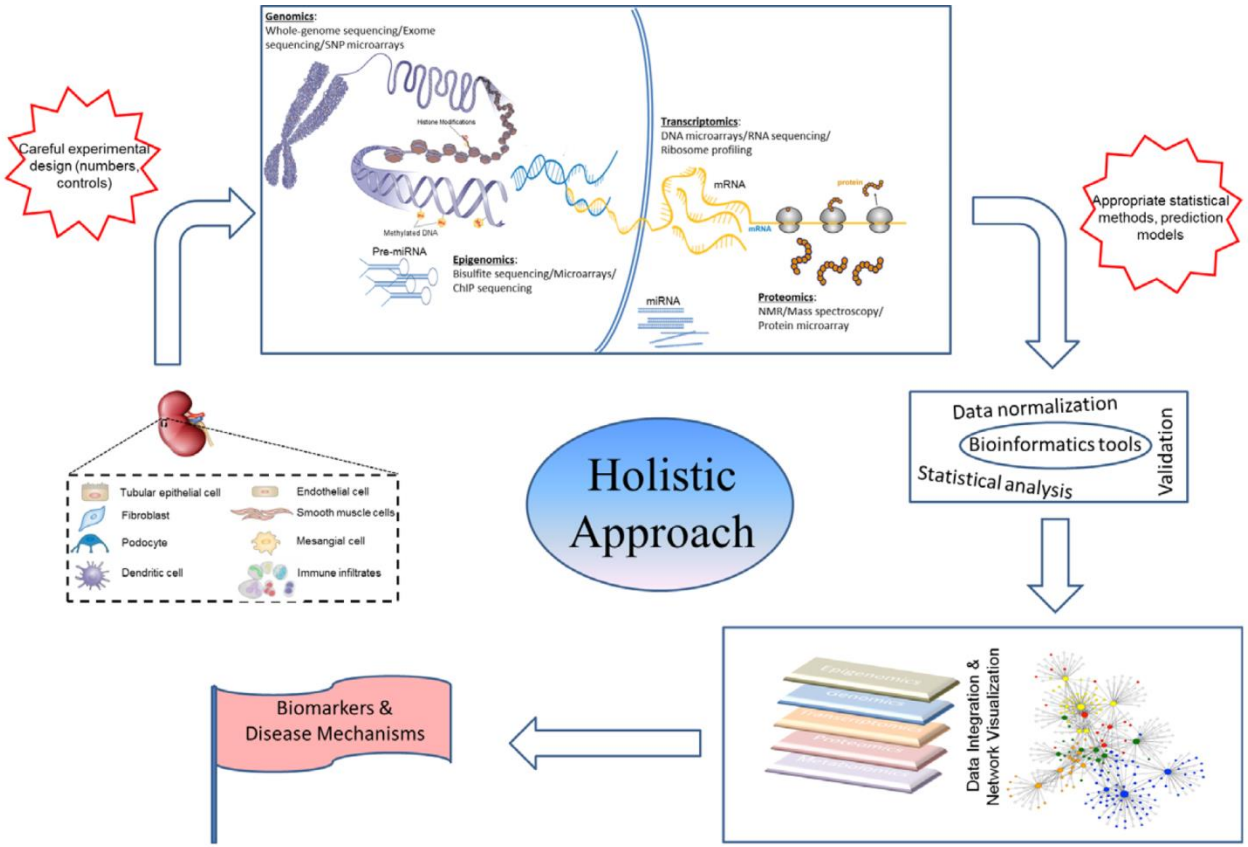

Figure 1: Systems biology approach for biomarker discovery and gaining mechanistic insights. Biomarker discovery when eval-

Relationships among injury, fibrosis, and time in human kidney transplants

Jeffery M. Venner,[1,2] Konrad S. Famulski,[1,3] Jeff Reeve,[1,3] Jessica Chang,[1] and Philip F. Halloran[1,2]

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Table 3. Distribution of fibrosis (ci scores) lesions by diagnosis in biopsies taken before or after 1 year after transplant (TxBx)

Diagnosis in 242 biopsies with ci score >1	Number of early biopsies with ci score >1 (% of column) *n* = 42	Number of late biopsies with ci score >1 (% of column) *n* = 200
Biopsies with potentially progressive diseases	**3 (7%)**	**119 (60%)**
Antibody-mediated rejection (ABMR)	2 (5%)	62 (31%)
C4d$^-$	1	46
C4d$^+$	1	16
Transplant glomerulopathy (TG)	0 (0%)	15 (7%)
Mixed rejection (M)	0 (0%)	13 (6%)
Glomerulonephritis (GN)	1 (2%)	29 (14%)
Other diagnoses	**28 (67%)**	**28 (14%)**
Borderline rejection (BD)	7 (17%)	14 (7%)
T cell–mediated rejection (TCMR)	11 (26%)	6 (3%)
Polyoma nephropathy (PVN)	8 (19%)	3 (1%)
Other	2 (5%)	5 (2%)
Biopsies with atrophy fibrosis with no specific disease diagnosis (IFTA)	**10 (24%)**	**49 (25%)**
Relatively normal biopsies	**1 (2%)**	**4 (2%)**
Acute kidney injury (AKI)	0 (0%)	0 (0%)
No major abnormalities (NOMOA)	1 (3%)	4 (2%)

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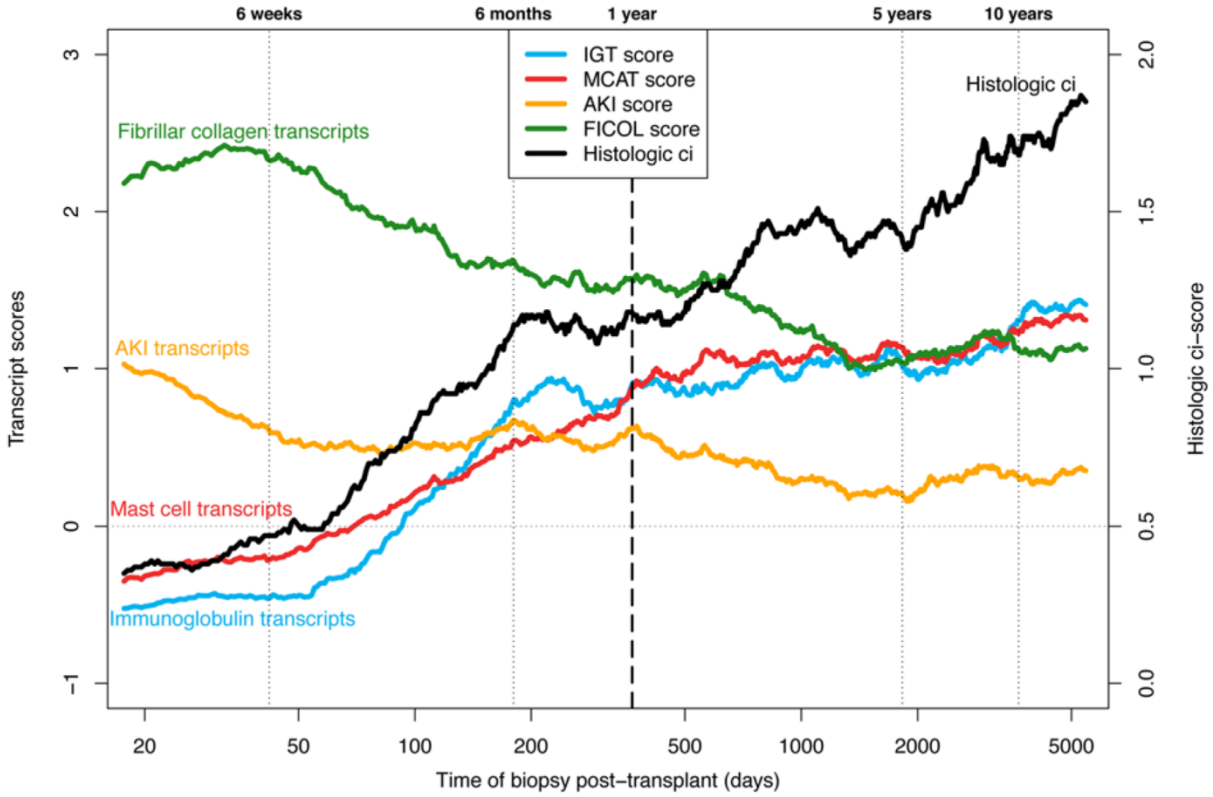

Figure 3. Moving averages for atrophy fibrosis–related features vs. time after transplant. The left *y* axis represents average molecular scores (immunoglobulin [IGT], mast cell [MCAT], acute kidney injury [AKI], and fibrillar collagen [FICOL] transcripts), and the right *y* axis average histologic ci scores (fibrosis). Biopsies are ordered by time after transplant and then the means (of both the *x* and *y* axis values) are plotted based on a sliding window of size 100 biopsies.

Monocyte-Secreted Inflammatory Cytokines Are Associated With Transplant Glomerulopathy in Renal Allograft Recipients

Sacha A. De Serres,[1] Nidyanandh Vadivel,[1] Bechara G. Mfarrej,[1] Monica Grafals,[1] Maura DeJoseph,[2] Christine Dyer,[1] Ciara N. Magee,[1] Anil Chandraker,[1] Lorenzo G. Gallon,[3] and Nader Najafian[1,4]

Background. Although there is ample evidence about the role of adaptive immunity in the development of chronic allograft dysfunction, little is known about the contribution of innate immunity to this process. Herein, we studied the relationship between inflammation, chronic biopsy scores, and anti-human leukocyte antigen (HLA) circulating alloantibodies in a cohort of 57 patients recruited at our center.

Methods. Available biopsies (n=27) were graded for chronic lesion scores according to Banff criteria. The production of cytokines by peripheral blood mononuclear cells after 48 hr of culture under resting conditions was quantified by Luminex. Tumor necrosis factor (TNF)-α secretion assay and depletion studies were used to identify the source of these cytokines.

Results. There was a high correlation between the levels of interleukin (IL)-1β, IL-6, and TNF-α ($r>0.8$, $P<0.001$ for all correlations). The levels of these cytokines were associated with transplant glomerulopathy (IL-1β, $P=0.019$; IL-6, $P=0.015$; and TNF-α, $P=0.006$) but not with other chronic lesions or anti-HLA circulating alloantibodies. TNF-α was predominantly secreted by monocytes (percent of TNF-α secreting cells: 20.4 ± 4.8 vs. 1.2 ± 0.5 vs. 1.4 ± 0.6 vs. 1.7 ± 0.5 for $CD14^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cells, respectively; all $P<0.01$ vs. $CD14^+$). The levels of all three proinflammatory cytokines were significantly reduced after monocyte depletion. Intriguingly, cytokine levels increased after ex vivo depletion of regulatory T cells (all $P<0.001$).

Conclusions. Taken together, these data suggest that in vivo–activated monocytes in peripheral blood spontaneously secrete proinflammatory cytokines in renal allograft recipients with transplant glomerulopathy and seem to be under the regulation of functional regulatory T cells in this setting.

Keywords: Renal transplantation, Transplant glomerulopathy, Innate immunity, Monocytes, Inflammation.

(*Transplantation* 2011;91: 552–559)

Pathogenesis base gene transcript expression in MVI negative TG with (G3A) and without DSA (G3B) to normal (G1)