# Chronic Active TCMR: i, iatr, i-IFTA, and the clinical implications

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Ruiz, 2002

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I have no financial relationships to disclose within the past 12 months relevant to my presentation

<u>and</u>

My presentation *does* include discussion of off-label or investigational use therapies

# The Etiology of Chronic Graft Injury ("CGI"): The Bench and Bedside Knowledge

- IF/TA—interstitial fibrosis and tubular atrophy
  - May also be associated with glomerular or arterial lesions
- Association with TGF$\beta$ and other growth factors
- Association with CNI toxicity (chronic)
- <u>Association with antibody mediated injury (allo, auto)</u>
- <u>Inflammation in unscared kidney "i" + IFTA</u>
- <u>**"Chronic inflammation"** in areas of atrophy "iatr"</u>
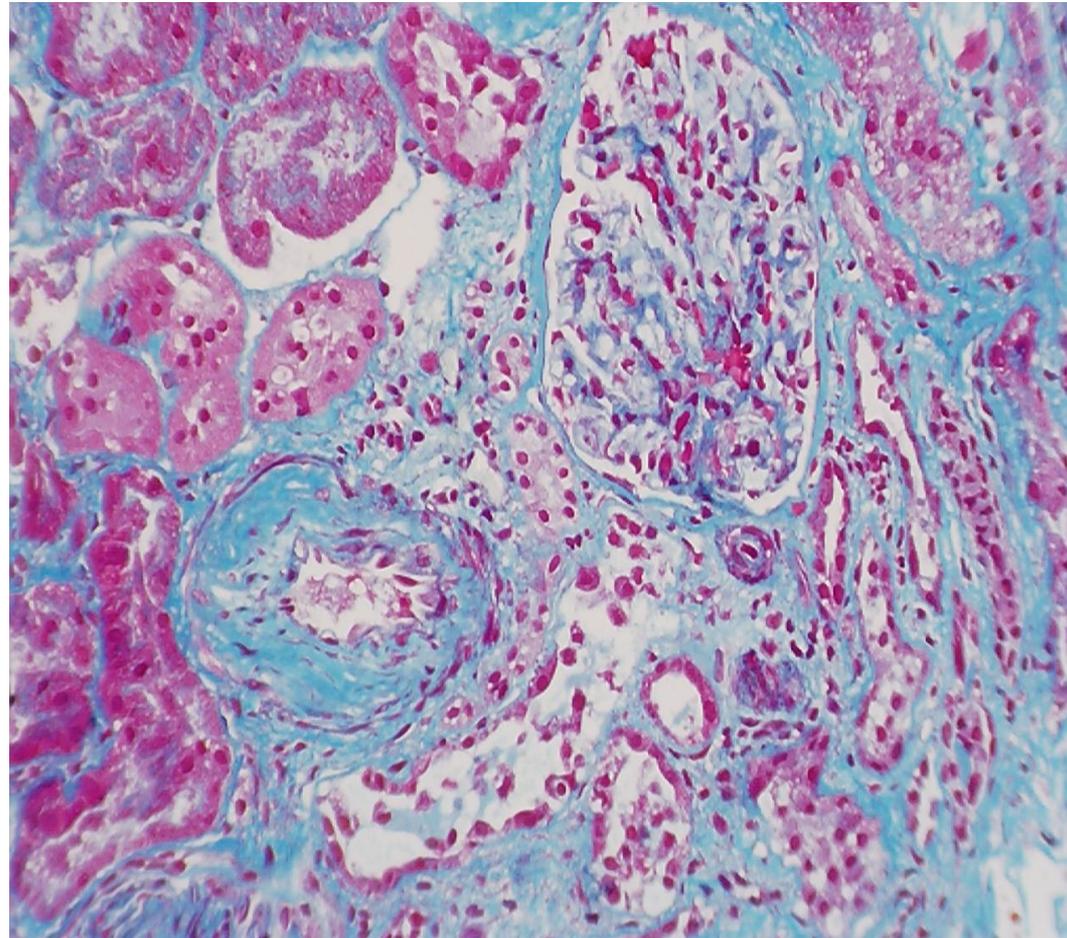


Final common pathway for many injuries

# Causes of Graft Loss Over Time
# T cell Mediated Rejection
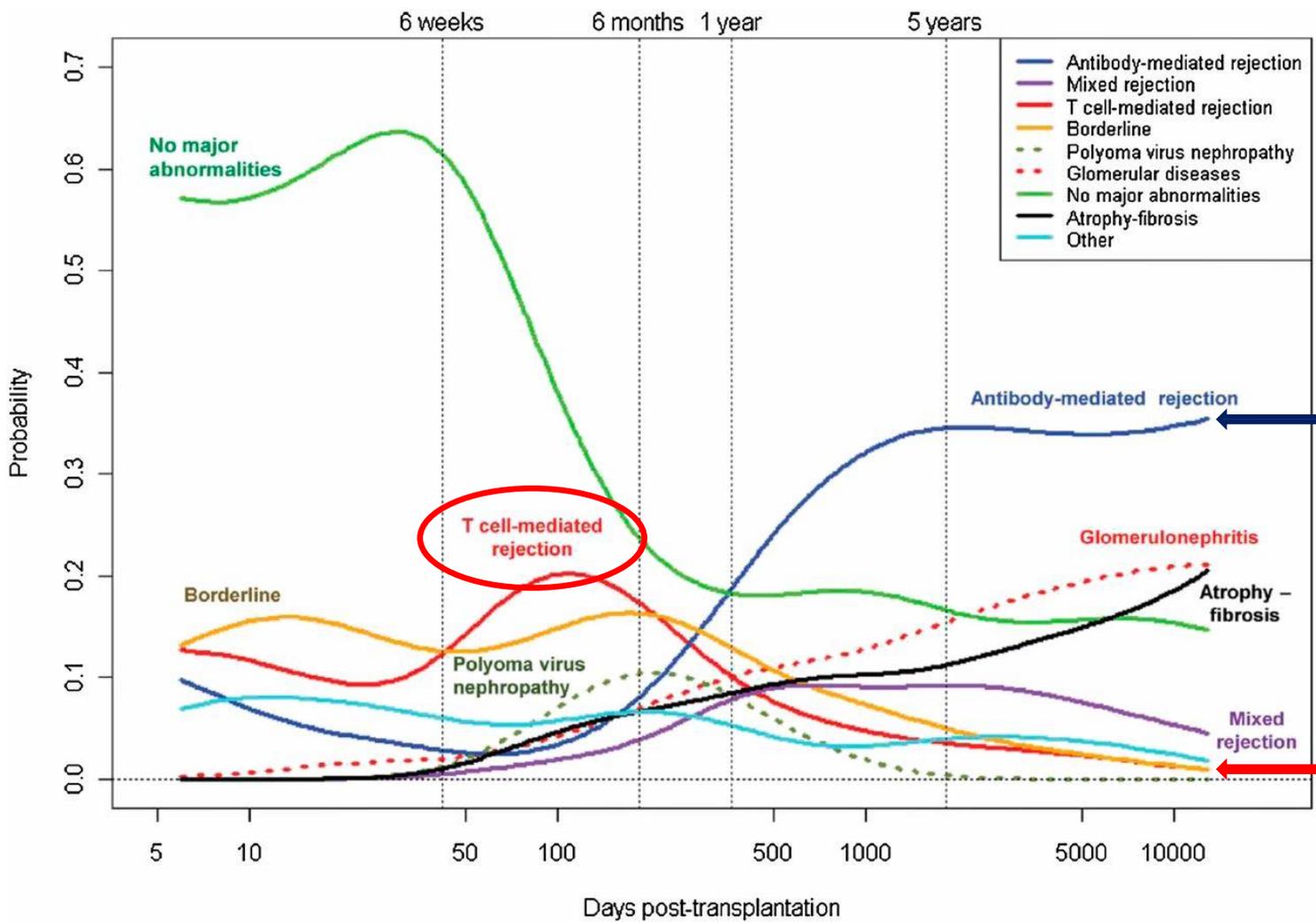


60/312 for cause biopsies developed allograft failure

# Cellular Rejection : Evolution of Criteria

| Dark Ages | Banff '91 (Kidney Int 1993; 44:411) | Banff '97 (Kidney Int 1999; 55:713) | Banff '97 AMR Update (AJT 2003; 3:706) | Banff 2005 (AJT 2007; 7: 518) | Banff 2007 (AJT 2008; 8:753) | Banff 2009, 2011, 2013, 2015 |
|---|---|---|---|---|---|---|
| **Acute TCM Rejection** | Grade I: i2– i3 and/ or t2 | Type IA: i2,i3, &t2 Type IB: t3 | ditto | Type IA i2 or i3 +t2 Type IB i2 or i3 +t3 | ditto | ditto |
| | Grade II: t3 and/or intimal arteritis: v1, v2 | Type IIA: mild-mod arteritis v1 Type IIB: severe intimal v2 | ditto | Type IA: v1 Type IIB severe intimal arteritis comprising >25% of luminal area v2 | ditto | ditto |
| | Grade III: transmural arteritis v3 | Type II: transmural arteritis V3 | ditto | Type III; transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells accompanying lymphocytic inflammation v3 | ditto | ditto |
| **"Chronic rejection"** | | | **Chronic TCM Rejection** | Chronic allograft arteriopathy: arterial intimal fibrosis with mononuclear cell infiltration; formation of neo-intima (ie. cv) | ditto | ditto |

# Grading Chronic TCMR
# Banff 2005

Chronic TCMR was defined by sclerosing transplant arteriopathy. This lesion is characterized by intimal widening due to the de novo accumulation of collagens I and III, lack of elastosis, and varying degrees of <u>intimal inflammation</u> with mononuclear inflammatory cells
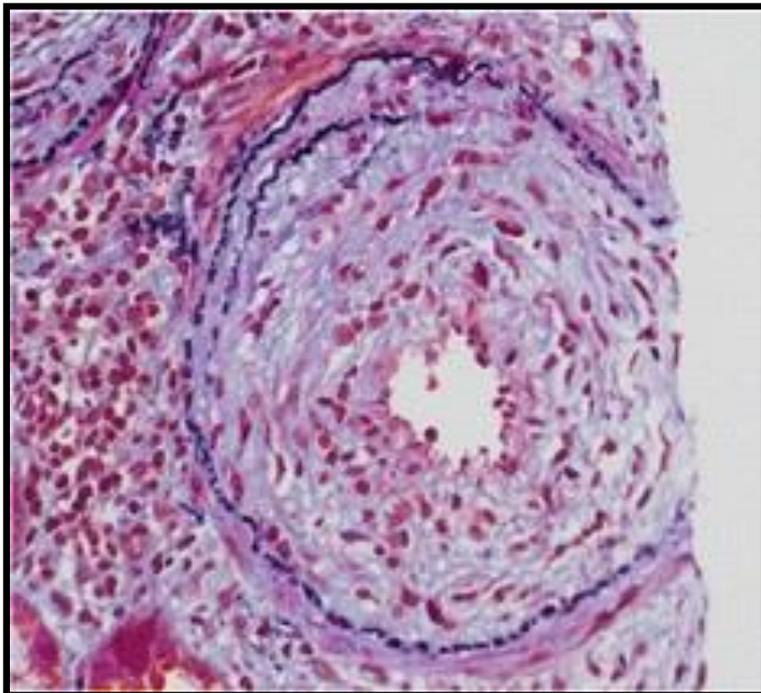
Arterial intimal thickening (cv)
% narrowing lumen of most
severely affected vessel

| Grade | | | |
|---|---|---|---|
| None | I | II | III |
| 0% | <25% | 26–50% | >50% |

*Am Jnl Transplant 2007; 7: 518*

# Donor Age and cv Score in Healthy Living Donors

**Table 3.  Matched Comparison of Arterial Fibrointimal Thickening (cv) Scores in Wedge and Core Biopsies***

| | Core cv Score | | | |
|---|---|---|---|---|
| Wedge cv Score | 0 | 1 | 2 | Total |
| **All arteries** | | | | |
| 0 | 12 | 9 | 7 | 28 |
| 1 | 1 | 1 | 6 | 8 |
| **Total** | 13 | 10 | 13 | 36 |
| **Interlobular arteries only** | | | | |
| 0 | 16 | 9 | 3 | 28 |
| 1 | 1 | 2 | 5 | 8 |
| **Total** | 17 | 11 | 8 | 36 |

* All arteries: odds ratio 7.97 (95% confidence interval, 3.58–17.78; $P < .001$). Interlobular arteries only: odds ratio 4.62 (95% confidence interval, 2.21–9.70; $P < .001$) by ordered multinomial logit regression. Odds ratios reflect the odds of a higher cv score on a core biopsy compared with a wedge biopsy from the same kidney.

**Table 4.  Association Between Arterial Fibrointimal Thickening (cv) Score and Other Parameters in Core and Wedge Biopsies**

| | Wedge | | Core | |
|---|---|---|---|---|
| Association of cv With | Odds Ratio* | P | Odds Ratio* | P |
| Donor age | 1.04 | .25 | 1.09 | .002 |
| Donor sex† | 1.25 | .79 | 1.28 | .69 |
| Donor race‡ | 1.52 | .72 | 1.00 | >.99 |
| Donor creatinine clearance | 0.98 | .38 | 0.98 | .20 |
| Donor family history of hypertension§ | 2.20 | .42 | 2.24 | .36 |
| Percentage of globally sclerotic glomeruli | 1.02 | .72 | 1.13 | .02 |
| Estimated percentage of interstitial fibrosis | 1.22 | .04 | 1.39 | .003 |
| Hyaline arteriosclerosis score | 4.72 | .06 | 2.89 | .07 |

* By ordered multinomial logit regression. Odds ratios reflect the incremental odds of a high cv score per year of donor age, 1 mL/min of creatinine clearance, 1% globally sclerotic glomeruli, 1% estimated interstitial fibrosis, and 1 unit of hyaline arteriosclerosis score.
† Odds ratios for female gender (male = reference).
‡ Odds ratios for white race (black = reference).
§ For donors to unrelated recipients only.

"The findings on core biopsy indicate that significant arteriosclerosis is often present in kidneys from normotensive donors with normal renal function, particularly those older than 40 years."

~Haas et al. Arch Pathol Lab Med 2008; 132:37

# Cluster Analysis of Lesions in Nonselected Kidney Transplant Biopsies:
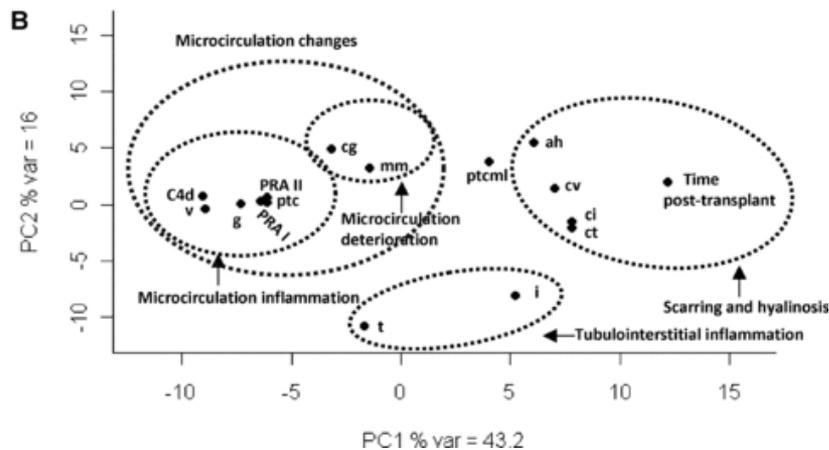## cv as a correlate of parenchymal scarring

234 for cause biopsies

PCA

PCA plus ptcml, C4d, Class I or II PRA, TPTx
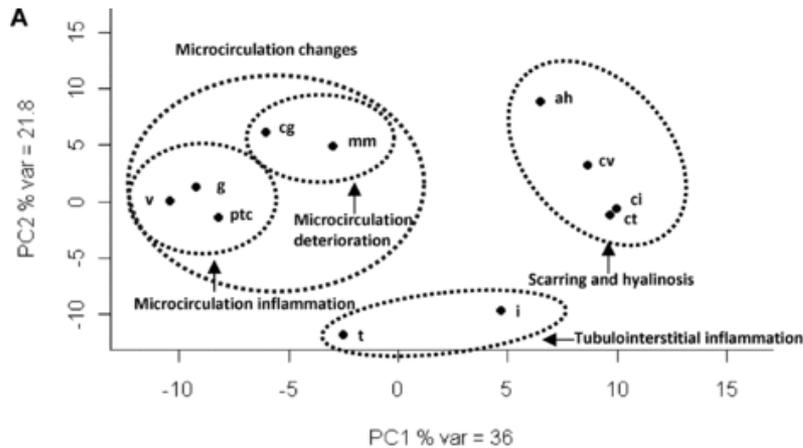


"cv lesion may be produced by various stresses, and acts as a nonspecific feature of time-dependent scarring rather than a feature of antibody-mediated injury"
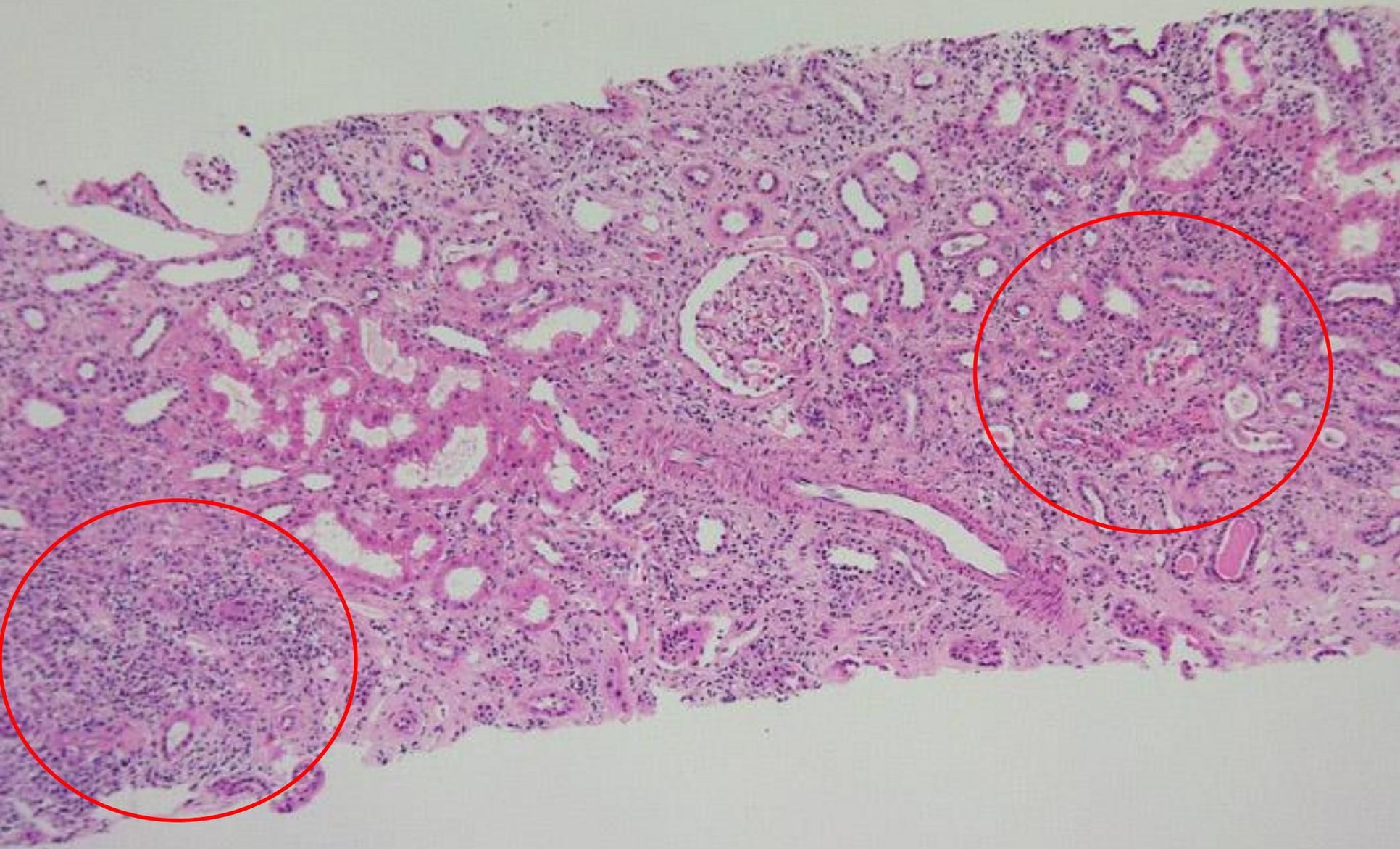
*Sis et al.  Am Jnl Transplant 2010;10(2): 421*

# cv Lesions and Donor Specific Antibody

- Progression of cv is associated with transplantation and accelerated in presence of DSA [Hill et al. JAMA 2011; 22(5): 975].
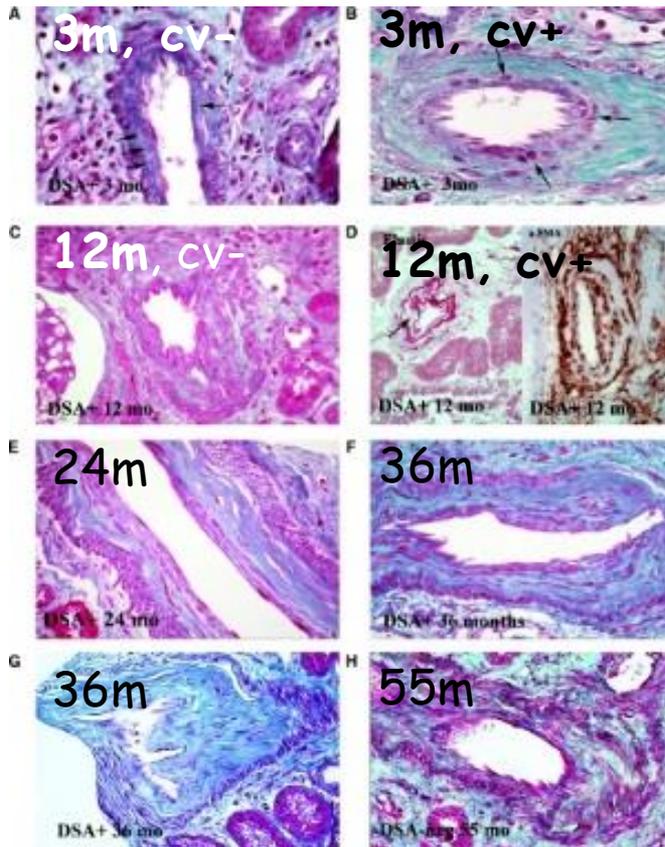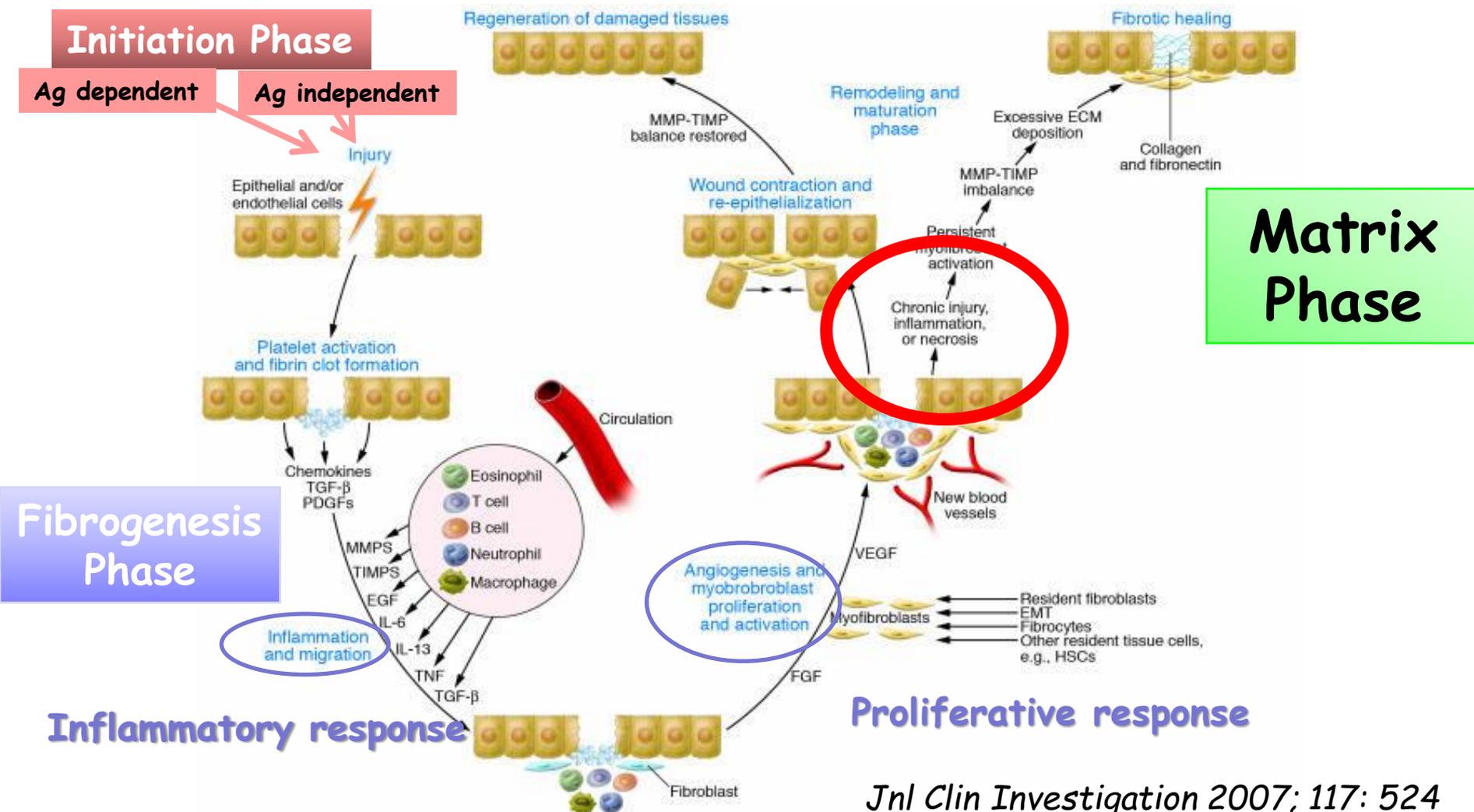


| | Preformed DSA | No Preformed DSA | |
| --- | --- | --- | --- |
| | | De Novo DSA | Persistent Negative DSA |
| Donor age at transplantation | 48 ± 4 | 50 ± 6 | 49 ± 4 |
| No. patients | 15 | 4 | 19 |
| 3-month Bx | 0.57 ± 0.17 | 0.50 ± 0.29 | 0.63 ± 0.17 |
| Last Bx | 2.23 ± 0.15 | 2.25 ± 0.25 | 1.32 ± 0.15 |
| Δ cv grade | 1.67 ± 0.20 | 1.75 ± 0.25 | 0.63 ± 0.23 |
| | P = 0.000012 | P = 0.0009 | P = 0.000006 |
| Months to last Bx | 39 ± 4 | 67 ± 16 | 49 ± 4 |
| Slope of Banff cv grade | 27.3 yr/yr | 16.2 yr/yr | 8.9 yr/yr |

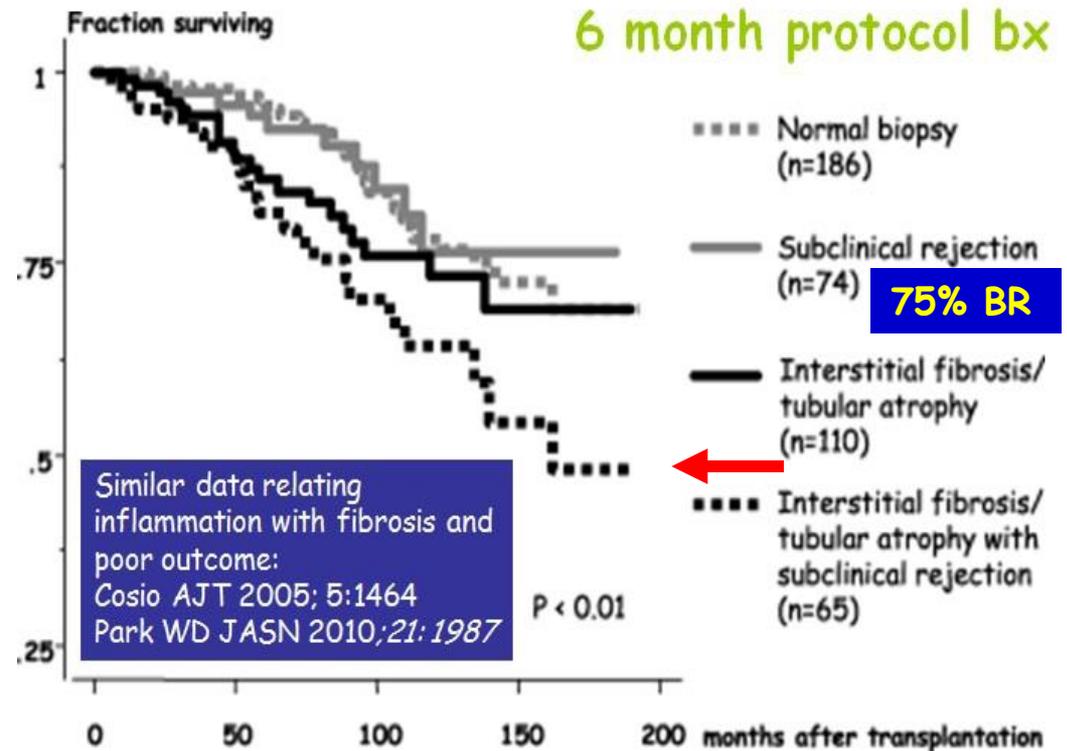Builds a case for cv as a chronic AMR feature

# Tissue Regeneration versus Fibrosis: The Process of Wound Healing



Initiation Phase

Ag dependent

Ag independent

Matrix Phase

Fibrogenesis Phase

Inflammatory response

Proliferative response

Regeneration of damaged tissues

Fibrotic healing

MMP-TIMP balance restored

Remodeling and maturation phase

Excessive ECM deposition

Collagen and fibronectin

Wound contraction and re-epithelialization

MMP-TIMP imbalance

Persistent myofibroblast activation

Chronic injury, inflammation, or necrosis

Injury

Epithelial and/or endothelial cells

Platelet activation and fibrin clot formation

Circulation

New blood vessels

Chemokines TGF-β PDGFs

Eosinophil
T cell
B cell
Neutrophil
Macrophage

MMPS
TIMPS
EGF
IL-6
IL-13
TNF
TGF-β

Inflammation and migration

Angiogenesis and myobrobroblast proliferation and activation

VEGF

FGF

Myofibroblasts

Resident fibroblasts
EMT
Fibrocytes
Other resident tissue cells, e.g., HSCs

Fibroblast

*Jnl Clin Investigation 2007; 117: 524*

# Graft Survival is Lower in Patients With SCR Associated with IFTA (i+IFTA)

- Tubulo-interstitial inflammation in early surveillance biopsies is associated with progression of IF and decreased allograft survival [*Nankivell et al. Transplantation 2004; 78:242; Choi et al. AJT 2005;5: 1354*].

- Surveillance biopsies with i in non-scarred areas and IFTA [IFTA + i] are associated with shorter graft survival.



**6 month protocol bx**

Fraction surviving

**75% BR**

- Normal biopsy (n=186)
- Subclinical rejection (n=74)
- Interstitial fibrosis/ tubular atrophy (n=110)
- Interstitial fibrosis/ tubular atrophy with subclinical rejection (n=65)

Similar data relating inflammation with fibrosis and poor outcome:
Cosio AJT 2005; 5:1464
Park WD JASN 2010;*21: 1987*

P < 0.01

months after transplantation

*Moresco et al. Am Jnl Transplant 2006; 6: 747-752*

# Banff 2007 Criteria:
## Scoring of Total Inflammation (ti) in the Allograft

- Total index of interstitial inflammation which uses the same semi-quantitative criteria used for determining the i score, for all cortical tissue present, including the sub-capsular cortex, perivascular cortex and areas of IF/TA.
- Cortical nodular infiltrates will be included in the i or ti score depending on their localization
- Am J Transplant. 2008 Apr;8(4):753-60

| Score | Criteria |
|-------|----------|
| ti 0 | No or trivial interstitial inflammation (<10% of parenchyma) |
| ti1 | 10–25% of parenchyma inflamed |
| ti2 | 26–50% of parenchyma inflamed |
| ti3 | >50% of parenchyma inflamed |

# Total i Score: Better Predictor of Outcome (and gene expression)
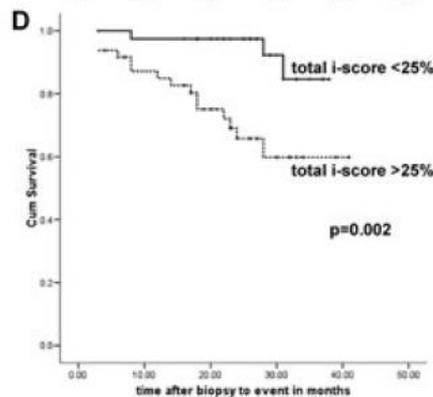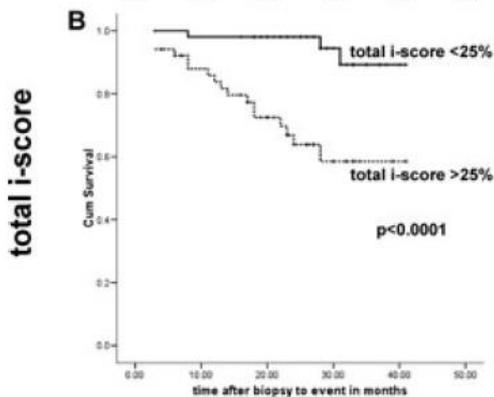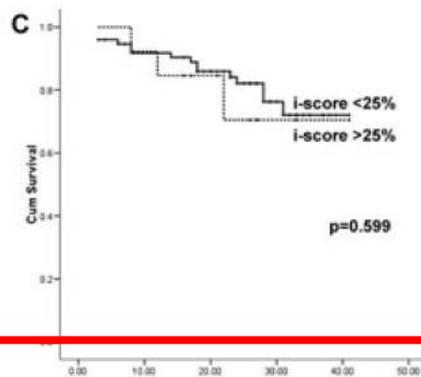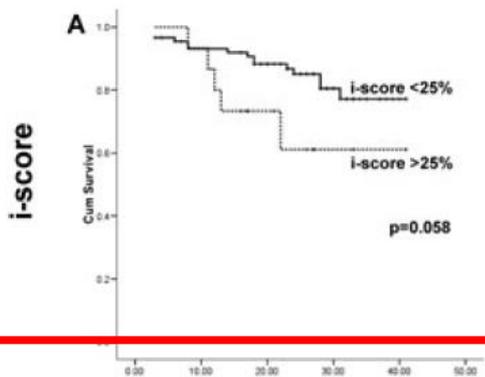


- 129 biopsies
- 2004-2006
- Total i=
  - *infiltrates in areas of nonscarred tubulointerstitium,*
  - *in areas of interstitial fibrosis and tubular atrophy (IFTA),*
  - *nodular infiltrates*
  - *perivascular infiltrates,*
  - *subcapsular infiltrates*

*Mengel at al. Am Journal Transplant 2009; 9: 1859*

# IFTA + i and DSA

- 598 kidney transplant recipients of low immune risk (CTX neg, PRA<20%, DSA neg)
  - Basiliximab, CNI based therapy
  - 6w and 12m biopsies with DSA measurements (LabScreen)/ MFI<1000 = negative
    - normal histology (i+t≤1 and ci+ct≤1)
    - inflammation (i+t≥2 and ci+ct≤1)
    - IFTA (i+t≤1 and ci+ct≥2)
    - IFTA+i (i+t≥2 and ci+ct≥2)
- Findings of IFTA+i @ 6w are independent risk for dnDSA (8.9% of pop at 1y)

| Factor | OR of dnDSA |
|---|---|
| HLADR MM | 1.95 (1.09-3.49) |
| "i" score at 6w biopsy | 5.49 (1.67-10.03) |
| IFTA+i at 6w | 4.09 (1.67-10.05) |

*Garcia-Carro et al. Transplantation 2016; PMID 27163535*

# Deterioration of Kidney Allograft Function (DeKAF) Study (NIH U01 AI58013)

7 transplant centers    Hennepin County Med Center (Kasiske)
Mayo Clinic (Cosio)
University of Alabama (Gaston/Mannon)
University of Alberta (Halloran/Gorishankar)
University of Iowa (Hunsicker)
University of Manitoba (Rush)
University of Minnesota (Matas)

Central pathology    Mayo Clinic (Grande)
Central anti-HLA antibody    UCLA (Cecka)
Central urine metabolomics    University of Manitoba (Rush)
Multicenter database and
   Biostatistics Core    University of Minnesota (Connett, Leduc, Fieberg)

# Deterioration of Kidney Allograft Function (DeKAF) Study

- Prospective cohort (N=3751)
  - Kidney or kidney-pancreas transplant with no other organs simultaneously transplanted
  - Enrolled within 10 days post-transplant
  - Clinical and biopsy data entered into the database

- Cross sectional cohort (N=440)
  - Enrolled as of 02/01/2006
  - sCR < 2.0 mg/dL prior to 01/01/06
  - Deterioration of function (>25% baseline) or new proteinuria, i.e. Biopsy for cause
  - Pathology, urine mass spec, serum for DSA
  - **Mean Creatinine - 1/2006 - 1.4 ± 0.3 mg/dl**

# Characteristics of Cohorts

| | CSC (N=422) | Prospective (N=2270) |
|---|---|---|
| Female | 211 (50%) | 864 (38%) |
| Race | | |
| Caucasian | 333 (79%) | 1740 (77%) |
| AA | 56(13%) | 376 (17%) |
| Mean age ± SD | 48±18 | 48±14 |
| Diabetes | 169 (41%) | 813 (37%) |
| Years Post Transplant to Biopsy | 7.4±6.1 (median 5.7y) | 1.0±0.6 (median 0.8y) |
| Living Donor | 262 (62%) | 1239 (59%) |
| % graft Survival (post enrollment) | | |
| 6m | 89.5% | 98% |
| 12m | 79% | 96% |
| 18m | 74% | 95% |

# Cross Sectional Cohort Local Biopsy Diagnoses
## * adds up to >100% as 2 diagnoses/biopsy

| Primary/Secondary DX | N=425 N (%) |
|---|---|
| Allograft nephropathy | 196 (48) |
| CNI toxicity | 116 (29) |
| Other (e.g., pyelo) | 91 (22) |
| Transplant glomerulopathy | 82 (20) |
| Acute cellular rejection | 76 (19) |
| Recurrent disease | 53 (13) |
| Art. nephrosclerosis | 33 (8) |
| Borderline change | 28 (7) |
| Acute antibody mediated rejection | 29 (7) |
| Glomerulonephritis (de novo) | 23 (6) |
| ATN | 18 (4) |
| Polyomavirus (BK) | 11 (3) |
| NPD | 9 (2) |
| Inadequate | 4 (1) |

# Impact of CAN (IF/TA):
## Graft Survival in CSC After Renal Biopsy



*Matas et al. Am J Transplant 2010; 10:324-337*
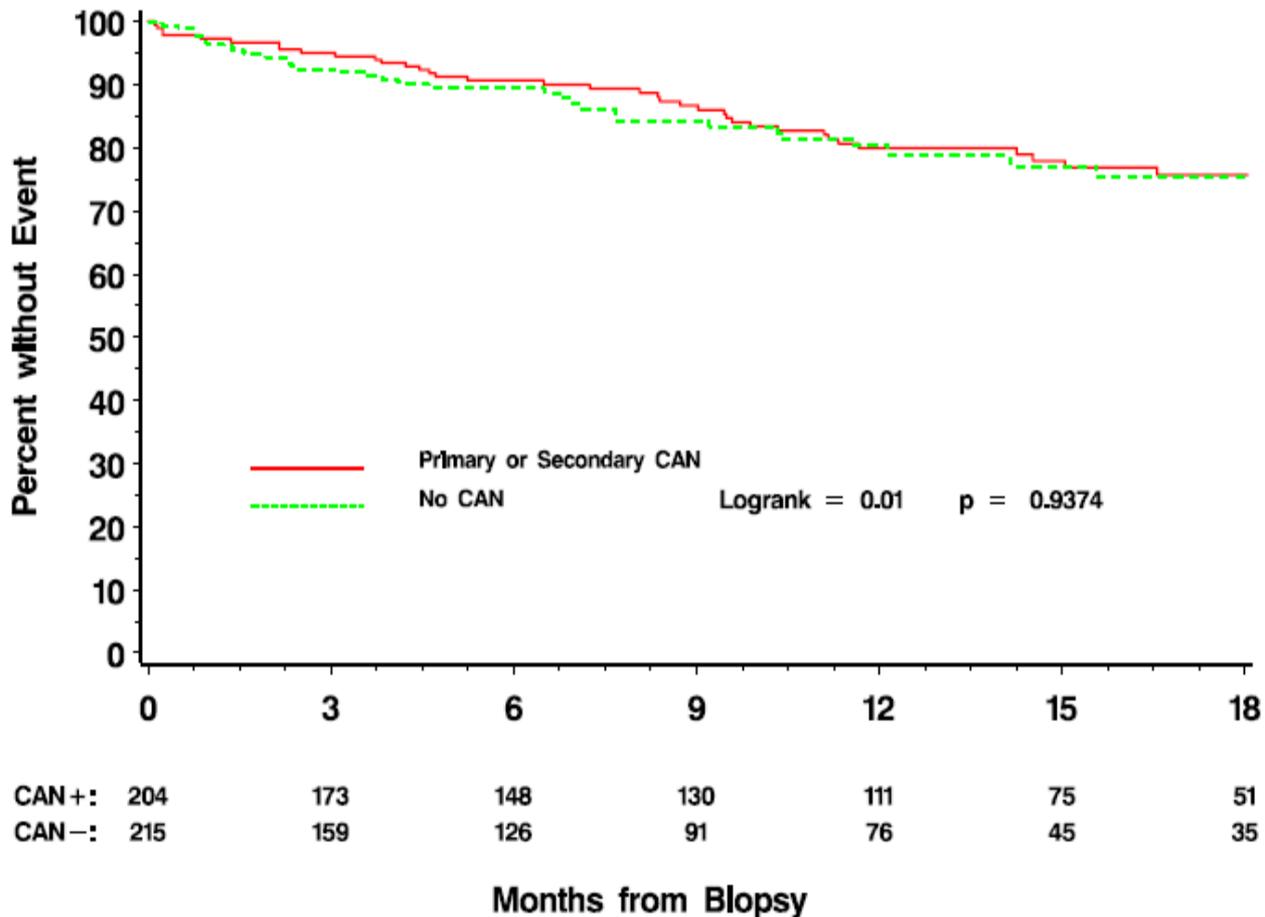
# DeKAF Cross Sectional Cohort: Graft Survival after Biopsy CNI Toxicity versus none



Time to Graft Failure – All CS Index Biopsies
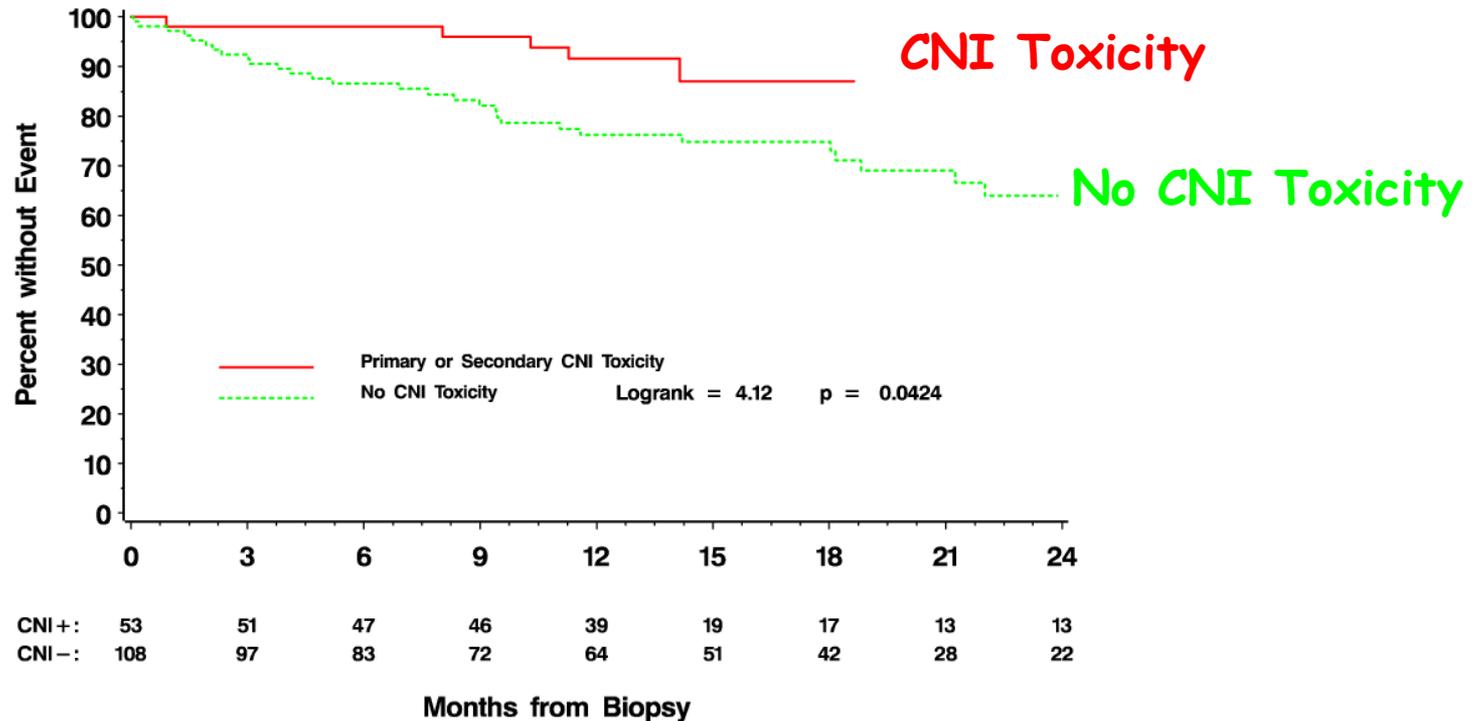
# Depiction of Clusters – "Cluster Clock" With additional histo scores

**Legend**

**Each spoke represents a Banff score**

**Length of spokes = % with finding**

............= Banff 1
- - - - = Banff 2
——— = Banff 3

**All BANFF shown**

Clustering based on Banff scores (**i, t, g, v, ct, ci, cv, cg, mm, ah) plus tatr, iatr, ptc**
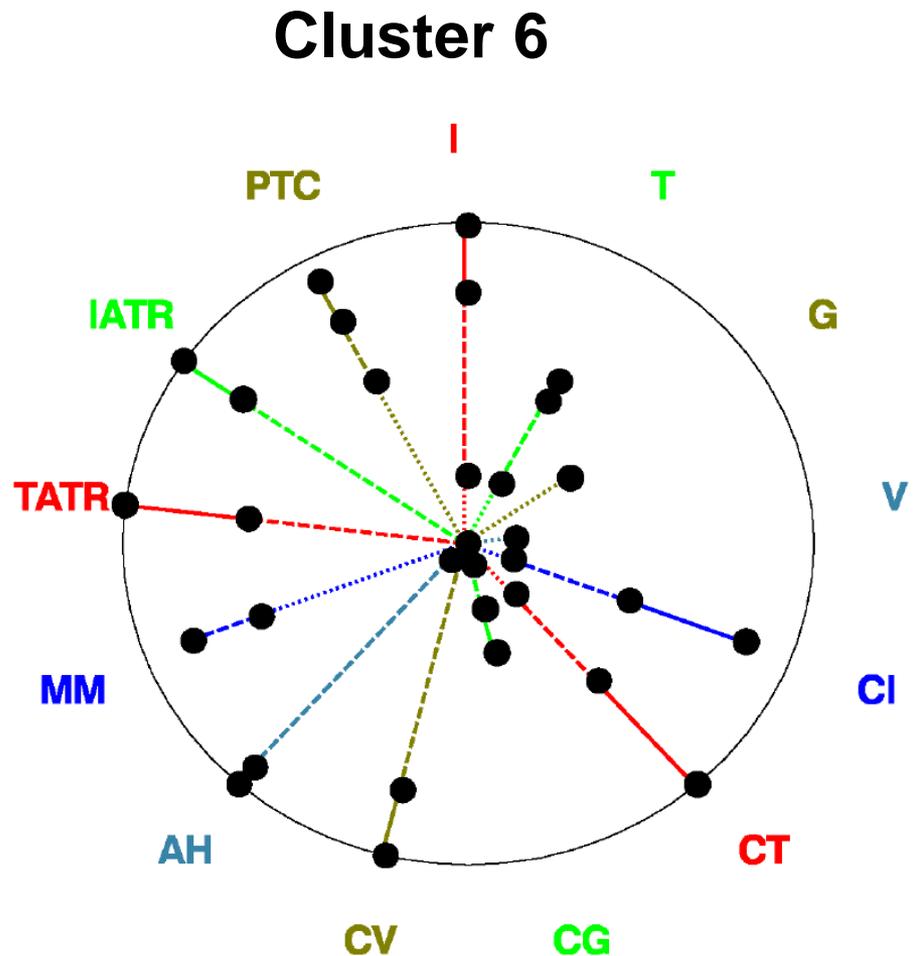
## Cluster 6

# Hierarchical Cluster Analysis of CS Biopsies Using Selected Banff Scores
# i, t, g, ct, ci, cv, mm, ah, and tatr
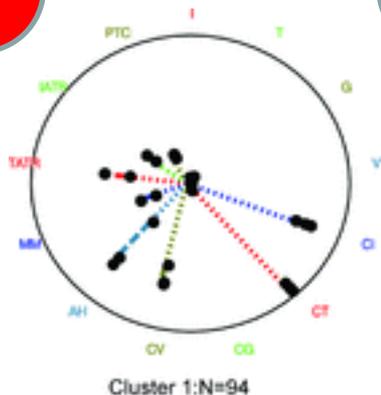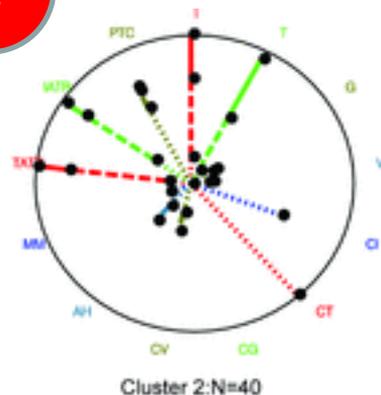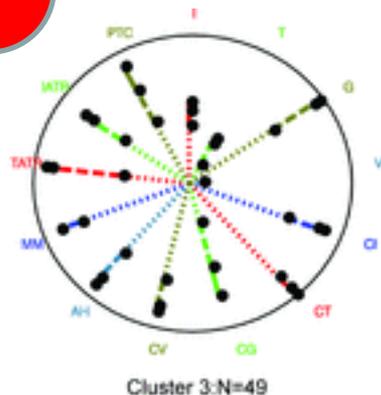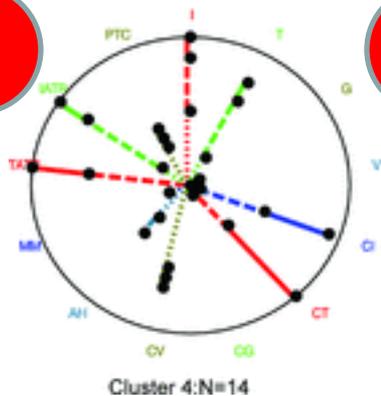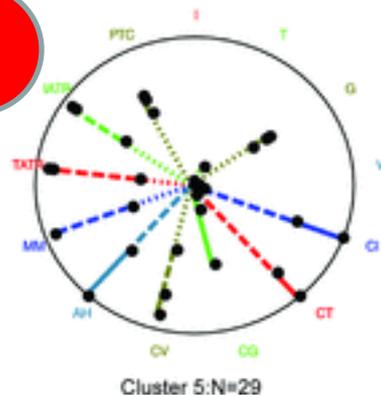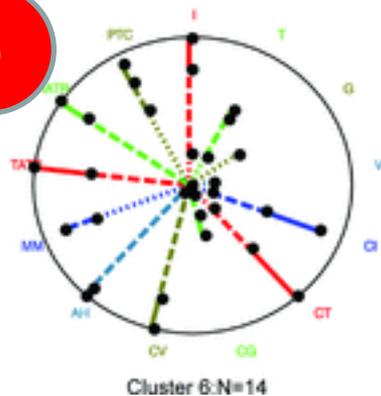


**Legend**

Length of spokes = %  with finding

…. = Banff 1

---- = Banff 2

— = Banff 3

Cluster 1:N=94

Cluster 2:N=40

Cluster 3:N=49

Cluster 4:N=14

Cluster 5:N=29

Cluster 6:N=14

**Clusters**
1—no inflamm, min ci and min mm
2—i, t
3 +4—mm, ah, cv
5 +6 —inflam, and 2,3 4

**Score Distributions for DeKAF Clusters**
N = 253. 13 Observations in 3 Clusters not Shown

# Demographics of Clusters

No major differences in:
- donor or recipient age
- race/ethnicity
- primary kidney disease
- living/deceased donor
- prior transplants
- transplant era
- initial immunosuppressive protocol

# Actuarial Graft Survival Based on Clustering
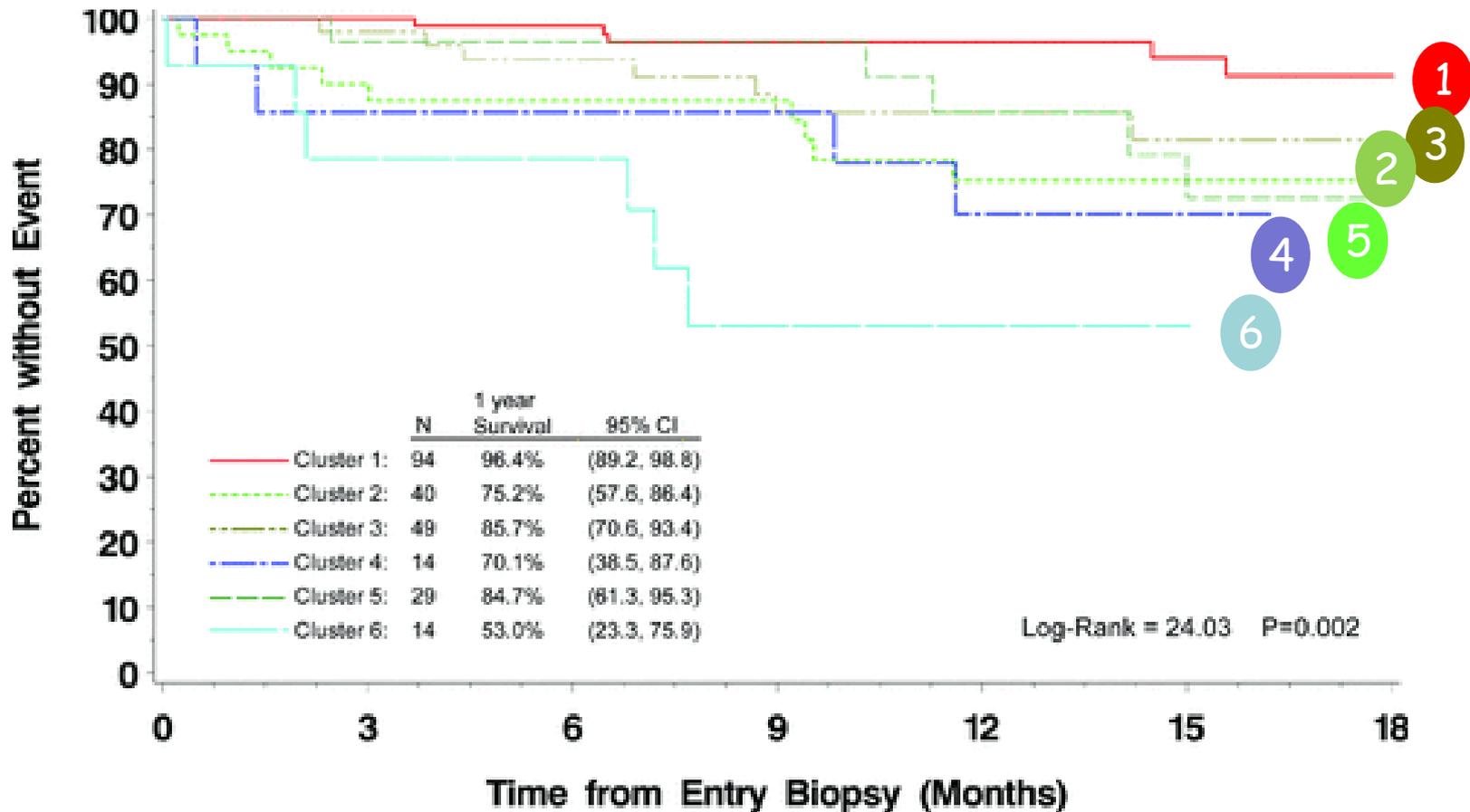
# Characteristics of the 6 Computer-Generated Clusters

**Table 6:** Local pathologists' primary or secondary diagnoses for biopsies in the 6 clusters[1]

| Characteristic | Cluster 1 (N — 94) | Cluster 2 (N — 40) | Cluster 3 (N — 49) | Cluster 4 (N — 14) | Cluster 5 (N — 29) | Cluster 6 (N — 14) |
|---|---|---|---|---|---|---|
| CAN (%) | 53% | 40% | 54% | 50% | 62% | 57% |
| Tx glomerulopathy (%) | 8% | 5% | 38% | 21% | 48% | 36% |
| CNI toxicity (%) | 45% | 8% | 21% | 7% | 41% | 21% |
| Acute cellular rejection (%) | 5% | 73% | 17% | 29% | 3% | 36% |
| Ab-mediated rejection (%) | 3% | 13% | 17% | 7% | 3% | 7% |

[1]25 biopsies were not clustered; of these, 52% had local pathologists diagnosis of CAN.
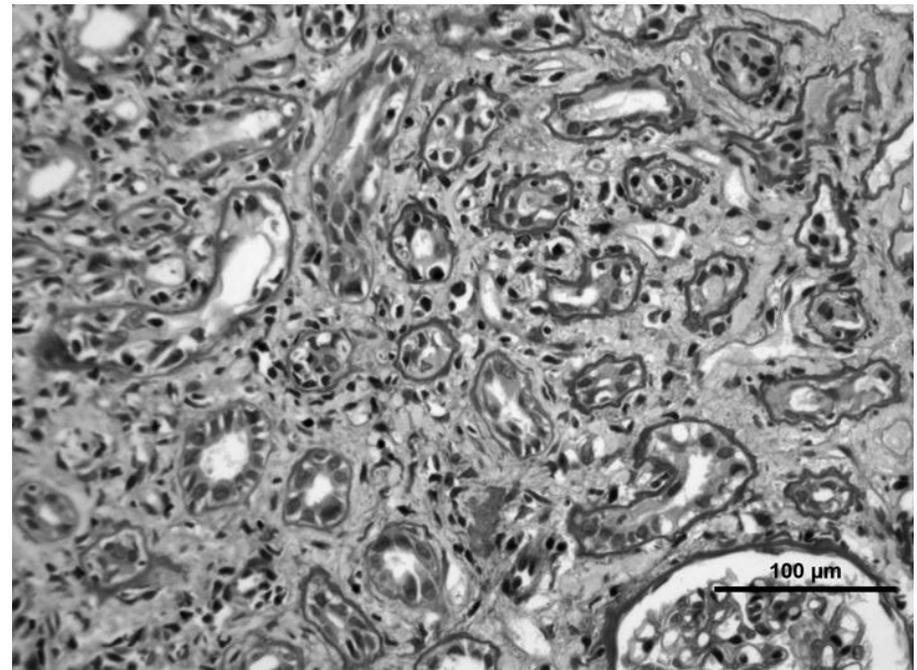CAN, chronic allograft nephropathy; Tx, transplant; CNI, calcineurin inhibitor toxicity; Ab, antibody.

# Findings in For Cause Biopsies in Late Allograft Dysfunction

## IATR
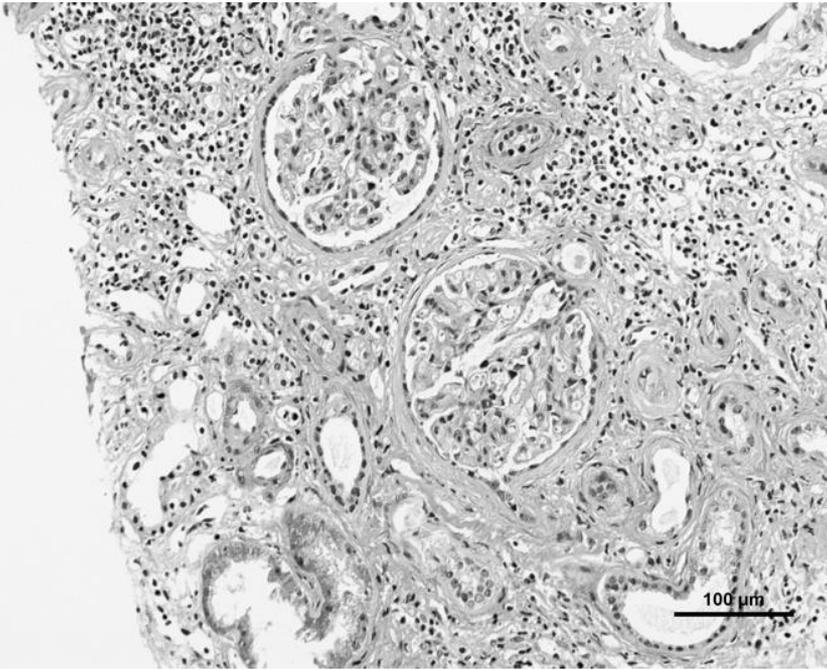
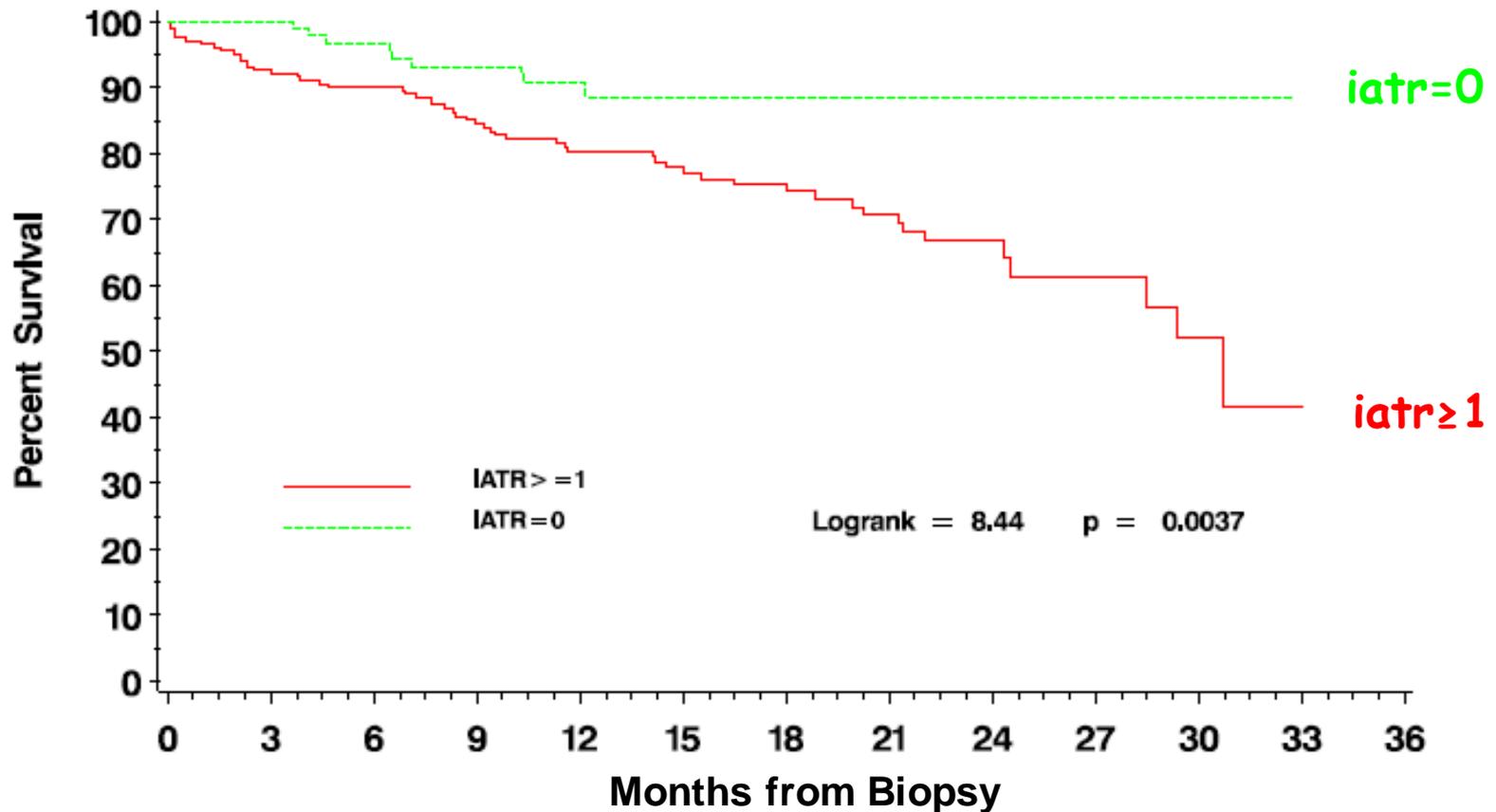## TATR



"iatr"—inflammation in areas of tubular atrophy
> 0 = inflammation in less than 10% of atrophic regions
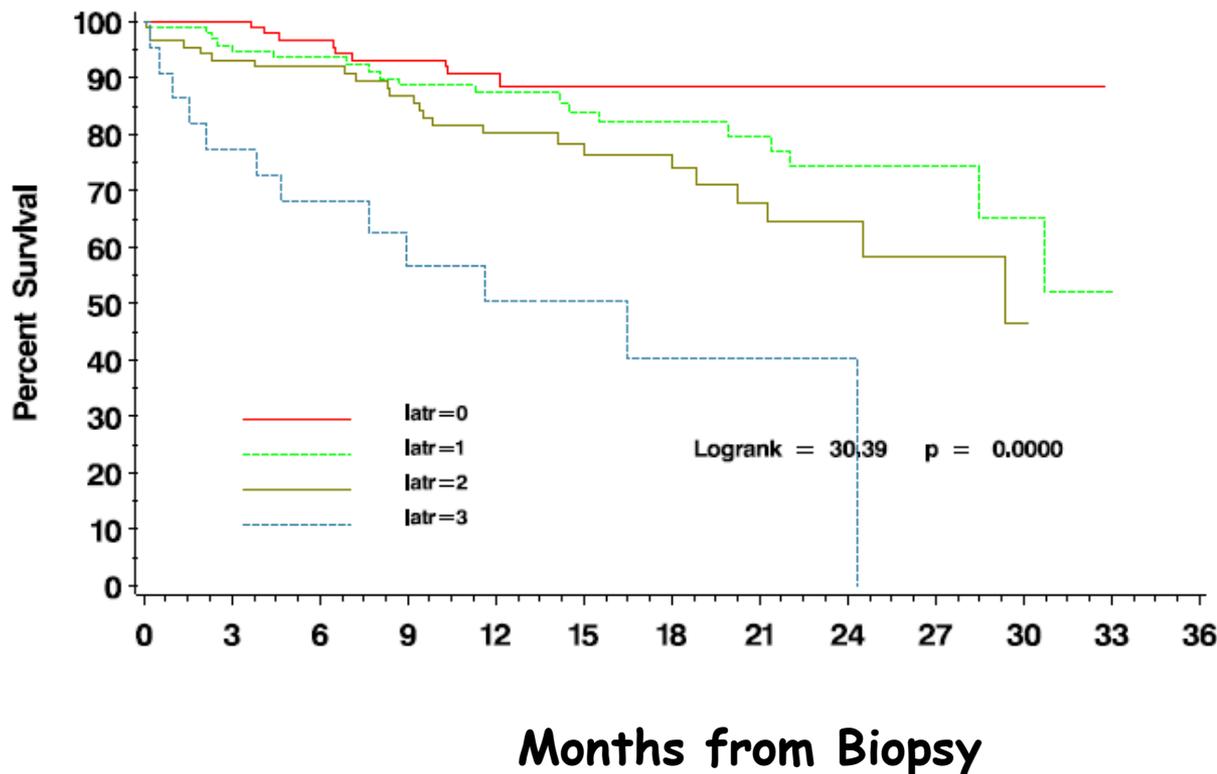> 1 = inflammation in 10-25% of atrophic regions;
> 2 = inflammation in 26-50% of atrophic regions;
> 3 = inflammation in >50% of atrophic regions.

*Mannon et al. Am Jnl Transplant 2010; 10: 2066*

# Impact on Presence of IATR on Graft Failure after Biopsy



*Mannon et al. Am Jnl Transplant 2010; 10: 2066-2073*

# Grade of IATR Impacts Time to Graft Loss



| iatr | Hazard Ratio [95% Confidence Interval]; P-value |
|------|---------------------------------------------------|
| 0 | REF* |
| 1 | 2.27 [0.891, 5.77]; 0.0860 |
| 2 | 2.98 [1.07, 8.34]; 0.0371 |
| 3 | 4.75 [1.58, 14.27]; p=0.0055 |

**Months from Biopsy**

*Mannon et al. Am Jnl Transplant 2010; 10: 2066-2073*

# IATR Has Independent Effect on Time to Death Censored Graft Failure
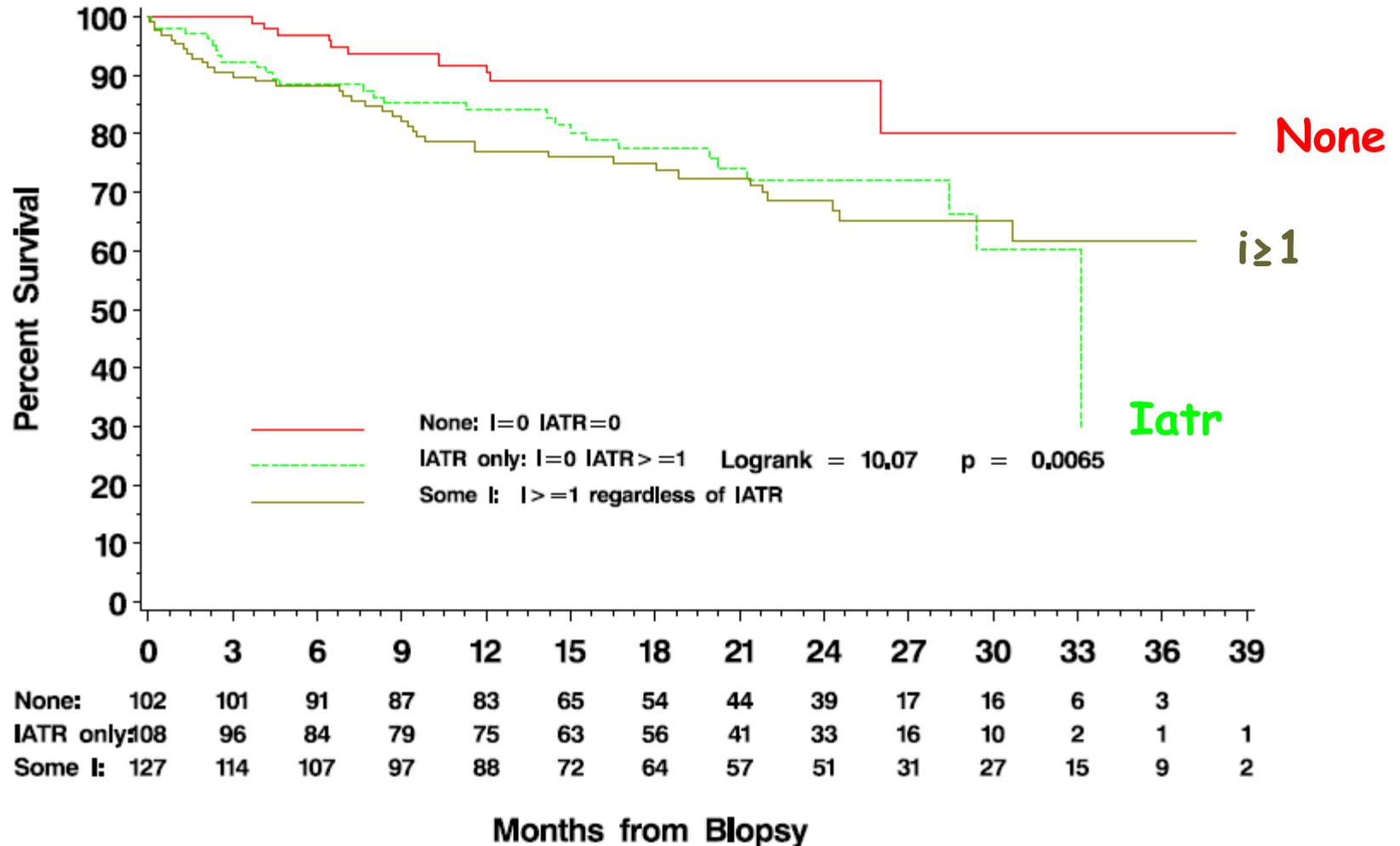
# Proportional Hazards Regression Models of Time to Death-Censored Graft Failure: IATR and Other Factors

| Group | Model 1 Adjusted for creatinine | Model 2 Adjusted for i and creatinine | Model 3 Adjusted for ci and creatinine | Model 4 Adjusted for ct and creatinine | Model 5 Adjusted for ci and ct and creatinine | Model 6 Adjusted for i, ci, ct, C4d$^+$, DSA$^+$ and creatinine |
|---|---|---|---|---|---|---|
| iatr=0 | REF* | REF | REF | REF | REF | REF |
| iatr=1 | 1.91 [0.95,3.90]; 0.075 | 2.47 [1.17,5.20]; 0.018 | 1.59 [0.77,3.30]; 0.212 | 1.68 [0.81,3.48]; 0.161 | 1.60 [0.77,3.32]; 0.207 | 3.36 [1.05,10.68]; 0.0403 |
| iatr=2 | 2.52 [1.26,5.02]; 0.009 | 4.38 [1.95,9.82] <0.001 | 2.12 [1.02,4.38]; 0.043 | 2.00 [0.96,4.16]; 0.065 | 2.07 [0.99,4.35]; 0.053 | 5.11 [1.44,18.07]; 0.0114 |
| iatr=3 | 6.35 [2.91,13.85]; <0.001 | 12.0 [4.4,32.61]; <0.001 | 3.36 [1.39,8.13]; 0.007 | 3.44 [1.42,8.33]; 0.006 | 3.23 [1.29,8.06]; 0.012 | 8.07 [1.71,38.07]; 0.0083 |
| Overall p-value for iatr | <0.0001 | <0.0001 | 0.0441 | 0.0543 | 0.0756 | 0.0450 |

# Summary

- Late allograft failure attributed to T cell rejection is less commonly described in the literature.
- Arteriosclerotic lesions may classify Banff Chronic TCMR but are in part donor derived, worsen during the post-transplant period, and accelerated in the setting of donor specific antibodies.
- Late cellular rejection can be seen in allograft biopsies and contributes to graft loss.
- In biopsies for late allograft dysfunction, inflammation in areas of atrophy is an independent risk factor for death-censored graft loss, even in the setting of antibody mediated injury features.

# Conclusions

The classification of Chronic TCMR needs updating and will need inclusion of both T and B cell activation reflecting contributions of both cellular (innate and adaptive) and humoral arms of the immune response.
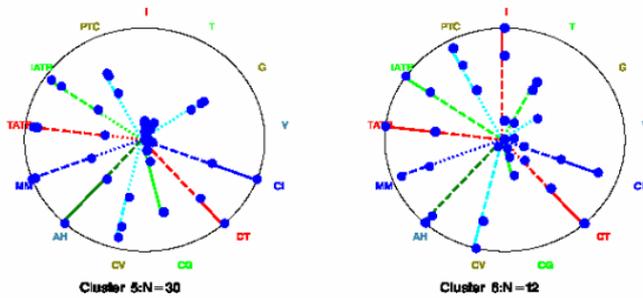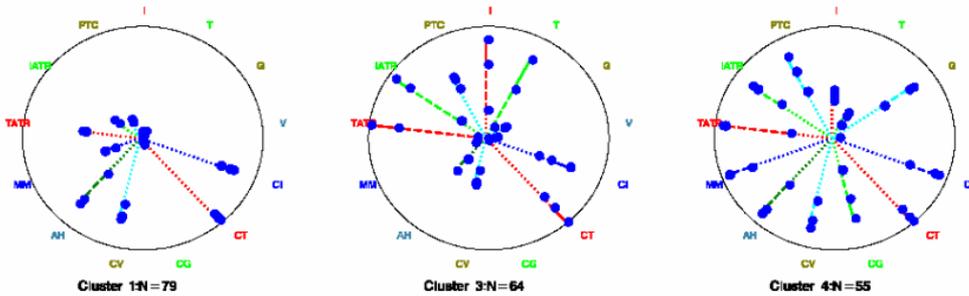
# Selection of Final Number of Clusters

Selection of the final number of clusters requires specification of objective criteria and clinical input
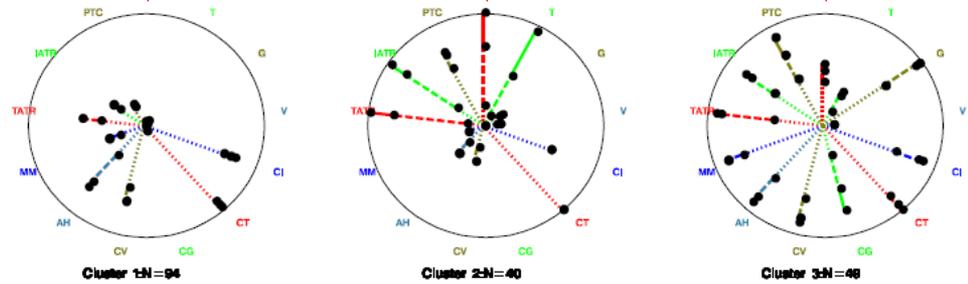
Heuristic measures are available depending on the specific clustering algorithm (pseudo-F, pseudo-$R^2$, cubic clustering criterion)

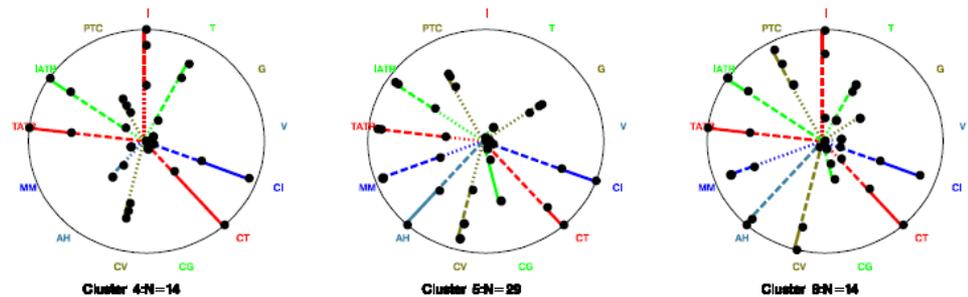When the 'true' number of clusters is unknown, one heuristic is to select a number close to Sqrt(N/2)
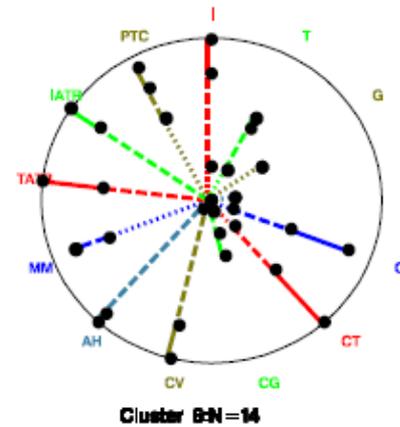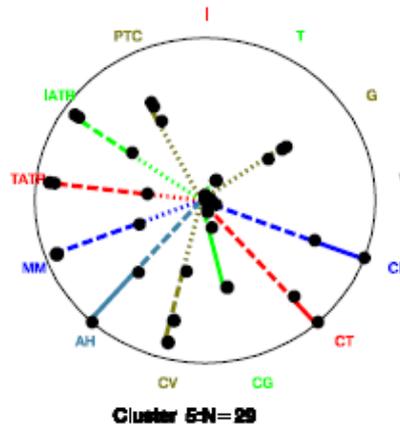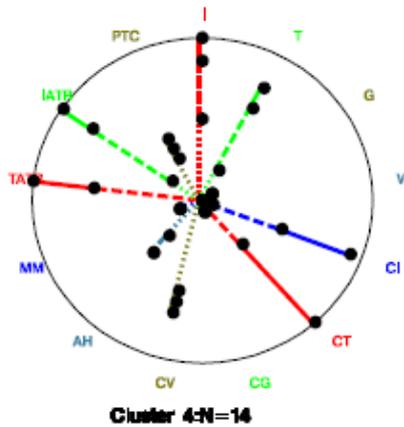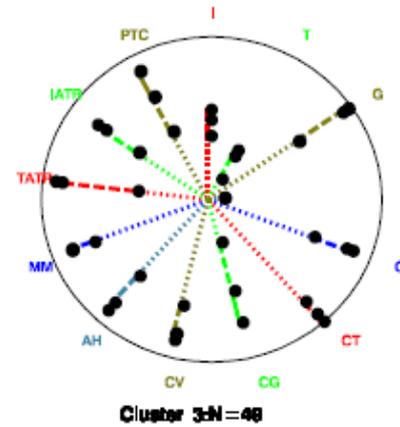
# 2 Clustering Analyses



February / 09

November / 08

<u>Selected Banff</u> – i, g, ct, cv, mm, ah, and tatr – used in clustering;

i - mononuclear cell interstitial infiltrate          mm - mesangial matrix

g - glomerulitis                                                    ah - arteriolar
   hyaline

ct - tubular atrophy
   thickening

cv- vascular fibro...

**All Banff (plus iatr, of**
**tatr, ptc) depicted:**

t - tubulitis

v- intimal arteritis

ci - interstitial fibrosis

cg - glomerulopathy

iatr - infl in areas of
        atrophy

<u>ptc</u> – peritubular capillary
        infiltrates



Cluster 1:N=94     Cluster 2:N=40     Cluster 3:N=48

Cluster 4:N=14     Cluster 5:N=29     Cluster 6:N=14

# Selected Banff – I, g, ct, cv, mm, ah, and tatr



DeKAF clusters (n=265); 25 observations not depicted

**Cluster**

**1** – no infl;
min ci;
min mm
**2** – I,T
**6** – infl &
severe
ci,ct

# Introduction

Majority of recipients with slow deterioration of function are labeled as having "chronic rejection", "chronic allograft nephropathy" (CAN), or "interstitial fibrosis with tubular atrophy" (IF/TA).

These diagnostic terms do not define specific entities from the etiologic, physiologic, pathologic, or prognostic point of view.

The above factors make development of treatment algorithms for care of recipients with persistent and/or progressive graft dysfunction difficult, if not impossible.

# Inflammation in Areas of Atrophy: Strong Negative Predictor of Outcome



DeKAF Study:
289 recipients in cohort
59 with graft loss
89 with i=0, and iatr>1

Percent Survival

IATR > =1
IATR = 0

Logrank = 8.44    p = 0.0037
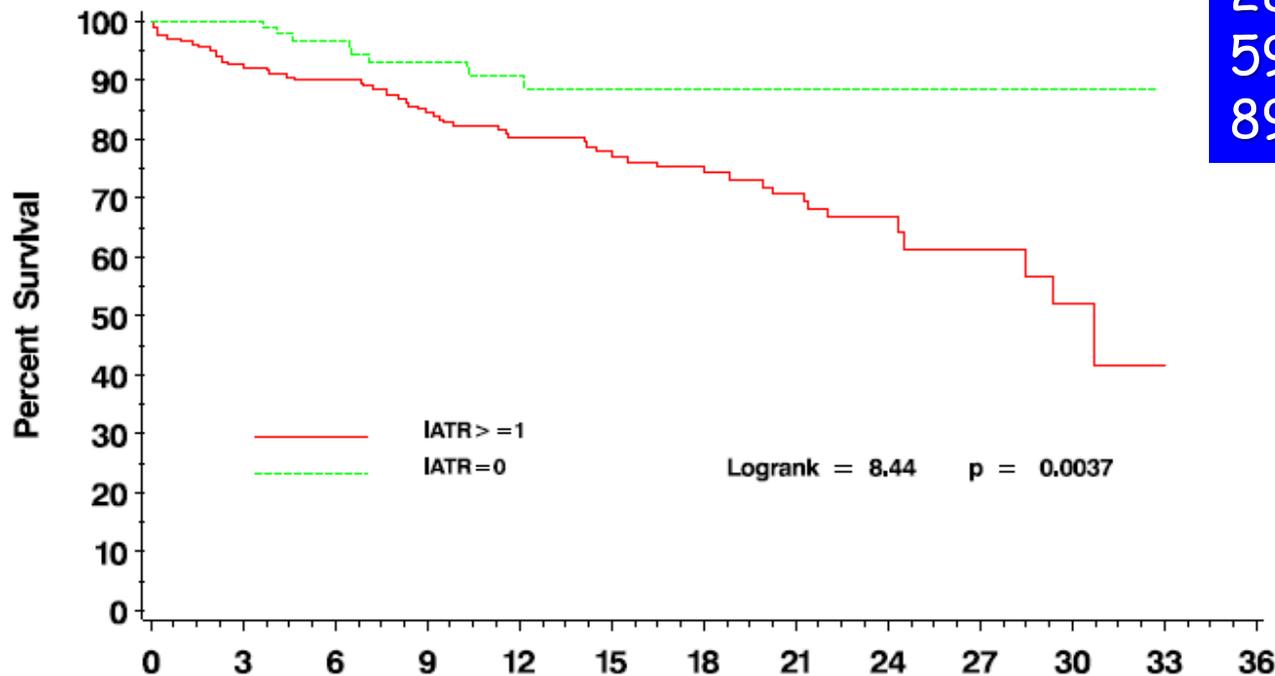
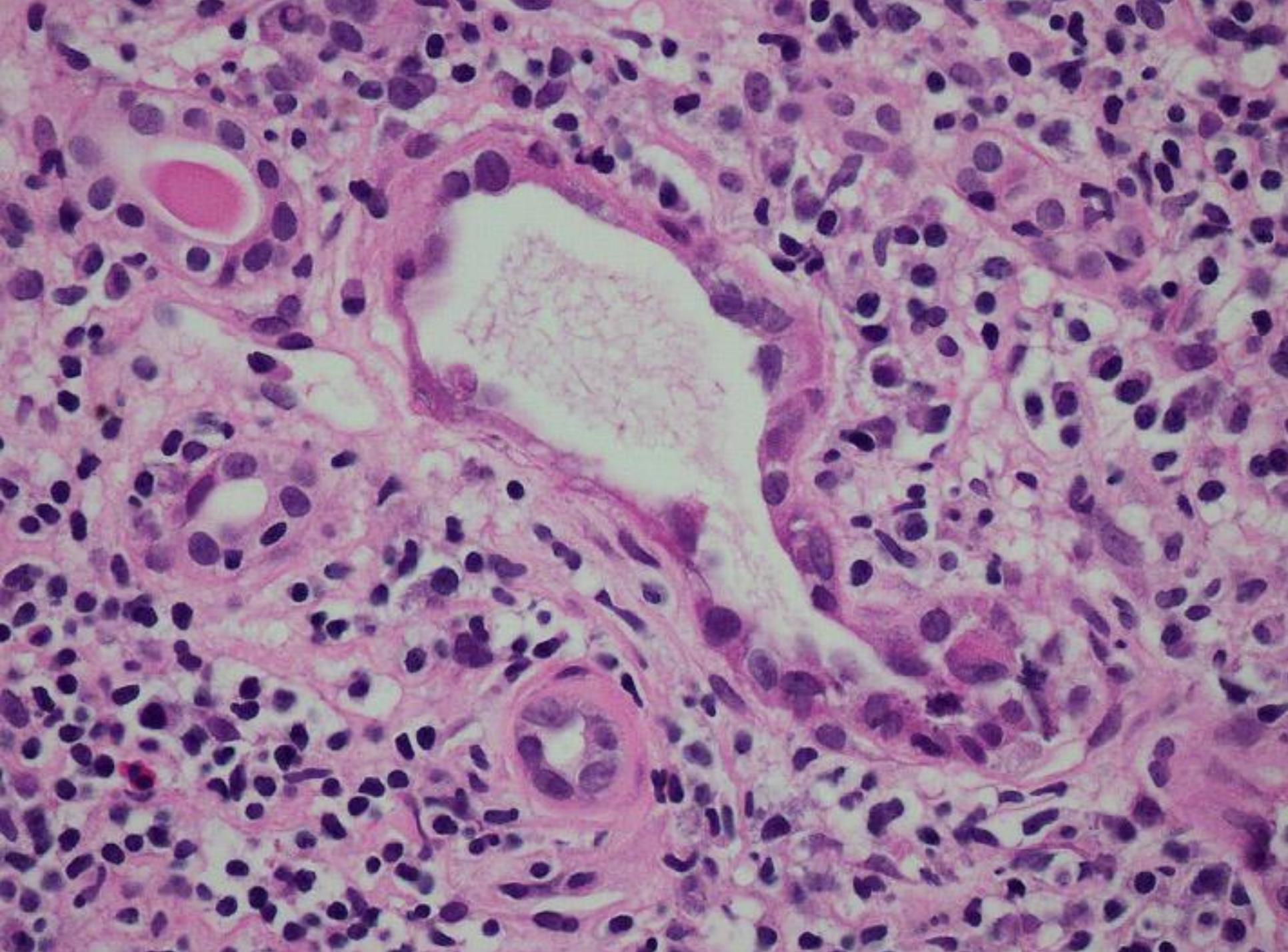"iatr"—inflammation in areas of tubular atrophy
0 = inflammation in less than 10% of atrophic regions
1 = inflammation in 10-25% of atrophic regions;
2 = inflammation in 26-50% of atrophic regions;
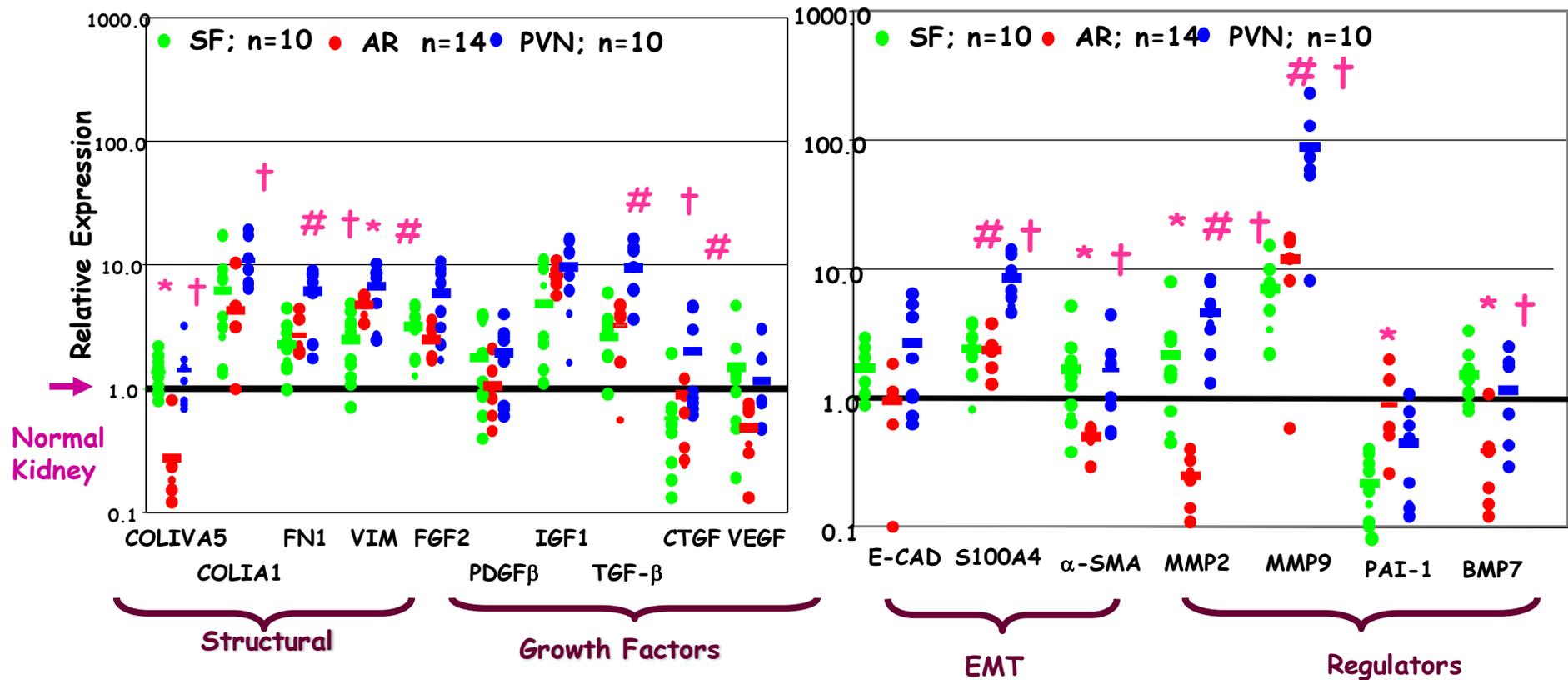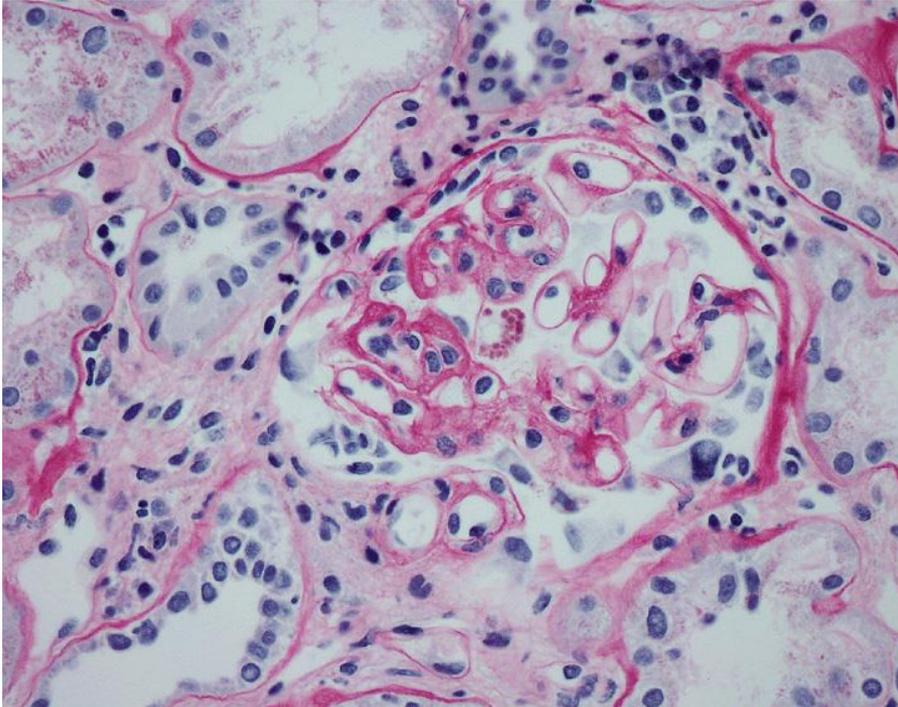3 = inflammation in >50% of atrophic regions.

*Grande et al. Banff Poster Session*
*Matas et al. Next lecture*

# Fibrosis and Fibrogenesis Transcripts in BK PVN Biopsies

# Alloantibody and Autoantibody Associations with CGI



- Endothelial injury mediated by antibody, complement, monocytes, leukocytes
- Outcomes impacted by presence of DSA.
- Effective treatment options?

# Summary/Conclusion

- Chronic graft injury is a considerable long term problem for solid organ transplant recipients.

- The etiologies are multi-factorial and include both antigen dependent and independent events, some of which are beyond clinical control.

- Regardless of insult, the response to inflammation is fibrosis. Primary injury may occur in the endothelium, microvasculature, or epithelium. In the kidney, epithelial injury occurs and may be associated with EMT.

- CNI toxicity contributes to allograft fibrosis, but is not the only factor.

- Identifying novel mediators and targets may provide for specific opportunities for therapy.

# Local Pathologists Primary or Secondary Diagnosis for Each Cluster

|  | #1 (n=94) | #2 (n=40) | #3 (n=49) | #4 (n=14) | #5 (n=29) | #6 (n=14) |
|---|---|---|---|---|---|---|
| CAN (%) | 53 | 40 | 54 | 50 | 62 | 57 |
| Transplant glomerulopathy (%) | 8 | 5 | 38 | 21 | 48 | 36 |
| CNI toxicity (%) | 45 | 8 | 21 | 7 | 41 | 21 |
| Acute cellular rejection (%) | 5 | 73 | 17 | 29 | 3 | 36 |
| Ab-mediated rejection (%) | 3 | 13 | 17 | 7 | 3 | 7 |

# Characteristics at Biopsy for Each Cluster

| | #1 (n=94) | #2 (n=40) | #3 (n=49) | #4 (n=14) | #5 (n=29) | #6 (n=14) |
|---|---|---|---|---|---|---|
| C4d positive (%) | 29 | 50 | 49 | 50 | 36 | 58 |
| Donor specific Ab+ (%) | 18 | 40 | 53 | 43 | 52 | 50 |
| Proteinuria ≥60 mg/g CR (%) | 19 | 35 | 51 | 50 | 55 | 50 |
| Time from tx to biopsy (mos) (± SD) | 85 (65) | 53 (52) | 71 (53) | 58 (32) | 134 (104) | 126 (78) |

# Other Analyses – Cross-sectional Cohort

1) Level of C4d+ staining of peritubular capillaries
   correlates with long-term graft survival
   Optimal" cutoff has not been determined; ≥ 10% strong predictor of graft    loss

2) Time to graft failure is significantly associated with C4d+ status but not AR (in late post-transplant biopsies)

# Depiction of Clusters – "Cluster Clock"

## Legend

**Each spoke represents a Banff score**

**Length of spokes = % with finding**

**…. = Banff 1**
**---- = Banff 2**
**⎯⎯ = Banff 3**

Clustering based on 6 Banff scores (**i, g, ct, cv, mm, ah) plus tatr**



Cluster 1: N = 94

# Depiction of Clusters – "Cluster Clock"
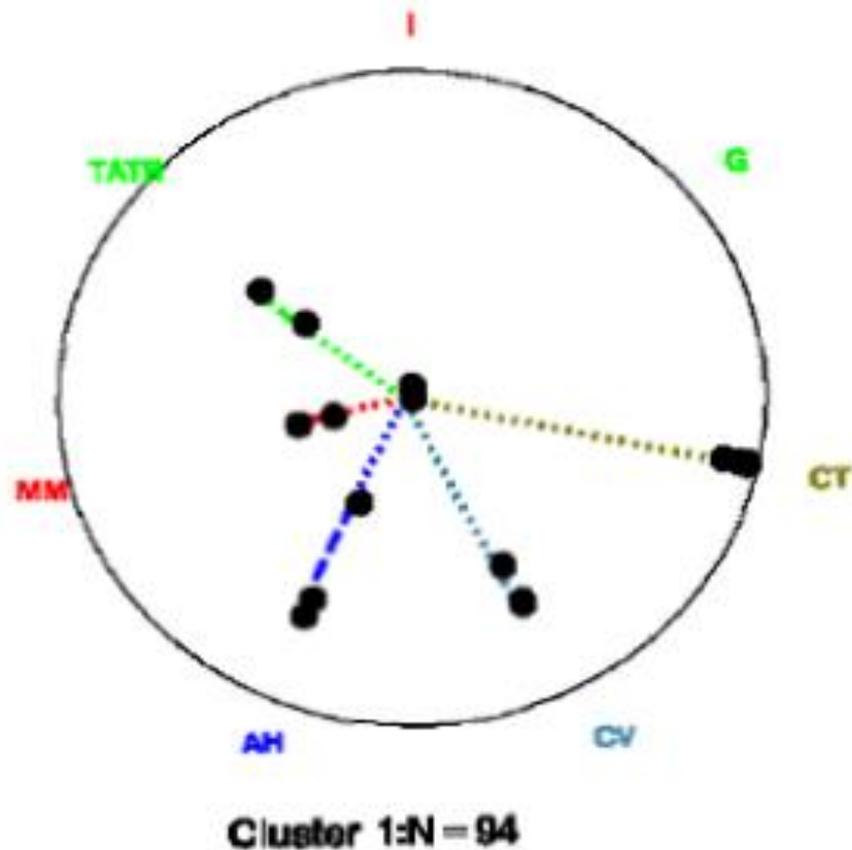
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Clustering based on 6 Banff scores (**i, g, ct, cv, mm, ah) plus tatr**
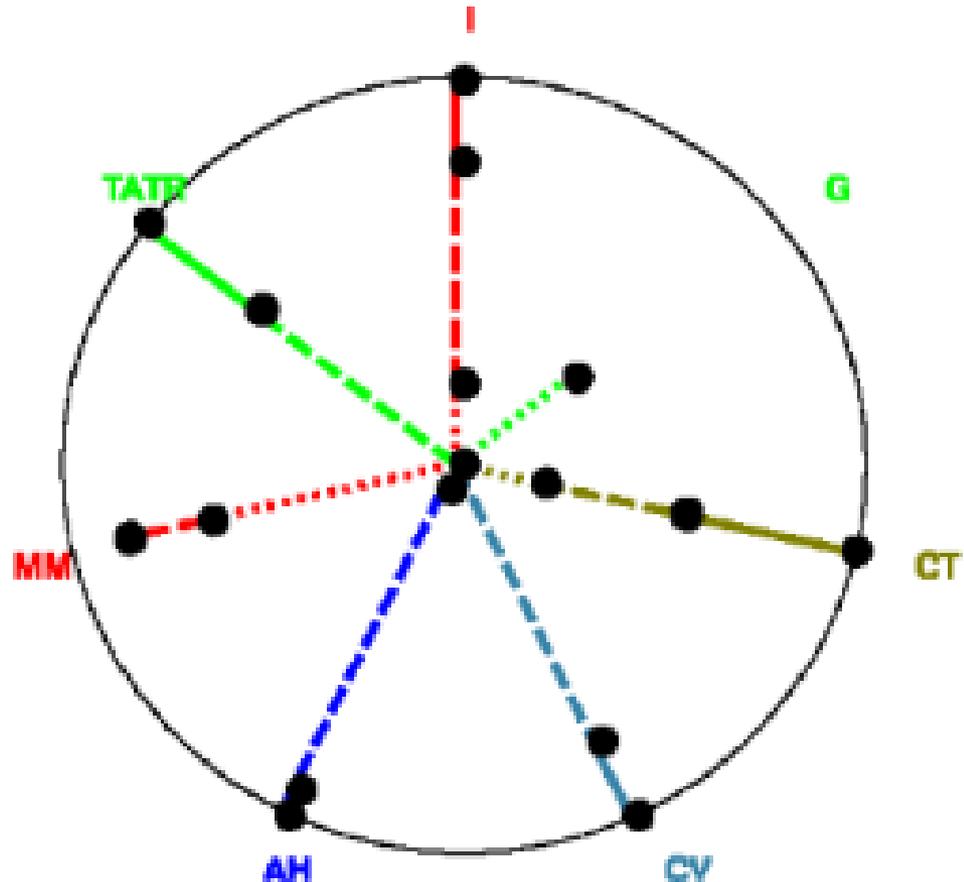


Cluster 6

# Cluster 1

# Cluster 6
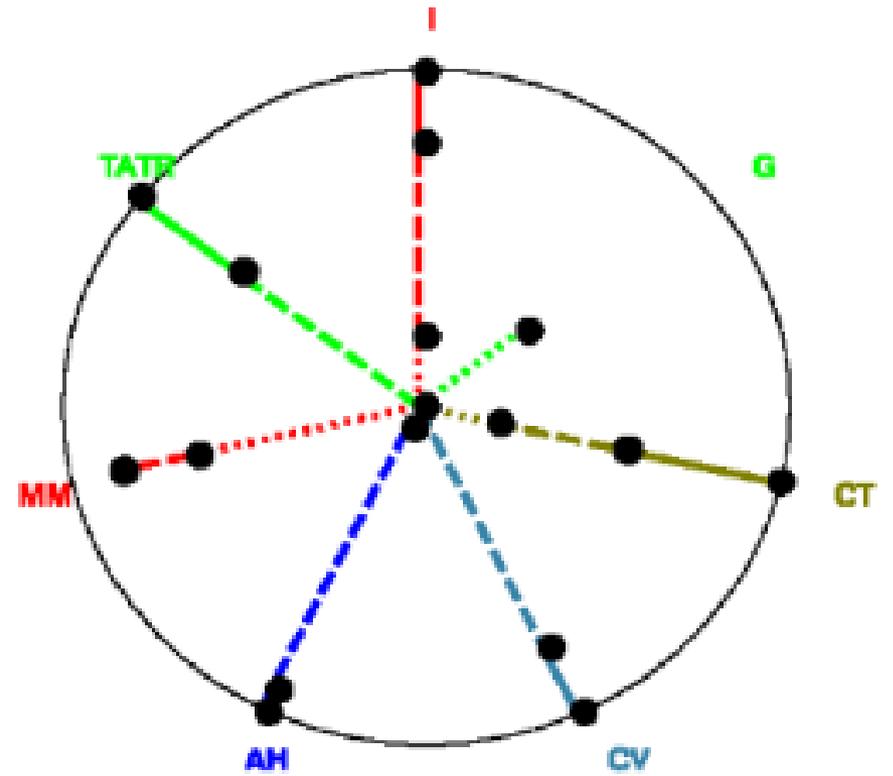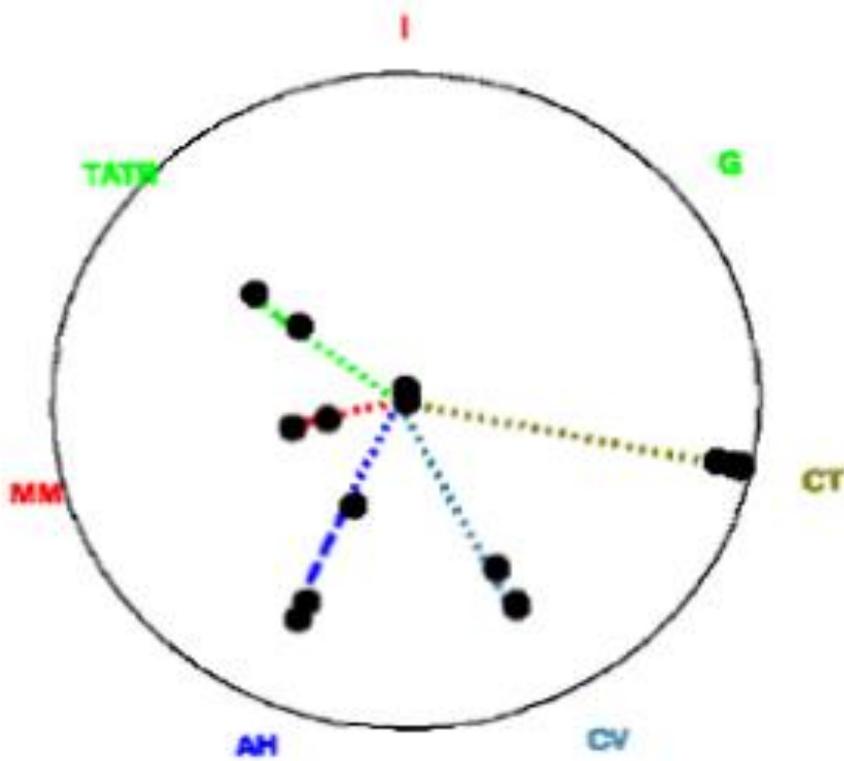
# Histopathologic Clusters Differentiate Subgroups Within the Nonspecific Diagnoses of CAN or CR: Preliminary Data from the DeKAF Study

25 observations not depicted
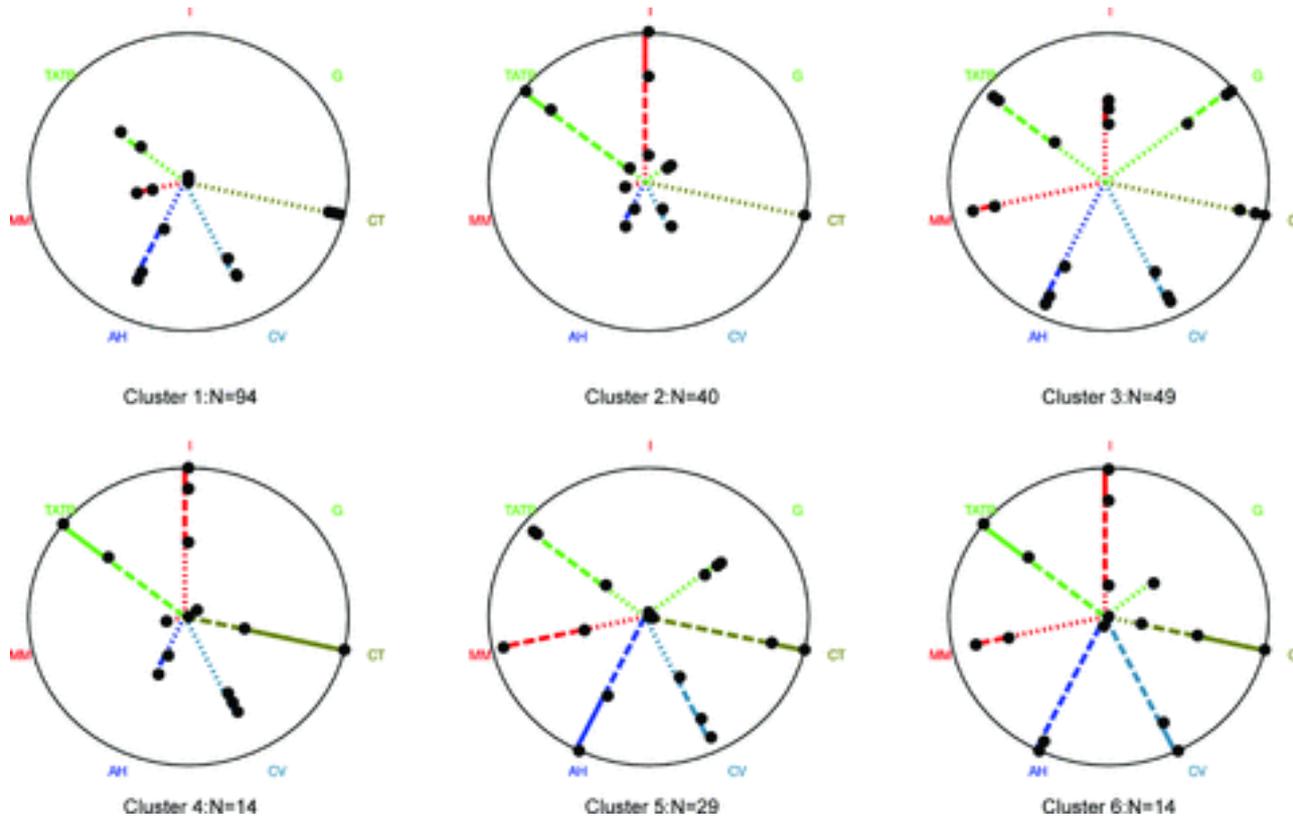


Cluster 1:N=94    Cluster 2:N=40    Cluster 3:N=49

Cluster 4:N=14    Cluster 5:N=29    Cluster 6:N=14

# Histopathologic Clusters Differentiate Subgroups Within the Nonspecific Diagnoses of CAN or CR: Preliminary Data from the DeKAF Study



Cluster 1:N=94

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Cluster 3:N=49

Cluster 4:N=14

Cluster 5:N=29

Cluster 6:N=14
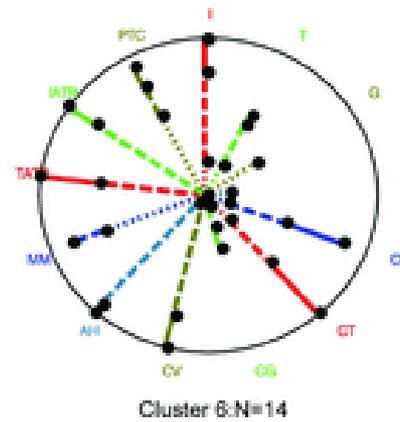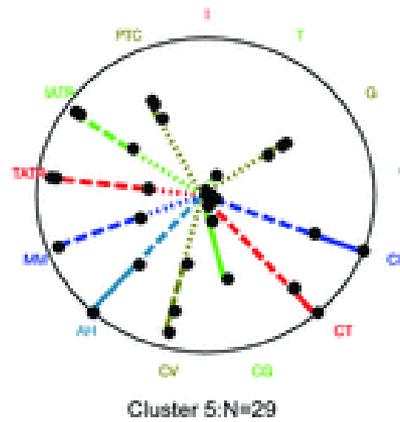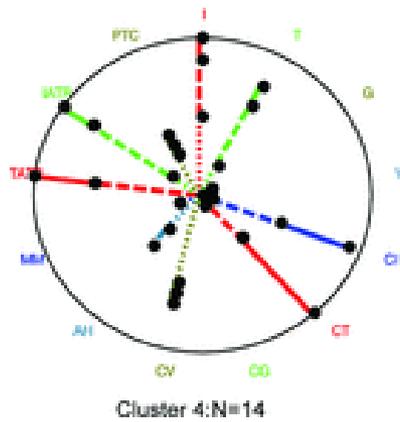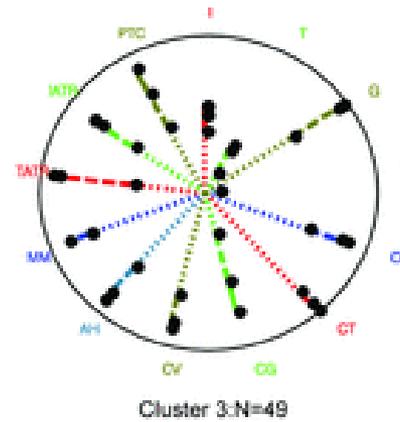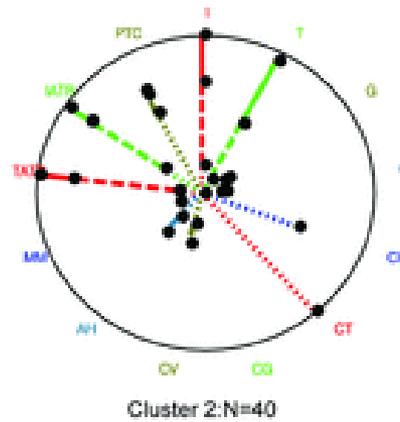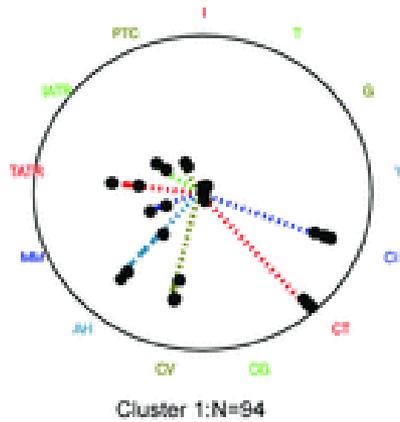
# C4d⁺ Progressed More Rapidly to Graft Failure than DSA⁺



DeKAF Study Graft Survival by C4d/DSA Group
Long–Term Cohort Entry Biopsies

Patients
with C4d or
DSA or both
had worse
outcomes
(*p*<0.0001)
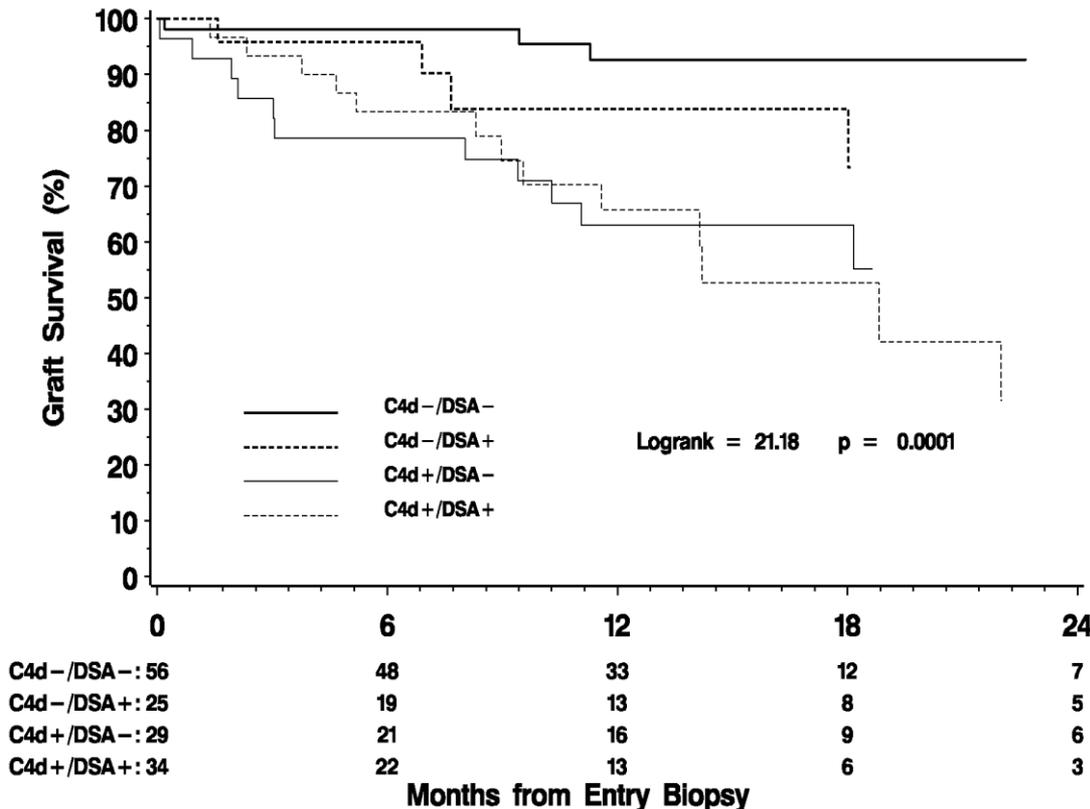
# Inflammation in Areas of Atrophy:
## Strong Negative Predictor of Outcome
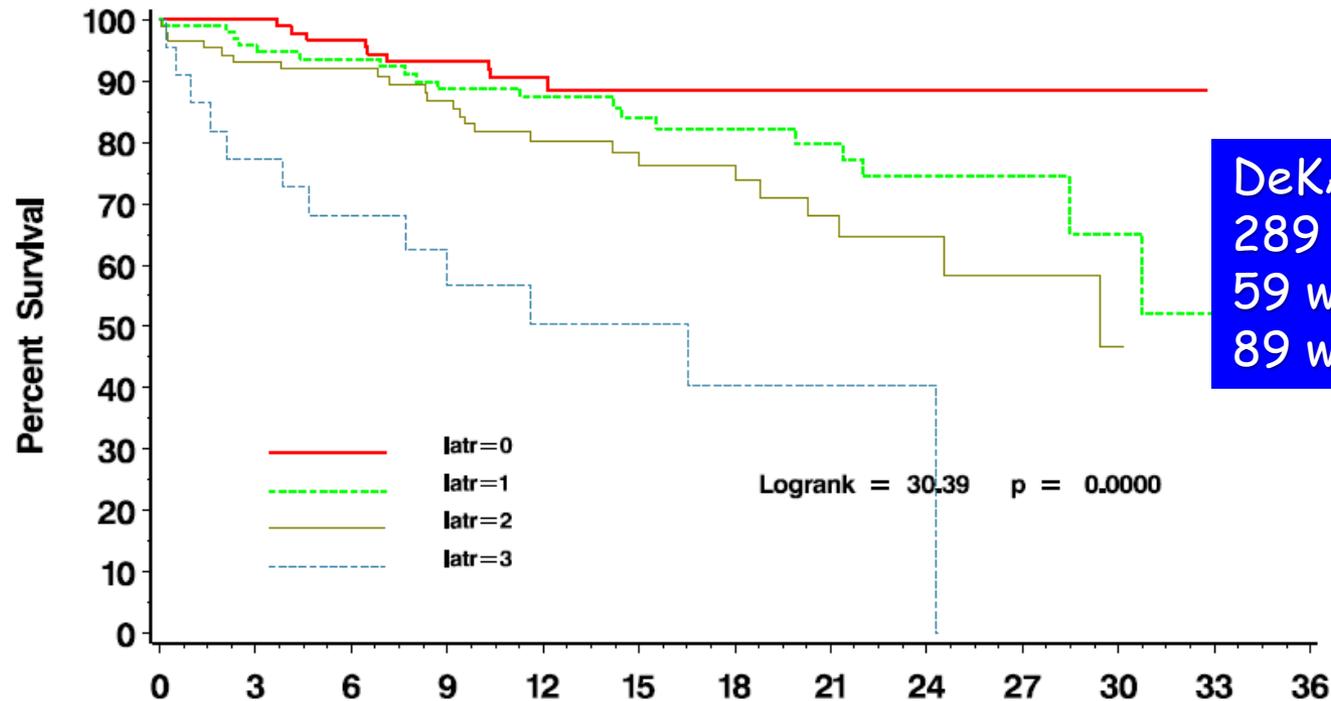


**DeKAF Study:**
289 recipients in cohort
59 with graft loss
89 with i=0, and iatr>1

Logrank = 30.39    p = 0.0000

iatr=0
iatr=1
iatr=2
iatr=3

"iatr"—inflammation in areas of tubular atrophy
        0 = inflammation in less than 10% of atrophic regions
        1 = inflammation in 10-25% of atrophic regions;
        2 = inflammation in 26-50% of atrophic regions;
        3 = inflammation in >50% of atrophic regions.

*Mannon RB.  Am Jnl Transplant 2010; 10:* 2066-2073

**6 month protocol bx**

Fraction surviving

Normal biopsy (n=186)

Subclinical rejection (n=74)

Interstitial fibrosis/ tubular atrophy (n=110)

Interstitial fibrosis/ tubular atrophy with subclinical rejection (n=65)

Similar data relating inflammation with fibrosis and poor outcome:
Cosio AJT 2005; 5:1464
Park WD JASN 2010;*21: 1987*

P < 0.01

*Moresco et al. Am Jnl Transplant 2006; 6: 747-752*
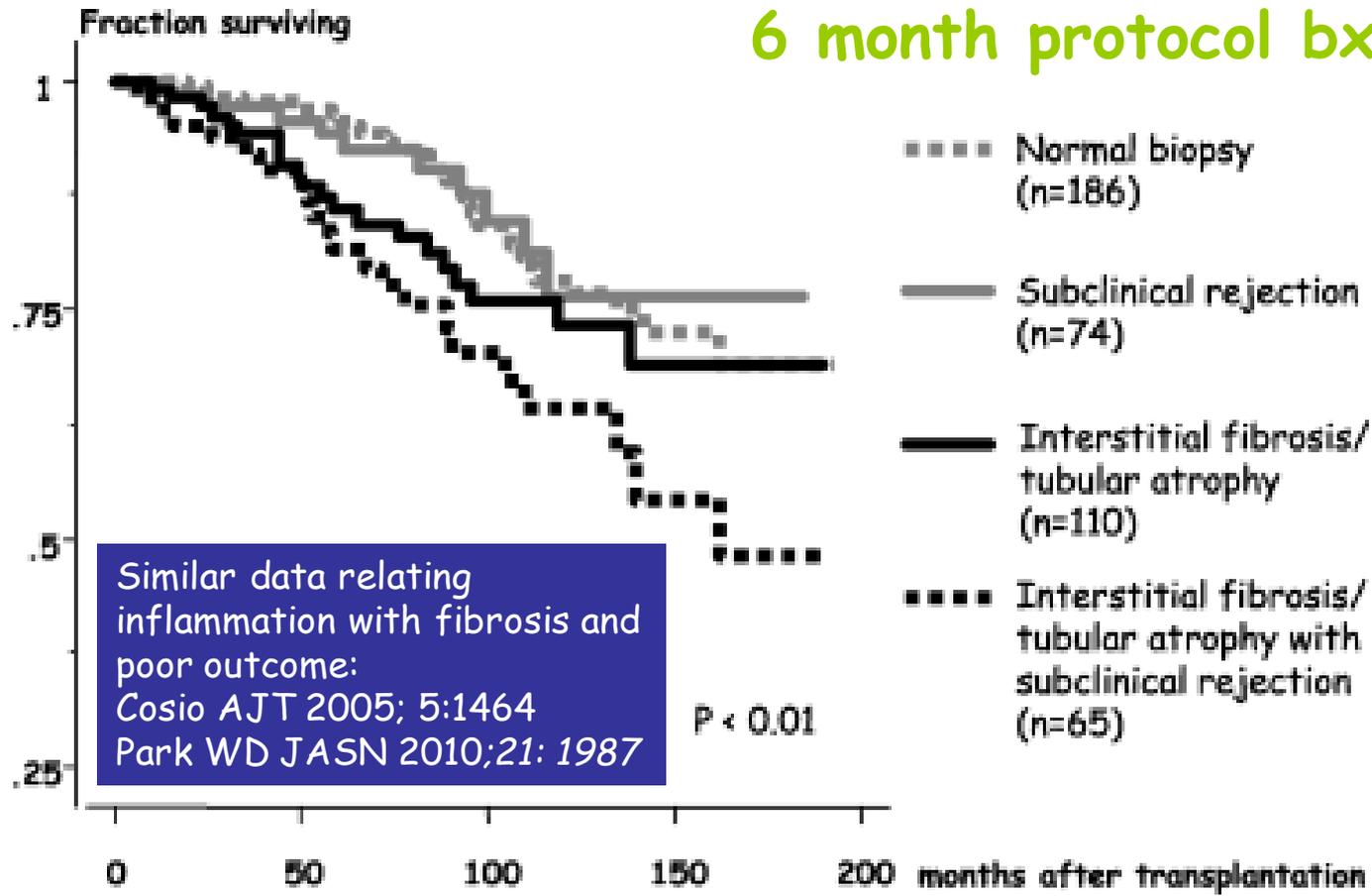
# Chronic TCMR

"Chronic TCMR was defined by sclerosing transplant arteriopathy. This lesion is characterized by intimal widening due to the de novo accumulation of collagens I and III, lack of elastosis, and varying degrees of intimal inflammation with mononuclear inflammatory cells.

   In sclerosing transplant arteriopathy, the intima usually contains varying numbers of myofibroblasts, occasional foam cells, and, in active disease stages, scattered, often clustered mononuclear inflammatory cells that may be most prominent along the inner elastic lamina. Endothelial cells are often enlarged with reactive nuclei sometimes overlying an ill-defined ring of smooth muscle cells: that is, so-called neomedia formation."

# Cohort Local Biopsy Diagnoses
## * adds up to >100% as 2 diagnoses/biopsy

| Primary/Secondary DX | CSC (N=425) N (%) | Prospective (N=227) N (%) |
|---|---|---|
| Acute antibody mediated rejection | 29 (7) | 18 (8) |
| Acute cellular rejection | 76 (19) | 77 (34) |
| ATN | 18 (4) | 25 (11) |
| Allograft nephropathy | 196 (48) | 61 (27) |
| Art. nephrosclerosis | 33 (8) | 5 (2) |
| Borderline change | 28 (7) | 18 (8) |
| CNI toxicity | 116 (29) | 25 (11) |
| Glomerulonephritis (de novo) | 23 (6) | 7 (3) |
| NPD | 9 (2) | 23 (10) |
| Polyomavirus (BK) | 11 (1) | 18 (8) |
| Recurrent disease | 53 (13) | 9 (4) |
| Transplant glomerulopathy | 82 (20) | 16 (7) |
| Inadequate | 4 (1) | 2 (1) |
| Other (e.g., pyelo) | 91 (22) | 48 (21) |

# Tissue Regeneration versus Fibrosis: The Process of Wound Healing



Initiation Phase

Ag dependent    Ag independent

Matrix Phase

Fibrogenesis Phase

Inflammatory response

Proliferative response

Regeneration of damaged tissues

Fibrotic healing

MMP-TIMP balance restored

Remodeling and maturation phase

Excessive ECM deposition

Collagen and fibronectin

Injury

Wound contraction and re-epithelialization

MMP-TIMP imbalance

Epithelial and/or endothelial cells

Persistent myofibroblast activation

Chronic injury, inflammation, or necrosis

Platelet activation and fibrin clot formation

Circulation

New blood vessels

Chemokines TGF-β PDGFs

Eosinophil
T cell
B cell
Neutrophil
Macrophage

VEGF

MMPS
TIMPS
EGF
IL-6
IL-13
TNF
TGF-β

Inflammation and migration

Angiogenesis and myobrobroblast proliferation and activation

Myofibroblasts

Resident fibroblasts
EMT
Fibrocytes
Other resident tissue cells, e.g., HSCs

FGF

Fibroblast

*Jnl Clin Investigation 2007; 117: 524*

# Graft Survival is Lower in Patients With SCR Associated with IFTA (i+IFTA)



6 month protocol bx

Fraction surviving

- Normal biopsy (n=186)
- Subclinical rejection (n=74) — **75% BR**
- Interstitial fibrosis/ tubular atrophy (n=110)
- Interstitial fibrosis/ tubular atrophy with subclinical rejection (n=65)

Similar data relating inflammation with fibrosis and poor outcome:
Cosio AJT 2005; 5:1464
Park WD JASN 2010;21: 1987

P < 0.01

months after transplantation

*Moresco et al. Am Jnl Transplant 2006; 6: 747-752*

# IFTA + i

- Tubulointerstitial inflammation in early surveillance biopsies is associated with progression of IF and decreased allograft survival [Nankivell et al. Transplantation 2004; 78:242; Choi et al. AJT 2005;5: 1354].

- Surveillance biopsies with i in non-scarred areas and IFTA [IFTA + i] are associated with shorter graft survival



Fraction surviving

6 month protocol bx

- - - - Normal biopsy (n=186)

—— Subclinical rejection (n=74)

—— Interstitial fibrosis/ tubular atrophy (n=110)

- - - - Interstitial fibrosis/ tubular atrophy with subclinical rejection (n=65)

Similar data relating inflammation with fibrosis and poor outcome:
Cosio AJT 2005; 5:1464
Park WD JASN 2010;21: 1987

P < 0.01

months after transplantation

Moresco et al. Am Jnl Transplant 2006; 6: 747-752

# IFTA + i and DSA

- 598 kidney transplant recipients of low immune risk (CTX neg, PRA<20%, DSA neg)
  - Basiliximab, CNI based therapy
  - 6w and 12m biopsies with DSA measurements (LabScreen)/ MFI<1000 = negative
    - normal histology (i+t≤1 and ci+ct≤1)
    - inflammation (i+t≥2 and ci+ct≤1)
    - IFTA (i+t≤1 and ci+ct≥2)
    - IFTA+i (i+t≥2 and ci+ct≥2)
- Findings of IFTA+i @ 6w are independent risk for dnDSA (8.9% of pop at 1y)

| Factor | OR of dnDSA |
|---|---|
| HLADR MM | 1.95 (1.09-3.49) |
| "i" score at 6w biopsy | 5.49 (1.67-10.03) |
| IFTA+i at 6w | 4.09 (1.67-10.05) |

*Garcia-Carro et al. Transplantation 2016; PMID 27163535*