"Omics" technologies and allograft injury

David Rush
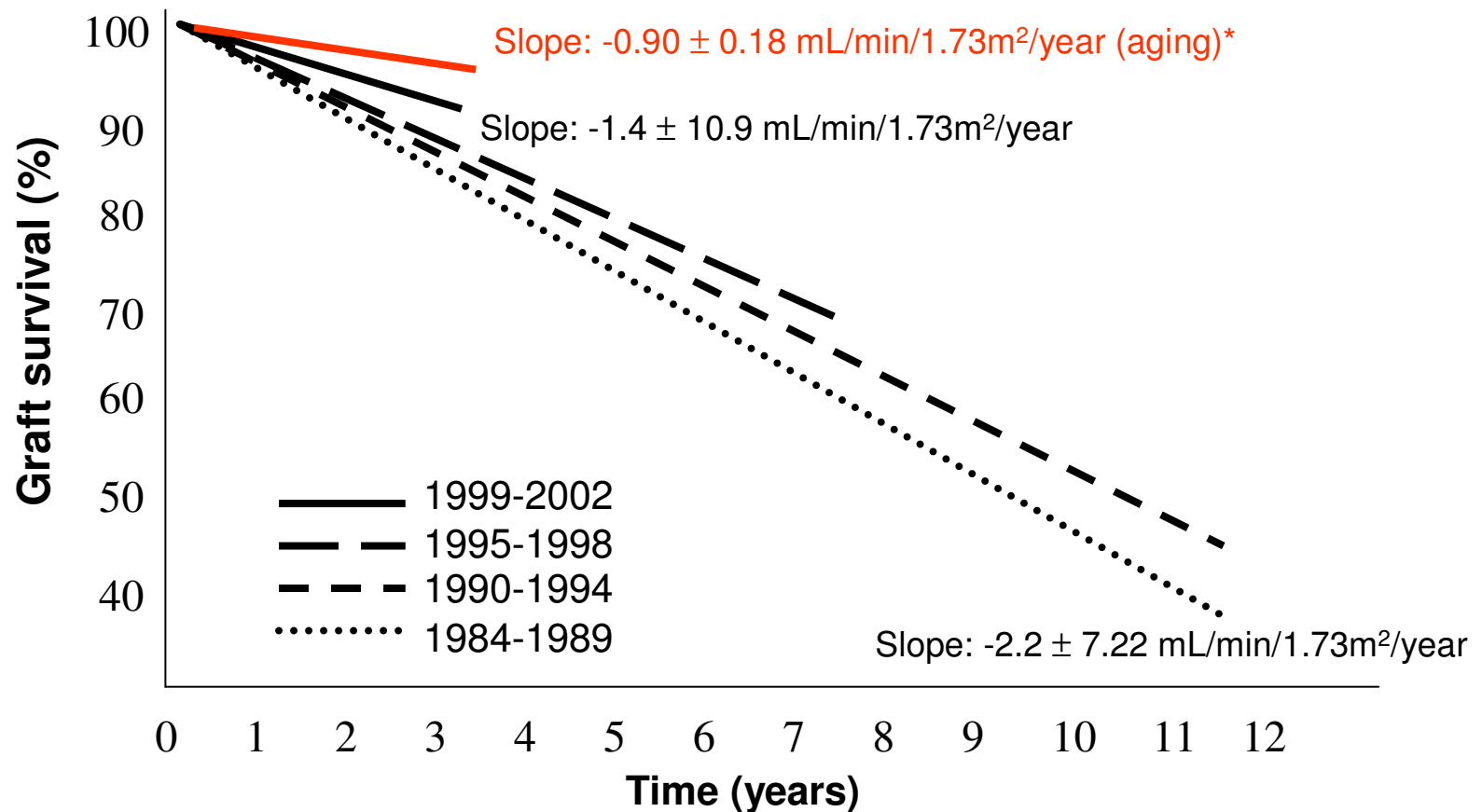
UNIVERSITY OF MANITOBA

Graft survival by year of transplantation

DeKAF (Decline in Kidney Allograft Function) Study Group
Matas, Kasiske, Hunsicker, Gaston, Mannon, Cecka, Gourishankar, Halloran, Rush

Slope: -0.90 ± 0.18 mL/min/1.73m²/year (aging)*

Slope: -1.4 ± 10.9 mL/min/1.73m²/year

Slope: -2.2 ± 7.22 mL/min/1.73m²/year

Legend:
- —— 1999-2002
- – – 1995-1998
- - - 1990-1994
- ···· 1984-1989

Graft survival (%)

Time (years)

Kasiske et al, Am J Transplant (2005); 5:1405
*Rowe et al, J Gerontol (1976); 31:155

Deterioration of Kidney Allograft Function

DeKAF Study

Prospective/cross-sectional study (~ 5000 patients)

- Participating centres: Minneapolis (Matas, PI; Kasiske), Mayo Clinic (Cosio, Grande), Iowa (Hunsicker), Alabama (Gaston, Mannon), UCLA (Cecka), Alberta (Gourishankar, Halloran), Manitoba (Rush)

- Renal Biopsies done "for cause" (n ~ 800) – (Mayo Clinic)

- Urine magnetic resonance done in Winnipeg

DeKAF: Hypotheses

1) Progressive graft dysfunction is due to ongoing active injury, and is not necessarily the consequence of past events;

2) There are discrete, definable entities responsible for injury, that lead to chronic graft deterioration and late graft loss;

3) These entities can be differentiated by means of clinical, laboratory, and pathologic studies;

4) Accurate diagnosis offers the best hope for the development of interventional trials.

Enrollment: Prospective and Cross-sectional cohorts
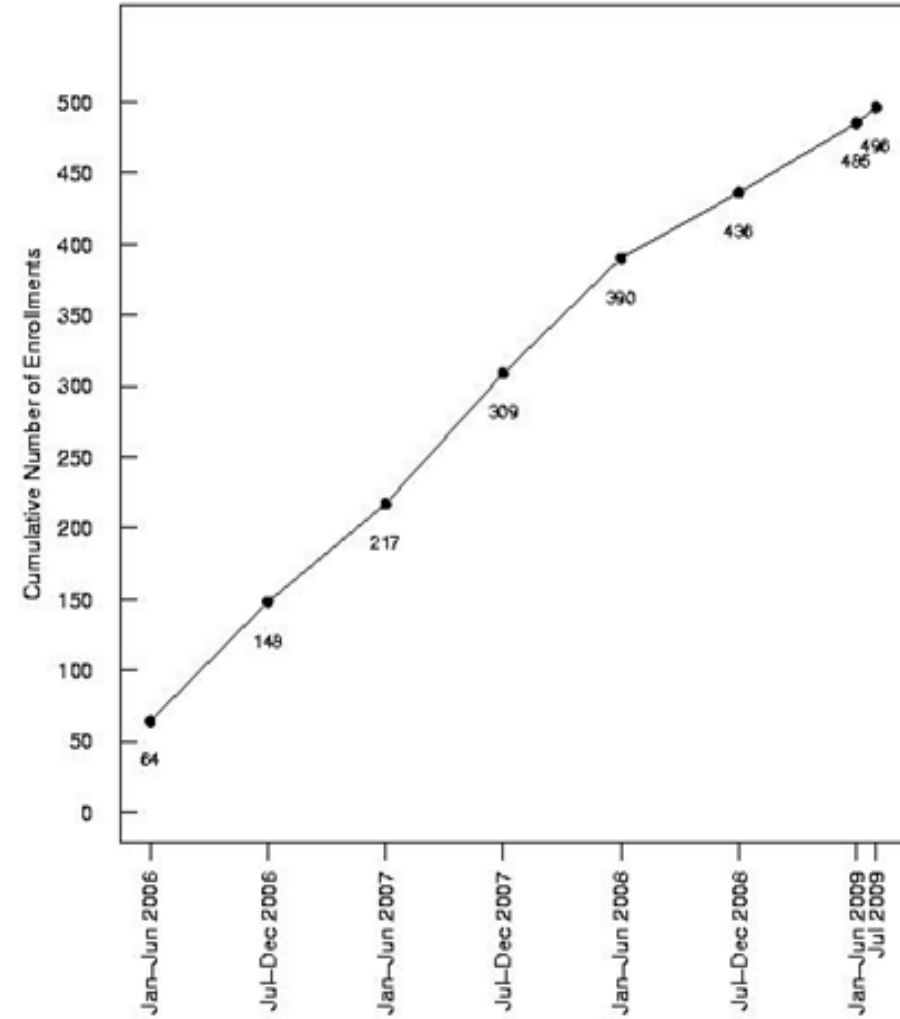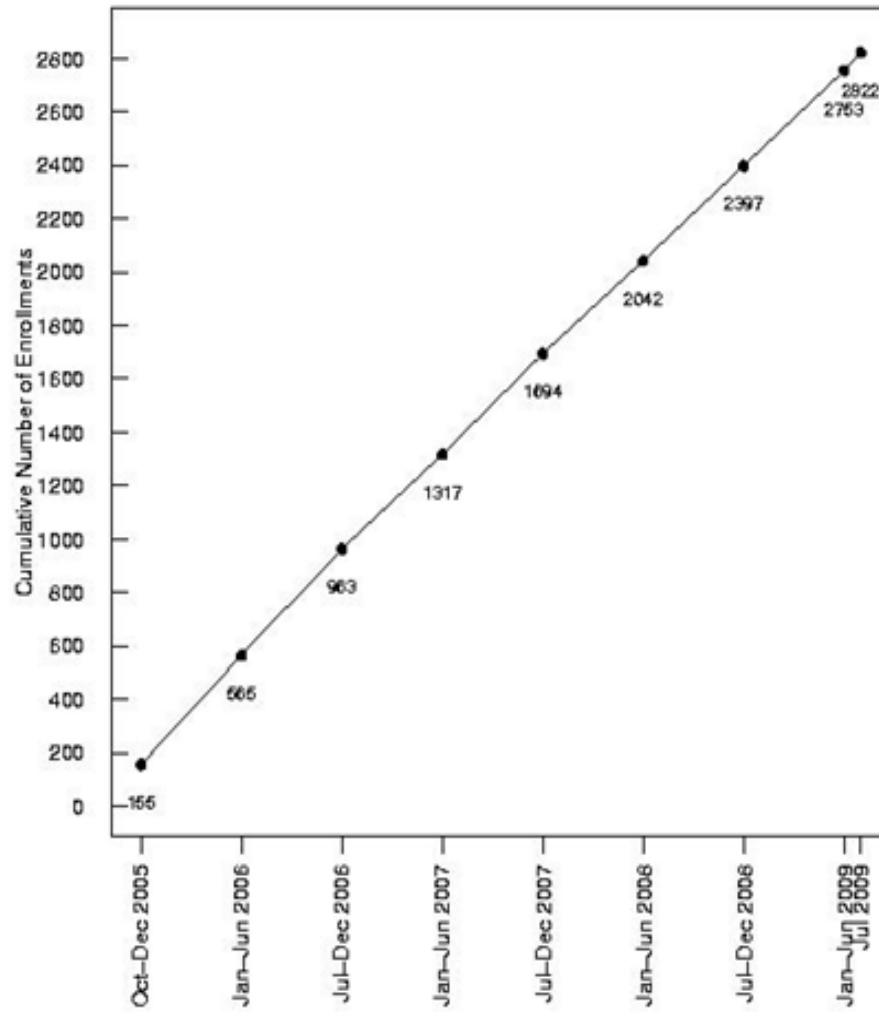
Depiction of clusters – "cluster clocks"

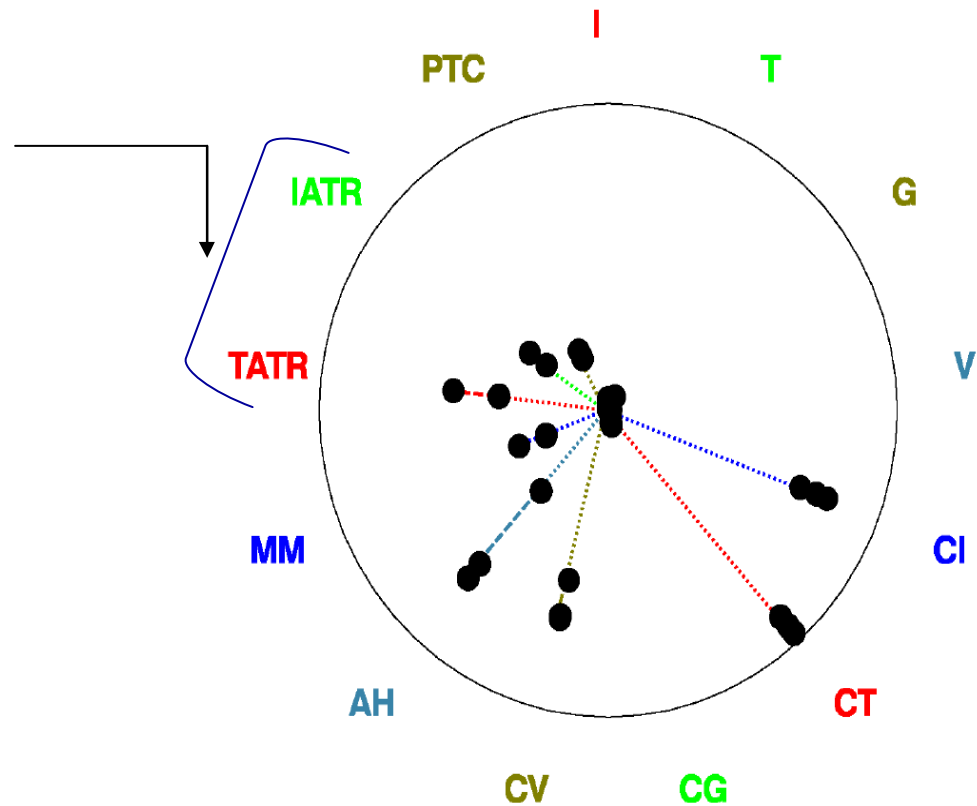**Legend**

Each spoke represents a Banff score, except

Length of spokes = % patients with finding
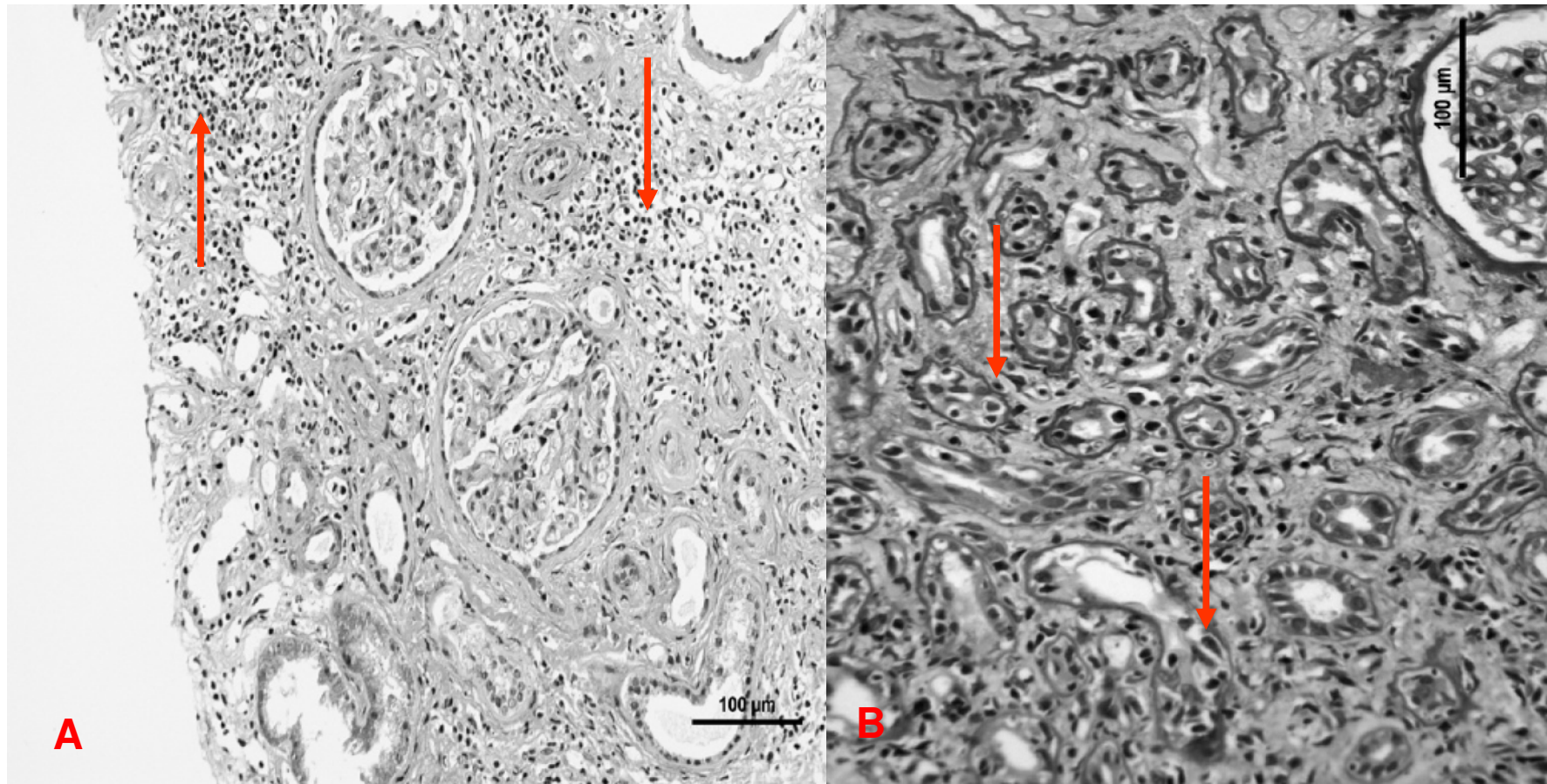
No spoke = Banff 0

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

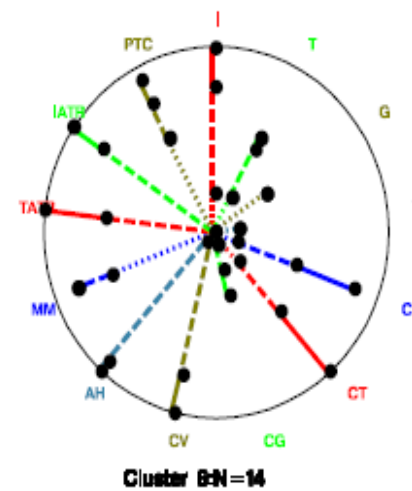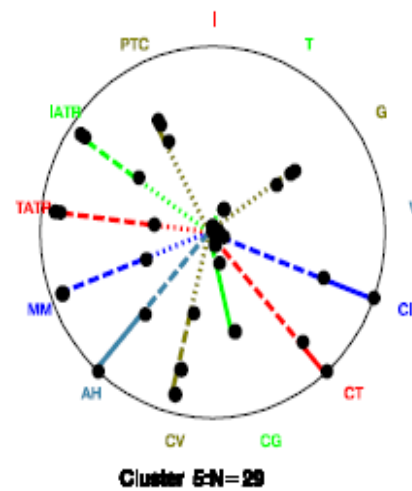# Inflammation in areas of fibrosis ("iatr"; A) and tubulitis in atrophic tubules ("tatr"; B)
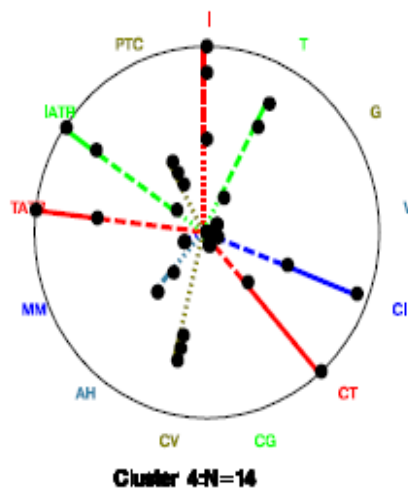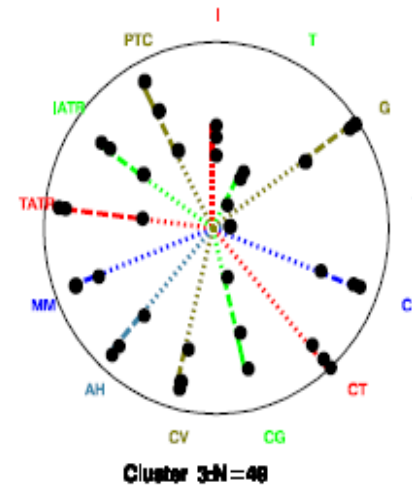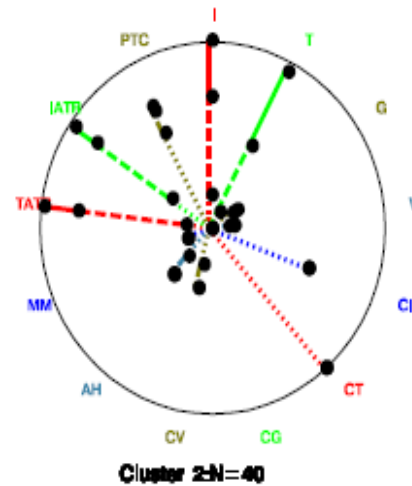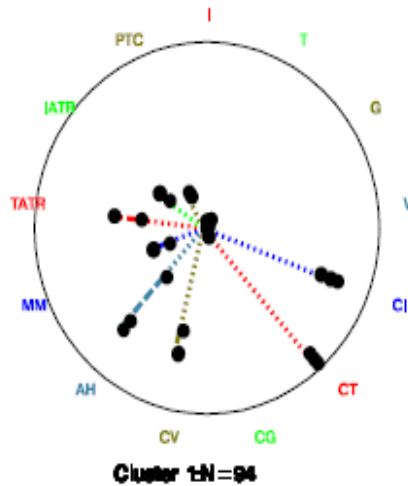
Inflammation in areas of scar

Has previously been excluded from Banff

Original DeKAF clusters (n = 265; now 370)

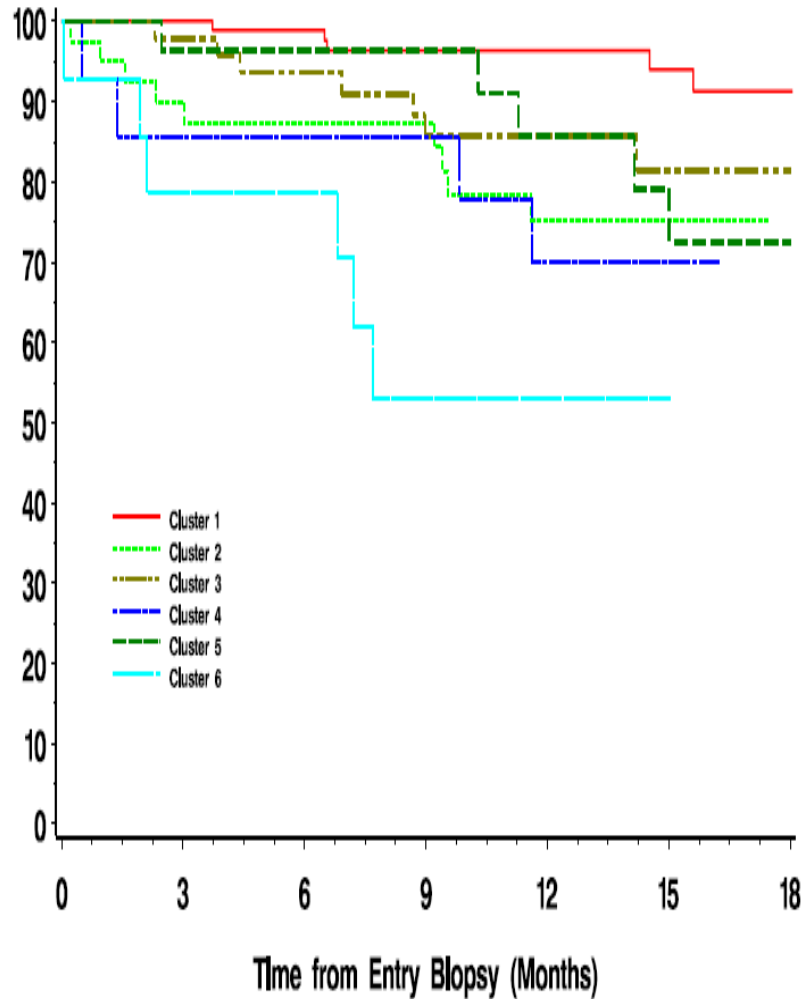Cluster 1 – mild inflammation; mild fibrosis

Cluster 2 – ai, at, iatr, tatr; mild fibrosis

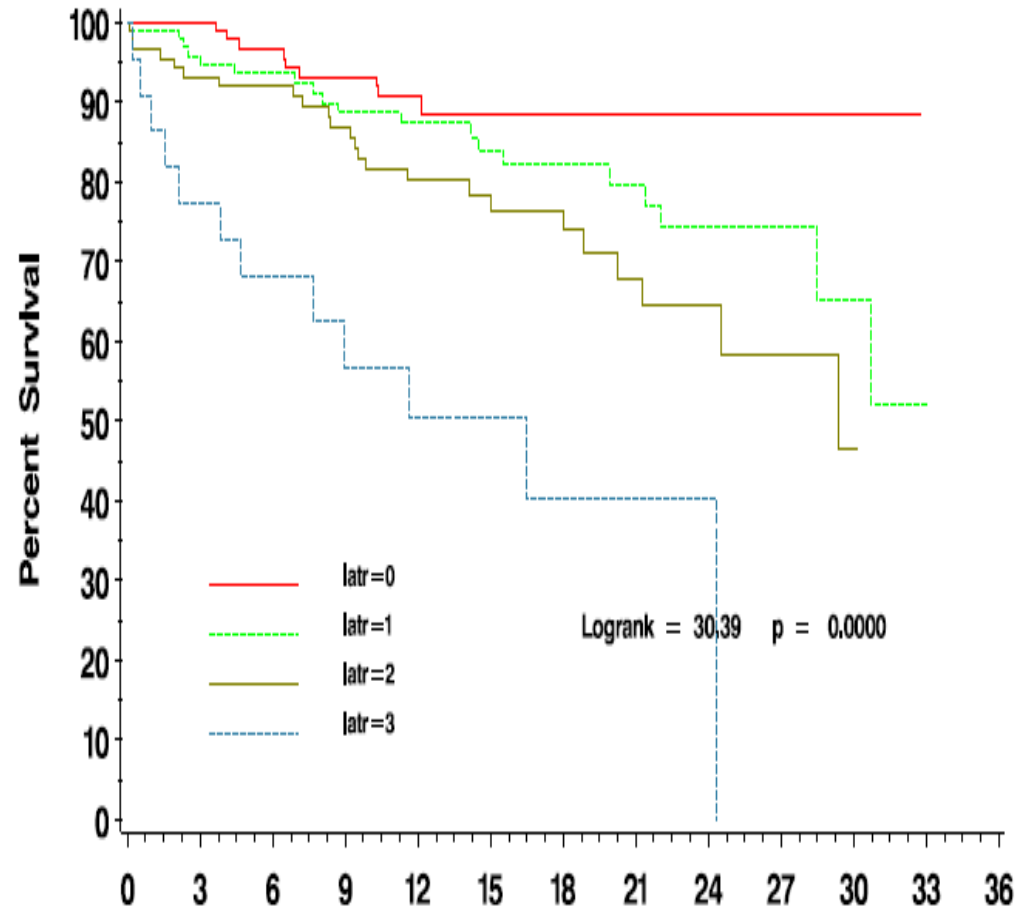Cluster 3 – ai, at, iatr, cg; fibrosis

Cluster 5 – no ai, at; only iatr, tatr; fibrosis

PTC in several clusters

Actuarial Graft Survival
by Cluster and by "iatr" score

Matas et al Am J Tansplant (2010)

Mannon et al Am J Tansplant (2010)

Systems Biology Approach:
Profiling all Components in a Sample
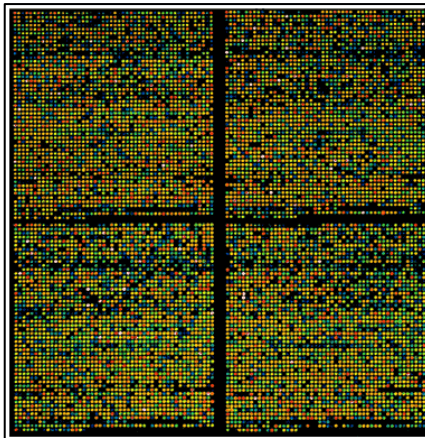
gDNA → mRNA → Protein → Metabolism

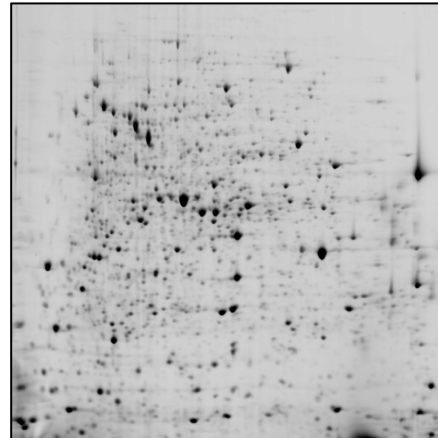| 20,000–30,000 Genes | > 100,000 mRNAs | > 1,000,000 Proteins | Low MW Compounds (<5kD) |

acgtacca
aggtaacg
cggtttttcgt
gtatctccctt

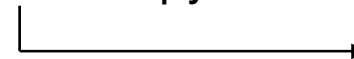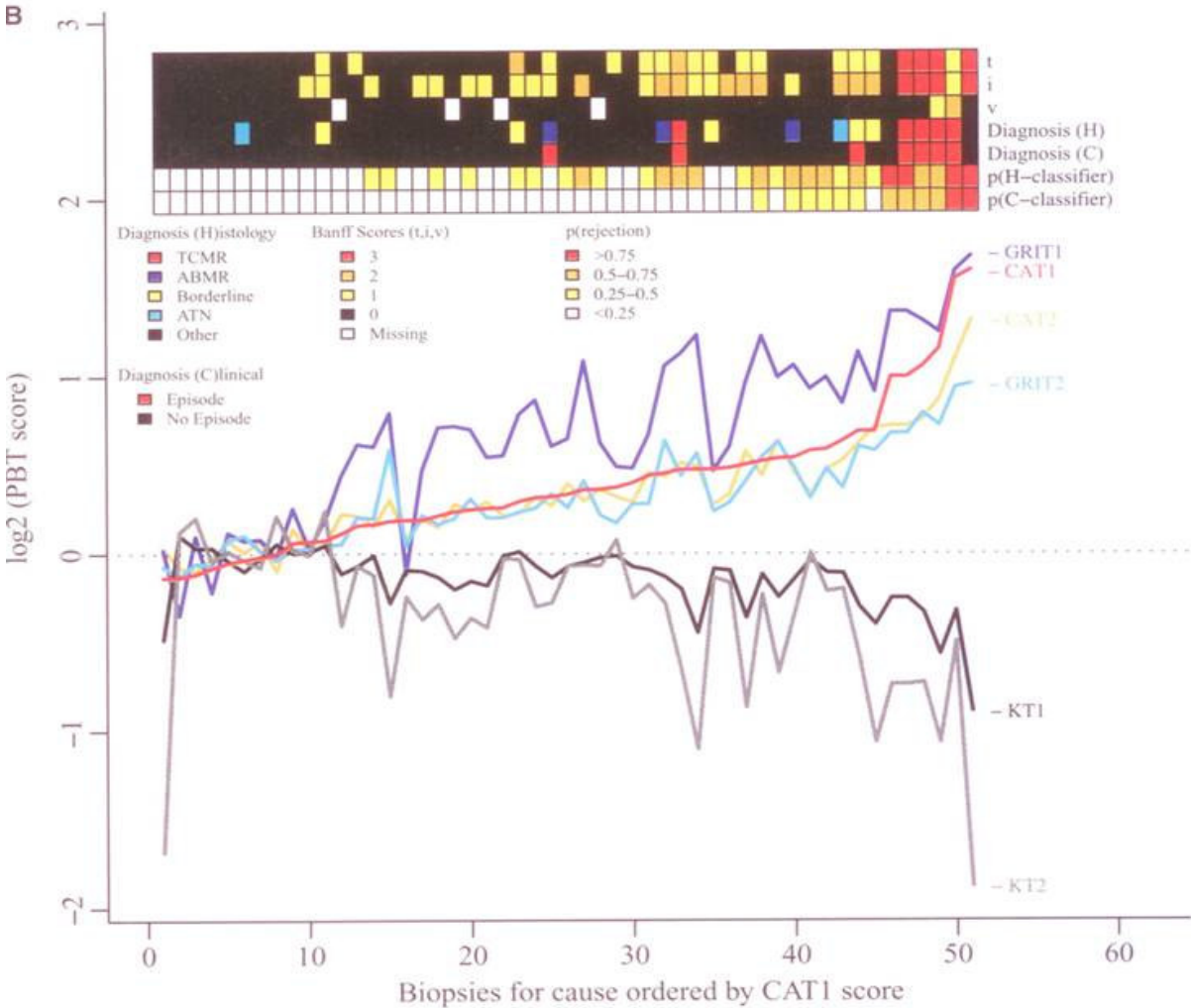cDNA Microarrays   Mass Spectroscopy ^{1}H Magnetic Resonance Spectroscopy
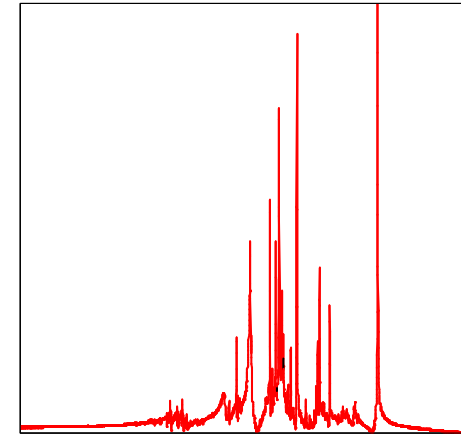
Microarray analysis of rejection using pathogenesis based transcripts (PBT)

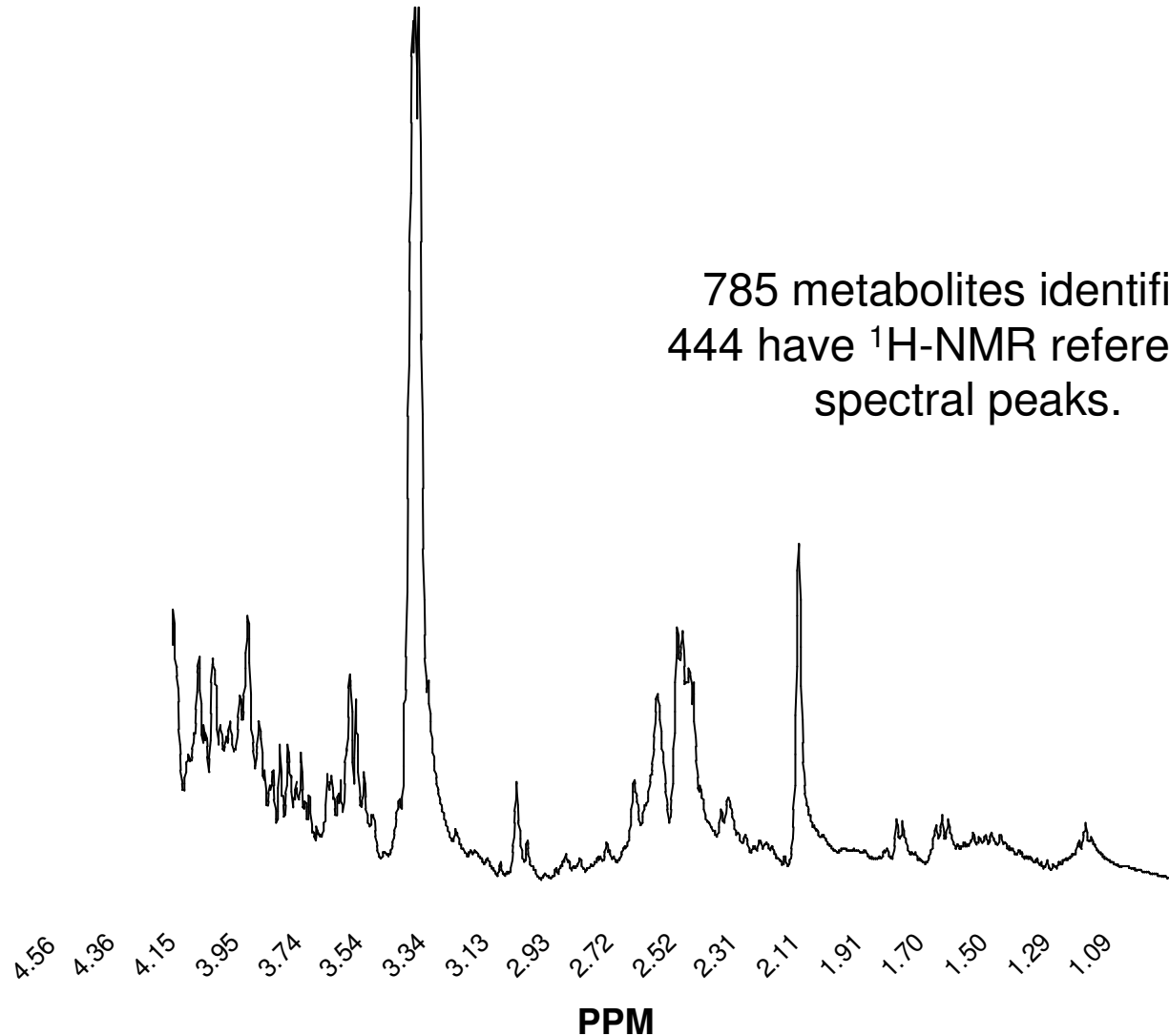Mueller et al, Am J Transplant (2007); 7: 2712-2722

The Human Metabolome Database: Biofluid Urine

785 metabolites identified.
444 have ^{1}H-NMR referenced spectral peaks.

Wishart et al, Nucleic Acids Research; 37: D603-D610, 2009 (updated 2011)

Urine Metabolomics: Methodology

- The large number of data points in MR spectra requires an informatics approach:

- The strategy for "pattern recognition" has 4 stages:
  - 1) Pre-processing : Area normalization, peak alignment
  - 2) Feature selection: Identification of maximally discriminating averaged subregions of the spectra;
  - 3) Classifier development: With these subregions, crossvalidated linear discriminant analysis classifiers are developed
  - 4) Accurate visualization of results

Somorjai RL et al. *Artificial Intelligence Methods and Tools for Systems Biology (Dubitzky W and Azuaje F, (eds.))*, Computational Biology Series, Vol. 5 Springer pp. 67-85 (2004)

Urine spectra from DeKAF patients

- Matching urine spectra/biopsy pairs (u/b) studied to date are 457:
  - 102 u/b from patients with varying degrees of fibrosis but no inflammation;
  - 150 u/b from patients with varying degrees of fibrosis and **severe inflammation** in both normal and atrophic parenchyma;
  - 108 u/b from patients with varying degrees of fibrosis and **minor inflammation** mostly in atrophic parenchyma;
  - 97 u/b from patients with transplant glomerulopathy.
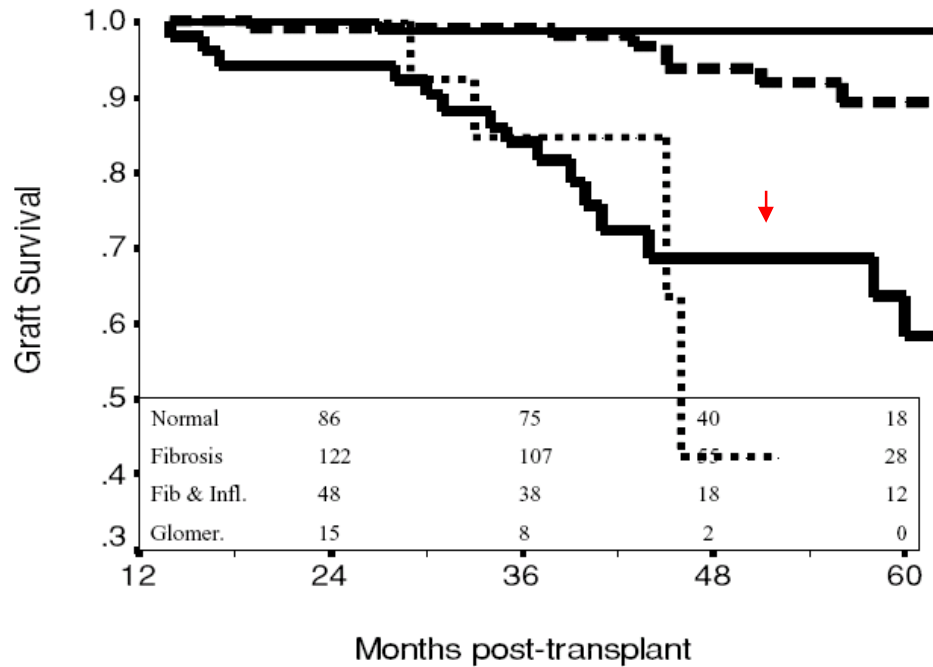
Cosio et al, Am J Transplant (2005) 5: 2464

The combination of inflammation and fibrosis detected on protocol biopsy identifies grafts at ↑ risk

Moreso et al, Am J Transplant (2006), 6:747
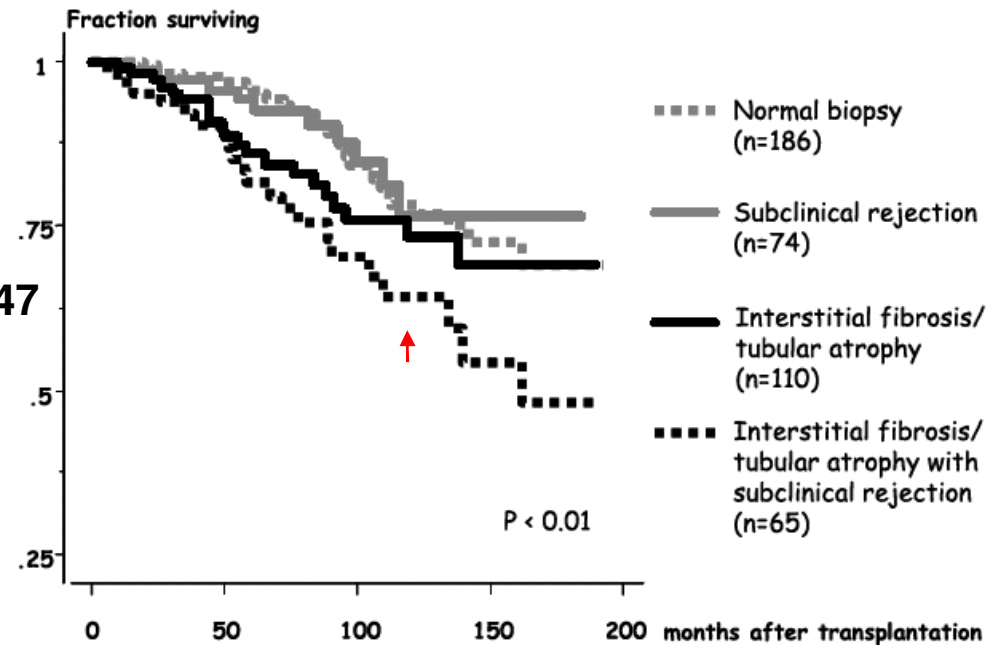
1. Can Urine MR spectra distinguish between IF <u>with</u> severe inflammation and IF <u>without</u> inflammation

- One hundred (100) patients with IF plus severe inflammation and 68 patients with IF minus inflammation were used for the training set; and 50 and 34 independent patients, respectively, were used as the test set.

- The 3,300 data point data set of the average spectra was analyzed (100 points at a time) and the best classifier was developed on the training set. The classifier was validated with the independent test set.

- Visualization of the data was done using the "class-proximity plane" graphic.

Conclusions

- Urine magnetic resonance spectroscopy (UMRS) distinguishes IF with severe inflammation from IF without inflammation with ~90% accuracy.

- The extent of inflammation (severe vs. minor) can also be accurately determined by UMRS with ~90% accuracy.

- Similarly, IF without inflammation can be distinguished from minor inflammation by UMRS with ~ 90% accuracy.

- Validation of UMRS signatures for other allograft pathologies – e.g. transplant glomerulopathy – is in progress.

- The non-invasive nature of UMRS will allow for repeat testing to evaluate changes in spectra and their correlation with specific interventions and their outcomes in prospective studies.

Thank you!