



Gene Expression Profiling

Advantages and Disadvantages of
Monitoring Risk rather than Injury

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I have no disclosures related to this presentation

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Gene expression Profiling- Rationale

Current techniques for monitoring the lung allograft

Clinical symptoms/
Spirometry

Radiographic findings

Bronchoscopy with
BAL/ transbronchial
biopsies

Current diagnostic tests

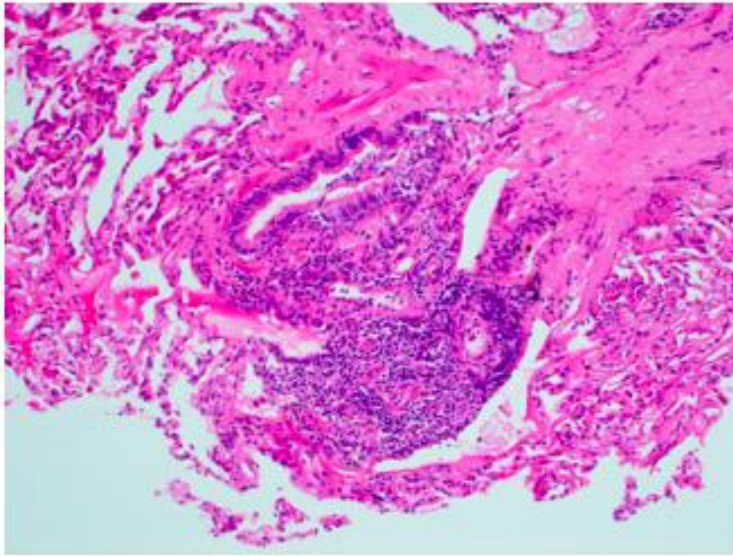
Low sensitivity

Low specificity

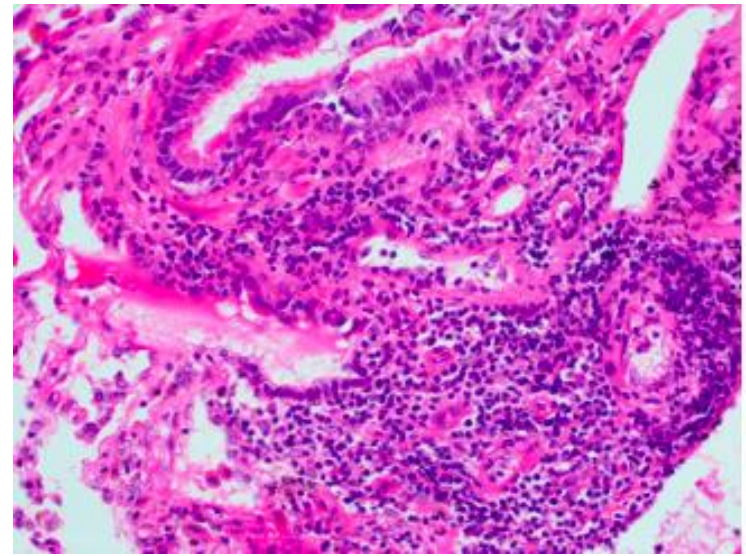
Do not address
mechanism/
endotypes

Diagnosis of Acute Cellular Rejection

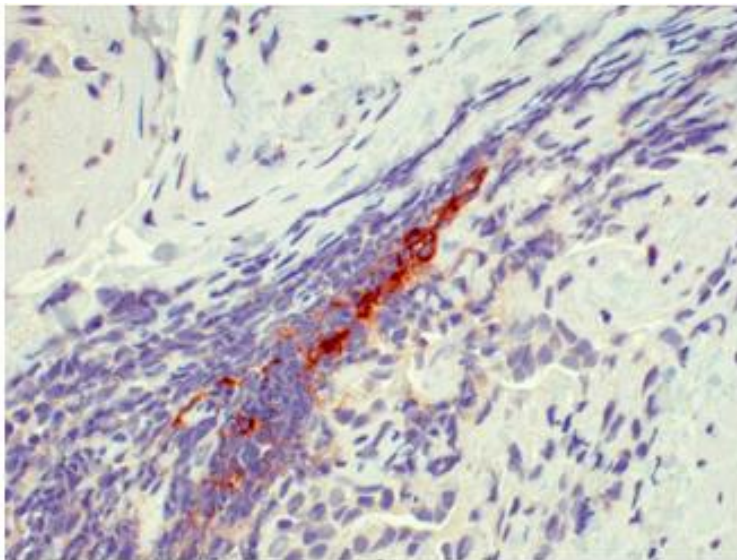
A



B



C



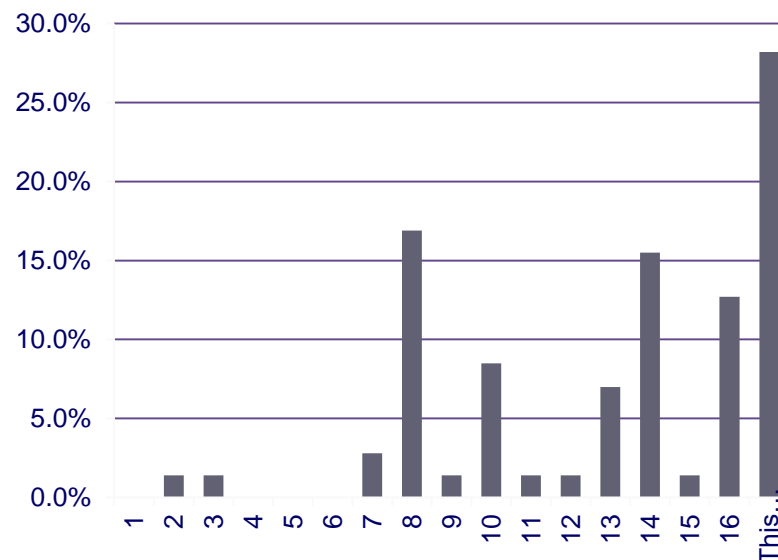
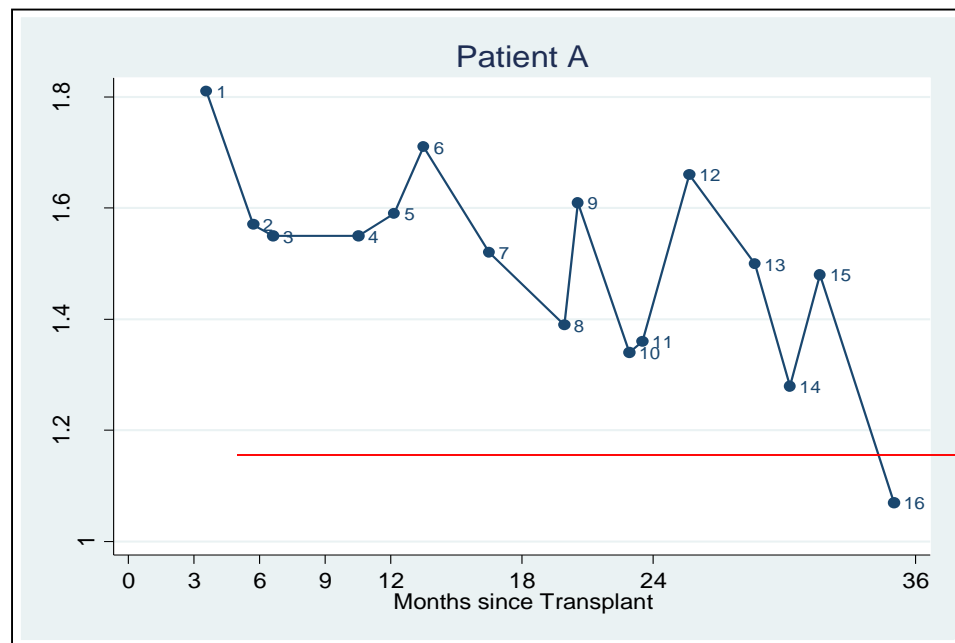
Bronchoscopy with Biopsies:

- Invasive
- Variability in sampling
- Inter-observer variability in reading the biopsies
- Not predictive

FEV1 decline in CLAD/ BOS

Inter-observer variability

- Clinical gestalt
- Personal experience
- Definition is difficult to operationalize

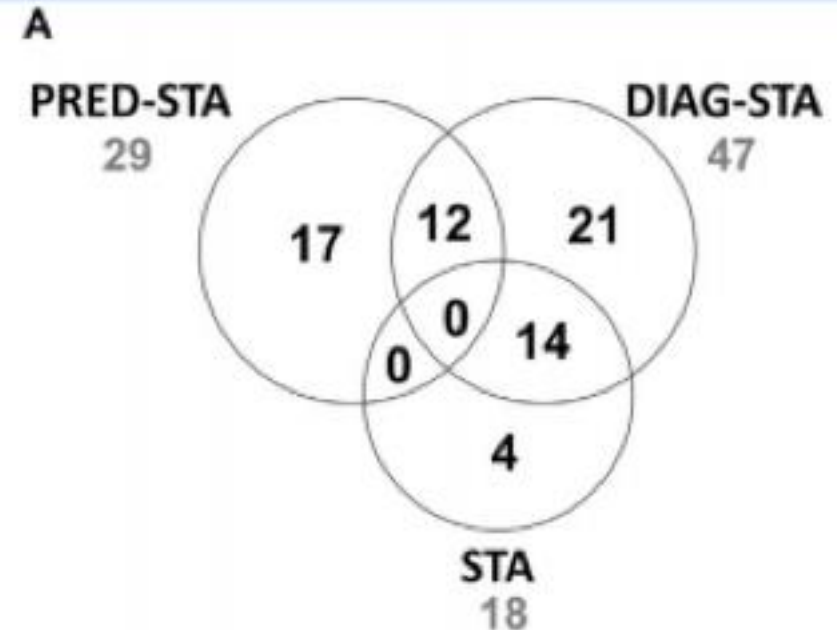


Gene Expression Profiling

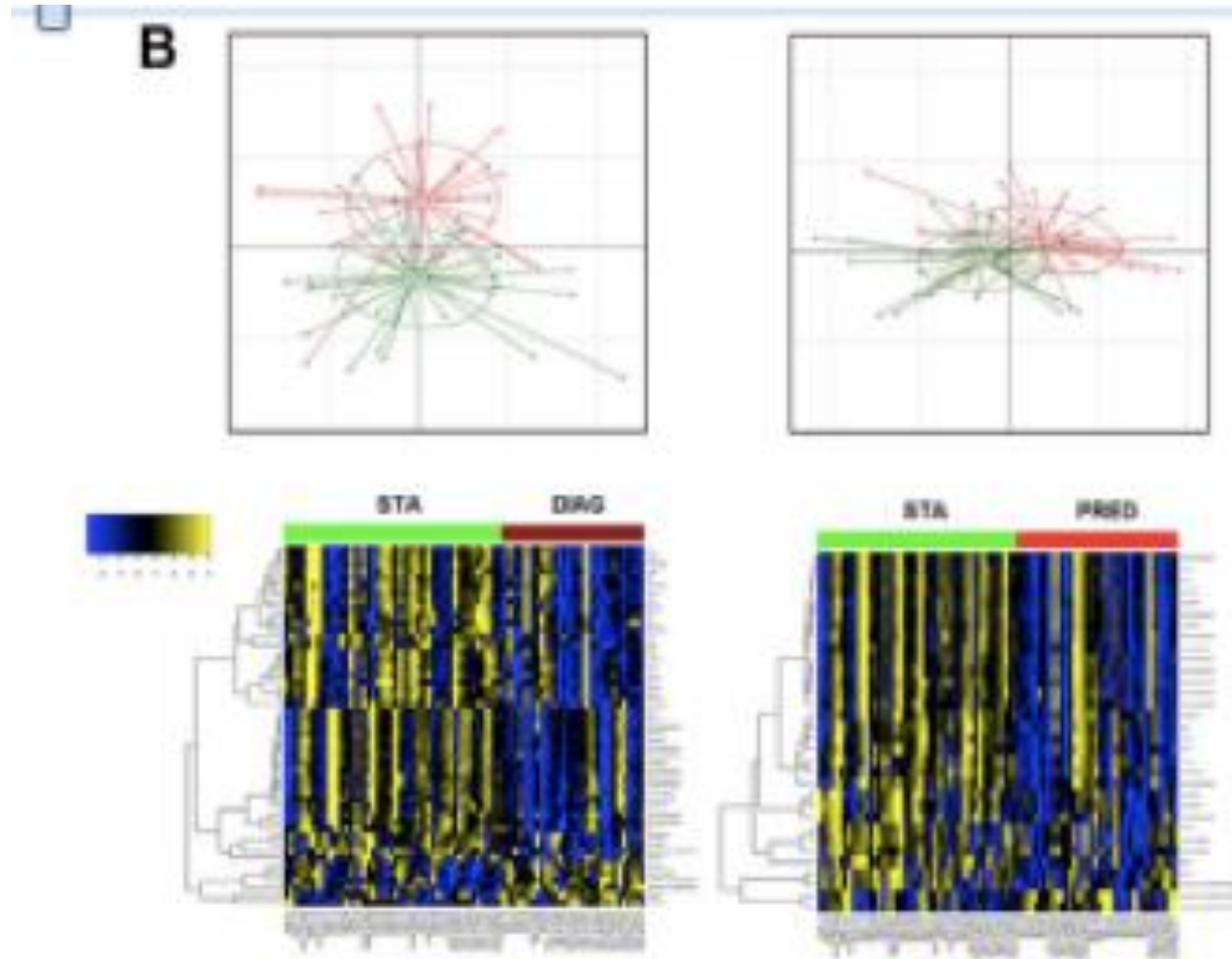
- Promising Tool to identify transcriptomic markers associated with allograft function
- Gene signatures and “molecular microscope”
- Successfully used in heart, kidney and liver transplant recipients as predictors of rejection
- Interest in lung transplantation to develop a gene signature for predicting rejection
- Sampling in lung transplant recipients may occur from
 - Peripheral blood
 - Bronchoalveolar lavage
 - Transbronchial biopsies

Blood Gene Expression Predicts BOS

- 107 peripheral blood samples from 89 lung transplant recipients in the COLT study
- 49 patients with stable lung function
- 40 patients with BOS at 3 years post transplant
 - Samples 6 months prior to BOS (PRED)
 - Samples at BOS onset (DIAG)
- 50 gene transcripts were differentially expressed between the two groups



Blood Gene Expression Predicts BOS

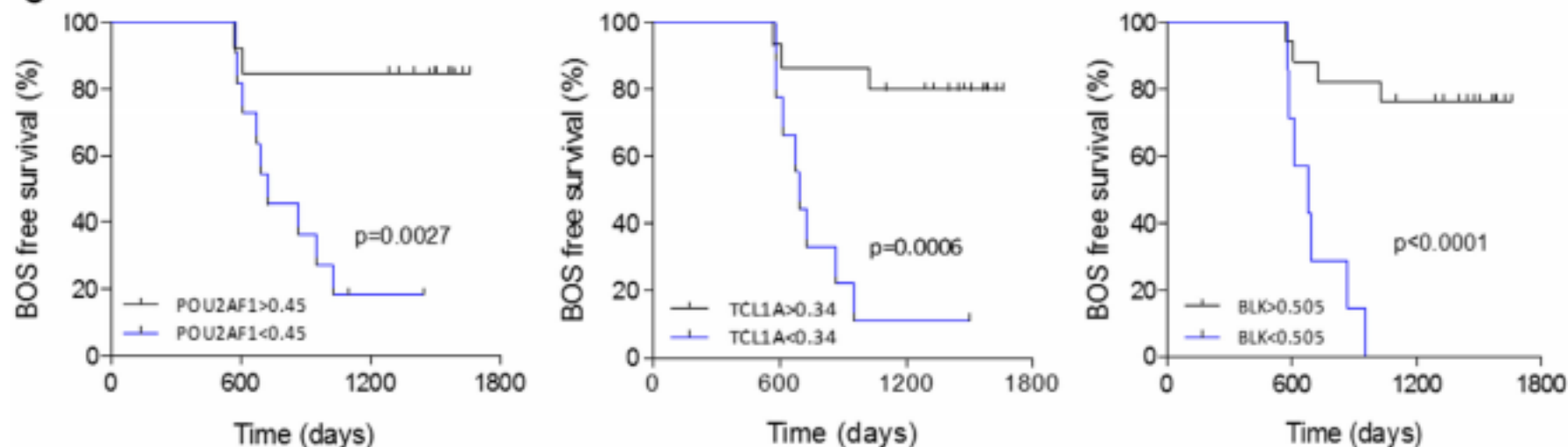


Blood Gene Expression Predicts BOS

B

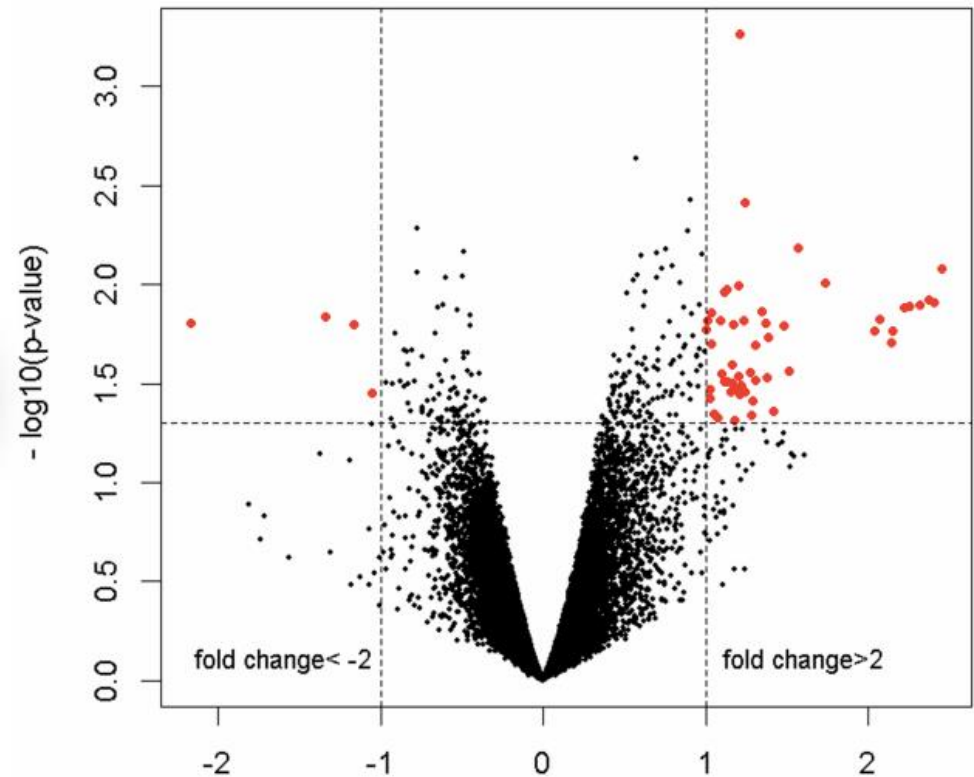
	<i>POU2AF1</i>	<i>TCL1A</i>	<i>BLK</i>	<i>POU2AF1</i> + <i>TCL1A</i> + <i>BLK</i>
sensitivity	82%	73%	68%	73%
specificity	85%	92%	100%	92%
ppv	82%	89%	100%	89%
npv	85%	80%	76%	80%
accuracy	83%	83%	83%	83%

C



Gene Expression preceding CLAD

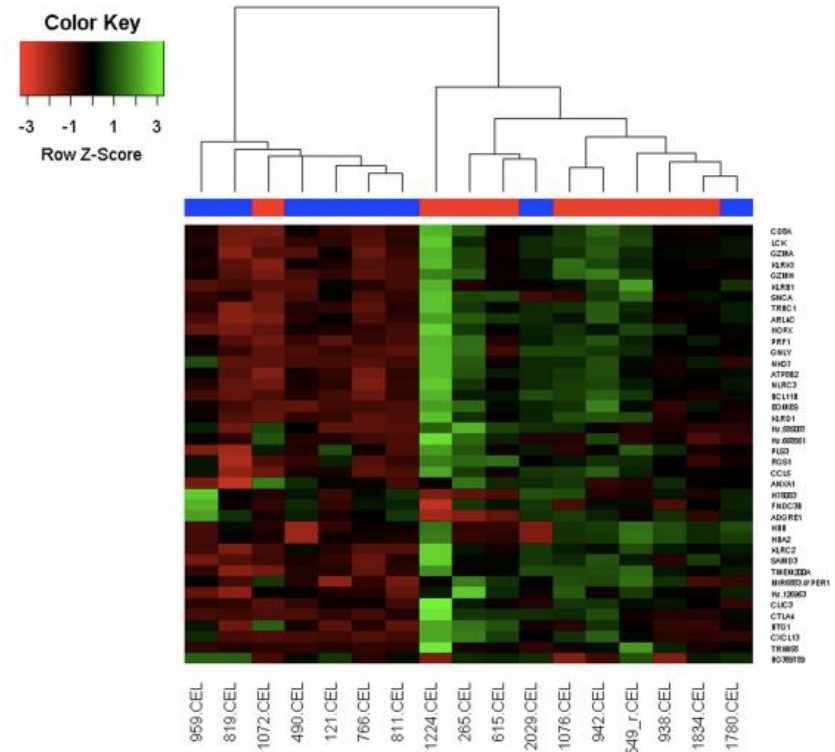
- Hypothesized the BAL cell pellet may provide a larger “window” as it may contain several compartments of the lung allograft
- 9 patients with CLAD/ BOS (3yrs)
- 8 stable patients up to 4 years
- BAL cell pellets at one year surveillance bronchoscopy
 - 55 dysregulated genes
 - 51 upregulated genes
 - 4 downregulated genes



Gene expression preceding CLAD

- 55 differentially expressed genes
 - Retention, activation and proliferation of cytotoxic lymphocytes
 - Innate and adaptive cytotoxic response
- Hierarchical clustering and supervised machine learning correctly categorized:
 - 82% CLAD
 - 94% non CLAD

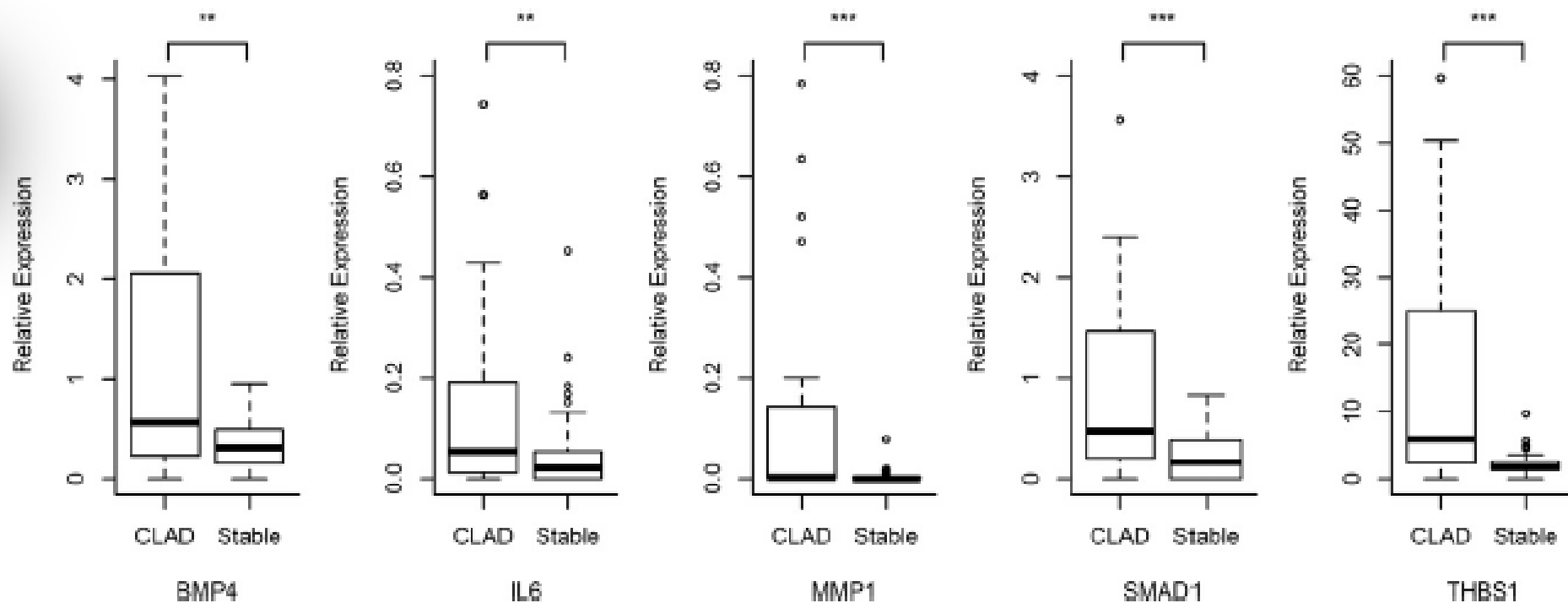
Hypothesized that AR and CLAD may both represent allorecognition by specific T cell signatures



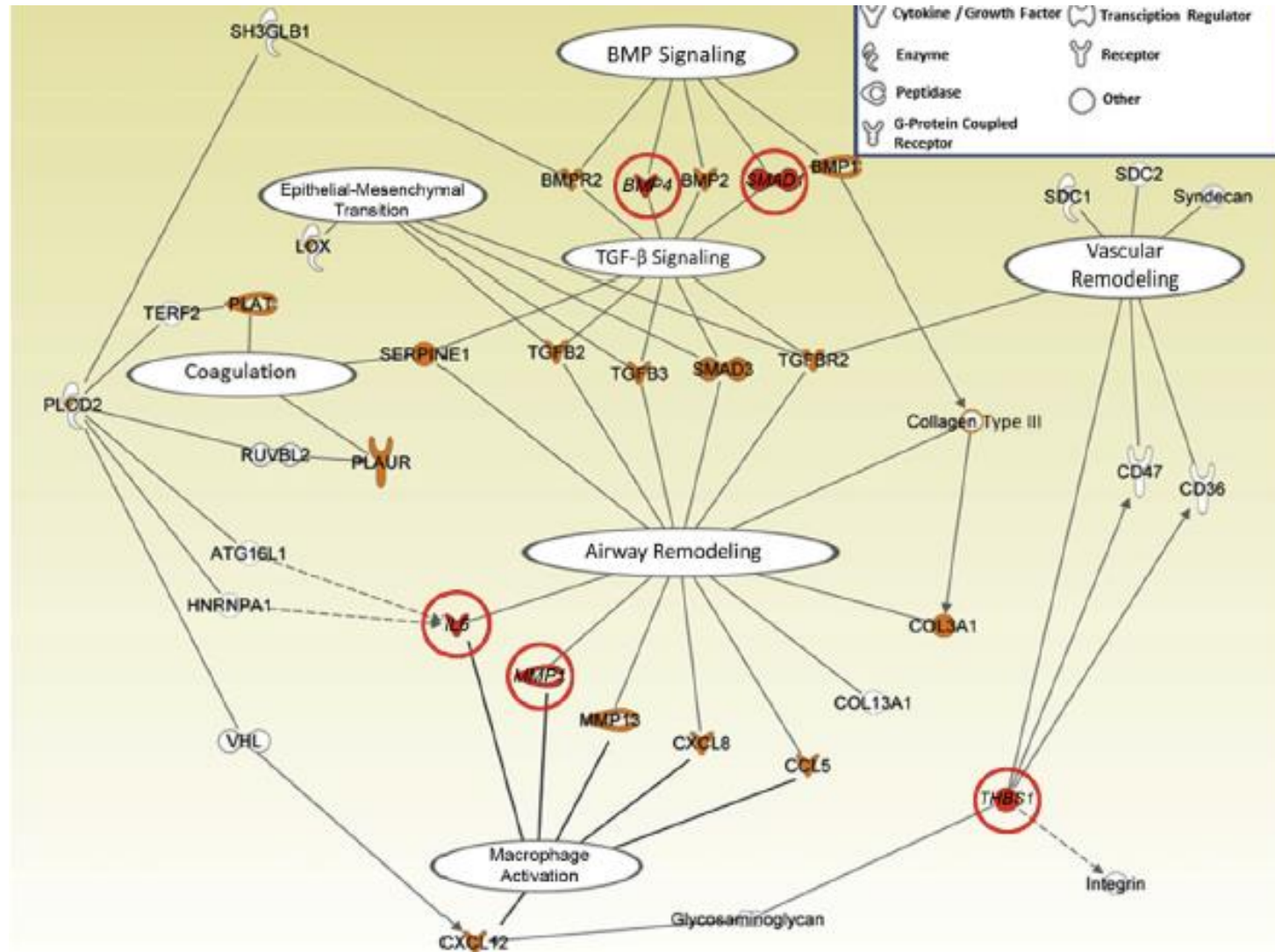
Molecular profiling in lung biopsies to predict CLAD

- Hannover group hypothesized that airway fibrosis of CLAD is due to myofibroblasts and excessive matrix production/deposition.
- They analyzed the cellular composition and differential expression of 45 tissue remodeling associated genes
 - 18 patients with CLAD (within 3 years)
 - 18 patients without CLAD
 - 22 genes were significantly upregulated in the CLAD group

Biopsy Molecular profiling in CLAD



Biopsy Molecular profiling in CLAD



Common rejection model in Chronic Lung Rejection

01

Several studies have suggested similar injury mechanisms in all solid organ transplantation resulting in identification of a chronic rejection module (CRM)

02

CRM (11 genes) obtained from a meta- analysis of public microarray gene expression data for biopsy confirmed acute rejection in all 4 organ transplants
Heart
Liver
Kidney
Lung

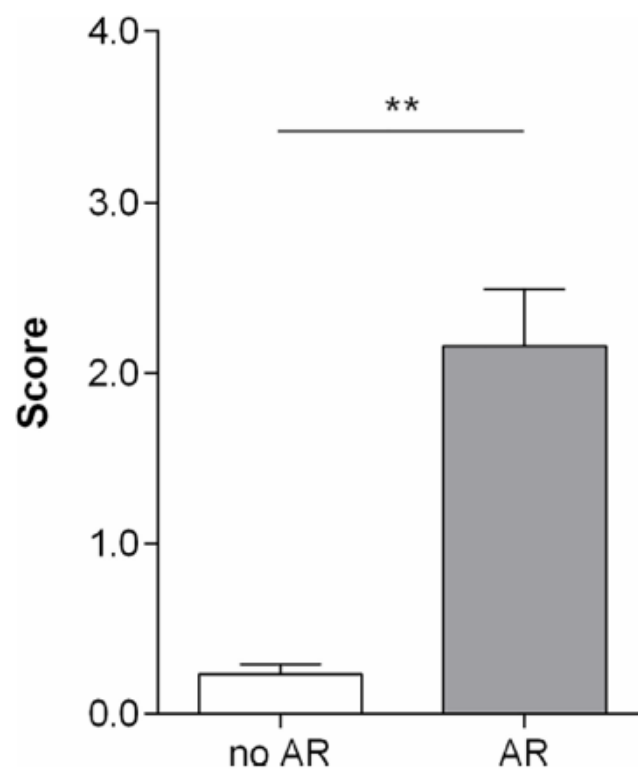
03

CRM genes obtained from:

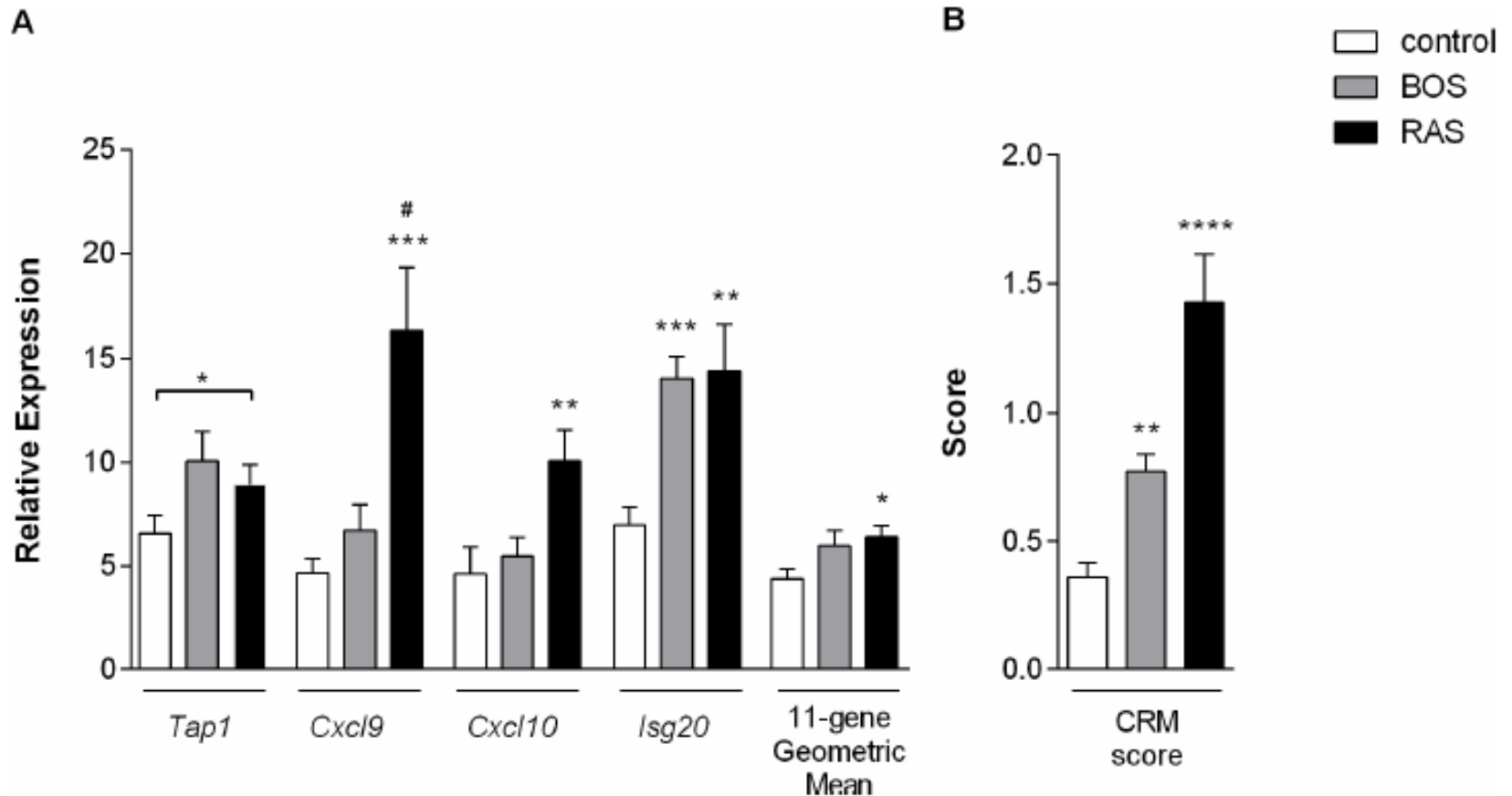
- Transbronchial brushings
- Lung tissue
- BAL

Common rejection model in Chronic Lung Rejection

no AR	AR
0.71	2.73
1.84	9.49
1.44	34.81
1.24	17.28
0.8	1.92
5.94	17.34
2.51	5.19
0.46	2.56
3.92	13.37
1.06	2.83
3.84	6.61
<i>1.69 (1.35–1.95)</i>	<i>6.61 (4.26–7.04)</i>
<i>0.17 (0.12–0.30)</i>	<i>2.19 (1.51–2.79)</i>



Common rejection model in Chronic Lung Rejection



Sequential Gene Expression prior to CLAD

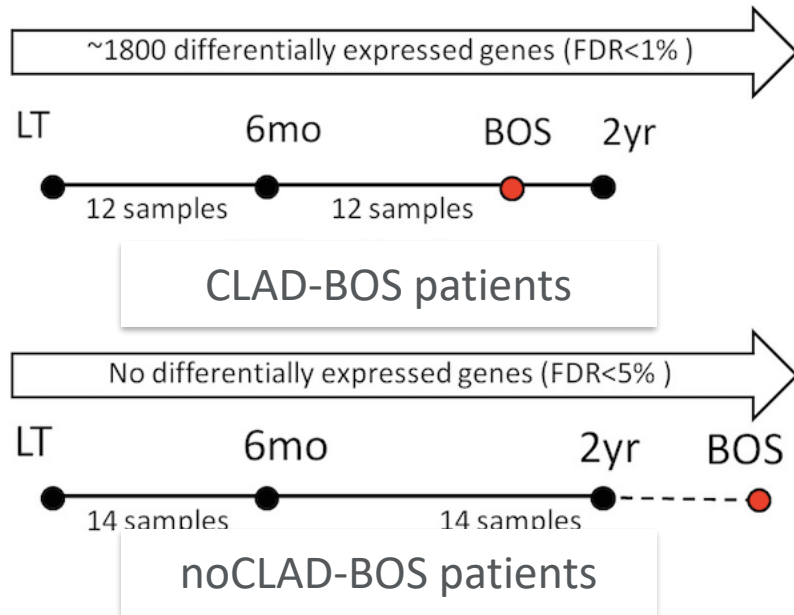


Figure 2a

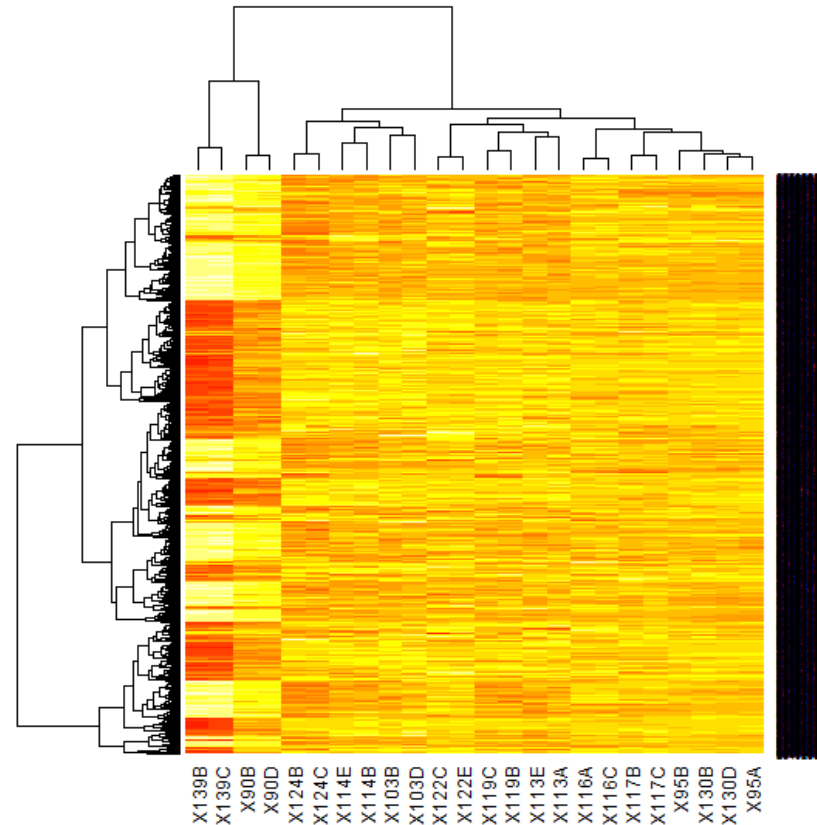
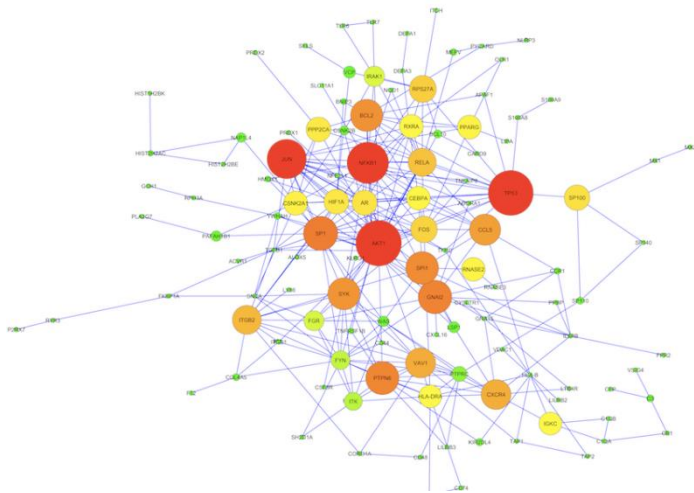
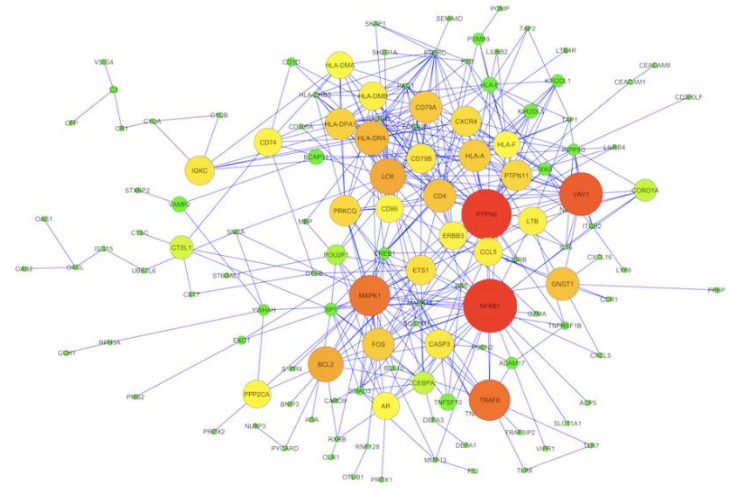


Figure 2b

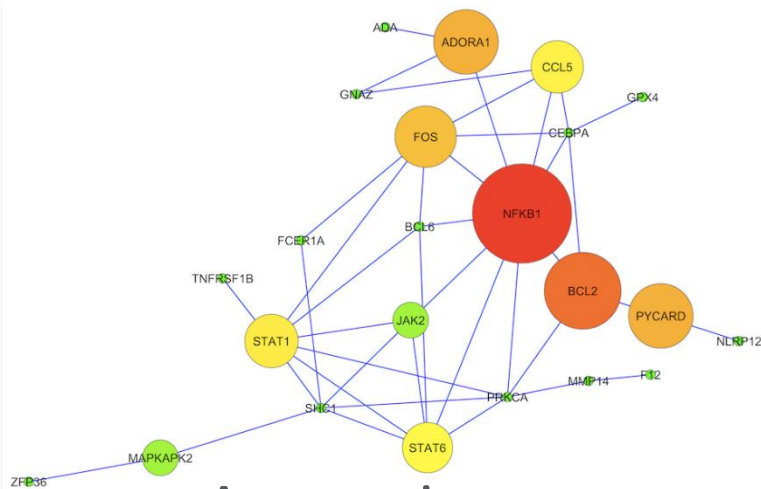
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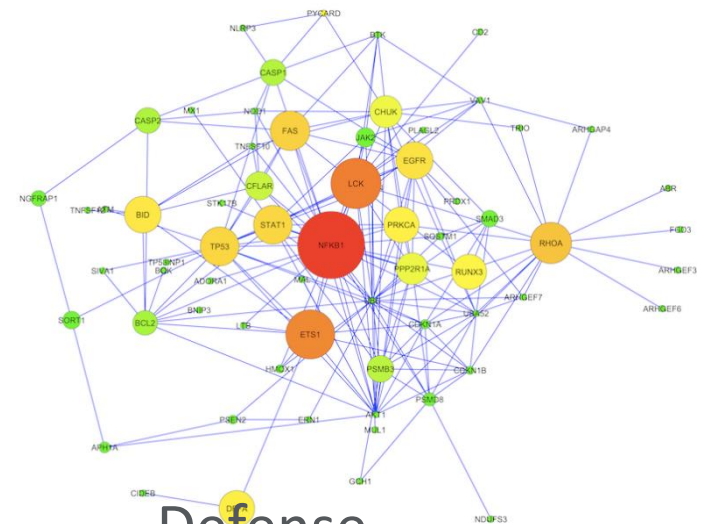
Inflammatory



Immune

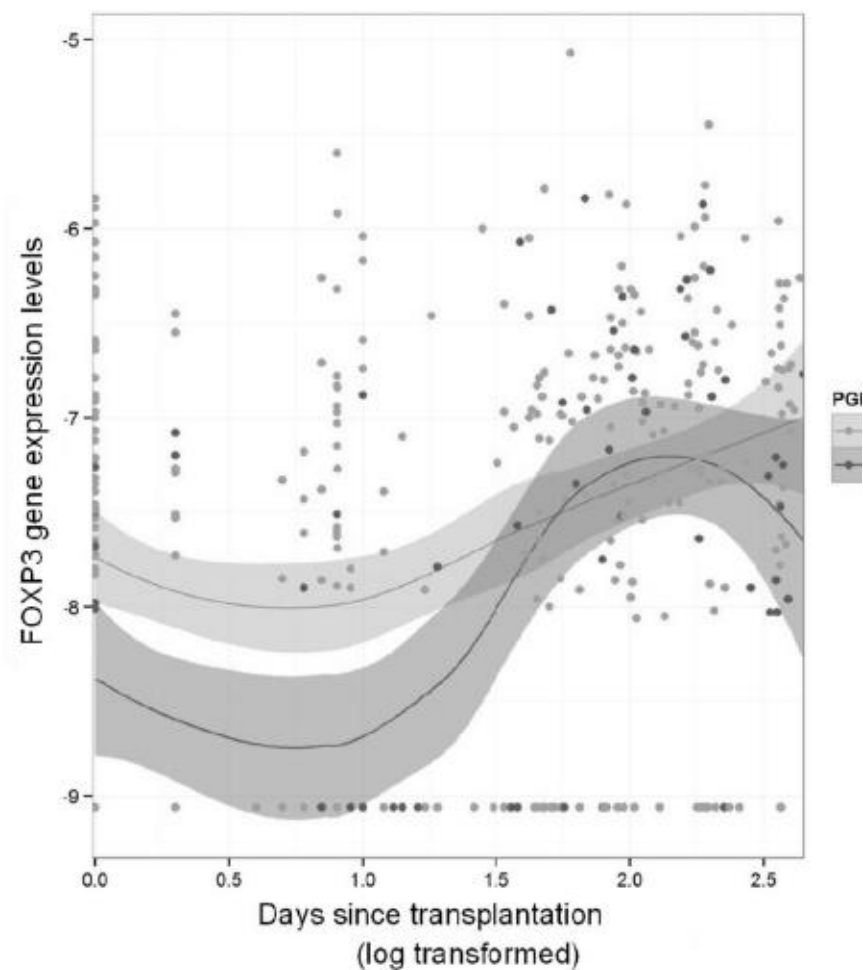
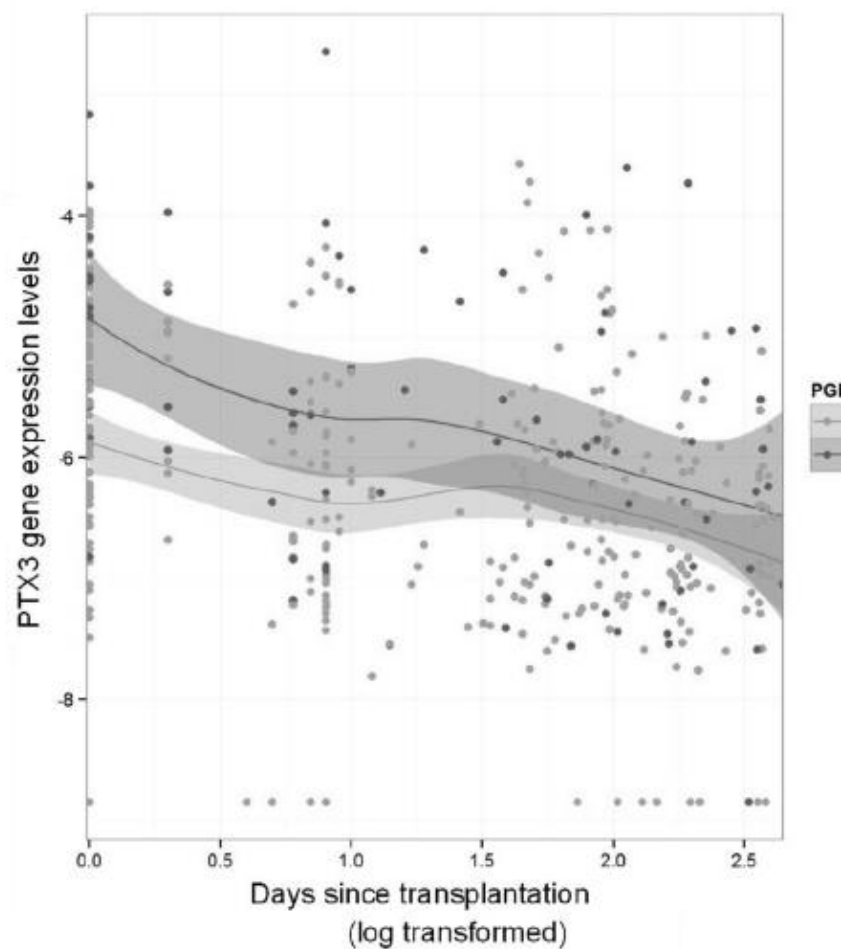


Apoptotic



Defense

Peripheral Gene expression in PGD after Lung Txp



Differences among Gene expression studies

Where	When	Why	How
Peripheral blood	Early	Predictiion	Appropriate BAL technique
BAL	Late	Risk stratification	Microdissection of biopsy lesions
Biopsy	Multiple	Mechanisms	
Brushings	Longitudinal	Phenotypes	
		Targeted str	

The promise of molecular profiling in lung transplantation

- Develop specific disease phenotypes/endotypes
- Risk stratification of patients
- Identify potential mechanisms with targeted therapeutic strategies