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

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

Danger R et al. Frontiers in Immunology 2018
Blood Gene Expression Predicts BOS

Danger R et al. Frontiers in Immunology 2018
Blood Gene Expression Predicts BOS

<table>
<thead>
<tr>
<th></th>
<th>POU2AF1</th>
<th>TCL1A</th>
<th>BLK</th>
<th>POU2AF1 + TCL1A + BLK</th>
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<tbody>
<tr>
<td>sensitivity</td>
<td>82%</td>
<td>73%</td>
<td>68%</td>
<td>73%</td>
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<tr>
<td>specificity</td>
<td>85%</td>
<td>92%</td>
<td>100%</td>
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<tr>
<td>ppv</td>
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<td>89%</td>
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<td>accuracy</td>
<td>83%</td>
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</tbody>
</table>

C

![Graphs showing BOS free survival](image)

**BOS free survival (%)**

- **POU2AF1 > 0.45** vs **POU2AF1 < 0.45**
  - *p* = 0.0027
- **TCL1A > 0.34** vs **TCL1A < 0.34**
  - *p* = 0.0006
- **BLK > 0.505** vs **BLK < 0.505**
  - *p* < 0.0001
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 (3 yrs)
- 8 stable patients up to 4 years
- BAL cell pellets at one year surveillance bronchoscopy
  - 55 dysregulated genes
  - 51 upregulated genes
  - 4 downregulated genes

Weigt S et al. PLOS One 2017
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 allore cognition by specific T cell signatures

Weigt S et al. PLOS One 2017
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

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

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.

CRM genes obtained from:
- Transbronchial brushings
- Lung tissue
- BAL
Common rejection model in Chronic Lung Rejection

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<th>no AR</th>
<th>AR</th>
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<tr>
<td>1.84</td>
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<td>1.44</td>
<td>34.81</td>
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<td>3.92</td>
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<tr>
<td>1.06</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>3.84</td>
<td>6.61</td>
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</tbody>
</table>

1.69 (1.35–1.95) 6.61 (4.26–7.04)

0.17 (0.12–0.30)  2.19 (1.51–2.79)

**
Common rejection model in Chronic Lung Rejection

A. Relative Expression

B. Score

- Tap1
- Cxcl9
- Cxcl10
- Isg20

11-gene Geometric Mean

- control
- BOS
- RAS
Sequential Gene Expression prior to CLAD

~1800 differentially expressed genes (FDR<1%)

LT 6mo BOS 2yr
12 samples 12 samples
CLAD-BOS patients

No differentially expressed genes (FDR<5%)

LT 6mo 2yr BOS
14 samples 14 samples
noCLAD-BOS patients

Figure 2a

Figure 2b
Inflammatory

Immune

Apoptotic

Defense
Peripheral Gene expression in PGD after Lung Txp

### Differences among Gene expression studies

<table>
<thead>
<tr>
<th>Where</th>
<th>When</th>
<th>Why</th>
<th>How</th>
</tr>
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<tbody>
<tr>
<td>Peripheral blood</td>
<td>Early</td>
<td>Prediction</td>
<td>Appropriate BAL technique</td>
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<tr>
<td>BAL</td>
<td>Late</td>
<td>Risk stratification</td>
<td>Microdissection of biopsy lesions</td>
</tr>
<tr>
<td>Biopsy</td>
<td>Multiple</td>
<td>Mechanisms</td>
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<tr>
<td>Brushings</td>
<td>Longitudinal</td>
<td>Phenotypes</td>
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</tbody>
</table>

**Note:**
- **Where:** Peripheral blood, BAL, Biopsy, Brushings
- **When:** Early, Late, Multiple, Longitudinal
- **Why:** Prediction, Risk stratification, Mechanisms, Phenotypes
- **How:** Appropriate BAL technique, Microdissection of biopsy lesions, 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