Translational Studies of Primary Graft Dysfunction

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Disclosure

No relevant commercial interests

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I have also received Institutional Grant Funding from GSK to study ARDS and critical illness, unrelated to the content of today’s lecture.
Two Patients

• Patient #1
  – 46 yo F PAH with progressive white out of allografts on POD#1
Two Patients

• Patient #1
  – 46 yo F PAH with progressive white out of allografts on POD#1
  – Never off vent, died on POD # 17 of MSOF
Two Patients

• Patient #2
  – 60 yo F COPD, bilateral
  – Hazy infiltrates, hypoxia, days 1 and 2
Two Patients

• Patient #2
  – 60 yo F COPD, bilateral
  – Hazy infiltrates, hypoxia, days 1 and 2
  – Extubated day #3, gets BOS at 26 months
Primary Graft Dysfunction

• Acute lung injury due largely to IRI
  – donor brain death/critical illness
  – organ explantation
  – storage
  – re-implantation

• Hypoxemia, lung edema, CXR infiltrates
• 10-25% of lung transplants
• ~50% of deaths in first 30 days after transplant
Outline

• PGD Definition
• Clinical Risk Factors
• Translational studies
• Precision Therapies
Why do we care about PGD definition

GOALS

• Identify donor and recipient risk factors
• Gain mechanistic inferences
• Predict PGD
• Define PGD subgroups “endotypes”
• Design therapies
Why do we care about PGD definition

GOALS

• Identify donor and recipient risk factors
• Gain mechanistic inferences
• Predict PGD
• Define PGD subgroups “endotypes”
• Design therapies

• What, exactly, are we studying?
ISHLT Working Group

Participants

Jackie ABRAMS  Patrick EVRARD  Octavio PAJARO
Vivek AHYA     Andy FISHER    Glenda PATTERSON
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Remzi BAG      Grisha GUENTHER Yaron SHARGALL
Mark BARR      Ramsey HACHEM  Arun SINGHAL
Robert BONSER  Marshall HERTZ  Josh SONETT
Heidi BOTTCHER  Steven KAWUT  Dirk VAN RAEMDONCK
Martin CARBY   Rosemary KELLY  Geert VERLEDEN
Stephen CASSIVI Shaf KESHAVJEE Wickii VIGNESWARAN
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Paul CORRIS    David McGIFFIN  Lorraine WARE
Peter DAHLBERG Rebecca MENZA   David WEILL
John DARK      Jonathan ORENS  Timothy WHELAN
Marc de PERROT Hyo Chae PAIK


# ISHLT GRADING

<table>
<thead>
<tr>
<th>GRADE</th>
<th>PaO2/FiO2</th>
<th>X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;300</td>
<td>NO Diffuse infiltrates</td>
</tr>
<tr>
<td>1</td>
<td>&gt;300</td>
<td>Diffuse infiltrates</td>
</tr>
<tr>
<td>2</td>
<td>200&lt;P/F&lt;300</td>
<td>Diffuse infiltrates</td>
</tr>
<tr>
<td>3</td>
<td>&lt;200</td>
<td>Diffuse infiltrates</td>
</tr>
</tbody>
</table>

Christie, JHLT 2005
TIME COURSE – “T” SCORE

• **T-zero (T0)**
  – within 6 hours of final lung reperfusion.

• **T24, T48, T72**
  – Later times measured at multiple time points 24 to 72 hours.
Figure 1. Cohort study: Bias as a function of sensitivity and specificity. Disease incidence (cumulative) in populations A and B, .10 and .05, respectively. True relative risk (of A to B) equals 2.0.
Validity of “PGD”

- No gold standard
- We and others have performed discriminant validity studies
  - Prediction of mortality
  - Concurrent markers of lung injury
Validation of the Proposed International Society for Heart and Lung Transplantation Grading System for Primary Graft Dysfunction After Lung Transplantation

Matthew E. Prckker, MD, D. S. Nath, MD, A. R. Walker, MD, A. C. Johnson, MD, M. I. Hertz, MD, C. S. Herrington, MD, D. M. Radosevich, PhD, and Peter S. Dahlberg, MD, PhD

Table 3. Ninety-day Mortality and Severity of Lung Dysfunction

<table>
<thead>
<tr>
<th>P/F grade</th>
<th>T0</th>
<th>T24</th>
<th>T48</th>
<th>T(0–48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>10%</td>
<td>8%</td>
<td>7%</td>
<td>10%</td>
</tr>
<tr>
<td>Grade 2</td>
<td>11%</td>
<td>7%</td>
<td>12%</td>
<td>8%</td>
</tr>
<tr>
<td>Grade 3</td>
<td>18%</td>
<td>28%</td>
<td>33%</td>
<td>17%</td>
</tr>
</tbody>
</table>

*p = 0.12  *p < 0.0001  *p < 0.0001  *p = 0.05

(b)

<table>
<thead>
<tr>
<th>Survival</th>
<th>P/F Grade 1</th>
<th>P/F Grade 2</th>
<th>P/F Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>162</td>
<td>109</td>
<td>133</td>
</tr>
<tr>
<td>0.8</td>
<td>48</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>0.6</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>0.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

p = 0.0007 Log Rank

JHHT, 2006
Whitson, JHLT, 2007
## Association with Mortality

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Risk in those with PGD</th>
<th>Risk in those without PGD</th>
<th>Risk Ratio (95%CI)</th>
<th>Risk Difference (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 day mortality</td>
<td>23%</td>
<td>5%</td>
<td>4.8 (3.3, 7.0)</td>
<td>18% (12, 24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 year mortality</td>
<td>34%</td>
<td>11%</td>
<td>3.0 (2.3, 3.9)</td>
<td>22% (15, 30)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Whitson, JHLT, 2007
Impact of Immediate Primary Lung Allograft Dysfunction on Bronchiolitis Obliterans Syndrome

Shiraz A. Daud¹, Roger D. Yusen¹, Bryan F. Meyers², Murali M. Chakinala¹, Michael J. Walter¹, Aviva A. Aloush², G. Alexander Patterson², Elbert P. Trulock¹, and Ramsey R. Hachem¹

![Graph showing freedom from BOS stage 1 over time after transplantation, days.](AJRCCM 2007)
MEAN PLASMA PAI-1 LEVEL BY PGD GRADE

Christie, JHLT 2010
MEAN PLASMA ICAM-1 LEVEL BY PGD GRADE

Plasma ICAM-1 Level (mg/dL)

STUDY TIME (hours)

* P<0.01

Christie, JHLT 2010
Normothermic Ex Vivo Lung Perfusion in Clinical Lung Transplantation

Marcelo Cypel, M.D., Jonathan C. Yeung, M.D., Mingyao Liu, M.D., Masaki Anraku, M.D., Fengshi Chen, M.D., Ph.D., Wojtek Karolak, M.D., Masaaki Sato, M.D., Ph.D., Jane Laratta, R.N., Sassan Azad, C.R.A., Mindy Madonik, C.C.P., Chung-Wai Chow, M.D., Cecilia Chaparro, M.D., Michael Hutcheon, M.D., Lianne G. Singer, M.D., Arthur S. Slutsky, M.D., Kazuhiro Yasufuku, M.D., Ph.D., Marc de Perrot, M.D., Andrew F. Pierre, M.D., Thomas K. Waddell, M.D., Ph.D., and Shaf Keshavjee, M.D.
Interim Conclusion on Definition

- ISHLT Definition seems to work
- Grade 3 seems bad and worth preventing
  - Moderate/Severe ARDS

- ISHLT update currently underway
  - Refine PGD definition
24 hour sRAGE and BOS

Log rank
p=0.003

Shah, AJT 2013
sRAGE with BOS - Cox model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sRAGE per 1 SD (n=107)</td>
<td>1.78 (1.16, 2.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3 PGD at 72 hours</td>
<td>1.99 (1.10, 3.61)</td>
<td>0.02</td>
</tr>
<tr>
<td>Any episode of ACR grade 2</td>
<td>1.80 (1.16, 2.80)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBP</td>
<td>2.12 (1.32, 3.38)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Recipient Diagnosis</td>
<td>1.98 (1.24, 3.16)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood (ml) during first 24 hrs</td>
<td>2.41 (1.44, 4.01)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transplant type</td>
<td>2.14 (1.35, 3.39)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Multivariable Model</strong></td>
<td>2.73 (1.41, 5.30)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*adjusted for PGD, ACR, CBP, diagnosis, PRBC, transplant type
Outline

• PGD Definition
• Clinical Risk Factors
• Translational studies
• Precision Therapies
<table>
<thead>
<tr>
<th>Category</th>
<th>Risk Factor for PGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor variables</td>
<td></td>
</tr>
<tr>
<td>(inherent):</td>
<td>Age &gt;45</td>
</tr>
<tr>
<td></td>
<td>Age &lt;21</td>
</tr>
<tr>
<td></td>
<td>African American race</td>
</tr>
<tr>
<td></td>
<td>Female gender</td>
</tr>
<tr>
<td></td>
<td>History of smoking &gt;10 pack-years</td>
</tr>
<tr>
<td>Donor variables</td>
<td>Prolonged mechanical ventilation</td>
</tr>
<tr>
<td>(acquired):</td>
<td>Aspiration</td>
</tr>
<tr>
<td></td>
<td>Trauma</td>
</tr>
<tr>
<td></td>
<td>Hemodynamic instability post–brain death</td>
</tr>
<tr>
<td>Recipient variables:</td>
<td>Diagnosis of idiopathic pulmonary arterial hypertension</td>
</tr>
<tr>
<td></td>
<td>Elevated pulmonary arterial pressure at time of surgery</td>
</tr>
<tr>
<td></td>
<td>Diagnosis of diffuse parenchymal lung disease</td>
</tr>
<tr>
<td>Operative variables:</td>
<td>Preservation solution and flush technique</td>
</tr>
<tr>
<td></td>
<td>Prolonged ischemic time</td>
</tr>
<tr>
<td></td>
<td>Use of cardiopulmonary bypass</td>
</tr>
<tr>
<td></td>
<td>Blood product transfusion</td>
</tr>
</tbody>
</table>
Lung Transplant Outcomes Group (LTOG)

• Initially formed in 2002
• Twelve centers throughout the U.S.
  • Penn, Columbia, UAB, Vanderbilt, JHU, Stanford, Michigan, Pittsburgh, Duke, UC, IUPUI, UCLA
  • Relational database with detailed donor, recipient, and perioperative phenotypes
• Over 2100 lung transplants to date
• Over 8000 buffy coats donor & recipient
• Over 24000 sequential plasma aliquots
Clinical Risk Factors Study Design

• PGD definition
  – Grade 3 PGD at 48 or 72 hours
  – Two independent readers

• Statistical Methods
  – Center as fixed effect in conditional logistic regression
  – Multiple imputation for missing data
  – Marginalized standardized risks based on final model

Diamond, AJRCCM 2013
Subject enrollment

Total transplants
n=2,011

Excluded: n=756
• Refused n=16
• Not Consented n=414
• Consented but not enrolled due to logistics n=326

Enrolled
n=1,255

PGD
n=211

Non-PGD
n=1,044

Diamond, AJRCCM 2013
## Multivariable Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR for PGD</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Lung Transplant</td>
<td>2.0</td>
<td>1.2, 3.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Cardiopulmonary Bypass Use</td>
<td>3.4</td>
<td>2.2, 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recipient gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female without prior pregnancy</td>
<td>0.9</td>
<td>0.4, 2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Female with one prior pregnancy</td>
<td>0.6</td>
<td>0.2, 1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Female with 2 or greater pregnancies</td>
<td>1.3</td>
<td>0.9, 2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Recipient BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>1.3</td>
<td>0.6, 2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>25-30</td>
<td>1.8</td>
<td>1.2, 2.7</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;30</td>
<td>2.3</td>
<td>1.3, 3.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Total ischemic time per hour</td>
<td>1.1</td>
<td>1.0, 1.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPF</td>
<td>1.2</td>
<td>0.8, 1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>CF</td>
<td>0.7</td>
<td>0.3, 1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>2.5</td>
<td>1.1, 5.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Pulmonary Arterial Hypertension</td>
<td>3.5</td>
<td>1.6, 7.7</td>
<td>0.002</td>
</tr>
<tr>
<td>PRBC transfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>up to 1L</td>
<td>1.1</td>
<td>0.7, 1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>&gt;1L</td>
<td>1.9</td>
<td>1.1, 3.2</td>
<td>0.01</td>
</tr>
<tr>
<td>mPAP per 10 mmHg</td>
<td>1.3</td>
<td>1.1, 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reperfusion FiO₂ per 10% increase</td>
<td>1.1</td>
<td>1.0, 1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Any donor smoking</td>
<td>1.8</td>
<td>1.2, 2.6</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Diamond, AJRCCM 2013
Standardized PGD Risk

- Any Donor Smoking
- Donor Smoking >20 pack-years
- Reperfusion FiO₂
- Recipient BMI
- Pulmonary Hypertension
- Cardiopulmonary Bypass Use
- PRBC Transfusion Volume
- Gender and Parity: Female with 1 Pregnancy, Female with 2+ Pregnancies, Female with No Pregnancy, Male

Diamond, AJRCCM 2013
Next Steps: Clinical Risk Factors

• Build a predictive model for PGD
  – Selectively match donors and recipients
    • Rupal Shah, ATS 2013

• Ask Why?
  – Evaluate potential mechanisms
  – Lead to therapies
Predictive Model Objectives

• Develop a simple, clinically useful tool using recipient and donor variables to predict PGD

• Validate the tool in a separate cohort
Study Population

• Derivation cohort
  – 1255 subjects
  – Lung Transplant Outcomes Group (LTOG)
    • 10 centers, transplanted between 2002-2010
    • Multiple imputation for missing data

• Validation cohort
  – 372 subjects from LTOG
    • 11 centers
    • Transplanted between 2011-2012
    • Complete case analysis
Prediction Model-Derivation

• Bootstrap resampling for variable inclusion
• Logistic regression for standardized risks

• Significant factors:
  – Recipient BMI (WHO Class)
  – Pre-transplant diagnosis
  – Mean PA pressure (WHO Class)
  – Donor smoking (yes/no)
Results – Recipient Risk Stratification

• Low risk (24%)
  – BMI 18-25 and
  – COPD/CF and
  – mPAP < 40

• Higher risk (76%)
  – Overweight or obese (BMI > 25) or
  – non-COPD/CF or
  – mPAP > 40
Results - Prediction Model

Derivation n=1255

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>n</th>
<th>Predicted risk of PGD</th>
<th>Validation n=372</th>
<th>Observed risk of PGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>315</td>
<td>6% (2, 10%)</td>
<td>81</td>
<td>6% (0.3, 13%)</td>
</tr>
<tr>
<td>Low risk + Smoking Donor</td>
<td>128</td>
<td>9% (4, 15%)</td>
<td>20</td>
<td>15% (0, 31%)</td>
</tr>
<tr>
<td>Higher risk</td>
<td>940</td>
<td>16% (13, 19%)</td>
<td>291</td>
<td>13% (8, 17%)</td>
</tr>
<tr>
<td>Higher risk + Smoking Donor</td>
<td>356</td>
<td>26% (21, 31%)</td>
<td>66</td>
<td>29% (19, 40%)</td>
</tr>
</tbody>
</table>
Interim Conclusions about prediction

• Predictors for PGD include:
  – Overweight or obese,
  – Diagnoses other than COPD or CF
  – Moderate-severe pulmonary hypertension

• Adding a donor with smoke exposure significantly increased the risk of PGD in the higher-risk group

• This is a simple, clinically useful tool may be helpful in predicting PGD
  – Base for improvement
Outline

• PGD Definition
• Clinical Risk Factors
• Translational studies
• Precision Therapies
Why PH?

- Hemodynamic
  - Shearing forces on endothelium → more ROS
  - RV/LV relationship → pulmonary edema
- use of CPB and/or blood products
- Shared recipient mechanism
  - Autoimmune, platelet, coagulation/inflammation
    - Steve Kawut/Josh Diamond
Why Obesity?

• Adipose tissue “inflammation”
• Immune activation
• LTBC study (Lederer, Singer)
  – Sampling lung, ITAT during the surgery
    • ATM functional profile
    • Adipose lymphocyte function
  – Imaging for adiposity
  – Systemic markers of adipose inflammation
    • Leptin, resistin, adiponectin
Why PRBCs?

- PRBCs
  - Confounding by indication
  - Augmentation of inflammation
    - RBC degradation products as DAMPs
      - AGEs
    - Heme
  - Nilam Mangalmurti
    - AGEs, HMGB1, and endothelial RAGE
      - Christie, AJRCCM 2011
Why FiO2?

• Confounding by indication
• Worse oxidative burst with reperfusion
• Measuring ROS markers, genes
  – Lorraine Ware, ISHLT 2014
  – Higher isoprostanes in PGD and high FiO2
  – Now also linking with genetics
    • NRF-2 (Cantu, JTCVS 2014)
Standardized PGD Risk

- Any Donor Smoking
  - Donor Smoking >20 pack-years
- Reperfusion FiO₂
- Recipient BMI
  - <18.5
  - 18.5-25
  - 25-30
  - >30
- Pulmonary Hypertension
  - Normal
  - Mild
  - Moderate
  - Severe
- Cardiopulmonary Bypass Use
- PRBC Transfusion Volume
  - None
  - <1 L
  - >1 L
- Gender and Parity
  - Female with 1 Pregnancy
  - Female with 2+ Pregnancies
  - Female with No Pregnancy
  - Male

Diamond, AJRCCM 2013
Why not just exclude Smokers?

Bonser, Lancet 2012
Why not just exclude Smokers?

Bonser, Lancet 2012
Why not just exclude Smokers?

• A strategy of excluding lungs from donors with smoke exposure leads to greater wait list mortality

• HL087115 goals:
  – Better quantify smoke exposure
  – Understand the mechanisms of PGD risk
How do we measure donor smoke exposure?
How do we measure donor smoke exposure?

• Ask proxies
How do we measure donor smoke exposure?

• Ask proxies

• Much missing data

• Not very accurate
  – Passive smoke exposure
  – Time since exposure
Smoke exposure biomarkers?

• Cotinine
  – 14 hour half life

• 3-HC
  – Different metabolizers

• NNAL
  – 14 day half life
  – Calfee measured in critically ill
Distribution of NNAL by proxy donor smoking

P=0.01

NNAL (pg/mg Cr)

Non-smokers

Smokers
Smoking Mechanisms?

- Literature is protean
- Relied on our prelim data
Nested case control study (N=106)

Donor lung before procurement

1 hour after reperfusion in recipient

BAL Fluid Gene Expression

23 PGD Cases

23 Matched Controls

Matched on
• Donor age
• Recipient diagnosis

Cantu, AJT 2013
Methods

• Genes ranked to reflect greatest changes to post-implantation from donor using log transformation

• The resulting ranked gene list was tested for networks of gene sets using GSEA
## Results

<table>
<thead>
<tr>
<th>Gene Sets (Pathway)</th>
<th>Source</th>
<th>NOM p-val</th>
<th>FWER p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD-like receptor signaling</td>
<td>KEGG</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TOLL-like receptor signaling</td>
<td>KEGG</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL1R</td>
<td>BIOCARTA</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>MYD88 cascade</td>
<td>REACTOME</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>NTHI</td>
<td>BIOCARTA</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Activated TLR4 signaling</td>
<td>REACTOME</td>
<td>&lt;0.001</td>
<td>0.008</td>
</tr>
<tr>
<td>TLR9 cascade</td>
<td>REACTOME</td>
<td>&lt;0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>TOLL</td>
<td>BIOCARTA</td>
<td>&lt;0.001</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Cantu, AJT 2013
DAMPs

PAMPs

Haemopoietic or resident cell

NALP

Pro-IL-1β

IL-1β

?- ?

ASC

Casp1

NBD

PYD

CARD

TIR

TLR2

TLR4

IL-1Ra

IL-1β

IL-1R1

MyD88

NF-κB activation

IL-6, TARC, pro-IL-1β

MMPs, TIMP-1

IL-1β

KC

Neutrophils

Lymphocytes

Collagen deposition

Fibroblast proliferation

Resident cell: epithelial cell or fibroblast

vi Inflammation

vii Pulmonary fibrosis

Gasse, AJRCCM. 179. p.903–913, 2009
Smoke exposure and Donor BAL

Donor lung before procurement

- Current Smokers
- Non-smokers
## Smoke exposure and Donor BAL

### VIRAL mRNA TRANSLATION

<table>
<thead>
<tr>
<th>mRNA transcript</th>
<th>Rank metric score</th>
</tr>
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<tbody>
<tr>
<td>RPS25</td>
<td>2.421</td>
</tr>
<tr>
<td>RPL41</td>
<td>2.386</td>
</tr>
<tr>
<td>RPL4</td>
<td>2.269</td>
</tr>
<tr>
<td>RPS3A</td>
<td>1.789</td>
</tr>
<tr>
<td>RPL27A</td>
<td>1.696</td>
</tr>
<tr>
<td>RPL18A</td>
<td>1.638</td>
</tr>
<tr>
<td>RPS18</td>
<td>1.456</td>
</tr>
<tr>
<td>RPS27</td>
<td>1.403</td>
</tr>
<tr>
<td>RPS27A</td>
<td>1.402</td>
</tr>
<tr>
<td>RPL10</td>
<td>1.342</td>
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### CYTOKINE PATHWAY

<table>
<thead>
<tr>
<th>mRNA transcript</th>
<th>Rank metric score</th>
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<tbody>
<tr>
<td>IL12B</td>
<td>3.133</td>
</tr>
<tr>
<td>IL6</td>
<td>2.866</td>
</tr>
<tr>
<td>IL15</td>
<td>2.511</td>
</tr>
<tr>
<td>IL5</td>
<td>2.326</td>
</tr>
<tr>
<td>IL9</td>
<td>2.237</td>
</tr>
<tr>
<td>IL2</td>
<td>1.698</td>
</tr>
<tr>
<td>IL18</td>
<td>1.665</td>
</tr>
<tr>
<td>IL4</td>
<td>1.333</td>
</tr>
<tr>
<td>IL1A</td>
<td>1.030</td>
</tr>
<tr>
<td>IL17A</td>
<td>0.924</td>
</tr>
</tbody>
</table>
Why?

• Innate immunity, inflammatory cytokines
• Inherent to donor lung
• Epithelial barrier innate immunity
• Interplay with pathogens and DAMPs
Innate Lymphoid Cells

Adaptive Immunity
Helper T cell subsets

- TH1
- TH2
- TH17
- TH22
- Treg

IFN-γ
IL-13, IL-4
IL-6, IL-5
IL-17, IL-22
TGF-β, IL-10
IL-35

Innate Immunity
Innate lymphocyte subsets

- ILC1
  Natural killer (NK)

- ILC2
  Nuocytes, Natural helper

- ILC17
  Lymphoid tissue inducer (LTi)

- ILC22
  NKp46+ ILC

Rankin, Frontiers Immunology, 2013
Innate Lymphoid Cells in Lung Injury

Wills-Karp, Nature Immunology 2011
Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus

Laurel A Monticelli\textsuperscript{1,2}, Gregory F Sonnenberg\textsuperscript{1,2}, Michael C Abt\textsuperscript{1,2}, Theresa Alenghat\textsuperscript{1,2}, Carly G K Ziegler\textsuperscript{1}, Travis A Doering\textsuperscript{1}, Jill M Angelosanto\textsuperscript{1}, Brian J Laidlaw\textsuperscript{1}, Cliff Y Yang\textsuperscript{3}, Taheri Sathaliyawala\textsuperscript{4}, Masaru Kubota\textsuperscript{4}, Damian Turner\textsuperscript{4}, Joshua M Diamond\textsuperscript{5}, Ananda W Goldrath\textsuperscript{3}, Donna L Farber\textsuperscript{4}, Ronald G Collman\textsuperscript{5}, E John Wherry\textsuperscript{1} & David Artis\textsuperscript{1,2}
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Laurel A Monticelli, Gregory F Sonnenberg, Michael C Abt, Theresa Alenghat, Carly G K Ziegler, Travis A Doering, Jill M Angelosanto, Brian J Laidlaw, Cliff Y Yang, Taheri Sathaliyawala, Masaru Kubota, Damian Turner, Joshua M Diamond, Ananda W Goldrath, Donna L Farber, Ronald G Collman, E John Wherry & David Artis

Lineage negative gating strategy

Nature Immunology, 2011
Lung ILCs in Donors

Pre-procurement | Post-reperfusion

Non-smoker

CRTH2

0.5

1.69

0.8

5.1

CD127

0.2

0.9

1.3

6.9

Smoker

Gated on live, CD45^{pos}, Lineage^{neg} cells
Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers

Emily S. Charlson\textsuperscript{1,3}, Jun Chen\textsuperscript{2}, Rebecca Custers-Allen\textsuperscript{1}, Kyle Bittinger\textsuperscript{1}, Hongzhe Li\textsuperscript{2}, Rohini Sinha\textsuperscript{1}, Jennifer Hwang\textsuperscript{1}, Frederic D. Bushman\textsuperscript{1\*}, Ronald G. Collman\textsuperscript{1,3\*}

PloS One, 2010
Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers

Emily S. Charlson¹,³, Jun Chen², Rebecca Custers-Alen¹, Kyle Bittinger¹, Hongzhe Li², Rohini Sinha¹, Jennifer Hwang¹, Frederic D. Bushman¹*, Ronald G. Collman¹,³*

PloS One , 2010

• aberrant bacterial populations in URT
  – looser community structures
  – more gram negative and anaerobic bacteria,
    • actinobacteria, bacteroidetes, fusobacteria

• Most Lung Donors intubated >48 hours
LRT Bacteria increase with 48 hrs
DONOR SMOKE EXPOSURE

PGD
DONOR SMOKE EXPOSURE

RESIDENT IMMUNE CELL CHANGES

PGD
DONOR SMOKE EXPOSURE

ALTERED MICROBIOME

RESIDENT IMMUNE CELL CHANGES

PGD
DONOR SMOKE EXPOSURE

ALTED MICROBIOME

RESIDENT IMMUNE CELL CHANGES

PGD
Outline

• PGD Definition
• Clinical Risk Factors
• Translational studies
• Precision Therapies
Assessment
Antimicrobials
ILC2 (IL33R)
NLRP3
Inflammasome
## Pharmacogenomic loci

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Function</th>
<th>Potential Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTX3</td>
<td>AJRCCM 2013</td>
<td>Innate Immunity</td>
<td>development</td>
</tr>
<tr>
<td>TLR4</td>
<td>ATS 2013</td>
<td>Innate Immunity</td>
<td>TLR4 antagonist</td>
</tr>
<tr>
<td>ANGPT2</td>
<td>AJRCCM 2011</td>
<td>Vascular permeability</td>
<td>PEG-TIE</td>
</tr>
<tr>
<td>PTGER4</td>
<td>AJRCCM 2014</td>
<td>Treg function</td>
<td>PGE2</td>
</tr>
<tr>
<td>IL1RN</td>
<td>AJRCCM 2013</td>
<td>Inflammation</td>
<td>hrIL1RA</td>
</tr>
</tbody>
</table>
Individual Lung D-R pairs by inferred ancestry
Freedom from BOS, %

TIME, DAYS

"NEAR" G.D.

"FAR" G.D.

P = 0.033
Acknowledgements

• Lung Transplant Outcomes Group
  – Selim Arcasoy, M.D.
  – Jonathan Belperio, M.D.
  – Sangeeta Bhorade, M.D.
  – Jason Christie, M.D., M.S.
  – Maria Crespo, M.D.
  – Ejigayehu Demissie, M.S.N.
  – Steven Kawut, M.D., M.S
  – Benjamin Kohl, M.D.
  – Vibha N. Lama, M.D., M.S.
  – David Lederer, M.D., M.S.
  – Jonathan Orens, M.D.
  – Lorraine B. Ware, M.D
  – Scott Palmer, M.D., M.H.S
  – John Reynolds, M.D.
  – Ashish Shah, M.D.
  – Rupal Shah, M.D.
  – Joshua Sonett, M.D.
  – Pali D. Shah, M.D.
  – David Weill, M.D.
  – Ann Weinacker, M.D.
  – Keith Wille, M.D.
  – David Wilkes, M.D.

• HL081619, HL087115, HL096845, HL115354, HL114626
• NIAID CTOT – Meyer, Lederer

• Penn Lung Transplant Team
## Pharmacogenomic loci

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</tr>
<tr>
<td>IL1RN</td>
<td>AJRCCM 2013</td>
<td>Inflammation</td>
<td>hrIL1RA</td>
</tr>
</tbody>
</table>
Long Pentraxin 3 (PTX3)

Innate Immunity → Adoptive Immunity

Microbial components
LPS, Omp A, PGN, Poly I:C, CpG ODN, Candidia, Flagellin

TLRs → MyD88

Antigen presenting

PTX3

Naïve T Cell

Costimulatory molecules

IL-12, IL-18

IL-10, IL-4

IL-13

Th2

Th1

TNFα, IL-1, IFNγ, L-12
PTX3 and PGD

![Graph showing changes in PTX3 concentration over time]

- Pre-Transplant
- 6h Post-Transplant
- 24h Post-Transplant

Diamond, AJT 2011
## Genetic Association with PGD

<table>
<thead>
<tr>
<th>rs number</th>
<th>Risk Allele</th>
<th>MAF PGD</th>
<th>MAF Non-PGD</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9289983</td>
<td>A</td>
<td>0.42</td>
<td>0.51</td>
<td>0.8 (0.6, 1.0)</td>
<td>0.03</td>
<td>5’</td>
</tr>
<tr>
<td>rs1456099</td>
<td>T</td>
<td>0.44</td>
<td>0.52</td>
<td>0.8 (0.6, 1.0)</td>
<td>0.03</td>
<td>5’</td>
</tr>
<tr>
<td>rs2120243</td>
<td>A</td>
<td>0.47</td>
<td>0.40</td>
<td>1.5 (1.1, 1.9)</td>
<td>0.004</td>
<td>5’</td>
</tr>
<tr>
<td>rs2305619</td>
<td>T</td>
<td>0.53</td>
<td>0.45</td>
<td>1.4 (1.1, 1.9)</td>
<td>0.007</td>
<td>Intron</td>
</tr>
<tr>
<td>rs3816527</td>
<td>G</td>
<td>0.43</td>
<td>0.40</td>
<td>1.3 (1.0, 1.7)</td>
<td>0.08</td>
<td>Coding</td>
</tr>
<tr>
<td>rs3845978</td>
<td>T</td>
<td>0.06</td>
<td>0.06</td>
<td>0.7 (0.4, 1.3)</td>
<td>0.3</td>
<td>Intron</td>
</tr>
</tbody>
</table>

Diamond, AJRCCM 2013
24 hours post-transplant PTX3 level vs. rs2305619 genotype

PTX3 concentration (ng/ml)

C/C

C/T

T/T

p = 0.047
PGD survivors have higher PTX3 mRNA
Genetic PTX3 Deficiency and Aspergillosis in Stem-Cell Transplantation

Cristina Cunha, Ph.D., Franco Aversa, M.D., João F. Lacerda, M.D., Ph.D., Alessandro Busca, M.D., Oliver Kurzai, M.D., Matthias Grube, M.D., Jürgen Löffler, Ph.D., Johan A. Maertens, M.D., Ph.D., Alain S. Bell, Ph.D., Antonio Inforzato, Ph.D., Elisa Barbati, Ph.D., Bruno Almeida, Ph.D., Pedro Santos e Sousa, M.D., Anna Barbui, M.D., Leonardo Potenza, M.D., Ph.D., Morena Caira, M.D., Ph.D., Fernando Rodrigues, Ph.D., Giovanni Salvatori, Ph.D., Livio Pagano, M.D., Mario Luppi, M.D., Ph.D., Alberto Mantovani, M.D., Andrea Velardi, M.D., Luigina Romani, M.D., Ph.D., and Agostinho Carvalho, Ph.D.
Different patterns

T0

T72
Different patterns

T0

T72
PGD Grade 3 phenotypes

Figure 1a. Class 1 (55%)

Estimated PGD probability

- PGD (grade 3)
- Equivocal (grade 2, 1)
- No PGD (grade 0)

Study Day

Shah, Chest 2013
Kaplan-Meier survival estimates

Hazard of Death

Analysis Time (Days)

Class 1
Class 2
Class 3

p<0.001

Shah, Chest 2013
<table>
<thead>
<tr>
<th></th>
<th>Class 1 (n=197)</th>
<th>Class 2 (n=25)</th>
<th>Class 3 (n=139)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient Age</td>
<td>52.9±12.3</td>
<td>51.5±14.6</td>
<td>53±12.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Native Disease</td>
<td></td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Prior Pregnancy</td>
<td>73 (79%)</td>
<td>6 (60%)</td>
<td>41 (67%)</td>
<td>0.15</td>
</tr>
<tr>
<td>BMI Recipient</td>
<td>26.8±5.5</td>
<td>24.6±5.0</td>
<td>26.1±4.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Female Donor</td>
<td>91 (46%)</td>
<td>14 (56%)</td>
<td>56 (41%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Donor Age</td>
<td>35.6±14.6</td>
<td>27.8±10.4</td>
<td>35.7±13.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Donor Race</td>
<td></td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Donor Smoking</td>
<td>100 (51%)</td>
<td>6 (24%)</td>
<td>61 (44%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Donor Mode of Death</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Bilateral Transplant</td>
<td>135 (69%)</td>
<td>15 (60%)</td>
<td>91 (66%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Cardiopulmonary Bypass</td>
<td>120 (61%)</td>
<td>10 (40%)</td>
<td>64 (46%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Intra-Op Crystalloids (mL)</td>
<td>1056.3±1593</td>
<td>1550±2114</td>
<td>1547.8±2083.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Tidal Volume (mL/kg)</td>
<td>7.0±3.2</td>
<td>8.0±2.6</td>
<td>7.0±2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>PRBC (mL)</td>
<td>1142.3±1547</td>
<td>694±9831</td>
<td>914.1±1255.5</td>
<td>0.003</td>
</tr>
<tr>
<td>PASP (mmHg)</td>
<td>51.6±24.1</td>
<td>43.5±20.7</td>
<td>43.2±17.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Shah, Chest 2013, in press
sRAGE

• Indicates innate immune receptor activation
• May interact with AGE in blood products
  – other DAMPs and PAMPs
• Highly expressed on Type I pneumocytes
  – Epithelial injury marker in PGD
• We previously showed high sRAGE during PGD
24 hour sRAGE and BOS

Log rank
p=0.003

Shah, AJT 2013
### sRAGE with BOS - Cox model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sRAGE per 1 SD (n=107)</strong></td>
<td>1.78 (1.16, 2.71)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Adjusted for</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3 PGD at 72 hours</td>
<td>1.99 (1.10, 3.61)</td>
<td>0.02</td>
</tr>
<tr>
<td>Any episode of ACR grade 2</td>
<td>1.80 (1.16, 2.80)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBP</td>
<td>2.12 (1.32, 3.38)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Recipient Diagnosis</td>
<td>1.98 (1.24, 3.16)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood (ml) during first 24 hrs</td>
<td>2.41 (1.44, 4.01)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transplant type</td>
<td>2.14 (1.35, 3.39)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Multivariable Model</strong>*</td>
<td>2.73 (1.41, 5.30)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*adjusted for PGD, ACR, CBP, diagnosis, PRBC, transplant type

Shah, AJT 2013