Molecular Signature in the Peripheral Blood Detects Sub-Clinical Acute Kidney Rejection

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DISCLOSURES
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I have relevant financial relationship(s) with respect to the content of this presentation/session as follows:
1. Transplant Genomics Inc (consulting, equity); SK, JL, MA and DS have similar disclosures.
2. Pfizer (grant support)
3. Sanofi (non-branded CME development)
Background

- Sub-clinical acute rejection (SCAR) is defined as histologic acute cellular rejection in the presence of a normal/stable serum creatinine, and is associated with worse long term graft survival\(^1\)

- Creatinine is a lagging indicator of renal injury

![Stages of chronic kidney disease](image)

Cleveland Clinic Journal of Medicine 2011;78:189-195

- Currently, protocol surveillance kidney biopsies are performed at some centers to detect SCAR, with variable frequency and for variable periods of time post transplant

\(^1\)American Journal of Transplantation, Volume 13, Issue 9, pages 2334–2341, September 2013
Background

• Our group recently published a peripheral blood gene expression profile that can distinguish kidney recipients with normal function and biopsy (TX) from clinical acute rejection (AR) and from acute dysfunction but no rejection (ADNR)\(^1\).

Figure 2: Performance of the 200-probeset nearest centroids (NC) classifier discovered and locked in the discovery cohort tested on the validation cohort based on area under the curve (AUC).

Study Objectives

- To determine whether a gene expression profile exists in the peripheral blood of patients with acute cellular rejection on a surveillance protocol biopsy (SCAR – normal/stable creatinine) that is different from that of patients with normal protocol surveillance biopsies (TX – normal creatinine).

- To determine whether this gene expression profile (SCAR) differs from a previously validated peripheral blood profile/signature for patients with clinical acute cellular rejection (AR - elevated creatinine) found on a “for cause” biopsy.
Methods

• Single center, prospective trial at the Comprehensive Transplant Center at Northwestern Medicine
• Approved by Northwestern University’s IRB
• At the time of either “for cause” or protocol biopsy, peripheral blood was obtained in PAX gene tubes
• The patients were precisely phenotyped by: a) histology read by 2 blinded pathologists using Banff criteria; b) histologic diagnosis reconciled with laboratory data and clinical course by 2 clinicians prior to biomarker analyses.
Patient Demographics

• 69 Patients were phenotyped: SCAR (23 – 16 “Borderline” and 7 Banff 1A); AR (21); TX (25)

• Demographics: Mean age 49.3 yrs (range 22-71); 35% female; 52% from deceased donors

• Time to Biopsy (days post transplant)

<table>
<thead>
<tr>
<th></th>
<th>SCAR</th>
<th>AR</th>
<th>TX</th>
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<tbody>
<tr>
<td>Mean</td>
<td>287.6</td>
<td>921.2</td>
<td>267.8</td>
</tr>
<tr>
<td>Range</td>
<td>8-748</td>
<td>15-2876</td>
<td>84-2228</td>
</tr>
</tbody>
</table>
Methods

• Peripheral blood samples were profiled using Affymetrix HG-U133 PM Peg microarrays
• 3-way ANOVA analysis was performed between the 3 Groups (SCAR, AR, and TX)
Results

• Using a False Discovery Rate (FDR) cut-off of <10% we detected over 2,500 significantly differentially expressed probesets.

• For the diagnostic signature, we used only the top 200 differentially expressed probesets as ranked by p values.

• These 200 top probesets have FDR values of <0.05%.
**Results**

**Blood expression profiling: 3-way classifier (SCAR, AR, TX)**

- We used 3 different predictive algorithms: Nearest Centroid (NC), Support Vector Machines (SVM) and Diagonal Linear Discriminant Analysis (DLDA) to build predictive models.

- NC, SVM and DLDA picked classifier sets of 188, 192 and 200 probesets as the best classifiers, respectively.

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Predictors</th>
<th>Comparison</th>
<th>AUC</th>
<th>Predictive Accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tbody>
<tr>
<td>NC</td>
<td>200</td>
<td>SCAR vs. TX</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>SCAR vs. AR</td>
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<tr>
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<td>AR vs. TX</td>
<td>0.932</td>
<td>93</td>
<td>96</td>
<td>90</td>
<td>92</td>
<td>95</td>
</tr>
</tbody>
</table>
Results

**Blood expression profiling : 2 way classifier (SCAR, TX)**

- We also tested a 2-way prediction of SCAR vs. TX to further validate that a phenotype as potentially subtle clinically as SCAR can be truly distinguished from TX.

- At a p-value <0.001, there were 33 probesets whose expression signals highly differentiated SCAR and TX, a result in marked contrast with the >2500 probesets differentially expressed between AR vs. TX at that same p-value.
SCAR Probesets

SMPO3
RLBP1
TAC4
TRO
C10f1182
TMEM221
ZNF627
SPATA6
...
C16o140
LOC729296
PLIN5
MCTP2
LOC540090
CDH11
...
ERWWE1
RAPGEF5
SHAP2
MBL1P
TUSC3
ARHGAP44
TA52R5
...
ZNF775
DARS2
LOC442421 L
TAF8
SLC26A2
C3orf31
USP37
ZNF663
WHSC1
Summary

• We have discovered a gene expression profile in the peripheral blood of patients with acute cellular rejection on a surveillance protocol biopsy (SCAR) that is different from that of patients with a normal protocol surveillance biopsies (TX – normal creatinine).

• This gene expression profile (SCAR) differs from a previously validated peripheral blood profile/signature for patients with clinical acute cellular rejection (AR - elevated creatinine) found on a “for cause” biopsy.
Conclusions and Next Steps

• Peripheral blood gene expression profiling may prove to be a useful, minimally invasive method for monitoring kidney transplant recipients.
  – Informing when to do a biopsy in a patient with normal renal function, and/or
  – Replacing surveillance protocol biopsies
  – Less invasive
  – Can be done more frequently than kidney biopsies
Conclusions and Next Steps

• We are currently validating this SCAR signature in the multi-center NIH-funded (CTOT-08) study.
  – External validation with SCAR samples from CTOT-08; multi-center, variable IS, etc.
  – What happens to this signature after treatment of SCAR? Can the signature be used to monitor treatment of SCAR? Does resolution of SCAR signature result in better long-term outcome?
  – Comparison with peripheral blood gene expression profiles that precede AR – are these similar to SCAR?
Thank You

Questions?