Biomarkers to assess risk and guide immunosuppression in kidney transplantation

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Disclosure

Faculty: Peter Heeger

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Learning Objectives

To differentiate a biomarker from a surrogate endpoint

To explain the clinical utility of urinary CXCL9 testing among other biomarkers to diagnose kidney transplant rejection during CNI withdrawal

To explain the utility of pretransplant biomarkers as risk assessment tools for guiding CNI withdrawal post transplant
Definitions

• **Biomarkers** are anatomic, physiologic, biochemical, or molecular parameters that indicate, or are associated with an alteration in physiology and are of clinical significance (this doesn’t necessarily mean they are clinically useful)

• **Surrogate Markers** can be defined as biomarkers that have established clinical utility

• **Surrogate Endpoints** are biomarkers used (in clinical trials) to evaluate the safety or effectiveness of a therapy and serve as alternatives to traditional endpoints.
The need for biomarkers in transplantation

Pre-transplant Risk assessment
HLA typing
Cross matching
Implantation biopsy
Clinical risk factors

Induction
ATG
Alemtuzumab
Anti-IL2R

CNI/belatacept
anti-prolif agent
+/-steroids

Level of immunosuppression

Transplant Day 0
6 months
24 months

Post-transplant monitoring
Serum creatinine

Long term outcomes are not optimal
Protocolized treatment
Biomarkers - potential uses

• Surrogate endpoints for clinical trials
• Risk assessment for post transplant outcomes
  – who is most likely to do badly (rejection/graft loss) and might require more/different immunosuppression
  – who is most likely to tolerate decreasing immunosuppression?
• Noninvasive diagnosis graft injury
  – Prevent morbidity of biopsy
  – Detect subclinical or incipient injury and or fibrosis
  – safety net for drug withdrawal studies
  – long term monitoring to detect changes in status
• Predict DGF
• Detect Immune tolerance
Biomarkers can support drug development & approval

- Trials cannot be done using hard endpoints of graft or patient survival because they would take too long, so we need surrogates of these hard endpoints
- AR is the only approved surrogate endpoint but
  - a) it occurs relatively infrequently and
  - b) graft failure occurs in the absence of AR
- Are there viable alternatives?
Candidate surrogate endpoints

• De novo class II DSA
• Changes in eGFR during the first 2 years (kidney transplant)
• iBOX score (kidney transplant)
• IVUS measurements of cardiac vasculopathy (heart transplant)
• others
Beyond clinical trials
Moving Biomarkers Toward Clinical Implementation in Transplantation

Menon, Murphy, Heeger, JASN 2017
Multicenter validation and assay standardization are crucial

- Representative example of CTOT biomarker standardization
- Rigorous analytical validation is critical
- Inter-laboratory reproducibility important
Pre-transplant biomarkers for risk stratification in transplantation

• Are there biomarkers that can be measured pre-transplant that can predict risk of post transplant rejection and/or graft function (beyond DSA)?

• If yes, one implication is that treatment strategies for high vs low risk patients could be individualized prior to transplant to optimize outcomes

• Yesterday we heard about pre transplant gene expression/non HLA mismatches
  – Sarwal, UCSF
  – Murphy, Mount Sinai
Donor-reactive Memory T cells and transplant outcome

- Memory cells are resistant to most immunosuppressant meds, are present at high frequency, have high functional avidity and respond rapidly to antigenic challenge.
- Hypothesis: high frequencies of memory T cells reactive to donor HLA negatively impact transplant outcomes.
Pre-transplant donor-reactive T cells and post-transplant outcome

IFNγ ELISPOT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative (&lt;25/300 K)</th>
<th>Positive (&gt; 25/300 K)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute cellular rejection</td>
<td>17%</td>
<td>50%</td>
<td>.036</td>
</tr>
<tr>
<td>GFR (MDRD) 12 months</td>
<td>55±20 ml/min/1.73 m²</td>
<td>37±16 ml/min/1.73 m²</td>
<td>.006</td>
</tr>
<tr>
<td>DGF</td>
<td>23%</td>
<td>31%</td>
<td>NS</td>
</tr>
</tbody>
</table>
Validation sets

• Other independent validation:
  • Donor reactive IFNγ ELISPOT assays pre- and post-transplant correlate strongly with AR and 1 y eGFR
    – Berlin group (Volk, Reinke)
    – Barcelona (Grinyo, Bestard)
    – Results from multicenter CTOT-01 study
Status: Pre-transplant donor-reactive IFN\(\gamma\) ELISPOT as a biomarker for post-transplant outcome

- Tested and validated by multiple groups
- Utility of using marker to guide therapy unknown
- Complex assay
- Requires customization (donor reactive)
- Some commercial interest
HLA EPITOPE Analysis (molecular mismatch) as a biomarker for developing DSA

Epitope MM Load associated with *de novo* DR or DQ Donor specific antibody (DSA)
Can pre-transplant biomarkers predict those at highest risk for poor outcomes during changes in immunosuppression?
CTOT09
TAC withdrawal in low risk, stable recipients of first living donor kidneys

- Living donor transplants
- DSA neg PRA<30%
- ATG induction
- TAC, MMF, Pred

Randomize at 6 mo if:
- No ACR
- No DSA
- Surveillance biopsy normal
- No BKV, on 1500/day MMF
(<50% of enrollees reached randomization)
Study terminated due to absence of equipoise

Confirms standard clinical risk assessment is inadequate!
Pre-transplant risk assessment
High epitope load associates with development of de novo DQ DSA in the CTOT09 cohort

Pre transplant risk assessment
ACR/DSA upon withdrawal associated with high pre-transplant anti-donor IFNγ ELISPOTs in the CTOT-09 cohort

Do peripheral blood gene expression profiles obtained before and after withdrawal provide insight?

Thymo

MMF, steroids, Tac

Randomization*

Months 0 6 9

PBMC RNA Affimetryx Array analysis

Work done in collaboration with Dan Salomon, Scripps
Few Differences in Transcriptional Programs at Baseline (before randomization)

Differential Gene Expression

N = 8856 genes

NW: No withdrawal (n=4)
WR: Withdrawal AR (n=8)
WS: Withdrawal stable (n=6)

Upregulation logFC > 1.5
Downregulation logFC < -1
p < 0.001 (no adjustment)
FcgR2b has coinhibitory functions on CD8+ T cells

Work done in collaboration with Mandy Ford, Emory
Evolution of the transcriptional program between 0 and 3 months post-randomization

- The three groups have distinct upregulated transcriptional programs.
- The Withdrawal Stable group is characterized by a large downregulatory transcriptional program.

<table>
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<th>Upregulated genes</th>
<th>Downregulated genes</th>
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<td>NW: No withdrawal (n=4)</td>
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<tr>
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</table>

- NW: No withdrawal (n=4)
- WR: Withdrawal AR (n=8)
- WS: Withdrawal stable (n=6)
Evolution of the transcriptional program between 0 and 3 months post-randomization

**Upregulated genes**
- Cell Death
- Apoptosis
- MAPK cascade activation
- Activation of Protein Kinase Activity
- T cell and Lymphocyte Activation through Antigen-presenting cells.

**Downregulated genes**
- SOCS2
- FKP
- RAP1
- TAF11

Gene Ontology (GO) Term Enrichment:
- No enrichment
- NW
- WS
- WR
Evolution of the transcriptional program between 0 and 3 months post-randomization

Upregulated genes

- Cell Death
- Apoptosis
- MAPK cascade activation
- Activation of Protein Kinase Activity

Downregulated genes

- T cell and Lymphocyte Activation through Antigen-presenting cells.

CellCODE analysis (SPV estimation + interaction model + GO term enrichment)
Gene expression profiling prior to and during Tac withdrawal has potential to guide decision-making.
Post transplant biomarkers

- **Urine**
  - Gene expression (PCR/nanostring)
  - cfDNA
  - Protein (chemokines)

- **Blood**
  - Gene expression patterns
  - cfDNA
  - others

Identification of Common Blood Gene Signatures for the Diagnosis of Renal and Cardiac Acute Allograft Rejection

Li Li1, Kiran Khush2,3, Szu-Chuan Hsieh1,3, Lihua Ying1, Helen Luikart2, Tara Sigdel1,3, Silke Roedder1,3, Andrew Yang2, Hannah Valentine2,3, Minnie M. Sarwal1,3

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Peripheral blood T cell exhaustion as a biomarker for posttransplant outcome

- A differentiation state that prevents immunopathology in situations of persistently high antigen load and inflammation
- Exploited by pathogens and tumors to dampen or silence potentially protective immunity
- Associated with PD1 expression (target of checkpoint blockade)
- Functionally: progressively decreased proliferative capacity and interleukin-2 (IL-2) production followed by a reduced ability to secrete tumor necrosis factor α (TNFα) and interferon γ (IFNγ)
- Role in transplantation is unclear but more exhaustion is hypothesized to be associated with better outcomes (opposite of tumors)
Exhaustion and kidney transplant outcomes

Paolo Cravedi and Miguel Fribourg

Kidney International 2019, in press
26 CTOT01 subject samples (frozen) studied at 0, 3 and 6 mo post-transplant

Kidney International 2019, in press
Identification of T cell exhaustion subsets

Kidney International 2019, in press
Increased CD4$^+$ T$_{EXH}$ cells post-transplant, inverse correlation with ATP production and association with graft fibrosis

Kidney International 2019, in press
26 CTOT01 subject samples (frozen) studied at 0, 3 and 6 mo post transplant: CD8⁺ T cell subsets

Kidney International 2019, in press
T cell exhaustion phenotype associates with ATG induction

CTOT01 was an observational study and ATG given at discretion of investigator

Kidney International 2019, in press
50 CTOT19 subjects: flow cytometry using PD1 and CD57
texh at 6 mo associates with better eGFR

Kidney International 2019, in press
What do we do now?

Pre-transplant
Risk assessment
HLA typing
Cross matching
Implantation biopsy
Clinical risk factors

Post-transplant monitoring
Serum creatinine

Current approach

Induction
ATG
Alemtuzumab
Anti-IL2R

Level of immuno-suppression

Transplant Day 0
6 months
24 months

CNI/belatacept
anti-prolif agent
+/-steroids
Precision/Individualized Care

Clinical events
Post-transplantation

Conventional risk assessment:
- Demographics
- Cross-match
- PRA

- Biopsy-transcriptome
- PBMC-transcriptome
- Urinary cell RNA
- Urinary CXCI9
- IFN-γ ELISPOT (PRT)
- HLA-Antibody (luminex)
- Epitope mismatch
- Recipient SNP analysis
- Donor SNP analysis

Pre-Tx 0d 3m 6m 12m 24m

Immunosuppression Levels by protocol
- TCMR
- ABMR

Drug levels, serial renal function, urinalysis adherence monitoring, screening viral PCRs.
Need to perform controlled trials to assess utility of biomarker-directed changes in therapy

- Incorporate validated biomarkers into clinical trial designs
  - Test whether biomarker based changes in therapy including during drug withdrawal detect subclinical injury and improve outcomes
  - Randomized controlled trials
    - One arm standard of care
    - One arm treat based on biomarker status
    - Is outcome better in the biomarker guided group?
Heeger Consortium CTOT Collaborators

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Thank you

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