kSORT™: A novel biomarker for early detection of kidney rejection

November 7 and 8, 2016

Flavio Vincenti, M.D.
Professor of Clinical Medicine
University of California, San Francisco
School of Medicine
Dear Dickie,

This is a reminder that you are registered to attend: “Answers to Your Questions About Blood Bank Proficiency, Competency and QC” which will begin in 1 Hour on:

November 7, 2016

Add to Calendar: Outlook® Calendar | Google Calendar™ | iCal®

Please send your questions, comments and feedback to: dnichols@immucor.com

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To register, please copy and paste the following link into your web browser to access the registration site:

https://www.surveymonkey.com/r/kSORT

DEADLINE TO REGISTER FOR CE IS November 25, 2016. NO REGISTRATION WILL BE ACCEPTED AFTER THAT DATE. Certificates of attendance will be sent out by December 6, 2016.
Handouts
Continuing Education

• PACE, California DHS
  – 437-307-16

• Florida BPR
  – 20-552235

• ABHI
  – TBD

• 1.5 Contact Hours

• Each attendee registers at:
  https://www.surveymonkey.com/r/kSORT
Dear Dickie,

This is a reminder that you are registered to attend: “Answers to Your Questions About Blood Bank Proficiency, Competency and QC” which will begin in 1 Hour on:

Thursday, June 23, 2016

Add to Calendar: Outlook® Calendar | Google Calendar™ | iCal®

Please send your questions, comments and feedback to: dnichols@immucor.com

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Continuing Education

• Each attendee must register for CE
• Registration deadline is November 25, 2016.
• No other form of CE registration will be accepted
• Certificates will be sent via email by December 6, 2016
• Session is being recorded and will be posted on LEARN in about 2 weeks
  – All registrants will be notified when recording is available
  – No CE will be issued for participating in recorded session
• You are all muted
• Q&A following session – as time allows
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kSORT
KIDNEY SOLID ORGAN RESPONSE TEST

IMMUCOR®
OBJECTIVES

1. Understand what kSORT is.
2. Elaborate on why kSORT was developed to address unmet needs in transplantation.
3. Describe the development of kSORT.
4. Describe the clinical data to support the use of kSORT.
5. Review potential clinical applications of kSORT.
AGENDA

• The Unmet Need
• What is kSORT
• Development of kSORT
The Number of ESRD Patients Continues to Rise

Annual Number of ESRD Cases

Percentage of Dialysis Patients Wait-Listed

United States Renal Data System (USRDS)
Annual Data Report 2015
Transplant is more Cost-Effective than Hemodialysis

Total Medicare Expenditures

United States Renal Data System (USRDS)  
Annual Data Report 2015
Survival Benefit of Transplantation

Wolfe et al. NEJM 1999
Renal Transplant Outcomes Have Been Stagnant

Deceased Kidney Transplants

Living Kidney Transplants
Post Transplant Surveillance - Guidelines
Too little, too late?

Slide from Maarten Naesens, MD, PhD via slideshare.net
Graft Injury and Rejection Are Driven By Ongoing, Subclinical Events

Naesens et al, Kid Int, 2011
Fehr et al Kidney Int, 2011

“Subtle inflammation”
What is kSORT?
kSORT™
kidney Solid Organ Response Test

kSORT (kidney Solid Organ Response Test) is a non-invasive, whole blood derived, molecular expression assay that can establish an immune risk index for enhanced post-transplant surveillance of graft health and immune quiescence in immunosuppressed, renal transplant patients. In conjunction with standard clinical care guidelines, kSORT assists in assessing the overall dynamic immune risk profile of post-renal transplant patients thereby improving risk stratification and patient management.
kSORT Measures Gene Expression

kSORT measures mRNA (i.e. expression of target genes) using qRT-PCR
# kSORT™ PCR Gene Set

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<th>FUNCTION</th>
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<tr>
<td>18S</td>
<td>18s ribosomal RNA</td>
<td>Internal Control</td>
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<tr>
<td>CEACAM4</td>
<td>Carcinoembryonic antigen related cell</td>
<td>function unclear, may play a role in phagocytosis</td>
</tr>
<tr>
<td>CFLAR</td>
<td>Caspase-like apoptosis regulatory protein</td>
<td>programmed cell death</td>
</tr>
<tr>
<td>DUSP1</td>
<td>Dual specificity phosphatase 1</td>
<td>cellular response to environmental stress</td>
</tr>
<tr>
<td>EPOR</td>
<td>Erythropoietin receptor</td>
<td>erythroid cell survival</td>
</tr>
<tr>
<td>GZMK</td>
<td>Granzyme K</td>
<td>cytolytic function of cytotoxic lymphocytes</td>
</tr>
<tr>
<td>ITGAX</td>
<td>Integrin subunit α</td>
<td>phagocytosis</td>
</tr>
<tr>
<td>MAPK9</td>
<td>Mitogen-activated protein kinase 9</td>
<td>role in a variety of processes, including T-cell differentiation</td>
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</table>

<table>
<thead>
<tr>
<th>GENE</th>
<th>NAME</th>
<th>FUNCTION</th>
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</thead>
<tbody>
<tr>
<td>NKTR</td>
<td>Natural killer cell triggering receptor</td>
<td>Facilitates NK cell target binding</td>
</tr>
<tr>
<td>NAMPT</td>
<td>Nicotinamide phosphoribosyl-transferase</td>
<td>metabolism and stress response</td>
</tr>
<tr>
<td>PSEN1</td>
<td>Presenilin 1</td>
<td>protease activation</td>
</tr>
<tr>
<td>RARA</td>
<td>Retinoic acid receptor α</td>
<td>cell differentiation and apoptosis</td>
</tr>
<tr>
<td>RHEB</td>
<td>Ras homolog enriched in brain</td>
<td>regulation of cell cycle</td>
</tr>
<tr>
<td>RXRA</td>
<td>Retinoid X receptor α</td>
<td>transcriptional regulation</td>
</tr>
<tr>
<td>RYBP</td>
<td>RING1 and YY1 binding protein</td>
<td>function unclear</td>
</tr>
<tr>
<td>SLC25A37</td>
<td>Solute carrier family 25 member 37</td>
<td>essential iron importer for synthesis of mitochondrial heme</td>
</tr>
</tbody>
</table>

AART Study, Plos 2014
kSORT™ Score via kSAS Analytics

- GENES
- ΔΔCt

- MODELS

- HIGH RISK

- INDETERMINATE

- LOW RISK

ΔΔCt values are color-coded and correspond to risk levels:

- Red: High Risk
- Orange: Indeterminate
- Blue: Low Risk
kSORT™ Results

**HIGH RISK**
Indicative of increased immune response and inflammation, which may lead to graft injury

**INDETERMINATE**
Indicates that the patient cannot be classified at this time

**LOW RISK**
Indicative of immune quiescence
Development of kSORT
kSORT Genesis Timeline

- **Organ-i Acquisition**
  - AART Study (kSORT Genesis)
    - Method Finalized
    - Optimal Gene Selection
    - kSAS Models Locked

- **Immucor Dx Established**
  - TITRATE Study (interventional) ongoing

- **kSORT Launched**
  - SAILOR Study: (kSORT-AR) ongoing
  - PRISM Study: (highly sensitized) ongoing
  - SITAR Study: (kSORT surveillance)

- **2017**
  - Planned kSORT Interventional Study
  - IMMUCOR DX Accuracy and Precision Studies
  - ESCAPE Study: (subclinical rejection)
History of kSORT Development

1. Gene discovery in whole genome microarrays:
   n=2382 genes AR vs. No-AR (FDR<5%)
   - Increased level of significance
   - Biological relevance in AR cell specific immune response
   - Literature confirmed

2. Gene validation in QPCR: n=32 genes
   - Best combinations of genes to predict class: AR/No-AR

3. Gene discovery, validation, prediction in cross-organ rejection analysis (Lung, Liver, Heart, Kidney)
   - Meta-analysis n=1030 AR/No-AR
   - AR vs. No-AR p<0.01
   - Banff Score Correlation p<0.05
   - Injury prediction

QPCR Refinement: n=43 unique kSORT candidates

4. Training: AART143
   - Machine learning techniques for variable selection + classification
   - Differential Expression AR vs. No-AR (p-value, fc)

17 genes
   (AUC 0.94, CI 0.91-0.98)

Validation: AART124
   - Independent validation for AR classification
   - (plsDA with equal prior probability) AUC=0.9379

kSORT 17-gene selection

Algorithm Development Phase

Cross-Validation: AART100
   - Correlation to known AR/No-AR kSORT profiles (Pearson Correlation)

Gene Refinement Phase

II. Refinement and Development Phase

kSAS

- No batch effect
- Not affected by Sample Processing Variation

Proof-of-Concept Study:
   n=377 samples: pediatric, homogenous, SOP 1

kSORT Gene Identification Triage

• AR vs. No-AR (FDR<5%)
• Literature Review
• Internal control genes
• Best 10 gene subset

• Fc (Immune cell vs. 84 tissues) >10; p<0.05
   (BioGPS)

• Meta-analysis n=1030 AR/No-AR
• AR vs. No-AR p<0.01
• Banff Score Correlation p<0.05
• Injury prediction
Assessment of Acute Rejection in Renal Transplantation Study

Assessment of 558 Samples from 436 Patients in the **AART Study** (Patient/Sample Flow):

**Cross-Sectional Multi-Center Study** (338 Patients; n=367 Blood Samples)
(8 Centers: Barcelona, CPMC, Emory, Mexico, Stanford, UCLA, UCSF, UPMC)

1. **135 Patients**
   - Training
   - **AART143 (n=143)**
     - AR (n=47), No-AR (n=96)
     - Blood Samples

2. **107 Patients**
   - Validation
   - **AART124 (n=124)**
     - AR (n=23), No-AR (n=101)
     - Blood Samples

3. **96 Patients**
   - Cross-Validation
   - **AART100 (n=100)**
     - AR (n=43), No-AR (n=57)
     - Blood Samples

**Longitudinal Multi-Center Study** (98 Patients; n=191 Blood Samples)
(5 Centers: CPMC, Emory, Stanford, UCLA, UPMC)

4. **98 Patients**
   - Prediction
   - **AART191 (n=191)**
     - AR (n=74), pre-AR (n=65), post-AR (n=52)
     - Blood Samples

---

1. **kSORT Gene Identification**
   - For AR detection/prediction using QPCR

2. **kSAS Development**

**Roedder et al. 2014 PLOS Medicine**
kSAS Results in AART100

Risk Category
- High Risk
- Indeterminate
- Low Risk

kSORT Score

- n=39
  - No-AR (biopsy proven)
    - n=58
  - AR (biopsy proven)
    - n=42

- n=15
  - No-AR (biopsy proven)
  - AR (biopsy proven)

- n=46
  - No-AR (biopsy proven)
  - AR (biopsy proven)

Roedder et al. 2014 PLOS Medicine
kSORT™ and Current Functional Measurements

Risk Category

- High Risk
- Indeterminate
- Low Risk

$p=0.0005$
AART191: Prediction Cohort

Increasing probabilities (>50%) of AR

Roedder et al. 2014 PLOS Medicine
The AART Study: Summary

- Development of kSORT
  - Locked gene set to differentiate AR vs Stable
  - Established algorithm to identify patients at high risk of AR

- kSORT is not confounded by demographic factors

- kSORT detects both TCR and AMR

- kSORT may be able to detect graft dysfunction prior to detection by biopsy
Steroid Avoidance and Low-Dose CNI by ATG-Induction in Renal Transplant (SAILOR)
SAILOR Study

- Randomized multicenter trial of 222 renal transplant recipients
- Blood drawn at day 0, 10, months 3, 6, 12 and at graft dysfunction
- Protocol biopsies at engraftment and 12 mo
- Interim results
  - First 79 enrolled patients = 338 samples
  - 98 blood samples were matched with protocol or indicated biopsies
  - 22 patients had clinically suspected acute rejection (AR) of which
    18 were biopsy confirmed
    - All were DSA negative
  - 80 biopsy matched blood samples without histological AR

kSORT was evaluated for its accuracy in diagnosing and predicting biopsy confirmed AR.
Interim results of kSORT assay confirm:

- 93.3% sensitivity
- 90% specificity
- 98.6% NPV
- not confounded by BK viremia

“73% of AR could have been diagnosed by the kSORT assay days to months prior to biopsy.”
Evaluation of Sub-Clinical Acute Rejection Prediction (ESCAPE)
The observational trial of Evaluation of Sub-Clinical Acute Rejection PrEdiction (ESCAPE); retrospective, single-center study in Barcelona, Spain, prospectively collecting serial PB samples from 75 patients matched with a surveillance graft Bx collected at mo 6 (stable serum creatinine) and at times of clinically indicated AR suspicion.

Objectives: validate the ability of kSORT alone and/ or in combination with the functional donor specific IFN-y T-cells ELISPOT assay to detect sub-clinical AR
ESCAPE Study

kSORT (kSAS algorithm):
- High Risk (HR)
- Indeterminate (IR)
- Low risk (LR)

Donor-specific IFN-γ ELISPOT
- POSITIVE: >25 spots/300,000 PBMC
- NEGATIVE: <25 spots/300,000 PBMC

- 75 consecutive KTR
- 6-mo d-s IFN-γ ELISPOT / kSORT
- 6-months Protocol Biopsy
  - Analysis of basic histological lesions
- 24-months Follow-up (Graft function)

Assessment of on-going 6-mo Subclinical Rejection

⇒ The discrimination ability of each biomarker further repeated 1000 times using bootstrap samples to derive 95% confidence intervals

Bestard O, Sarwal MM, Transplantation 2016 In Press
kSORT Detection of Subclinical Rejection at 6mo
Predictive Probabilities of Subclinical Rejection combining the Two Assays

- **HR-kSORT / DSA +** → **ABMR**
- **HR-kSORT / ELISPOT +** → **TCMR**
- **LR-kSORT / ELISPOT** - → **STABLE**
ESCAPE Study: Summary

- A novel molecular signature (kSORT/kSAS) in peripheral blood has shown very high accuracy (PPV) to identify “at-risk” patients

- The assessment of alloreactive immune memory seems to be a promising approach to refine both the humoral and cellular effector responses using ELISPOT based platforms

- The combination of Biomarkers may increase their predictive capacity and provide insight of the effector mechanisms of allograft rejection
# kSORT Performance in Three Independent Clinical Trials

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## Performance

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<tr>
<td>Sensitivity</td>
<td>92.3%</td>
<td>93.3%</td>
<td>70.0%*</td>
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<tr>
<td>Specificity</td>
<td>93.5%</td>
<td>91.8%</td>
<td>97.78%</td>
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<td>Bx Correlation</td>
<td>92.9%</td>
<td>92.1%</td>
<td>89.23%</td>
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<tr>
<td>Indeterminate</td>
<td>15%</td>
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**Validation in the Clinical Laboratory**

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<tr>
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<tr>
<td><strong>Total</strong></td>
<td>28</td>
<td>56</td>
<td>84</td>
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- **Agreement Rate**: 87%
- **IND Rate**: 11%
- **Sensitivity**: 100%
- **Specificity**: 96%
IMMUCOR DX IS POSITIONED TO PROVIDE:

- Accelerated availability of novel technologies to laboratories, clinicians and patients

**kSORT** can be ordered worldwide NOW!!!

immucordx@immucor.com
Ordering and Reporting Process

Complete Lab Requisition

Draw Blood in Paxgene Tubes

Report to Clinician
A non-invasive molecular diagnostic assay for rejection is finally a reality!!!
kSORT
KIDNEY SOLID ORGAN RESPONSE TEST
IMMUCOR
Reminder

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• Registration deadline is November 25, 2016.
Thank You for joining us!