Molecular Diagnostics:

Stanford University Medical Center

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Agenda

- About XDx and Molecular Diagnostics
- R&D and Product Development
- AlloMap Molecular Expression Testing
- Challenges & Lessons Learned
About XDX and Molecular Diagnostics

Subtitle
### XDx Mission

#### Mission

- Improve patient care by developing molecular diagnostics that translate an individual’s immune status into clinically actionable information.

#### Goals

- Understand the immune system on the molecular level.
- Dynamically monitor the immune system using genomics technologies and simple blood test.
- Deliver clear and precise information to clinicians making critical decisions where no such information is currently available.
Product Opportunities

- Immune Mediated Inflammatory Diseases (IMID)
  - Immune-system activation causes many inflammatory diseases:

  - Heart Transplant: Invasive biopsy
  - Lung Transplant: Bronchial Lavage, Invasive biopsies
  - Renal Transplant: Blood tests followed by biopsy
  - Crohn’s Disease: Colonoscopy, biopsy
  - Multiple Sclerosis: MRI imaging, clinical
  - Lupus: Unpredictable course

Autoimmunity: 22,000,000

Alloimmunity: 400,000
IMID Challenge

- Delicate balance between benefits and side effects of immunosuppressive therapy
- Finding the “sweet spot” has been a challenge since the advent of immunosuppressive therapy
- Lack of diagnostic innovation
### Molecular Diagnostics

- **Definition**: the use of DNA, RNA, and proteins to test for specific states of health or disease

- Enabled by genomic technologies and information
  - **Measurement technologies**
    - DNA: PCR, hybridization
    - RNA: microarray, PCR
    - Protein: antibodies, mass spec

- Explosion of “analytes”—genes and gene products

- Allows detection of almost any biological state
The Business of Molecular Diagnostics

- Diagnostics are valued based on how well the information provided improves clinical decisions.

- Traditional diagnostics:
  - Known biology or associations of single analytes (glucose, cholesterol, etc.)
  - Cheapest and most efficient and convenient technology wins...

- Molecular diagnostics:
  - Complicated biology of critical clinical decisions can be addressed
  - Most innovative science and technical implementation wins...
Examples of Successful Molecular Diagnostics

- **Myriad Genetics:** BRACAnalysis®
  - Assess inherited risk for breast cancer
  - Mutations in BRCA1 and BRCA2 by DNA sequencing

- **Genomic Health:** Oncotype DX®
  - Predicts benefit of chemotherapy and risk of recurrence in breast cancer patients
  - Gene expression levels of 21 genes from tumor tissue

- **Genentech:** HER2/neu
  - Predicts response to Herceptin in breast cancer patients

  FISH (or IHC) detection of epidermal growth factor 2 in tumors
Opportunity to Change Medical Practice

- Care focused on late-stage treatment: pathology, severe clinical manifestations, organ dysfunction

- New diagnostic technologies: opportunity to reduce patient morbidity and mortality, and health care costs by optimizing the application of medical care

Figure 2. The development of chronic disease and opportunities for intervention.

Disease burden generally increases over time and is correlated with less reversibility and greater cost of intervention.
R&D and Product Development
Subtitle
Interdisciplinary Approach

- Developing molecular diagnostics requires understanding the structure and properties of medical and biological information:
  1. Collecting and analyzing genomic data
  2. Collecting and analyzing clinical data
  3. Correlating genomic signatures with clinical patterns
  4. Distilling molecular signatures to provide maximal clinical and commercial value
**The Science: Gene Expression Profiling**

A. Trafficking of WBCs through tissues with acute rejection

- Peripheral blood mononuclear cells (PBMCs)

B. DNA - fixed genetic programming

**RNA - dynamic gene expression**

1. Usually, a gene in DNA is copied into a messenger RNA.

2. The messenger RNA leaves the cell’s nucleus and finds a ribosome, a protein-making structure that reads along the messenger, converting its information into the protein specified by the gene.

DNA Microarrays

Quantitative real-time PCR

Exponential amplification

\[ 2^x = 4 \text{ copies} \]
\[ 2^{x+2} = 8 \text{ copies} \]
\[ 2^{x+3} = 16 \text{ copies} \]
\[ 2^{x+4} = 32 \text{ copies} \]
\[ 2^{x+5} = 64 \text{ copies} \]

Agilent Whole Human Genome Array
DNA Microarrays

- Used for Discovery (and confirmation of biomarkers)
- Hybridization to array of oligonucleotides sequences
- Thousands of genes ("whole genome") tested in single experiment
- Low sensitivity for rare genes; low specificity for

Quantitative real-time PCR

- Used for Diagnostic Test (Development and Validation)
- Count PCR steps required to amply from sample
- Individually designed specific and robust assays
- Increased sensitivity and reproducibility
- Accepted test
XDx Diagnostic Development Process

Discovery
- Product profile; whole genome analysis and literature; candidate gene selection and verification

Development
- Classifier development; robustness and performance; biomedical interpretation

Validation
- Analytical and Clinical

Commercialization
- Establish clinical utility & reimbursement
AlloMap® Molecular Expression Testing

Subtitle
Management of the Heart Transplant Recipient

PATIENT
- History & Physical Exam

HEART
- Hemodynamics

CELLULAR
- Endomyocardial Biopsy

MOLECULAR
- AlloMap Molecular Expression Testing

The Transplant Patient Management Challenge

ADVERSE EFFECT

Quiescence
- Rejection
- Cancer
- Toxicity
- Infections

IMMUNOSUPPRESSIVE THERAPY
Endomyocardial Biopsy
### ISHLT Standardized Cardiac Biopsy Grading

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histopathological Findings</th>
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<tbody>
<tr>
<td>0R</td>
<td>0 No rejection</td>
</tr>
<tr>
<td>1A</td>
<td>Focal perivascular and/or interstitial infiltrate without myocyte damage</td>
</tr>
<tr>
<td>1B</td>
<td>Diffuse infiltrate without necrosis</td>
</tr>
<tr>
<td>2</td>
<td>One focus of infiltrate with associated myocyte damage</td>
</tr>
<tr>
<td>3A</td>
<td>Multifocal infiltrate with myocyte damage</td>
</tr>
<tr>
<td>3B</td>
<td>Diffuse infiltrate with myocyte damage</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ± hemorrhage, + vasculitis</td>
</tr>
</tbody>
</table>

Additional information (required when present): biopsy <4 pieces, humoral rejection, “Quilty” effect, ischemia, infection present, lymphoproliferative disorder, other.
Limitations of Endomyocardial Biopsy

- Interpretive variability
  - Intra- and inter-reader variability
  - Over calling of ≥3A

- Tissue sample inadequacy
  - May miss focal areas of rejection
  - Repetitive biopsy leads to fibrosis

- Invasive
  - Percutaneous catheterization
  - Risk (0.2-2.3%) includes:
    - Right ventricular perforation
    - Tricuspid valve damage
    - Arrhythmias
    - Bleeding

Example of reader variability

Agreement of Study Pathologists with Local Pathologists (n=1,356)

Average Study Pathologist Agreement

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

<table>
<thead>
<tr>
<th>Local ISHLT Grade</th>
<th>0</th>
<th>1A, 1B</th>
<th>2</th>
<th>3A, 3B</th>
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</thead>
<tbody>
<tr>
<td>Average</td>
<td>100%</td>
<td>50%</td>
<td>20%</td>
<td>30%</td>
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CARGO Study

- Hypothesis
  - Peripheral blood gene expression profiles can differentiate between the absence and presence of acute cellular rejection

- Study Overview
  - 9 center observational study
    - Conducted 2001-2005
  - 737 subjects enrolled
  - 5,837 post transplant encounters
  - Centralized biopsy grading
    - 3 expert heart transplant pathologists read biopsies
    - Use of central reads to define Rejection/No rejection (R/NR)

- Columbia University (New York)
- Cleveland Clinic (Cleveland)
- Kaiser Permanente (San Jose)
- Ochsner Clinic (New Orleans)
- Stanford University (Palo Alto)
- Temple University (Philadelphia)
- UCLA (Los Angeles)
- University of Florida (Gainesville)
- University of Pittsburgh (Pittsburgh)
# AlloMap Discovery

<table>
<thead>
<tr>
<th>I</th>
<th>Definition of rejection and non-rejection classes</th>
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<tr>
<td></td>
<td>Centralized pathology data</td>
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<table>
<thead>
<tr>
<th>II</th>
<th>Candidate Gene Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leukocyte microarray analysis</td>
</tr>
<tr>
<td></td>
<td>285 samples</td>
</tr>
<tr>
<td></td>
<td>8,000 gene probes</td>
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<table>
<thead>
<tr>
<th>III</th>
<th>Literature review, database mining, expert consultation</th>
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<tbody>
<tr>
<td></td>
<td>252 candidate genes selected</td>
</tr>
<tr>
<td></td>
<td>68 biomarkers for rejection verified by</td>
</tr>
</tbody>
</table>
Genes that Distinguish Rejection

- Quantitative real-time PCR for 252 candidate genes
- 145 samples divided into ISHLT Grade 0 and ISHLT ≥3A by centralized pathologists
- 68 genes correlated with rejection (p <0.01) or were more than 25% up- or down-regulated
AlloMap Development

I. Multi-gene classifier development
   - Statistically rigorous

II. Robust
   - Biologically plausible

III. 20-gene linear classifier derived by Linear Discriminant Analysis (LDA)

IV. 11 “informative” genes in 7 rejection pathways or functional groups
   - 6 normalization genes
### Classifier Development

**β₀ + β₁ x Group 1**  
Steroid Responsive

**β₂ x Group 2**  
Platelet Activation

**β₃ x Group 3**  
Hematopoiesis

**β₄ x Gene 1**  
Morphology

**β₅ x Gene 2**  
T cell activation

**β₆ x Gene 3**  
T cell trafficking

**β₇ x Gene 4**  
B cell/T cell activation
AlloMap Validation

I. Clinical Verification
   - 62 high-grade rejection samples
   - 86 mild rejection samples

II. Analytical Validation
   - 122 no rejection samples
   - Reproducibility
   - Confidence intervals

III. Over 800 additional samples in order to characterize the clinical performance
   - NPV, PPV and score distribution

IV. Longitudinal analysis
CARGO Clinical Validation Study Results

- AlloMap results significantly distinguish grade ≥3A rejection from grade 0 (p < 0.0001)

- Patients with low scores have very low risk of grade ≥3A rejection on biopsy (e.g., beyond year 1, scores below 34 have an NPV of >99%)

- Patients with high scores have an increased but moderate risk of current rejection (e.g., beyond year 1, scores above...
The sample needed for AlloMap testing can be obtained from a routine phlebotomy procedure. The prepared sample is shipped, along with the completed test requisition form, to the XDx clinical laboratory for testing. Results are typically reported to the ordering physician in 1-2 business days.
AlloMap Value Proposition

- AlloMap HTx addresses major clinical need in the long-term management of heart transplant patients
  - Regular assessment of risk for acute cellular rejection used to adjust management strategy (e.g. immunosuppression)
  - Patients underwent frequent invasive endomyocardial biopsies as only option pre-AlloMap

- AlloMap HTx has substantial market potential (U.S. alone)
  - Over 15,000 commercial tests provided to date
  - Over 5,000 patients tested (~19,000 living recipients)
  - 75 transplant centers have ordered AlloMap (~150 in U.S.)
Challenges & Lessons

Subtitle
“Crossing the Chasm”

- **Challenge:** adoption of new technology is never simple
  - Even CARGO investigators didn’t immediately integrate AlloMap

- **Solutions & Lessons**
  - Understand the perspectives (and psychology!) of all stake-holders
    - Patient, Clinic, Laboratory, Reimbursement
  - Continue to show clinical value with strong science (IMAGE trial)
  - Education and training is the best marketing tool
  - Provide solutions or alternatives to your customer
Regulatory

- Challenge: AlloMap was introduced as a Laboratory Developed Test (LDT) under CLIA, but the FDA now wants to regulate as IVDMIA
  - Faced double regulatory burden starting in late 2006
  - Choice to engage or resist...

- Solutions & Lessons
  - FDA clearance in August 2008
  - Reduce uncertainty through proactive engagement
  - You don’t need to be afraid if your science is strong
## Reimbursement

- **Challenge:** CMS changed a simple rule causing a reimbursement upheaval
  - Date-of-service change meant hospitals had to bill Medicare for tests on blood collected in the clinic
  - Financial risk shifted from XDx to hospital

### Solutions & Lessons
- Patient Service Centers (short term) and 3rd party solution
- Stick to your core mission and business model
- Consider all solutions
- If you can’t change it, feature it!
## Conclusions

- A compelling mission and strong scientific culture serve as the compass to navigate turbulent times
  - Invest your foundation and future in good times, and focus on what’s important for survival during bad times

- Innovation = Invention + Implementation
  - Good science and clinical development are the foundation of any biotechnology effort, but usually strategic and operational excellence determines eventual success

- An integrated, interdisciplinary approach is required at multiple levels
Thank You!

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