PROOF of the Pudding in Canada

2010 ITMAT International Symposium

Wednesday, October 27, 2010

Bruce McManus
**PROOF Centre Background**

*Who are we?*

- Not-for-profit Society established with competitive federal funding from the NCE secretariat in 2008
- Created as an NCE CECR devoted to developing useful biomarker products that provide socioeconomic benefits for Canada
- Based at St. Paul’s Hospital (Institute for Heart + Lung Health) in Vancouver, Canada
- Hosted by the University of British Columbia

[www.proofcentre.ca](http://www.proofcentre.ca)
Biomarkers

Distinct biological indicators (cellular, biochemical or molecular) of a process, event or condition that can be measured reliably in tissues, cells or fluids.
Integration of whole blood genomics and plasma proteomics adds value as they reflect different biomarker compartments.

- DNA
- RNA
- Protein
- Metabolite

Epigenetics
Genetics / Genotype
Genomics / Transcriptomics
Proteomics
Metabolomics
**PROOF Centre Mission**

*Can we do better?*

- Discover
- Develop
- Commercialize
- Implement

**BIOMARKER SOLUTIONS**

- Prevent
- Predict
- Diagnose
- Manage
- Treat

**Improved Healthcare Economic Development**

- Heart failure
- Lung failure
- Kidney failure

*Biomarkers in Transplantation* is the lead project of the Centre. Programs are also underway in heart, lung, and kidney failure.
Biomarker Journey

Our end-to-end approach to biomarkers

Clinical Question
Where can a new blood test improve patient care and create socio-economic value?

Biomarker Discovery
Computational strategies discover sets of genes and proteins to diagnose a type of patient

Biomarker Development
- Biomarker Refinement / Validation / Qualification
- Diagnostic Assay Development
- Health Economics
- Regulatory Filing
- Reimbursement

Clinical Implementation
Work with physicians, healthcare organizations, governments and private partners to implement biomarker tests in clinical settings
Our Community of Partners

Patient Cohorts

Technology Platforms

Computation

Financial Resources

Biomarker Science

Health Economics

Health Systems

Commercialization
Biomarker Programs

**Clinical Question**

- **Chronic Kidney Disease**
  Blood tests that predict rate of progression of kidney disease

- **Chronic Obstructive Pulmonary Disease**
  Blood tests for lung function endpoints to develop therapies

- **Chronic Heart Failure**
  Blood tests that diagnose diastolic versus systolic heart failure

- **Acute Heart Failure**
  Blood tests that guide ventricular assist device removal

**Biomarker Discovery**

- **Biomarkers in Transplantation**
  Diagnostic / predictive blood tests for acute and chronic rejection

**Biomarker Development**

- **“Cured” Organ Failure**
  Blood tests to determine when a therapy is working

- **New Biomarker Technology**
  Multiplex peptide and gene blood tests
The Life Cycle of Organ Failure

- Baseline Risk
- Disease Presence
- Disease Progression
- “Recovered” Organ Function
- Improved Organ Function
- Earlier Intervention
- End-stage Organ Failure
- Recurrent Organ Failure

Organ Function (%)

Time (years)

Transplantation/Assist Devices
Acute Organ Rejection

Current diagnostic approaches

Tissue biopsies remain the gold standard for diagnosis of *acute rejection*

- Highly invasive
- Not timely
- Expensive
- Diagnostic only, not prognostic
- Uncomfortable and fear-evoking
- Prone to sampling error
- Subject to interpretative variability
Chronic Organ Rejection

Current diagnostic approaches

- A major hurdle for the long-term survival of cardiac allograft transplant recipients is development of cardiac allograft vasculopathy (CAV) as an expression of chronic rejection.

- The current (gold) standard for diagnosis of CAV is invasive:
  - Coronary Angiography
  - Intravascular Ultrasound
Timeline...Transplant Patient’s Life

- **End-Organ Failure**
  - HLA, PRA, viruses
  - Predictive genes and proteins

- **Transplant**
  - Heart: Protocol biopsies
    - Kidney: Creatinine, GFR, For-cause biopsies
  - Diagnostic genes and proteins

- **Chronic Rejection / Recurrence**
  - Heart: Angiography, IVUS, Echocardiography
    - Kidney: Creatinine, GFR, For-cause biopsies
  - Diagnostic genes and proteins

- **12 months**
- **6 months**
Reflection on improvement of care for heart transplant patients

Maxine’s presentation and first year post-transplant

- Acute Viral Myocarditis
- Sudden Death
- Ventricular Assist Device
- Heart Transplant
- ~12-14 Heart Biopsies During 1st Year Post-transplant; “Standard” Immunosuppressive Therapy

First steps for implementing test

- Heart Transplant
- Blood Test to Guide Need for Biopsy
- +/- Biopsy
- “Standard” Immunosuppressive Therapy

Future implementation

- Blood Test to Predict if Rejection Will Occur
- +/- Pre-dose Immunosuppressive Therapy
- Heart Transplant
- Blood Test to Replace the Need for Biopsy
- Altered Immunosuppressive Therapy
Biomarkers in Transplantation

2004 → 2009

Discovery and internal validation of blood-based biomarkers:
- Genomic
- Proteomic

Patient Cohorts:
- Acute heart rejection
- Chronic heart rejection
- Acute kidney rejection
- Chronic kidney rejection

Eight Potential Tests:
- Diagnostic
- Predictive

Funded by Genome Canada, IBM, Novartis, Vancouver Hospital Foundation, St. Paul’s Hospital Foundation, UBC, Genome BC, The James Hogg iCAPTURE Centre, BC Transplant Research Institute, Affymetrix, and Eksigent
Biomarkers in Transplantation

**Discovery strategy**

**PATIENT COHORT**

**DATA**

- ~36,000 Probe Sets are normalized and pre-filtered
- Apply protein group code algorithm to ~2,000 Peptides

- ~10,000 Probe Sets and ~200 Protein Groups assessed by multiple robust and classical t-tests for differences among patient groups

- ~250 Genes, Proteins, & Clinical Variables combined into a discriminative score by support vector machine classification

**BIOMARKER PANEL**

Biologically validated with ELISA and qPCR
Statistically validated with leave-one-out cross validation to estimate sensitivity, specificity and AUC

**INTERNALLY VALIDATED BIOMARKER PANEL**

IO Informatics Knowledge Explorer
(www.io-informatics.com)

**SOP DRIVEN**

- **BIOLOGICAL SAMPLES**
- **CLINICAL DATA**
- **Patient Review / Sample Selection**

- **TRANSCRIPTOMICS**
  - Whole blood RNA (PAXgene)
  - Affymetrix Microarray

- **PROTEOMICS**
  - Depleted Plasma
  - iTRAQ Mass Spectrometry

- **METABOLOMICS**
  - Plasma, Serum, Urine
  - NMR, Mass Spectrometry

Include known biomarkers or essential clinical variables
Predictive Markers – Acute Heart Rejection

Whole blood genomics

Biological Processes
- Regulation of actin cytoskeleton organization
- Regulation of actin filament-based process
- Protein amino acid dephosphorylation
- Dephosphorylation
- Regulation of cytoskeleton organization
- Regulation of organelle organization
- Regulation of protein kinase cascade
- Negative regulation of catalytic activity
- Regulation of hydrolase activity
- Regulation of biological quality

Sensitivity 83%
Specificity 88%
Diagnostic Markers – Acute Heart Rejection

What value does the endomyocardial biopsy add?

- Whole Blood Samples
  - Affymetrix U133 Microarray
    - 54,675 PROBE SETS
  - 17,610 PROBE SETS

- Endomyocardial Biopsy Tissues
  - Affymetrix U133 Microarray
    - 54,675 PROBE SETS
  - 2,186 PROBE SETS

WHOLE BLOOD + BIOPSY PROBE SETS

BIOMARKER PANEL

Sensitivity 100%
Specificity 100%

Elastic Nets

Leave-one-out Cross-Validation
Diagnostic Markers – Acute Heart Rejection

What value does the endomyocardial biopsy add?

Blood
AUC = 0.60

Biopsy
AUC = 0.85

Biopsy-targeted blood
AUC = 0.83

(Hollander Z et al Transplantation, in press, December 2010)
Proteomic Signatures in Plasma during Early Acute Renal Allograft Rejection


Acute graft rejection is an important clinical problem in renal transplantation and an adverse predictor for long-term graft survival. Plasma biomarkers may offer an important avenue for post-transplant monitoring, and permit timely and effective therapeutic intervention to minimize graft damage. The case-control discovery study (n = 38) used targeted liquid chromatography-tandem mass spectrometry to evaluate protein expression in 16 plasma samples from patients with confirmed acute rejection. A total of 18 proteins were upregulated in acute rejection, including reduced expression of 4 proteins: tumor necrosis factor alpha, tumor necrosis factor beta, IL-2, and IL-12. The AUC of the plasma biomarker panel for acute rejection was 0.89. The study provides a potential biomarker panel for acute rejection in renal allograft recipients.

From the Mission of Organ Failure (PROOF) Centre of Excellence, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, Department of Medicine, University of British Columbia and Vancouver General Hospital, Vancouver, British Columbia, V6H 3M5, Department of Surgery, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, Department of Pathology, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, Department of Transplantation Surgery, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, Department of Immunology, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, Department of Pathology, University of British Columbia, Vancouver, British Columbia, V6T 1Z5, and Department of Transplantation Surgery, University of British Columbia, Vancouver, British Columbia, V6T 1Z5.

Although advances in immunosuppressive have increased the success of renal transplantation, improvements in graft survival remain a critical issue. The study of molecular mechanisms underlying acute rejection is essential for the development of more effective therapeutic strategies.
Diagnostic Markers – Acute Renal Rejection

Effect of time post-transplant on diagnosis by biomarkers

(Gunther O et al, *Transplantation*, in press)
Cardiac Allograft Vasculopathy

Combinatorial biomarker panel

GENOMIC BIOMARKER PANEL
- Sensitivity = 83%
- Specificity = 83%

PROTEOMIC BIOMARKER PANEL
- Sensitivity = 83%
- Specificity = 83%

COMBINATORIAL BIOMARKER PANEL
- Sensitivity = 100%
- Specificity = 83%

Genes:
- CLEC2B
- CHPT1
- 242907_at
- GBP3
- CFHR1
- CPN1
- C1QB
- GC
Cardiac Allograft Vasculopathy

**Correlation with severity of coronary artery stenosis?**

Example: % (maximum) stenosis of the left anterior descending artery was investigated.

- % of coronary artery stenosis as predicted by new protein biomarker panel (‘Predicted’ Stenosis)
- % of coronary artery stenosis based on clinical, coronary angiography-based assessment (‘True’ Stenosis)

![Graph showing correlation between predicted and true stenosis percentages with Pearson's correlation coefficient R = 0.79.](image)

**Pearson’s Correlation (R) = 0.79**

*(between the ‘predicted’ and the ‘true’ stenosis)*
Biomarkers in Transplantation

Moving from development to the clinic

2009

External qualification of genomic and proteomic blood-based biomarkers for heart and kidney rejection

International Biomarker Trial (BiT2) - 350 kidney transplant patients and 150 heart transplant patients

Biomarker Panel Refinement – improved AUCs to >0.90 for acute kidney and heart rejection

Assay Development

2011

In vitro diagnostic regulatory submissions

Funded by PROOF Centre of Excellence, Genome British Columbia, Astellas, St. Paul’s Hospital Foundation, UBC, BC Transplant, Luminex
Biomarker Trial Sites for Validation
Computational Excellence

Cornerstone for value

- Pre-filtering
- Uni-variate ranking
- Uni-variate filtering
- Multi-variate ranking
- Multi-variate filtering
- Classifier generation

Bio-IT World Best Practices Award in Personalized & Translational Medicine
April 22, 2010
Biomarker Panel Refinement

Improving the AUC for diagnosis of acute renal rejection

| Pre-filtering | 1) k samples above absolute threshold  
|              | 2) First half using inter-quartile range  
|              | 3) First half using empirical central mass range |
| Uni-variate ranking | 1) Maximum of LIMMA, robust LIMMA and SAM  
|                  | 2) LIMMA  
|                  | 3) Robust LIMMA |
| Uni-variate filtering | 1) FDR cut-off (FDR<0.01)  
|                      | 2) Size cut-off: Top 50 probe-sets  
|                      | 3) Combination rule: FDR<0.05 but at least 50 and at most 500 probe sets |
| Multi-variate ranking | 1) Stepwise Discriminant Analysis  
|                        | 2) SVM-based ranking (one step)  
|                        | 3) Recursive Feature Elimination (multi-step)  
|                        | 4) Elastic Net-based (coefficients) |
| Multi-variate filtering | 1) Significance of improvement cut-off  
|                          | 2) Top 50 (as returned by multi-variate ranking)  
|                          | 3) Non-zero coefficients (Elastic Net) |
| Classifier Generation | 1) Linear Discriminant Analysis  
|                      | 2) Support Vector Machine  
|                      | 3) Random Forest  
|                      | 4) Elastic Net  
|                      | 5) Logistic regression |

Biomarker Panel Pipeline

From 54,615 probe-sets to biomarker panels with 1 to 500 probe-sets

Classifier Generation

>100 classifiers were generated during the refinement period
Biomarker Panel Refinement

Improving the AUC for diagnosis of acute renal rejection

![ROC Curve](image)

- Elastic Net: AUC = 0.9983
- SVM: AUC = 0.9863
- LDA: AUC = 0.9863
- Random Forest: AUC = 0.9957
Combining Classifier Panels

Harvesting the art and science of the ensemble

- Genomics Biomarkers
  - Genomics Classifiers
  - Clinical Biomarkers
  - Clinical Classifiers
  - Proteomics Biomarkers
  - Proteomics Classifiers

Proteogenomic Ensemble Classifiers
Network Analysis of Predictive Signatures

Early acute renal transplant rejection

The human protein interaction network (PIN)

Map on 128 significant PROOF Centre genes onto PIN, search for sub-networks

Sergio Baranzini
UCSF Department of Neurology
Integrated View of Predictive Genes

Acute kidney rejection

Sub-network in PIN of 157 genes included 98/128 of our genes, then enriched
Diagnostic Assay Development

**Biomarker Discovery**
- **Proteomics**
  - iTRAQ
  - MALDI-TOF-TOF Mass Spectrometry
- **Genomics**
  - Affymetrix Microarray

**Biomarker Development**
- Initial Validation
- Refinement / Validation
- Assay Evaluation
- Assay Migration

**Clinical Implementation**
- Multiplex Protein Assays
- Multiplex Gene Assays

**Selected platform AND selected panel**

**Establish assays that are high throughput for many proteins and genes but cost effective**

*For proteins / genes with available assays*
Transplant Clinics as Beachheads

Value for the largest global healthcare needs

Cardiac Allografts

Heart Failure

Coronary Artery Disease

Renal Allografts

Chronic Kidney Disease

Diabetes
The Life Cycle of Organ Failure

- Baseline Risk
- Disease Presence
- Disease Progression
- "Recovered" Organ Function
- Earlier Intervention
- Improved Organ Function
- End-stage Organ Failure
- Recurrent Organ Failure
- Transplantation/Assist Devices
Heart Failure (HF)

Chronic Systolic HF
“Weak Heart”

Chronic Diastolic HF
“Stiff Heart”

Acute HF
“Stressed Heart”

Less blood is pumped out of the ventricles.

Weakened heart muscle can’t squeeze as well.

Diagnostic biomarkers distinguish Diastolic from Systolic Heart Failure.

Ventricular Assist Device (VAD)

Diagnostic markers determine if or when the VAD can be removed.

Biomarker signatures that return to normal after treatment.
COPD Biomarker Program

- **Problem:** 50-80% of COPD patients are under-diagnosed
  - The current functional biomarker, FEV1, is insensitive
  - Lack of surrogate endpoints inhibit development of new therapies

- **Goal:** Using a non-targeted biomarker discovery approach, identify novel blood-based biomarkers to...
  - Risk-stratify patients for exacerbations
  - Develop and qualify new compounds and drugs for the treatment of patients with COPD

- **Cohort:** GlaxoSmithKline ECLIPSE Cohort (~2600 COPD patients and controls)

- **Outcomes:**
  - Simple, early and accurate diagnosis of COPD to allow for effective treatment and earlier management of the disease
  - Screening tool or surrogate marker to shorten clinical trials or create a new drug target
Chronic Kidney Disease

CLINICAL PRESENTATION

CKD

STABLE

F

Organ Function

Time

DEATH

DIALYSIS/TRANSPLANTATION

CVD-RELATED DEATH

Biomarker panel to predict progression versus non-progression reduces unnecessary evaluation and medication.

Biomarker panel to monitor response to medication reduces unnecessary drug use.
PROOF Centre Business Model

A collaborative, flexible approach

Contract Services

Discover
Develop
Commercialize
Implement

Prevent
Predict
Diagnose
Manage
Treat

Heart failure
Lung failure
Kidney failure

Improved
Health Care
Economic
Development

BIOMARKER SOLUTIONS

In-license
Technology co-development
Companion diagnostics
Validation trials

Out-license
Spin-offs
Thank You