PROOF
Centre of | Centre d'
EXCELLENCE

Biomarqueurs – Solutions en soins de santé.

Biomarker solutions for health care.

PROOF of the Pudding in Canada

2010 ITMAT International Symposium

Wednesday, October 27, 2010

Bruce McManus

Government of Canada Networks of Centres of Excellence
Gouvernement du Canada Réseaux de centres d'excellence
a place of mind THE UNIVERSITY OF BRITISH COLUMBIA

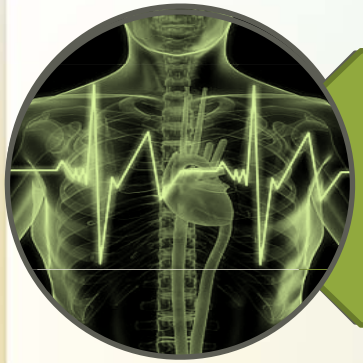
PROOF Centre Background

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

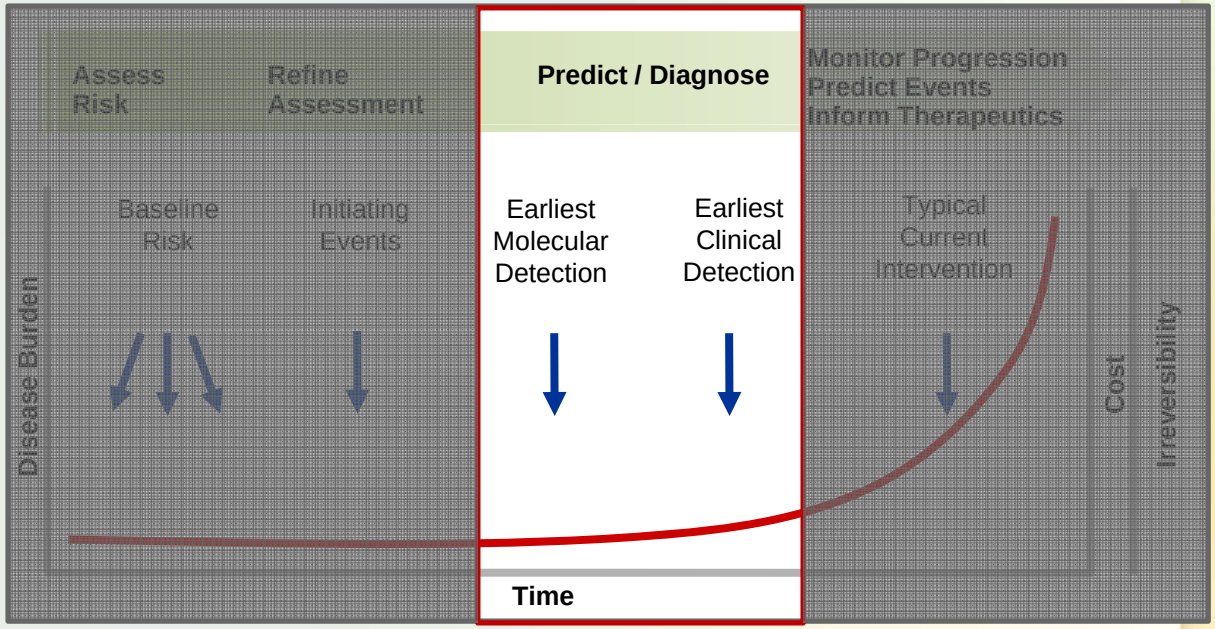
www.proofcentre.ca

Biomarkers



Distinct biological indicators (cellular, biochemical or molecular) of a process, event or condition that can be measured reliably in tissues, cells or fluids

- Sensitive and specific
- Reproducible and cost effective assay + platform
- Temporal relationship with clinical status
- Add value to current clinical tools

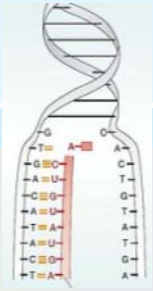


PROOF Centre Focus on “-Omics”

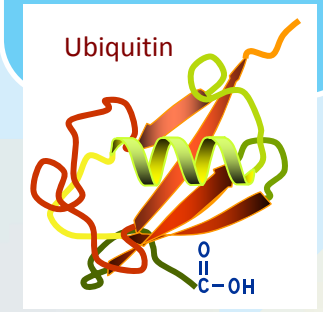
Integration of whole blood genomics and plasma proteomics adds value as they reflect different biomarker compartments

DNA

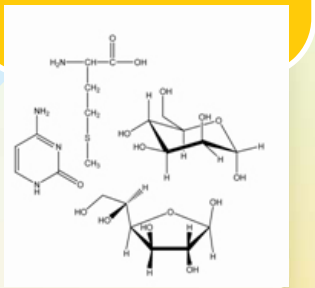
RNA



Protein



Metabolite



Epigenetics

Genetics / Genotype

Genomics / Transcriptomics

Proteomics

Metabolomics

PROOF Centre Mission

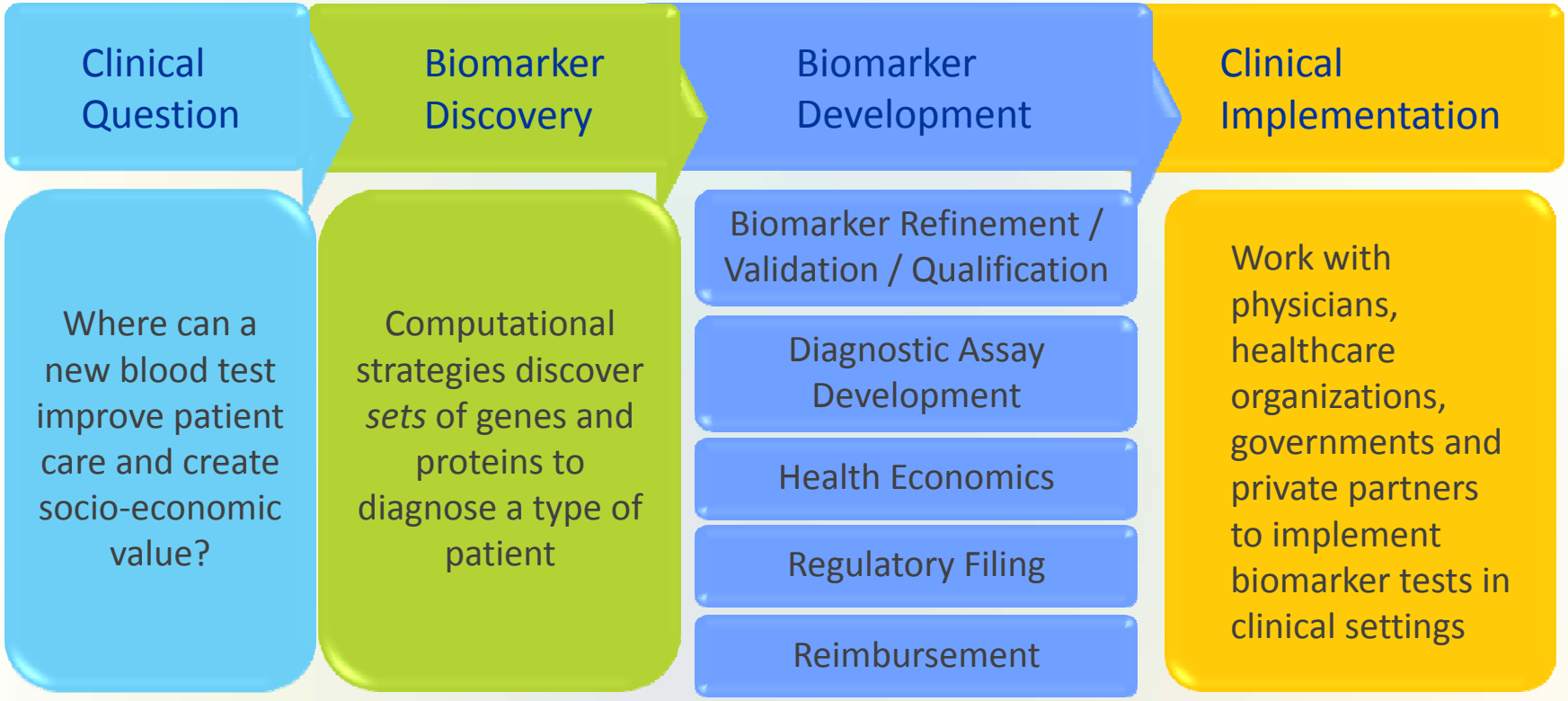
Can we do better?

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



Our Community of Partners

Patient Cohorts

Technology Platforms

USC/CHLA Microarray Core

Computation

Financial Resources

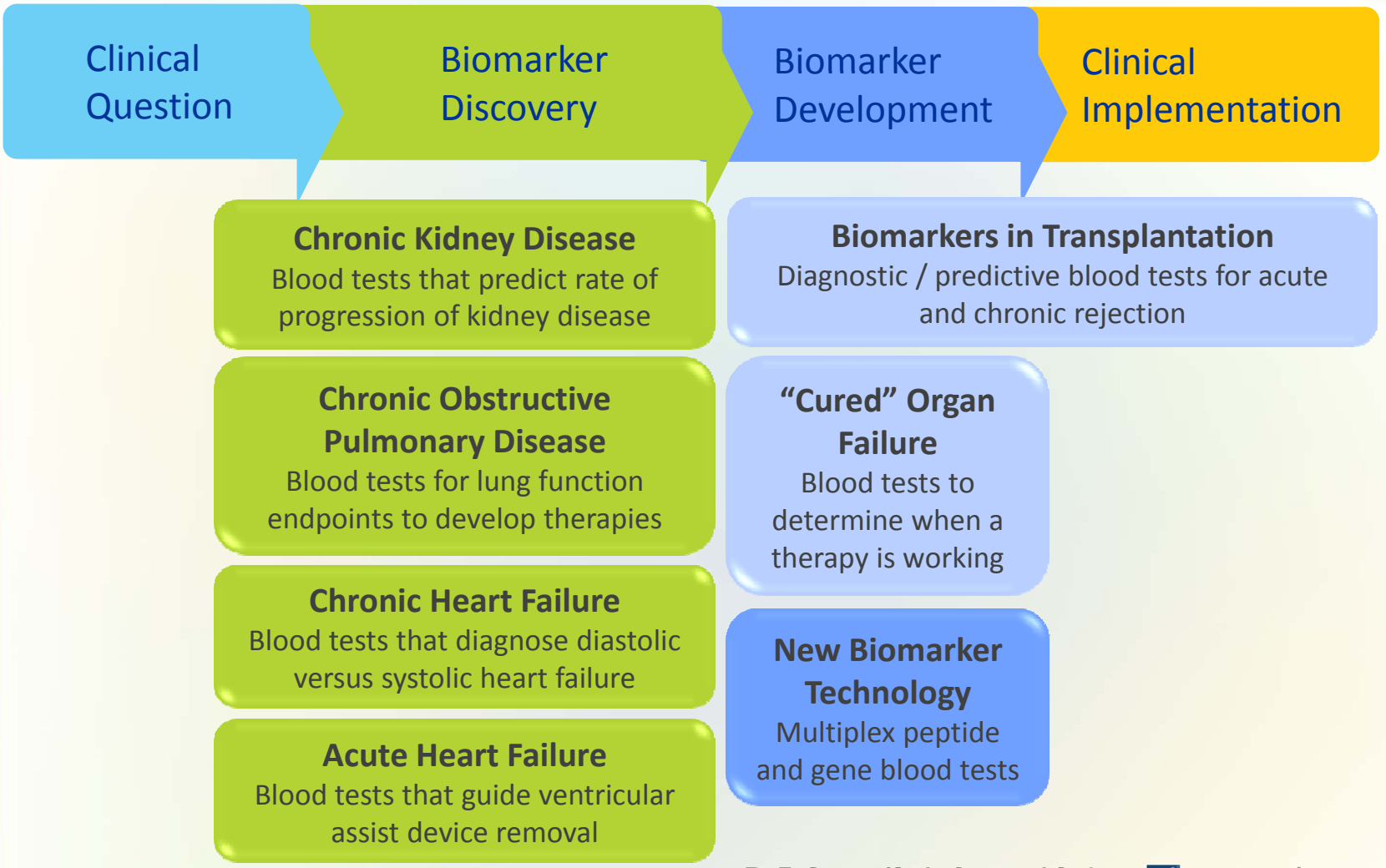
Biomarker Science

Health Economics

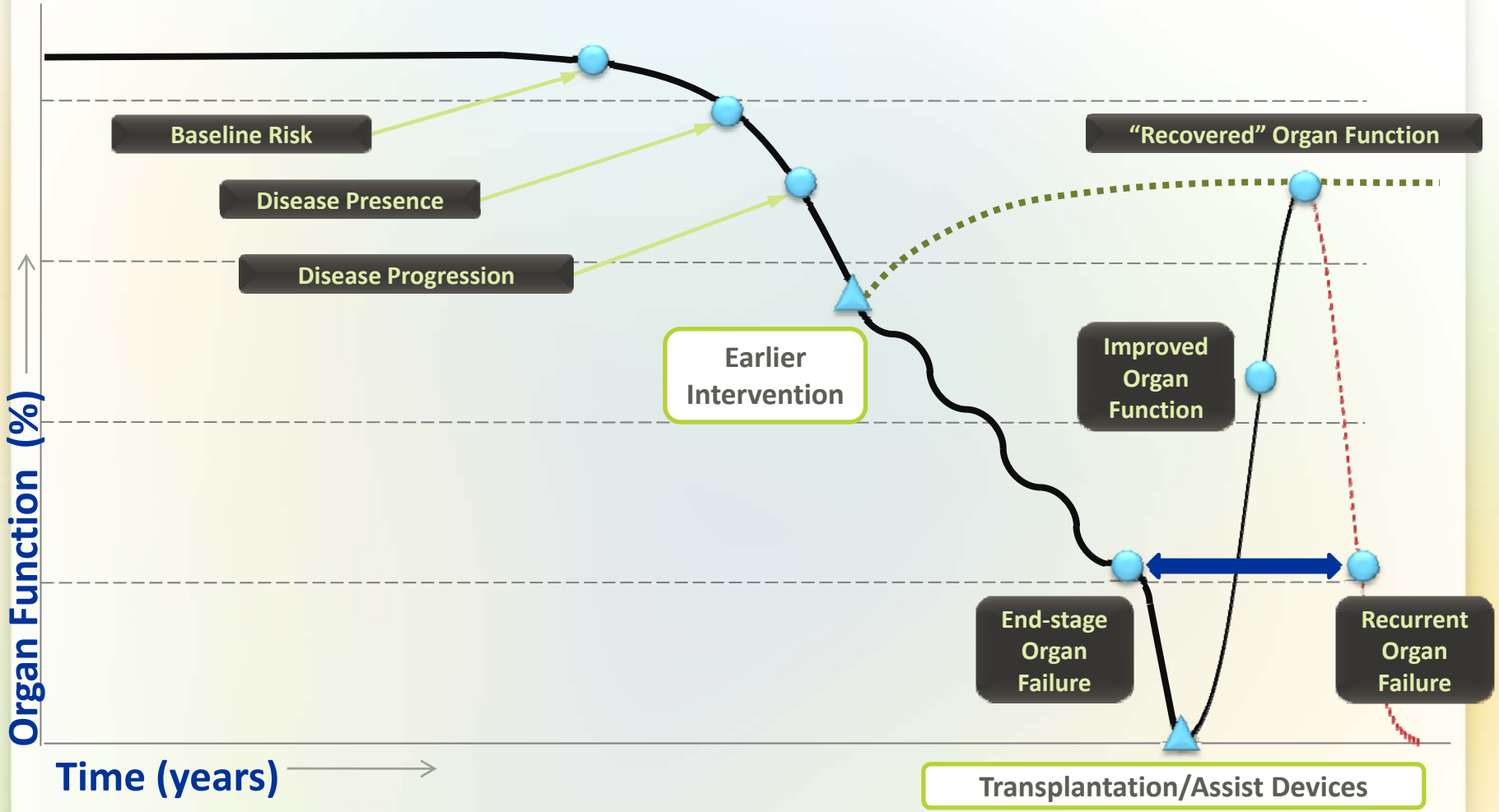
Health Systems

Commercialization

Biomarker Programs

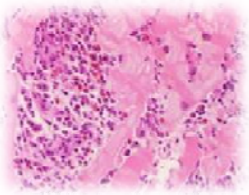


The Life Cycle of Organ Failure

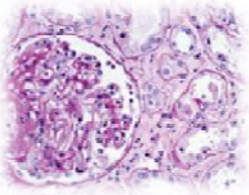


Acute Organ Rejection

Current diagnostic approaches



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

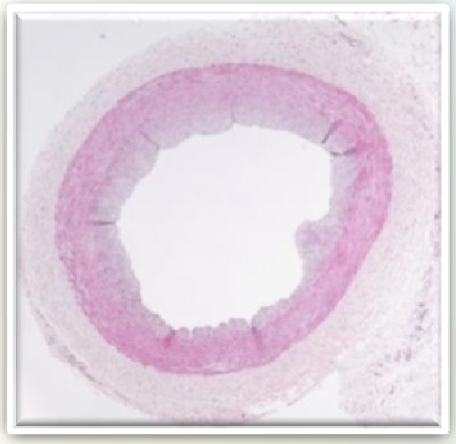


- HIGHLY INVASIVE
- NOT TIMELY
- EXPENSIVE
- DIAGNOSTIC ONLY, NOT PROGNOSTIC
- UNCOMFORTABLE AND FEAR-EVOKING
- PRONE TO SAMPLING ERROR
- SUBJECT TO INTERPRETATIVE VARIABILITY

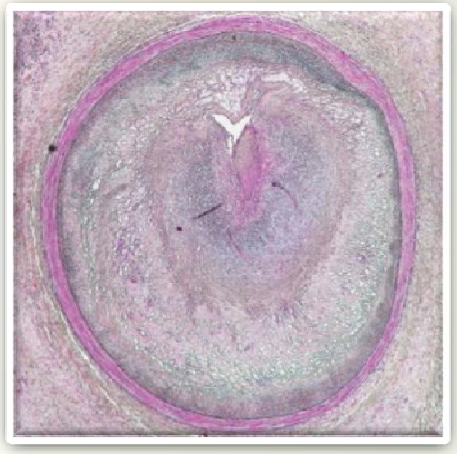
Chronic Organ Rejection

Current diagnostic approaches

Normal Artery

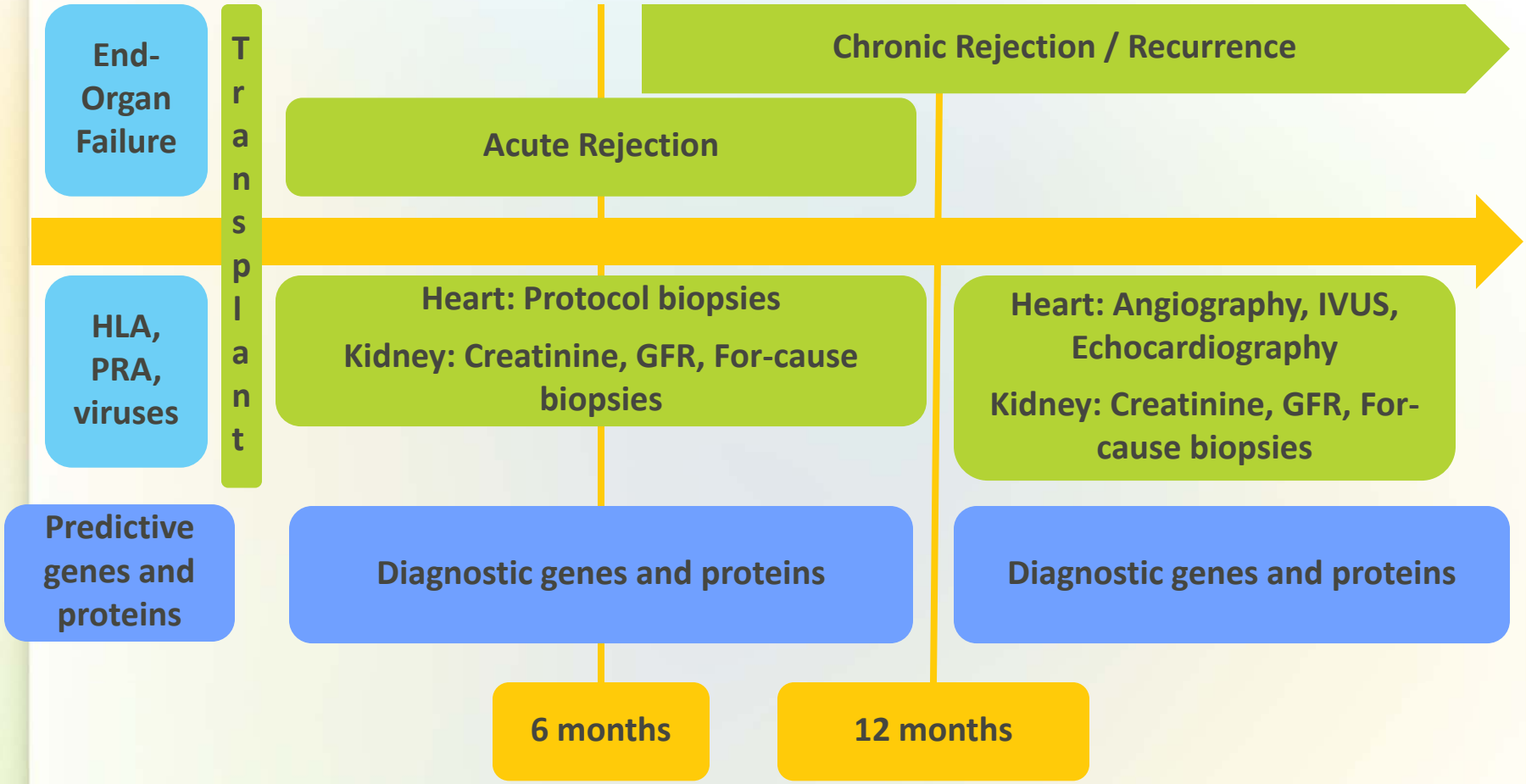


CAV



- 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



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

FDA Voluntary eXploratory Data Submission (VXDS)

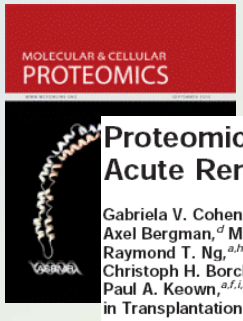
Functional Genomic Analysis of Peripheral Blood During Early Acute Renal Allograft Rejection

Oliver P. Günther,[1,2] Robert F. Balshaw,[1,3] Andreas Scherer,[4] Zsuzsanna Hollander,[1,2] Alice Mui,[1,5,6] Timothy J. Triche,[7] Gabriela Cohen Freue,[1,3] Guiyun Li,[8] Raymond T. Ng,[1,9] Janet Wilson-McManus,[1,10] W. Robert McMaster,[1,5,11] Bruce M. McManus,[1,2,10] and Paul A. Keown[1,8,10,12,13]; for the Biomarkers in Transplantation Team

ORIGINAL PRE-CLINICAL SCIENCE

Whole Blood Genomic Biomarkers of Acute Cardiac Allograft Rejection

David Lin, BSc,* Zsuzsanna Hollander, MSc,* Raymond T. Ng, PhD, Carol Imai, BSN, Andrew Ignaszewski, MD, Robert Balshaw, PhD, Gabriela Cohen Freue, PhD, Janet E. Wilson-McManus, BSc, Pooran Qasimi, MSc, Anna Meredith, BSc, Alice Mui, PhD, Tim Triche, MD, PhD, Robert McMaster, D.Phil, Paul A. Keown, MD, and Bruce M. McManus, MD, PhD, for the Biomarkers in Transplantation Team and the NCE CECR Centre of Excellence for the Prevention of Organ Failure



Proteomic Signatures in Plasma during Early Acute Renal Allograft Rejection*

Gabriela V. Cohen Freue,[a,b,c] Mayu Sasaki,[a,d] Anna Meredith,[e,f] Oliver P. Günther,[a,e] Axel Bergman,[d] Mandeep Takhar,[a,e] Alice Mui,[a,d,g] Robert F. Balshaw,[a,b] Raymond T. Ng,[a,h] Nina Opushneva,[a,e] Zsuzsanna Hollander,[a,e,f] Guiyun Li,[i] Christoph H. Borchers,[j] Janet Wilson-McManus,[a,e,f] Bruce M. McManus,[a,e,f] Paul A. Keown,[a,f,i,k] and W. Robert McMaster[a,d,l,m] for the Genome Canada Biomarkers in Transplantation Group

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

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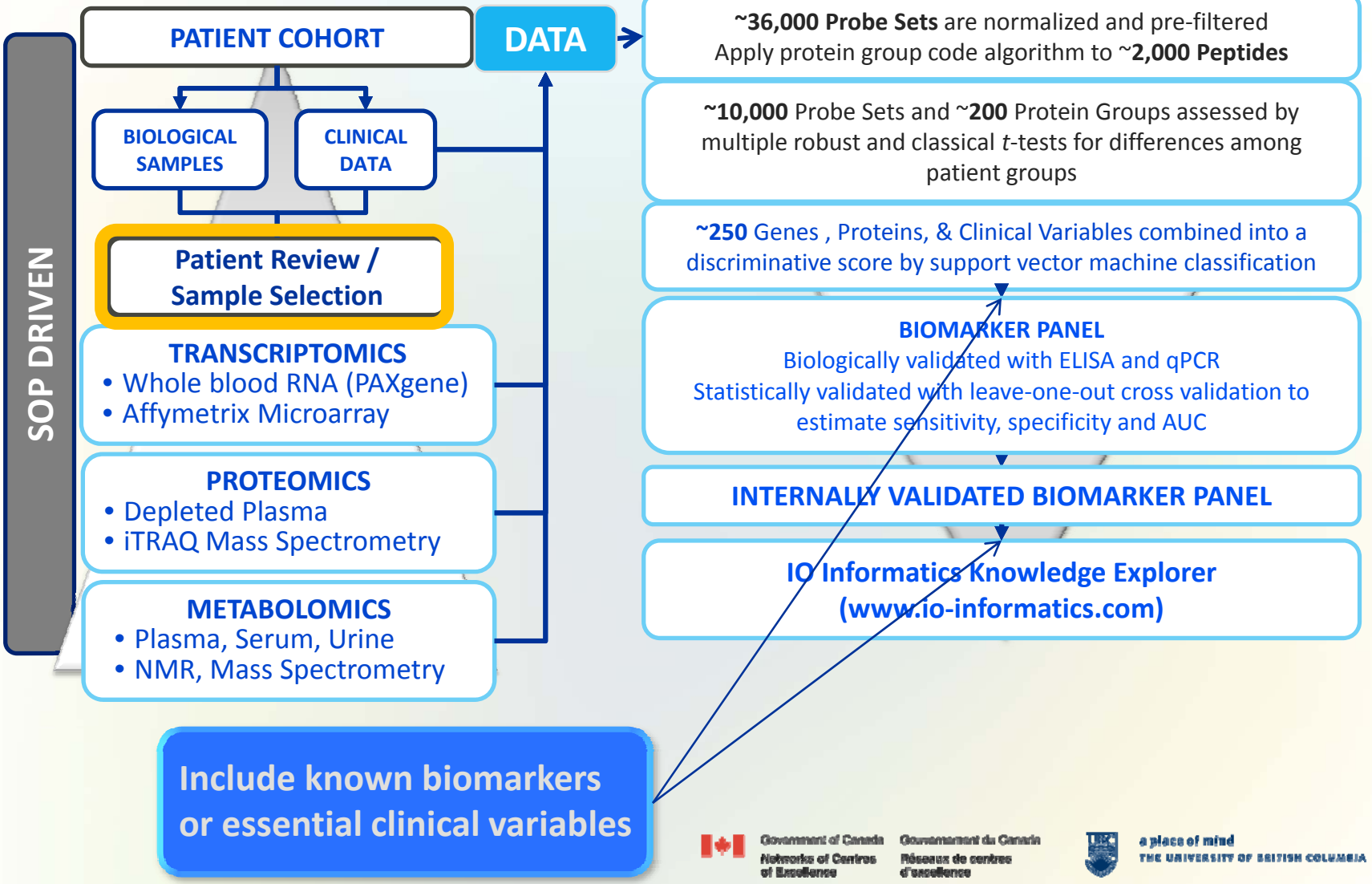
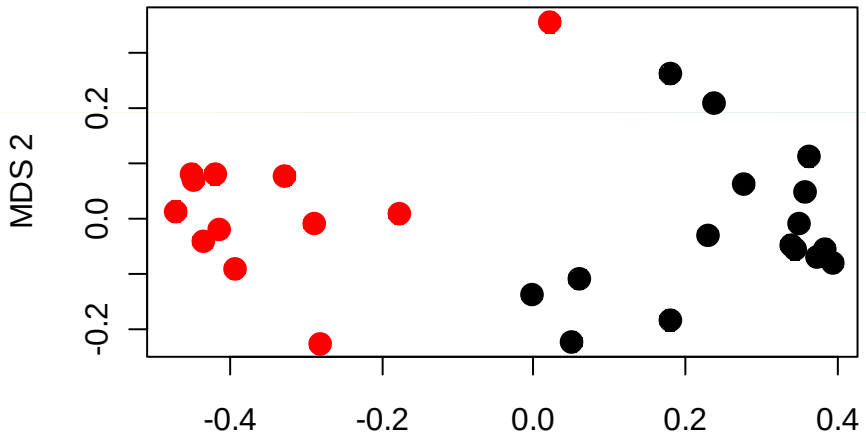
Biomarkers in Transplantation

Discovery strategy



Predictive Markers – Acute Heart Rejection

Whole blood genomics



Sensitivity 83%
Specificity 88%

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

Diagnostic Markers – Acute Heart Rejection

What value does the endomyocardial biopsy add?

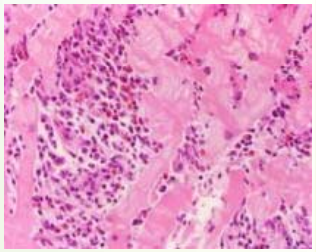
Sensitivity 83%
Specificity 88%

Whole Blood Samples

Endomyocardial Biopsy Tissues

Affymetrix U133 Microarray
54,675 PROBE SETS

Affymetrix U133 Microarray
54,675 PROBE SETS



17,610 PROBE SETS

2,186 PROBE SETS

Elastic Nets

WHOLE BLOOD + BIOPSY PROBE SETS

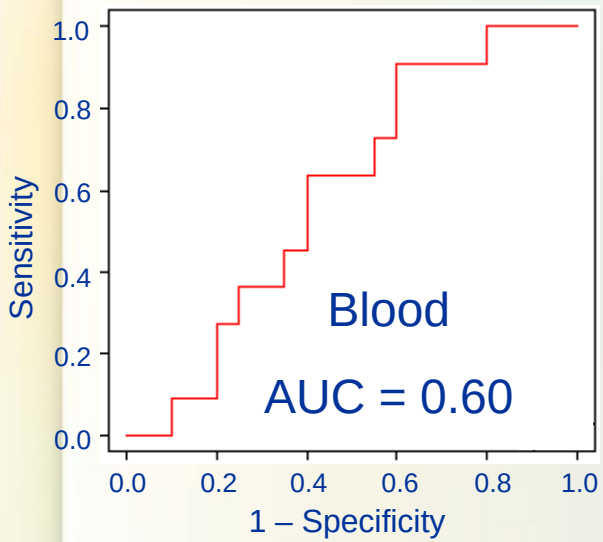
Leave-one-out Cross-Validation

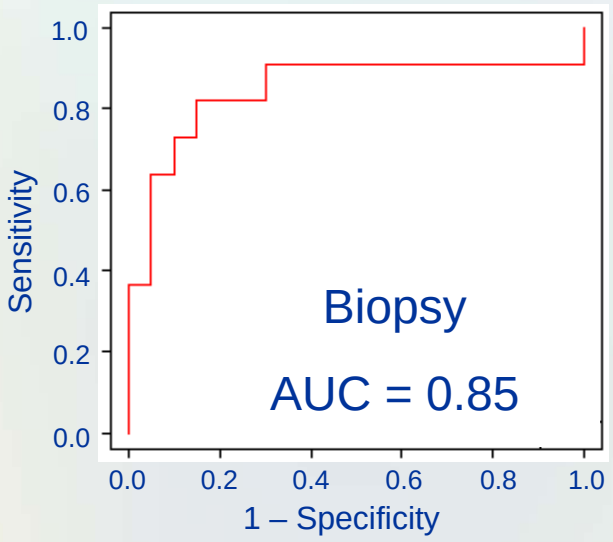
BIOMARKER PANEL

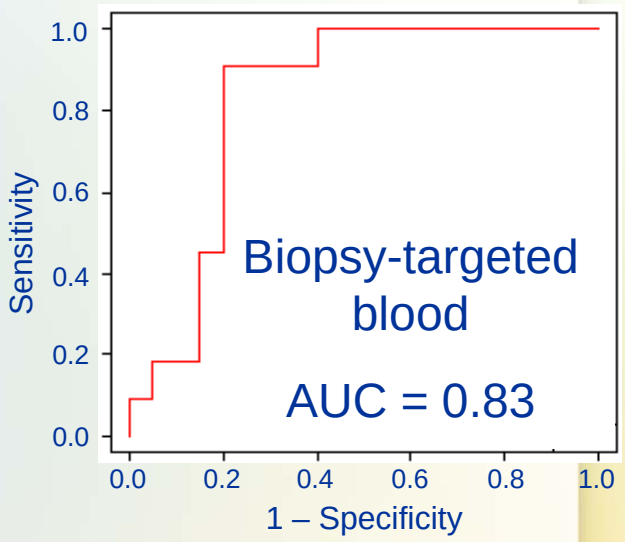
Sensitivity 100%
Specificity 100%

Diagnostic Markers – Acute Heart Rejection

What value does the endomyocardial biopsy add?







(Hollander Z et al ***Transplantation***, in press, December 2010)

Author's Choice

Proteomic Signatures in Plasma during Early Acute Renal Allograft Rejection*

Gabriela V. Cohen Freue,[a,b,c] Mayu Sasaki,[a,d] Anna Meredith,[a,f] Oliver P. Günther,[a,e] Axel Bergman,[d] Mandeep Takhar,[a,e] Alice Mui,[a,d,g] Robert F. Balshaw,[a,b] Raymond T. Ng,[a,h] Nina Opushneva,[a,e] Zsuzsanna Hollander,[a,e,f] Guiyun Li,[i] Christoph H. Borchers,[j] Janet Wilson-McManus,[a,e,f] Bruce M. McManus,[a,e,i] Paul A. Keown,[a,f,i,k] and W. Robert McMaster[a,d,l,m] for the Genome Canada Biomarkers in Transplantation Group

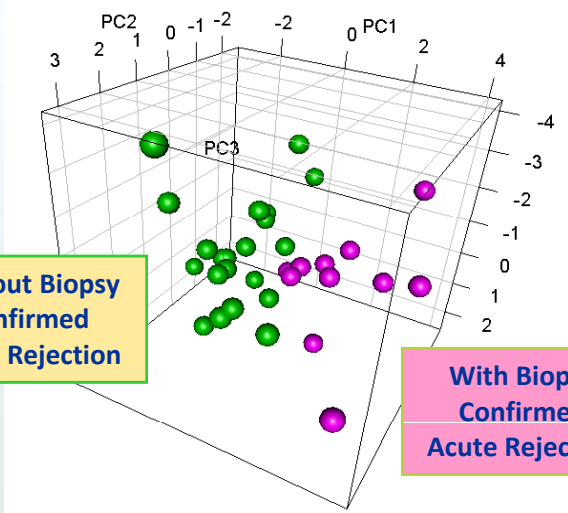
Acute graft rejection is an important clinical problem in renal transplantation and an adverse predictor for long term graft survival. Plasma biomarkers may offer an important option for post-transplant monitoring and permit timely and effective therapeutic intervention to minimize graft damage. This case-control discovery study ($n = 32$) used isobaric tagging for relative and absolute protein quantification (ITRAQ) technology to quantitate plasma protein relative concentrations in precise cohorts of patients with and without biopsy-confirmed acute rejection (BCAR). Plasma samples were depleted of the 14 most abundant plasma proteins to enhance detection sensitivity. A total of 18 plasma proteins that encompassed processes related to inflammation, complement activation, blood coagulation, and wound repair exhibited significantly different relative concentrations between patient cohorts with and without BCAR (p value <0.05). Twelve proteins with a fold-change ≥ 1.15 were selected for diagnostic purposes: seven were increased (titin, lipopolysaccharide-binding protein, peptidase inhibitor 16, complement factor D, mannose-binding lectin, protein Z-dependent protease and β_2-microglobulin) and five were decreased (kininogen-1, afamin, serine protease inhibitor, phosphatidylcholine-sterol acyltransferase, and sex hormone-binding globulin) in patients with BCAR. The first three principal components of these proteins showed clear separation of cohorts with and without BCAR. Performance improved with the inclusion of sequential proteins, reaching a primary asymptote after the first three (titin, kininogen-1, and lipopolysaccharide-binding protein). Longitudinal monitoring over the first 3 months post-transplant based on ratios of these three proteins showed clear discrimination between the two patient cohorts at time of rejection. The score then declined to baseline following treatment and resolution of the rejection episode and remained comparable between cases and controls throughout the period of quiescent follow-up. Results were validated using ELISAwhere possible, and initial cross-validation estimated a sensitivity of 80% and specificity of 90% for classification of BCAR based on a four-protein ELISA classifier. This study provides evidence that protein concentrations in plasma may provide a relevant measure for the occurrence of BCAR and offers a potential tool for immunologic monitoring. *Molecular & Cellular Proteomics* 9:1954–1967, 2010.

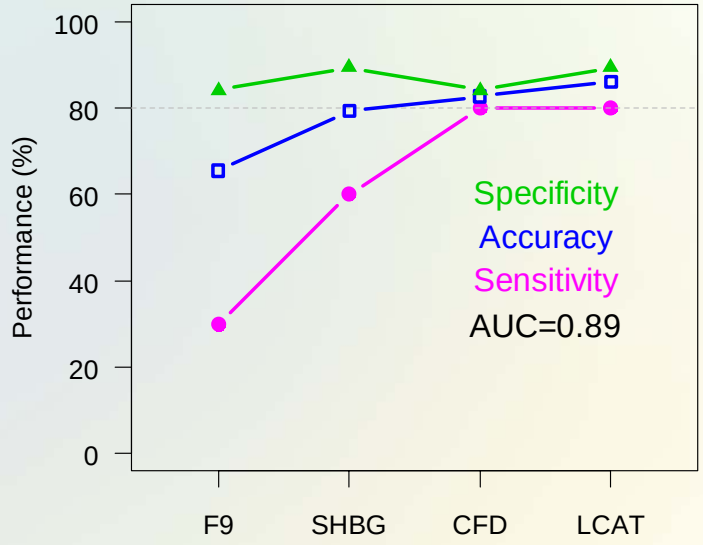
From the [a]Prevention of Organ Failure (PROOF) Centre of Excellence, Vancouver, British Columbia V6Z 1Y6, [b]Department of Statistics, University of British Columbia, Vancouver, British Columbia V6T 1Z2, [c]Immunity and Infection Research Centre, Vancouver, British Columbia V5Z 3J5, [d]James Hogg Imaging, Cell Analysis, and Phenotyping Toward Understanding Responsive, Reparative, Remodelling, and Recombinant Events (iCAPTURE) Centre, Vancouver, British Columbia V6Z 1Y6, [e]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia V6T 2B5, [f]Department of Surgery, University of British Columbia, Vancouver, British Columbia V5Z 4E3, [g]Department of Computer Science, University of British Columbia, Vancouver, British Columbia V6T 1Z4, [h]Department of Medicine, University of British Columbia, Vancouver, British Columbia V5Z 1M9, [i]University of Victoria Genome BC Proteomics Centre, Victoria, British Columbia V8Z 7X8, [j]Immunology Laboratory, Vancouver General Hospital, Vancouver, British Columbia V5Z 1M9, and [k]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

*Author's Choice—Final version full access.

Received, May 7, 2010
Published, MCP Papers in Press, May 25, 2010, DOI 10.1074/mcp.M110.000554

Although advances in immunosuppression have increased the success of renal transplantation continuously during the past decades, immunological injury to the graft remains a critical barrier to long term survival (1–4). Both innate and immune responses are implicated in the process of graft rejection (5–7). Major and minor histocompatibility antigens expressed on graft tissue are quickly identified following implantation through direct or indirect pathways of the innate response, and consequent activation of T-cell and B-cell components of the host adaptive immune response leads to cellular and antibody-mediated injury to numerous structural components of the grafted organ (8). The resulting inflammatory sequence comprising cellular infiltration, antibody production, complement deposition, and activation of the coagulation cascade can be identified by histological changes on allograft biopsy (biopsy-con-





Plasma biomarkers measured by ELISA

Molecular & Cellular Proteomics 9, Sept 2010

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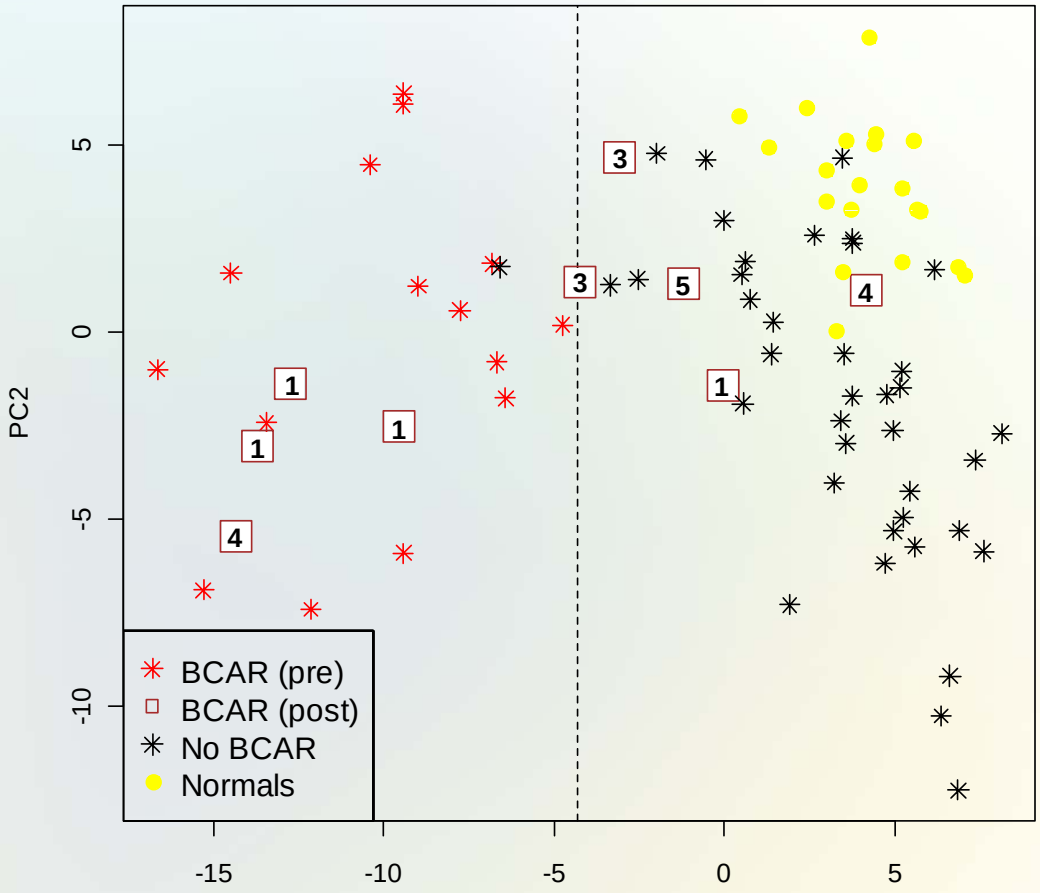
Diagnostic Markers – Acute Renal Rejection

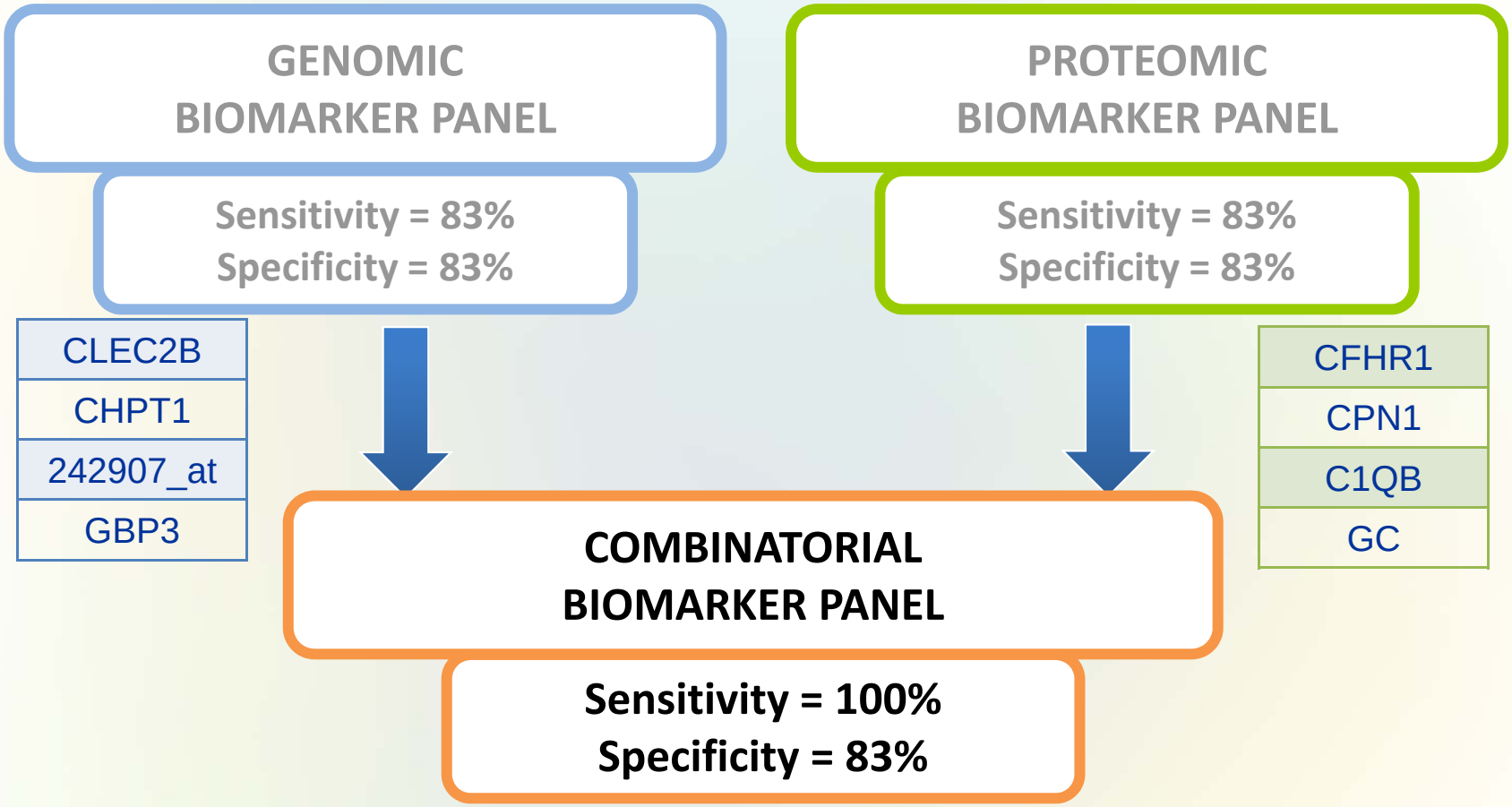
Effect of time post-transplant on diagnosis by biomarkers



(Gunther O et al, ***Transplantation***, in press)

Cardiac Allograft Vasculopathy

Combinatorial biomarker panel



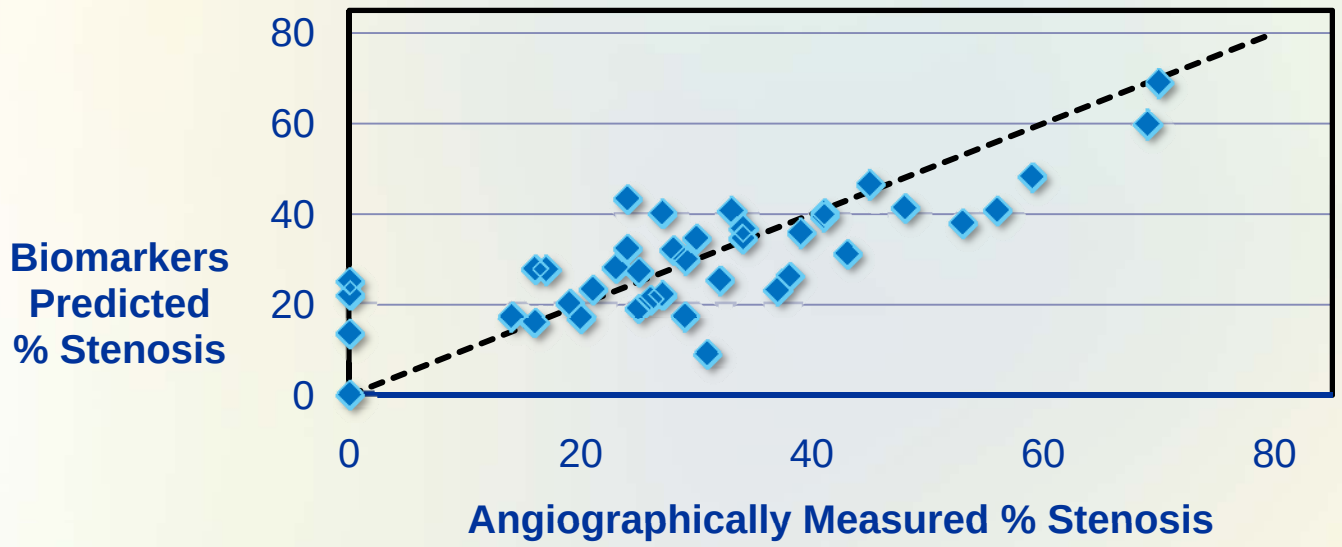
Cardiac Allograft Vasculopathy

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)



The dotted line represents where the ‘predicted’ and the ‘true’ % stenosis are exactly the same

Pearson’s Correlation (R) = 0.79

(between the 'predicted’ and the ‘true’ stenosis)

Biomarkers in Transplantation

Moving from development to the clinic

2009 → 2011

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

In vitro diagnostic regulatory submissions

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

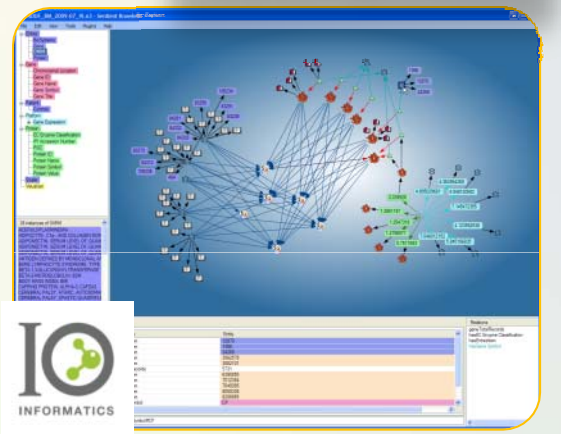
Funded by PROOF Centre of Excellence, Genome British Columbia, Astellas, St. Paul's Hospital Foundation, UBC, BC Transplant, Luminex

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

Classifier Generation	1) Linear Discriminant Analysis 2) Support Vector Machine 3) Random Forest 4) Elastic Net

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

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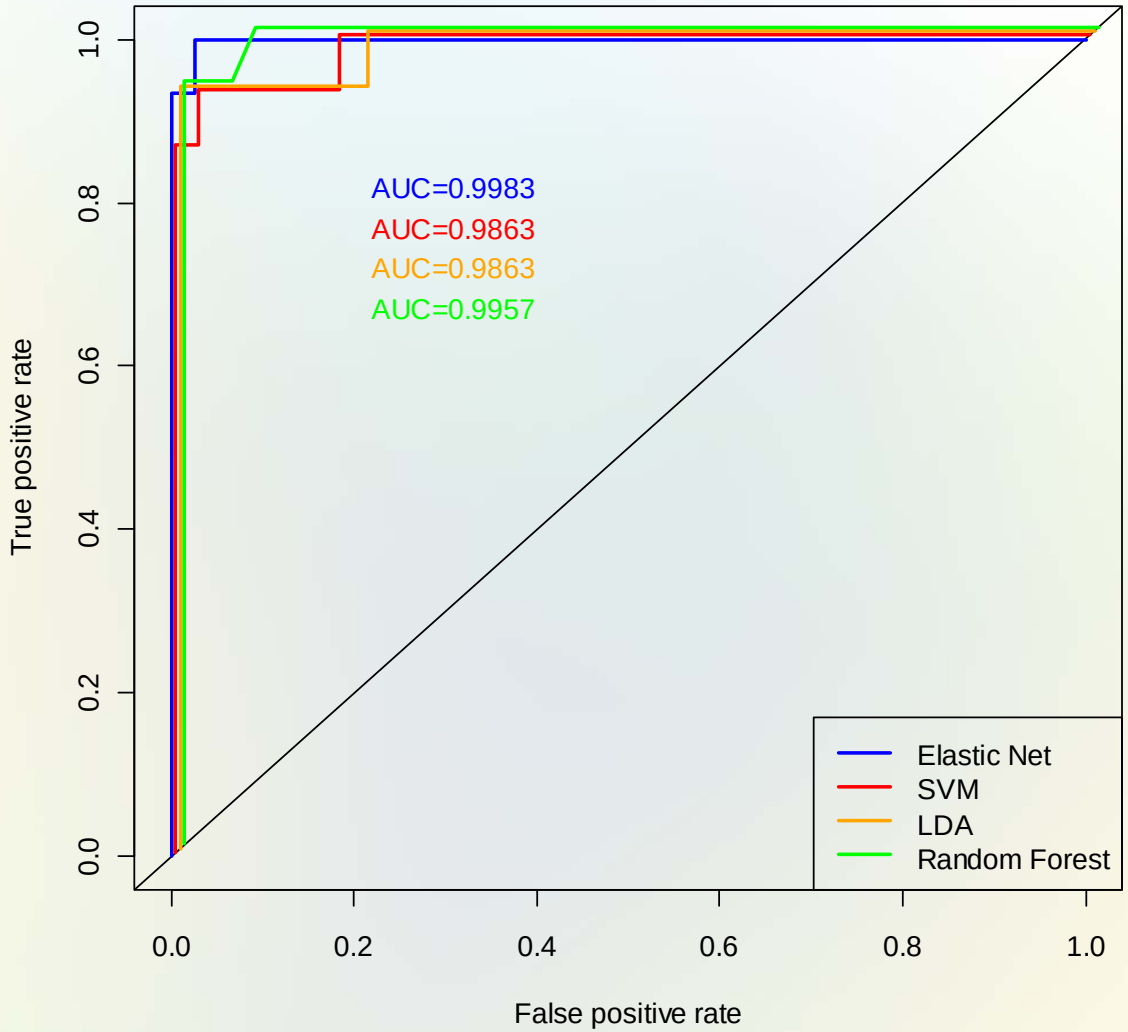
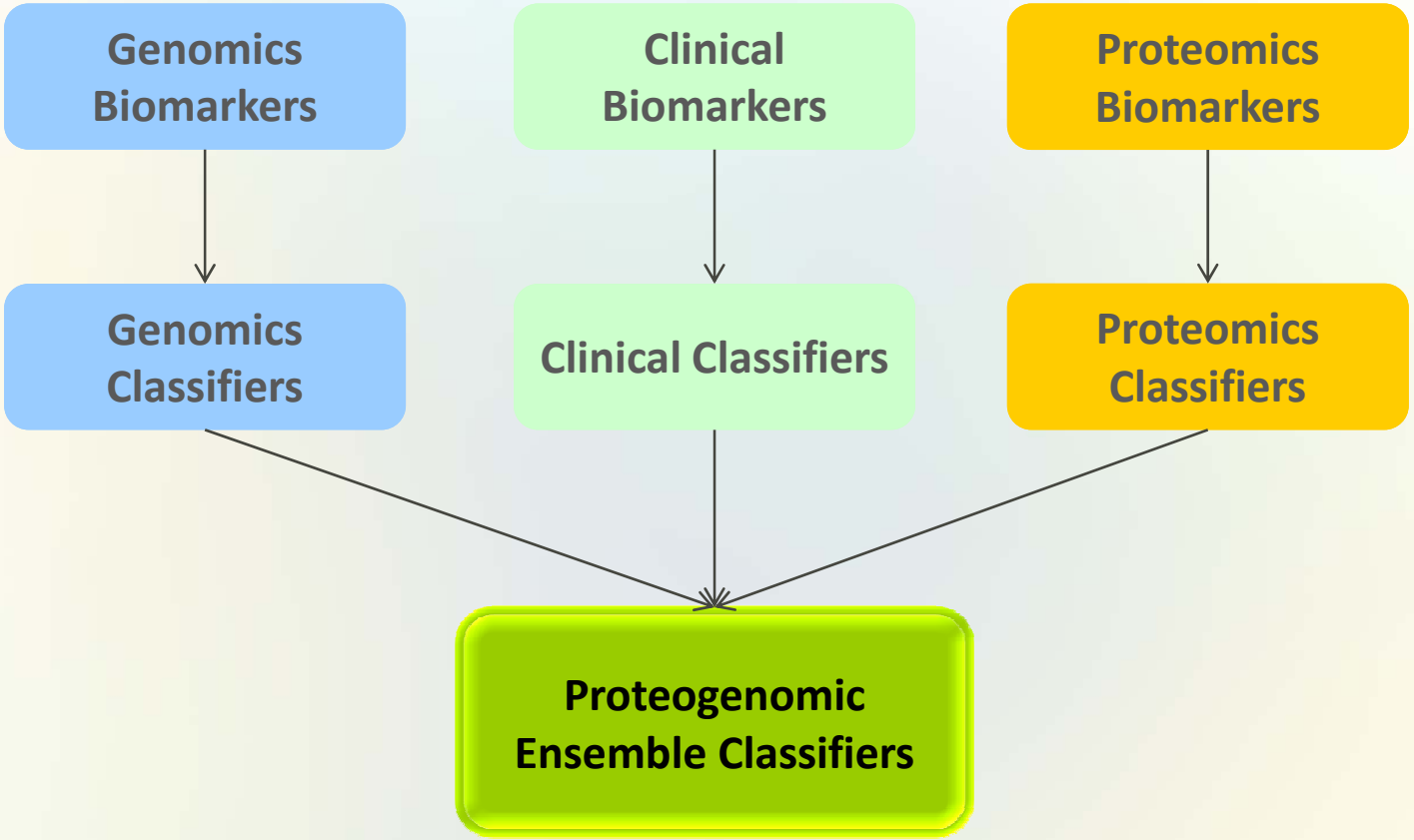
Biomarker Panel Refinement

Improving the AUC for diagnosis of acute renal rejection



Combining Classifier Panels

Harvesting the art and science of the ensemble

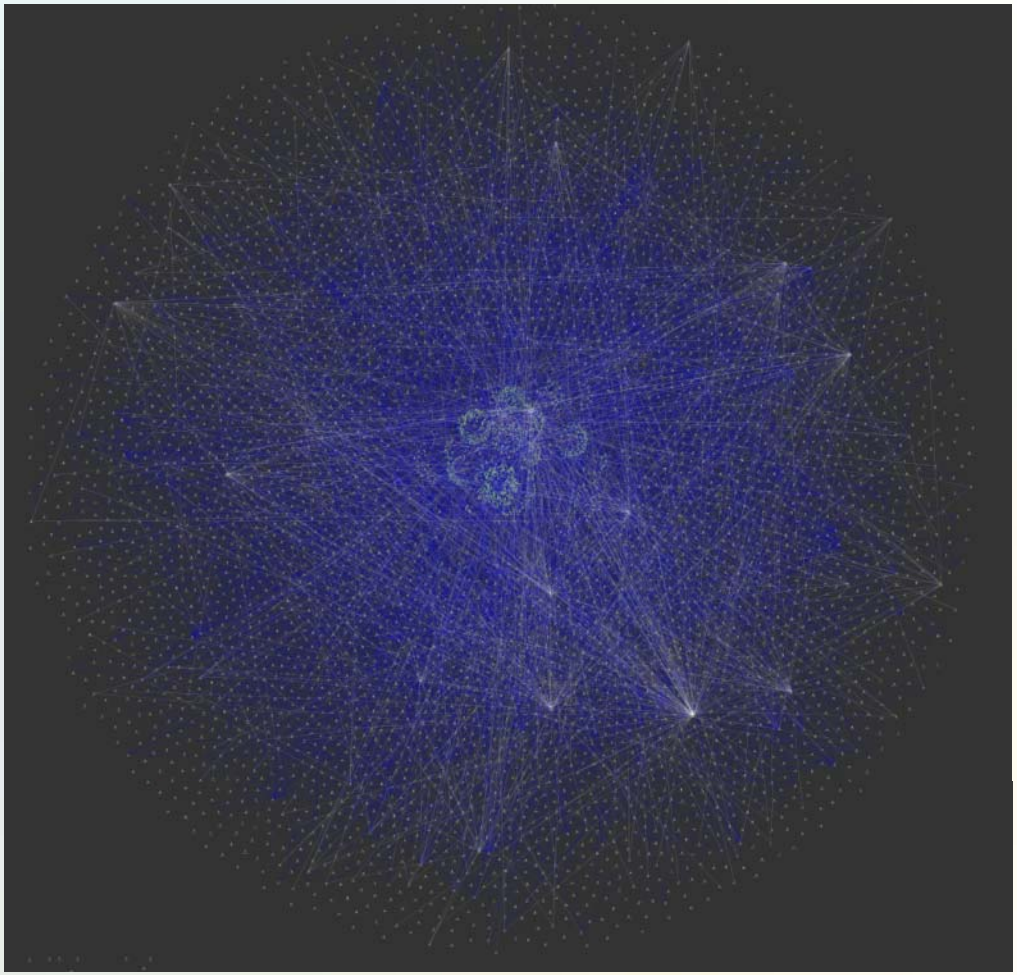


Network Analysis of Predictive Signatures

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

Gene (PROOF genes in yellow)

Disease (or phenotype)

Drug

Tissue or organ

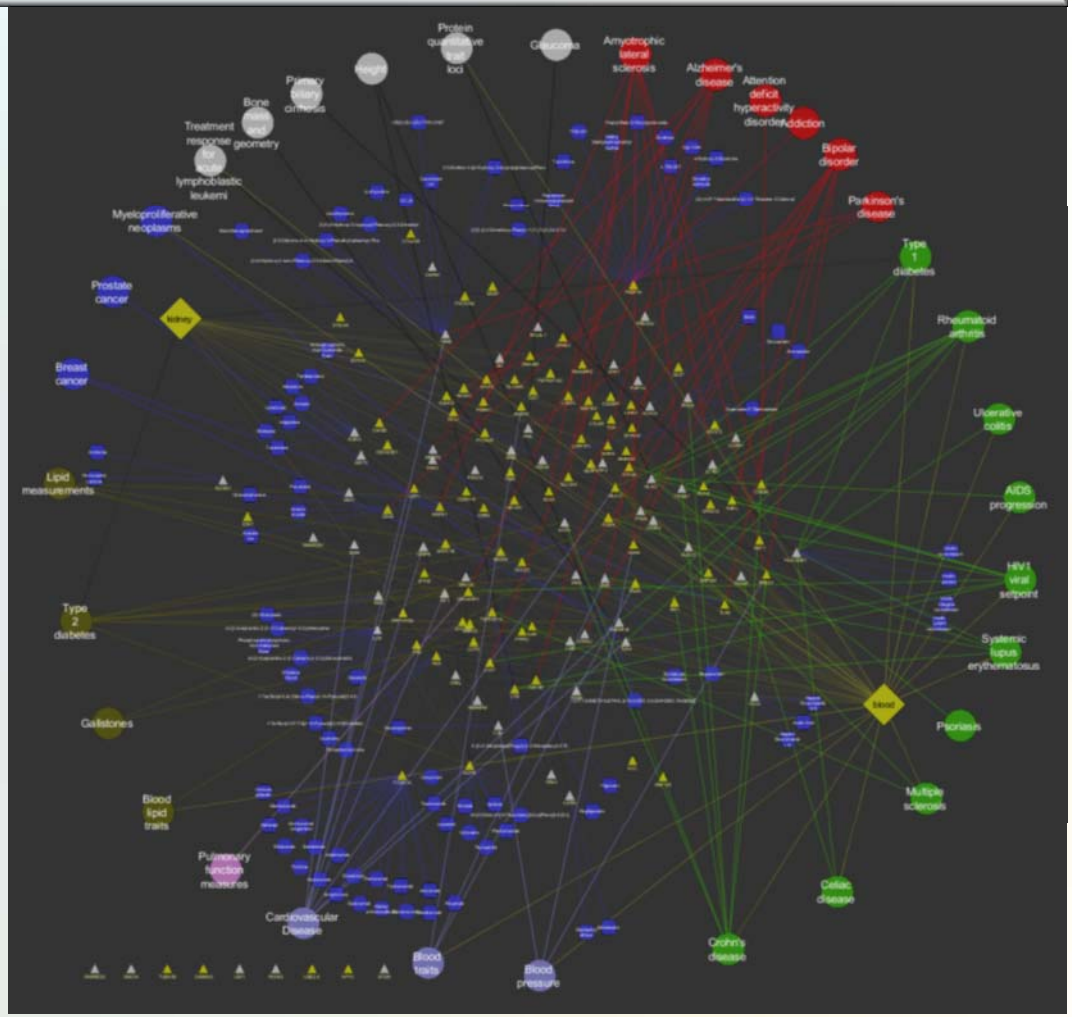
Protein-protein

Protein-protein

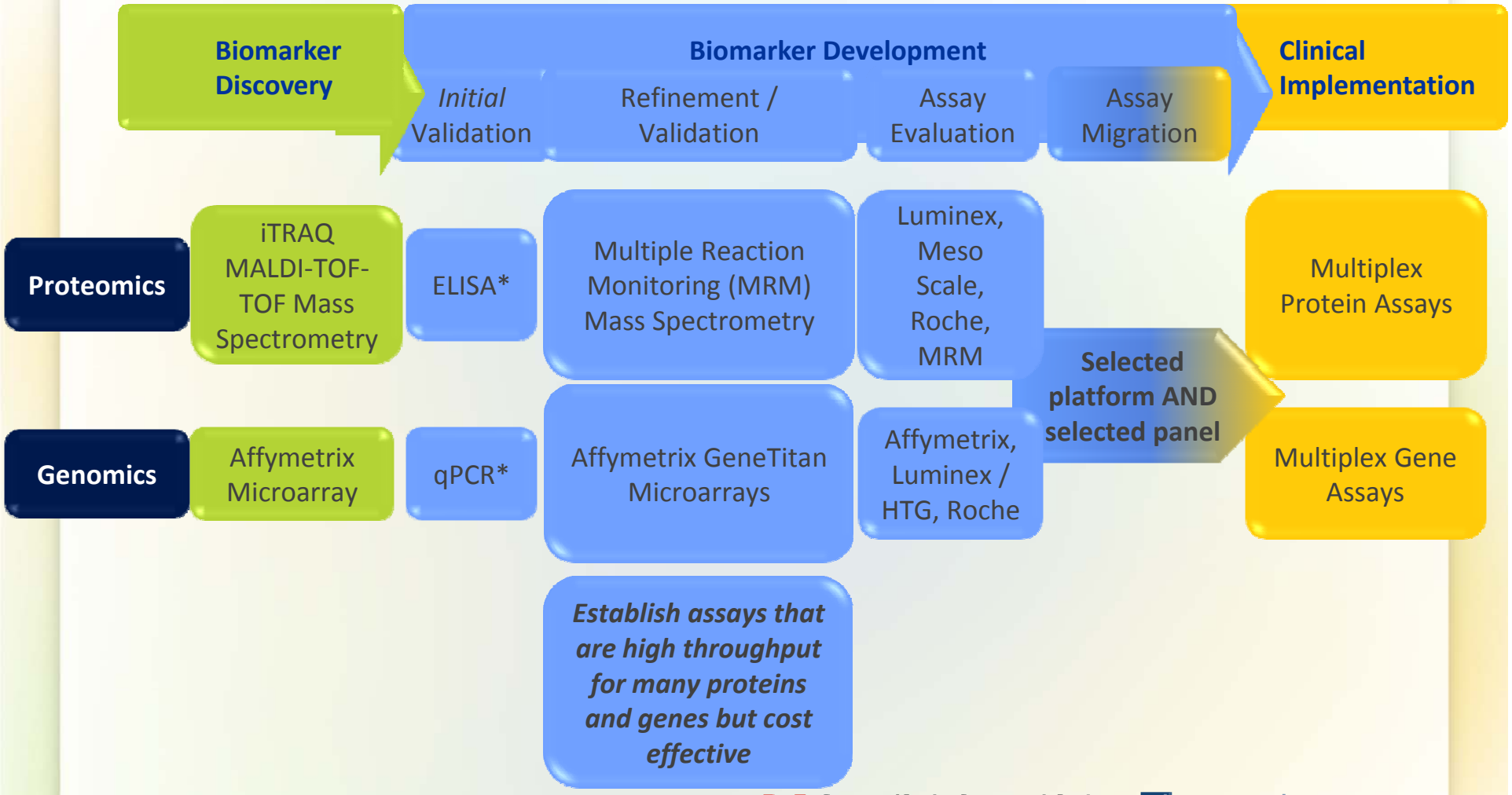
Disease-gene (color indicates disease class)

Tissue-gene

Drug-target



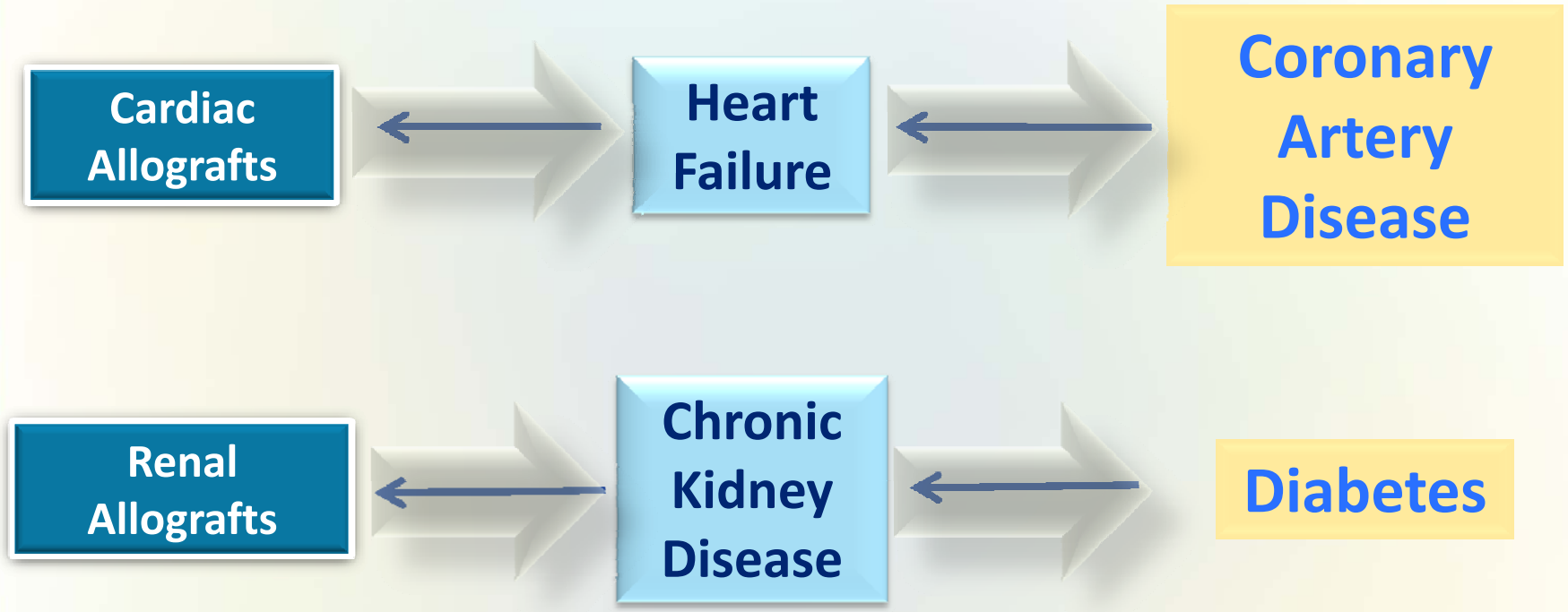
Diagnostic Assay Development



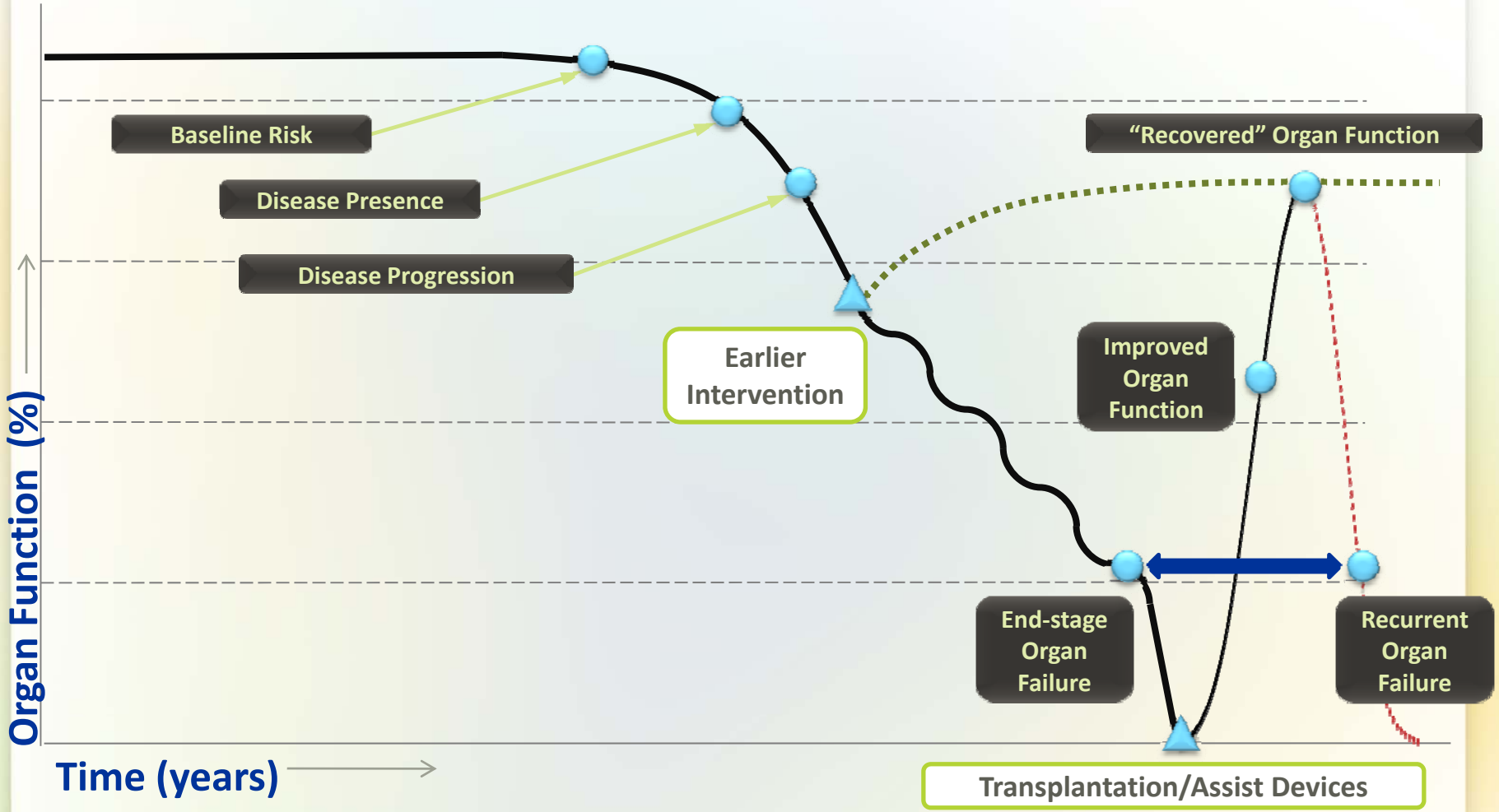
*For proteins / genes with available assays

Transplant Clinics as Beachheads

Value for the largest global healthcare needs

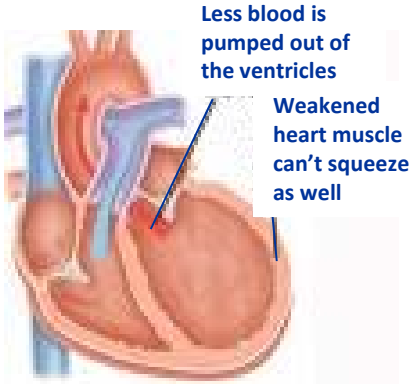


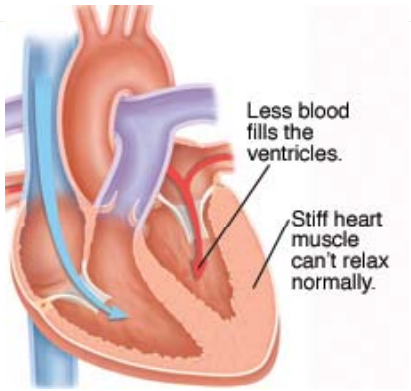
The Life Cycle of Organ Failure

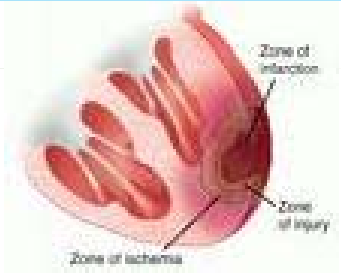


Heart Failure (HF)

Chronic Systolic HF "Weak Heart"	Chronic Diastolic HF "Stiff Heart"	Acute HF "Stressed Heart"







Ventricular Assist Device (VAD)

Diagnostic biomarkers distinguish Diastolic from Systolic Heart Failure

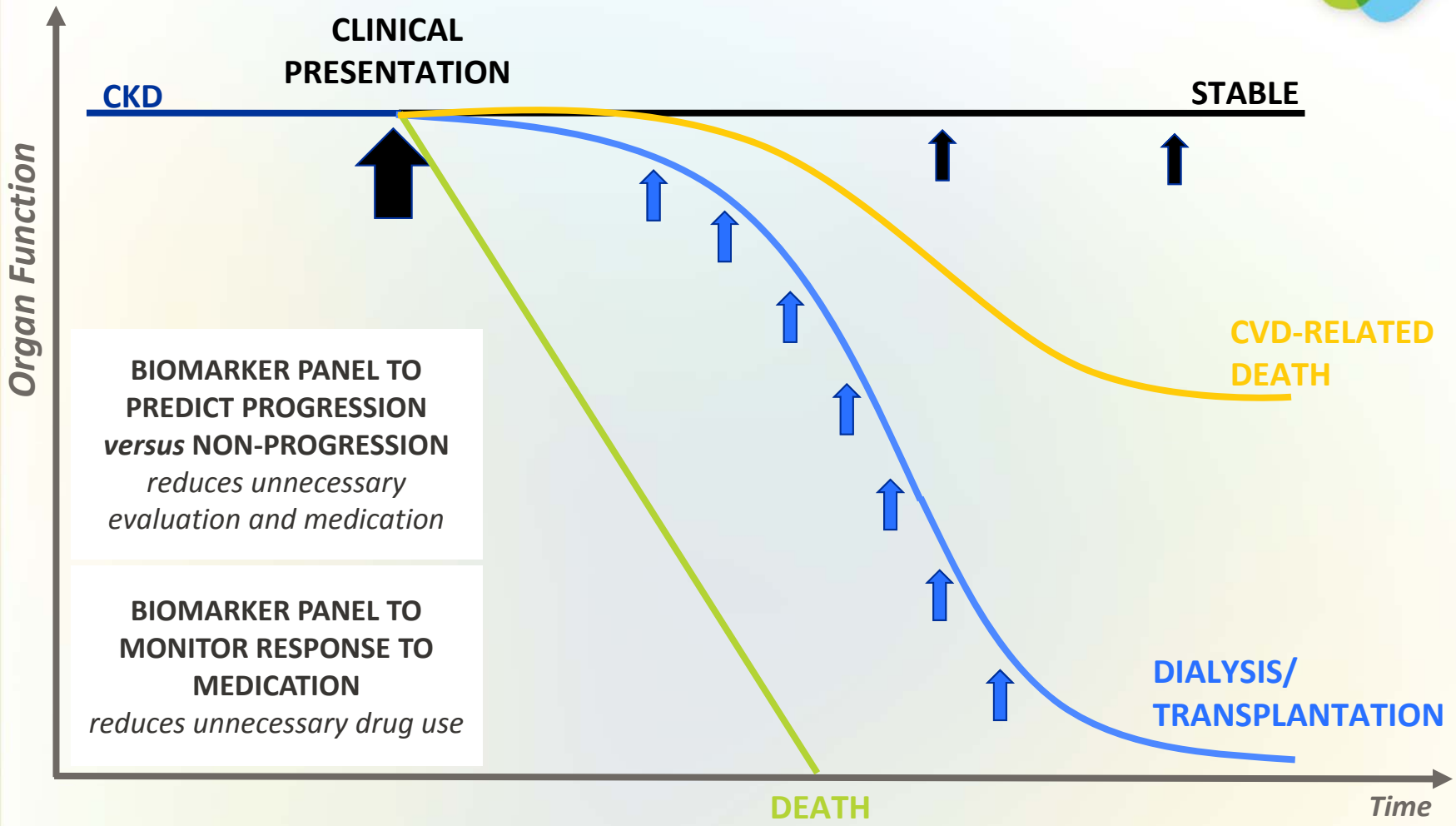
Diagnostic markers determine *if or when* the VAD can be removed

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

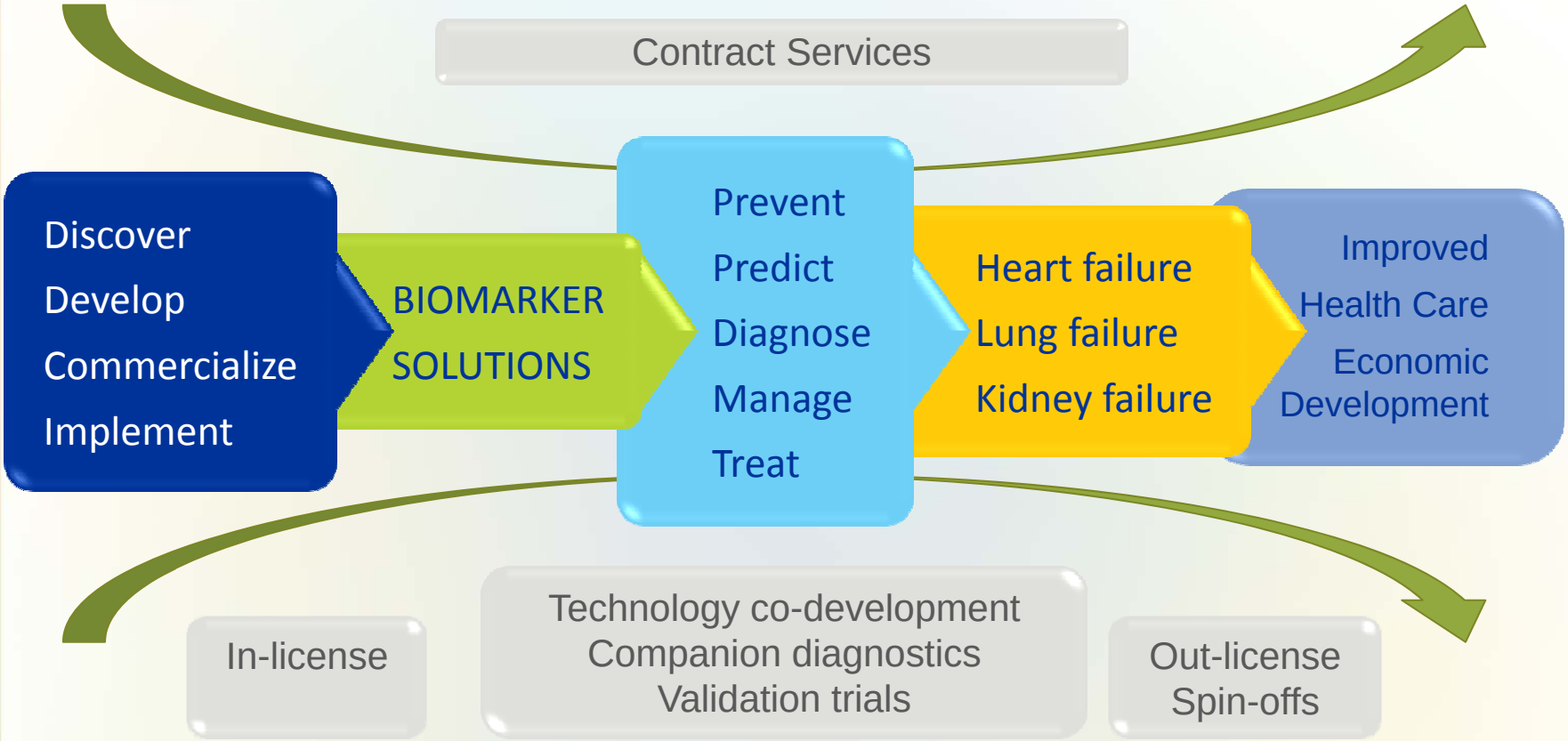
Chronic Kidney Disease



Government of Canada Networks of Centres of Excellence
Gouvernement du Canada Réseaux de centres d'excellence
a place of mind THE UNIVERSITY OF BRITISH COLUMBIA

PROOF Centre Business Model

A collaborative, flexible approach



Thank You