• **Acute Rejection** – acute immune attack with cellular or antibody mediated injury and associated inflammation

• **Chronic Rejection** – persistent immune attack with injury, repair, remodeling, fibrosis and failure

• **Accommodation** – immune acceptance of the transplanted organ maintained by immunosuppression

• **Tolerance** – immune acceptance of the transplanted organ comparable to the recipient’s own tissues
DESIGN OF BIOMARKERS PROGRAM

Pre-transplant Blood, Tissue and Data (Baseline Controls)

Solid Organ Transplant

Acute Rejection  Accommodation  Chronic Rejection

Post-transplant Blood, Urine, Tissue, and Data Collection

Tissue  Lymphocytes  Serum  Urine

Genomics  Proteomics
TRANSPLANT PATIENTS
Pre-transplant through 3 years post-transplant

CLINICAL DATA

BIOLIBARY SAMPLES
• Blood, Urine, Tissue

Transcriptomics

Proteomics

Metabolomics

BIOMARKER DATABASE

54,000 Probe Sets
2,000 Peptides or Metabolites
Normalization, Filtering
Protein Dictionary

10,000 Probe Sets / 200 Protein Groups or Metabolites
Single Time point and Time course
Multivariate Analysis within a Platform

100-500 Genes / Proteins / Metabolites / Clinical Variables
Classification and Pathway Analysis

Biomarker Panel
## Patient Enrollment

### Total Number

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>De Novo</th>
<th>Existing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients enrolled</td>
<td>562</td>
<td>459</td>
<td>103</td>
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<tr>
<td>Timepoints collected</td>
<td>3761</td>
<td>3649</td>
<td>112</td>
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<tr>
<td>Samples available</td>
<td>23045</td>
<td>22302</td>
<td>743</td>
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### Number of Kidney Transplants

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<th>Total</th>
<th>De Novo</th>
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<tbody>
<tr>
<td>Patients enrolled</td>
<td>407</td>
<td>335</td>
<td>72</td>
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<tr>
<td>Timepoints collected</td>
<td>2713</td>
<td>2637</td>
<td>76</td>
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<td>Samples available</td>
<td>16351</td>
<td>15854</td>
<td>497</td>
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### Number of Heart Transplants

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<th>Total</th>
<th>De Novo</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Patients enrolled</td>
<td>63</td>
<td>48</td>
<td>15</td>
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<tr>
<td>Timepoints collected</td>
<td>410</td>
<td>390</td>
<td>20</td>
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<tr>
<td>Samples available</td>
<td>2832</td>
<td>2693</td>
<td>139</td>
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### Number of Liver Transplants

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<th></th>
<th>Total</th>
<th>De Novo</th>
<th>Existing</th>
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</thead>
<tbody>
<tr>
<td>Patients enrolled</td>
<td>92</td>
<td>76</td>
<td>16</td>
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<tr>
<td>Timepoints collected</td>
<td>638</td>
<td>622</td>
<td>16</td>
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<tr>
<td>Samples available</td>
<td>3862</td>
<td>3755</td>
<td>107</td>
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</table>
CST

IMMUNE RESPONSE IN TRANSPLANTATION

- Transplant
- 1 week
- 3 months
- 1 year

Primary Disease State

Implantation

Transition

Equilibrium

Rejection

Acceptance

Tolerance
1. Total RNA
   Reverse Transcription
   cDNA
   Biotin-labeled cRNA
   in vitro Transcription
   Fragmentation
   GeneChip Expression Array

2. Wash and Stain
   Scan and Quantitate

3. CST
   GENE EXPRESSION PROFILING
GENE TRANSCRIPTS AND INFLAMMATION IN UREMIA
• Anova on 16877 probe sets
• Bonferroni correction;
• Result: Unsupervised ANOVA reveals inter-group variance difference

547 probe sets, 
FDR<0.01

25 probe sets, 
FDR<5x10^{-6}
DIFFERENTIAL EXPRESSION BY PRIMARY DISEASE
INCREASED GENE EXPRESSION AT WEEK 1

Differential expression

GO higher level categories

1. Chemotaxis and cell migration
2. Inflammation and innate immunity
3. Adaptive immunity (T- and B-cell)
4. Wounding and tissue healing
5. Other biological, cellular processes
DECREASED GENE EXPRESSION AT WEEK 1

Differential expression

GO higher level categories

1. Defense response to infection
2. Embryonic growth and development
3. Innate immune response
4. Adaptive immunity
5. Other biological, cellular processes
Acute Rejection  Borderline Rejection  No Rejection
183 significant probe-sets (<1%; 3-method intersection)
<table>
<thead>
<tr>
<th>Probe ID</th>
<th>Description</th>
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<tr>
<td>238320_at</td>
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<td>211454_x_at</td>
<td>Protein of unknown function DUF741</td>
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<td>244752_at</td>
<td>Zinc finger protein 438</td>
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<td>204978_at</td>
<td>splicing factor, arginine/serine-rich 16</td>
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<td>1558448_a_at</td>
<td>CDNA FLJ35687 fis</td>
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<tr>
<td>210787_s_at</td>
<td>calcium/calmodulin-dependent protein kinase kinase 2, beta</td>
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<tr>
<td>211251_x_at</td>
<td>nuclear transcription factor Y, gamma</td>
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<tr>
<td>209060_x_at</td>
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<tr>
<td>200805_at</td>
<td>lectin, mannose-binding 2</td>
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<td>226266_at</td>
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<td>202150_s_at</td>
<td>neural precursor cell expressed, developmentally down-regulated 9</td>
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<td>227510_x_at</td>
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<td>240057_at</td>
<td>-</td>
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<td>210184_at</td>
<td>integrin, alpha X (complement component 3 receptor 4 subunit)</td>
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<td>217436_x_at</td>
<td>hypothetical protein Human MHC class I HLA-J gene, exons 1-8</td>
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<td>200709_at</td>
<td>FK506 binding protein 1A, 12kDa</td>
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<td>210514_x_at</td>
<td>HLA-G histocompatibility antigen, class I, G</td>
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<td>203748_x_at</td>
<td>RNA binding motif, single stranded interacting protein 1</td>
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<tr>
<td>205921_s_at</td>
<td>solute carrier family 6 (neurotransmitter transporter, taurine), member 6</td>
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</tbody>
</table>
BIOMARKER EXPRESSION IN BPAR

Gene names

classification accuracy
1 probe set of 17338 with 78% correct classification rate
CST

PREDICTION OF REJECTION PRE-TRANSPLANT

183 significant probe sets AR vs NR at W1

126 significant probe sets AR vs NR pre-transplant
Gene categories altered

1. Energy and transport regulation
2. Immune defense and antibodies
3. Nuclear transport and signaling
4. Control of intermediary metabolism
5. Other biological, cellular processes
GENE SIGNATURES IN MURINE KIDNEY POST TRANSPLANT

Famulski et al; Am J. Transplant 2006; 6: 1342
Endstage Heart Failure

7,368 probe sets were significantly differentially expressed in heart failure versus normal.

Endstage Kidney Failure

10,484 probe sets were significantly differentially expressed in kidney failure versus normal.

45 protein groups were significantly differentially expressed in heart failure versus normal.
T cell activation
MOLECULAR ACTION OF CURRENT IMMUNE SUPPRESSANTS

TCR CD4/8

HLA CD-40 CD-80 CD-86

CD-2 CD-154 CD-28 CTLA4

Protein Tyrosine Kinases: Src, lck, fyn, Zap 70

PIP2
PLC
γ1

[Ca2+] ER
Calcineurin

NFATc

NFAT
NFkB
CD28RC

Oct1/2 AP-1 Oct1/2

IP3
ER

TOR

PP70^65k
P70^56k

Cdk2/cycE
aCdk2/cycE
Rb/E-F2
pRb + E-F2

Cell-cycle progression
Protein synthesis
Cell proliferation

Intermediate-early genes: fos, jun.

Map Kinase

Raf-1

Mek

Ras

X PO4

PKC

DG

PIP2

PLC

γ1
MOLECULAR ACTION OF NOVEL IMMUNE SUPPRESSANTS

HLA  CD-40  CD-80  CD-86

TCR  CD4/8  IL2R

CD-2  CD-184  CD-28  CTLA4

Protein Tyrosine Kinases
Src, Ick, fyn, Zap 70

PKC  DG  PIP2

PLCγ1

X PO4
Ras
Raf-1
Mek
Map Kinase

Protein Tyrosine Kinases
Src, Ick, fyn, Zap 70

IP3

ER

[Ca2+]i
Calcineurin
NFATC

Intermediate-early genes: fos, jun.

NFAT  NFKB  CD28RC

Oct1/2  AP-1  Oct1/2

Cell-cycle progression
Protein synthesis
Cell proliferation
SUMMARY AND NEXT STEPS

• **Confirmation** – validation of the initial discoveries in a multi-centre Canadian study

• **Application** – development of simple and rapid biomarkers using routine laboratory methods

• **Exploration** – understanding of the mechanisms controlling accommodation and tolerance

• **Translation** – use of these discoveries to develop new drugs and treatment to improve graft outcome
**The GENOME Team**

**Steering Committee**
Paul Keown, Robert McMaster, Bruce McManus, Raymond Ng, Robert Balshaw, Alice Mui

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**Translation and IP**
- JP Heale, University Industry Liaison Office, Steering Committee, Advisory Committee, Novartis, Genome BC

**Funding Partners**
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