Personalised Risk stratification for patients with prostate cancer: PRONTO
“Natural history” of prostate cancer

Popiolek et al., EUR UROL 63 (2013) 428
## Prostate Cancer: Screening

**Release Date:** May 2012

### Recommendation Summary

<table>
<thead>
<tr>
<th>Population</th>
<th>Recommendation</th>
<th>Grade (What’s This?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, Screening with PSA</td>
<td>The U.S. Preventive Services Task Force (USPSTF) recommends against prostate-specific antigen (PSA)-based screening for prostate cancer.</td>
<td>D</td>
</tr>
</tbody>
</table>
Pure low grade

- Death from prostate cancer
- Death from other causes
Let’s not treat low grade cancers

NEARLY 1/2

Adapted from Eggener et al., J. Urol. 2011;185:869
“Active Surveillance”
Attrition on Active Surveillance

Laurence Klotz et al. JCO 2015;33:272-277
Diagnosed with pure low grade prostate cancer?
Diagnosed with harmless prostate cancer?
Biopsy sampling error
Pathologist disagreement
Biomarkers for prostate cancer
Biomarkers for prostate cancer
PTEN loss in low grade biopsies

Genotypic biomarker

Prostate cancer has few mutations

Lawrence et al. Nature 499, 214–218
PRONTO – A pan-Canadian PCC TEAM Grant

• Our objective is to develop and fully validate novel diagnostic approaches to risk stratification of early CaP to enable patients to make informed choices as to the optimal treatment for their individual disease.

• Molecular tests for prostate cancer have not been validated in the active surveillance setting
  • patients lack key information to direct choices to active surveillance versus prostatectomy etc.

• PRONTOs diagnostic test will:
  • Integrate gene expression, copy number variation, and methylation
  • Undergo technical and clinical validation in active surveillance patients
### PRONTO Project Plan

**Phase I**

Discovery

- **TRAIN**

  - Discovery Cohort 1

**Phase II**

Integrated Biomarker Validation

- Validate

  - Discovery Cohort 2

  - Cohort 1

**Phase III**

Integrated Biomarker Validation in Population Based Cohorts

- Validate

  - Active Surveillance Cohort 2

  - Radiotherapy Cohort 3

  - Validated Integrated Biomarker
PRONTO – A pan-Canadian PCC TEAM Grant

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Ants Toi (Mount Sinai)

Axel Thompson (McGill)
Jacques Lapointe (McGill)
Anne Marie Mes-Masson/Fred Saad (CHUM)

Harman Sekhon (Citrus)

22
Cancer Metabolism

RNA

DNA

PARSE

Epithelial plasticity/EMT

Promoter hypermethylation

Stromal development/CAF

Copy Number Alteration

VALIDATE
PRONTO

400 Gene Panel

- Stromal Signaling
- Cancer Metabolism
- Epithelial Plasticity

Copy number variants
DNA methylation

mRNA Expression
Project 1: PTEN (Lapointe)

- DNA Copy number aberrations in key genes (e.g., PTEN loss) define molecular subtypes
- Linked to recurrence, death and prognostication

121 genes submitted to PARSE Core
- 32 genes associated with grade (p<0.05)
- 40 genes with recurrence (p<0.05)
- Max AUC (single gene = 0.708)
Projects 2 and 3: Epithelial Plasticity and EMT (Buttyan, Park)

Plasticity
• Gene expression profile associated with \textit{in vitro} model for prostate cancer de-differentiation
• 14 genes associated with biochemical relapse submitted to PARSE

EMT
• Expression profiling identified key genes associated with EMT and correlated with grade
• Validated in separate cohort
• 37 genes submitted to PARSE core
Project 4: Prostate cancer stromal signature (Thomson)

- Stroma produces factors that support cancer
- Factors were identified by profiling patient matched CAFs and normal fibroblasts
- Identified key pathways associated with prostate morphogenesis and cell cycle
- Validated through IHC, microarray and RNASeq
- 37 genes submitted to PARSE core
- Developed a stromal content classifier
Project 5: Cancer metabolism (Venkateswaran)

• Hypothesis: Metabolic pathways as risk factors
• Comparative expression profiling between GP3 and GP4 foci

• 22 genes submitted to PARSE core
• 5 genes discriminate low grade from higher grade with AUC 0.83
Project 6: CPC-GENE (Boutros)

- 99 DNA copy number aberrations correlated with treatment failure
- AUC 0.746, specificity 0.908
- Shrunk to 40 gene panel
  - AUC 0.739, specificity 0.908

40 genes submitted to PARSE core
Project 7: DNA Methylation (Berman)

- 14 Genes associated with outcome in literature
- of 14 Genes have AUC above 0.9
- 509 Cases
- >1000 samples
- 62,000 Methylation-Specific PCR reactions
- 4 genes with AUC >0.9 for cancer vs. benign
Integrated Biomarker: ANALYSIS

1° Endpoint: GLEASON SCORE Classifier
2° Endpoint: BIOCHEMICAL RECURRENCE

For Both:

a) Univariate
b) Multivariate
- Age
- Biopsy tumour volume
- Pre op PSA
- Clinical Stage
Analytical Plan

Reviewed Cases

250/250

McGill University Health Centre

139/276

TRAIN

Validate
**DISCOVERY Phase - Progress**

**Biopsy Gleason grade**
3+3=6 or 3+4=7

- 250 Cases
- 2 samples/case
  - High grade
  - Low grade

**Extractions**

- **778 RNA samples**
  - Completed

**Gene list submitted**

- Nanostring 393 mRNA
  - Queen's Completed
  - McGill Completed

**Extracts DNA**

- **778 DNA samples**
  - Completed

**Assay**

- **MSP**
  - 14 gene
  - Queen's Completed
  - McGill Completed

- **MLPA**
  - 15 target regions
  - Queen's and McGill Complete by Nov 2017

**Extractions**

- **172 target regions**
  - Nanostring

**Bx and RP REVIEW**

- Completed

**RP Gleason grade**
3+3=6 or 3+4=7

- 139 Cases
- 2 samples/case
  - High grade
  - Low grade

- 250 Cases
- 2 samples/case
  - High grade
  - Low grade
Overcoming Biopsy Sampling Error

1 H core
High grade cores
For extraction and TMA

4+3=7

3+3=6

1 L core
Low grade cores
For extraction and TMA
Tissue and nucleic acid harvest optimised

MLPA
Method for assessing copy number variation with low input (50ng) DNA.

NANOSTRING
Enables larger gene panels
Higher DNA Input and cost

1. Hybridise
2. Purify and Immobilise
3. Count
Preliminary Nanostring Analysis (Anna Lee - Boutros group)

Genes associated with Grace

Genes associated with Treatment failure
Publication progress

• Published/in press
  1. Nouri, Mannan et al. (2016) Therapy-Induced Developmental Reprogramming of Prostate Cancer Cells and Acquired Therapy Resistance *Oncotarget*
  3. Rochette et al., (2016) Asporin is a stromally expressed marker associated with prostate cancer progression. *BJC*

• Under Review
  • Nash et al., 2016 Genome-wide analysis of AR binding and comparison with transcript expression in primary human fetal prostate fibroblasts and cancer associated fibroblasts. Submitted to *MCE*

• In preparation
  1. Park/Thomson (EMT)
  2. Lapointe (MLPA/PTEN)
  3. Berman (Methylation)
  4. Thomson (Stroma)
  5. Vasu (Metabolism)
PHASE II - Integrated Biomarker Validation
PRONTO
Project Plan

Phase II

Integrated Biomarker Validation

• Objective 1: Validate assays in small input DNA/RNA from core biopsies

• Objective 2: Validate in Lawson Cohort

Validate
Technical Validation

1. Dilution Experiments
   **Goal:** to assess the minimum amount of DNA/RNA required to avoid signal loss in low expressing genes
   **COMPLETED**
   Analysis in progress

2. Specificity and Sensitivity of biomarker on Biopsy samples
   **In progress**
   COMPLETE by Nov 2017

3. Compare biomarker to clinical nomograms
   **COMPLETE**
   by Dec 2017

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Dr. Antonio Finelli’s Team
PHASE III - Biomarker Validation in population based cohorts
**PRONTO Project Plan**

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**Integrated Biomarker Validation**
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- **Project 1**: PTEN loss and downstream signaling
- **Project 2**: Epithelial Plasticity - Stem Cell Signature
- **Project 3**: Epithelial-mesenchymal transition (EMT) Signature
- **Project 4**: Influence of stroma on CaP progression
- **Project 5**: Tumour cell Metabolism
- **Project 6**: Validating diagnostic assays from CPC-GENE
- **Project 7**: CpG Island hypermethylation
Clinical biopsy cohorts: Finelli/Earle

25,427 = All prostate cancer cases diagnosed in Ontario 2004-6

14,973 had a procedure for early-stage disease that should result in a usable pathology specimen

Link to centralized pathology reports held at CCO

Sequentially request biopsy specimens until the following PRONTO cohorts are complete:

<table>
<thead>
<tr>
<th>1: Core bx + RP specimen</th>
<th>2: Core bx Gleason &lt;6</th>
<th>3: Core bx Gleason 7</th>
</tr>
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<tbody>
<tr>
<td>Gleason 6</td>
<td>1000 active surveillance</td>
<td>1000 EBRT</td>
</tr>
<tr>
<td>Gleason 7</td>
<td>1000 active surveillance</td>
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Chart reviews at local institutions to abstract missing data elements (e.g., PSA levels)

Review pathology reports starting in January 2004 to identify those with Gleason 6-7

Figure 3: PRONTO Cohort Building – Case Selection
Clinical Data Collection Teams

- GTA and surrounding area
- UHN team
  - Community hospitals up to 10 sites
PHASE III - Pathology Core
PRONTO Summary

- Promising biomarkers for active surveillance
- Teams built for molecular pathology and interdisciplinary research
- Addresses important clinical problem
- Best is yet to come