NCI’s Clinical Proteomic Technologies for Cancer:
“Restructuring Proteomics to Succeed in Discovering Cancer Biomarkers”
BSA Update Progress Report
June 2009

Joe Gray (moderator)
Lawrence Berkeley National Laboratory
Thus far, there are only 9 FDA-approved cancer protein biomarkers in blood.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>US Food and Drug Administration-approved cancer biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarker</strong></td>
<td><strong>Type</strong></td>
</tr>
<tr>
<td><em>α-Fetoprotein</em></td>
<td>Glycoprotein</td>
</tr>
<tr>
<td><em>Human chorionic gonadotropin-β</em></td>
<td>Glycoprotein</td>
</tr>
<tr>
<td><strong>CA19-9</strong></td>
<td>Carbohydrate</td>
</tr>
<tr>
<td><strong>CA125</strong></td>
<td>Glycoprotein</td>
</tr>
<tr>
<td><strong>Pap smear</strong></td>
<td>Cervical smear</td>
</tr>
<tr>
<td><strong>CEA</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>Epidermal growth factor receptor</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>KIT</strong></td>
<td>Protein (IHC)</td>
</tr>
<tr>
<td><strong>Thyroglobulin</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>PSA (total)</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>PSA (complex)</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>PSA (free PSA %)</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>CA15-3</strong></td>
<td>Glycoprotein</td>
</tr>
<tr>
<td><strong>CA27-29</strong></td>
<td>Glycoprotein</td>
</tr>
<tr>
<td><strong>Cytokeratins</strong></td>
<td>Protein (IHC)</td>
</tr>
<tr>
<td><strong>Oestrogen receptor and progesterone receptor</strong></td>
<td>Protein (IHC)</td>
</tr>
<tr>
<td><strong>HER2/NEU</strong></td>
<td>Protein (IHC)</td>
</tr>
<tr>
<td><strong>HER2/NEU</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>HER2/NEU</strong></td>
<td>DNA (FISH)</td>
</tr>
<tr>
<td><strong>Chromosomes 3, 7, 9 and 17</strong></td>
<td>DNA (FISH)</td>
</tr>
<tr>
<td><strong>NMP22</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>Fibrin/FDP</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>BTA</strong></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>High molecular weight CEA and mucin</strong></td>
<td>Protein (Immunofluorescence)</td>
</tr>
</tbody>
</table>

Where Clinical Proteomics Is Today

Few biomarker candidates translating into clinical utility

- Lack of new discoveries
- Questionable discoveries (claims)
- Lost opportunities

Source: Based on data from FDA and Plasma Proteome Institute
Understanding the Issues

NCI listens to experts

Experts identify barriers (issues)

1. Experimental design
2. Technical barriers (platform evaluation / optimization)
   - Discovery (survey) stage
   - Verification (targeted) stage
3. Biospecimen collection, handling, storage and processing
4. Data acquisition, analysis and reporting

Need to address sources of variability and bias
Addressing the Issues

- NCI establishes CPTC Oct. 2006 to Support Biomarker Development
  - Develop bias-free biospecimen procedures and repositories.
  - Evaluate and standardize performance of proteomic discovery platforms and standardize their use.
  - Evaluate and standardize proteomic validation platforms for analysis of cancer-relevant proteomic changes in human clinical specimens.
  - Develop and implement uniform algorithms for sharing bioinformatics and proteomic data and analytical/data mining tools across the scientific community.
  - Develop standard/reference materials and reagents for the proteomic community.

CPTC components:
- a) CPTAC Center Network  $35.5M Total
- b) Individual PI – Adv. Proteomic Platforms & Computational Sciences  $56M Total
- c) Reagents & Resources  $12.5M Total

CPTAC Centers: multidisciplinary team network
## CPTAC Center Network Presentation

### Outline

- **Technical Barriers (Discovery and Verification)**
  - *Daniel Liebler*: Discovery (survey) proteomics – Refining discovery
  - *Steven Carr*: Verification (targeted) proteomics – Filling the gap

- **Experimental Design and Biospecimens**
  - *David Ransohoff*: Addressing chance and bias

- **CPTC Additional Highlights and Data Analysis/Sharing**
  - *Henry Rodriguez*

- **Wrap-up**
  - *Joe Gray*

### Bio-Specimens
- Plasma
- Tissue
- Proximal fluids

### Discovery
- Tissue
- Proximal fluids

### Verification
- Blood
- Population

### Clinical Validation
- Blood
- Population
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Wrap-up

Joe Gray
Lawrence Berkeley National Laboratory
Program Goals for next 2 years

Biospecimens
• Establish plasma biorepository of BRCA/normal, with specific effort to avoid bias by collecting prior to diagnosis

Discovery Studies (inter-lab)
• Evaluate relative quantification methods in discovery proteomic technologies using cancer cell model (proteins and PTMs)
• Establish ability to detect cancer-relevant differences in tissue or proximal fluid specimens

Verification Studies (inter-lab)
• Define performance of MRM-MS at ~100-plex level for cancer-relevant proteins at ng/mL range in plasma and conduct “blinded” study
• Develop training course and reagent kits to aid widespread adoption
• With FDA, vendors move MRM-MS of peptides toward clinical acceptability
Projected outcomes of CPTAC program

Large, unbiased plasma collection for breast cancer BMD and “best practices” for collection for proteomic studies

Establish a robust pipeline for biomarker candidate discovery through pre-clinical verification
  • Clear understanding of relative merits and performance characteristics of best MS platforms for proteomic biomarker discovery
  • Robust, transferable MRM-MS technology for verification of biomarker candidates in blood at ng/mL levels with near clinical assay performance

Build bridge between “Discovery Omics” and Clinical Validation
  • Proteomics Community poised to apply technologies for real BMD and Verification in patient samples
Accomplishments
slides
Accomplishments: Experimental design and biospecimens

- Plasma samples from 2,000 patients with breast lesion being accrued (current >590)
- Collection prior to diagnosis from biopsy, therefore strongly unbiased
- Expect 500 breast cancers, 1500 benign disease
- Multi-site biospecimen tracking database (DB) developed, with strong pathology annotation (in alpha testing)
- Centralized biorepository identified (NCI-Frederick); will link their DB with CPTAC’s biospecimen DB

Bio-Specimens
- Plasma
- Tissue
- Proximal fluids

- untargeted proteomics
- genomics
Accomplishments: Discovery-stage

- First quantitative assessment of discovery proteomics technology platforms across laboratories
- Development of standard proteomes and performance mixtures for technology assessment
- Development of performance metrics “toolkit” for QC and standardization of proteomics technology platforms
Accomplishments: Verification-stage

- First large-scale evaluation of targeted MS technology (MRM-MS) for sorting through large lists of biomarker candidates to identify the most promising ones to advance to clinical validation.
- Demonstrated that multiplexed, quantitative MRM-MS-based assays can be rapidly and robustly configured and deployed for measurement of proteins in plasma.
- Near-clinical assay performance with respect to reproducibility can be achieved.
- Reagents, methods and multi-laboratory datasets produced.
- Aid acceptance and adoption by proteomics and clinical communities.