http://proteomics.cancer.gov

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

NCI's Clinical Proteomic Technologies for Cancer:

"Restructuring Proteomics to Succeed in Discovering Cancer Biomarkers"

BSA Update Progress Report

June 2009

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NATIONAL CANCER INSTITUTE

Thus far, there are only 9 FDA-approved cancer protein biomarkers in blood

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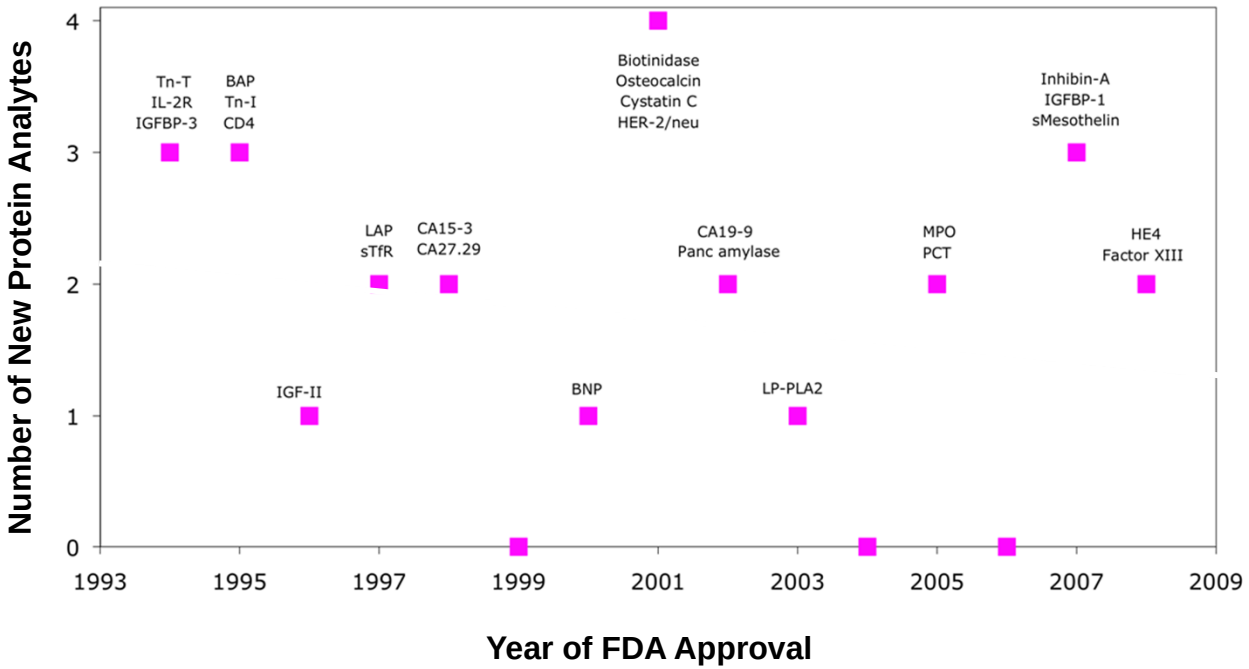
Table 1 | **US Food and Drug Administration-approved cancer biomarkers**

Biomarker	Type	Source	Cancer type	Clinical use
α-Fetoprotein	Glycoprotein	Serum	Nonseminomatous testicular	Staging
Human chorionic gonadotropin-β	Glycoprotein	Serum	Testicular	Staging
CA19-9	Carbohydrate	Serum	Pancreatic	Monitoring
CA125	Glycoprotein	Serum	Ovarian	Monitoring
Pap smear	Cervical smear	Cervix	Cervical	Screening
CEA	Protein	Serum	Colon	Monitoring
Epidermal growth factor receptor	Protein	Colon	Colon	Selection of therapy
KIT	Protein (IHC)	Gastrointestinal tumour	GIST	Diagnosis and selection of therapy
Thyroglobulin	Protein	Serum	Thyroid	Monitoring
PSA (total)	Protein	Serum	Prostate	Screening and monitoring
PSA (complex)	Protein	Serum	Prostate	Screening and monitoring
PSA (free PSA %)	Protein	Serum	Prostate	Benign prostatic hyperplasia versus cancer diagnosis
CA15-3	Glycoprotein	Serum	Breast	Monitoring
CA27-29	Glycoprotein	Serum	Breast	Monitoring
Cytokeratins	Protein (IHC)	Breast tumour	Breast	Prognosis
Oestrogen receptor and progesterone receptor	Protein (IHC)	Breast tumour	Breast	Selection for hormonal therapy
HER2/NEU	Protein (IHC)	Breast tumour	Breast	Prognosis and selection of therapy
HER2/NEU	Protein	Serum	Breast	Monitoring
HER2/NEU	DNA (FISH)	Breast tumour	Breast	Prognosis and selection of therapy
Chromosomes 3, 7, 9 and 17	DNA (FISH)	Urine	Bladder	Screening and monitoring
NMP22	Protein	Urine	Bladder	Screening and monitoring
Fibrin/FDP	Protein	Urine	Bladder	Monitoring
BTA	Protein	Urine	Bladder	Monitoring
High molecular weight CEA and mucin	Protein (Immunofluorescence)	Urine	Bladder	Monitoring

Ludwig & Weinstein, *Nature Reviews Cancer* (2005) 5, 845-856.

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

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NCI listens to experts

Dec 2005	▪ Proteomic Affinity/Capture Methods Workshop
Feb 2005	▪ Proteomic Technologies Informatics Workshop
Jan 2005	▪ Clinical Proteomics Technologies Team Initiative proposal
Nov 2004 Sept 2004	▪ Clinical Proteomics and Biomarker Discovery in Cancer Research
June 2004	▪ Initial draft proposal for a Clinical Proteomics/Biomarker Discovery Initiative
April 2003	▪ Proteomic Technologies for Early Cancer Detection
April 2002	▪ Proteomics Planning Workshop *(NCI/NHGRI/NIGMS)*

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.

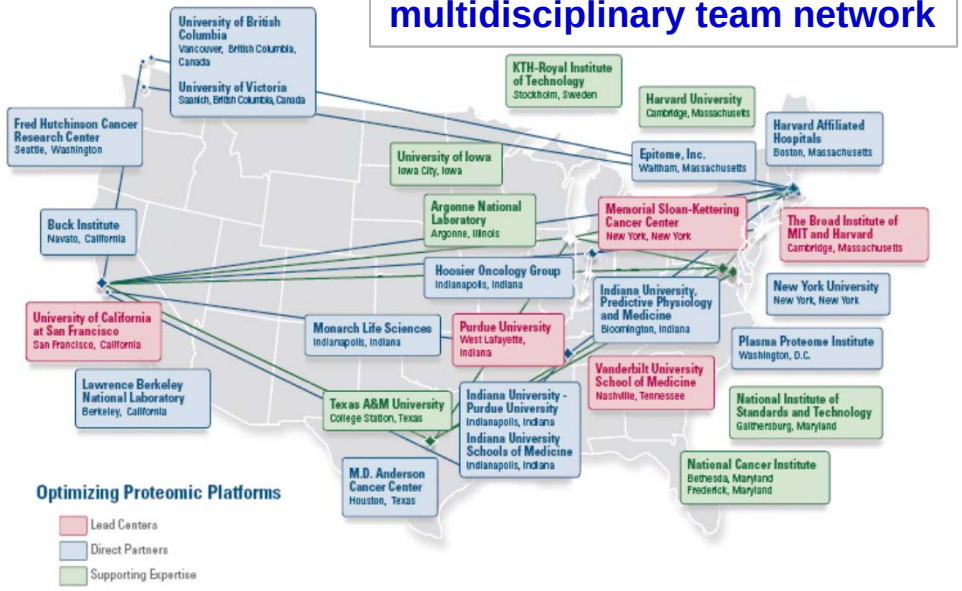
CPTC components:

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

Bio-Specimens
- **Plasma**
- **Tissue**
- **Proximal fluids**

Discovery
- **Tissue**
- **Proximal fluids**

Verification
- **Blood**
- **Population**

Clinical Validation
- **Blood**
- **Population**

- **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*

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Wrap-up

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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

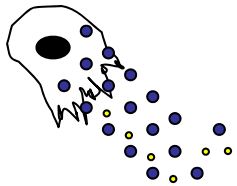
Build bridge between "Discovery Omics" and Clinical Validation

- Proteomics Community poised to apply technologies for real BMD and Verification in patient samples

Accomplishments slides

Accomplishments:
Experimental design and biospecimens

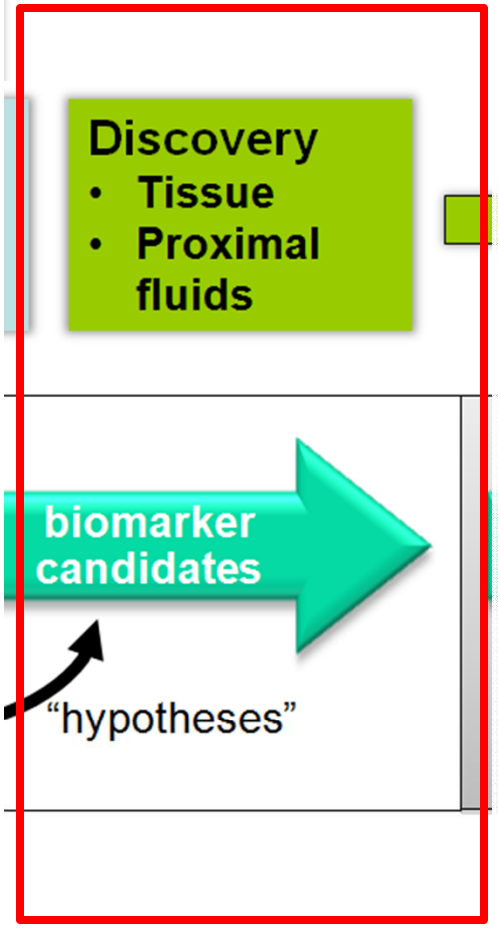
Bio-Specimens
- **Plasma**
- **Tissue**
- **Proximal fluids**



- **untargeted proteomics**
- **genomics**

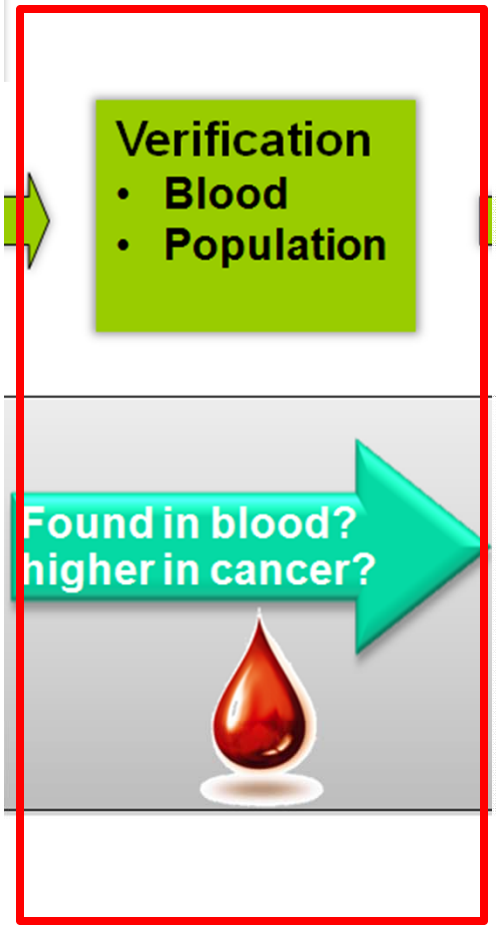
- Plasma samples from 2,000 patients with breast lesion being accrued (current >590)
- Collection <u>prior</u> to diagnosis from biopsy, therefore strongly <u>unbiased</u>
- Expect 500 breast cancers, 1500 benign disease
- Multi-site biospecimen tracking database (DB) developed, with strong pathology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

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