

## NCI FDA Clinical Proteomics Program

Co Directors L. Liotta (NCI) and E. Petricoin (FDA CBER)



**Mission:** Develop protein micro analysis technology for direct molecular analysis of tissue cells and serum.

- Mechanistic investigation
- Immediate ongoing clinical applications

### Discovery of molecules involved in pathogenesis

- LCM combined with 2-D gels and mass spec: >400 proteins isolated and mass spec sequenced

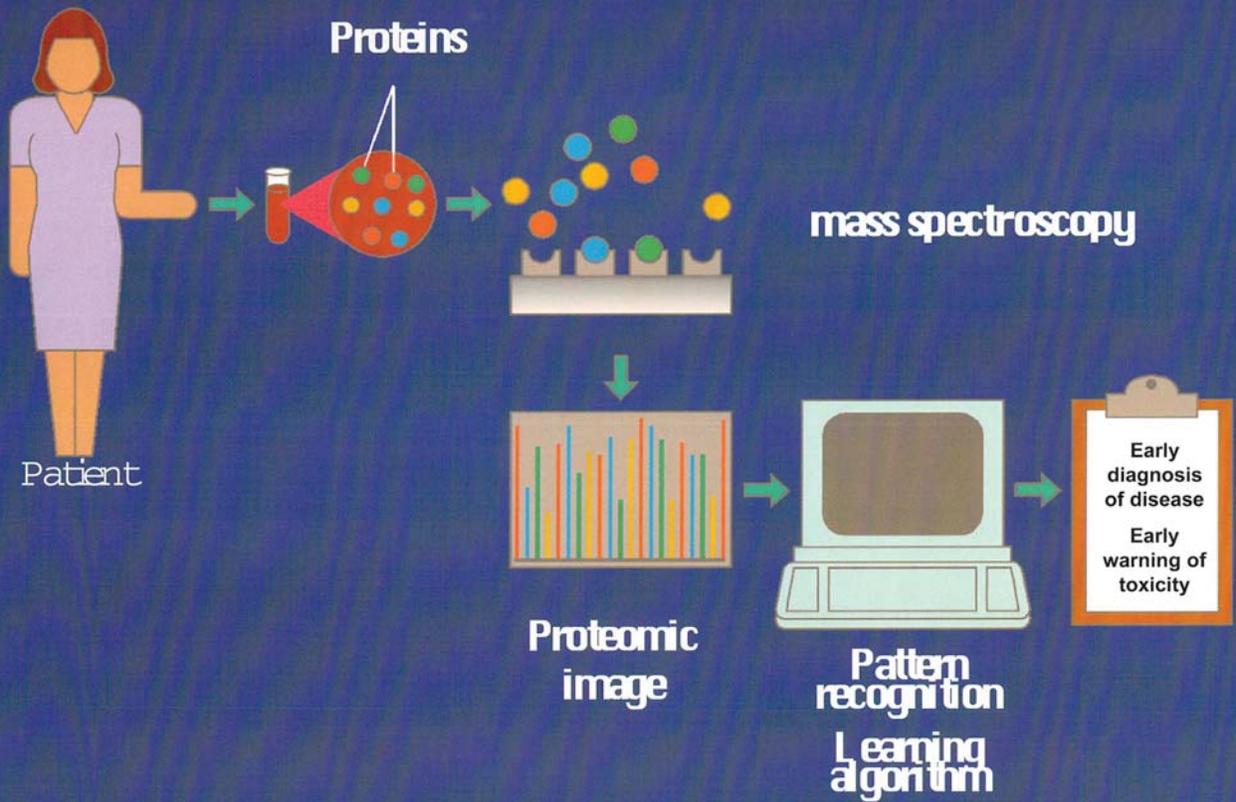
### Profiling the phosphorylated state of signaling pathway node proteins

- LCM combined with reverse phase protein microarrays: Phase I clinical trials: molecular profiling before and after therapy.

### Serum protein patterns (mass spec ions) reflect pathologic state

- Application to early diagnosis of ovarian and prostate cancer

# Serum Protein Pattern Diagnostics



**WCX SELDI CHIP PBSII  
Proteomic Pattern Diagnostics  
Ovarian Cancer Results**

<b>BLINDED TEST DATA</b>	<b>( N= 250 PATIENTS)</b>
NED (5 Year follow up)	<b>66/67 (99%)</b>
Benign gynecologic and non-gyn inflammatory (cysts, fibroids) (RA, colitis, sinusitis)	<b>70/71 (99%)</b>

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**TOTAL SPECIFICITY: 136/138 = 99%**

Ovarian cancer Stage II, III, IV **75/76 (99%)**

Ovarian cancer Stage I **36/36 (100%)**

**TOTAL SENSITIVITY: 111/112 = 99%**

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All spectra data:[www.clinicalproteomicssteem.com](http://www.clinicalproteomicssteem.com)

## Proteomic Pattern Diagnostics

### Ongoing Research Studies:

**AIM #1:** Serum based test to distinguish benign lesions from malignancy in conjunction with existing imaging modalities

- **Lung Cancer:** Collaboration with David Harpole, Sam Wells, Kim Lyerly, DUKE

Study set: Spiral CT positive, biopsy proven pathology N= 67 Benign, 48 Squamous Ca, 38 Adeno Ca (115 total)

Preliminary Results : **94 % Sensitivity, 97 % Specificity for squamous Ca,  
100% Sensitivity, 95% Specificity for adenocarcinoma**

- **Breast Cancer:** Collaboration with Gordon Mills, MD Anderson and John Olson, Sam Wells and Kim Lyerly, DUKE

Study set: Positive imaging finding, biopsy proven pathology N= 142 Benign, 165 Ca (317 total)

Preliminary Results : **90% Sensitivity  
71% Specificity**

**AIM #2:** Serum based test to distinguish cancer where existing biomarker levels lose PPV

- **Prostate Cancer:** Collaboration with David Ornstein, UNC, Walter Rayford, LSU

Study Set: PSA 4-10 ng/ml, biopsy proven, or 2X sextant biopsy negative N= 98 Cancer, 40 Benign (138 total)

Preliminary Results: **Sensitivity: 98%  
Specificity: 63%**

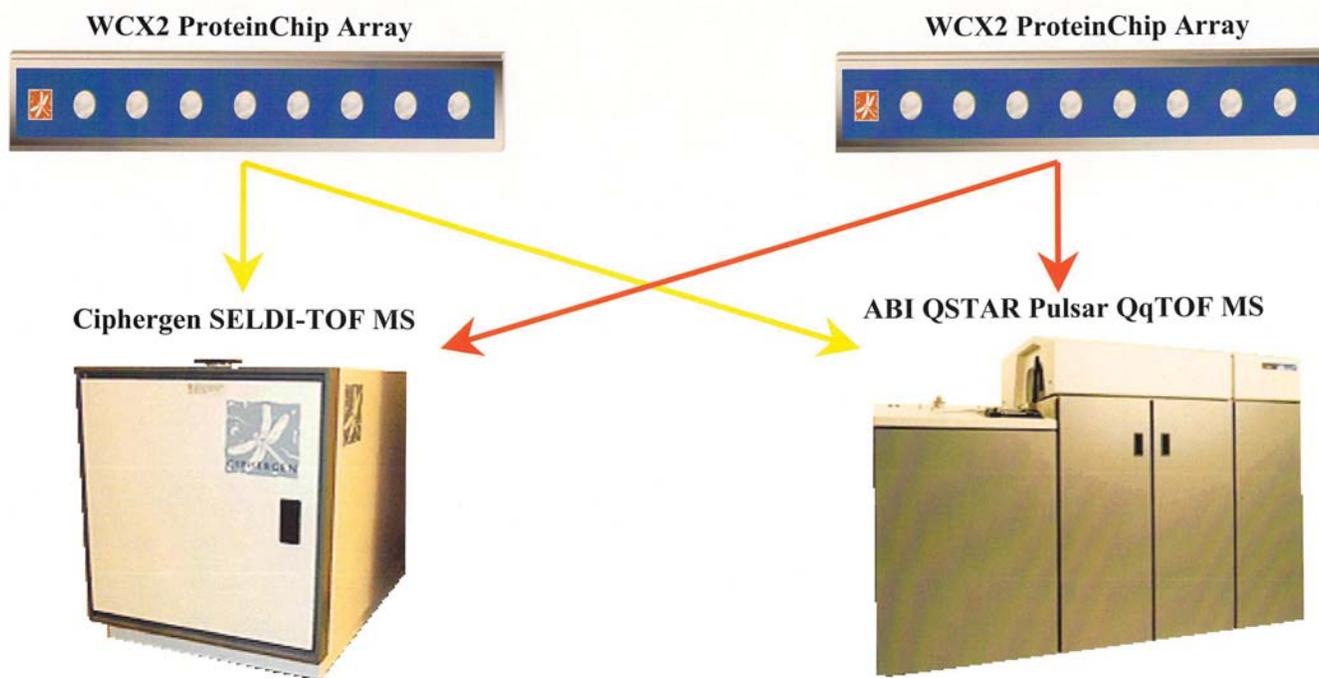
**AIM #3:** Serum based test to distinguish cancer from normal where no clinically useful marker exists for early detection and high co-morbidity with other diseases

- **Pancreatic Cancer:** Collaboration with Kristin Anderson and Myron Gross, U. of Minn

Study Set: 61 cancers, 55 controls (N= 116) 31 controls without diabetes; 30 controls with diabetes; 31 cancers without diabetes; 19 cancers with diabetes; and 5 subjects with pancreatitis.

Preliminary Results: **Sensitivity: 95%  
Specificity: 97%**

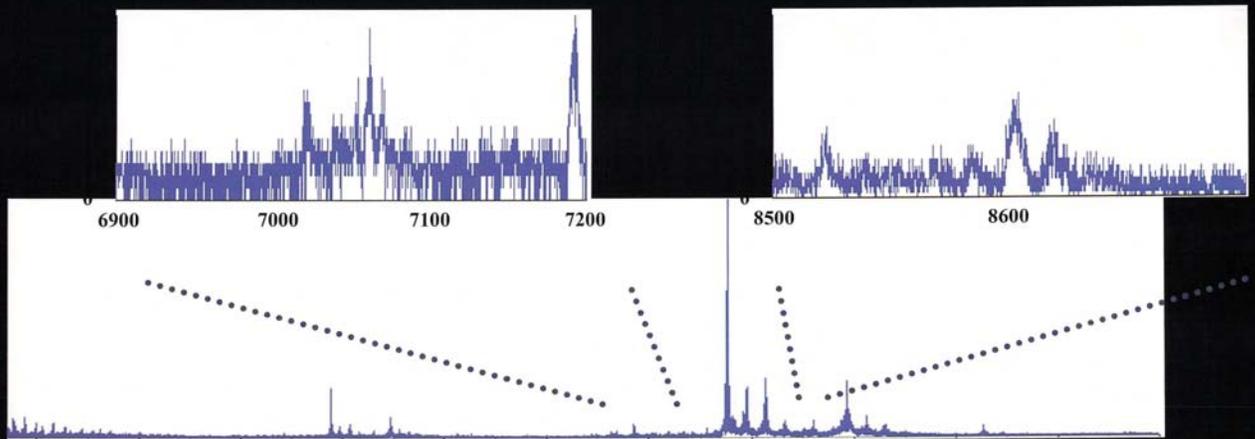
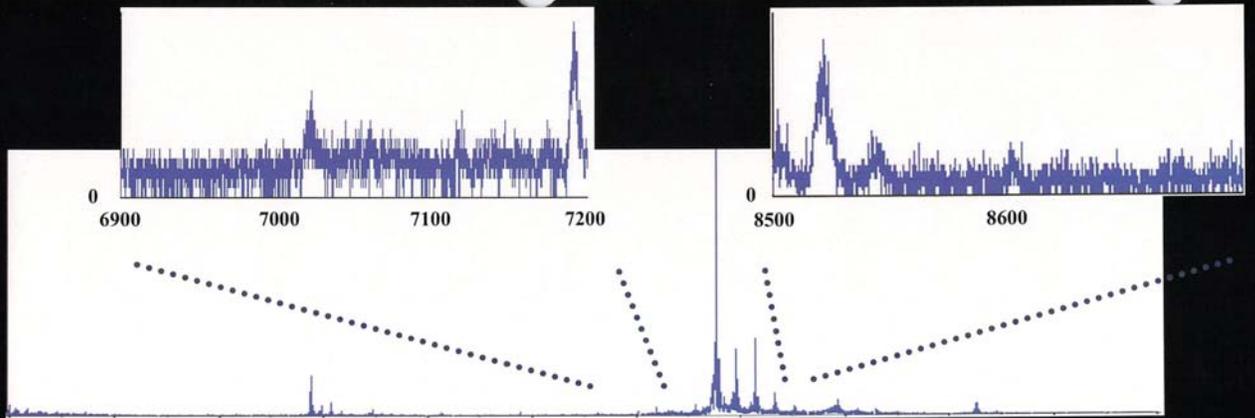
## Validation and Implementation of SELDI-QqTOF for Diagnostic Proteomics



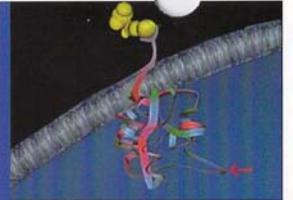
Widely accessible research instrument  
Extensive  $m/z$  range (5-300,000)  
Low Resolution (~ 100-200)  
Low Mass Accuracy (~1000 ppm)

More specialized knowledge required  
Limited  $m/z$  range (5-12,000 – XL to 40,000)  
High resolution (>9000 at  $m/z$  1500)  
High mass accuracy (>50 ppm - external cal)  
***Able to conduct CID of peptides***

<p><b>Model 1</b></p> <p>Test Specificity            100%  Test Sensitivity            100%  Validation Sensitivity      100%  Validation Specificity      100%</p> <p><b>Result Breakdown</b></p> <table border="1"> <thead> <tr> <th>State</th> <th>Testing Results</th> <th>Validation Results</th> </tr> </thead> <tbody> <tr> <td>Normal</td> <td>30/30 (100%)</td> <td>37/37 (100%)</td> </tr> <tr> <td>Ovarian Cancer</td> <td>57/57 (100%)</td> <td>40/40 (100%)</td> </tr> </tbody> </table> <p><b>Distinguishing <i>m/z</i> values</b></p> <table border="1"> <tbody> <tr><td>1</td><td>1276.8612</td></tr> <tr><td>2</td><td>2374.2444</td></tr> <tr><td>3</td><td>4292.9</td></tr> <tr><td>4</td><td><b>7060.121</b></td></tr> <tr><td>5</td><td><b>8605.678</b></td></tr> <tr><td>6</td><td><b>8706.065</b></td></tr> <tr><td>7</td><td>9870.9375</td></tr> </tbody> </table>	State	Testing Results	Validation Results	Normal	30/30 (100%)	37/37 (100%)	Ovarian Cancer	57/57 (100%)	40/40 (100%)	1	1276.8612	2	2374.2444	3	4292.9	4	<b>7060.121</b>	5	<b>8605.678</b>	6	<b>8706.065</b>	7	9870.9375	<p><b>Model 2</b></p> <p>Test Specificity            100%  Test Sensitivity            100%  Validation Sensitivity      100%  Validation Specificity      100%</p> <p><b>Result Breakdown</b></p> <table border="1"> <thead> <tr> <th>State</th> <th>Testing Results</th> <th>Validation Results</th> </tr> </thead> <tbody> <tr> <td>Normal</td> <td>31/31 (100%)</td> <td>37/37 (100%)</td> </tr> <tr> <td>Ovarian Cancer</td> <td>63/63 (100%)</td> <td>40/40 (100%)</td> </tr> </tbody> </table> <p><b>Distinguishing <i>m/z</i> values</b></p> <table border="1"> <tbody> <tr><td>1</td><td>818.4801</td></tr> <tr><td>2</td><td>6352.7227</td></tr> <tr><td>3</td><td>6548.771</td></tr> <tr><td>4</td><td><b>7060.121</b></td></tr> <tr><td>5</td><td>7096.9224</td></tr> <tr><td>6</td><td>8540.536</td></tr> <tr><td>7</td><td><b>8605.678</b></td></tr> <tr><td>8</td><td><b>8706.065</b></td></tr> </tbody> </table>	State	Testing Results	Validation Results	Normal	31/31 (100%)	37/37 (100%)	Ovarian Cancer	63/63 (100%)	40/40 (100%)	1	818.4801	2	6352.7227	3	6548.771	4	<b>7060.121</b>	5	7096.9224	6	8540.536	7	<b>8605.678</b>	8	<b>8706.065</b>
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# Serum Proteomic Pattern Diagnostics



- TISSUE PATHOLOGIC STATES ARE REFLECTED IN HIDDEN SERUM PROTEOMIC PATTERNS UNCOVERED USING AN ARTIFICIAL INTELLIGENCE BASED BIOINFORMATICS TOOL THAT LEARNS THE MOST FIT SOLUTION
- WE HYPOTHESE THAT SERUM PROTEOMIC PATTERNS ARE PRODUCT OF THE UNIQUE TUMOR-HOST MICROENVIRONMENT AND REFLECT TUMOR AND HOST INTERACTION

## CURRENT STRATEGY:

### TWO INDEPENDENT TRACKS:

- NCI-BASED NATIONAL CLINICAL TRIAL ON SERUM PROTEOMIC PATTERN DIAGNOSTICS WHERE IDENTITY IS NOT NEEDED
- SCIENTIFIC INVESTIGATION INTO SPECIFIC SOURCE AND IDENTITY OF THE CLASSIFIERS

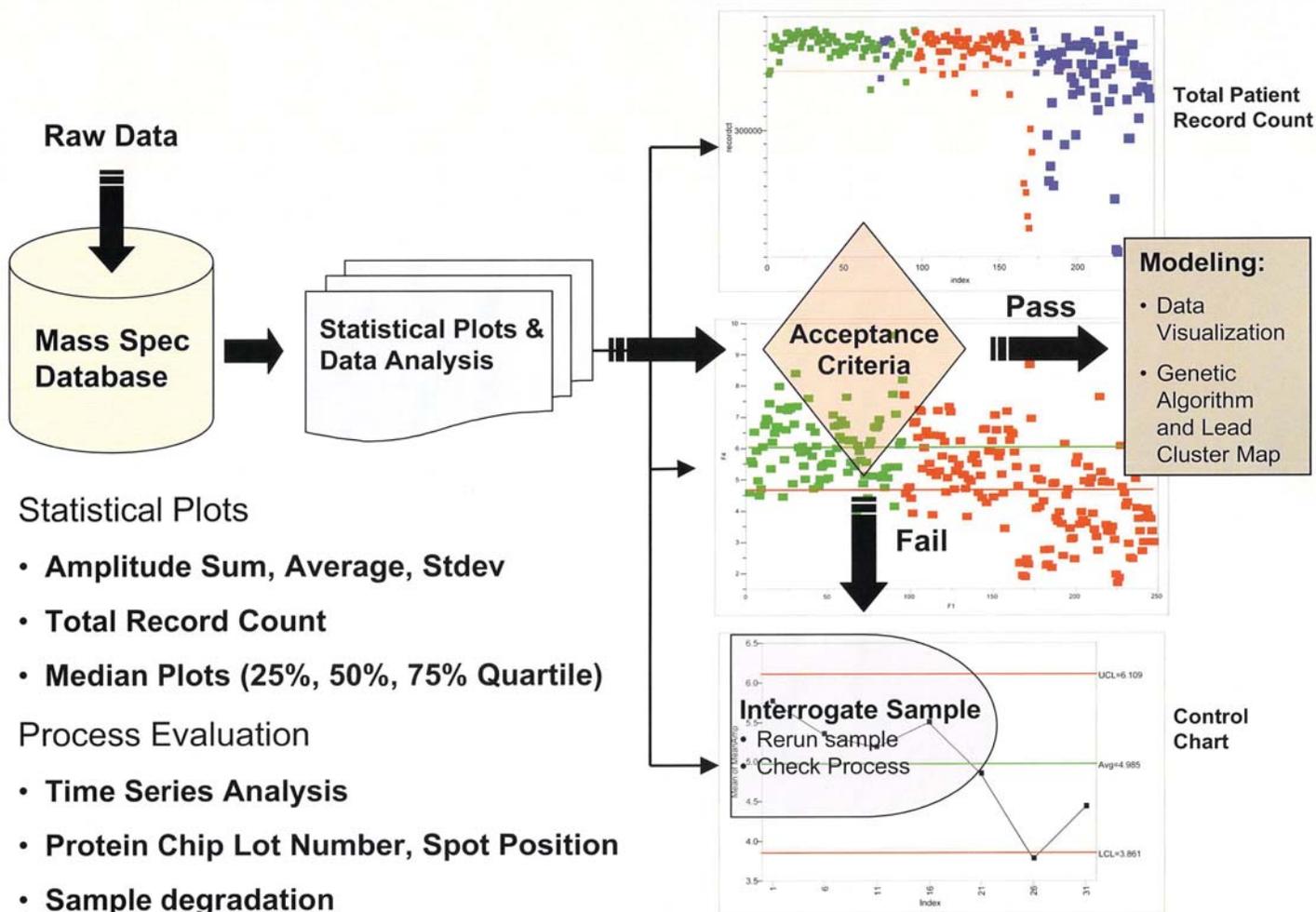
# Clinical Proteomics Reference Lab

## Progress to Date

- Completed acquisition of space - Jan. 1, 2003
- Outfitted laboratory
- Quality System Drafts Completed (per 21CFR820 subpart c)
- Retrofit laboratory with back-up systems - in progress
- Acquisition of Tecan robotic processor - March, 2003
- Acquisition of 1 of 3 Q-Star instruments - May, 2003
- Installation of Ciphergen front end - May, 2003
- First instrument qualification - June 1, 2003
- Pooled serum for initial reproducibility studies - March, 2003
- Software evaluation for validation in progress



# NCI-FDA Clinical Proteomics Statistical Sample Validation



# Clinical Proteomics Reference Lab

## Regulatory Strategy

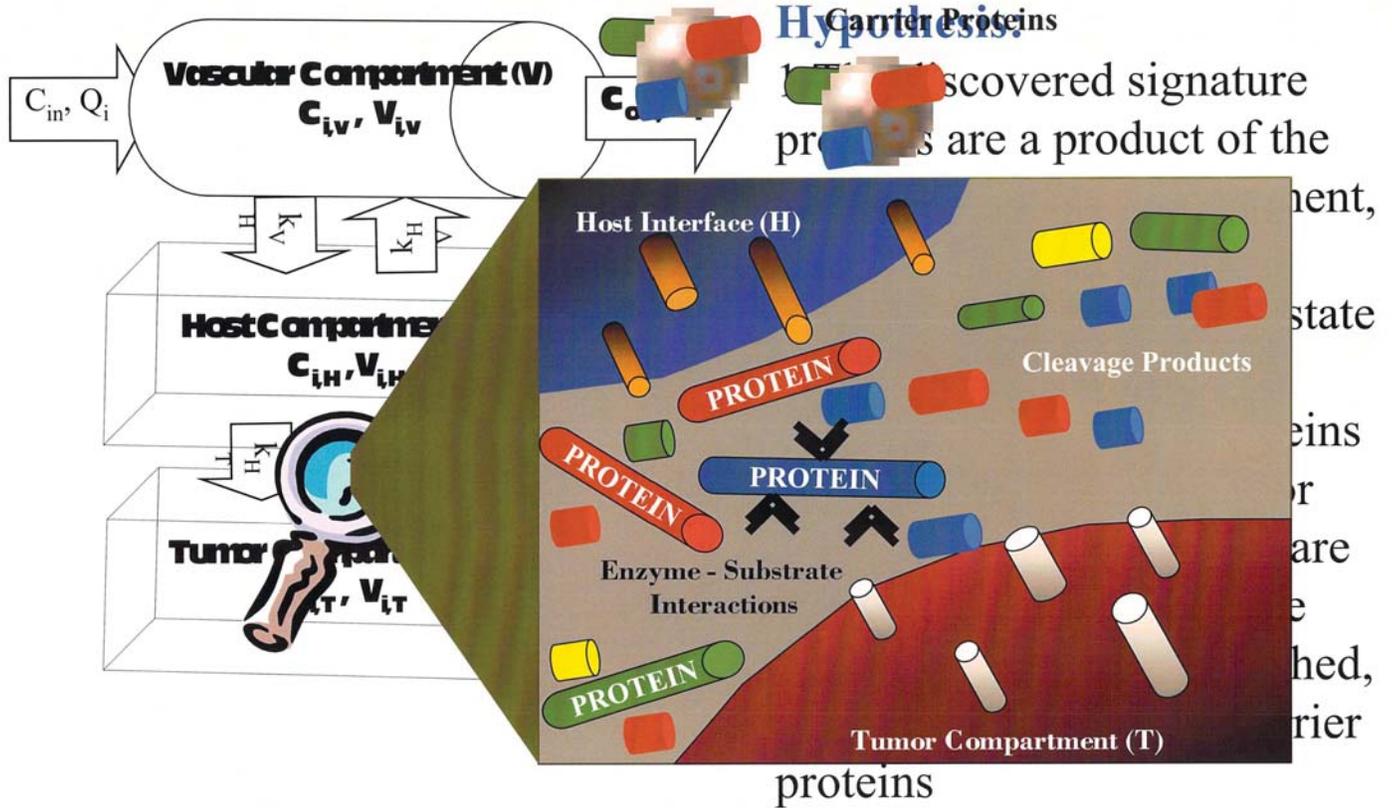
- **Design and implement Quality System (mandated by law)**
- **CAP/ CLIA inspection (extension of current LP CAP certification)**
- **File 510(k) for monitoring claim equivalent to CA125**
- **File validation data as a PMA for OBGYN POS screening**
- **Validate screening in high risk women for use as a “home brew” and for PMA filing**

# **Clinical Proteomics Reference Lab**

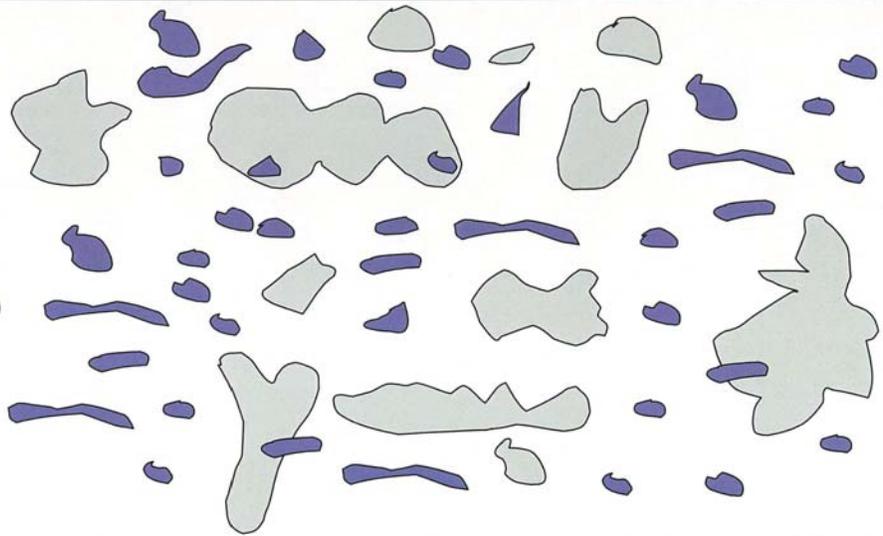
## **Time Line**

- **Complete feasibility phase by end of this year**
- **Bring facility to cGMP and CLIA standards by next February**
- **Aggressive schedule to file 510(k) by next June 2004**
- **Produce pre-clinical data for PMA at the same time**
- **GYN imaging positive indication trial to begin in parallel**

## Source of Ions Comprising Diagnostic Signature Subset

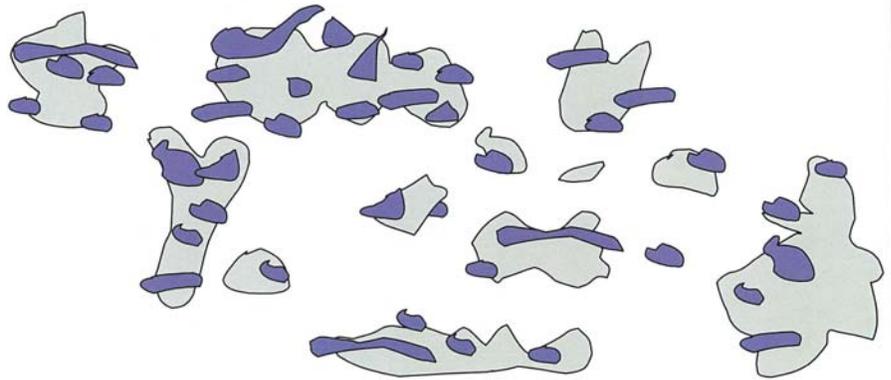


OLD VIEW  
of the  
SERUM PROTEOME



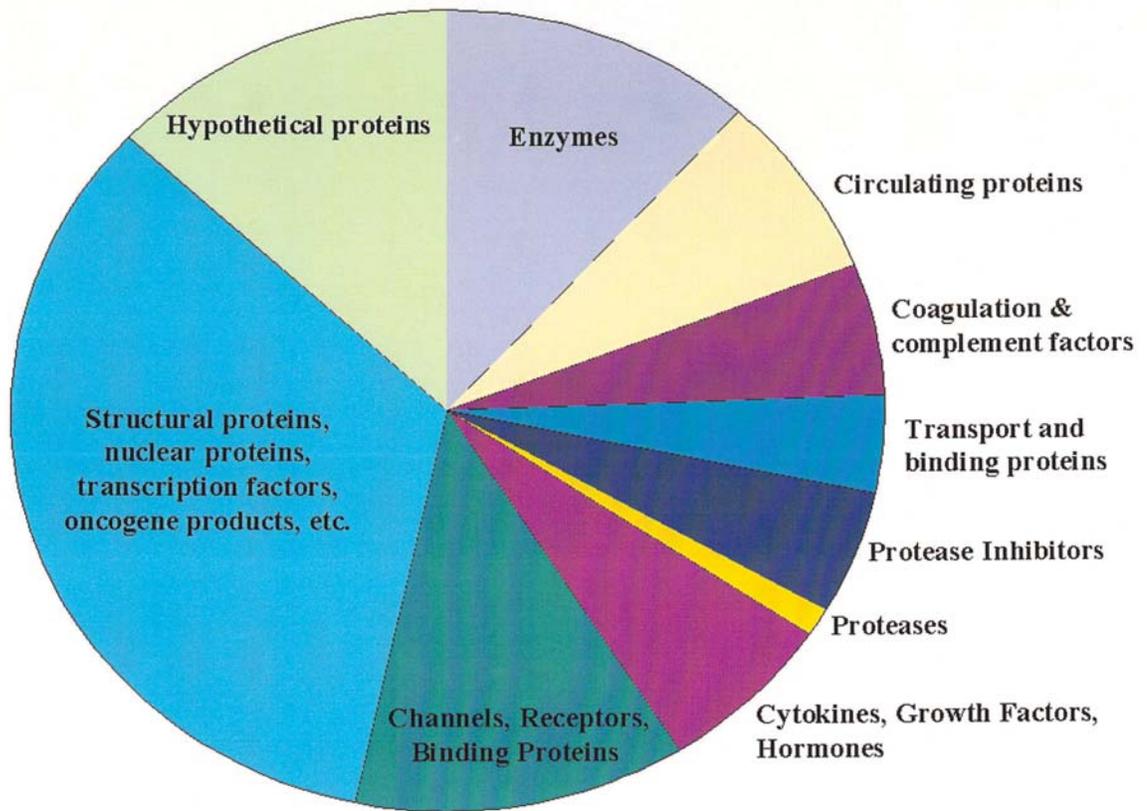
Mehta et al 2003

NEW VIEW  
of the  
SERUM PROTEOME

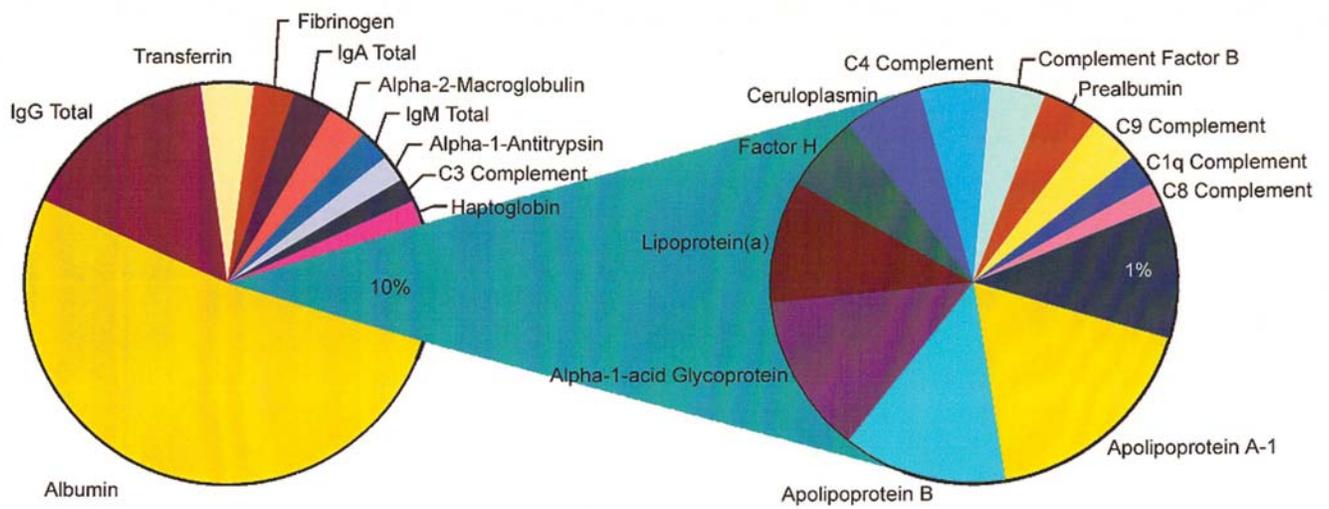


Biomarkers are bound and amplified by carrier pr

## Low Molecular Weight Serum Proteome



Tirumalai et al., Figure 6



Tirumalai et al., Figure 1

## The Albumin Serum Carrier Protein's Binding Partner Classes

Intracellular	Organ / System	Extracellular
Apoptosis/Survival	Bone Marrow	Antibody
Cytoskeleton	Brain	Cell Adhesion
Golgi	Cardiac	Complement/ Clotting
Cell Surface/Membrane	Endocrine	Immunoglobulins
Mitochondria	-Steroid metabolism	Infectious Diseases
Nucleus	-Hormone receptor	-HIV
-Chromatin	-Hormone steroid	-Rotavirus
-Transcription	Fetal	
Inhibitors Protease/Proteinase	Lipid	Inflammation
Signal transduction	Liver	Motility
-Kinases	-Iron Metabolism	Oncogene
-Phosphatase	Ovary	Secreted
Ribosome	Skin/Epithelium	Stress/ Hypoxia
	-Keritinocyte	Unknown/New

# Nano-particle Harvesters Collect Diagnostic Biomarkers

