Technology For Proteomics
Translation to Clinical Research Studies

Lance A. Liotta MD PhD
George Mason University

A. Novel one step preservative for tissue phosphoproteins
B. Protein Microarrays: 200 signal pathway phosphoproteins
   - Translation to clinical research trials
   - The universal tissue preservative: obviate frozen storage

C. Smart nanoparticles for one step in-solution molecular size sieving, affinity capture, biomarker preservation and amplification of effective sensitivity.
There is a need to measure the state of activity of the actual drug targets (the proteins) in a patient’s individual cancer.

Proteomics is the missing link for designing individualized therapies

Concurrent phosphorylation of kinases and kinase substrates indicates functional linkage
Genetic or epigenetic defects are selected during cancer progression because they cooperate to orchestrate alterations in protein networks generating a survival advantage for the target cell.

Post-translational modifications, such as phosphorylation, reflect the activity state of cellular signaling networks.

Patterns of phosphorylation indicate docking events and infer protein-protein interactions.
Pre-analytical Variables: The tissue is alive!

• The tissue is alive and reactive post excision
• During the post excision delay time, tissue signal pathway biomarkers fluctuate upward and downward as the tissue undergoes hypoxia, metabolic acidosis, wounding, hypotension, hypoglycemia, and dehydration.
• Fixation chemistries of the future that stabilize ex vivo reactive biomarkers
• Universal preservation of tissue biomarkers without freezing
PROTEIN MICROARRAYS

Circuit Mapping in the Tissue Microenvironment

Patient

Biopsy → Microdissection

Protein microarray
State of phosphorylated cell signaling proteins
Thousand of times more sensitive compared to IHC
Minimization of pre-analytical variables: novel phosphoprotein preservative

Comparison of \textit{in vivo} effects of molecular targeted therapy before and after therapy.

Establish workflow for multi-site trial w/o freezing for sample shipment.
US Oncology 05-074
Phase II Randomized Trial of Neoadjuvant Trastuzumab
and/or Lapatinib plus Chemotherapy

Target enrollment: 99  Enrolled to date: 82

 Eligibility:  Her2+ invasive breast cancer, Stage II/III, adequate cardiac function

Randomize Treatment

Trastuzumab (T)
Lapatinib (L)
Trastuzumab + Lapatinib (T+L)

Continuation of  T, L, or T+L
plus FEC75

Continuation of  T, L, or T+L
plus Paclitaxel

Surgery

Pre-Treatment
Core Needle Biopsy
Day 1

Post-Treatment
Core Needle Biopsy
Day 14

LCM and RPPA analysis pre-treatment

LCM and RPPA analysis post-treatment
Phase II Randomized Trial of Neoadjuvant Trastuzumab and/or Lapatinib plus Chemotherapy (Sequential FEC75 and Paclitaxel) in Women with ErbB2 (HER2/neu) Over-expressing Invasive Breast Cancer

Laser Capture Microdissection Pre and Post Treatment Core Needle Biopsies

Reverse Phase Protein Microarrays
Sensitivity: <500 cells
Built-in calibrators and controls
Precision: CV 3-10%
CAP CLIA compliant

Gallagher R, et al AACC 2009 abstract #2756
Concordance of Total Her2 RPMA Measurements with Her2 Central FISH Data  
n=63  

95% concordance  
(critical IHC data was 93% concordant)  

FISH+/RPMA Her2+  
FISH-/RPMA Her2-  

Wulfkuhle J, Petricoin EF ASCO 2009 Abstracts 11009/11014
Are patients with elevated phosphoHer2 likely to benefit from ErbB inhibition?

Patients with high phospho Her2 levels but low FISH scores may be eligible, or benefit from, anti-ErbB therapy.
Clinical research trials using phosphoprotein signal pathway profiling for stratification of tyrosine kinase inhibitors

A. Breast Cancer: (USO, Inova TKI: EGF/HER2 combination therapy
Status: Started Sept 2007 Target Completion Dec 2009

B. Multiple Myeloma: (Hem Oncol Assoc, Inova) targeted inhibitor screening
Status: Started May 2007 Target Completion May 2010

C. Breast Cancer Carcinoma in Situ: DCIS cancer stem cells
Status: Started Sept 2007 Target Completion Dec 2009

D. Breast Cancer Carcinoma in Situ: Treatment
Status: Will start in Dec 2009 Target Completion Dec 2011

E. Colon Cancer Liver Metastasis: Stratified combination therapy
Status: Started Aug 2009 Target Completion Dec 2010

F. Breast Cancer Stage IV: Side Out sponsored trial individualized therapy based on genomic and proteomic analysis
Status: Will start in Dec 2009 Target Completion 2010
Core needle biopsy

One Step Molecular Preservation and Fixation
a) Preserves morphology for histopathologic diagnosis
b) Stabilizes proteins, phosphoproteins and PTMs
c) Stabilizes RNA, miRNA, and DNA
d) Can be frozen for frozen section diagnosis or used for Flow Cytometry
e) Paraffin embedding for indefinite storage at room temperature

Multipurpose Molecular Preservation, Histologic Fidelity, and RT Storage

Paraffin Block

Archive

Laser Capture Microdissection

Protein Microarray

Genomics Microarray
22 proteins constitute 99% blood protein mass.

- A stage I cancer with a diameter of less than 0.5 cm: biomarker conc: **picogram/ml**

- Current mass spec methods can not detect less than **10 nanograms/ml** ms/MRM does not have sufficient sensitivity, precision or dynamic range.

Biomarkers exist in very low concentration: Significantly below the detection limits of mass spectrometry

Obscured by abundant resident blood proteins such as albumin

Rapidly degraded by enzymes post collection
Novel technology to overcome biomarker physiologic barriers

Smart” Core Shell Affinity Bait Nanoporous Particles

• Three independent functions within minutes, in one step, in solution:
  – a) Molecular size sieving
  – b) Affinity capture of all solution phase target molecules
  – c) Complete protection of harvested proteins from enzymatic degradation
  – d) Amplify the effective concentration of very low abundance molecules
• Particles can be produced in large quantities
• Stable at room temperature indefinitely
• Low cost
• Uniform in size (0.7 micron)
• Reproducibility among batches
Hydrogel NIPAm/ Core Synthesis Bait Covalent Binding
<table>
<thead>
<tr>
<th>Bait</th>
<th>Target</th>
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<tbody>
<tr>
<td>Acrylic acid</td>
<td>Cationic proteins and polypeptides</td>
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<tr>
<td>allylamine, 1-vinylimidazole</td>
<td>Anionic proteins and polypeptides</td>
</tr>
<tr>
<td>Cibacron blue F3G A, Procion Red H8BN</td>
<td>Proteins and polypeptides</td>
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<tr>
<td>Cyclodextrins</td>
<td>Small molecules, cholesterol</td>
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<tr>
<td>p-vinylphenyl boronic acid, N-acryoyl-(m)-aminophenyl boronic acid</td>
<td>Polysaccharides, glycopeptides, RNA</td>
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<tr>
<td>TiO(_2) nanoparticles incorporated in NIPAm beads</td>
<td>Phosphopeptides</td>
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</table>
In-solution harvesting

Smart particles amplify the biomarker concentration

Nanoparticles in vacutainer blood collection tubes

5 ml

50 µl

100 fold amplification

Alessandra Luchini, 2009 AACR Annual Meeting
Capture Efficiency >99.95%

Bait specific high affinity protein biomarker binding achieves virtually 100% sequestration of all solution phase analyte molecules. Elution yield is 100%

Analyte remaining in solution following introduction of nanoparticles

Urine Acld Black 48

Cibacron Blue F3G-A Reactive Blue 2

Immulite detection limit 50 picograms/mL
Size sieving & complete albumin exclusion

Concentration: High Precision %C.V. <3.0

Complete protection from degradation

Rapid 100 %uptake: minutes
Human Growth Hormone

Levels in the blood: 1-10 ng/mL

Levels in the urine: 1000 fold less than serum

Below the detection limits of clinical immunoassays

Nanoparticles amplify the effective concentration of hGH 50 fold to achieve routine measurement in urine

CERES NANOSCIENCE
License of ISS/GMU IMAT IP
### Amplification of Low Abundance Labile Biomarker Proteins by Smart Nanoparticles

**100 fold Effective Amplification of Mass Spec Peptide Sensitivity**

**Amplification of Clinical ELISA Sensitivity:** Linear Measurement of serum PDGF for levels that are 100 fold below the ELISA detection limit.

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<th>Dilution</th>
<th>Particle Eluate Peptide</th>
<th>Peptide (Hits) [Ions]</th>
<th>Starting Solution Peptide</th>
<th>Peptide (Hits) [Ions]</th>
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<td>15/18</td>
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<td>20/30</td>
<td>R.TNANFLWPPCVEVQR.C</td>
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**PDGF was not detected**

For dilutions 1:6000 and 1:600000, PDGF was not detected.
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<th>P (pro)</th>
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<th>Score</th>
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<td>alpha—2-HS-glycoprotein [Homo sapiens]</td>
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<td>platelet—derived growth factor beta isoform 1, preproprotein [Homo sapiens]</td>
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<td>80.22</td>
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<td>platelet—derived growth factor beta isoform 2, preproprotein [Homo sapiens]</td>
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<td>80.22</td>
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<td>insulin-like growth factor 1 (somatomedin C) [Homo sapiens]</td>
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<td>RAP1A, member of RAS oncogene family [Homo sapiens]</td>
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<td>116470.7</td>
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</table>
ISS Sponsored GMU Translational Research Program

Istituto Superiore di Sanità – Rome
George Mason University Virginia USA

Professor Enrico Garaci
Ruggero DeMaria
Claudio Belluco

Participating Centers:
- IEO - Milan
- INT – Milan
- IST Genova
- CRO - Aviano
- IRE - Rome
- IRCCS Oncol. - Bari
- Univers. - Brescia
- Ospedale Maggiore - Milan
- Surgery and Pediatric Depts. - Padova
- S. Camillo Hosp - Rome

NCI USA- Italy Bilateral Agreement
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<th>TUMOR TYPE</th>
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<td>Target: 10085 at present 4474</td>
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Prostate  (3600 serum samples expected: at present 858)
NanoTrap
MRM

Biomarker Discovery Workflow Employing Nanoparticle capture

High Throughput,
High Sensitivity Discovery,
Preservation and Validation
Melanoma

Prostate Cancer

Ovarian Cancer

100 uL serum pilot
Smart Nanoparticles for Biomarker Harvesting

Example application to skin patch for diagnostic marker (proteins and metabolites) harvesting

- Water resistant cover
- Harvesting Nanoparticles
- Porous membrane
- Permeation enhancer

- User friendly non invasive
- Amplifies low abundant markers over time of patch duration
- Protects biomarkers from degradation
- Mail-in room temperature shipping
Center for Applied Proteomics and Molecular Medicine
Co-Directors: Lance Liotta and Emanuel Petricoin

IMAT Technology Development

Phosphoprotein Smart Nanoparticles

Tissue Preservative

Virginia Espina
Claudius Mueller
Alex Reeder
Kirsten Edmiston
Lindsay Wescott
Sally Rucker

Alessandra Luchini
Virginia Espina
Claudia Fredolini
Caterina Longo
Davide Tamburro
Barney Bishop
Alexis Patanaraut
Ben Espina
Linday Wescott
Alex Reeder

Licensed to Theranostics Health Licensed to Ceres Nanoscience
Sponsors of Breast Cancer Research at GMU

GlaxoSmithKline/US Oncology
DOD Breast Cancer Research Program

NIH/National Cancer Institute

Susan G. Komen Foundation

Collaborating Organizations

University of California San Francisco

Inova Health System (Inova Fairfax Hospital)

Side Out Foundation
“Smart” Nanoporous Particles / Biomarker publications


