Theranostics Health

Drug Target Activation Mapping Through a CLIA Assay: A Companion Diagnostic Engine for Personalized Medicine Trials

Dr. Emanuel F Petricoin
DISCLOSURES:
Co-Founder/shareholder Theranostics Health, Inc
Co-Inventor/licenssee, RPPA Technology and associated biomarkers
DNA: The blueprints

PROTEINS: The Working Machinery
- Most often the drug target itself
- Sometimes the therapy
Gene domino effect behind brain, pancreatic tumors

By LAURAN NEERGAARD
AP Medical Writer

WASHINGTON (AP) -- Scientists have mapped the cascade of genetic changes that turn normal cells in the brain and pancreas into two of the most lethal cancers. The result points to a new approach for fighting tumors and maybe even catching them sooner. Genes blamed for one person's brain tumor were different from the culprits for the next patient, making the puzzle of cancer genetics even more complicated.

But Friday's research also found that clusters of seemingly disparate genes all work along the same pathways. So instead of today's hunt for drugs that target a single gene, the idea is to target entire pathways that most patients share. Think of delivering the mail to a single box at the end of the cul-de-sac instead of at every doorstep.
Cancer is driven by hyperactive or defective protein circuits

The components of these circuits contain the drug targets of the future.

Each patient’s cancer is different. A drug that works for one patient may not work for another patient with the same cancer.
Delivering Personalized Therapy Data for Cancer: Technical Barriers

- Tissue input: Tiny FNA or Core Needle Biopsy: hundreds to thousands of cells not millions to billions
- Tissue preservation and fixation at tertiary sites limited to formalin and lack of proper storage temp.
- Tissue heterogeneity
- Need to deliver both quantitative and multiplexed information about signaling network activation
Core needle biopsy 16 gauge needle
12 mm x 2.5 mm tissue

Clinical Proteomics: Some practical issues

$10^9$ cells in tissue section

15,000 – 30,000 tumor cells for microdissection

Fine Needle Aspirate

2 needle passes

1000 – 25,000 cells
Phosphoprotein Drug Targets as Biomarkers

Measuring the presence and \textit{activation status} of the proteins and drug targets in key cancer-driving signaling pathways.

- A comprehensive assessment of a tumor’s active signaling architecture, assessing multiple pathways and drug targets, in a single assay.
  - If a drug target and its pathway are inactive, then the drug should not work.
  - If a drug target and its pathway are active, this information should be considered when devising a therapeutic regime or seeking a clinical trial.
- Allows for the association of the functional consequences of genomic alterations at level of signal transduction protein activation; i.e. drug targets.
Trained Mills Lab
Trained Nishizuka/Weinstein Lab
100 laboratories worldwide


Idea Technology Proof of principle Basic science applications Pre-clinical/translational Clinical trials

- Miniaturized ligand immunoassay
  Ekins R & Chu FW (1991)
- Molecular profiling
- Propose antigen-down protein microarray
  Liotta LA & Petricoin EF (2000)

- Robotic arrayer
- Protein binding substratum
- Commercially available antibodies
- Phospho-specific antibodies
- Laser Capture Microdissection

Patent 6,969,614
Liotta LA, et al.
Feb. 16, 2000

Commercialization
Wide-spread adoption / Independent labs
Validation in leukemia
Tibes R, et al. (2006)

Enabling technologies
Phosphoprotein stabilization
Espina V, et al. (2008)

- Reverse Phase Protein Microarray
  Prostate phosphoprotein signaling
  Paweletz C, et al. (2001)
- NCI-60 cell line profile
- Follicular Lymphoma biomarkers
- Rhabdomyosarcoma, Breast cancer
  Lung cancer, Melanoma, Leukemia

- Predict treatment response
- Design rationale therapy
- Correlate outcome with phosphoproteomic profile
Reverse-Phase Protein Array (RPPA)

**Step 1.** Histopath sections, tumor cell enrichment (LCM), lysate prepared.

**Step 2.** Multiple sample lysates, positive/negative controls, and the calibrators* that define the linear range for each biomarker tested.

**Step 3.** Duplicate dilutions printed on replicate nitro-cellulose Slides using quadrant printing, to minimize spatial variability. Slides are stained to determine the protein loading (Cy3).

**Step 4.** Each slide is interrogated with a biomarker-specific Ab* using a programmable autostainer. After exposure to a 1° antibody, each slide is incubated with a universal 2° Antibody (biotin-HRP), followed by signal amplification (Cy5).

*FFPE CNBs, flash frozen sections, PBMCs, hair follicles, etc.

*Provides semi-quantitative measurement of each biomarker

Data analysis:

Intensities from QC-samples are used to assess each assay’s performance and the linearity of the calibrators confirmed.

Raw fluorescence intensity values from dilutions that fall in the linear range are then normalized to total protein and reported as Normalized Fluorescence Intensity.

**AKT S473**

**mTOR2 S2448**

*Over 450 Abs optimized for RPPA
Actionable molecular information for clinical applications:

- Oncologists
- Clinical Trials

Scoring Against Representative Patient Populations

Population Based Expression Level or Level of Activation

- Visual Scoring Representation
  - Low
  - High

- Mean
- Std. Dev. From Mean

Number of Individual Tumors

- Below the Population Mean
- Between the Population Mean and 1 STD above the Mean
- Between 1 STD and 2 STD Above the Population Mean
- Greater than 2 STD Above the Population Mean

ND – Value below the linear range of detection.

Linear Range of Assay

- Population Mean
- Patient Value
Second Generation TheraLink® Assay

Receptor Tyrosine Kinases

EGFR (HER1)
- p-EGFR-Y1068
- HER2
- p-HER2-Y1248
- HER3
- p-HER3-Y1249
- p-Met-Y1234-1235
- p-IGF-1R-Y1135
- P-FGF-1R-Y653-654
- p-VEGFR2-Y951
- p-ALK-Y1604
- p-Src-Y416
- Androgen Receptor (AR)
  - p-AR S650
  - p-Mek 1/2-S217-221
  - p-Erk 1/2 T202-Y204
  - p-Akt-SS473
  - p-Akt-T308
  - p-mTORC1-S2448
  - p-4E-BP1-S65
  - p-4EB-P1-T37-46
  - p-S6 Ribo. Protein-S235-236
  - p-Jak2-Y1007-1008
  - p-STAT3-Y705

Pathway Activation

JAK2

AKT

mTORC1

4EBP1

p70S6K

Mek1/2

Erk1/2

AR

Tumor Growth

Angiogenesis

Proliferation

Proliferation

Cytoplasm

Nucleus
Coverage of Targeted Therapies

**Cytoplasm**

- Cetuximab
- Panitumumab
- Erlotinib
- Gefitinib
- Vandetanib
- Lapatinib

**Pathway Activation**

- Trastuzumab
- Pertuzumab
- Afatinib
- Dasatinib
- Bosutinib
- Ponatinib
- Vandetanib
- Cetuximab
- Panitumumab
- Lapatinib
- Erlotinib
- Gefitinib
- Vandetanib

**Cytoplasm**

- Erk1/2
- Mek1/2

**Proliferation, Cell Growth, Angiogenesis, Migration, Survival**

- Everolimus
- Sirolimus
- Temsirolimus
- Trametinib
- Ruxolitinib
- Cabozantinib
- Ceritinib
- Ramucirumab
- Bevacizumab
- Alfiberecept
- Sorafenib
- Sunitinib
- Axitinib
- Carbozantinib
- Levatinib
- Ponatinib
- Dasatinib
- Bosutinib
- Axitinib
- Vandetanib
- Everolimus
- Sirolimus
- Temsirolimus
- Trametinib
- Ruxolitinib
- Cabozantinib
- Ceritinib
WHAT IS THE SCIENTIFIC AND CLINICAL EVIDENCE THAT MEASURING THE ACTIVATION AND LEVELS OF THESE PROTEINS ARE CLINICALLY IMPORTANT TO HELP GUIDE THERAPY?

WHAT DO THESE PROTEINS/PHOSPHOPROTEINS HAVE TO DO WITH HOW TARGETED THERAPEUTICS WORK??

UNIQUE AND POWERFUL ATTRIBUTE OF THE REVERSE PHASE PROTEIN ARRAY TECHNOLOGY:

MEASURE MULTIPLE (UP TO SEVERAL HUNDRED) PROTEINS/PHOSPHOPROTEINS AT ONCE IN A SINGLE ASSAY

The 14 Analytes Measured by the TheraLink™ HER Family Assay

<table>
<thead>
<tr>
<th>HER Family Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. EGFR</td>
</tr>
<tr>
<td>2. EGFR Tyrosine 1068*</td>
</tr>
<tr>
<td>3. HER2</td>
</tr>
<tr>
<td>4. HER2 Tyrosine 1248*</td>
</tr>
<tr>
<td>5. HER3</td>
</tr>
<tr>
<td>6. HER3 Tyrosine 1289*</td>
</tr>
<tr>
<td>Akt/mTOR Pathway</td>
</tr>
<tr>
<td>7. Akt Serine 473*</td>
</tr>
<tr>
<td>8. mTOR Serine 2448*</td>
</tr>
<tr>
<td>9. 4EBP1 Serine 65*</td>
</tr>
<tr>
<td>10. S6 Ribo. Prot. Serine 235/236*</td>
</tr>
<tr>
<td>MAPKinase Pathway</td>
</tr>
<tr>
<td>11. Mek1/2 Serine 217/221*</td>
</tr>
<tr>
<td>12. Erk 1/2 Threonine 202/Tyrosine 204*</td>
</tr>
<tr>
<td>Jak/Stat Pathway</td>
</tr>
<tr>
<td>13. Jak2 Tyrosine 1007/1008*</td>
</tr>
<tr>
<td>14. Stat3 Tyrosine 705*</td>
</tr>
</tbody>
</table>
HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use AFINITOR safely and effectively. See full prescribing information for AFINITOR.

AFINITOR (everolimus) tablets for oral administration
Initial U.S. Approval: 2009

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action
Everolimus is an inhibitor of mammalian target of rapamycin (mTOR), a serine-threonine protein kinase involved in the PI3K/AKT pathway. The mTOR pathway is dysregulated in several human cancers, including breast, colorectal, renal, and liver cancers. Everolimus reduces the activity of S6 ribosomal protein kinase (S6K1) and eukaryotic initiation factor 4E binding protein (4E-BP1), downstream effectors of mTOR, involved in protein synthesis. Inhibition of mTOR by everolimus has been shown to reduce cell proliferation in in vitro and/or in vivo studies.
RPPA Measures Signaling Pathway Modulation

Biomarker I

Biomarker II

Biomarker III

Biomarker IV

- Time
- Drug Dosage
- Treatment
RESPONSE TO EGFR THERAPY REGARDLESS of EGFR MUT STATUS IS PREDICTED BY PROTEOMICS (PHOSPHORYLATED EGFR Y1086 AND DOWNSTREAM AKT )
Case Study 1.

**Patient:** Female 50 years old  
**Diagnosis:** Triple negative chemo-refractory IBC  
**History:** 3 lines of neoadjuvant therapies prior to bilateral mastectomy  
**Sample received:** Right breast masectomy

**Interpretation:** The patient exhibits overexpressed HER2 and EGFR receptors. EGFR, HER2 and HER3 are activated. Phospho-mTOR is activated but downstream signaling (p-4E.BP1 and p-S6 Ribo. Prot.) is low.

**Therapy:** Patient was started on lapatinib and capecitabine.

**Outcome:** She completed treatment and remains with no recurrent disease.
Case Study 2.

**Patient:** Female 54 years old  
**Diagnosis:** ER+, HER2+  
**History:** Progressed on multiple HER2 therapies  
**Sample received:** Left breast skin punch biopsy

**Interpretation:** The patient exhibits high activity downstream in the mTOR pathway (with accumulation of p-4E.BP1 and p-S6. Ribo. Prot. S235-236). There is some MAPK activation with an accumulation of Erk ½ T202 Y204.

**Therapy:** Her therapy was changed to exemestane/everolimus.

**Outcome:** Objective response demonstrated by improvement in skin nodules and erythema for several months.
Case Study 3.

**Patient:** Female 44 years old  
**Diagnosis:** ER+, PR+, HER2 equivocal metastases IBC  
**History:** Refractory to all chemotherapy, trastuzumab and lapatinib  
**Sample received:** Left breast biopsy

**Interpretation:** The patient exhibits overexpression of EGFR, with both EGFR and HER2 activated. Downstream, the mTOR pathway is activated with accumulation of p-4E.BP1.  
**NGS:** PTEN deletion

**Therapy:** Her therapy was changed to afatinib.

**Outcome:** Her diffuse chest wall has responded but the leading edges are still advancing.
Identifying molecular targets and mechanisms of treatment resistance in Inflammatory Breast Cancer (IBC) using next-generation sequencing (NGS) and reverse-phase protein protein arrays (RPPA).

NGS alterations found in the mTOR signaling pathway include:
- PIK3CA, AKT1 mutations,
- PTEN loss, and
- AKT2 amplification

Austin, L, et al. 2015  AACR Abstract 4847
Molecular Profiling in Breast Cancers

Phosphoproteomic profiles* of 38 primary breast adenocarcinomas showing the heterogeneity in signaling activity in HER2-negative as well as HER2-positive tumors.

*Patients binned low to high based upon HER2 levels
Concordance of total Her2 RPMA Measurements with Her2 Central IHC Data
N=118

No false positives
93% concordance
Concordance of total Her2 RPMA Measurements with Her2 Central FISH Data

N=63

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

FISH+/RPMA Her2+

FISH-/RPMA Her2-

Ratio_Her2_to_CEP17

Her2 unamplified
Her2 borderline
Her2 amplified

Concordance of total Her2 RPMA Measurements with Her2 Central FISH Data
N=63
Correlation of Her2 FISH Data and phospho-Her2 Intensities Reveals a Population of FISH-/phospho-Her2+ Tumors

FISH-/phosphoHER2+
(ALSO COMMUNITY AND CENTRAL IHC -)
~10% of FISH/IHC- are pHER2+ and are pathway activated
this hypothesis. Available tissue blocks were examined at a central site by means of a Food and Drug Administration–approved HER2 FISH assay. We found no significant association between HER2 copy number and benefit (P = 0.60). Even patients with normal gene copy numbers appeared to benefit (relative risk for disease-free survival, 0.40; 95% confidence interval [CI], 0.18 to 0.89; P = 0.026).

HER2 status according to immunohistochemical analysis with the use of Herceptest (Dako) was also determined at a central site. Tumors that were negative on FISH and had an immunohistochemical staining intensity of less than 3+ were defined as “central HER2-negative,” as in our previous report. Among the 1787 patients with follow-up data, 174 patients had breast cancers that were found to be central HER2-negative (9.7%), yet these patients also appeared to benefit from trastuzumab (relative risk for disease-free survival, 0.34; 95% CI, 0.14 to 0.80; P = 0.014) (Table 1).

Ellis et al- Cancer Discovery

1-2% activating HER2 mutations without amplification

But higher percent (10%) of FISH/IHC- were seen as receiving benefit by HERCEPTIN

~10% were phosphoHER2+ !

Single readout:
Functional activation of the drug target itself (phosphoHER2)

PAIK ET AL, NEJM 2008
I-SPY 2 Adaptive Trial: Introduce several new agents for a given profile

Patient is on Study

HER 2 (+)
- Paclitaxel + Trastuzumab
- Paclitaxel + Trastuzumab* + New Agent A
- Paclitaxel + Trastuzumab* + New Agent B
- Paclitaxel + Trastuzumab* + New Agent C

HER 2 (-)
- Paclitaxel
  - Paclitaxel + New Agent C
  - Paclitaxel + New Agent D
  - Paclitaxel + New Agent E

AC: doxorubicin/cyclophosphamide

Learn, adapt from each patient as we go along

AC → Surgery

Key:
- MRI
- Residual Disease (Pathology)

*Or equivalent
I-SPY 2 Adaptive Trial: Learn, Drop, Graduate, and Replace Agents Over Time

Patient is on Study

HER 2 (+)

Paclitaxel+ Trastuzumab
Paclitaxel + Trastuzumab* + New Agent A
Paclitaxel + Trastuzumab* + New Agent B
Paclitaxel + Trastuzumab* + New Agent F

AC → Surgery

Learn, adapt from each patient as we go along

HER 2 (-)

Paclitaxel
Paclitaxel + New Agent F
Paclitaxel + New Agent GH
Paclitaxel + New Agent E

AC → Surgery

*Or equivalent

Key
- MRI
- Residual Disease (Pathology)
Evaluation of HER family protein signaling network as a predictive biomarker for pCR for breast cancer patients treated with neratinib in the I-SPY 2 TRIAL

JD Wulfkuhle et al. San Antonio Breast Cancer Conference 2013

<table>
<thead>
<tr>
<th>Signature</th>
<th>Estimated pCR Rate (95% probability interval)</th>
<th>Probability Neratinib is Superior to Control</th>
<th>Predictive Probability of Success in Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neratinib</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>HR- / HER2-</td>
<td>36% (29-43%)</td>
<td>30% (23-38%)</td>
<td>72%</td>
</tr>
<tr>
<td>HR- / HER2+</td>
<td>55% (46-64%)</td>
<td>32% (22-43%)</td>
<td>94%</td>
</tr>
<tr>
<td>HR+ / HER2-</td>
<td>14% (8-19%)</td>
<td>16% (10-21%)</td>
<td>39%</td>
</tr>
<tr>
<td>HR+ / HER2+</td>
<td>31% (24-37%)</td>
<td>17% (10-24%)</td>
<td>91%</td>
</tr>
</tbody>
</table>
### ALL NERATINIB pCR Y vs. NO

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Control</th>
<th>Neratinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT S473</td>
<td>0.963</td>
<td>0.308</td>
</tr>
<tr>
<td>AKT T308</td>
<td>0.284</td>
<td>0.645</td>
</tr>
<tr>
<td>EGFR total</td>
<td>0.897</td>
<td>0.127</td>
</tr>
<tr>
<td>EGFR Y1068</td>
<td>0.149</td>
<td>0.007</td>
</tr>
<tr>
<td>EGFR Y1148&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.530</td>
<td>0.932</td>
</tr>
<tr>
<td>EGFR Y1173</td>
<td>0.460</td>
<td>0.003</td>
</tr>
<tr>
<td>EGFR Y992</td>
<td>0.180</td>
<td>0.044</td>
</tr>
<tr>
<td>ERBB2 total</td>
<td>0.681</td>
<td>0.018</td>
</tr>
<tr>
<td>ERBB2 Y1248</td>
<td>0.380</td>
<td>0.013</td>
</tr>
<tr>
<td>ERBB3 total</td>
<td>0.132</td>
<td>0.905</td>
</tr>
<tr>
<td>ERBB3 Y1289</td>
<td>0.906</td>
<td>0.123</td>
</tr>
<tr>
<td>ERK1/2 T202/Y204</td>
<td>0.839</td>
<td>0.596</td>
</tr>
<tr>
<td>Heregulin total</td>
<td>0.912</td>
<td>0.740</td>
</tr>
<tr>
<td>mTOR S2448</td>
<td>0.789</td>
<td>0.204</td>
</tr>
<tr>
<td>mTOR total</td>
<td>0.241</td>
<td>0.188</td>
</tr>
<tr>
<td>PI3K p85 Y458/p55 Y199&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.937</td>
<td>0.059</td>
</tr>
<tr>
<td>PTEN S380&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.652</td>
<td>0.848</td>
</tr>
<tr>
<td>SHC Y317</td>
<td>0.493</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Distribution of pCR rates within EGFR Y1173 Low/High groups stratified by the graduating HR-/HER2+ signature in each treatment arm.

When EGFR Y1173 HIGH patients are added to the graduating HR-/HER2+ subset, the OR associated with treatment was 3.2 and is comparable to that in the HR-/HER2+ signature alone (OR=2.1).
When EGFR Y1173 HIGH patients are added to the graduating HR-/HER2+ subset (HR-/HER2+ OR EGFR Y1173 High), the predicted probability of Phase III trial success is 90%, which is comparable to that for the graduating signature alone, while increasing the prevalence of biomarker-positive patients by ~50%.

<table>
<thead>
<tr>
<th></th>
<th>Prob&gt;CTRL</th>
<th>Prob Ph III Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR-/HER2+</td>
<td>0.97</td>
<td>0.87</td>
</tr>
<tr>
<td>EGFR Y1173 High</td>
<td>0.99</td>
<td>0.93</td>
</tr>
<tr>
<td>HR-/HER2+ OR EGFR Y1173 High</td>
<td>0.99</td>
<td>0.9</td>
</tr>
<tr>
<td>HR-/HER2+ AND EGFR Y1173 High</td>
<td>0.99</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Protein activation mapping uncovers exploratory predictive markers for pCR in triple negative breast cancer patients treated with neratinib in the I-SPY 2 TRIAL

JD Wulfkuhle et al ASCO 2015
Protein activation mapping uncovers exploratory predictive markers for pCR in triple negative breast cancer patients treated with neratinib in the I-SPY 2 TRIAL

JD Wulfkuhle et al ASCO 2015

<table>
<thead>
<tr>
<th>Patient subset</th>
<th>Prob neratinib &gt; Ctr</th>
<th>Predictive probability of phase 3 success (300 pt)</th>
<th>Prevalence (% TN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected TN* (n=49)</td>
<td>0.76</td>
<td>0.42</td>
<td>100%</td>
</tr>
<tr>
<td>TN/EGFR.Y1173-high (n=27)</td>
<td>0.88</td>
<td>0.72</td>
<td>55%</td>
</tr>
<tr>
<td>TN/ERBB2.Y1248-high (n=30)</td>
<td>0.95</td>
<td>0.82</td>
<td>61%</td>
</tr>
<tr>
<td>TN/(EGFR.Y1173-high AND ERBB2.Y1248-high) (n=21)</td>
<td>0.99</td>
<td>0.95</td>
<td>43%</td>
</tr>
</tbody>
</table>

Table 5. Bayesian probabilities and biomarker prevalence
*estimate from a model with EGFR.Y1173 as the added biomarker.
# The Future of Companion Diagnostics:

## 2015-beyond:

- ~ 75 analytes measured
- 60 FDA cleared targeted therapies
- IMMUNOTHERAPEUTICS

What assay platform can quantitatively Measure 75 CDx targets at once from LCM material from a single core biopsy?

**Reverse Phase Protein Array**
Reclassification of Cancer by Functional Protein Pathway Activation

Classification by Location/Type of Cancer

Classification by Protein Activation Mapping

Adapted from WO 2009076472, US 20090155804: "Blume-Jensen"
CDx Report of the Future: Individualized Protein Pathway Activation Maps

Breast Cancer Patient 1

Breast Cancer Patient 2