Blood Biomarkers for Triple Negative Breast Cancer

Karen S. Anderson MD PhD
Associate Professor
Biodesign Institute, Arizona State University
Mayo Clinic Arizona
Disclosure Information

• I serve on the scientific advisory board and am a consultant for ProvistaDx.

• I hold patents on breast cancer biomarkers

• None of these biomarkers are approved for clinical use.
Moving Biomarkers From Discovery To Patients
## Uses of Biomarkers in Cancer Medicine

<table>
<thead>
<tr>
<th>Prior to Cancer</th>
<th>Diagnosis</th>
<th>After Cancer Diagnosis</th>
<th>Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Assessment</td>
<td>Diagnosis</td>
<td>Prognosis</td>
<td>Predicting Treatment Response</td>
</tr>
<tr>
<td>Am I at increased risk for cancer?</td>
<td>Do I have cancer? What type of cancer do I have?</td>
<td>What is the expected course of my cancer?</td>
<td>Will my cancer respond to this drug?</td>
</tr>
</tbody>
</table>

---

*Biomarkers in Cancer: An Introductory Guide for Advocates* is copyrighted by Research Advocacy Network – All rights reserved.
Early Detection of Breast Cancer

- Survival from breast cancer decreases with increasing stage at diagnosis
- Early detection is critical for improving morbidity and mortality of breast cancer
- Breast cancer progresses at different rates
Highly proliferative cancers frequently present as palpable masses

I-SPY-1 trial: >70% interval cancers!!

Esserman and Thompson JAMA 2009
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Official gene name*</th>
<th>Clinical use</th>
<th>Cancer type</th>
<th>Source type</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-fetoprotein (AFP)</td>
<td>AFP</td>
<td>Staging</td>
<td>Nonseminomatous testicular</td>
<td>Serum</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
<td>CGB</td>
<td>Staging</td>
<td>Testicular</td>
<td>Serum</td>
</tr>
<tr>
<td>Carbohydrate antigen 19–9 (CA19–9)</td>
<td>MUC16</td>
<td>Monitoring</td>
<td>Pancreatic</td>
<td>Serum</td>
</tr>
<tr>
<td>Carbohydrate antigen 125 (CA125)</td>
<td>MUC16</td>
<td>Monitoring</td>
<td>Ovarian</td>
<td>Serum</td>
</tr>
<tr>
<td>Carcinoembryonic antigen (CEA)</td>
<td>PSG2</td>
<td>Monitoring</td>
<td>Colorectal</td>
<td>Tissue</td>
</tr>
<tr>
<td>Epidermal growth factor receptor (EGFR)</td>
<td>EGFR</td>
<td>Prediction</td>
<td>Colorectal</td>
<td>Tissue</td>
</tr>
<tr>
<td>v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT)</td>
<td>KIT</td>
<td>Prediction</td>
<td>Gastrointestinal</td>
<td>Tissue</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>TG</td>
<td>Monitoring</td>
<td>Thyroid</td>
<td>Serum</td>
</tr>
<tr>
<td>Prostate specific antigen (PSA)</td>
<td>KLK3</td>
<td>Screening and monitoring</td>
<td>Prostate</td>
<td>Serum</td>
</tr>
<tr>
<td>Carbohydrate antigen 15.3 (CA 15.3)</td>
<td>MUC1</td>
<td>Monitoring</td>
<td>Breast</td>
<td>Serum</td>
</tr>
<tr>
<td>Carbohydrate antigen 27.29 (CA27.29)</td>
<td>MUC1</td>
<td>Monitoring</td>
<td>Breast</td>
<td>Serum</td>
</tr>
<tr>
<td>Estrogen receptor (ER)</td>
<td>ESR1</td>
<td>Prognosis and prediction</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>Progesterone receptor (PR)</td>
<td>PGR</td>
<td>Prognosis and prediction</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (HER2-neu)</td>
<td>ERBB2</td>
<td>Prognosis and prediction</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>Nuclear matrix protein 22 (NMP-22)</td>
<td></td>
<td>Screening and monitoring</td>
<td>Bladder</td>
<td>Urine</td>
</tr>
<tr>
<td>Fibrin/fibrinogen degradation products (FDP)</td>
<td></td>
<td>Monitoring</td>
<td>Bladder</td>
<td>Urine</td>
</tr>
<tr>
<td>Bladder tumor antigen (BTA)</td>
<td></td>
<td>Monitoring</td>
<td>Bladder</td>
<td>Urine</td>
</tr>
<tr>
<td>High molecular CEA and mucin</td>
<td></td>
<td>Monitoring</td>
<td>Bladder</td>
<td>Urine</td>
</tr>
</tbody>
</table>

* Human genes: AFP, α-fetoprotein; CGB, chorionic gonadotropin, beta polypeptide; MUC16, mucin 16, cell surface associated; PSG2, pregnancy specific beta-1-glycoprotein 2; EGFR, epidermal growth factor receptor; KIT, Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; TG, thyroglobulin; KLK3, kalliukin-related peptidase 3; MUC1, mucin 1, cell surface associated; ESR1, estrogen receptor 1; PGR, progesterone receptor; ERBB2, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/globlastoma derived oncogene homolog (avian).
Utility of Blood Biomarkers

- **CEA**: Monitoring patients with metastatic disease
  - Use in conjunction with history, physical exam, and imaging
  - 50-60% positive
  - Not recommended for screening, diagnosis, staging, or routine surveillance of patients after primary therapy

- **CA27.29; CA15-3**: Monitoring patients with metastatic disease
  - Use in conjunction with history, physical exam, and imaging
  - 75-90% positive
  - Not recommended for screening, diagnosis, staging, or routine surveillance of patients after primary therapy

- **Caution** for use within the first 4-6 weeks of therapy

Conclusions:

• CEA, CA27.29, and CA15-3 are used for monitoring patients with metastatic disease
• Not recommended for screening, diagnosis, staging, or routine surveillance of patients after primary therapy
• There is an emerging set of blood biomarkers for cancer that have potential for early detection, prediction, and prognosis
• The challenge of new biomarkers is **validation and integration** with existing clinical detection methods
Example: Early studies on breast cancer biomarkers:

The immune system as a sentinel for breast cancer
Finding p53 autoantibodies in the blood

Anderson and LaBaer, J. Proteome Res. 2008
Detecting Antibodies with Custom Protein Microarrays

Replicate microscopic arrays of proteins

Probe with sera

Cell free expression of target protein

Printed cDNA’s with cancer relevance

- ~10,000 fully sequenced human genes available in ready-to-print format
  - >1000 Breast cancer related genes
  - >300 GPCRs
  - >500 kinases
  - >700 transcription factors

Clone → Sequence Verify → Add to repository

www.Dnasu.edu
Detection of a Panel of 28-specific AAbs in Breast Cancer
A 28 Autoantibody Panel for Breast Cancer Detection

Stage 1: Pre-Screen 4988 Ag’s

- Screening mammography: 53 cases/53 controls

Stage 2: Training set 761 Ag’s

- Diagnostic Mammography:
  - 51 cases/39 controls (Benign breast disease)

Stage 3: Validation (Blinded)

- **28 antigen panel**
  - 51 cases/38 controls (Benign breast disease)

- 17 investigators
- 4 institutions
- Thousands of women donated blood

Anderson et al, J. Proteome Res. 2011
The Value of Biorepositories

- NCI/EDRN Reference Set for Breast Cancer Biomarker Discovery
- Multi-site collection of serum and plasma
- All collected from screening and diagnostic mammography clinics
- Well-annotated and stored at NCI/Frederick
- Available for use for biomarker evaluation
- Over 70 biomarkers from multiple labs (and companies) being tested

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>218</td>
</tr>
<tr>
<td>BBD without atypia</td>
<td>63</td>
</tr>
<tr>
<td>BBD with atypia</td>
<td>231</td>
</tr>
<tr>
<td>DCIS</td>
<td>48</td>
</tr>
<tr>
<td>LCIS</td>
<td>7</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>190</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>757</strong></td>
</tr>
</tbody>
</table>
NCI/EDRN/CPTAC Biomarker Validation Study: Triple Negative Breast Cancer

- TNBC cancers are aggressive
- Often not detected with screening mammography
  - Rapidly proliferative
  - Younger women
- Goal: Blood-based screening test
  - Will lead to further imaging

Mammography:
- Sensitivity is improving
- Challenge with high breast density

MRI:
- Highly sensitive
- High false positives

Need Randomized Controlled Trials To test and compare biomarkers
Breast Cancer Subtypes

<table>
<thead>
<tr>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Her2 positive</th>
<th>Triple negative (85% basal-like)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage at diagnosis</td>
<td>40%</td>
<td>20%</td>
<td>10-15%</td>
</tr>
<tr>
<td>Receptor expression</td>
<td>Estrogens and progesterone</td>
<td>Her2</td>
<td></td>
</tr>
<tr>
<td>Treatment strategies</td>
<td></td>
<td></td>
<td>Chemotherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Her2 targeted therapies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hormonal manipulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Novel targeted therapies</td>
</tr>
</tbody>
</table>

The Diversity of Triple-Negative Breast Cancers

- PIK3CA mutations
- AR expression
- Apocrine histology
- Low path CR chemotherapy

Basal keratin expression

- Claudin low gene expression phenotype
- Metaplastic histology
- Negative basal keratins

Mesenchymal-like

Lymphocytic infiltration
T-cell receptor deletions?

IM

Basal-like

Luminal AR

© 2013 American Association for Cancer Research

Biomarker Discovery: Basal-like Breast Cancer

**Discovery**
- Stage 1:
  - 45 cases
  - 45 controls
  - 10,000 human proteins

**Training**
- Stage 2:
  - 50 BLBC cases
  - 50 non-BLBC cases
  - 50 healthy controls
  - ~800 top hits from stage 1

**Validation**
- Stage 3:
  - 50 BLBC cases
  - 50 non-BLBC cases
  - 50 healthy controls
  - Blinded

With Jonine Figueroa, NCI and Josh LaBaer, ASU
*Wang et al, AACR 2014*
Antibody Biomarkers are Specific for BLBC

With Jonine Figueroa, NCI and Josh LaBaer, ASU

Wang et al, AACR 2014
Protein Biomarkers: FHCRC

Case or control plasma

Reference plasma

IgG and albumin removed

Cy5 labeling of case plasma

Concentrate & Pool samples

Cy3 labeling of reference plasma

Incubation

COMPARATIVE ANALYSIS
52 biomarkers selected

Protein Biomarkers: PNNL

- Sandwich ELISA microarrays of 24 antigens
- Tested 100 total samples; 20 ER-Her2- and 20 benign breast disease controls
- 7 markers selected for validation (including RANTES, VEGF)

Gonzalez et al, Cancer Epi Biomark and Prev. 2011
Our Biggest Challenge:
Getting from Discovery to the Bedside
NCI/EDRN/CPTAC Validation Study of Plasma Biomarkers for Detection of TNBC

**Biomarkers (n=80):**
- Protein biomarkers
- Autoantibody biomarkers

**Samples:**
- TNBC cases
  - 46 cases, 136 BBD controls
  - 18 cases, 54 BBD controls
  - 70 cases, 210 normal controls

**Target for composite biomarker panel**
- 98% specificity
- 60% sensitivity

**Team:**
- NCI/EDRN (~15 investigators, 5 sites)
- NCI/CPTAC (~12 investigators, 4 sites)
- Thousands of patients
A National Study for Blood Tests for TNBC cancers
<table>
<thead>
<tr>
<th>DUMC TNBC samples:</th>
<th>CPTAC-1 TNBC samples:</th>
<th>FCCC TNBC samples:</th>
</tr>
</thead>
<tbody>
<tr>
<td>46 cases</td>
<td>18 cases</td>
<td>70 cases</td>
</tr>
<tr>
<td>136 matched BBD controls</td>
<td>54 matched BBD controls</td>
<td>210 matched nl controls</td>
</tr>
<tr>
<td>Collected at Duke 1999-2011</td>
<td>Collected from four sites</td>
<td>Collected from one site</td>
</tr>
<tr>
<td>Collected from diagnostic mammography</td>
<td>Collected from diagnostic mammography</td>
<td>Collected prior to surgery</td>
</tr>
</tbody>
</table>
Validation of Plasma Biomarkers for Detection of TNBC

Inclusion Criteria

- Age<65
- 300 ul plasma available
- Cases:
  - Identify stage; histology; grade; ER, PR and HER2 receptor status.
  - Benign breast disease controls
    - Matched for age, study site, year of blood collection, and race.
- Secondary data elements when available:
  - Menopausal status; height; weight; hormone replacement therapy; use of oral contraceptives; BRCA1/2 status
Data Analysis Plan

• Prevalence of TNBC in the screening population is low (0.3%)
• 80 biomarkers tested (divided for statistical analysis into the A and B lists)
• Top biomarkers at 95% specificity were selected
• A Phase III validation study using pre-diagnostic samples is planned
A Blinded Multicenter Phase II Study of a Panel of Plasma Biomarkers for the Detection of Triple Negative Breast Cancer

Karen S. Anderson, Margaret Pepe, Jeffrey Marks, Paul Engstrom, Christos Patriotis, Richard Zangar, Steven Skates, Paul Lampe, Joshua LaBaer, and Christopher I. Li.

Center for Personalized Diagnostics, The Biodesign Institute, Arizona State University, Tempe, AZ; Fred Hutchinson Cancer Research Center, Seattle, WA; Duke University School of Medicine, Durham, NC; Fox Chase Cancer Center, Philadelphia PA; National Cancer Institute, Bethesda DC; Pacific Northwest National Laboratories, Richland WA; Massachusetts General Hospital, Boston MA

Data to be presented at San Antonio Breast Cancer Symposium 2014
The Future: Targeted Screening for Breast Cancer based on Risk

Standard screening

Biomarkers (annual) &/or Mammography (biennial)

Normal
Indeterminate
Abnormal

Serial Biomarkers
Imaging
Biopsy

High-risk screening

Biomarker every 3-6 months

Normal
Indeterminate
Abnormal

Mammography (annual)
MRI
Imaging
Biopsy
What we need to move biomarkers forward?

- An integrated team of scientists, clinicians, biomarker specialists, statisticians, and advocates

- Well-annotated biorepositories of patients followed *longitudinally* designed for **USE**

- Rapid, on-demand national biorepositories

- **Pipelines** to facilitate rapid biomarker validation throughout the scientific community

- Rapid, national testing of emerging biomarkers
Acknowledgements

**TNBC Team at EDRN**
- Christopher Li, FHCRC
- Richard Zangar, PNNL
- Jeffrey Marks, DUMC
- Paul Engstrom, FCCC
- Steve Skates, MGH
- Margaret Pepe, FHCRC

**ASU/Biodesign Institute**
- Josh LaBaer
- Ji Qiu
- Garrick Wallstrom
- Jie Wang
- Jonine Figueroa, NCI

**NCI/ Early Detection Research Network**
- Sudhir Srivastava
- Ian Thompson
- Christos Patriotis
- Jacob Kagan
- Paul Wagner
- Lynn Sorbara

**EDRN Advocate:** Elda Railey

- **Our Patients**
- **Our Advocates**