ASU BIODESIGN INSTITUTE
ARIZONA STATE UNIVERSITY

Virginia G. Piper Center for
Personalized Diagnostics

Blood Biomarkers for Triple Negative Breast Cancer

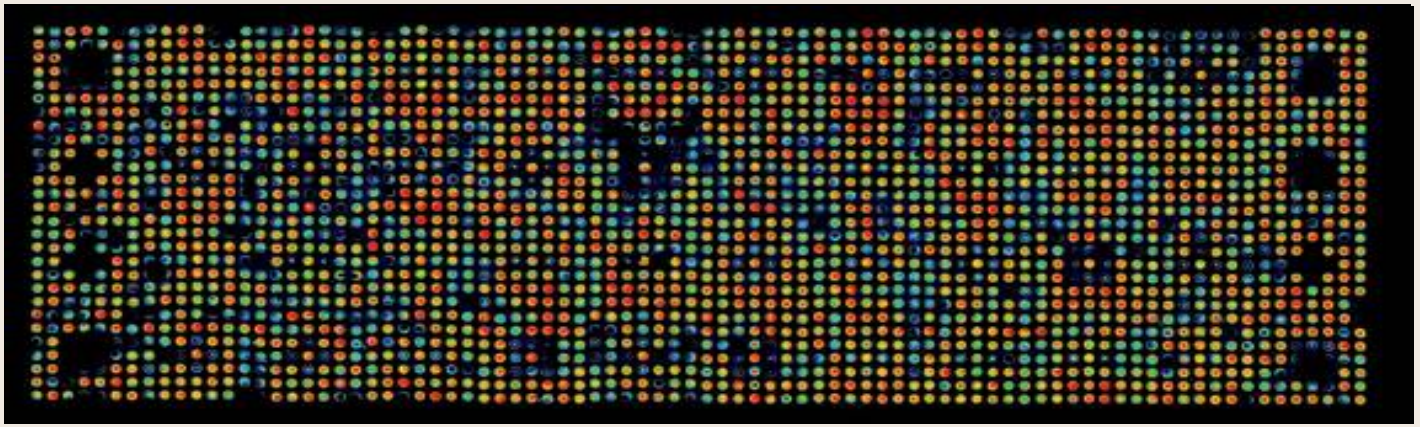
Karen S. Anderson MD PhD
Associate Professor
Biodesign Institute, Arizona State University
Mayo Clinic Arizona

Disclosure Information

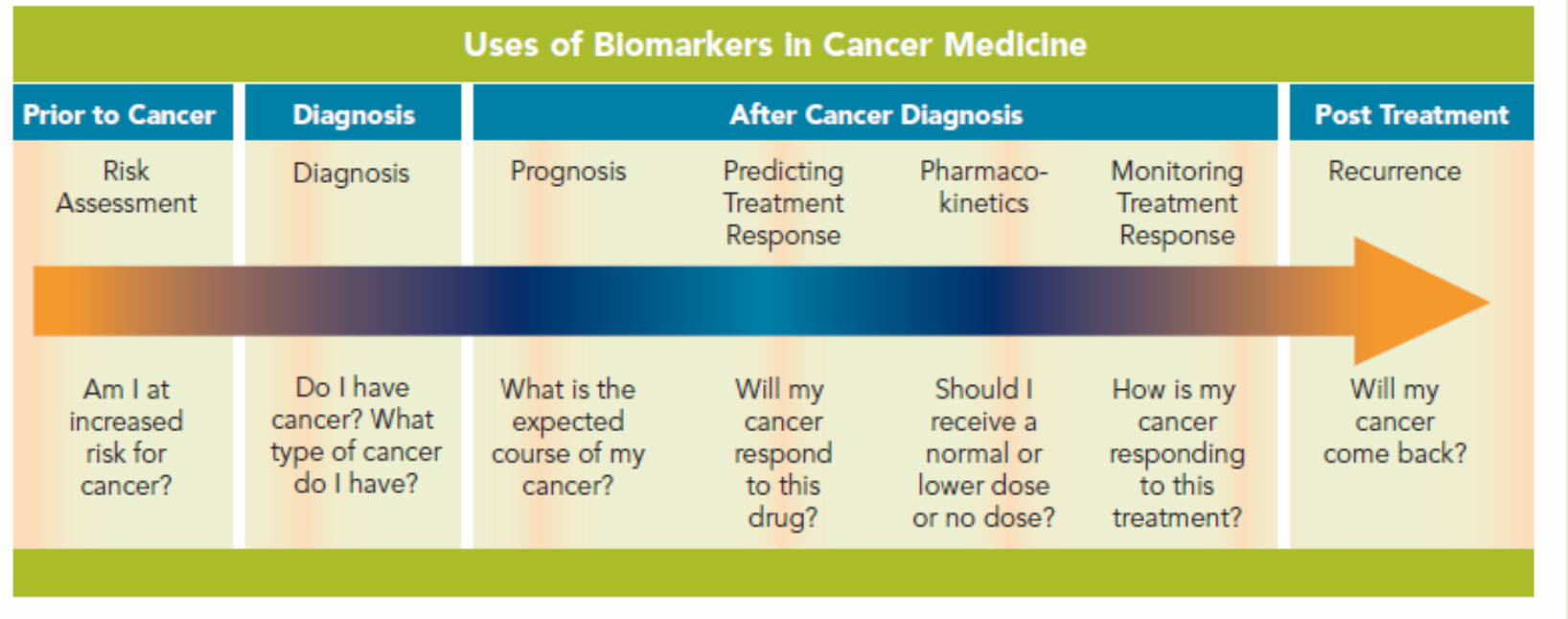
- I serve on the scientific advisory board and am a consultant for ProvistaDx.
- I hold patents on breast cancer biomarkers

- None of these biomarkers are approved for clinical use.

Moving Biomarkers From Discovery To Patients



Uses of Biomarkers in Cancer Medicine

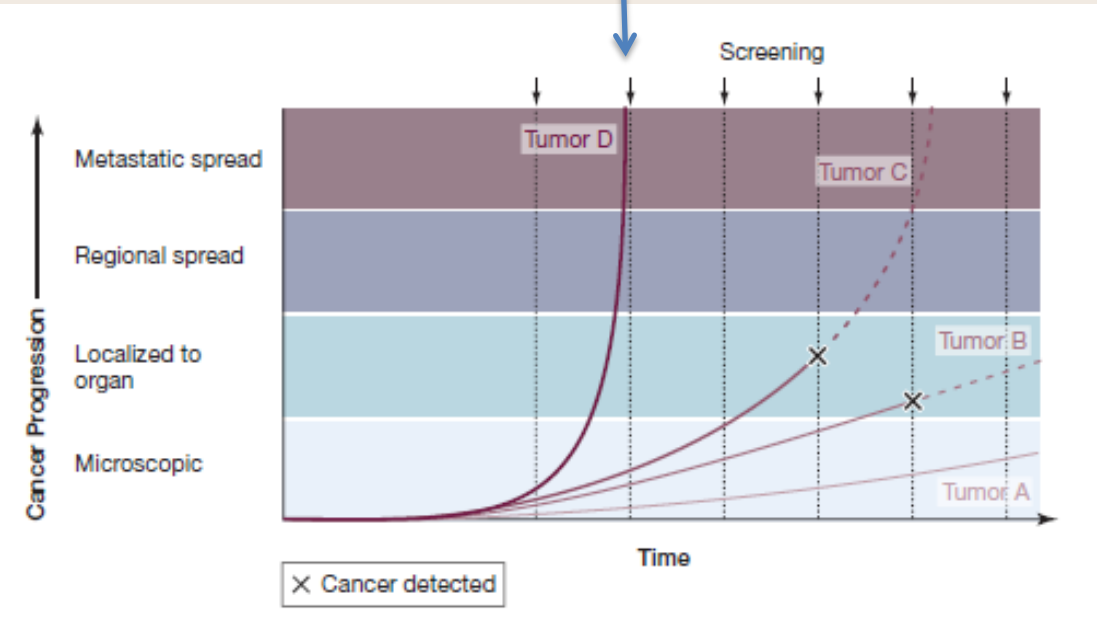


Early Detection of Breast Cancer

- 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 1. FDA-cleared protein cancer biomarkers.

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

[a] Human genes: *AFP*, alpha-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*, kallikrein-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/glioblastoma derived oncogene homolog (avian).

Pavlou and Blasutig, Clin Chem 2012

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

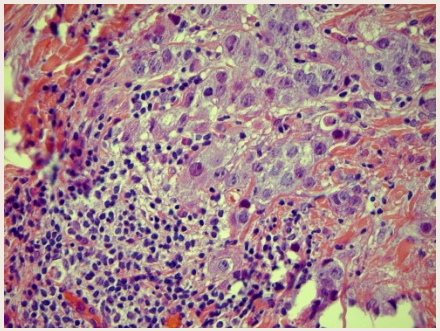
From: Harris et al, J. Clin.Oncol. 2007

Conclusions:

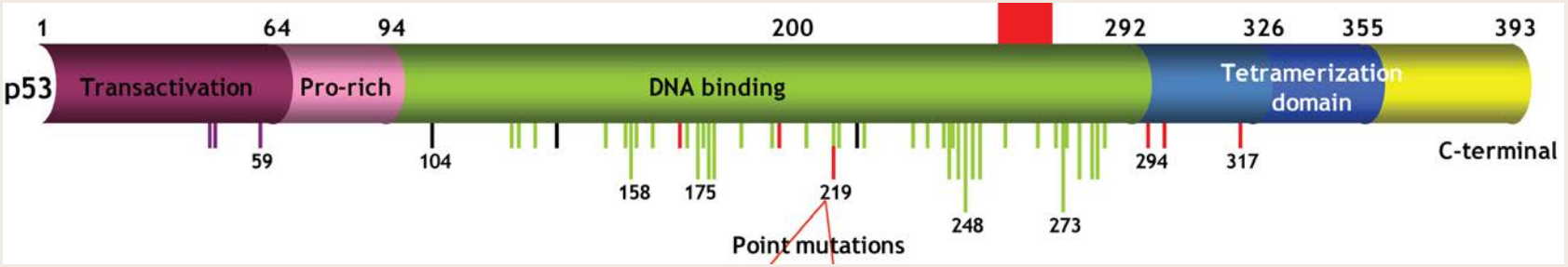
- CEA, CA27.29, and CA15-3 are used for monitoring patients with metastatic disease
- Not recommended for screening, diagnosis, staging, or routine surveillance of patients after primary therapy
- There is an emerging set of blood biomarkers for cancer that have potential for early detection, prediction,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

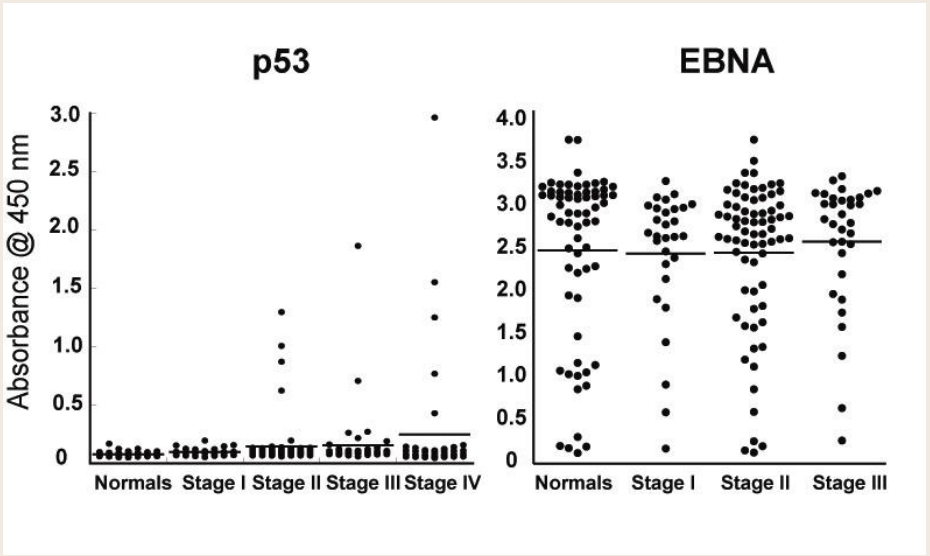
Example: Early studies on breast cancer biomarkers:

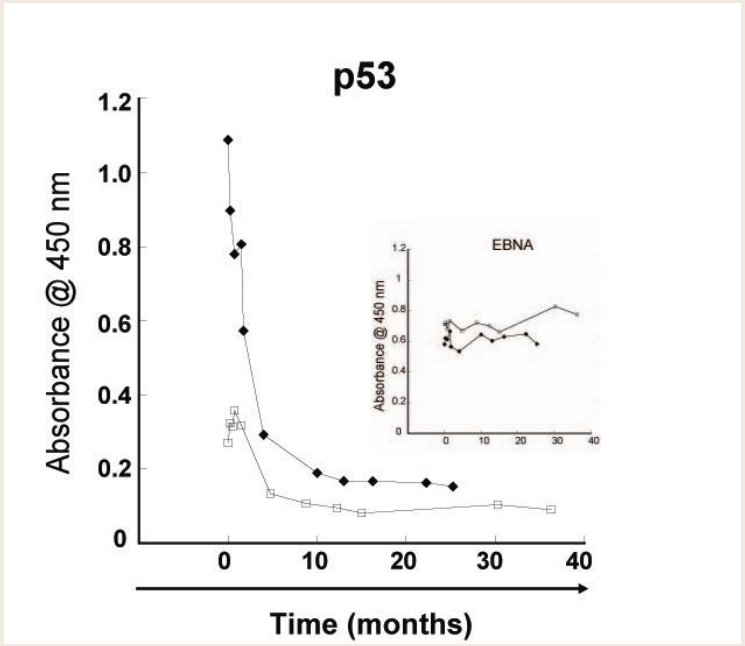
The immune system as a sentinel for breast cancer



Finding p53 autoantibodies in the blood

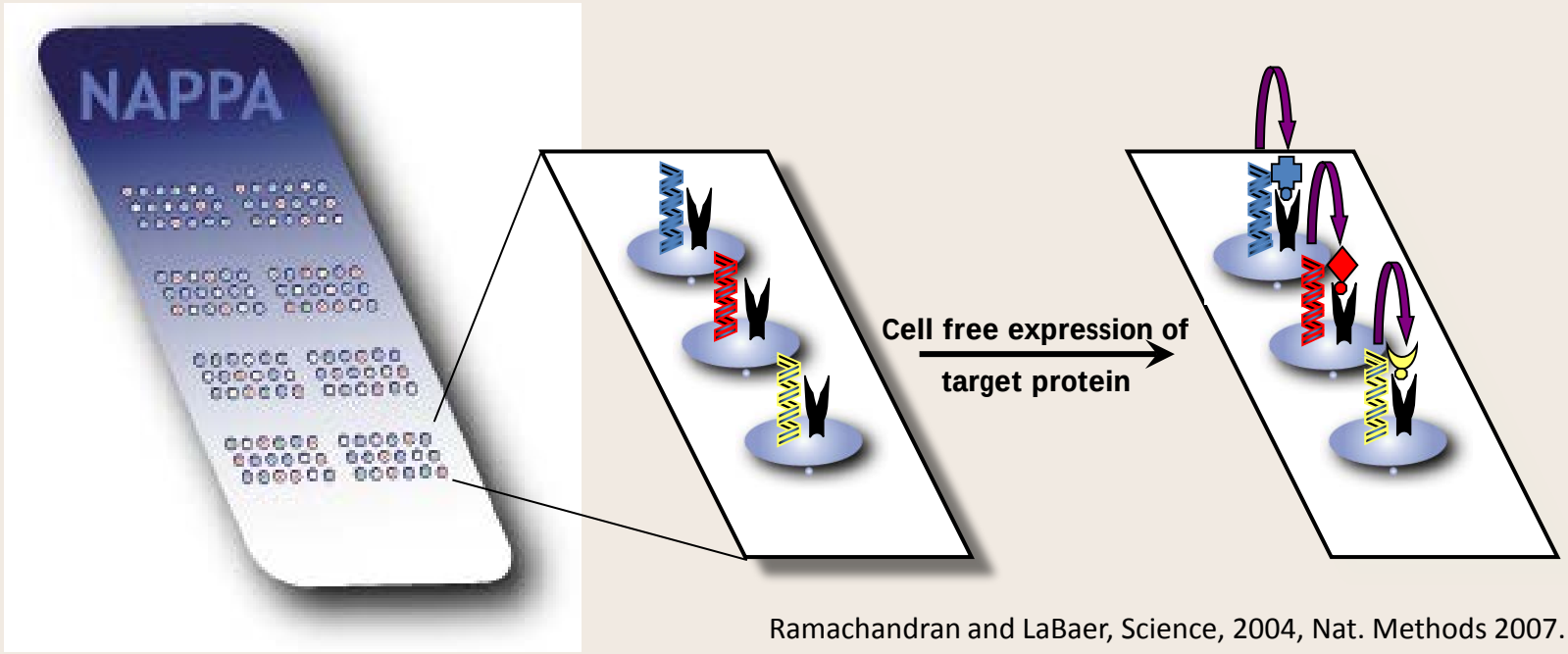




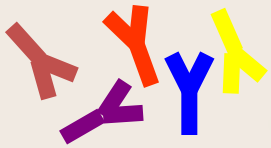


Anderson and LaBaer, J. Proteome Res. 2008

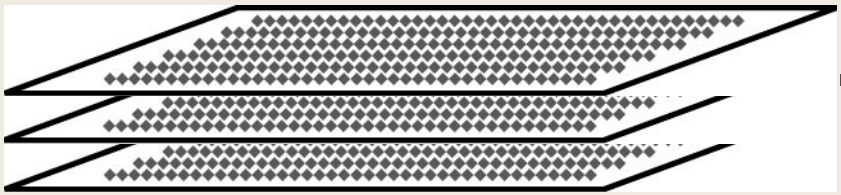
Detecting Antibodies with Custom Protein Microarrays

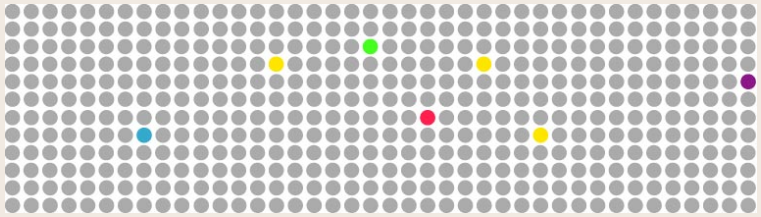


Ramachandran and LaBaer, Science, 2004, Nat. Methods 2007.



Probe with sera



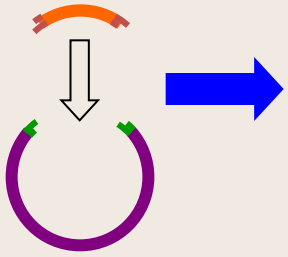


Replicate microscopic arrays of proteins

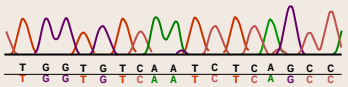
Printed cDNA's with cancer relevance

- ~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

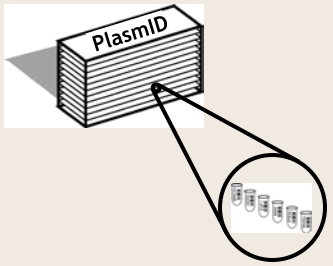


Sequence Verify

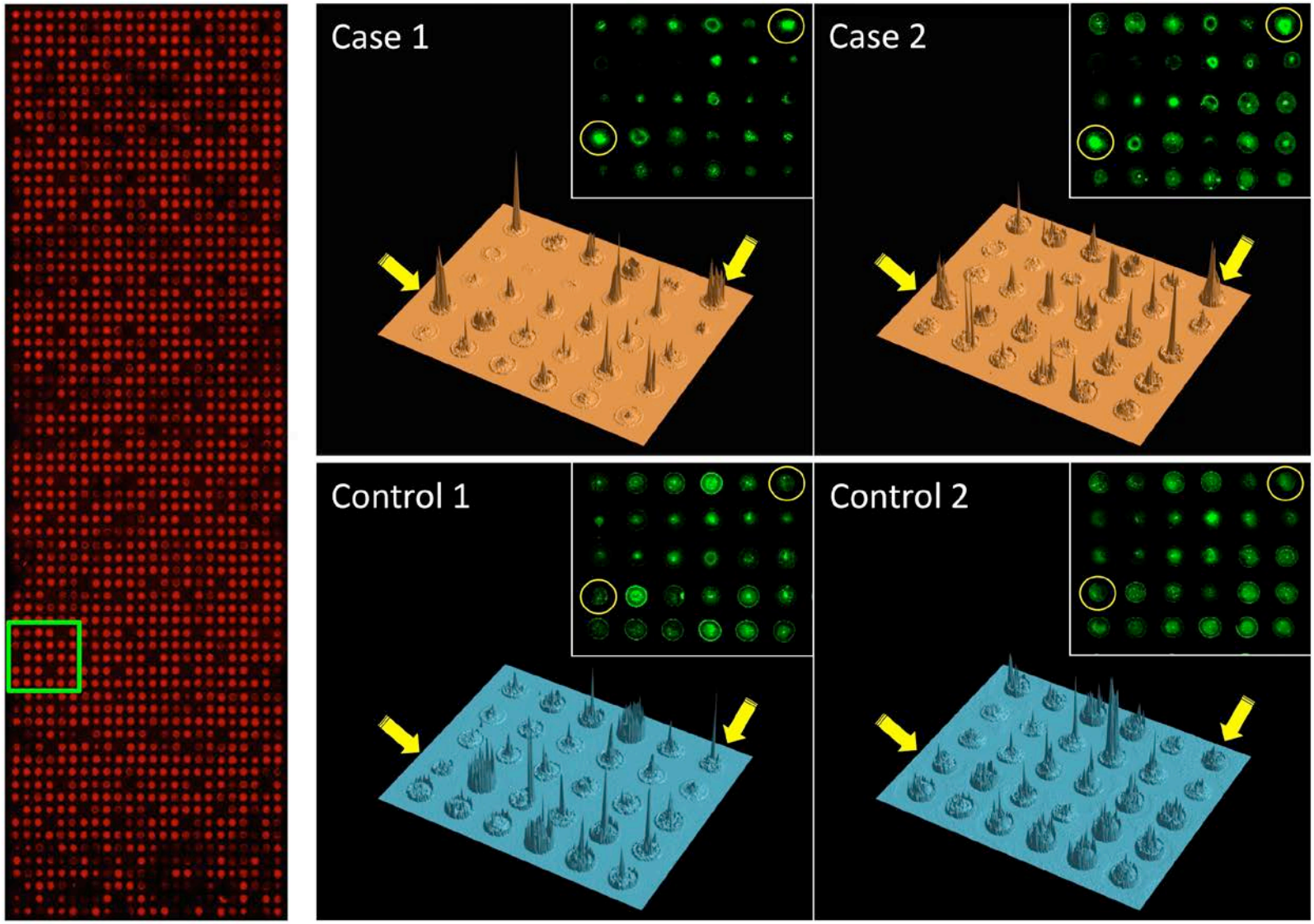


www. Dnasu.edu

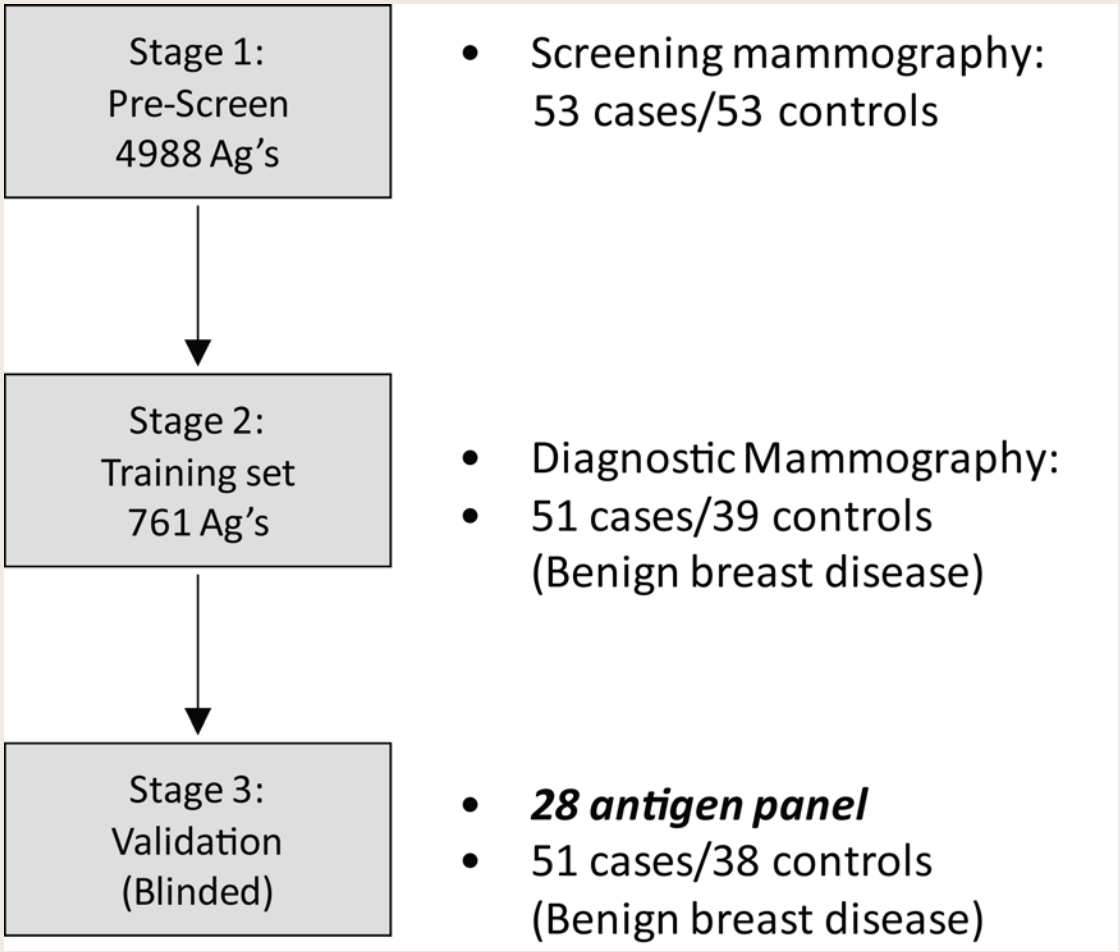
Add to repository



Detection of a Panel of 28-specific AAbs in Breast Cancer



A 28 Autoantibody Panel for Breast Cancer Detection



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

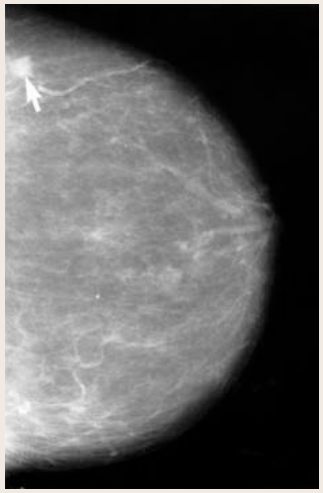
Anderson et al, J. Proteome Res. 2011

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

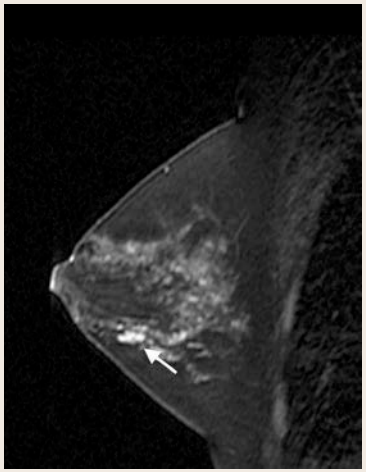
Diagnosis	Total
Normal Control	218
BBD without atypia	63
BBD with atypia	231
DCIS	48
LCIS	7
Invasive cancer	190
Total	**757**

NCI/EDRN/CPTAC Biomarker Validation Study: Triple Negative Breast Cancer



Mammography:
Sensitivity is improving

Challenge with high breast density

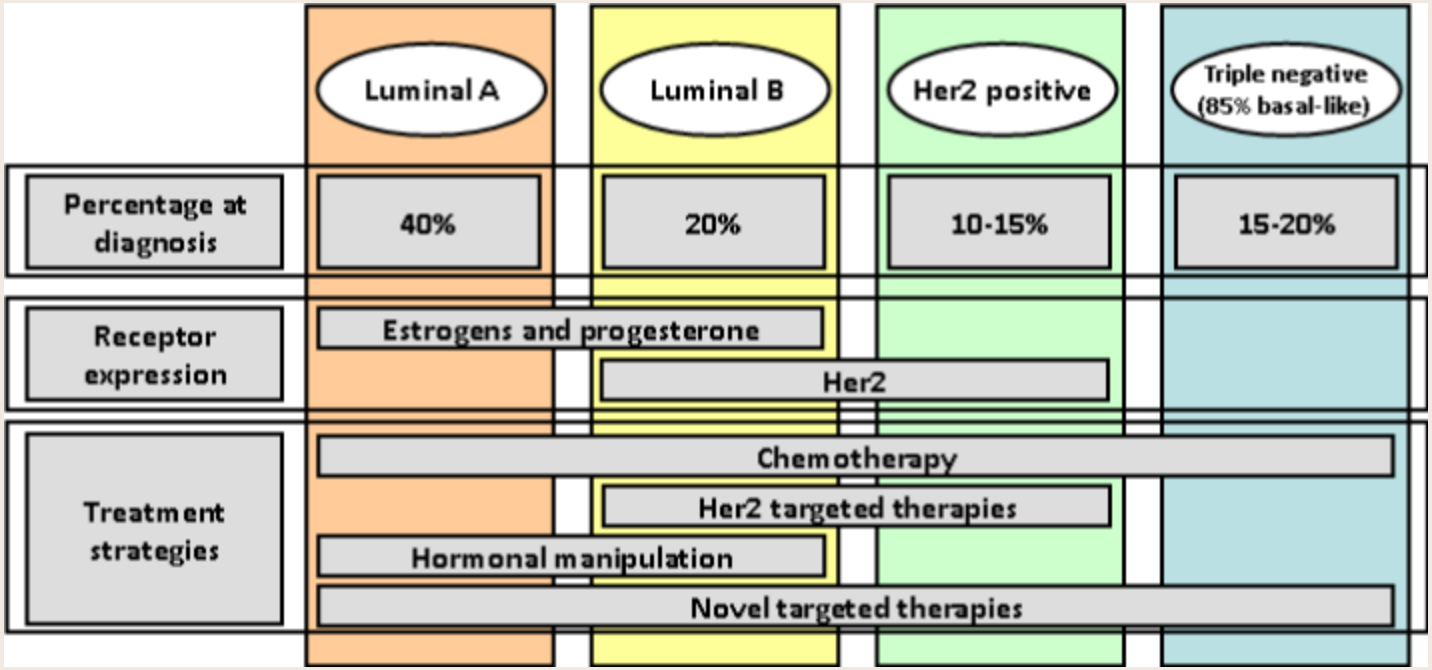


MRI:
Highly sensitive
High false positives

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

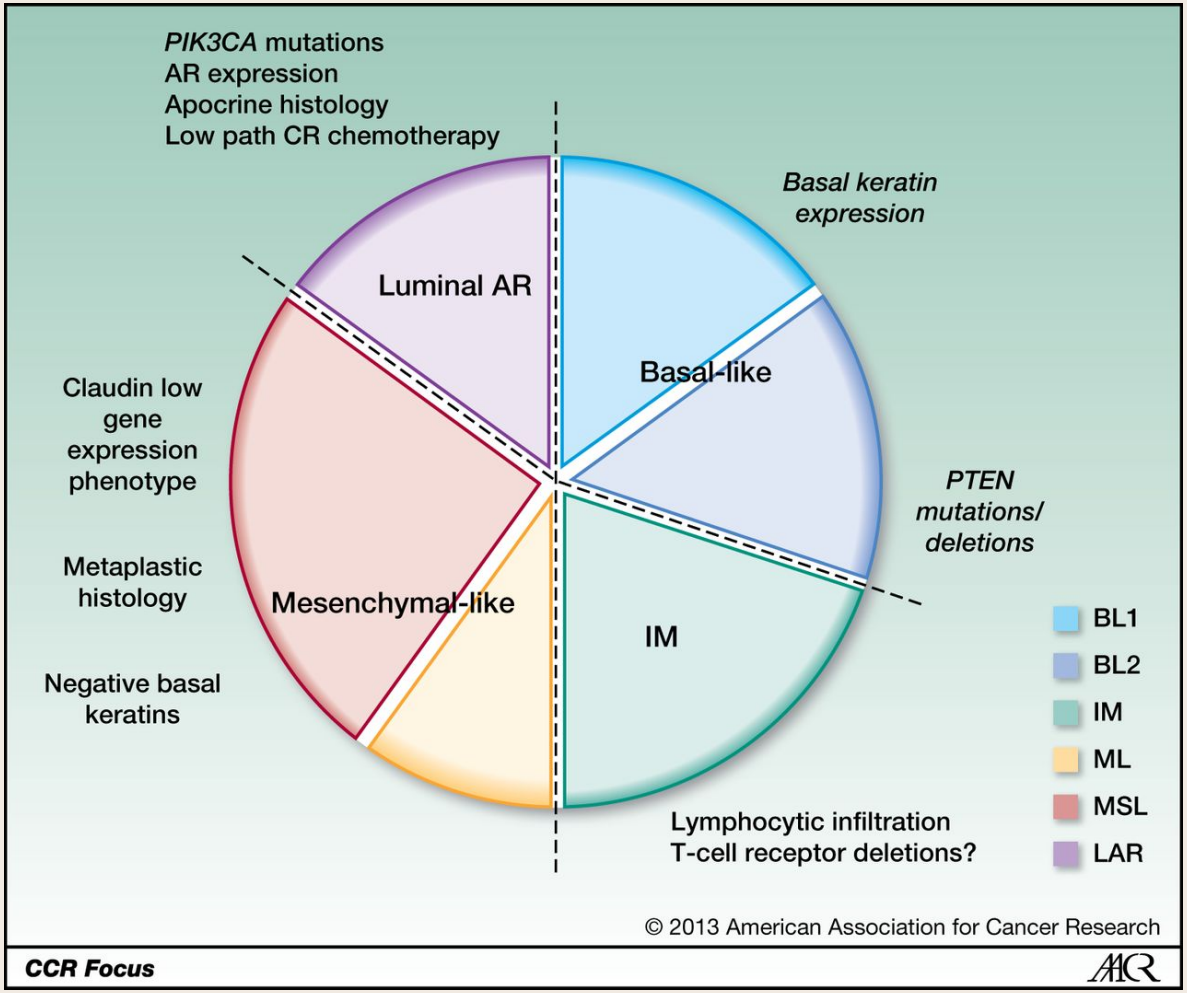
Need Randomized Controlled Trials
To test and compare biomarkers

Breast Cancer Subtypes



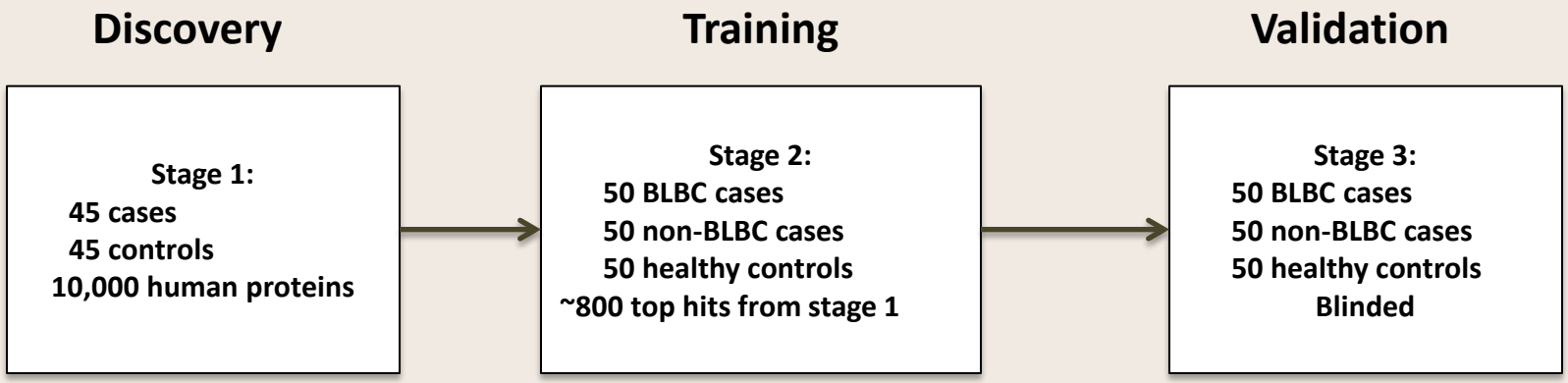
Sharp and Harper-Wynne, J. Cancer Biol. Res. 2014

The Diversity of Triple-Negative Breast Cancers



Turner N C , and Reis-Filho J S Clin Cancer Res 2013;19:6380-6388

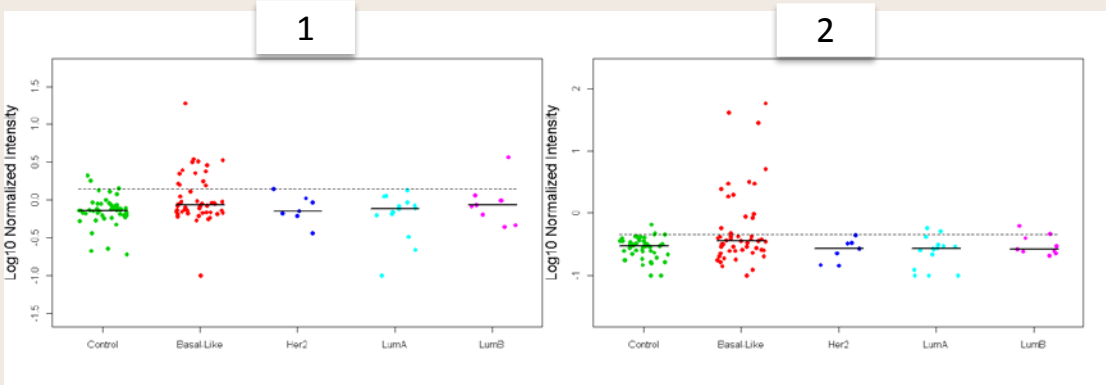
Biomarker Discovery: Basal-like Breast Cancer



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

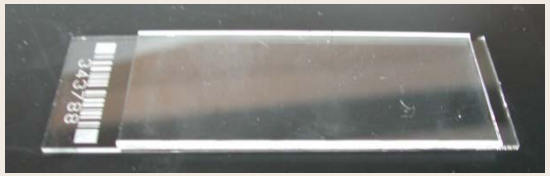
Reference plasma

IgG and albumin removed

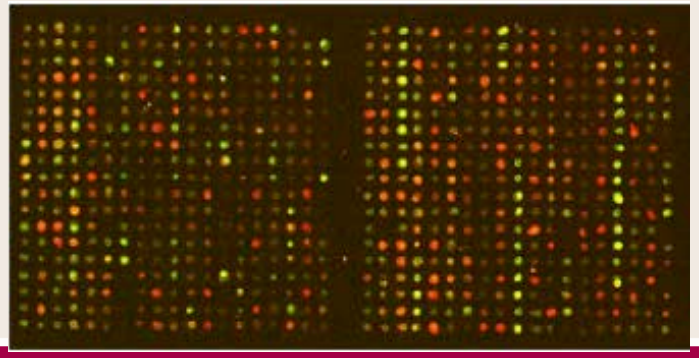
Cy5 labeling of case plasma

Cy3 labeling of reference plasma

Concentrate & Pool samples



Incubation



COMPARATIVE ANALYSIS
52 biomarkers selected

Li et al, Br. Cancer Res. Treat. 2012

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)

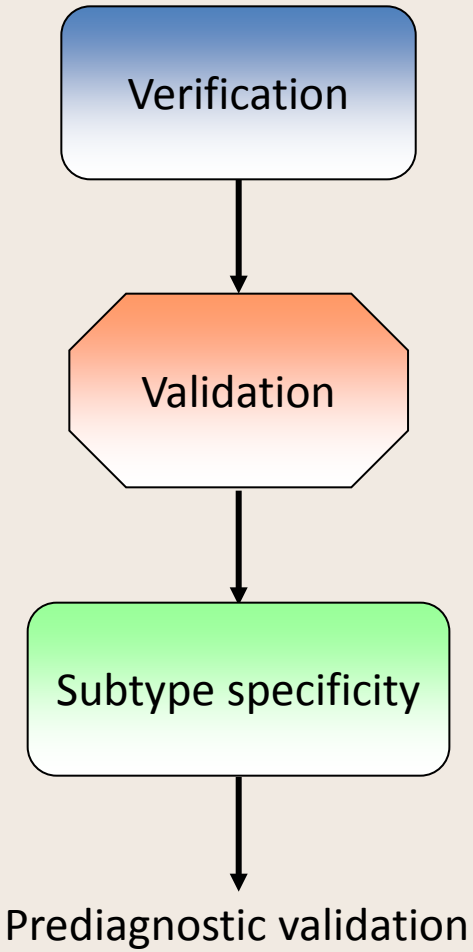
Gonzalez et al, Cancer Epi Biomark and Prev. 2011

Our Biggest Challenge:

Getting from Discovery to the Bedside

From Wiki Commons

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

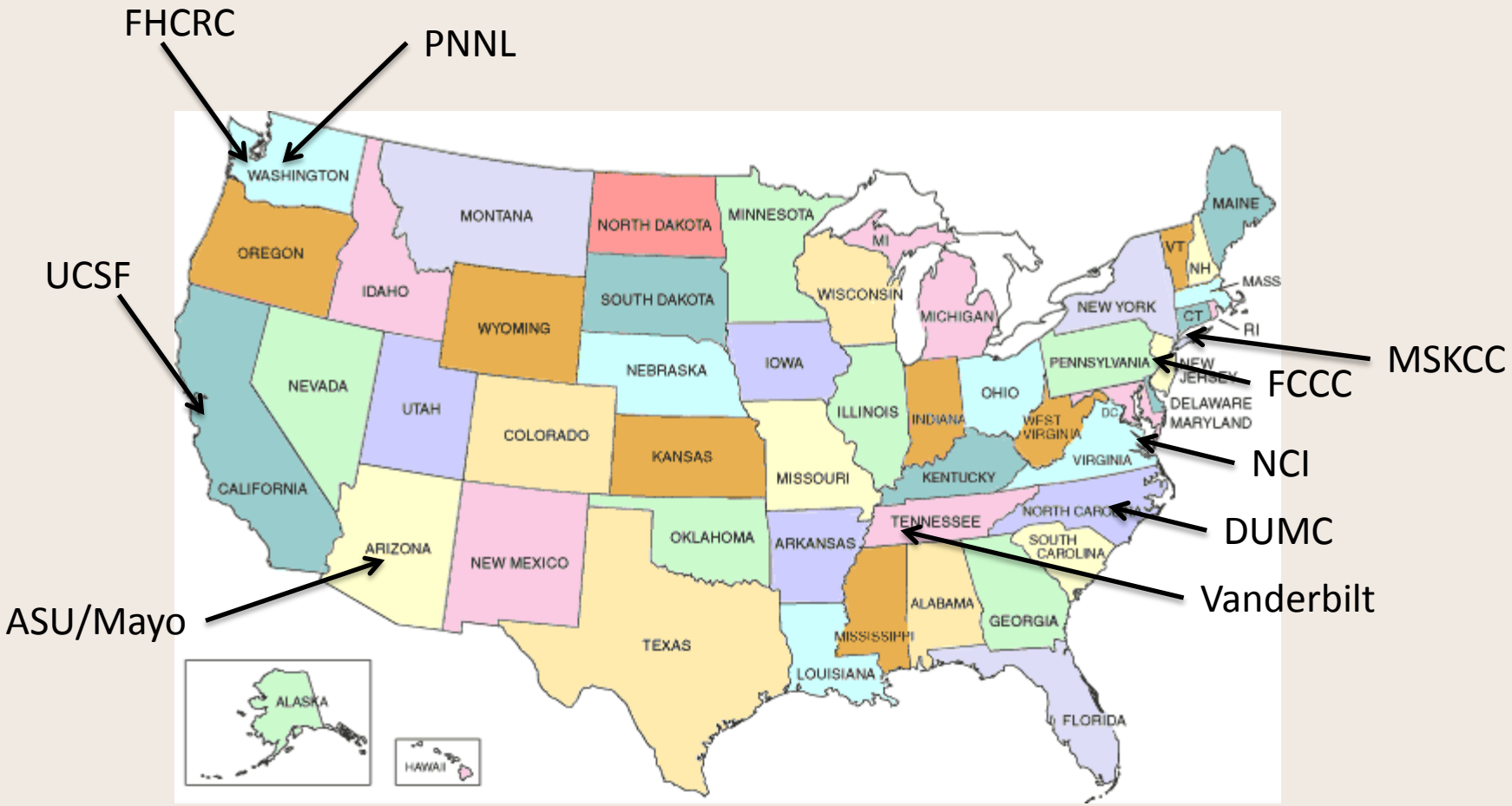
Target for composite biomarker panel

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



•DUMC TNBC samples:

- 46 cases
- 136 matched BBD controls
- Collected at Duke 1999-2011
- Collected from diagnostic mammography

CPTAC-1 TNBC samples:

- 18 cases
- 54 matched BBD controls
- Collected from four sites
- Collected from diagnostic mammography

FCCC TNBC samples:

- 70 cases
- 210 matched nl controls
- Collected from one site
- Collected prior to surgery

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

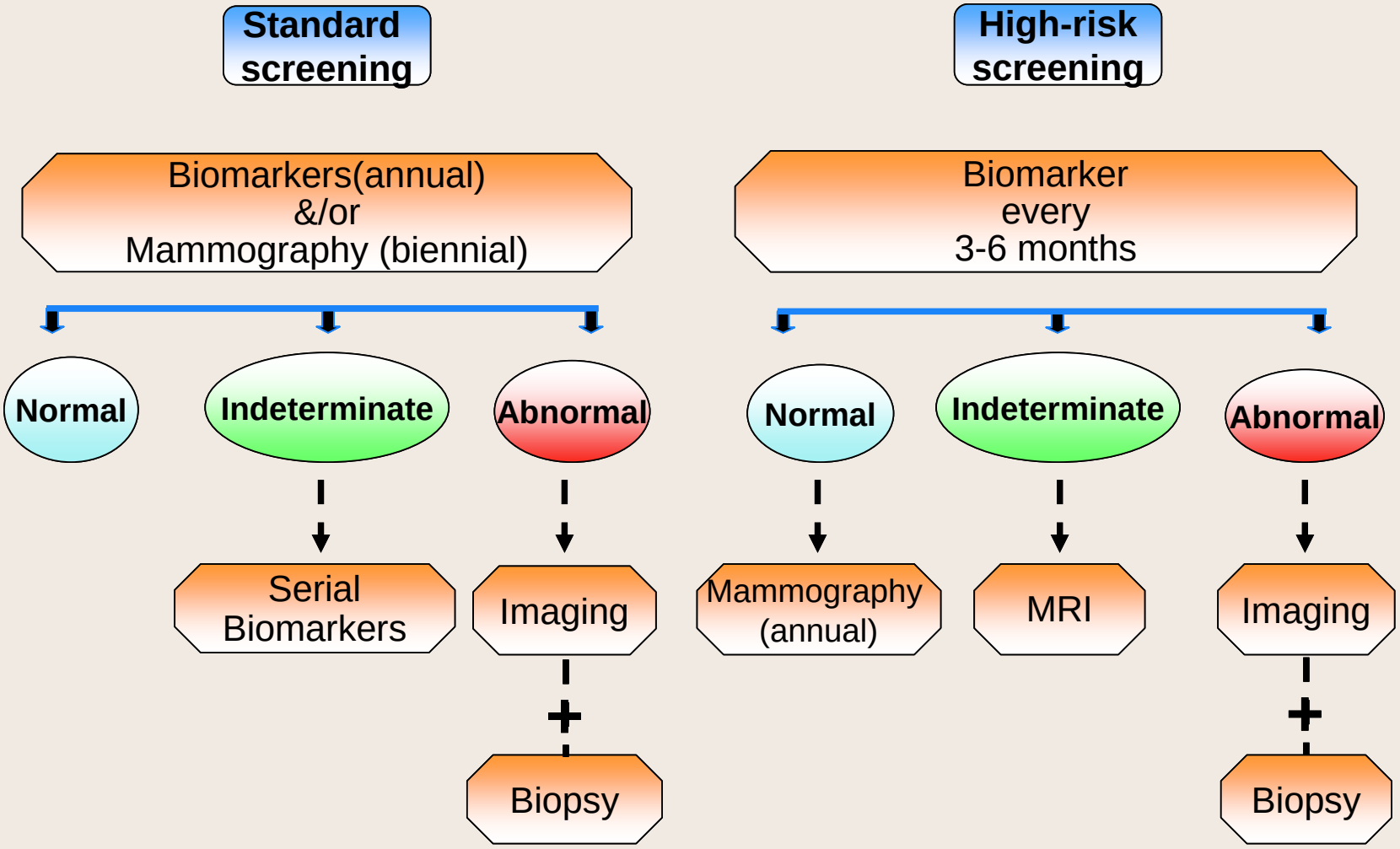
A Blinded Multicenter Phase II Study of a Panel of Plasma Biomarkers for the Detection of Triple Negative Breast Cancer

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



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

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

NCI/ Early Detection Research Network

- Sudhir Srivastava
- Ian Thompson
- Christos Patriotis
- Jacob Kagan
- Paul Wagner
- Lynn Sorbara

EDRN Advocate: Elda Railey