



# Biomarkers: Challenges and strategies for early detection of biologically aggressive breast cancer



Victoria Seewaldt, M.D.

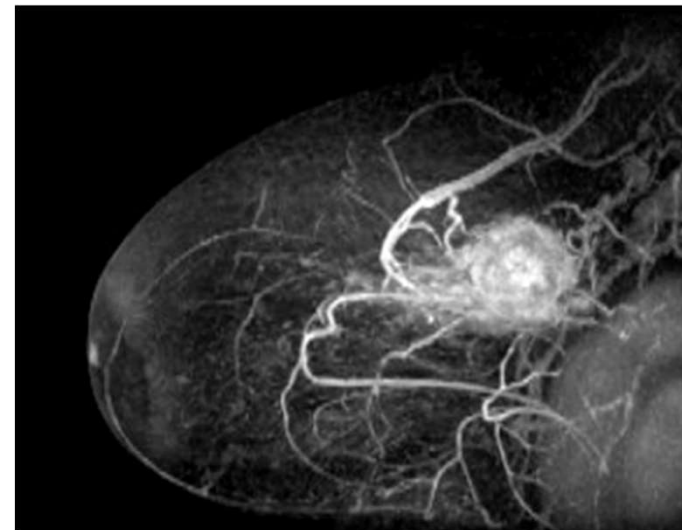
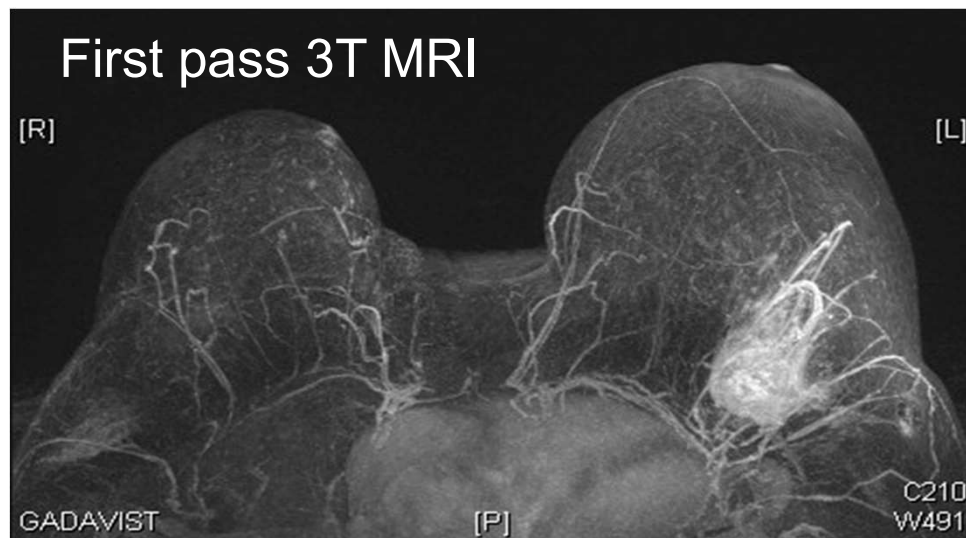
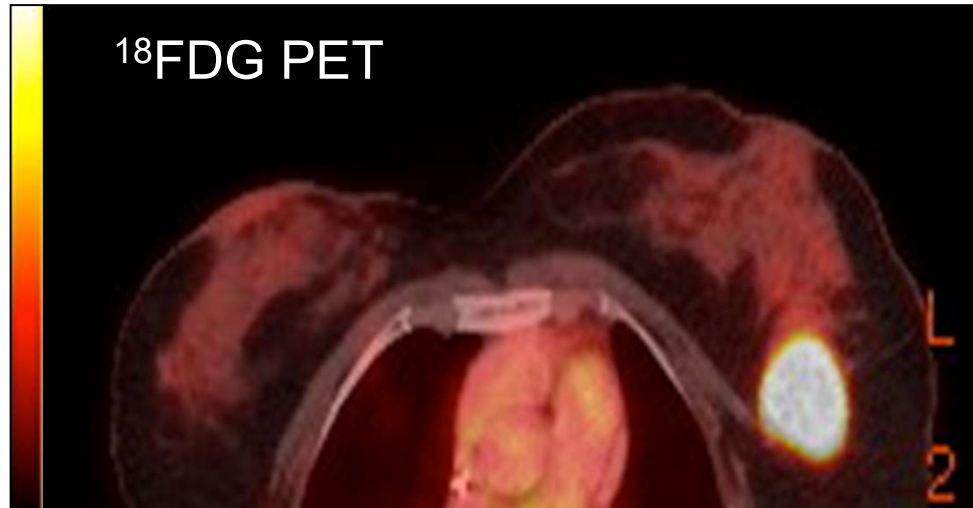
City of Hope, Comprehensive Cancer Center  
Beckman Institute, Los Angeles, California

# Interval TNBC high-risk AA woman, no BRCA mt

## Frequency BRCA1 mt

- 60% Ashkenazi
- 53% European American
- **29% Hispanic American**
- **26% Asian American**
- **20% African American**

Greenup et al. *Ann Surg Onc* 2013  
<http://www.cdc.gov/nchs/nvss.htm>

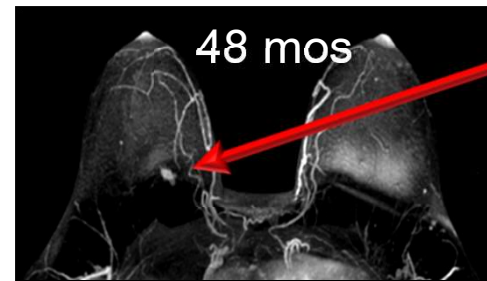
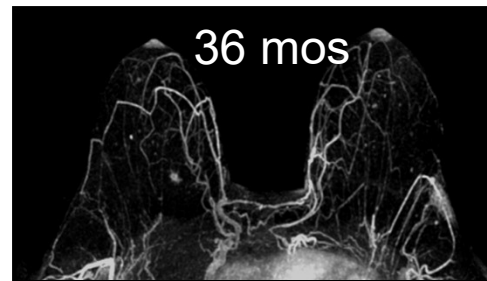
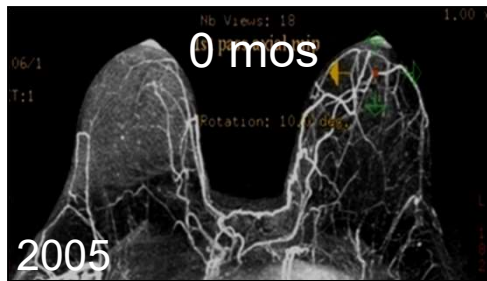


# Disparity MRI in High-Risk Women 2005-2015

- 621 premenopausal high-risk women
  - 352 African American
  - 269 Caucasian
- 48 mos observation, 51 breast cancers
  - 22 Focal / Age shifted
  - 29 Non-focal / Accelerated



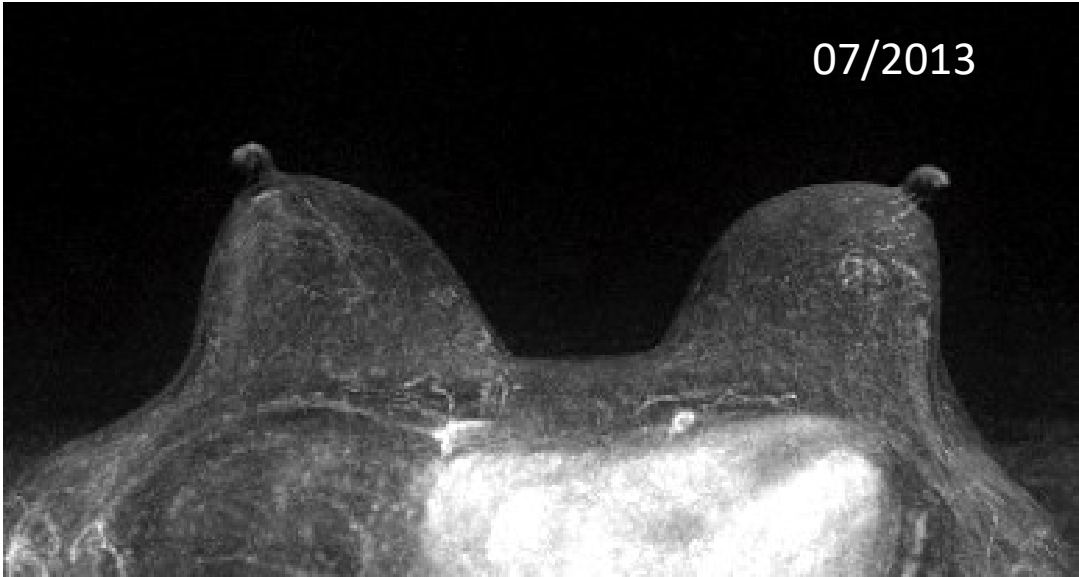
S. Riley



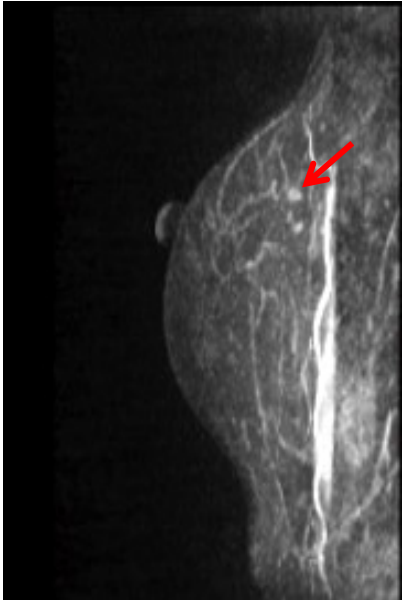
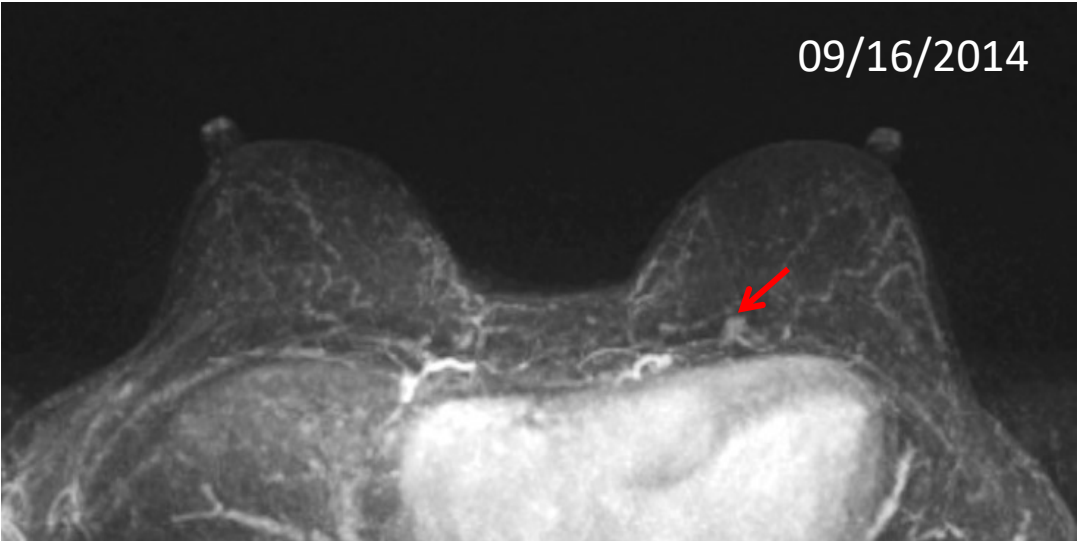
ER/PR+  
T1N0

Slow (22/51 women)  
Focal, benign biology

# Early detection in 47 yo BRCA2 mutation carrier.



0.7 cm  
ER+  
PR+  
Her2 2-3+  
FISH not amplified  
OncoDX low risk

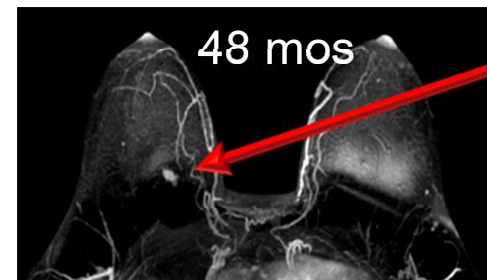
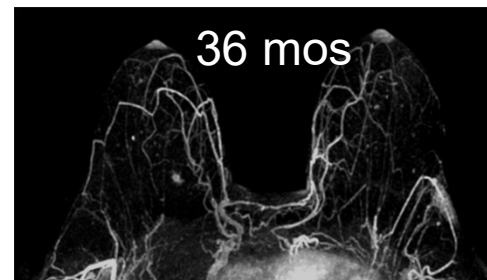


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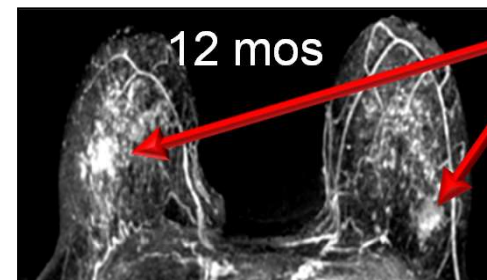
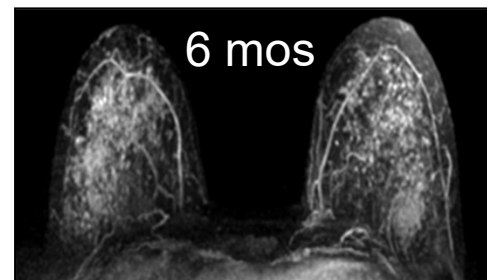
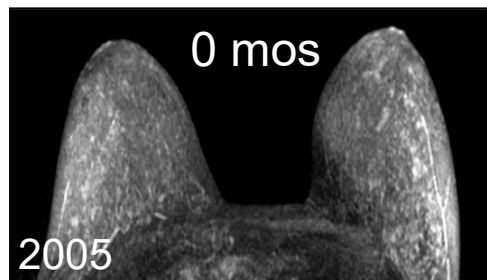


S. Riley



ER/PR+  
T1N0

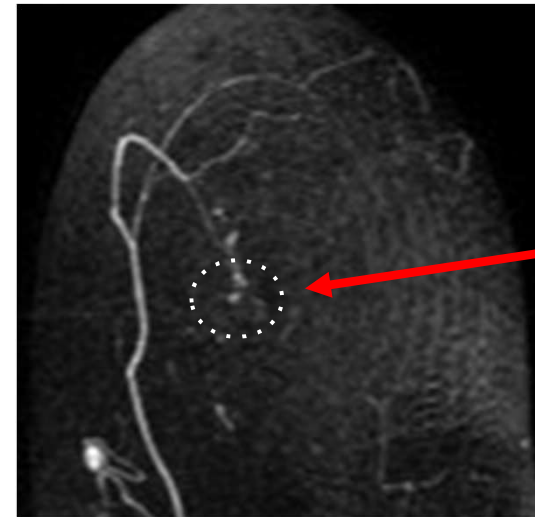
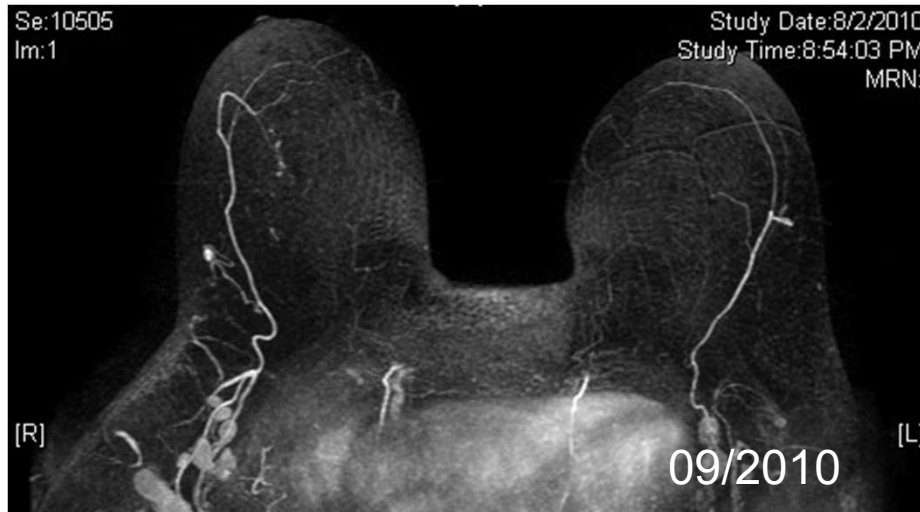
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Focal, benign biology



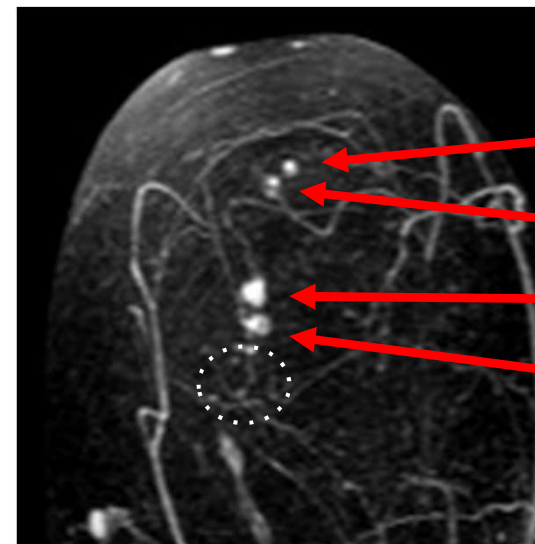
TNBC  
T1N0

Accelerated (29/51 women)  
Diffuse, aggressive biology

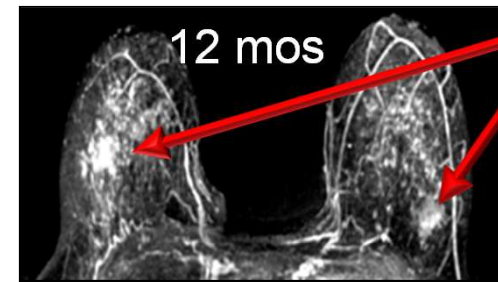
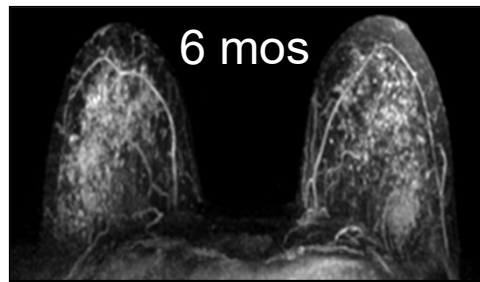
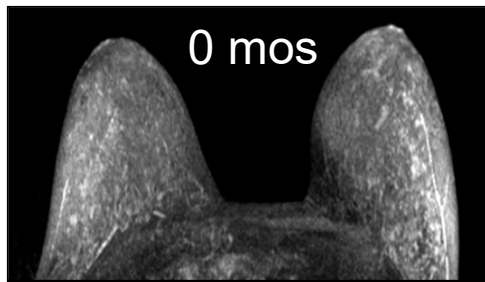
# BRCA1 mutation – 6 mos LN metastasis



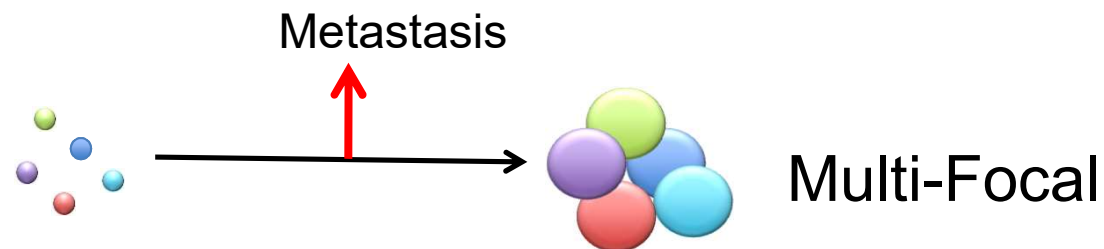
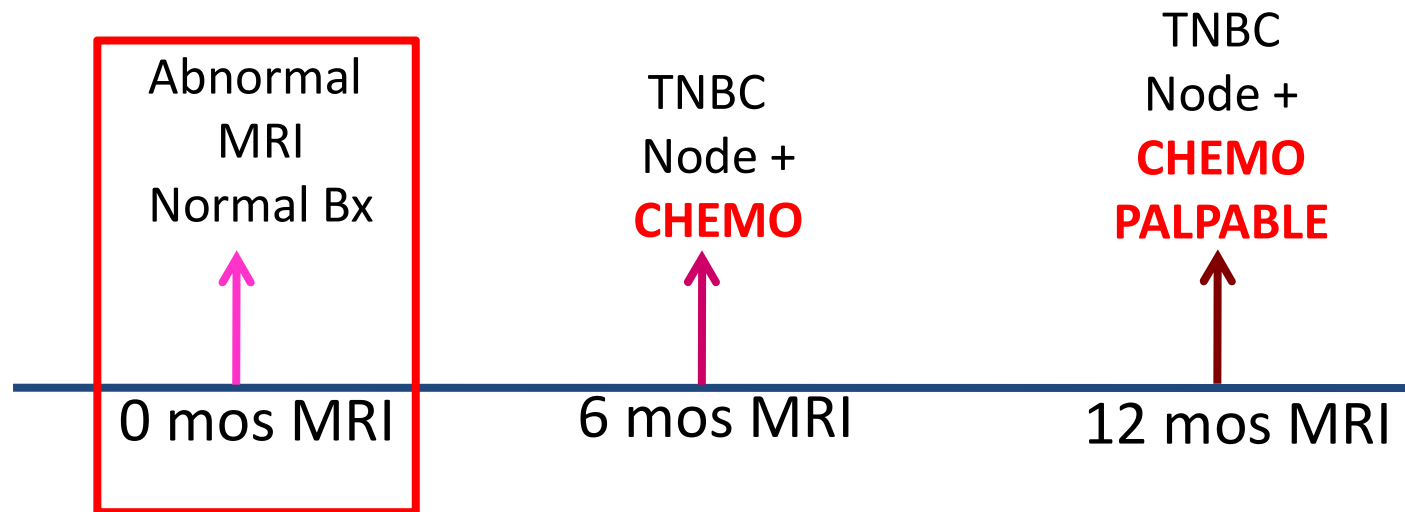
NL  
tissue



0.1 cm  
0.1 cm  
0.4 cm  
0.3 cm  
TNBC  
2+LN

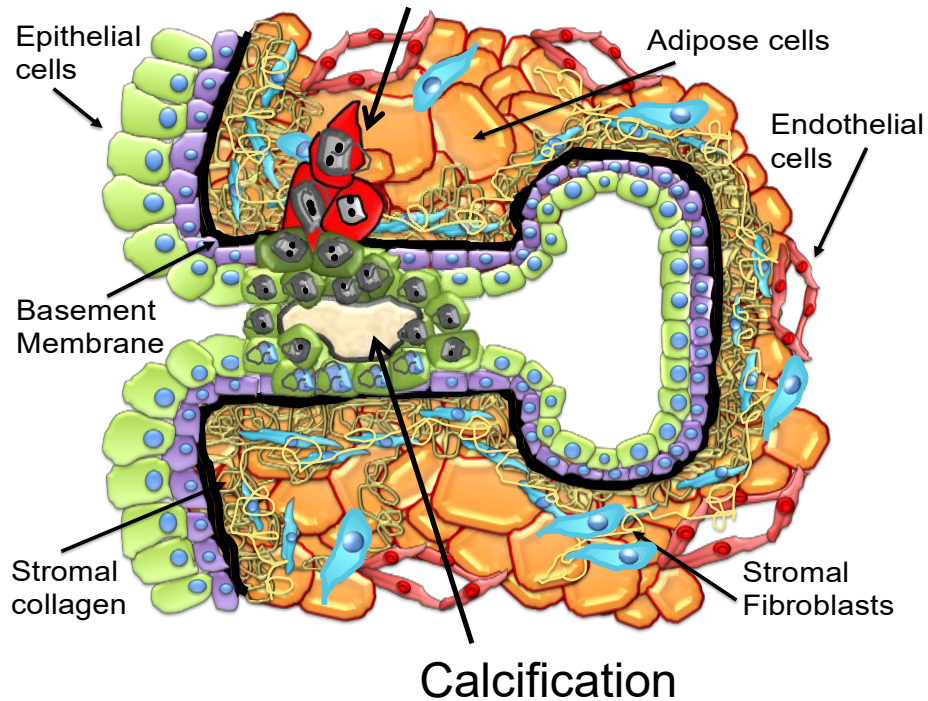


TNBC  
T1N0

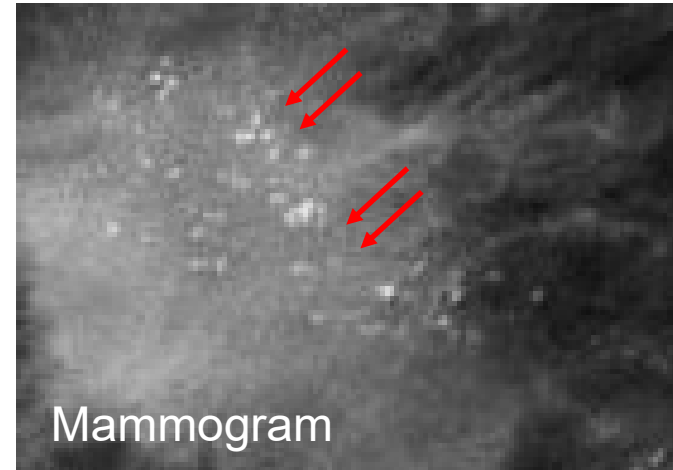


6 month screening interval ***NOT sufficient***

## DCIS with microinvasion



Frequent (>75%) calcification  
on mammogram



### Stepwise progression:

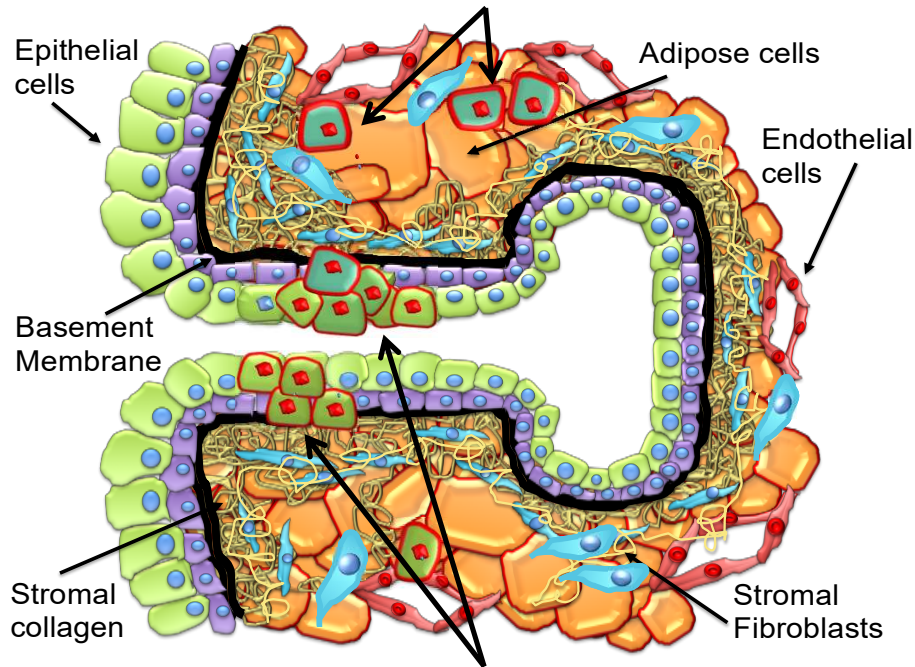
Normal → Hyperplasia → Atypia → DCIS → DCIS w/microinvasion → Invasive CA

**Clear morphological changes:** high nuclear/cytoplasmic ratio, chromatin condensation, prominent nucleoli, variation in cell size.

**Excellent prognosis:** 97% survival at 20 years (even w/ invasion or second CA)



# Morphologically Benign – Biochemically Abnormal



## Plasticity - EMT

### Rapid progression:

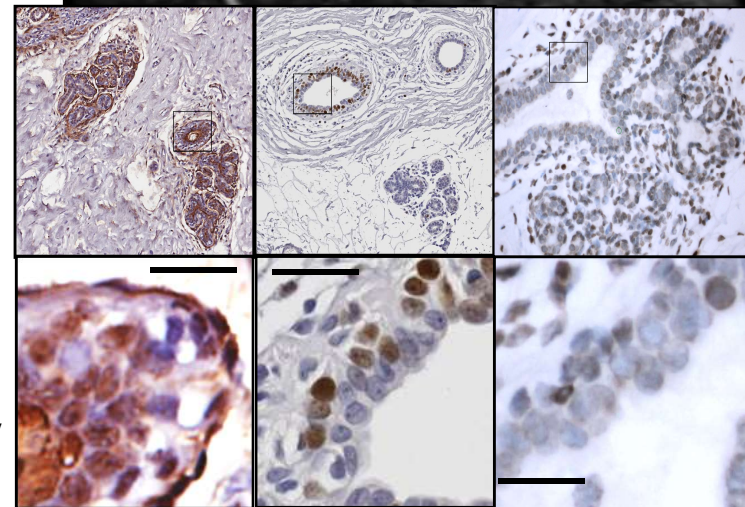
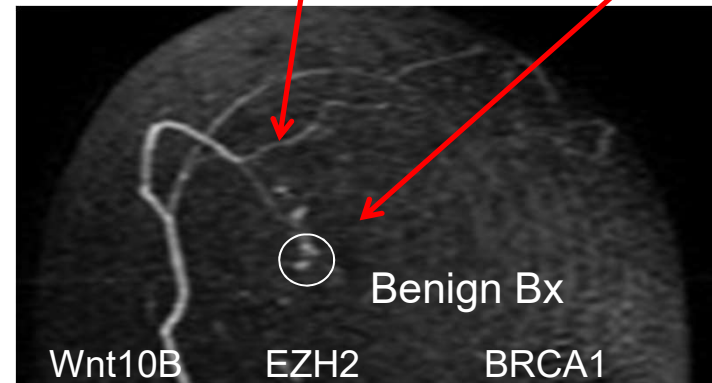
Normal → ? → ? → ? → Aggressive Interval CA

**Poor Prognosis:** 70-77% survival 5-years

**Aggressive biology** present in morphologically normal precancerous lesion

Rare (<15%) calcifications on mammogram

MRI - increased contrast uptake  
- increased vascularity



# Challenges, Needs, Tools

1. Tools for multi-spatial, multi-parametric, high-resolution profiling
2. Linked imaging/biomarker discovery: Non-contrast imaging to integrate with and help validate biomarker discovery
3. Challenge of Heterogeneity – in situ single cell transcript profiling.

# Challenges, Needs, Tools

## 1. Tools

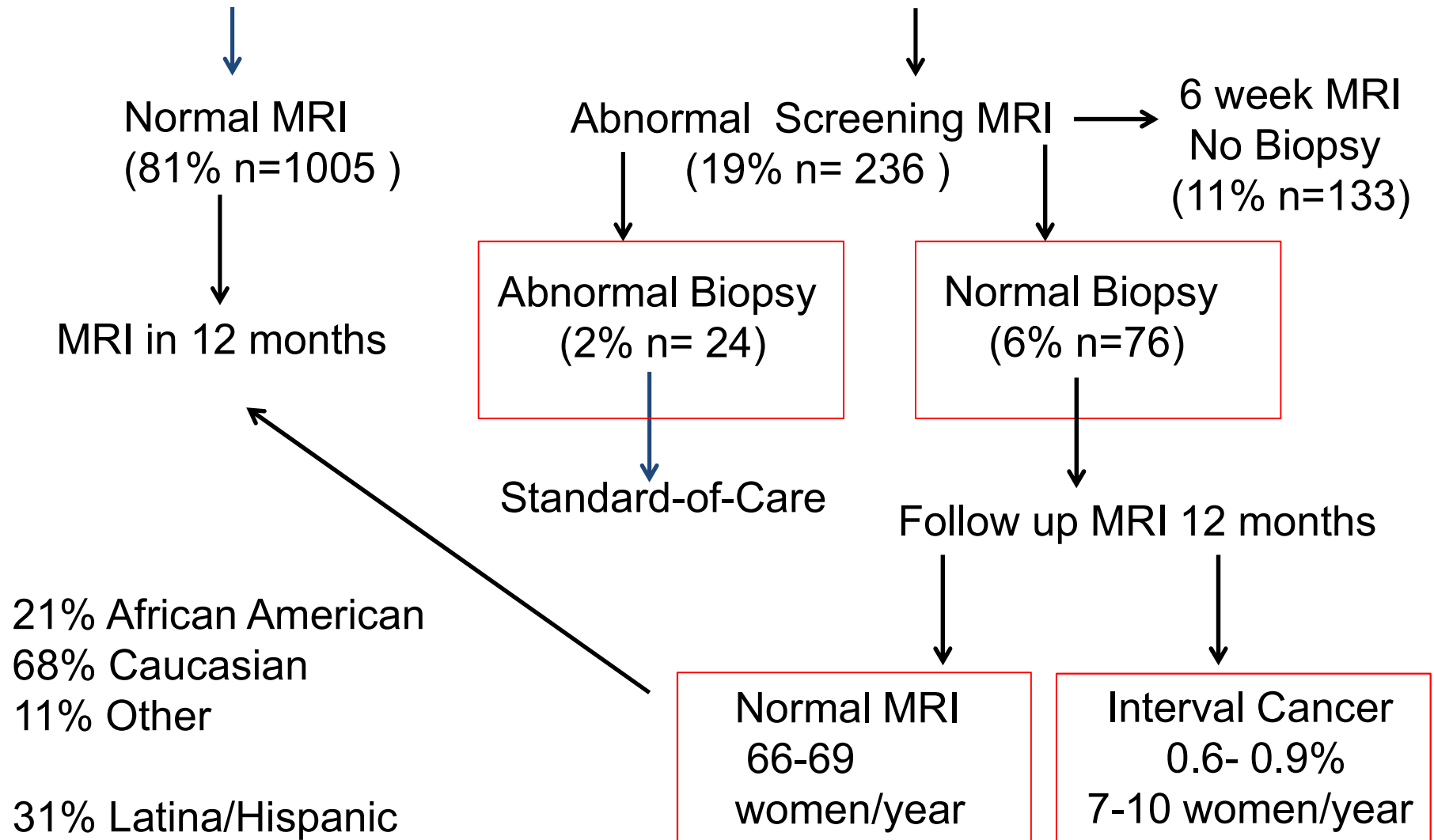
- Defined cohorts that are ethnically/racially diverse
- Consensus on best practices for sample preservation
- Statistical methods for multi-scale data integration

2. Linked imaging/biomarker discovery: Non-contrast imaging to integrate with and support/validate biomarker discovery

3. Challenge of Heterogeneity – in situ single cell transcript profiling.

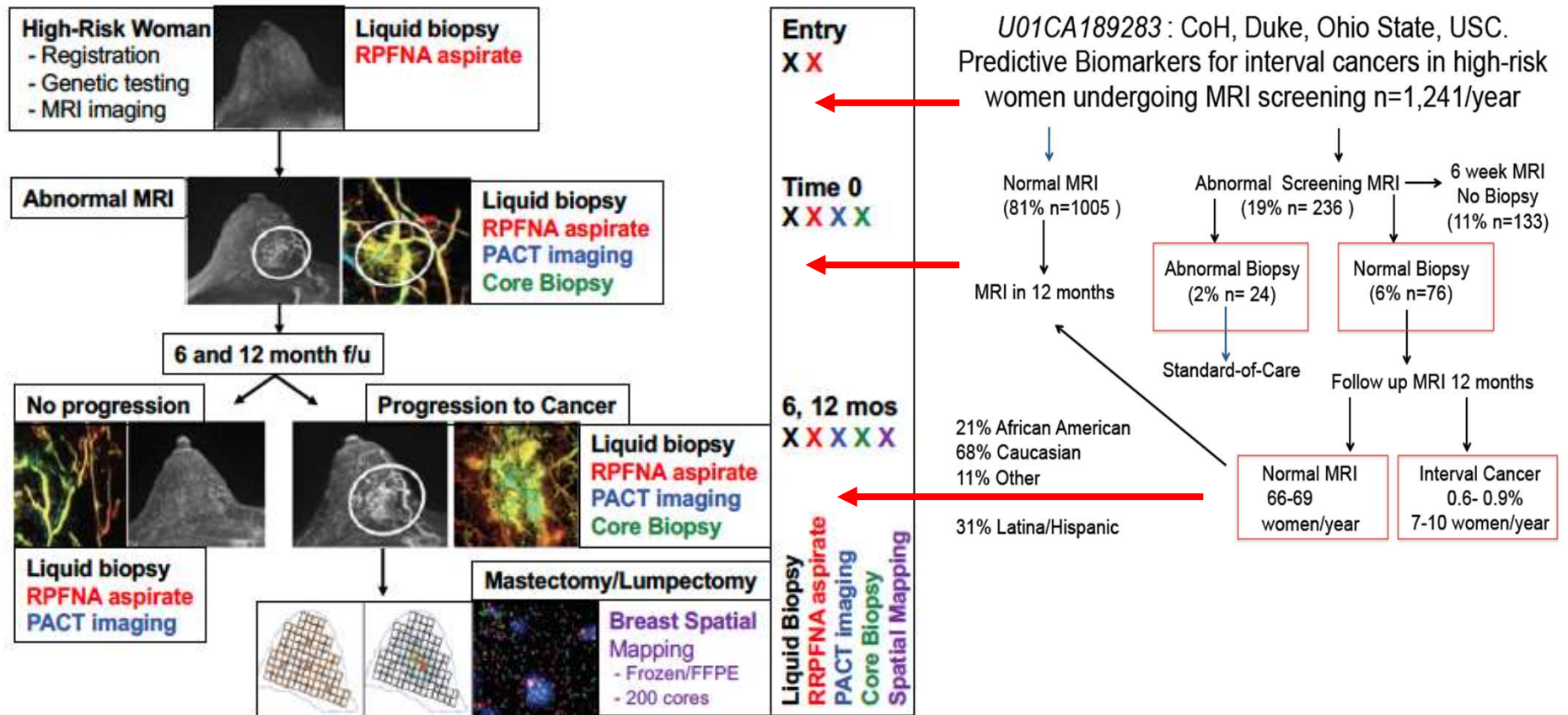
# Defined, Ethnically/Racially Diverse Cohorts

U01CA189283: CoH, Duke, OSU, USC. Predictive Biomarkers for interval cancers in high-risk women undergoing MRI screening n=1,241/year



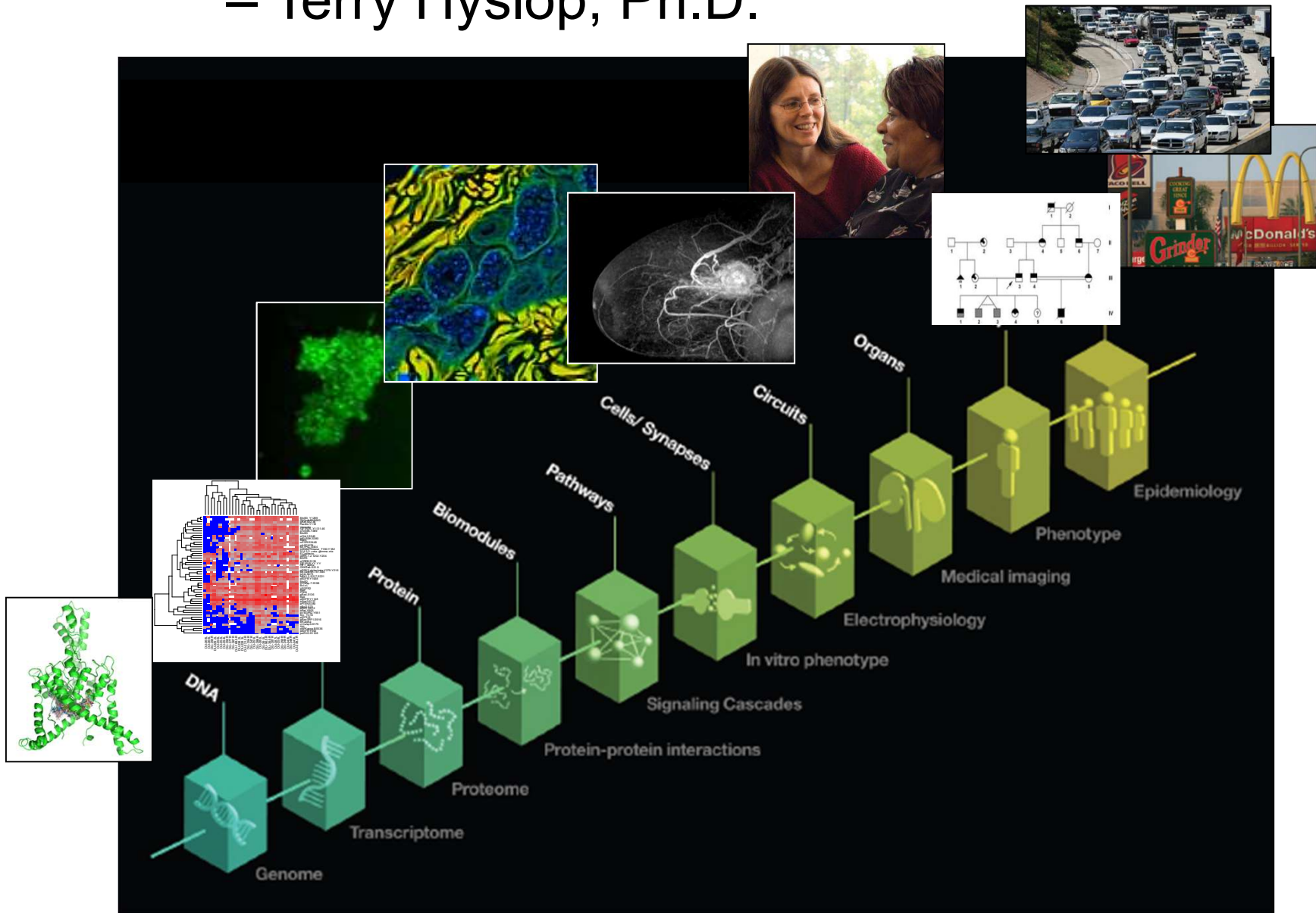
# Framework for multi-parametric, high-resolution biomarkers in small data sets:

- Consensus on best practices for sample preservation
- Statistical methods for multi-scale data integration

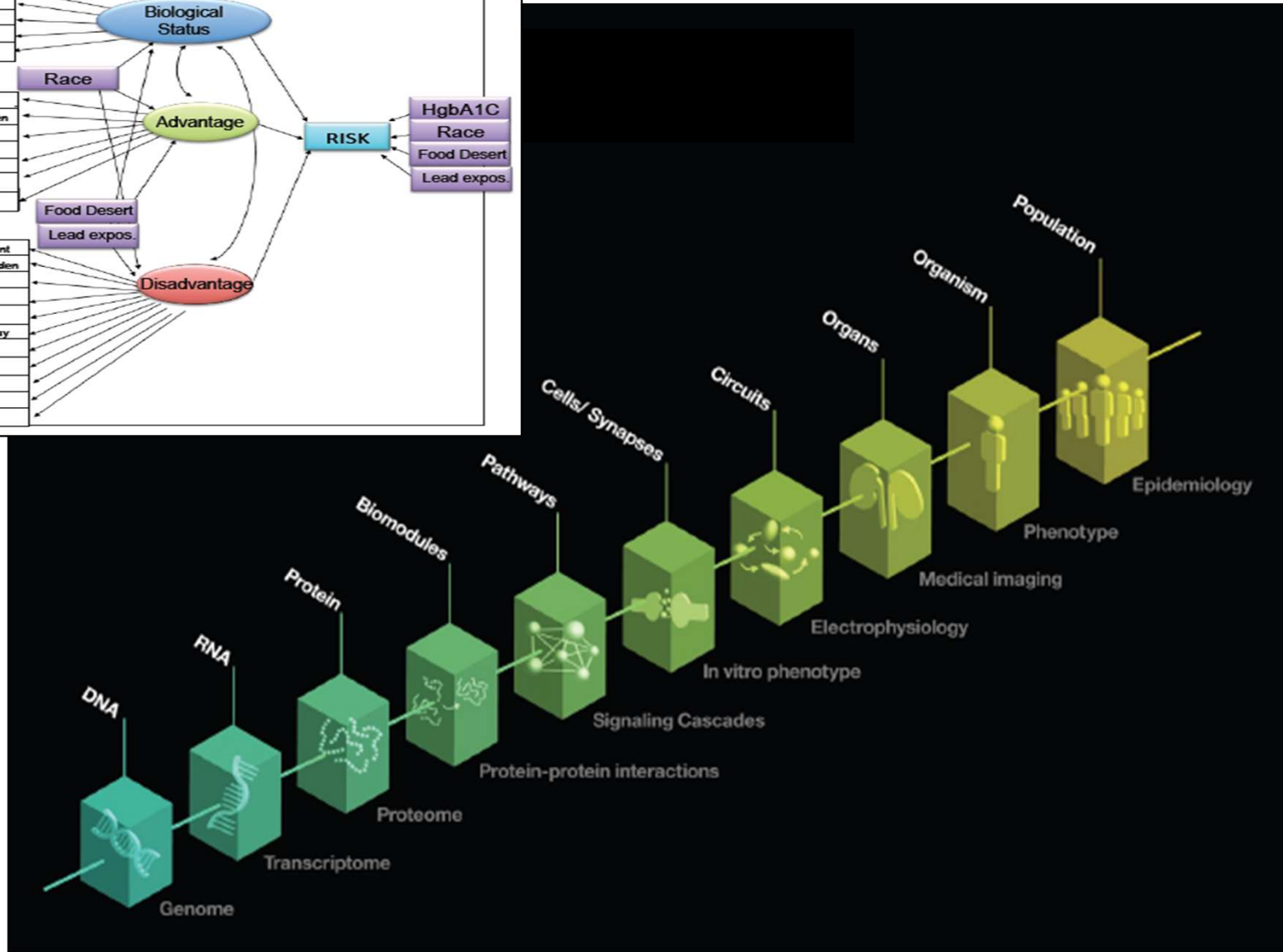
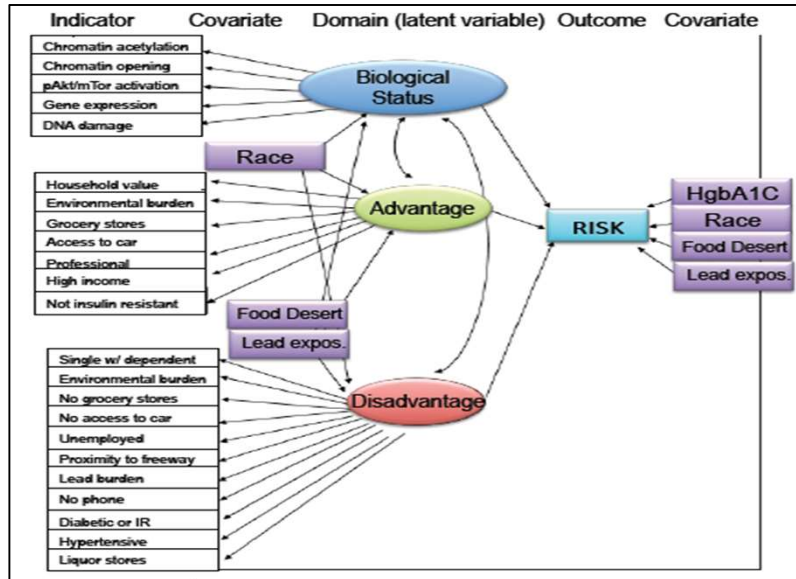


# Multi-Scale modeling of cancer initiation

– Terry Hyslop, Ph.D.



# Multi-Scale modeling of cancer initiation



# Challenges, Needs, Tools

1. Tools for multi-spatial, multi-parametric, high-resolution profiling

→ 2. Linked imaging/biomarker discovery: Non-contrast imaging to integrate with and support/validate biomarker discovery

*- Imaging and Biomarkers for Early Detection of Aggressive Cancer (U01)*

*Richard Mazurchuk, Ph.D. (AKA Maz)*

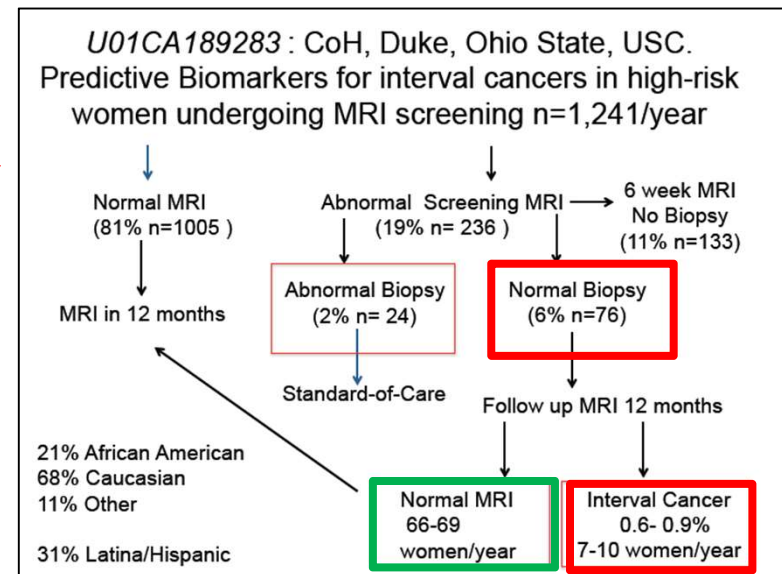
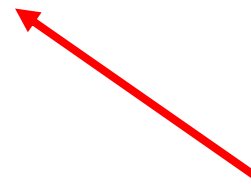
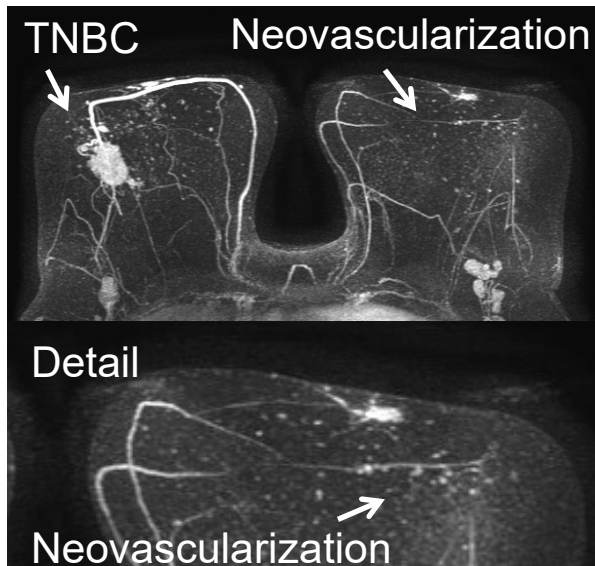
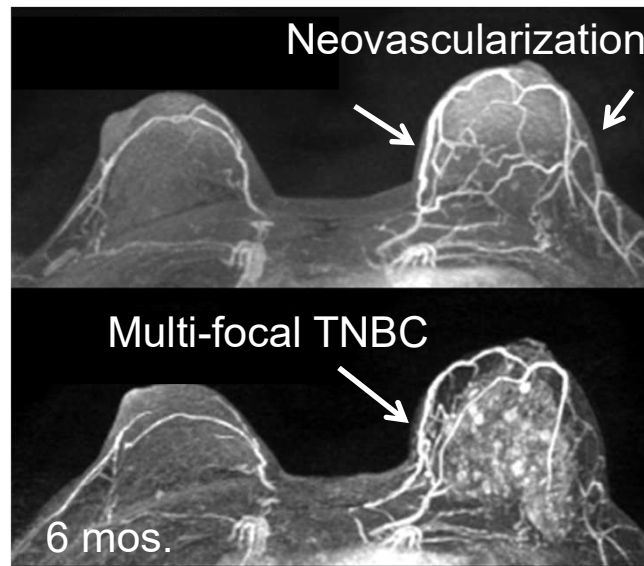
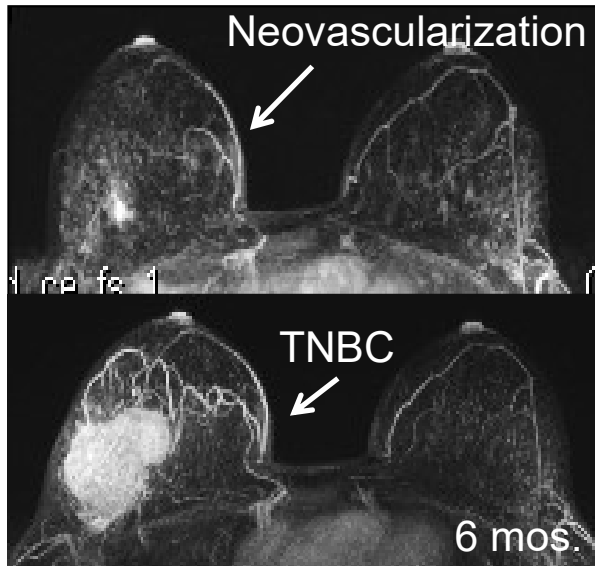
*Keyvan Farahani, Ph.D.*

3. Challenge of Heterogeneity – in situ single cell transcript profiling.



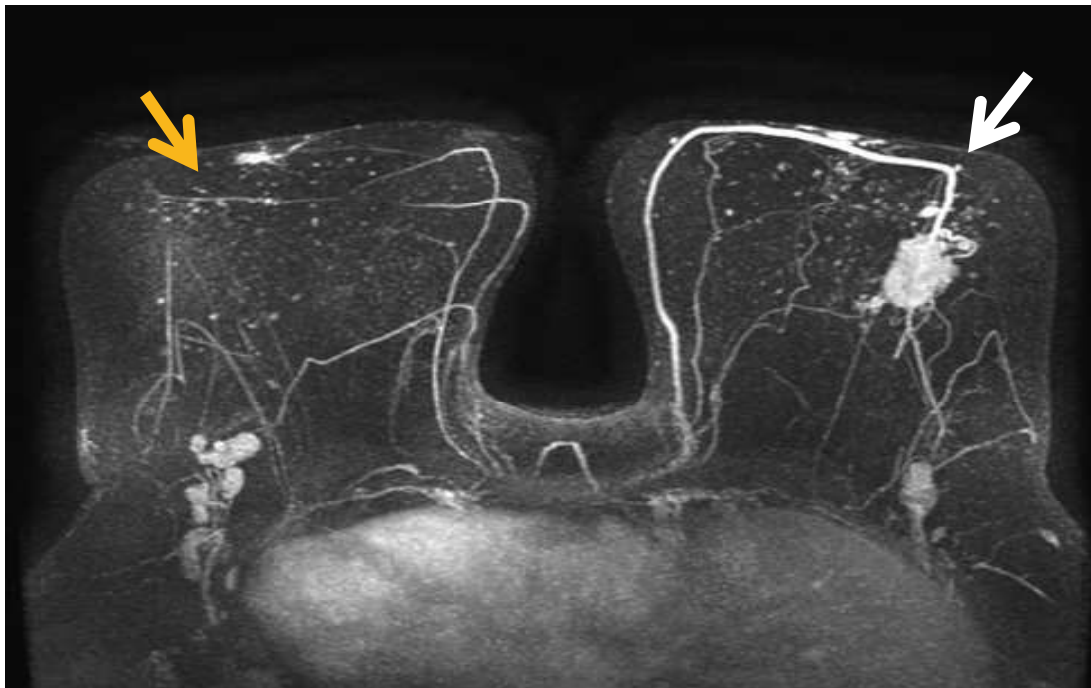
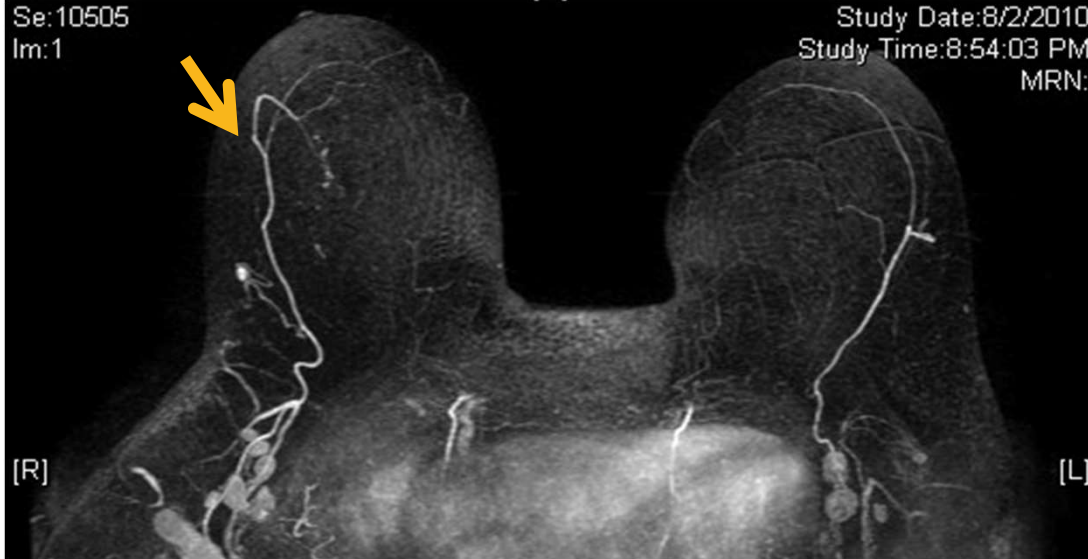
# Non-contrast imaging to correlate with biomarkers

## Neovascularization and progression

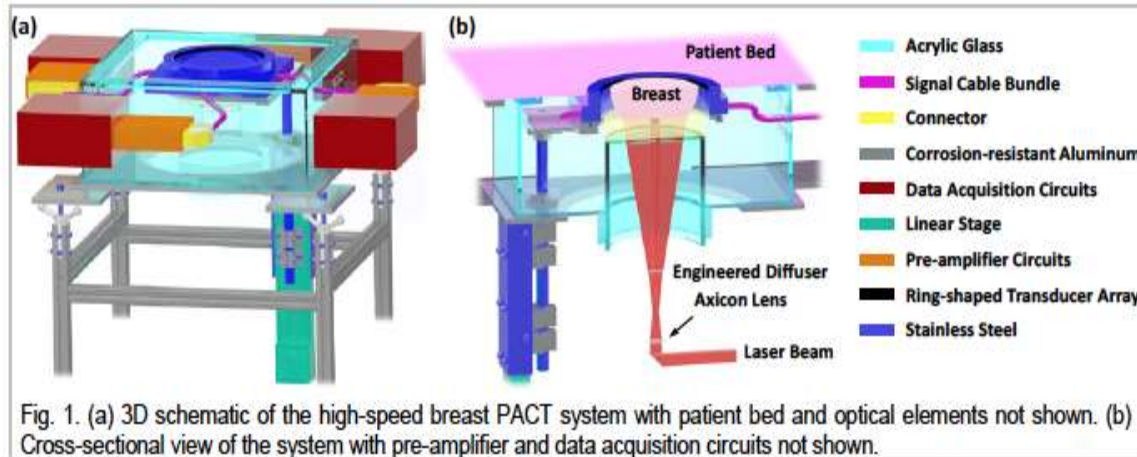


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Im:1

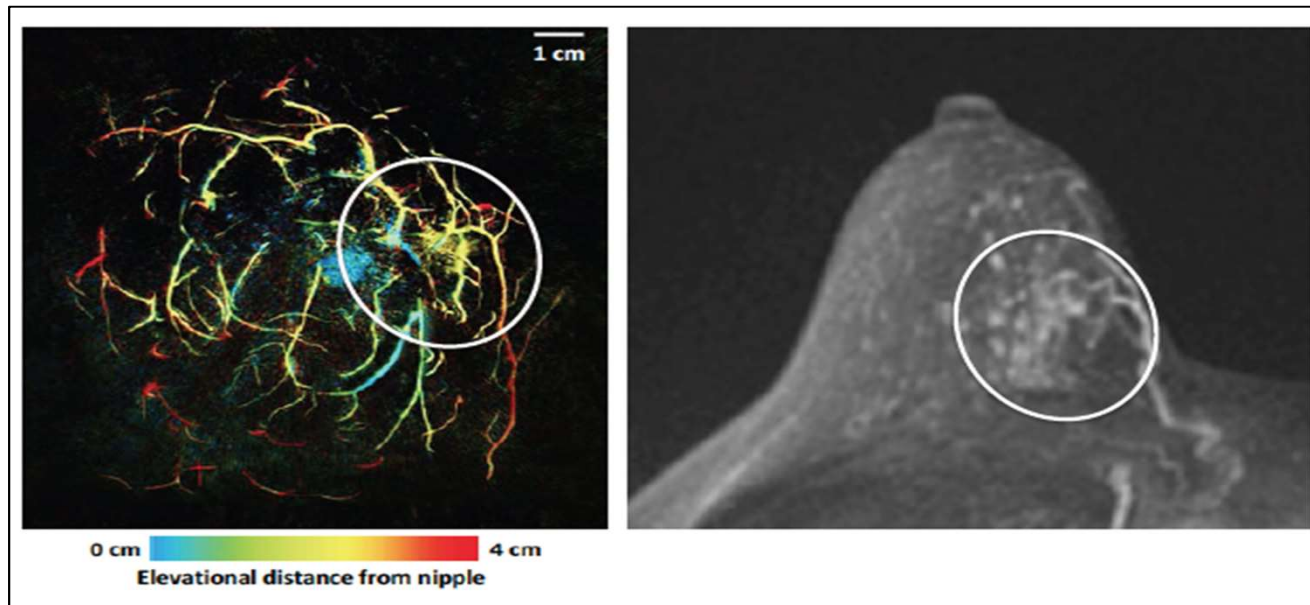
Study Date:8/2/2010  
Study Time:8:54:03 PM  
MRN:



# “Imagable Biomarkers” - Photoacoustic Tomography (PACT) - Caltech - Lihong Wang, Ph.D.



- 15 s image acquisition
- 250  $\mu$  in-plane resolution
- Endogenous fluorescence
- Repeat imaging
- Biological read out
- Multi-scale

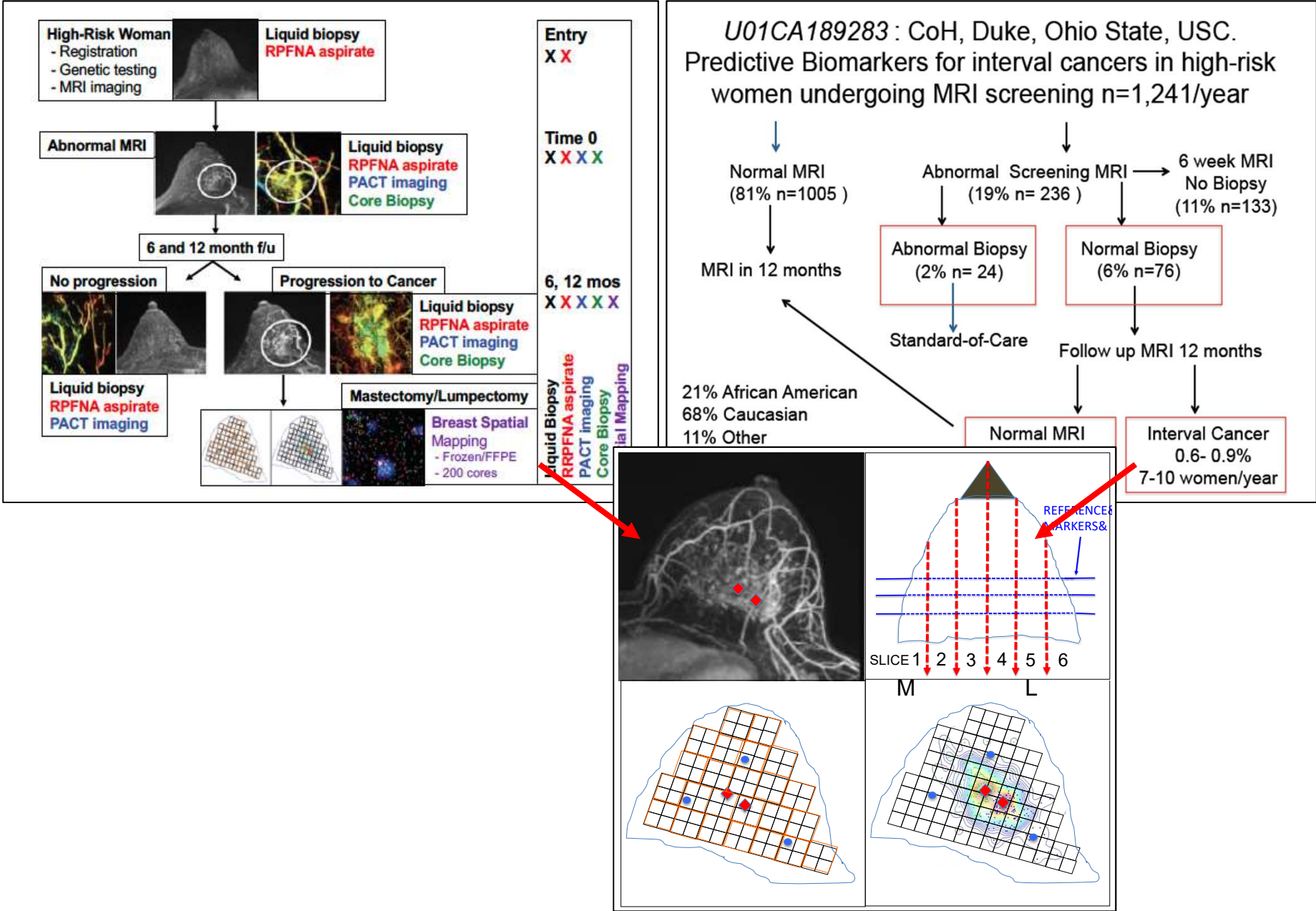


Visualization of blood vessels by PACT – combined US-optical tomography

# Challenges, Needs, Tools

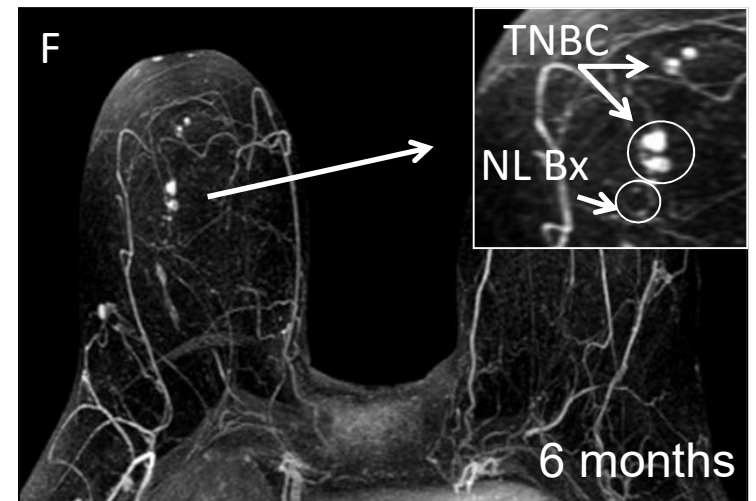
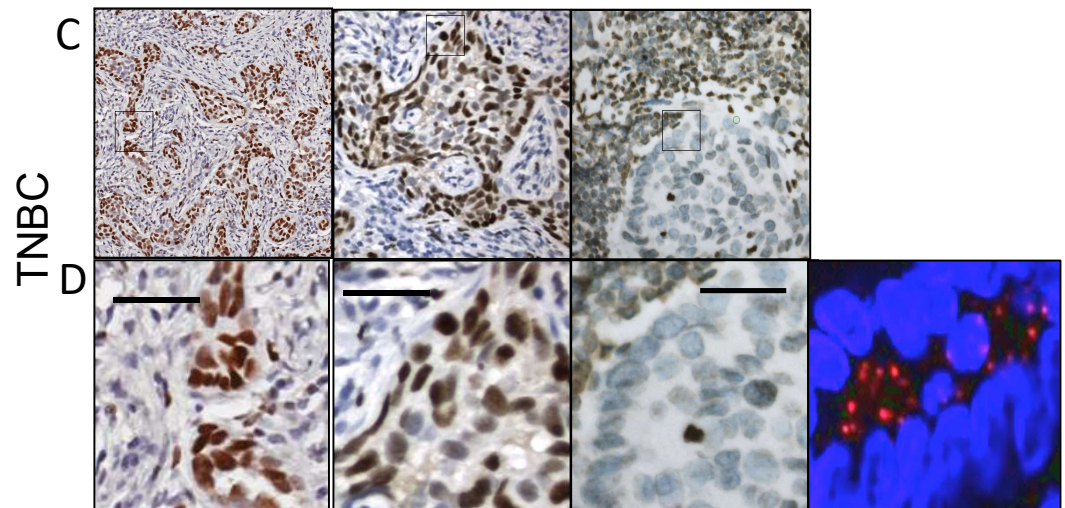
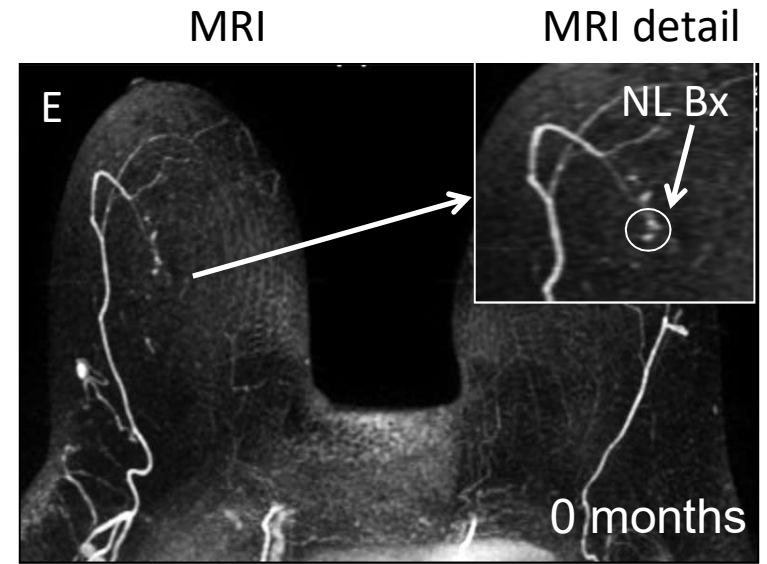
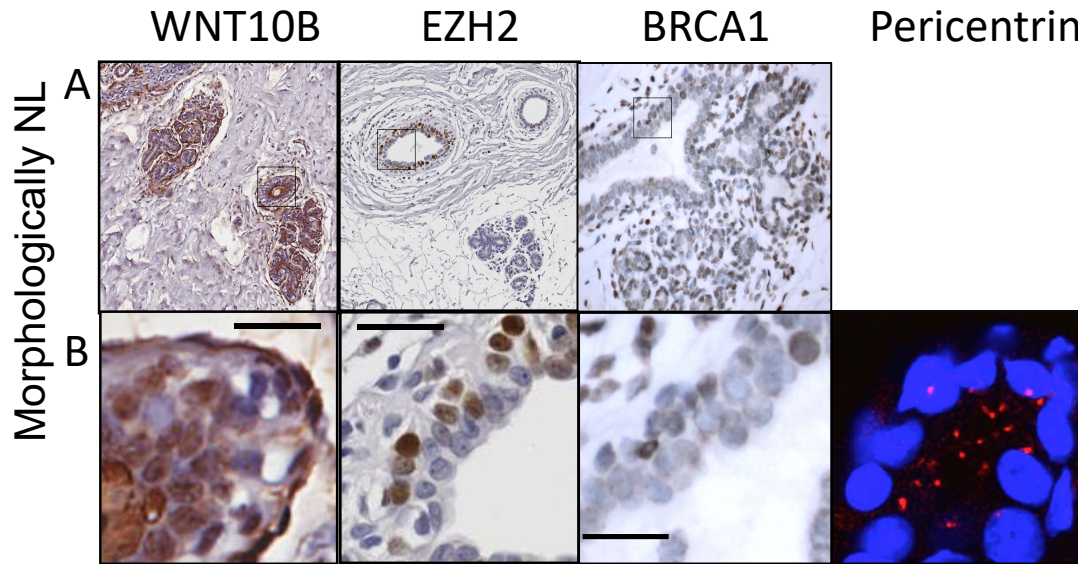
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# Temporal-Spatial Mapping



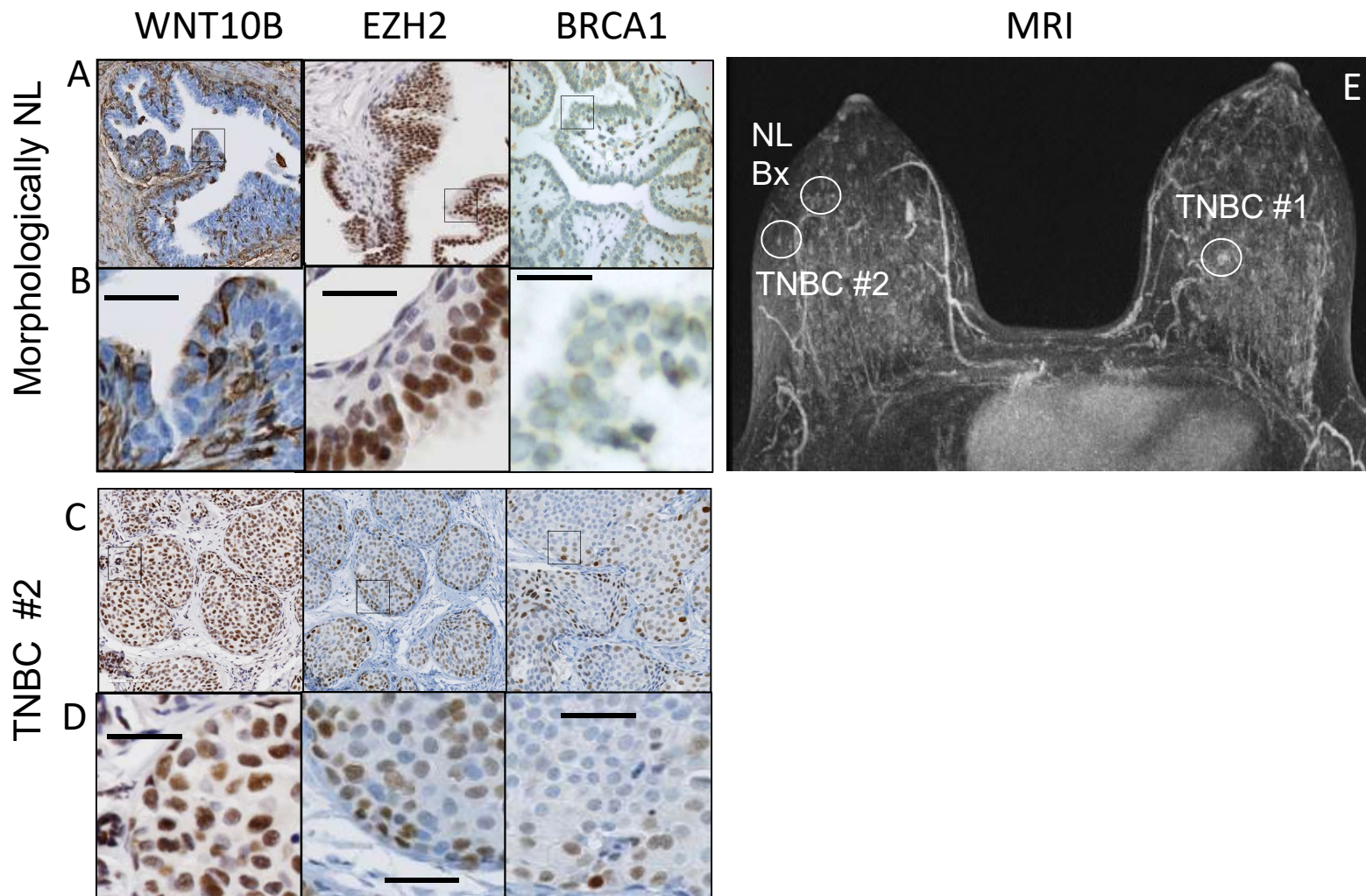
# Heterogeneity – *in situ* single cell transcript profiling.

DU703



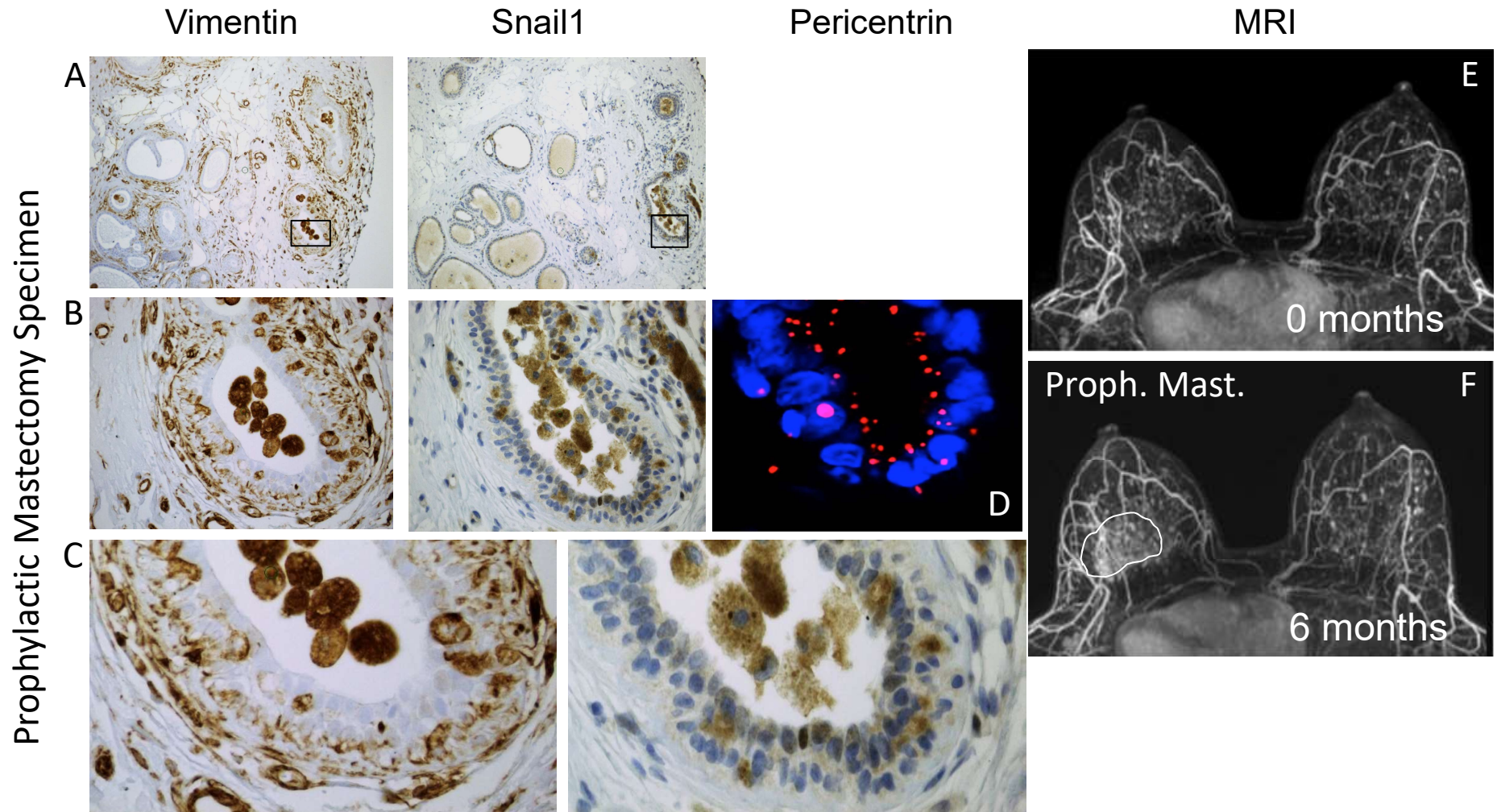
# Heterogeneity – *in situ* single cell transcript profiling.

DU390, Sister of DU368



# Heterogeneity – *in situ* single cell transcript profiling.

DU368, Sister of DU390





# Heterogeneity – *in situ* single cell transcript profiling.



Neuron  
NeuroResource



Long Cai PhD  
Caltech

## In Situ Transcription Profiling of Single Cells Reveals Spatial Organization of Cells in the Mouse Hippocampus

Sheel Shah,<sup>2,3,4</sup> Eric Lubeck,<sup>2,4</sup> Wen Zhou,<sup>1</sup> and Long Cai<sup>1,5,\*</sup>

<sup>1</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

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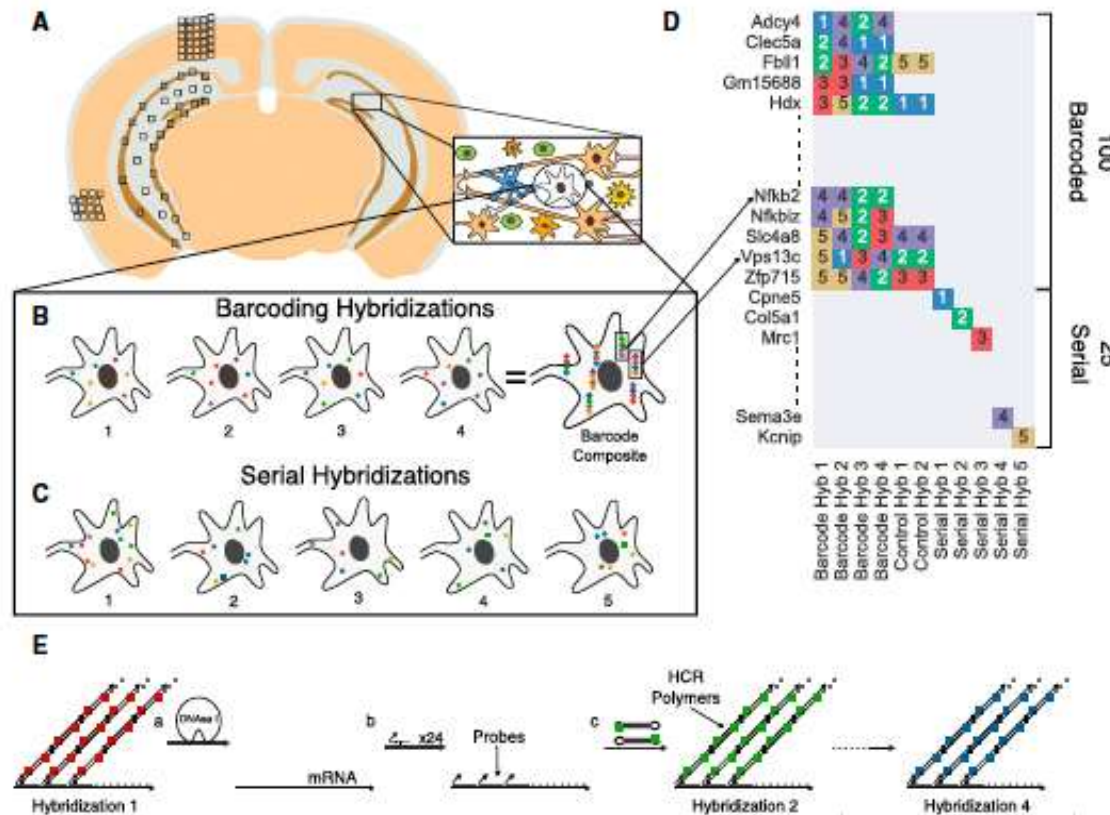
<sup>3</sup>UCLA-Caltech Medical Scientist Training Program, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095, USA

<sup>4</sup>Co-first author

<sup>5</sup>Lead Contact

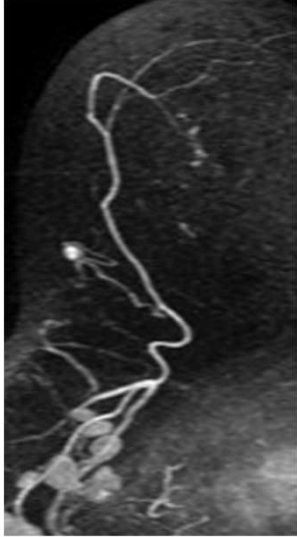
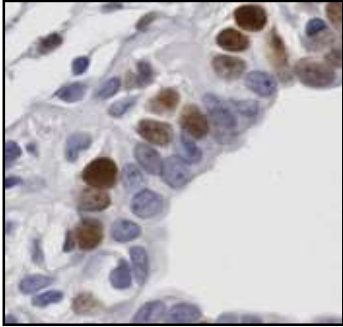
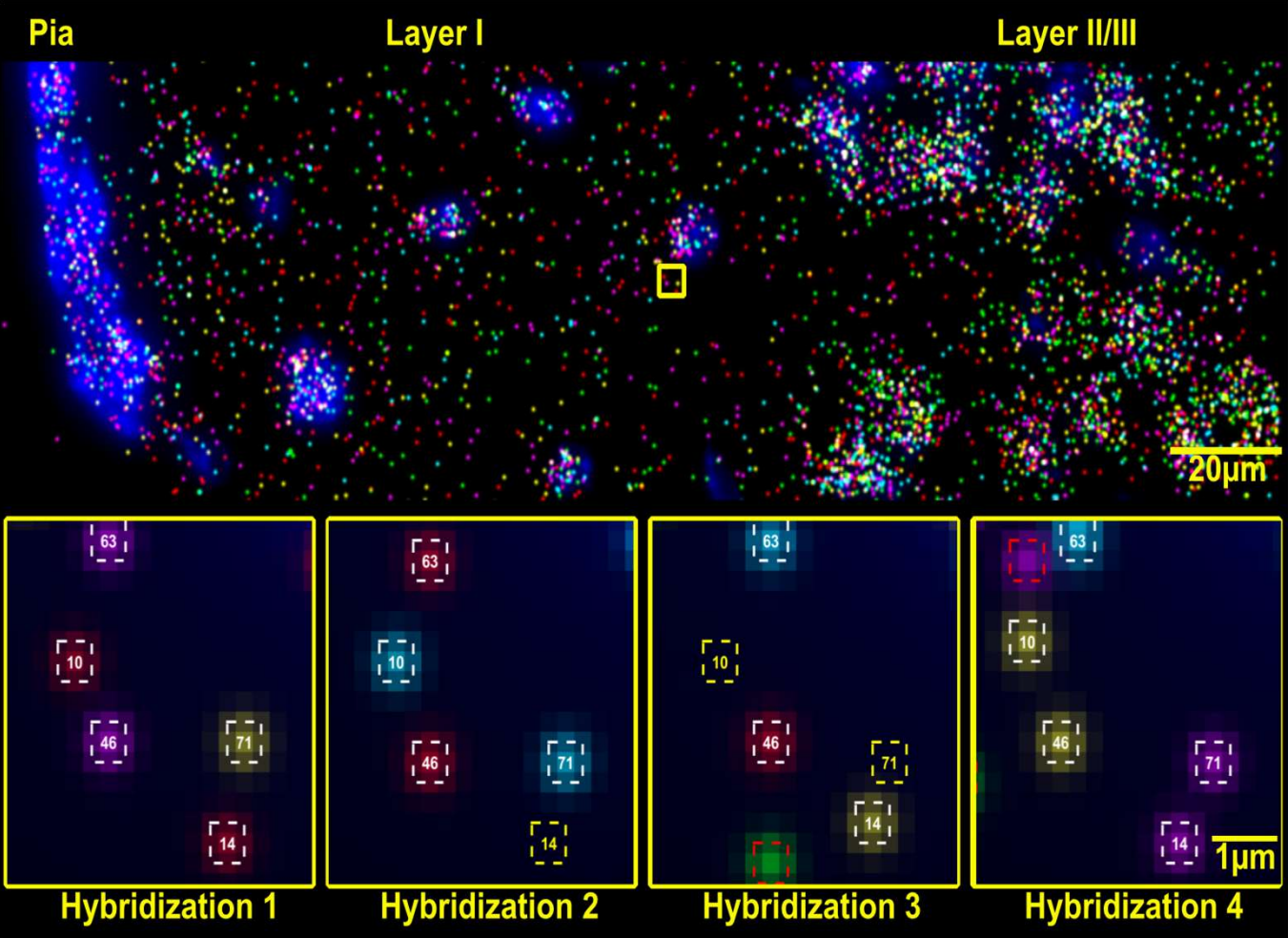
\*Correspondence: [lc@caltech.edu](mailto:lc@caltech.edu)

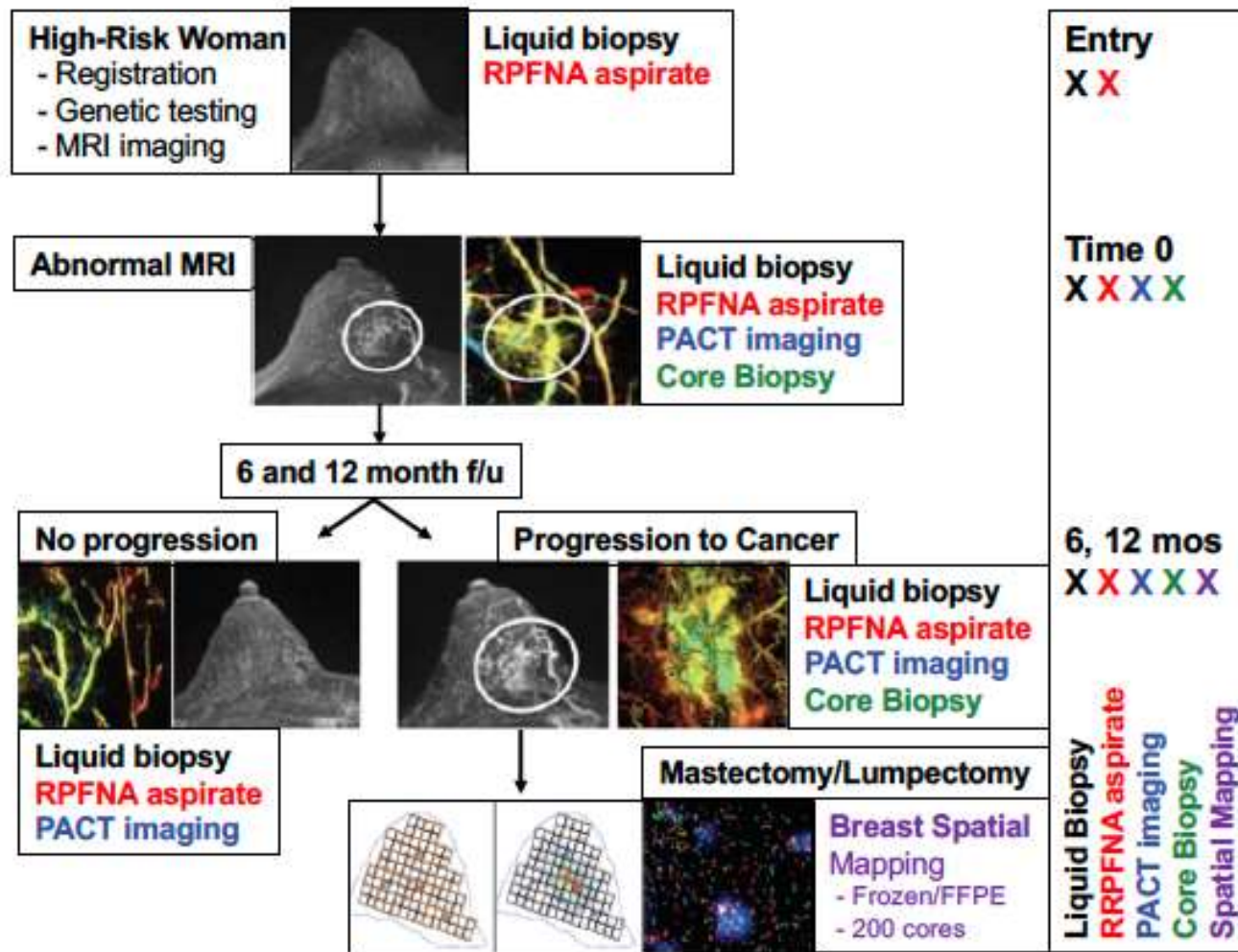
<http://dx.doi.org/10.1016/j.neuron.2016.10.001>



# In Situ Transcription Profiling of Single Cells seqFISH – 150-249 transcripts

Long Cai PhD  
Caltech





Serial MRI - analysis of imaging features that predict progression – compare with tissue and liquid biopsy – correlation with PACT imaging – can imaging/biomarkers predict aggressive biology?

Serial RPFNA breast aspiration - comparison with MRI imaging features, PACT imaging, circulating cells, core/excisional biopsy – do epigenetic dysregulation precede DNA mutations?

Liquid biopsy/single cell isolation – single cell transcript profiling – when during cancer progression is it possible to detect abnormal circulating cells? Is there concordance with breast chances?

Core/excisional biopsy and breast mapping – *in situ* spatial and temporal mapping of aggressive biology.



## Conclusions

- Aggressive precancer biology is challenging to capture for biomarker discovery
- Framework, cohort, and multi-parametric identification of aggressive biology.

## Future Directions

- Combined PACT-Biomarker
- *In situ* transcript profiling of archived and prospectively acquired breast biopsies.

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Laura Kruper MD

## Laboratory Team

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Kendall Kennedy

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Mayra Serrano  
Sharon Elliott-Bynum RN PhD

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### FHCRC

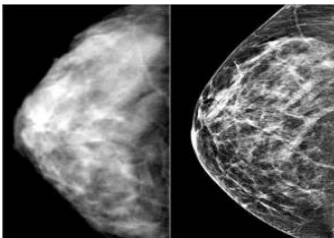
Jeffery Delrow PhD

### FDA Jefferson, Arkansas

Beverly Lyn-Cook PhD

### Duke

Terry Hyslop PhD  
Jay Baker MD





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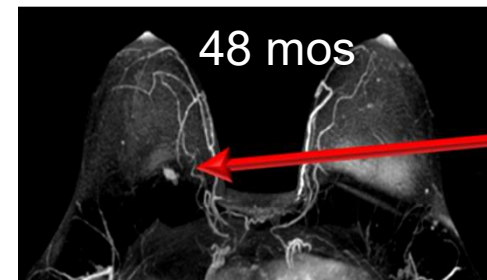
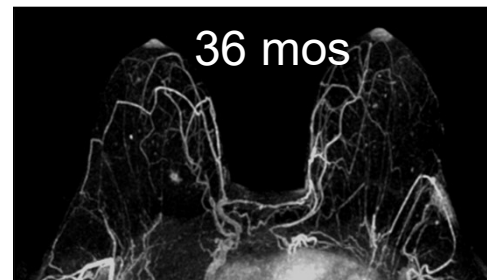
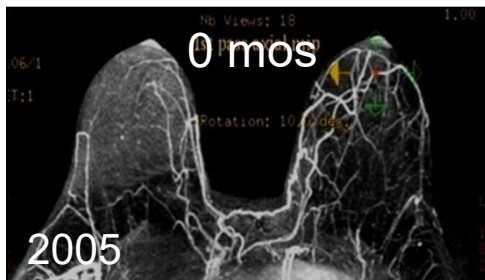
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S. Riley



ER/PR+  
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Focal, non-accelerated 22/51 women

