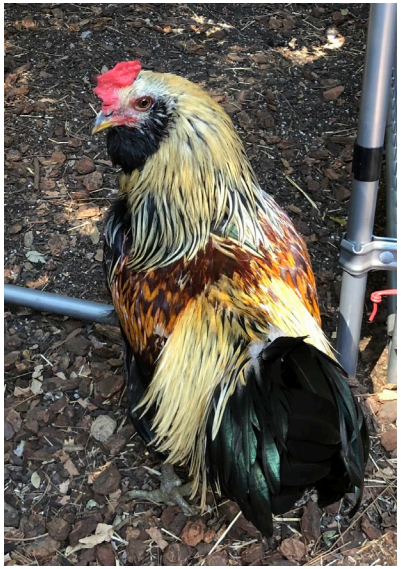
Breast Cancer Risk and Screening

Victoria Seewaldt, M.D.
…and Tom



Risk for BrCA aging vs. TNBC

Breast cancer risk - ER+ breast cancer of aging

- Age –age increases risk
- Parity – more children protects
- Early childbearing – protective
- Early obesity – thought to reduce risk
- Late obesity – increases risk ER+ breast cancer

TNBC risk – Carolina Breast Study – Bob Millikan

- Young – old age not a risk factor
- Parity – more children INCREASES risk
- Early childbearing – INCREASES risk
- Early obesity – controversial

[Carey, L et al., JAMA, 2006]

Risk models and Risk Assessment

- Gail Model
- Tyer-Cuzick Model
- Genetic Testing
- Mammographic density

Gail Model Risk Model

Risk Tool

(Click a question number for a brief explanation, or read all explanations.)

1. Does the woman have a medical history of any breast cancer or of ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) or has she received previous radiation therapy to the chest for treatment of Hodgkin lymphoma? — Select
2. Does the woman have a mutation in either the *BRCA1* or *BRCA2* gene, or a diagnosis of a genetic syndrome that may be associated with elevated risk of breast cancer? — Select
3. What is the woman's age? *This tool only calculates risk for women 35 years of age or older.* — Select
4. What was the woman's age at the time of her first menstrual period? — Select
5. What was the woman's age at the time of her first live birth of a child? — Select
6. How many of the woman's first-degree relatives - mother, sisters, daughters - have had breast cancer? — Select
7. Has the woman ever had a breast biopsy? — Select
   - 7a. How many breast biopsies (positive or negative) has the woman had? — Select
   - 7b. Has the woman had at least one breast biopsy with atypical hyperplasia? — Select
8. What is the woman's race/ethnicity? — Select
   - 8a. What is the sub race/ethnicity? — Select

Calculate Risk >

- Risk factors – age, menarche, menopause, biopsy, first degree relatives.
- Most accurate in non-Latina White women who receive mammograms
- Underestimates risk, particularly in African American women
- CARE study – better estimates risk in African American women
- Not appropriate for women with familial breast cancer

http://www.cancer.gov/bcrisktool/

Gail Model Risk Model

National Cancer Institute — U.S. National Institutes of Health | www.cancer.gov

Breast Cancer Risk Assessment Tool

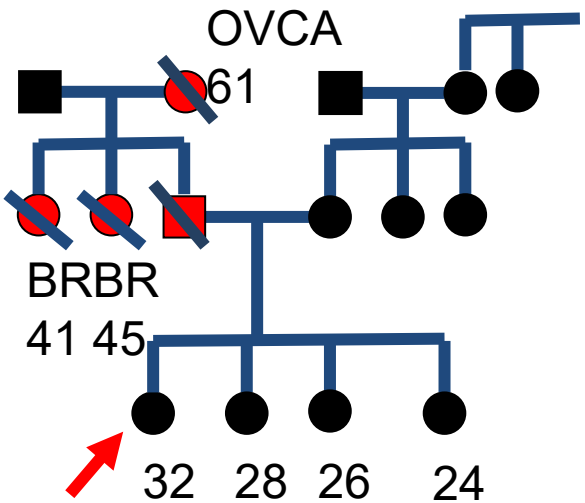
An interactive tool to help estimate a woman's risk of developing breast cancer

Risk Tool

(Click a question number for a brief explanation, or read all explanations.)

1. Does the woman have a medical history of any breast cancer or of ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) or has she received previous radiation therapy to the chest for treatment of Hodgkin lymphoma? — No
2. Does the woman have a mutation in either the *BRCA1* or *BRCA2* gene, or a diagnosis of a genetic syndrome that may be associated with elevated risk of breast cancer? — No
3. What is the woman's age? *This tool only calculates risk for women 35 years of age or older.* — 35
4. What was the woman's age at the time of her first menstrual period? — 7 to 11
5. What was the woman's age at the time of her first live birth of a child? — < 20
6. How many of the woman's first-degree relatives - mother, sisters, daughters - have had breast cancer? — 0
7. Has the woman ever had a breast biopsy? — No
   - 7a. How many breast biopsies (positive or negative) has the woman had? — Select
   - 7b. Has the woman had at least one breast biopsy with atypical hyperplasia? — No
8. What is the woman's race/ethnicity? — African American
   - 8a. What is the sub race/ethnicity? — Select

Calculate Risk >



Lifetime Risk of Developing Breast Cancer

- This woman (to age 90): 9.8%
- Average woman (to age 90): 10.1%

Explanation

Based on the information provided (see below), the woman's estimated risk for developing invasive breast cancer over her lifetime (to age 90) is 9.8% compared to a risk of 10.1% for a woman of the same age and race/ethnicity from the general U.S. population.

http://www.cancer.gov/bcrisktool/

Tyrer-Cuzick – or IBIS Tool

Likelihood of *BRCA1* or *BRCA2* mutation

Inform decision-making about genetic counselling and testing.

≥10% - mutation in *BRCA1, BRCA2,* - genetic counseling. [53]

Risk estimated based on :

- Body mass index
- Age at menarche, OB hx
- Age at menopause (if applicable)
- Benign breast biopsy - hyperplasia, atypical hyperplasia, LCIS
- Hx ovarian cancer
- Use of hormone replacement therapy
- Family history (including breast and ovarian cancer, Ashkenazi inheritance, genetic testing if done)

Tyrer–Cuzick - most consistently accurate, whereas the Gail, Claus, and Ford models significantly underestimate risk.

Boughey *et al* - Tyrer-Cuzick model significantly overestimated in women with atypical hyperplasia.

Amir E et al. J Med Genet. 2003; Boughey JC et al. JCO, 2010

BRCA1 - *BRCA2* and other germline mutations

BRCA1

- Chromosome 17 – discovered by Mary-Clair King
- High frequency TNBC – 40-60% lifetime
- Fallopian tube/Ovarian cancer, pancreas, skin, prostate
- True risk of developing breast cancer not known
- With MRI screening >60% need for chemotherapy

BRCA2

- Chromosome 13
- High frequency ER+ breast cancer – 40-55% lifetime
- Fallopian tube/Ovarian cancer, pancreas, skin, prostate
- Good prognosis for breast, not for prostate
- MRI screening <20% need for chemotherapy

Other germline mutation – *ATM, BARD1, CDH1, CHEK2, NF1, PALB2, PTEN, RECQL, STK11*, and *TP53*

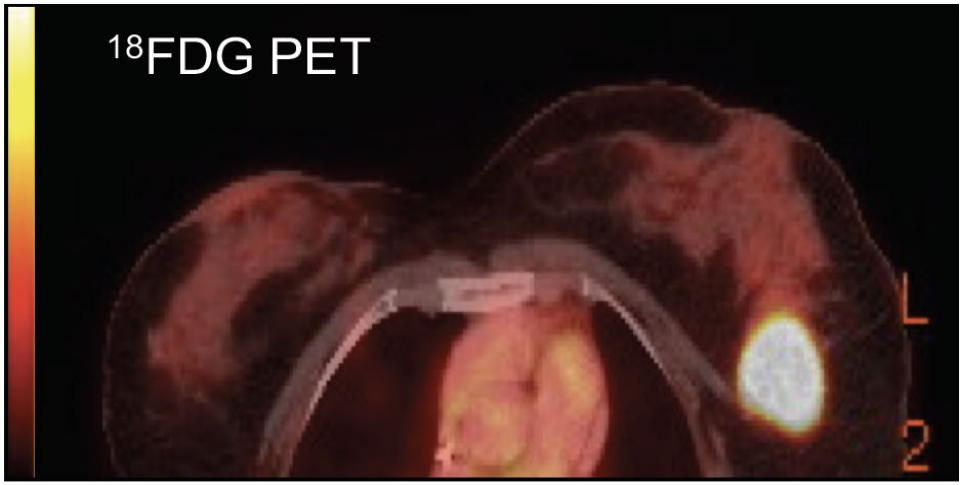
Roy, R. et al. Nature Reviews Cancer 2012; Wang, J et al. Frontiers, 2021

Triple-Negative Breast Cancer (ER/PR-/HERwt)

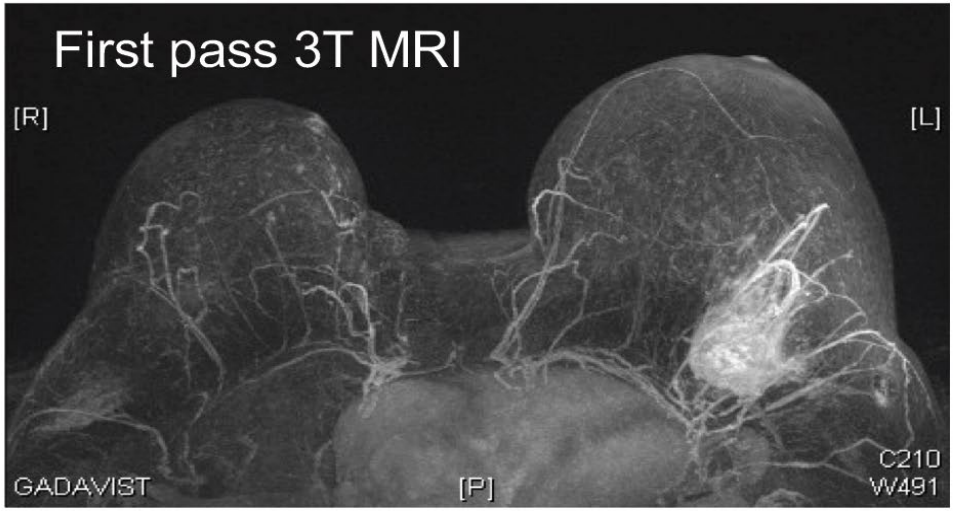
- majority low BRCA1 protein expr. - no germline or somatic mt

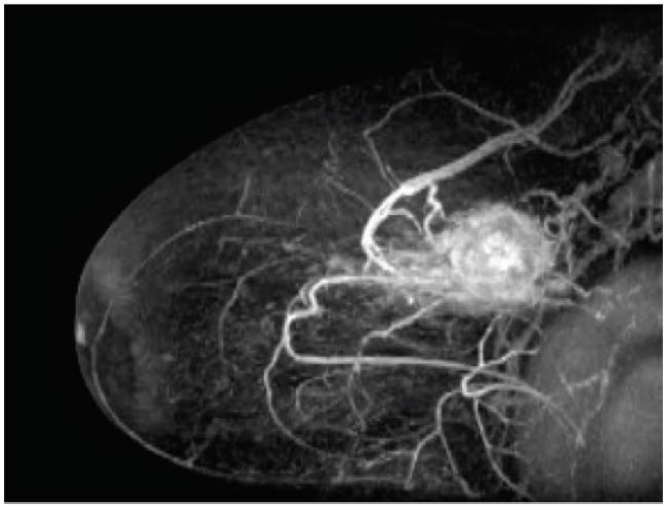
Frequency BRCA1 mt

- 51% Ashkenazi
- 38% European American
- **29% Latina**
- **26% Asian**
- **19% African American**
- **5.6% Lebanese**
- **0.6% Palestinian**



Greenup et al. *Ann Surg Onc* 2013
Jalkh et al. BMC Med Genomics, 2017





Genetic Studies – Cautionary Tale

Genomic analysis of inherited breast cancer among Palestinian women: Genetic heterogeneity and a founder mutation in TP53

Suhair Lolas Hamameh[1,2], Paul Renbaum[2], Lara Kamal[1], Dima Dweik[1], Mohammad Salahat[1], Tamara Jaraysa[1], Amal Abu Rayyan[1], Silvia Casadei[3], Jessica B. Mandell[3], Suleyman Gulsuner[3], Ming K. Lee[3], Tom Walsh[3], Mary-Claire King[3], Ephrat Levy-Lahad[2], and Moein Kanaan[1]

Hamameh SL et al. Int J. Cancer, 2017

875 Palestinian women with invasive breast cancer

453 dx $\leq$ 40, or Br/OV - mother, sister, grandmother, or aunt

422 women dx age >40 and with negative family history.

0.6% *BRCA1*, 1.1% *BRCA2*, 0.8% *TP53*

<0.1% *ATM, PTEN, BARD, BRIP1, PALB2, PTEN*

Most frequent mutation *TP53 p.R181C*

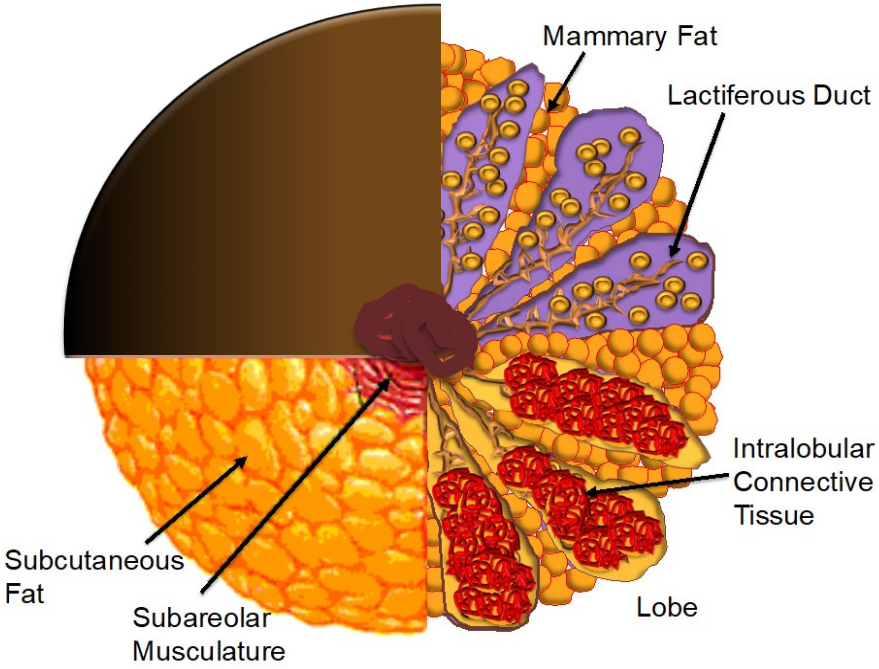
Issues

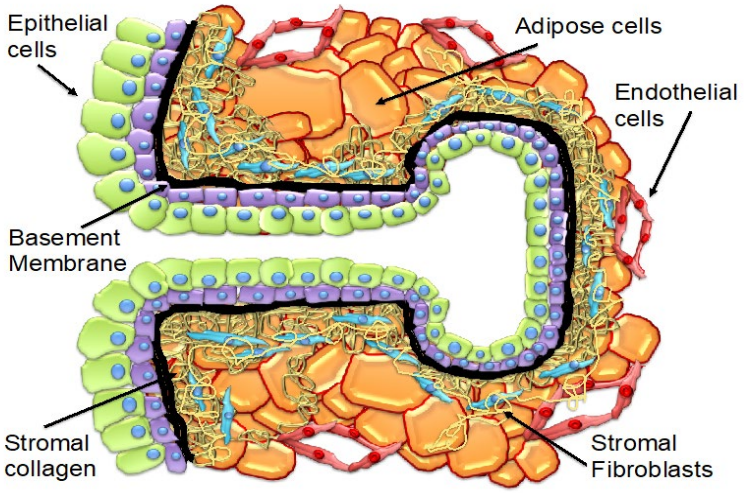
Women with *TP53* mutation - no Li Fraumeni pattern of cancers

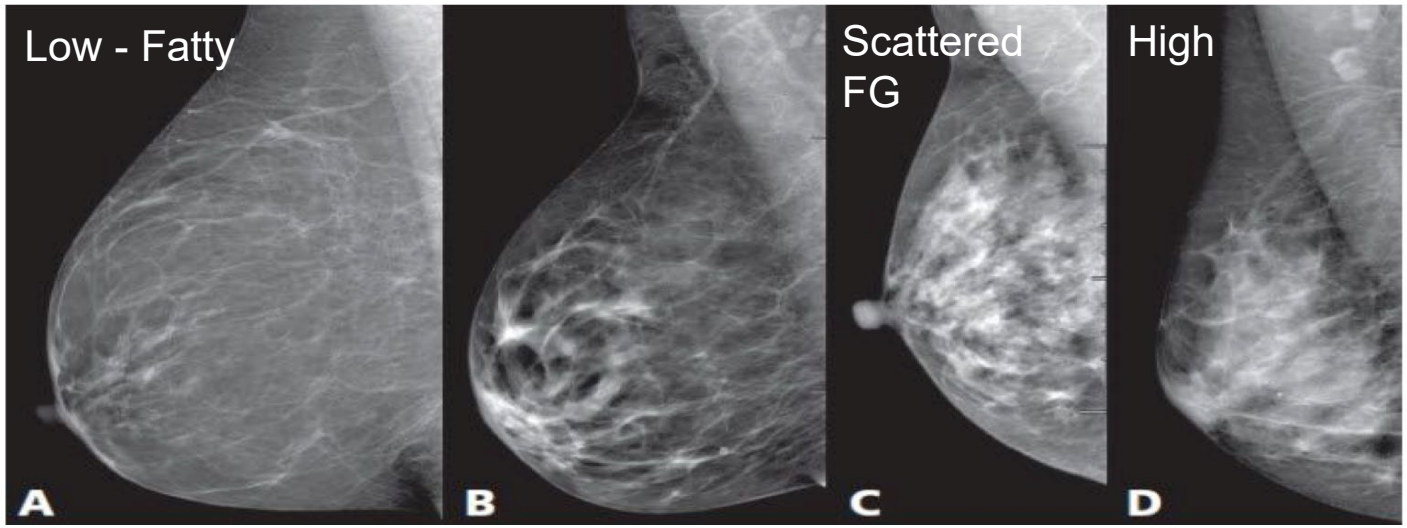
Tested post chemo – no skin biopsy

Did not inform women of dx – hence no family testing

Mammographic Density







Mammographic density and breast cancer risk for women of EUROPEAN descent

- NBSS 5.66 (2.8,11.3) p<0.001 (Boyd - Canada)
- OBSP 3.39 (1.1,10.3) p<0.001 (Boyd – Canada)
- SMPBC 4.52 (1.9,11.0) p<0.001 (Boyd – Canada)
- Combined 4.74 (3.0.7.4) p<0.001

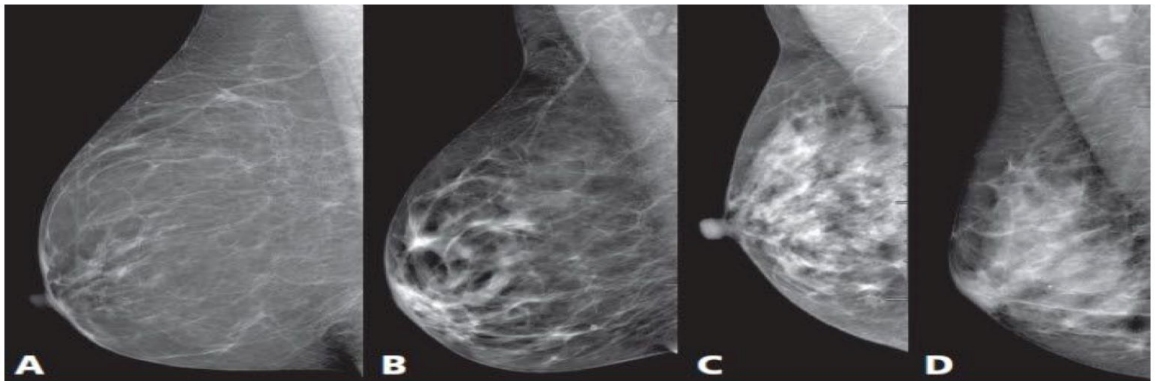
[Boyd et al. Breast Cancer Res, 2013]

African American women have low mammographic density

Race	No. (%) of Patients in BI-RADS Category[a]				Total No. of Patients (%)
	1	2	3	4	
Asian	4 (0.92)	69 (15.86)	244 (56.09)	118[b] (27.13)	435 (2.84)
White	831 (6.54)	3,463 (27.26)	6,825 (53.72)	1,585 (12.48)	12,704 (83.1)
African American	47 (8.38)	174 (31.02)	290 (51.69)	50 (8.91)	561[c] (3.67)
Other[d]	150 (9.42)	465 (29.21)	830 (52.14)	147 (9.23)	1,592 (10.4)
Total	1,032	4,171	8,189	1,900	15,292

Increasing Density →

Low Density
Asian 16%
EA 27%
AA 39%



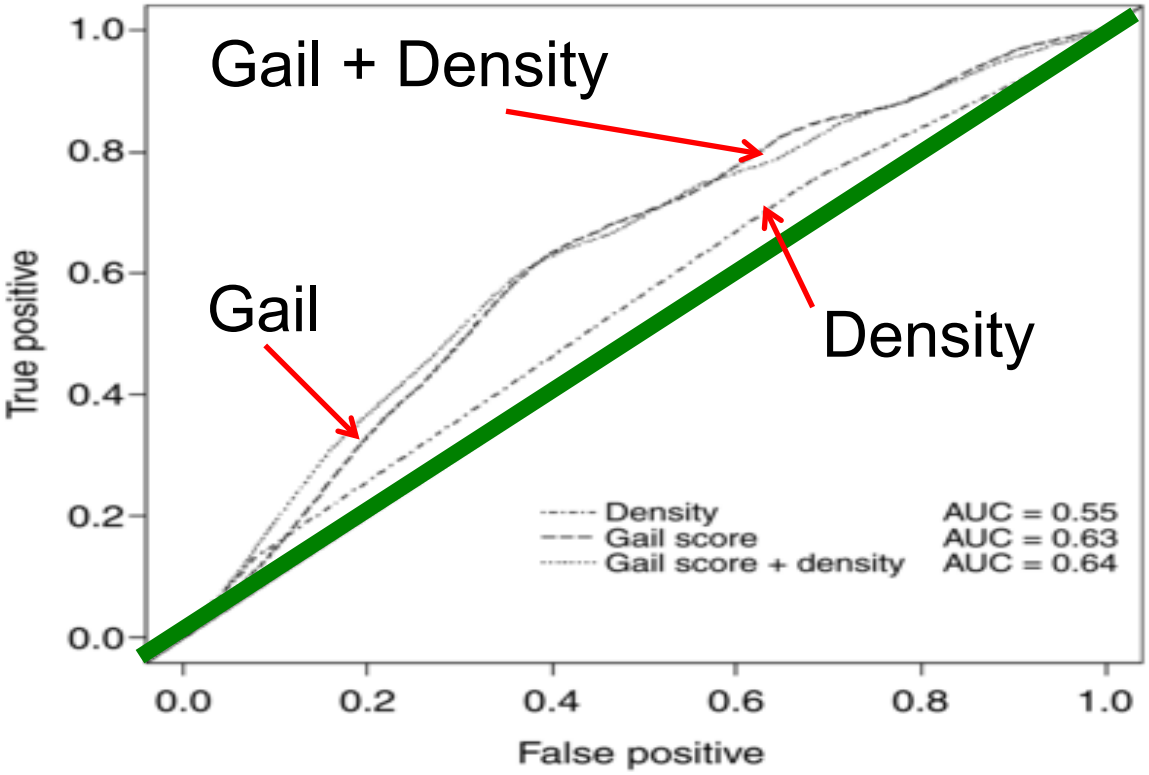
High Density
Asian 27%
EA 12%
AA 9%

[del Carmen et al AJR, 2007]

Mammographic density does not predict risk for individuals – only populations

Model	AUC (Cecchini)	AUC (Chen)	AUC (Tice)	AUC (Barlow)
Gail	0.63	0.596	0.67	0.605
Gail + density	0.64	0.643	0.68	0.624
Density	0.55	-	0.67	0.571

GREEN LINE= No better than a coin flip



[Cecchini et al. Can. Prev. Res. 5(11), 1321-9, 2012]

Low density predicts *death* from breast cancer

NCI-sponsored Breast Cancer Consortium

- 9,000 women
- January 1996 and December 2005, 6.6 years average
- 1,795 deaths, 889 breast cancer, 810 other causes.

Women with **high-density** had **increased risk of breast cancer** but high-density predicted **good survival**

Women with **low breast density** had **lower risk of breast cancer** but low-breast density predicted **poor survival**.

Gierach GL, et al. Relationship between mammographic density and breast cancer death in the Breast Cancer Surveillance Consortium. JNCI, 2012.

Screening

- Breast Self-Examination
- Mammography
- MRI
- Experimental Imaging – future discussion

Breast Self Exam – Shanghai Study

Factory owner

House
Pension
Health Care
One child policy

Woman w/ breast mass

Chinese Medicine 1989-1991
- No imaging
- ? Surgery
- ? Treatment

Conclusion:
Breast Self Exam NO difference cancer survival

Thomas et al. JNCI, 2002

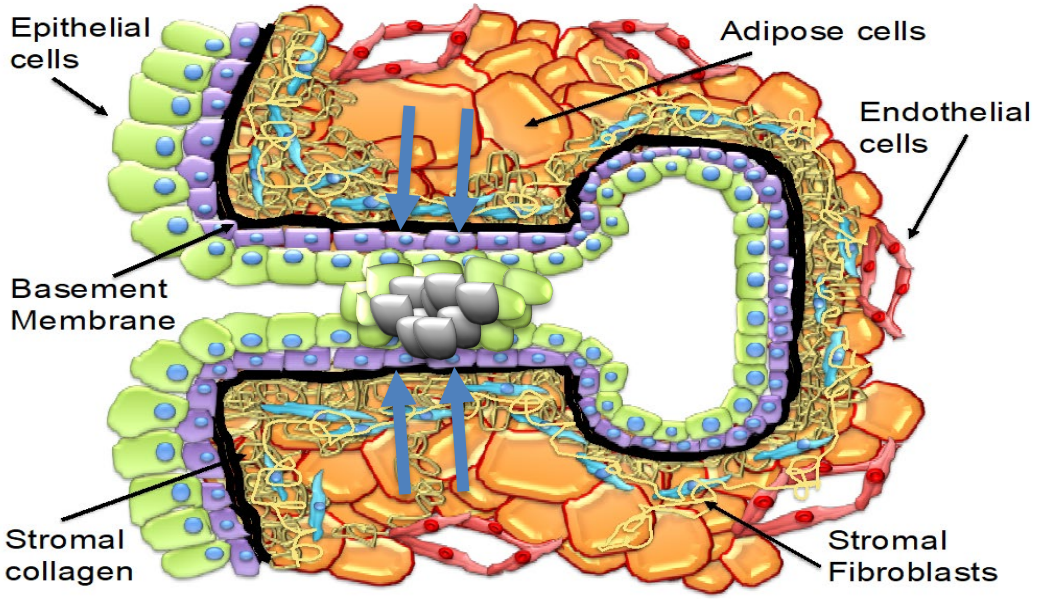
ER+ and HER2+ BrCa: high frequency mammographic calcifications

ER+ breast cancer

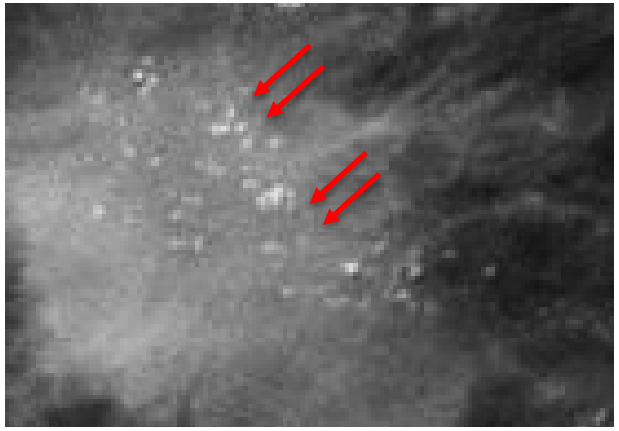
High frequency calcifications - 79%

HER2+ breast cancers

High frequency calcifications - 71%



Calcifications



[Ko ES, et al. Eur Radiol. 201]

TNBC BrCa low frequency calcifications

Triple-negative breast cancers

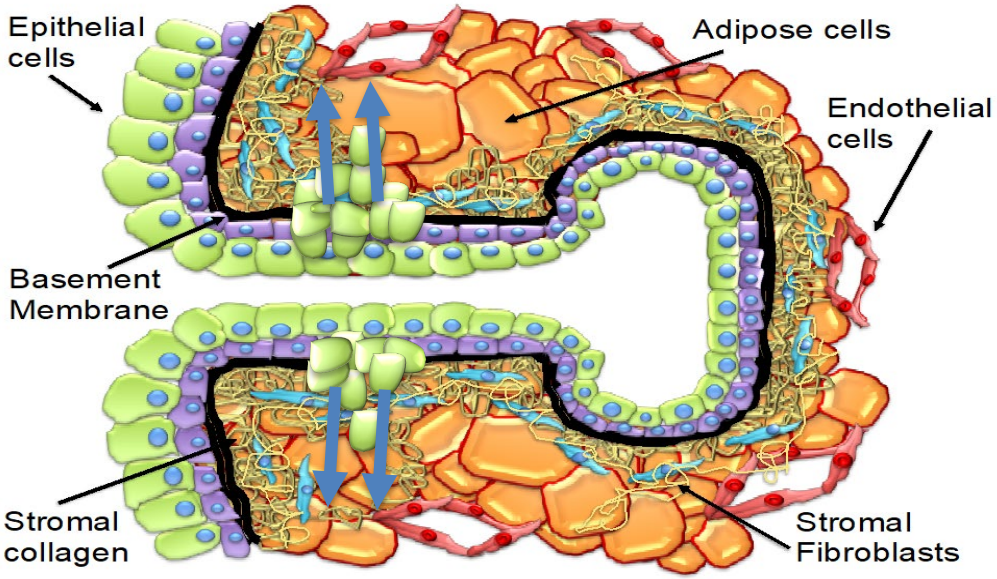
Low frequency calcifications - 15%

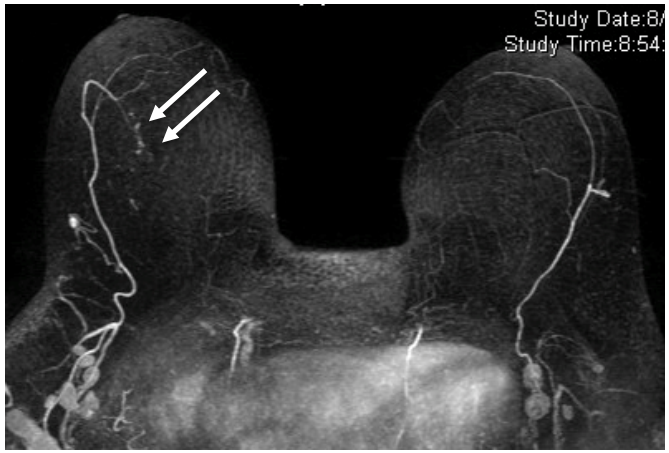
No findings - 30%

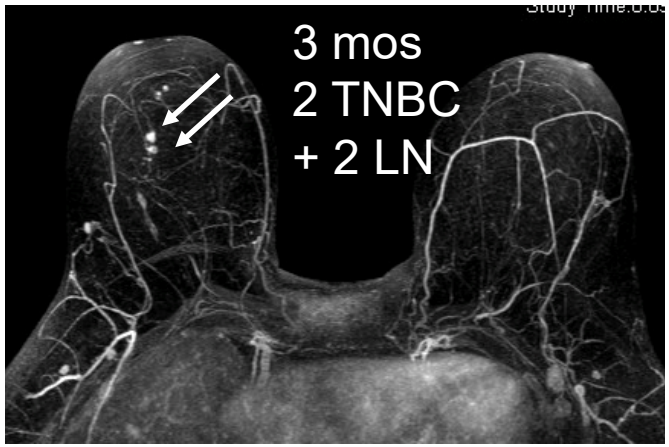
Mass/Focal asymmetry - 32%

Architectural distortion – 23%

TNBC?

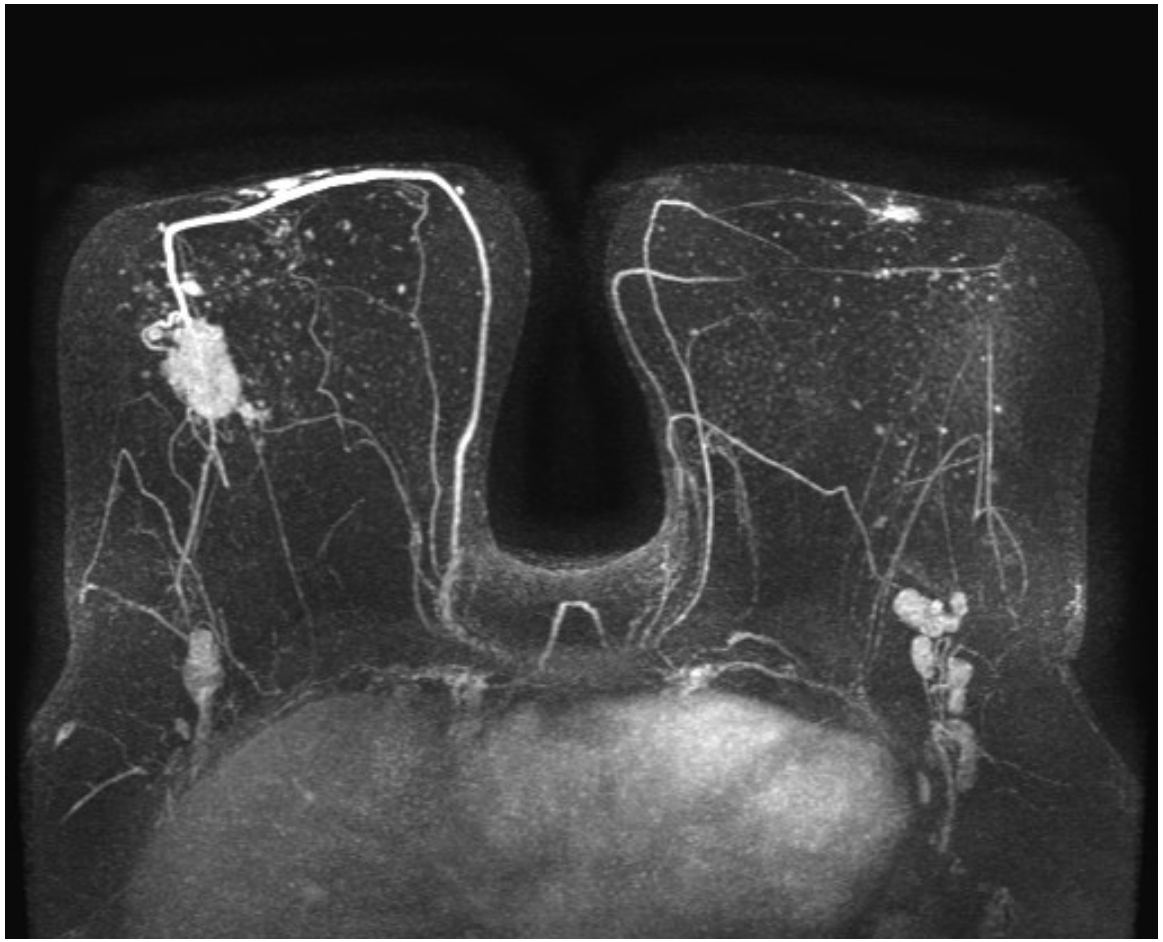






[Briedienė et al, ACTA MEDICA LITUANICA. 2011 REVIEW]

No BRCA mutation, normal mammogram 4 months prior – found by BSE.

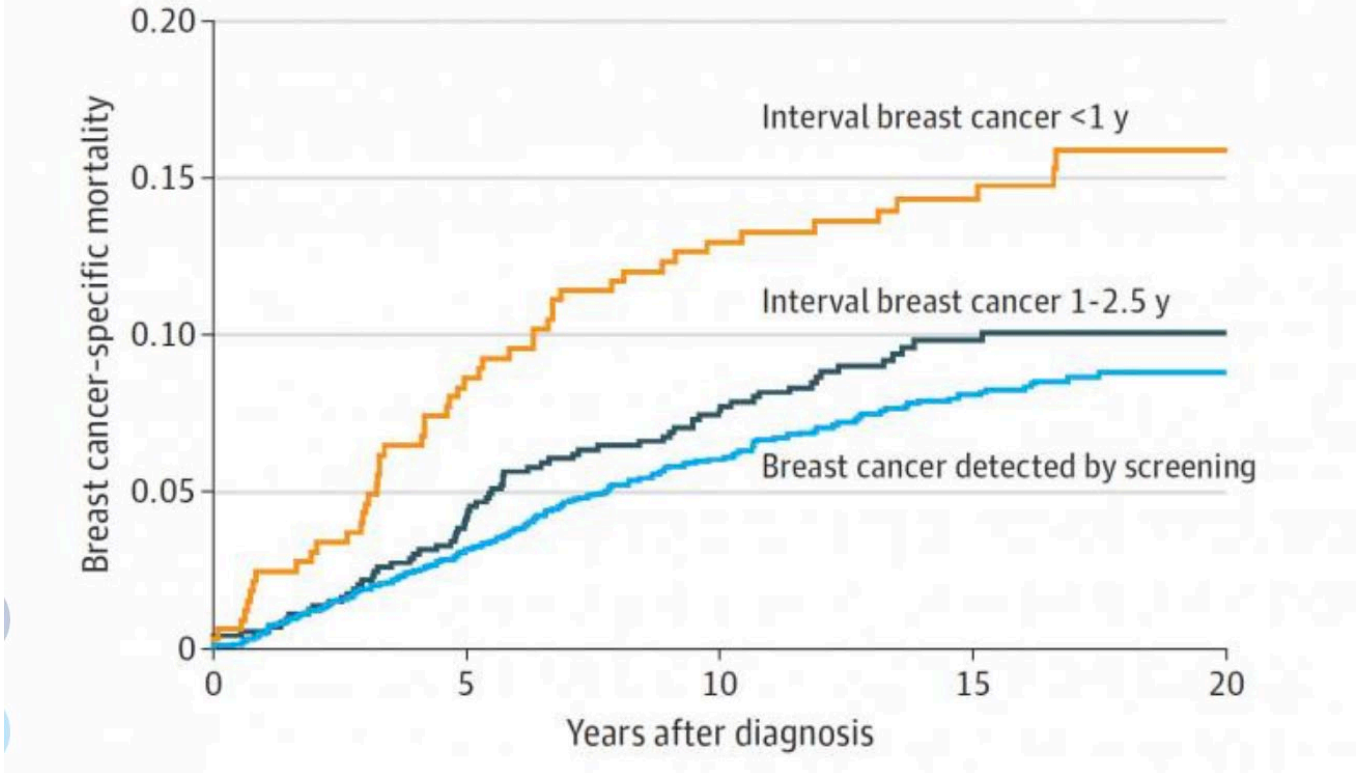


JAMA Network Open

Original Investigation | Oncology

Comparison of Mortality Among Participants of Women's Health Initiative Trials With Screening-Detected Breast Cancers vs Interval Breast Cancers

Veronica L. Irvin, PhD, MPH; Zhenzhen Zhang, PhD, MPH; Michael S. Simon, MD; Rowan T. Chlebowski, MD, PhD; Shiuh-Wen Luoh, MD, PhD; Aladdin H. Shadyab, PhD; Jessica L. Krok-Schoen, PhD; Fred K. Tabung, PhD; Lihong Qi, PhD; Marcia L. Stefanick, PhD; Pepper Schedin, PhD; Sonali Jindal, MD

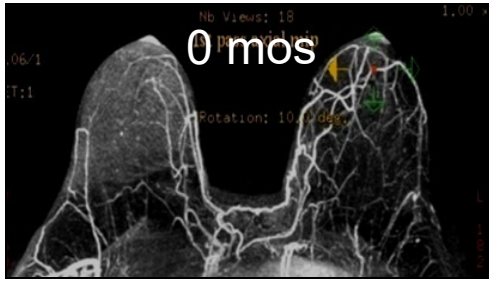


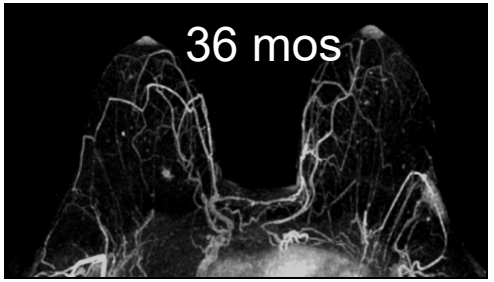
Breast MRI in High-Risk Women

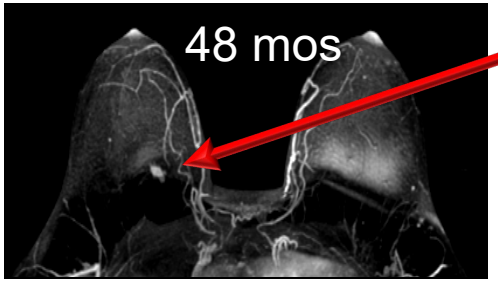
Stephanie Robertson

300 premenopausal high-risk women
- 152 African American
- 148 Caucasian

48 mos observation, 15 breast cancers
- 6 Focal / Age shifted
- 9 Non-focal / Accelerated

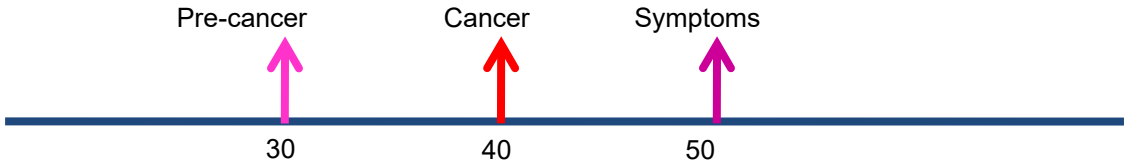


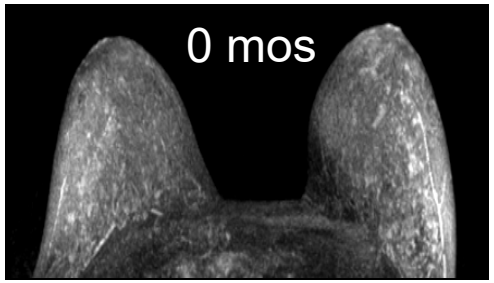


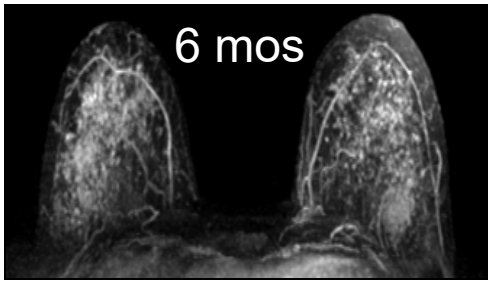


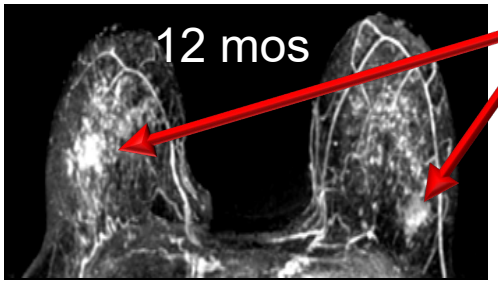
ER/PR+
T1N0

Focal
Age shifted







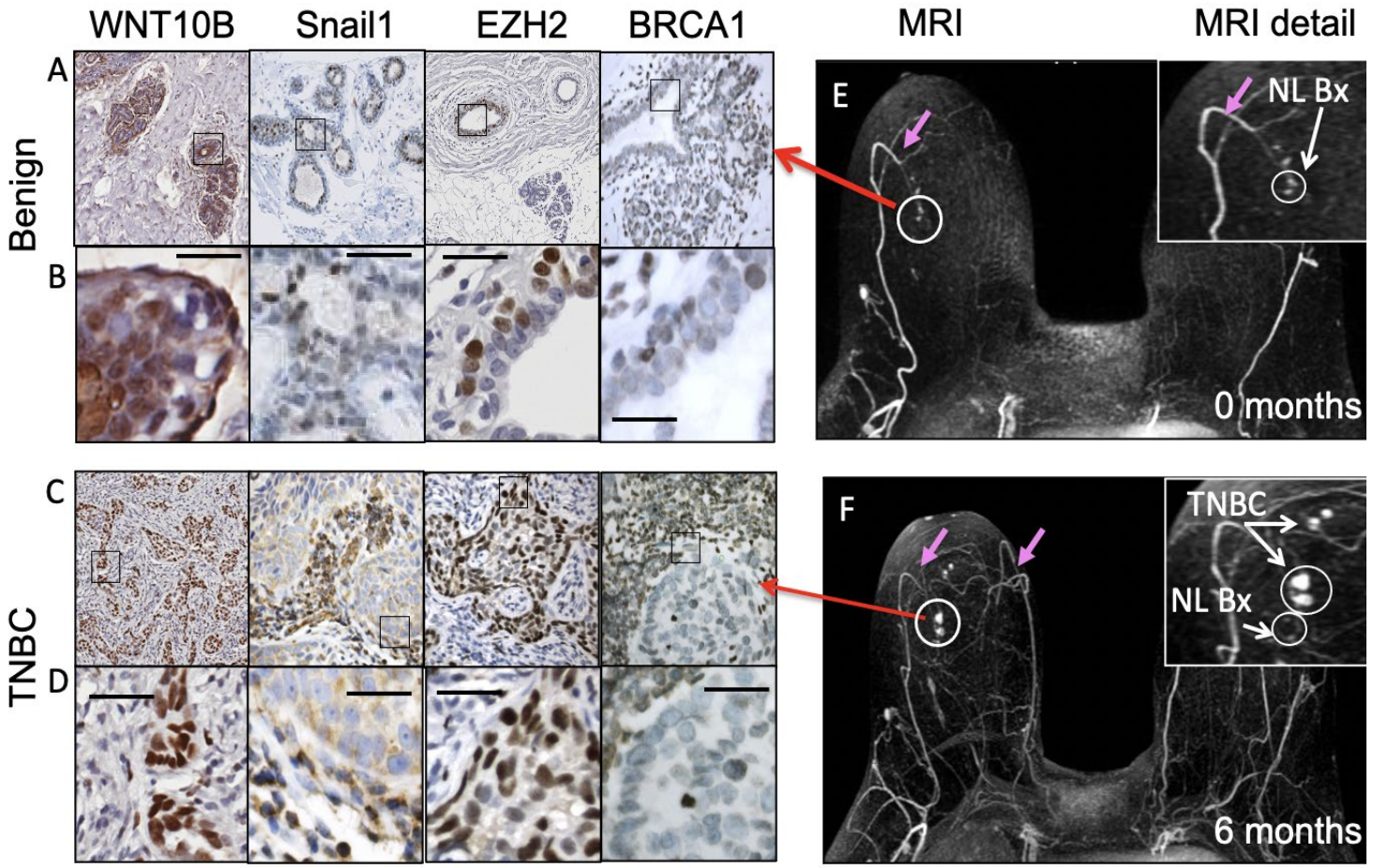


TNBC
T1N0

Non-stochastic?
Accelerated

Insulin-Driven Pre-Cancerous Biopsy

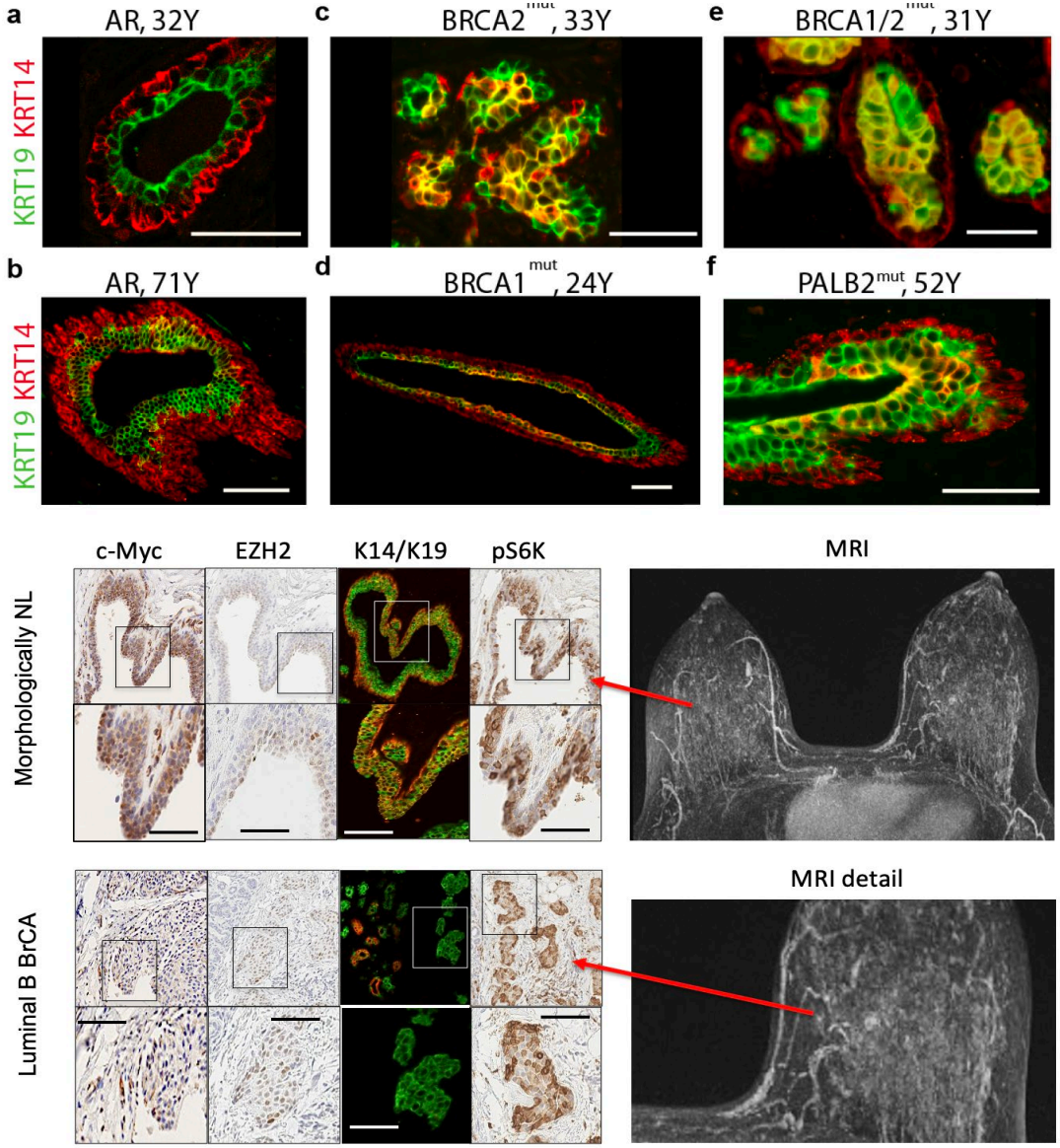
U01CA189283 - prospective (Duke, OSU, UT, USC, UW)

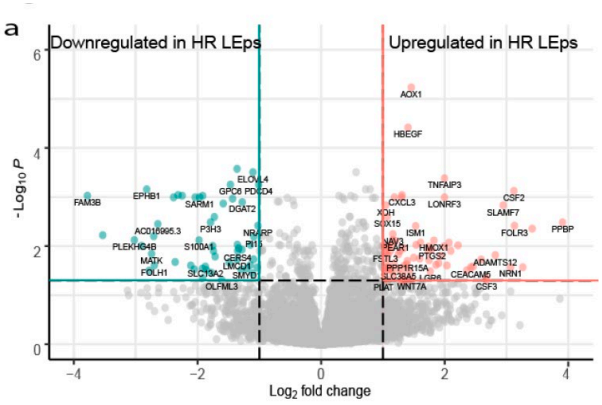


El Ayachi E et al. Cancer Res. 2019

Evidence for Loss of Lineage Fidelity – cKit+ K14/K19

U01CA189283 - prospective (Duke, OSU, UT, USC)





Expanded cKit+ K14/19 aging signature

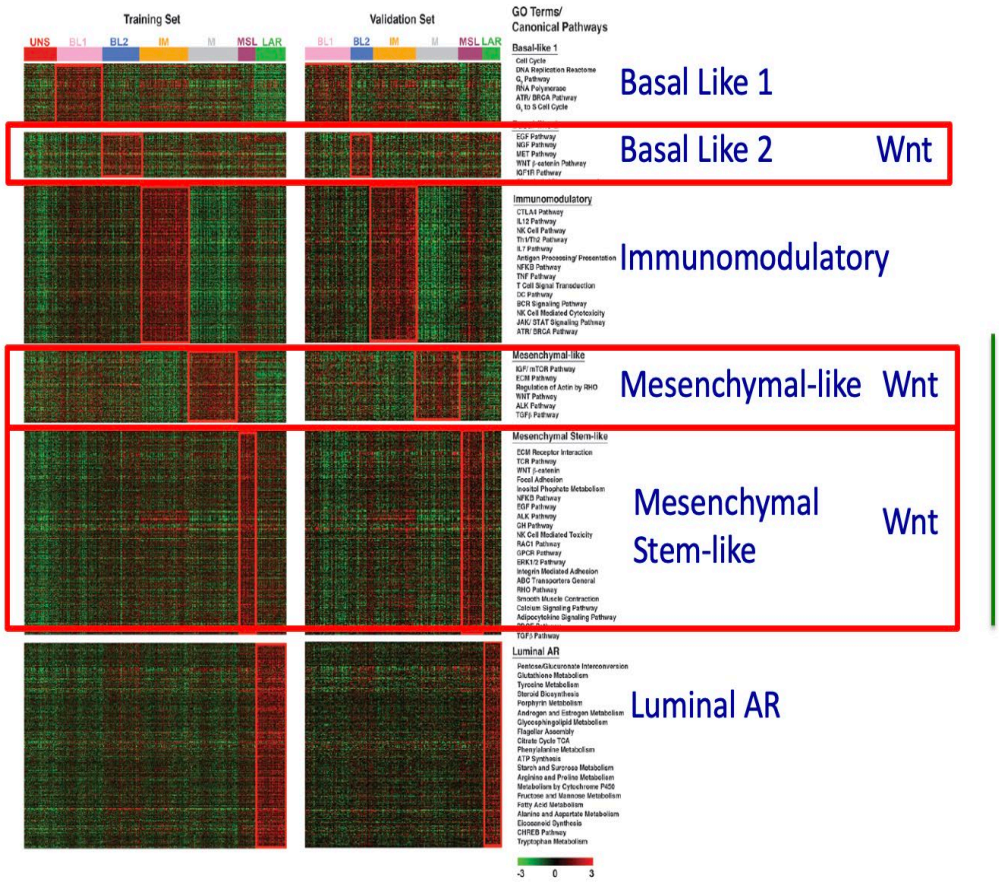
Wnt/β–catenin activation

EZH2/pAkt/cMYC

GeoMx in process

Shalabi S. et al. *Nature Aging* 2021

Wnt/beta-catenin, TNBC, immunosuppressive microenvironment



Lehmann et al, J Clin Invest. 2011.

Wnt/beta-catenin major driver of aggressive TNBC biology

Wnt10B regulates cell fate decisions – differentiation adipocytes

Wnt/beta-catenin
- MDSC homing
- PMN-MDSC activation
- CD8+ T-helper cell exhaustion

Spranger S Nature, 2015
Luke JJ et al. Clin Cancer Res., 2019
Li, X et al, Frontiers. 2019

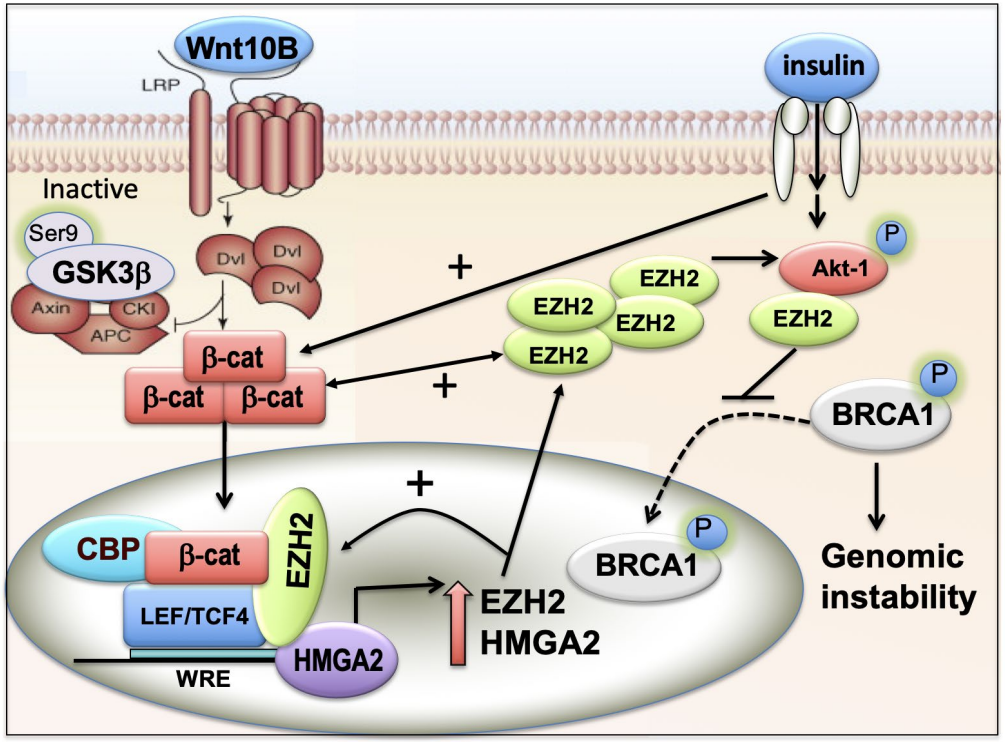
Gonzales et al. Cancer Res. 2011
Wend et al. EMBO, 2012
Ayachi et al. Cancer Res. 2019
Fatima et al. Cancers, 2020

Loss BRCA1 protein expression in TNBC

- Majority TNBC do not express nuclear BRCA1 protein
- Somatic mutation not early event
- Methylation inactivation is late not early

Celina Kleer – High EZH2/Akt1 block nuclear transport BRCA1

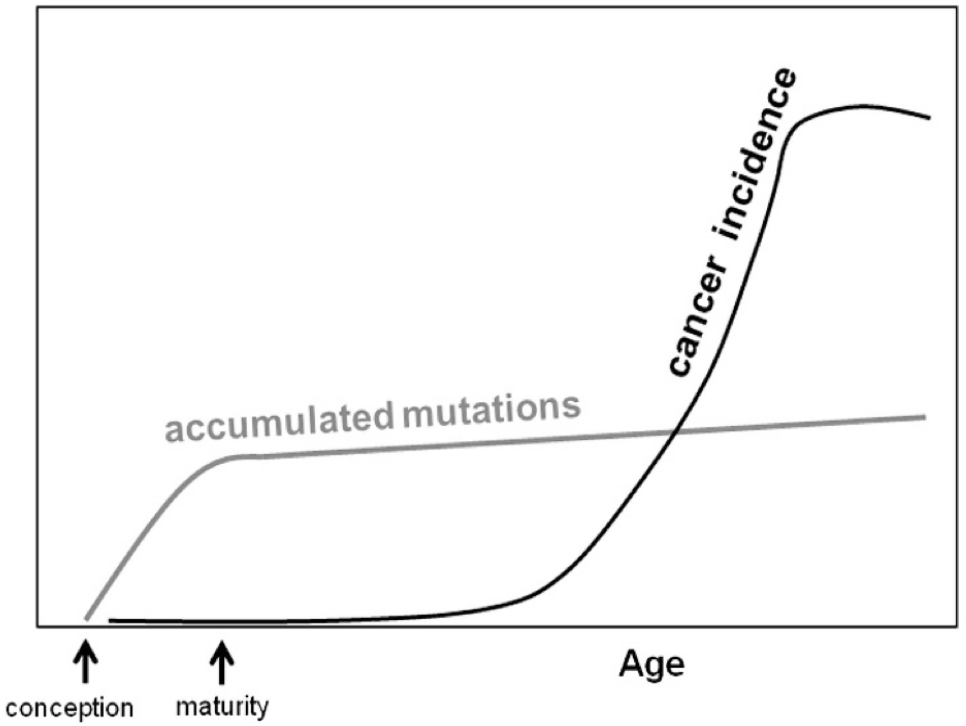
Wnt/βcatenin function in
+ feedback loop EZH2



Gonzales et al. Cancer Res. 2011
Wend et al. EMBO, 2012
Ayachi et al. Cancer Res. 2019
Fatima et al. Cancers, 2020

Breast Cancer Risk factors

- Aging – needs a full lecture
- Estrogen, E/P, BCP, Abortion, Alcohol
- Obesity
- Pregnancy



Rozhok AI and DeGregori J. Challenging the axiom: does the occurrence of oncogeneic mutation truly limit cancer development with age? Oncogene 2013

Obesity does not consistently increase risk of premenopausal TNBC in Black/AA women

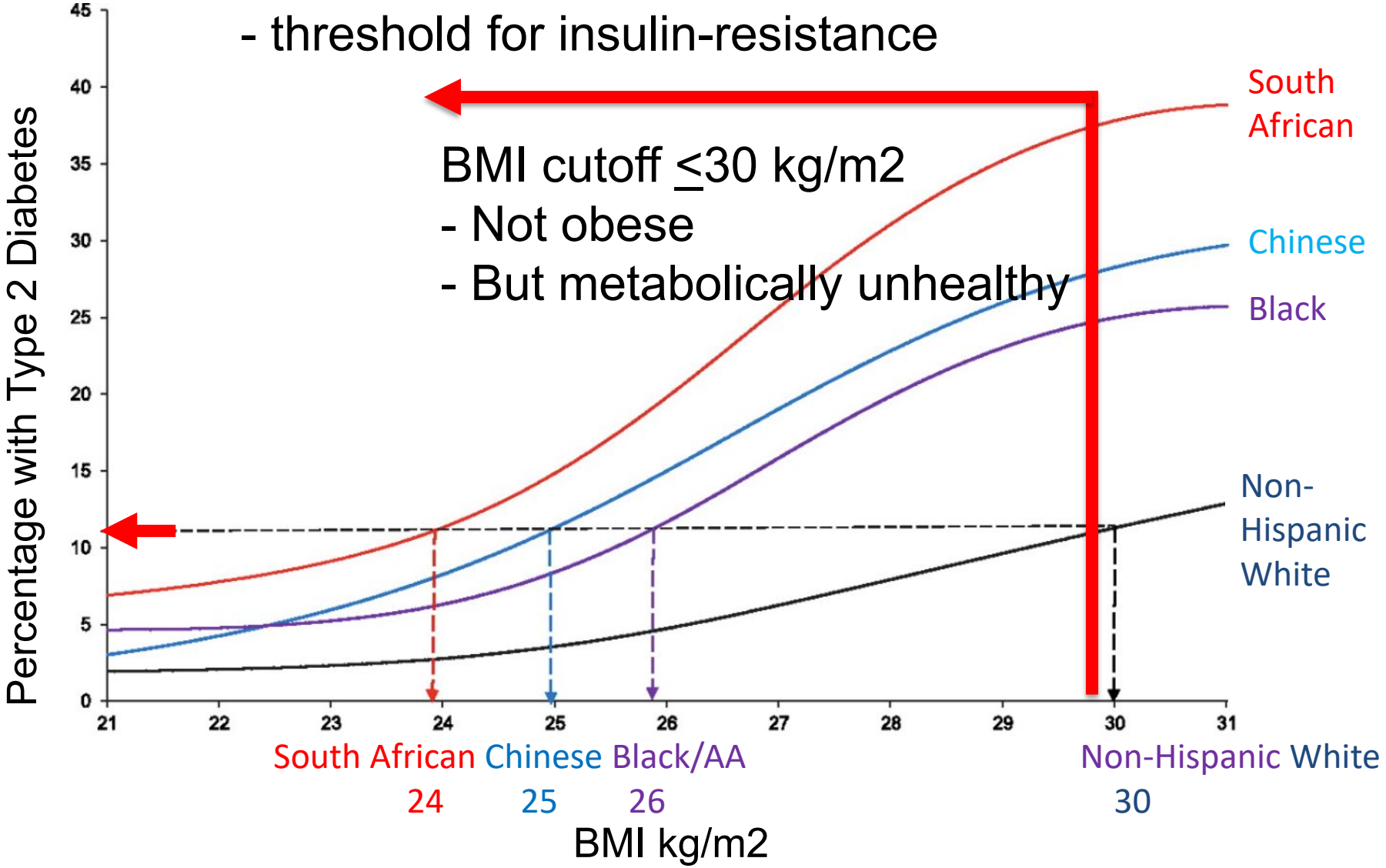
- Carolina Breast – Basal type TNBC increased in obese premenopausal Black/African American women (WHR).
- Women's Contraceptive and Reproductive Experience (CARE) (BMI ≥30) – No association
- Black Women's study (BMI) – ***Adams-Campbell*** - inverse association BMI and premenopausal cancer
- AMBER Association – consortium Carolina Breast, Multi-ethnic cohort, Black Women's study ***Adams-Campbell***, Women's Circle of Health - NO association (BMI, WHR)

Biphasic variable e.g. BMI ≥30 YES or NO

Bandera EV, Chandran U, Hong CC, Troester MA, Bethea TN, Adams-Campbell LL, et al. Abrosone C. Obesity, body fat distribution, and risk of breast cancer subtypes in African American women participating in the AMBER Consortium. *Breast cancer research and treatment* (2015)

BMI does not always equal metabolic potential

- importance of race/ethnicity
- threshold for insulin-resistance



Chiu M, et al. Diabetes Care 2011, 34:1741-8.

Significant individual variability in insulin resistance

Carolyn (sister)
58 years old
6’3” tall
239 lbs
BMI = 30
Diet – high carbs
Exercise – minimal, 30 pack year smoker
Steps per day <500

HgbA1c = 5.2
Cholesterol (total) = 168

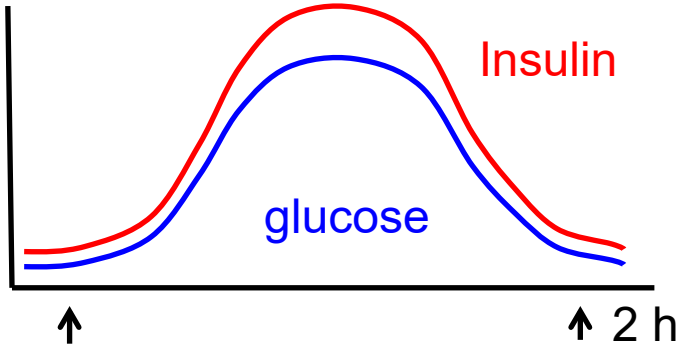
50 lb wt loss on Metformin

Vicky (AKA me)
62 years old
5’8” tall
125 lbs
BMI = 19
Diet – low carb vegetarian + salmon
Exercise – 90 min per day (swim, run)
Steps per day >5,000

HgbA1c = 6.0 on 2500 mg Metformin
Cholesterol (total) = 190 on Lipitor 40 mg

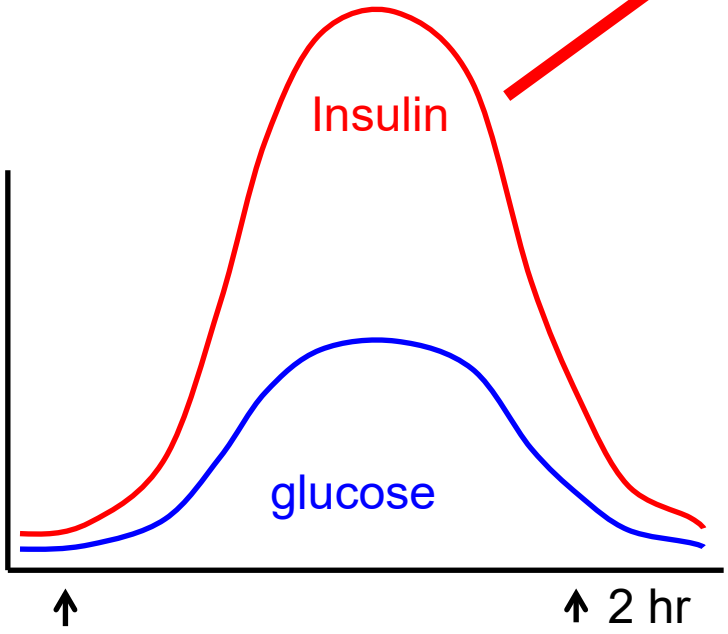
Insulin resistance and Type II Diabetes

- Increased Black/African American women
- Younger age of onset

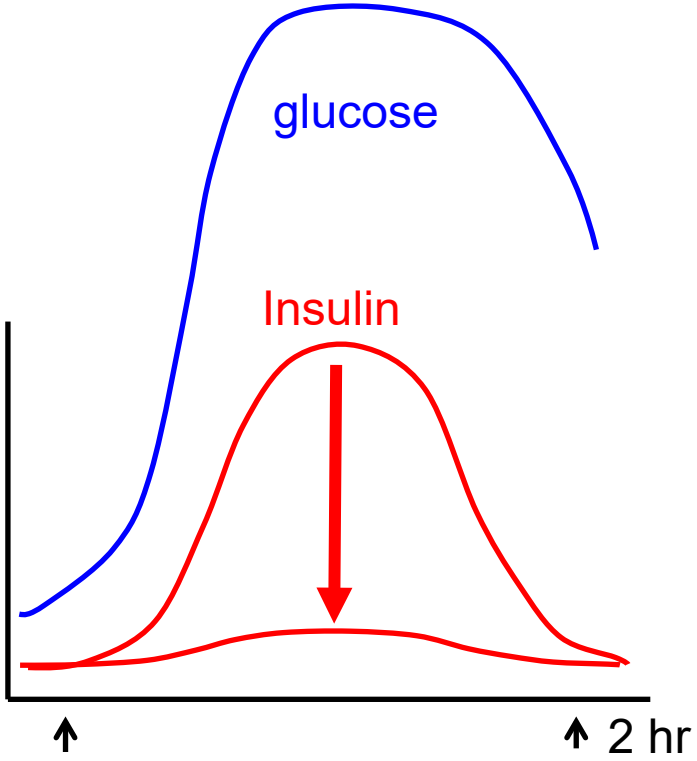


Healthy – HgbA1C = 5.3

- stimulates hunger
- promotes carbohydrate to fat
- prevents fat access (breast feeding)



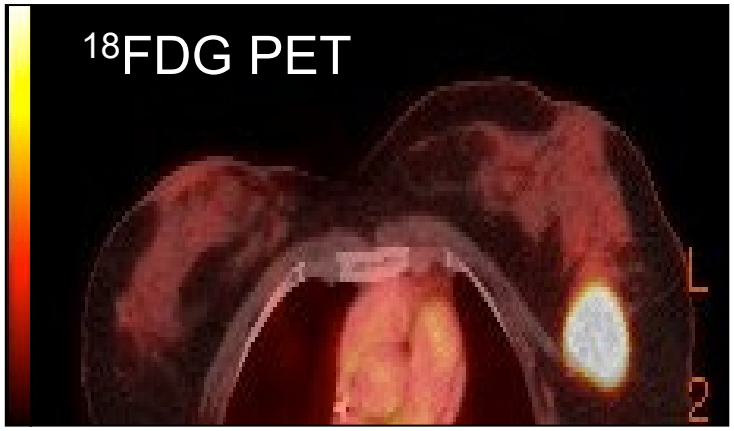
Resistant – HgbA1C = 5.7-6.3



Diabetes – HgbA1C > 6.3

Woman: Insulin Resistant

Insulin resistance, produces high insulin

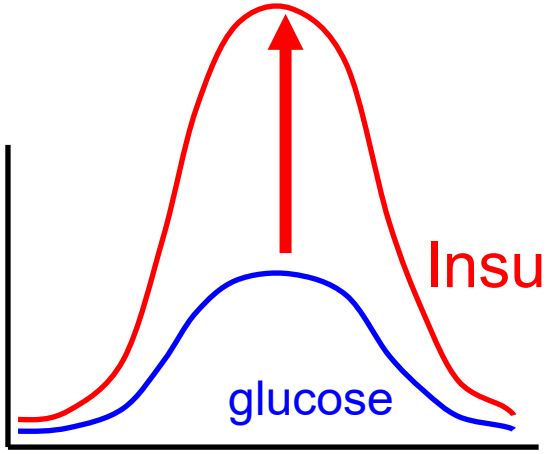


+

+

At risk breast tissue

Increased insulin sensitivity



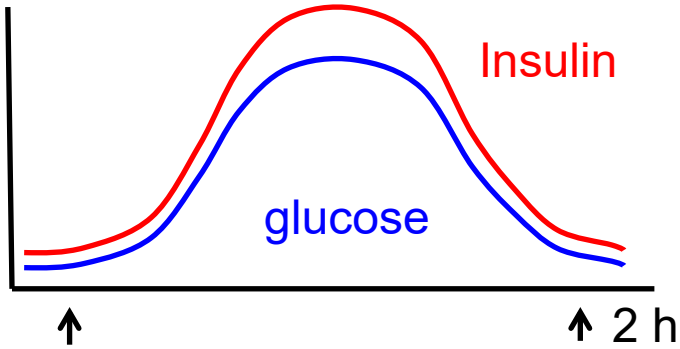
Resistant – HgbA1C = 5.7-6.2

- AKT/mTOR/glycolysis
- Wnt/beta-catenin
- EZH2/Notch/cMyc
- Stat3

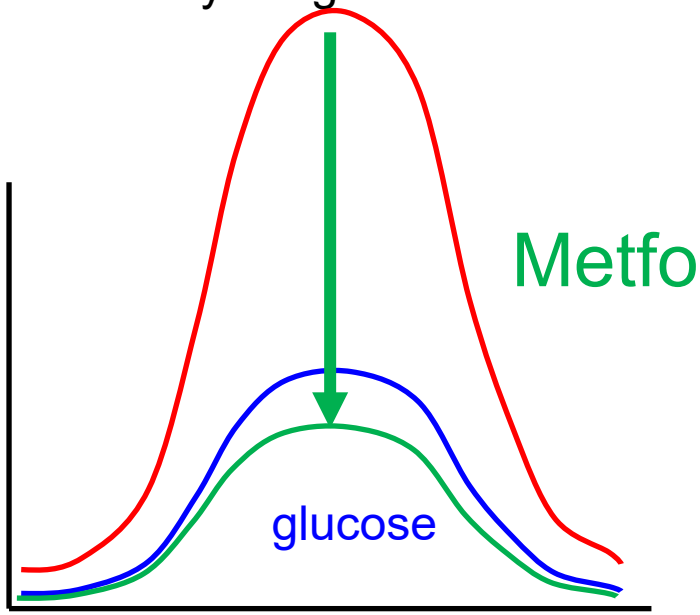
Metformin mobilizes fat, decreases hunger

Metformin - decreases serum insulin

- decreases hunger
- increases fat breakdown
- ***prevents >60% T2D***

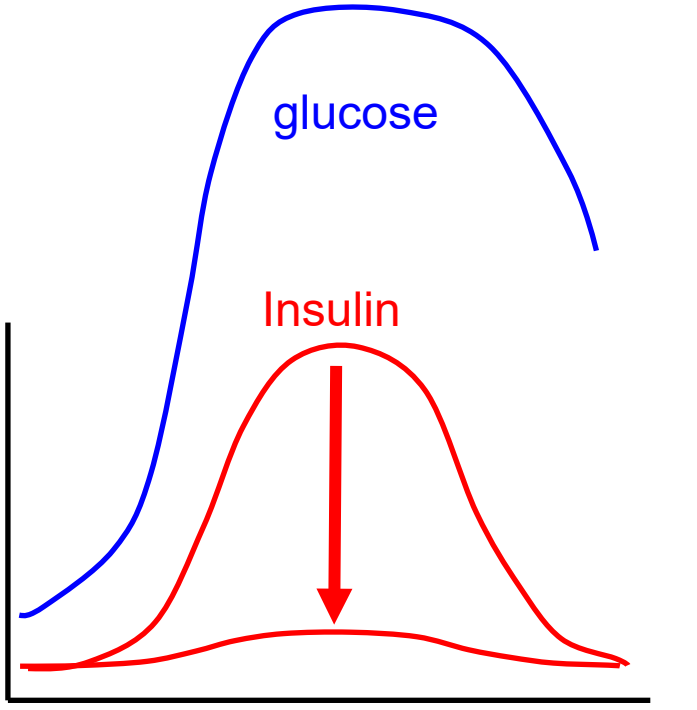


Healthy – HgbA1C = 5.3



Resistant – HgbA1C = 5.7-6.2

Metformin



Diabetes – HgbA1C > 6.3

Pregnancy and Breast Cancer

Pregnancy before age 35 in non-Latina Whites

- Ultimately, risk reduction
- Over age 35 increased risk
- Risk does not normalize for African American women

For all women – pregnancy, involution, postpartum

- All women increased initial risk – 1-3-10 years
- Risk highest for TNBC
- Women who self identify as African American, Black, etc. highest risk

Pepper Schedin – Schedin Nat Rev. Cancer, 2006; Vohra SN et al. CEBP, 2021

Bernhardt S et al. Nature Comm, 2021

Gooch JC et al. Pregnancy-associated breast cancer in a contemporary cohort of newly diagnosed women Breast Journal 2020

Pregnancy is a state of immune suppression

ARTICLE

Continuous activation of polymorphonuclear myeloid-derived suppressor cells during pregnancy is critical for fetal development

Mengyu Shi[1], Ziyang Chen[1], Meiqi Chen[1], Jingping Liu[2], Jing Li[3], Zhe Xing[1], Xiaogang Zhang[1], Shuaijun Lv[1], Xinyao Li[1], Shaowen Zuo[1], Shi Feng[1], Ying Lin[1], Gang Xiao[2], Liping Wang[4] and Yumei He[1,2,5,6]

MDSCs important for feto-maternal tolerance,
Continuous activation PMN-MDSCs during pregnancy - fetal growth

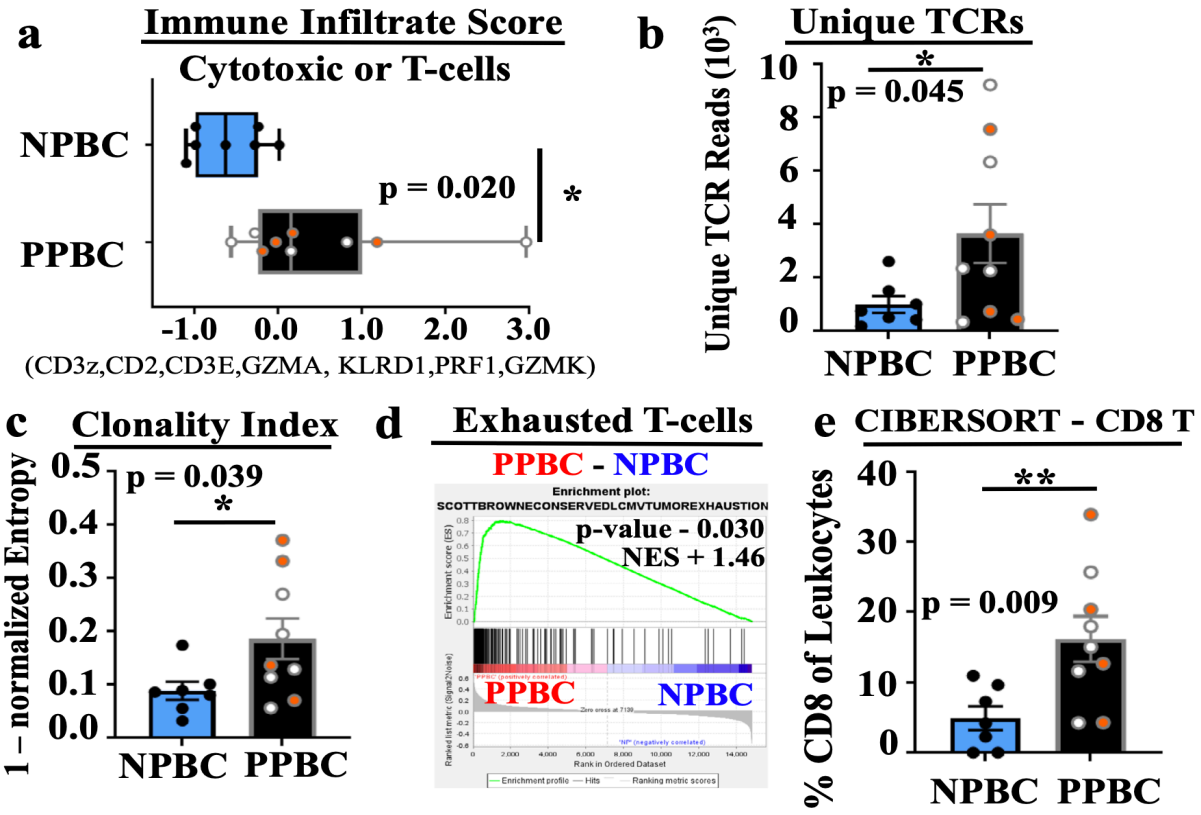
Mouse and human co-studies normal pregnancy
- Activation of class E scavenger receptor 1 (SR-E1)+ PMN-MDSCs was observed in all stages of human pregnancy
- ROS and arginase-1 activity, mediated by p-STAT6.

IUGR – poor outcome - mice and humans
- Lower SR-E1 PMN-MDSCs
- Lower arginase-1 activity, ROS, p-STAT6

Shi M et al. Cellular & Molecular Immunology (2021)

Pregnancy Associated BrCA n=16 – P.Schedin

PPBC increased activated T-cells that are PD-1+ and TOX1+
Enhanced signatures of exhaustion – GSEA/Tfh profile from CIBERSORT analyses
Mouse models PABR - T-cell suppression and tumor cell immune avoidance



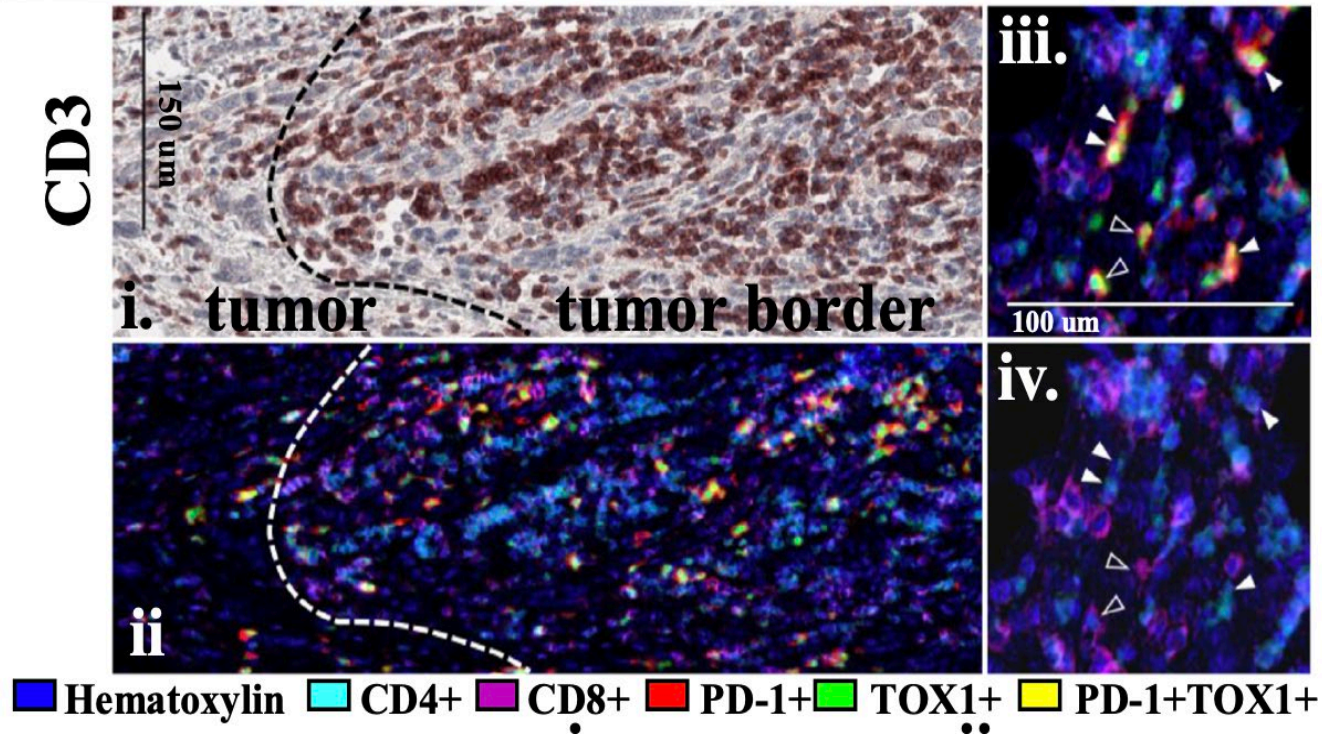
Bernhardt S et al. Nature Comm, 2021; Pennock ND et al. Immunother. Cancer, 2018

Khan O. TOX transcriptionally and epigenetically programs CD8+ T-cell exhaustion. Nature 2019

Pregnancy Associated BrCA n=16

PPBC increased activated T-cells that are PD-1+ and TOX1+
Increased signatures of exhaustion – GSEA/Tfh profile from CIBERSORT
Mouse models PABR - T-cell suppression and tumor cell immune avoidance

Leading edge of tumor +PD-1/+TOX1+

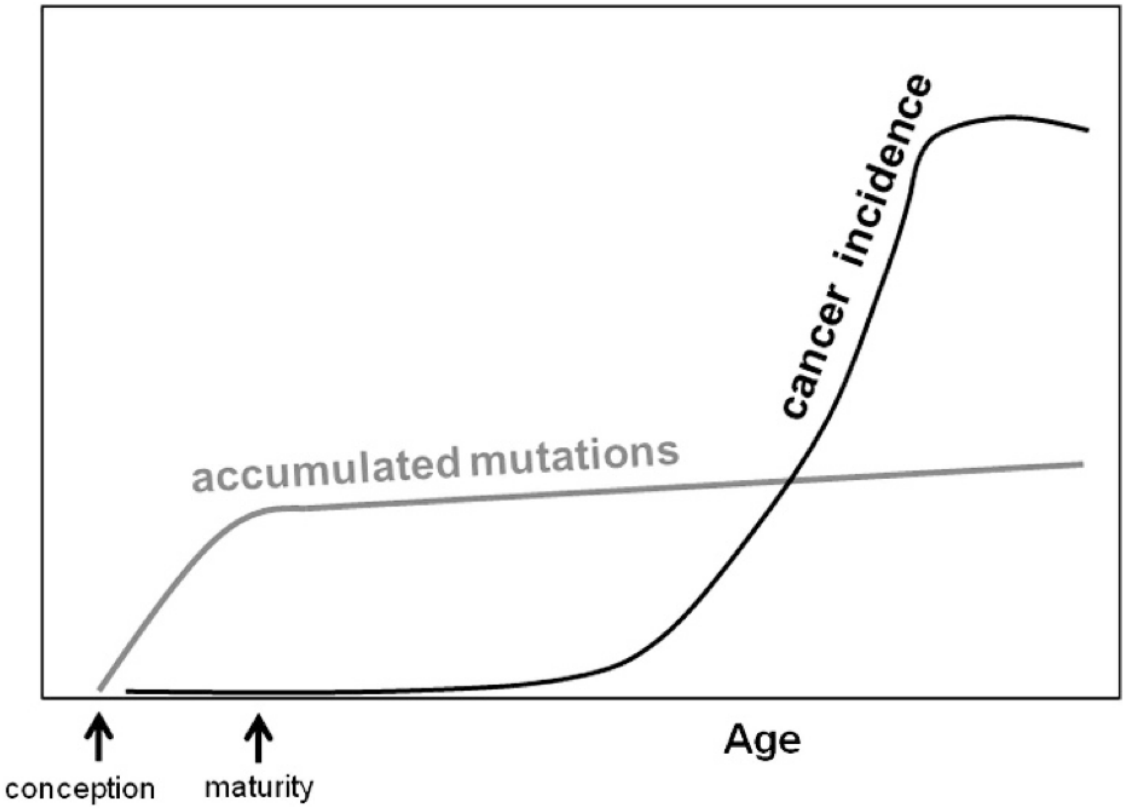


Bernhardt S et al. Nature Comm, 2021; Pennock ND et al. Immunother. Cancer, 2018

Khan O. TOX transcriptionally and epigenetically programs CD8+ T-cell exhaustion. Nature 2019

Thoughts for discussion

High throughput modeling

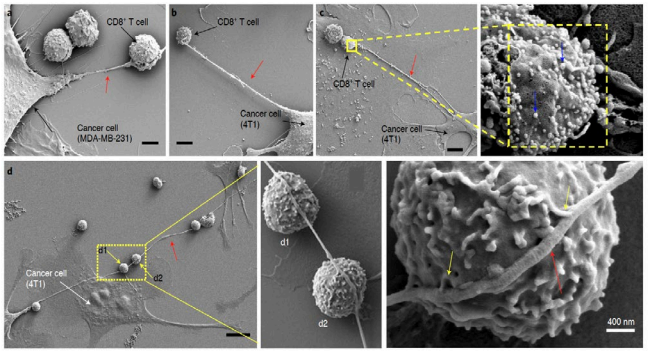


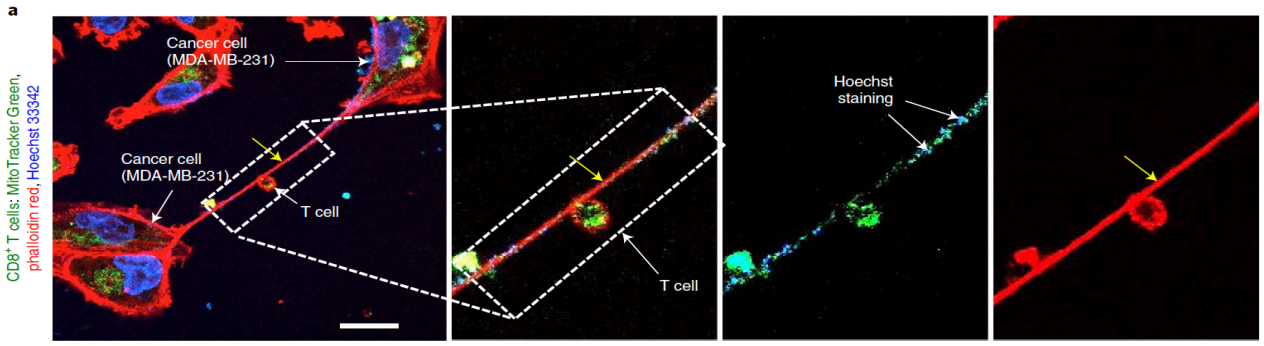
Rozhok AI and DeGregori J. Challenging the axiom: does the occurrence of oncogenic mutations truly limit cancer development with age? Oncogene 2013

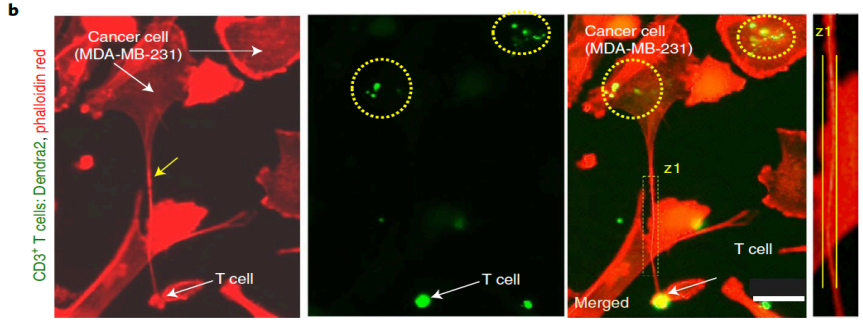
ARTICLES
https://doi.org/10.1038/s41565-021-01000-4
nature nanotechnology
Check for updates

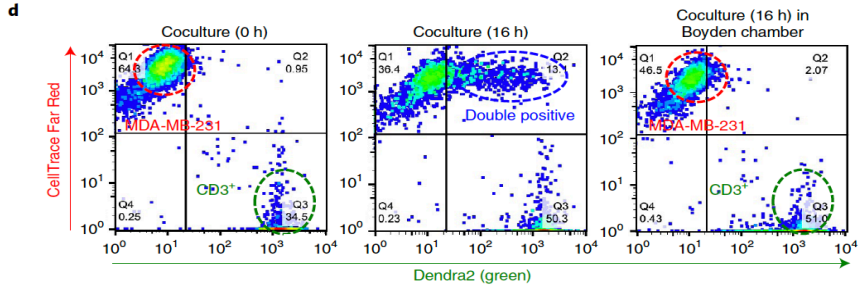
Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells

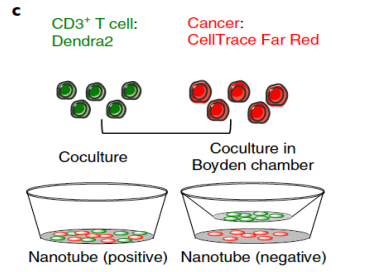
Tanmoy Saha[1,2], Chinmayee Dash[1,2], Ruparoshni Jayabalan[1,2], Sachin Khiste[1,2], Arpita Kulkarni[1], Kiran Kurmi[3], Jayanta Mondal[1,2], Pradip K. Majumder[4], Aditya Bardia[5], Hae Lin Jang[1] ✉ and Shiladitya Sengupta[1,2,6] ✉

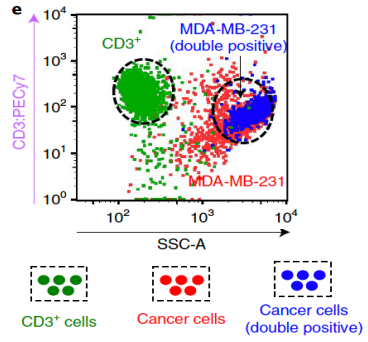










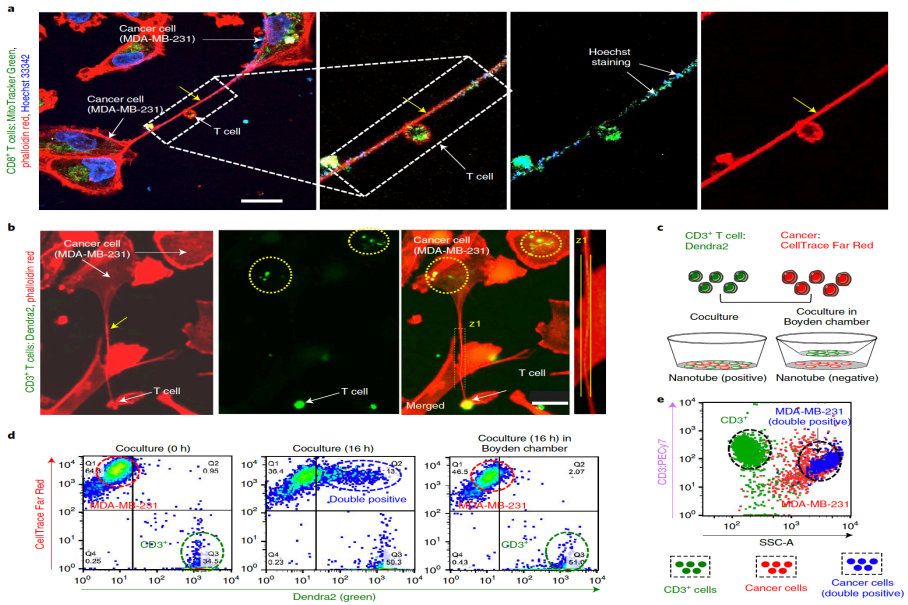


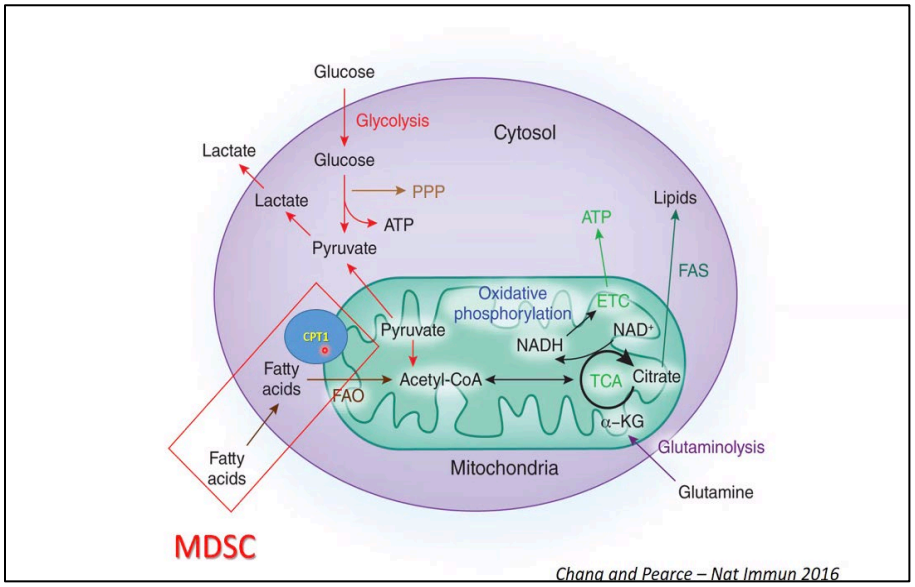
First Pass
- MDA231 Wnt10B+
- Label cells/mitoch.
- Co-culture Boyden
- Test for mitoch. transfer

Second Pass – Treatment with low-toxicity natural product or drug
- Roziglitizone
- MDSC inhibitor

- Wnt/beta-cat inhibitor

Modeling





First Pass

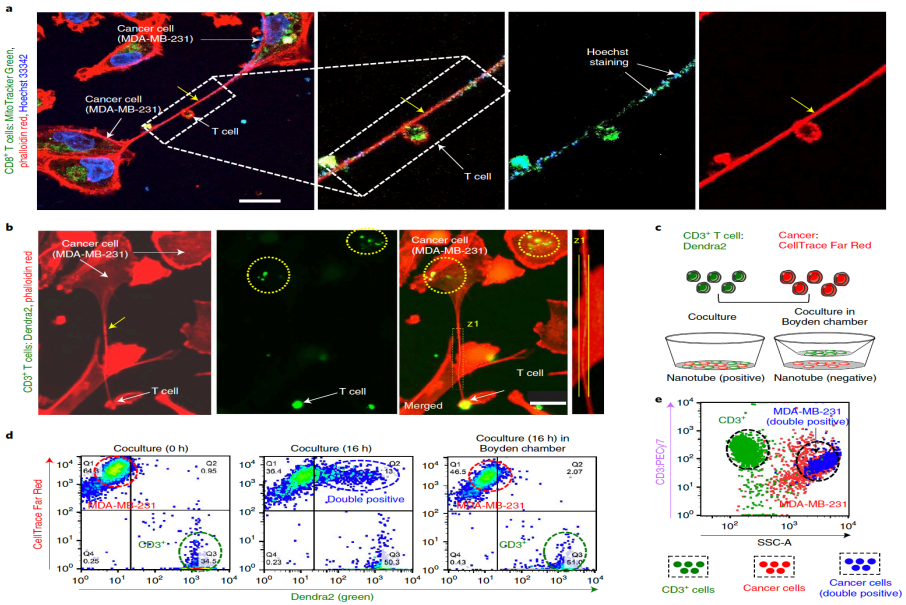
- MDA231 Wnt10B+
- Label cells/mitoch.
- Co-culture Boyden
- Test for nanotube transfer
- Seahorse OCAR/ECAR

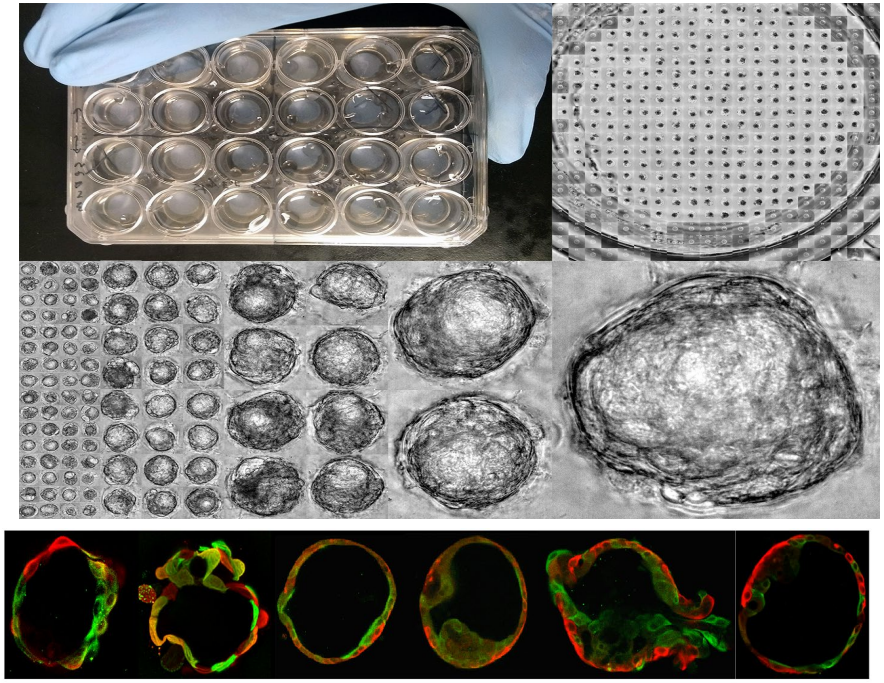
Second Pass – Treatment with low-toxicity natural product or drug

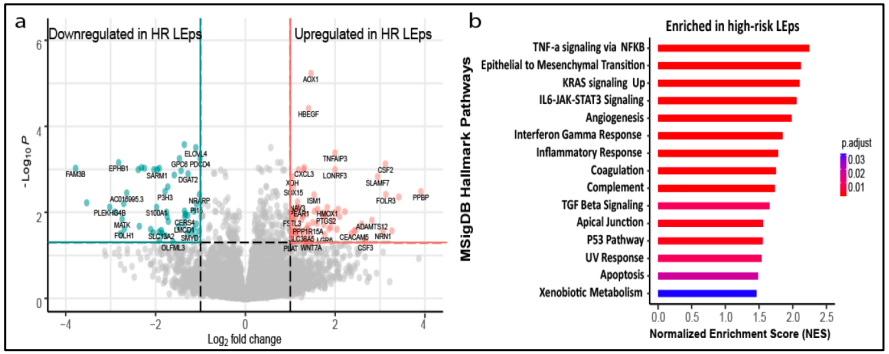
- Shut down FA transport – Roziglitizone
- Added FA, glutamine, glucose etc.
- MDSC inhibitor – GLPG1205
- Wnt/βcatenin inhibitor – Calotropin

  Calotropis gigantean (Asclepiadaceae)

Modeling





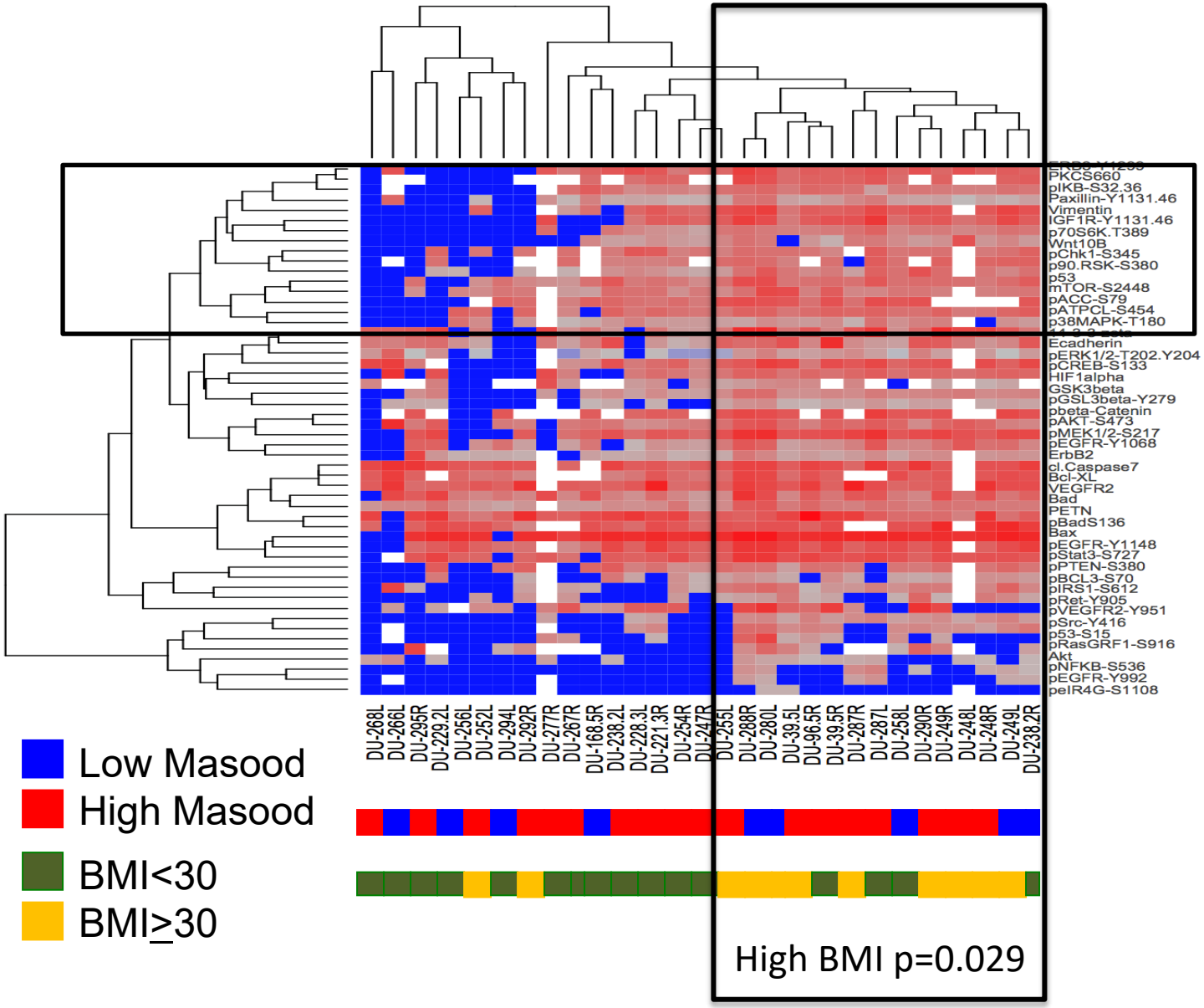


First Pass
- MDA231 Wnt10B+
- Label cells/mitoch.
- Co-culture Boyden
- Test for nanotube transfer
- Seahorse OCAR/ECAR

Second Pass – Treatment with low-toxicity natural product or drug
- Shut down FA transport - Roziglitizone
- MDSC inhibitor – PB1-405
- Wnt/βcatenin inhibitor – Calotropin

Third Pass – High throughput
HMEC/stromal cell culture
KI/KO target genes
With added MDSC or T-cells

Akt/mTor, beta-catenin, vimentin atypical breast aspirates- African American women contralateral TNBC



CLUSTER 1

ErbB3 Y1289

beta-catenin

Paxillin-Y118

Vimentin

IGF1R-1131

pStat3

p70S6K-T389

pChk1-S346

p90RSK-S380

Stat3

mTOR-Ser2448

pS6K-Thr389

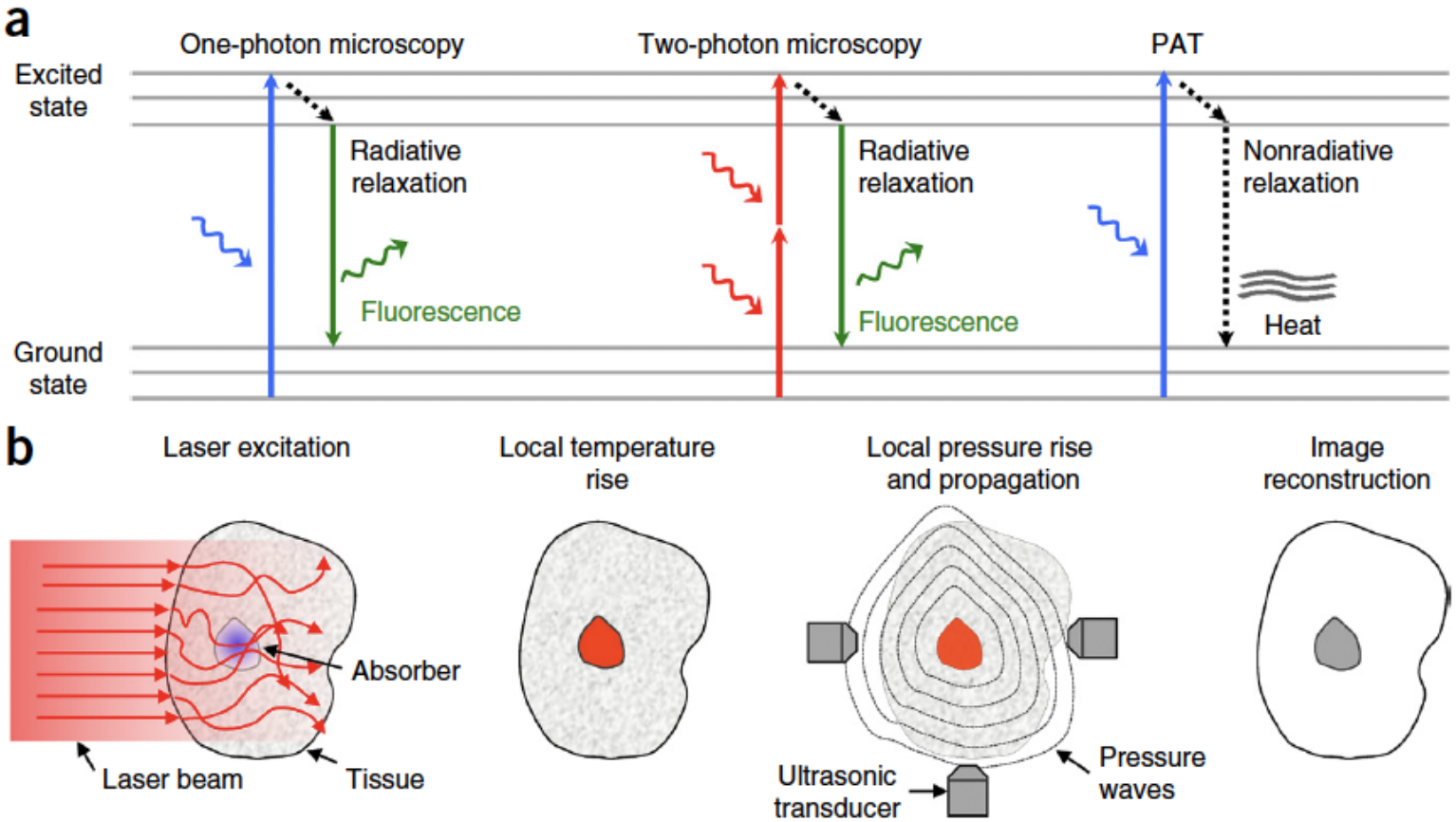
pATPCL-Ser454

pACC-Ser79

p38MAPK-Thr180/Tyr182

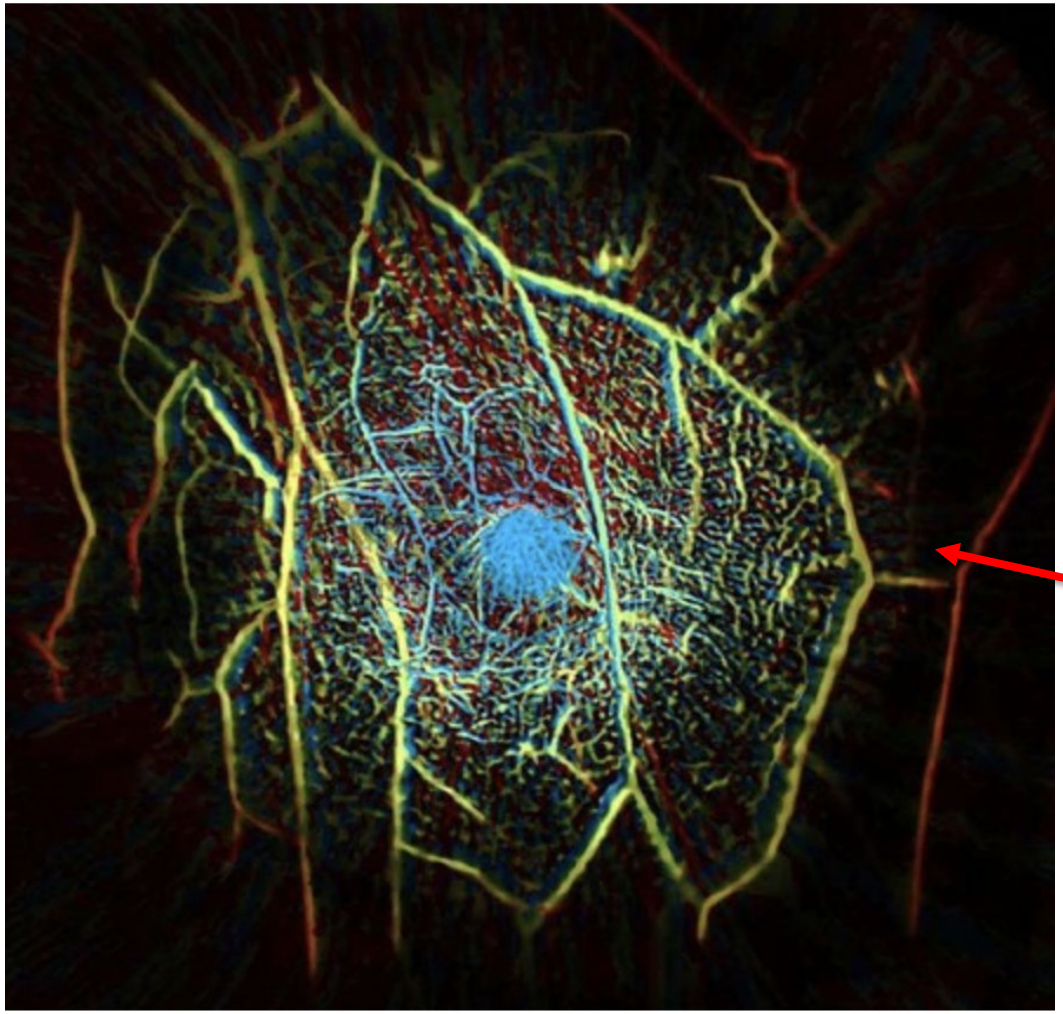
14-3-3-zeta

Experimental Imaging Vascular/Metabolic Imaging - Photoacoustic Tomography

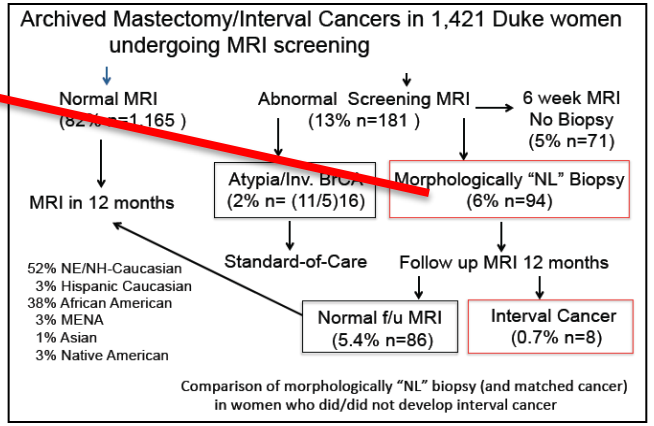


Short laser pulse Photons propagate	Photons absorbed by bio-molecules	Absorbed energy→ to heat	Heat-induced pressure wave propagates as ultrasound wave	US wave detected. Image reconstructed

Vascular/Metabolic Imaging - Photoacoustic Tomography (PACT) - Caltech - Lihong Wang, PhD



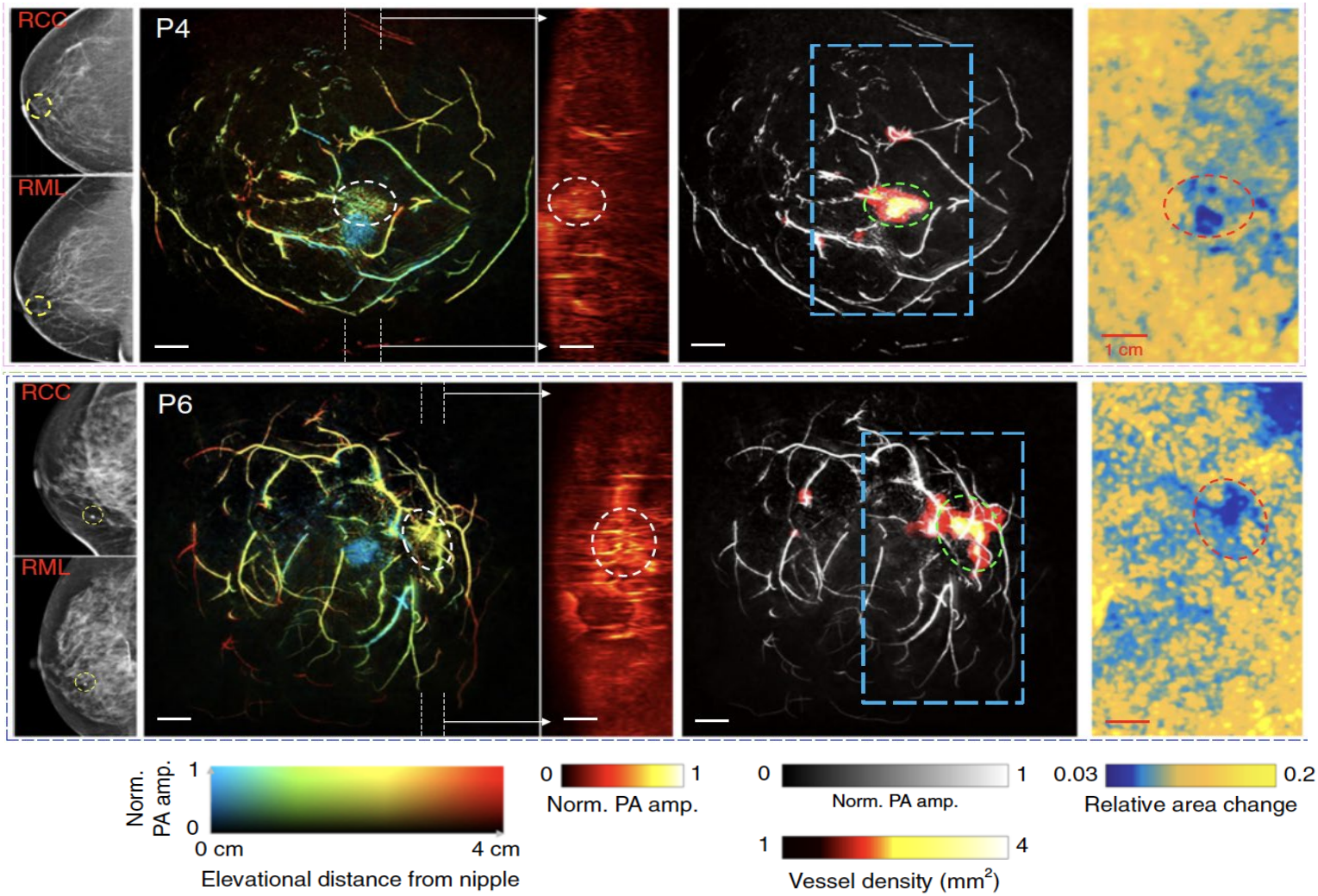
- 15 s image acquisition
- 250 u in-plane resolution
- Endogenous fluorescence
- Repeat imaging
- Biological read out
- No compression of breast



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

Lin et al. *Nature Communication*, 2018

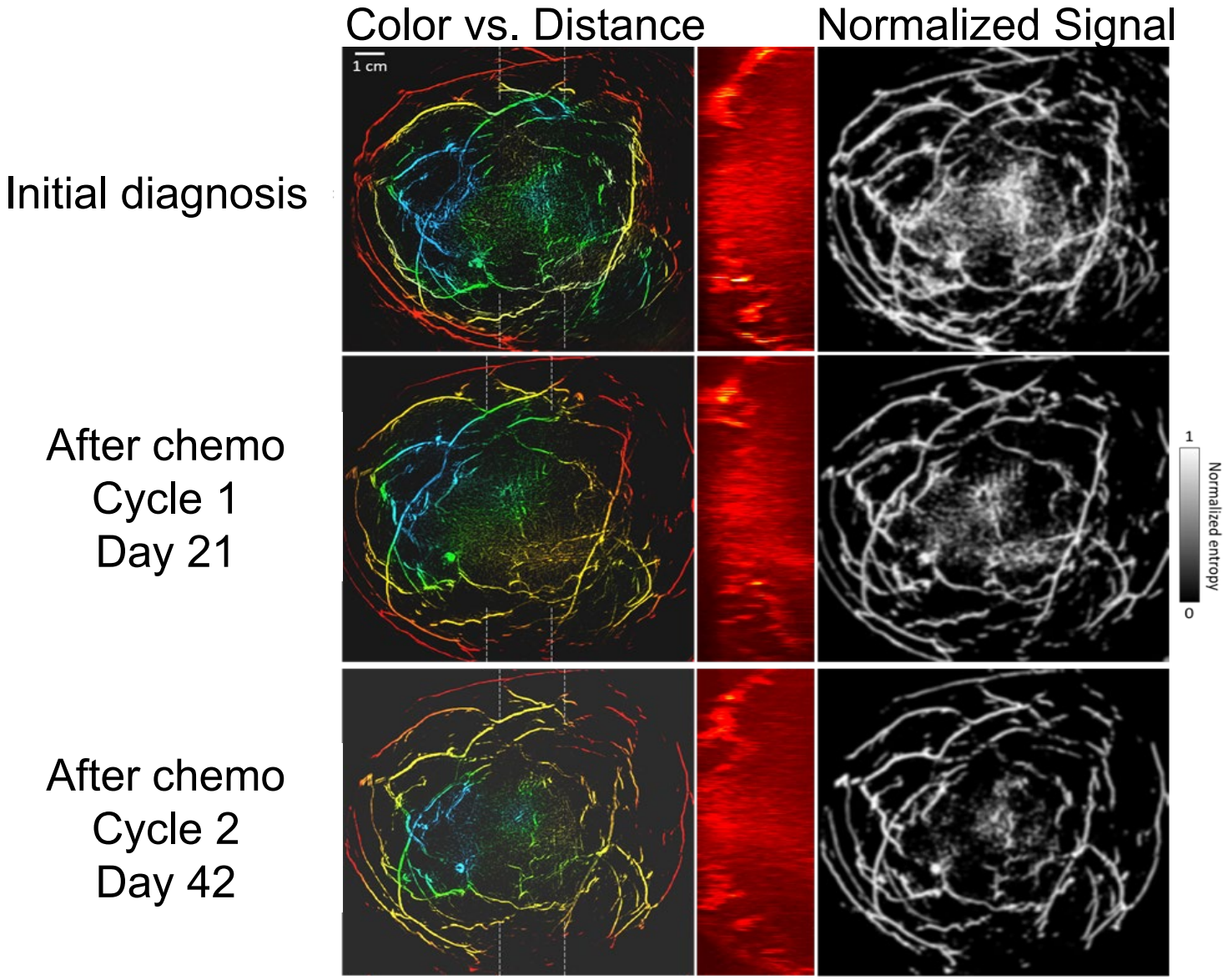
Photoacoustic Tomography (PACT) early detection of occult breast cancer and neovascularization



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

Lin et al. *Nature Communication*, 2018

Serial analysis neoadjuvant chemotherapy tx breast cancer



Lily Lai, MD
Lisa Yee MD

Lin et al. *Nature Communication*, 2018