

The Unexplored Human Breast

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Research with women by women for women (and a few good men)

What **CAUSES** breast cancer?

- The Biome Project
- Mapping the Breast Duct Study
- Mapping DCIS

At what **COST** breast cancer?

- Metastatic Breast Cancer Collateral Damage Project and Think Tank

Breast Cancer **INNOVATION**

- Self-Reading Portable Ultrasound

Breast Cancer **EDUCATION**

- The Health of Women (HOW) Study™
- ImPatient Science™ Video Series



380,000 enrolled

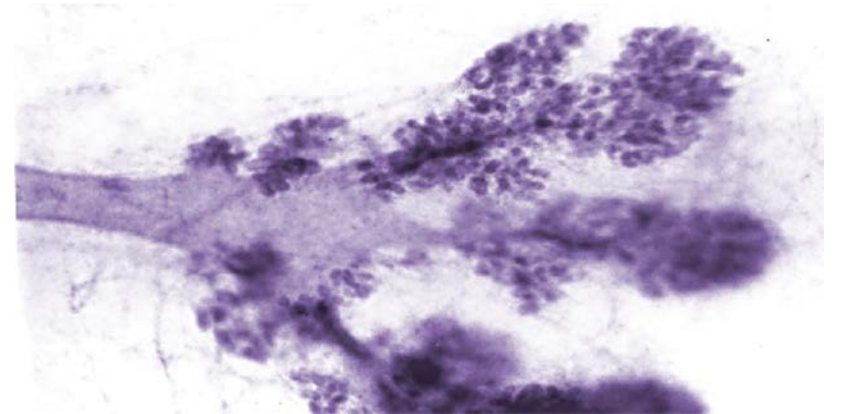
Dr. Susan Love Research Foundation Breast Cancer Research Philosophy

- a. Solving a clinical problem
- b. Multidisciplinary/multi-institutional
- c. Collaborative
- d. Whenever possible in humans rather than animal models

Most Research is on Animal Models

- Often rodents and animals with one duct per teat
- Breasts only develop for lactation and then regress
- Don't naturally get breast cancer (exception: domesticated dogs and macaque monkeys)

Even the stated site of initiation (ductal lobular Junction) is based on a study by Wellings in rats in 1975

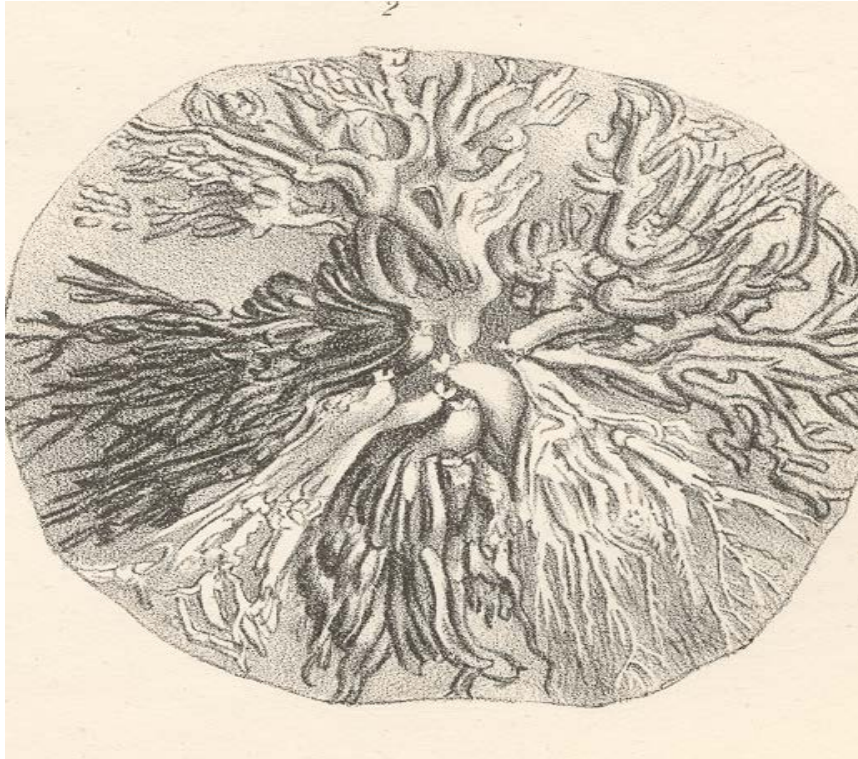


The Human Female Breast is a Unique Organ



- Does not develop until puberty
- Second phase of development with full term pregnancy
- Multiple roles in reproduction
 - Turns blood into milk
 - Mucosal immunity
- Involution after menopause
- Most common site of cancer in women

The Human Breast is Largely Unexplored



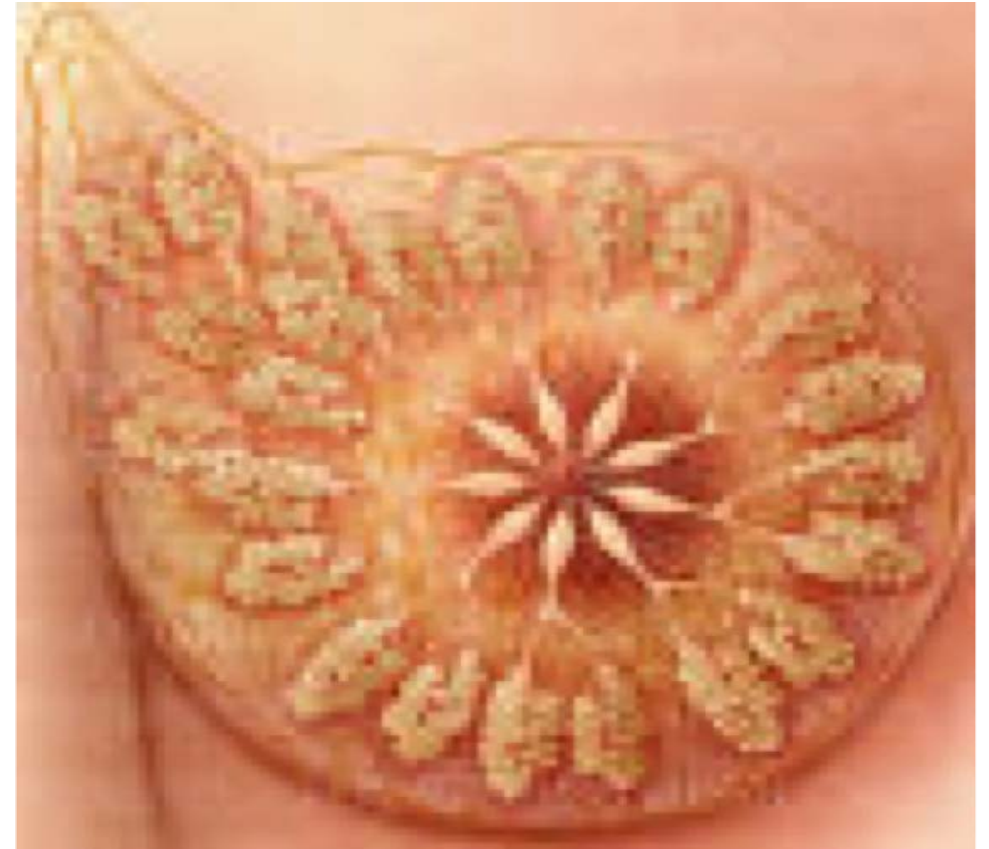
Ashley Cooper 1839

We still don't know

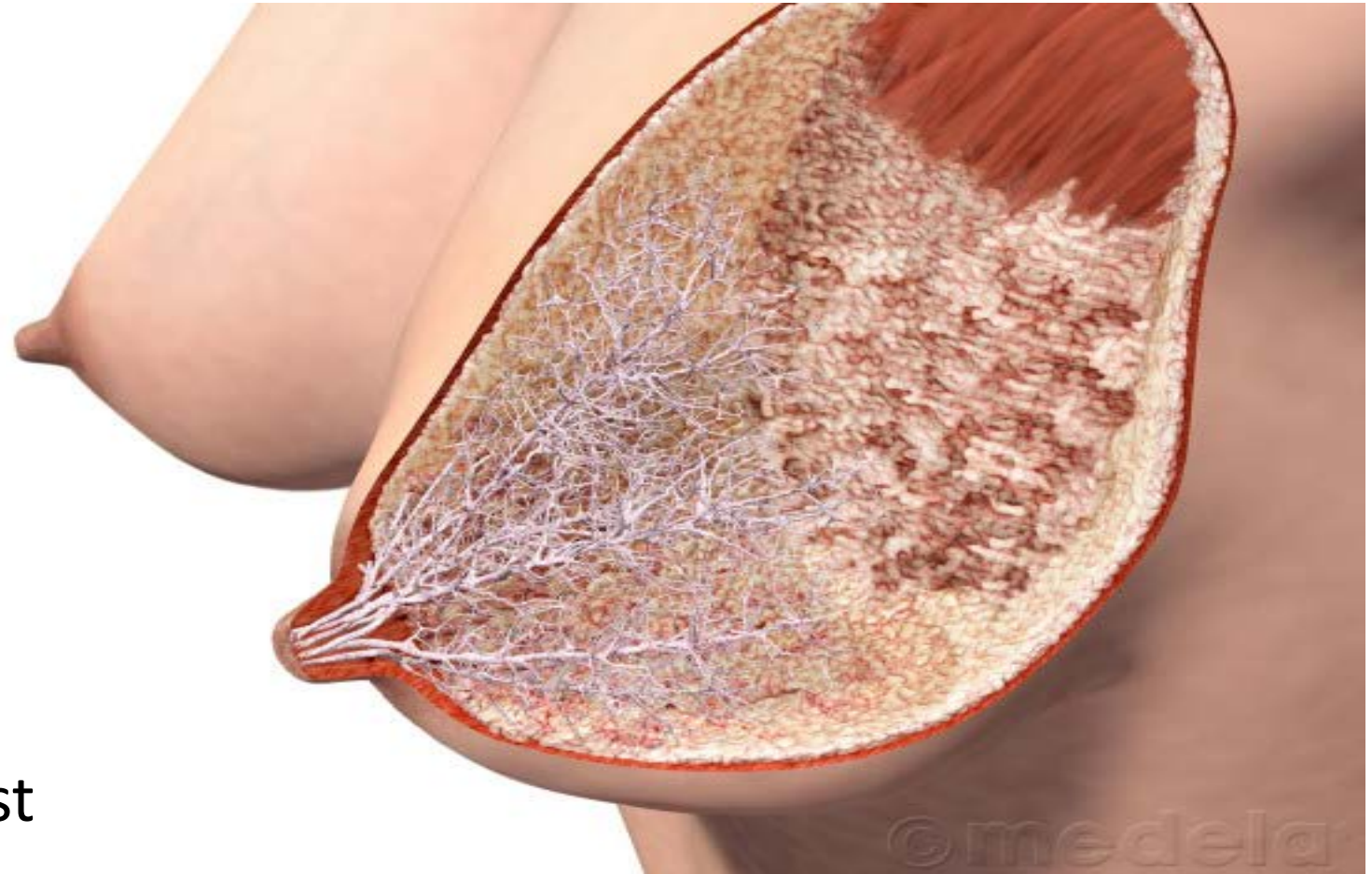
- Anatomy of the ductal systems in the human breast
- Repair/replacement? of ductal systems after involution
- Microbiome of the breast
- Whether the unit of study is the duct or the breast or both breasts
- The role of the stroma in carcinogenesis

Traditional Depiction of the Breast Anatomy

- 15-20 orifices in the nipple
- 15-20 ductal systems
- Radial distribution
- All ducts same size
- Two dimensional



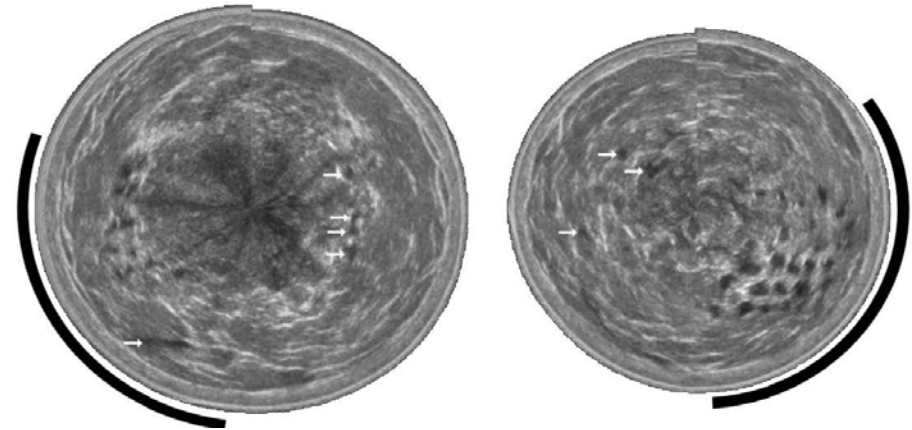
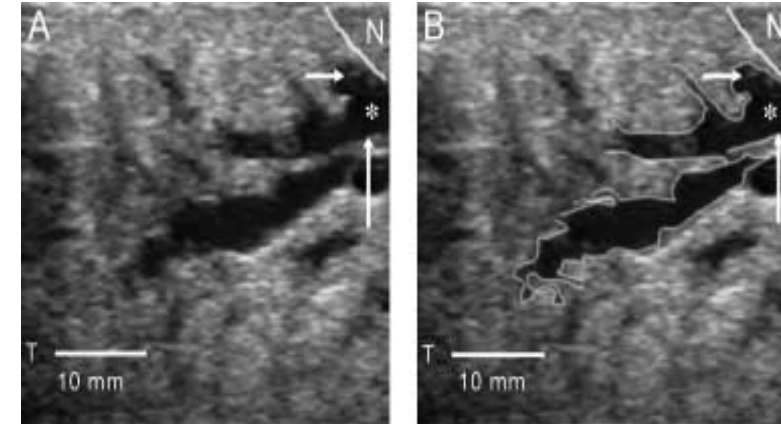
But the Human Breast is 3 Dimensional Not 2!



Artist reconstruction of
ducts in a lactating breast
based on handheld
ultrasound study (Ramsay)

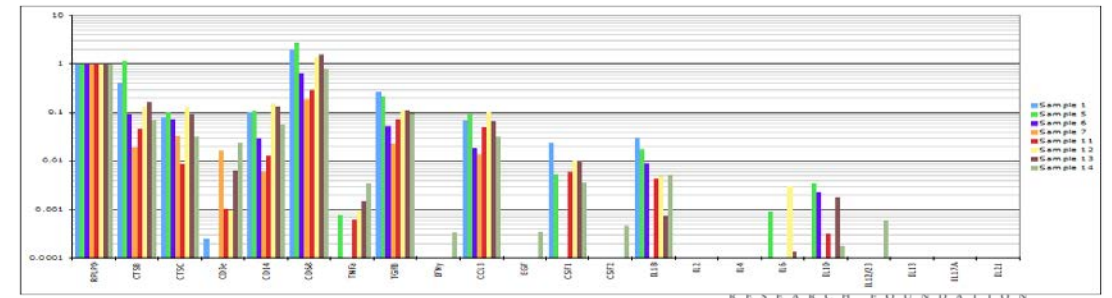
Need Contrast in Ducts to Allow Visualization

- Ducts are accessible in animals (rats, mice, rabbits) and women
- Ability to see ducts on ultrasound with contrast
 - Saline
 - Milk (Ramsay)
- Each ductal system appears to be independent and of variable size
- Ductal systems are non-anastomosing



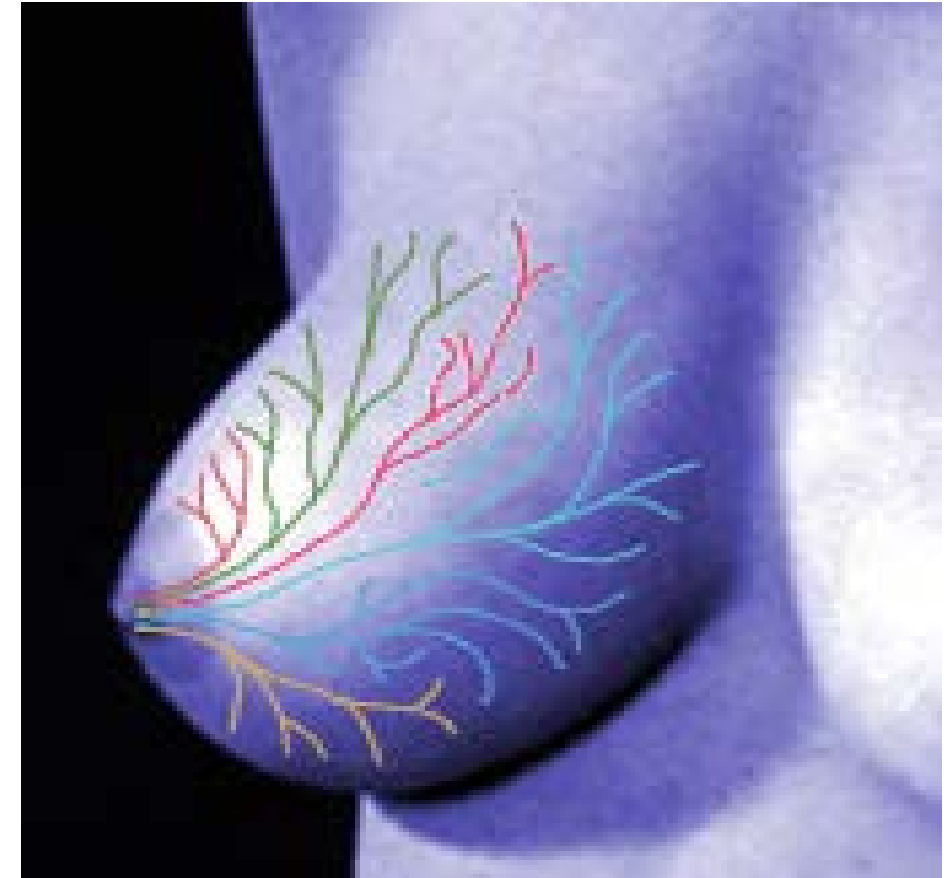
Our Research Suggests that Duct Physiology is Dynamic and that the Unit of Study is the Duct Not the Breast

- *Physiology of the normal breast*
 - Non-lactating women were given caffeine and cimetidine orally and its appearance in the breast duct fluid was measured over time
- *Study of the Correlation of Anatomy and Physiology*
 - Estrogen, progesterone and protein were studied in 3 ducts initially and after 6 months
 - There was no correlation between ducts, between breasts, or over time
- *Comparison of Inflammatory Cytokines in Different Ducts*
 - There was no correlation between ducts



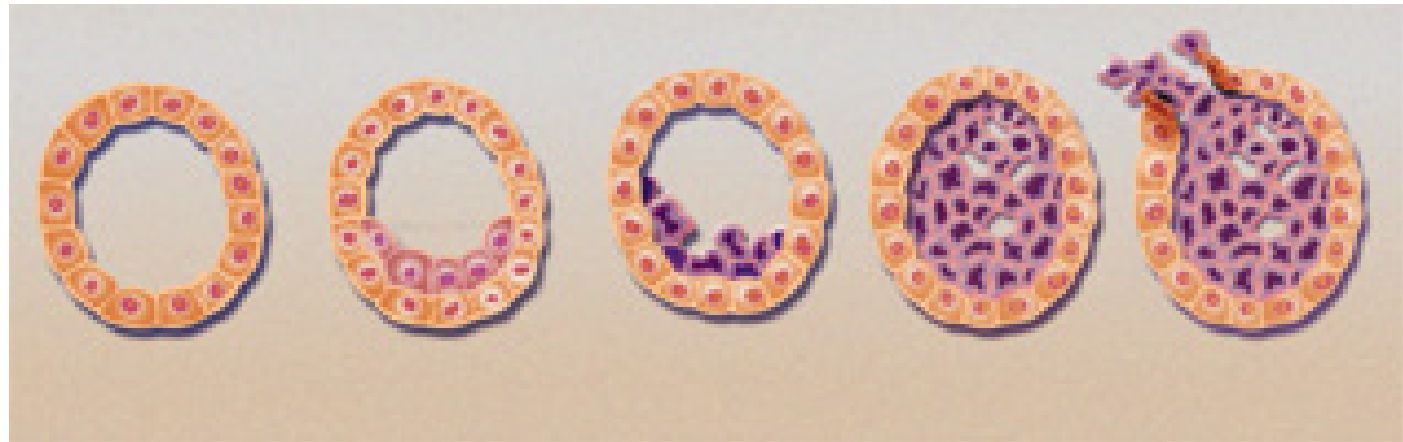
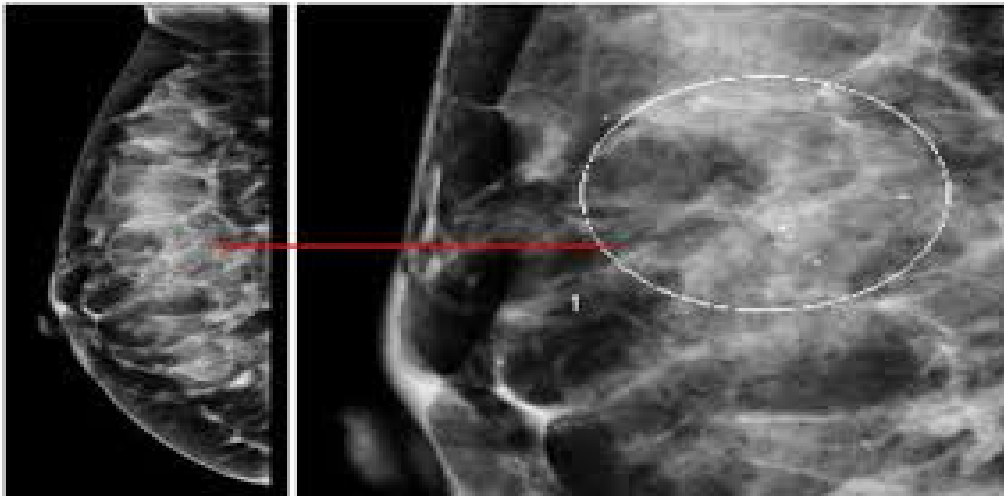
Conclusion: The Duct Should be the Unit of Study NOT the Breast!

- There are 6-9 ductal systems opening on the nipple which appear to be independent as well as “rudimentary ducts”
 - Appear to be independent
 - Uneven distribution
 - Central and peripheral
- Evidence suggests that carcinogenesis occurs in a duct not a breast
 - Local recurrences
 - Sick lobe
 - Field defect intraductal: genomic and transcriptive alterations mapped to duct (Done 2017)



There are early changes in the ducts/lobules that precede invasive cancer

- Most develop over a period of 8 -10 years before detection by standard methods.
- Ductal carcinoma in situ can be marked by microcalcifications that don't represent the extent of disease
- Tissue blindly removed surgically without attention to anatomy resulting in dirty margins, re-excisions, mastectomies. **We need a map!**



AOW Pilot Study: Collaboration with JPL to Map the Ducts!

- Purpose: document the most common distributional patterns of the breast ductal systems within the breast using automated 3D ultrasound (ABUS) to study lactating women
- End goal: create a functional map of the breast ducts so that doctors can better diagnose, treat, and – eventually – learn how to prevent breast cancer
 - A map of the common ductal distributions in the breast would allow for the removal of all the tissue at risk as well as allow intraductal image-guided therapy

Study Procedures

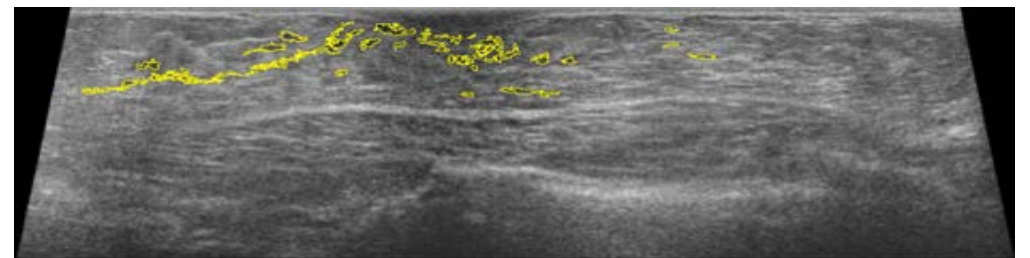
- Recruited lactating women
- Brief questionnaire – demographics and medical history (i.e. number of pregnancies and live births; history of breastfeeding)
- Whole breast 3D ultrasound on both breasts in the “full” state (e.g. prior to breastfeeding)
- Women breastfed their baby or pumped
- Whole breast 3D ultrasound on both breasts in the “in between” or “empty” state

Materials and Methods

Parameter	V1	V2	V3	V4	V5	V6
Age	29	32	32	33	36	39
Parity	2	1	1	3	3	3
Infant age, mo	2	6	12	11	2	18



Analysis: ductal patterns were generated from manual segmentation by expert breast ultrasonographer on anterior-posterior volumes

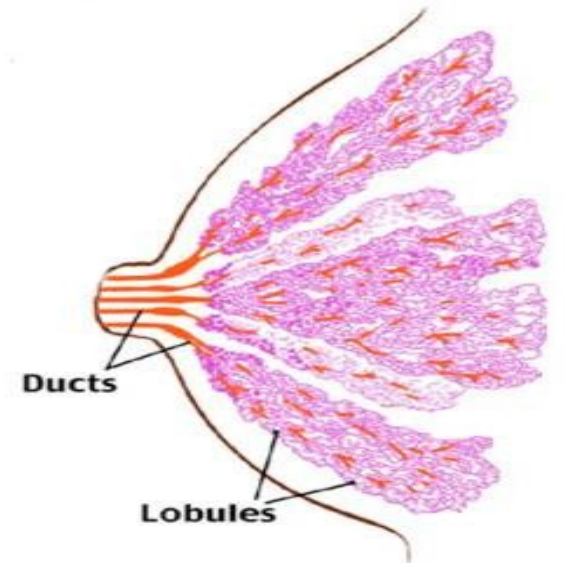


Results

- Feasible to obtain images
- Despite the fact that the women reported having full breasts and were studied supine, there was significant variability in the extent and number of ducts that were actually visualized:
 - 2/3 of the observed ducts were in the lower half of the breasts; only women who had the longest experience lactating (11-18 months) demonstrated ducts in the superior portion of the breast
 - Fewer ducts were observed in the right versus the left breast [difference ranged 10-80% ($P=0.03$)]
 - Significant inter- and intra-observer variability in tracing the ducts improved after training

What's next?

- Cannulate adjacent ducts in volunteers and instill saline prior to 3 D ultrasound
- Study women preoperatively with DCIS on 3D ultrasound to demonstrate that the disease overlies duct distribution and correlate with pathology
- Produce a 3D model of the breast anatomy



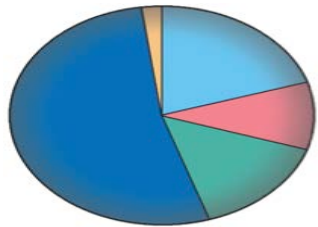
Could there be Microbes in the Breast?



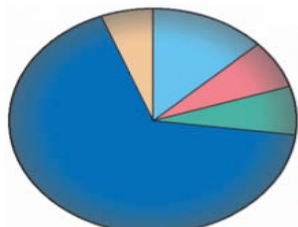
- Potential sources
 - Mouth/oral interaction: nursing, sexual activity
 - transmission of microbiome from gut
- Sampling opportunities
 - Nipple aspirate fluid
 - Ductal lavage
 - Breast tissue samples: core, paraffin blocks

The Breast Was Not Part of the Human Microbiome Project

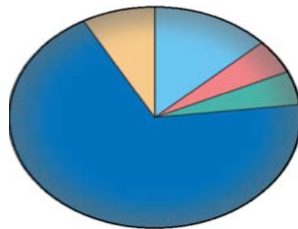
- Breast Microbiome Phyla:



Canada

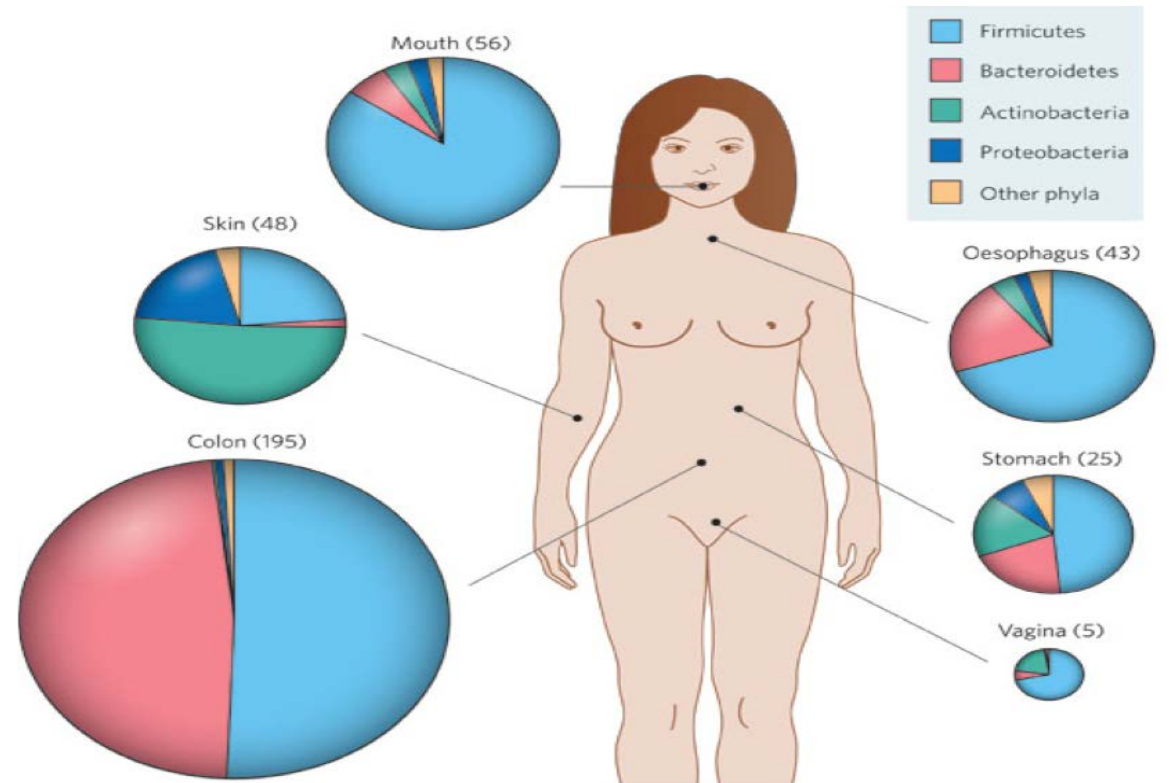


JWCC Tumor



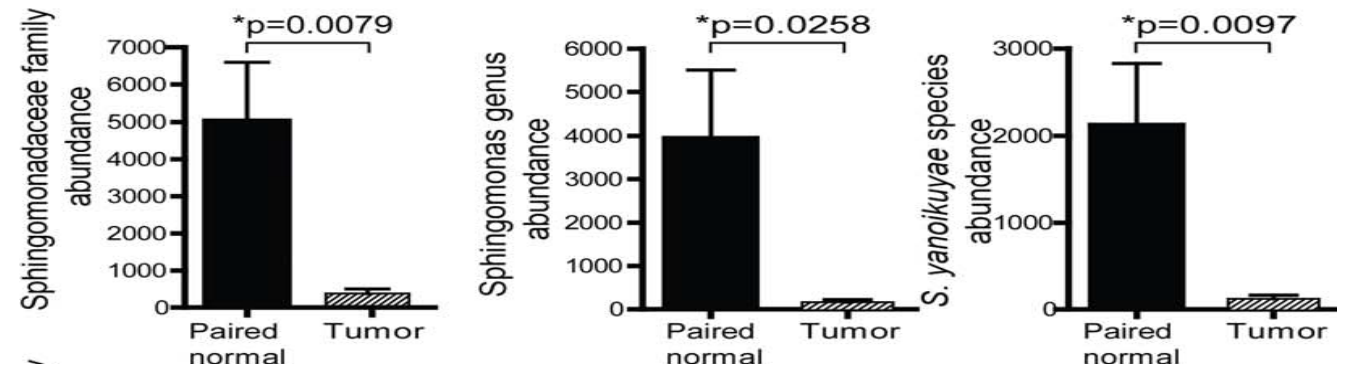
JWCC Normal

The Human Microbiome Project:



Fixed Tissue Exploratory Study: Xuan, Lee et al

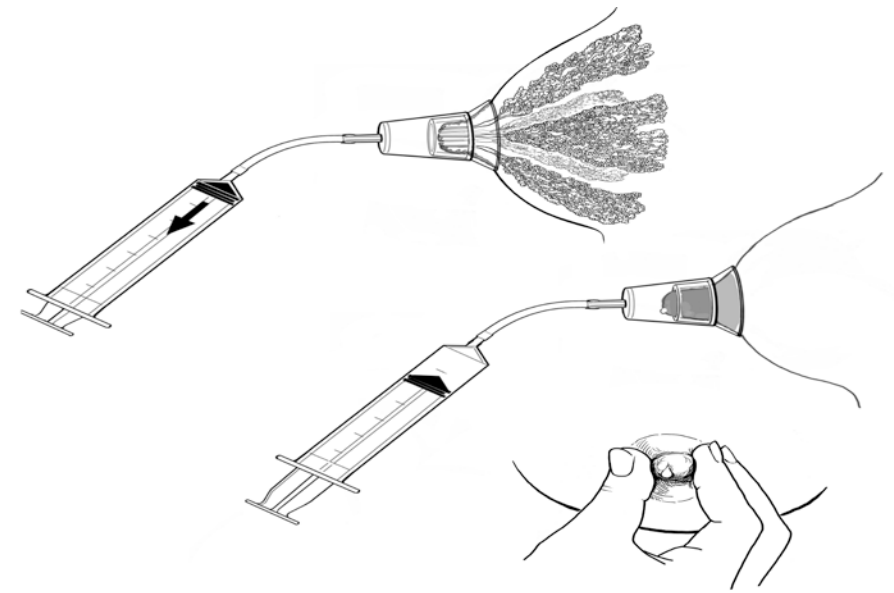
Lee et al John Wayne Cancer Institute studied fixed breast tissue from their bank compared to adjacent normal



- Findings

- *Spingomonas yanoikuyae* was the most significantly enriched in paired normal
 - 40% of paired normal and none in tumor tissue
- *M radiotolerans* was the most significantly enriched and most prevalent in tumor tissue (100%)
 - Found in both paired normal and tumor tissue equally

Microbiome of the Breast: Stroma/ductal fluid or both?



- Purpose
 - Are bacteria or viruses present in breast ductal fluid?
 - Do the types of bacteria and viruses present depend on breast cancer history?
- Opened to enrollment on November 14, 2012, closed on February 25, 2013
 - **211 Army of Women members signed up to be screened for participation**

Recruitment

Exclusion criteria

Stage IV breast cancer diagnosis

Had taken antibiotics in the last 6 months

OCP, HRT, any form of estrogen, any SERM, or any AI in the last 12 months

Currently lactating or had lactated within the last 12 months

Received chemotherapy or radiation in the last 12 months

Had any subareolar or other surgery within 2cm of the nipple

Had any active infections or inflammation in the breast

Study Procedures

Nipple aspirate fluid sterile collection

23 without history of breast cancer

25 with history of breast cancer

Questionnaire

MiSeq amplicon sequencing for 16S bacterial rRNA

Quality filtering

6 healthy subjects

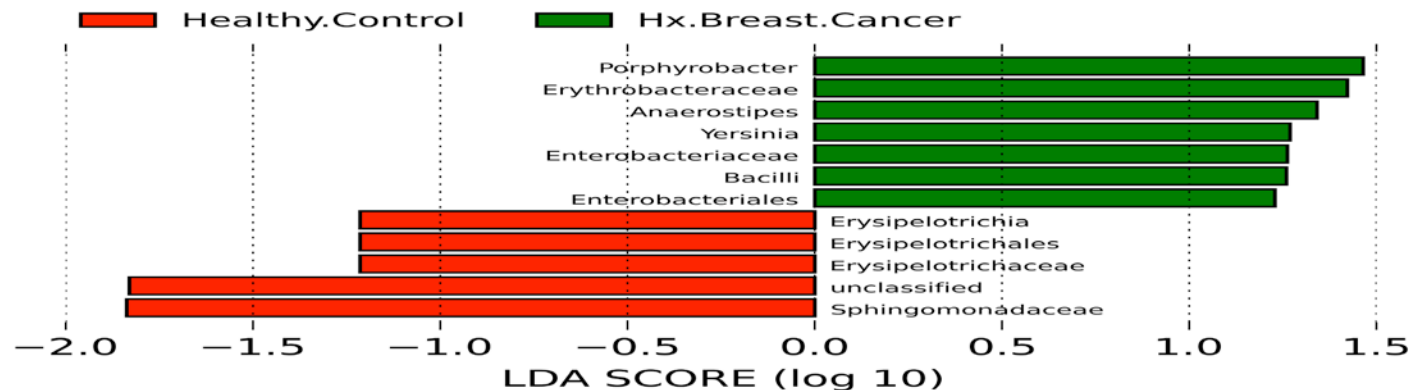
6 subjects with history of breast cancer

Army of Women Study – Results

- Bacteria are present in nipple aspirate fluid
- **There was a difference!**
 - The nipple aspirate fluid microbiome composition in the women with a history of breast cancer was different than that of the healthy controls
 - The types of bacteria found in the samples were mostly the same, but the amounts were different when comparing the two groups

Using Qiime filtered counts rarefied to 934 sampling depth.

Kruskal-Wallis pval < 0.10
log LDA score > 1.0



Clinical Correlation

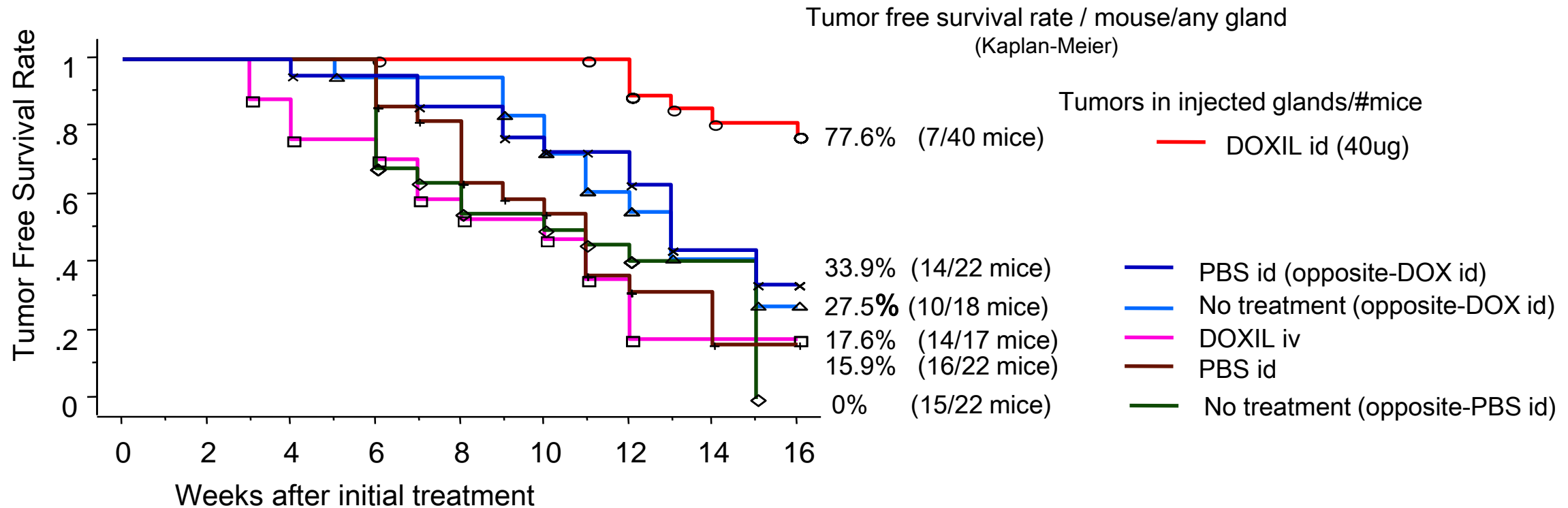
- High antibiotic use increases risk of breast cancer
 - Case control study of 10219 women enrolled at Group Health Cooperative
 - 2266 cases of breast cancer and 385 cases of in situ disease
 - One mechanism is an increase in estrogen metabolism in postmenopausal women
- Nulliparous women (nuns) and those who have not breast fed have increased rates of breast cancer

Clinical Correlation and Next Steps

- The existence of bacteria in the breast *tissue* has previously been described **BUT**
 - Until now, the potential role of the *local breast ductal microbiome* with breast cancer has not been explored
 - This study establishes the presence of bacterial DNA *in the breast ductal system* and thereby raises the question, “where do breast ductal bacteria originate?”
 - How do they relate to breast cancer?
- Determine whether all ducts have same microbiome
- Intraoperative study to Determine Whether There is a Different Microbiome in Duct with Cancer
 - Cannulate duct with DCIS/invasive cancer and additional ducts prior to surgery

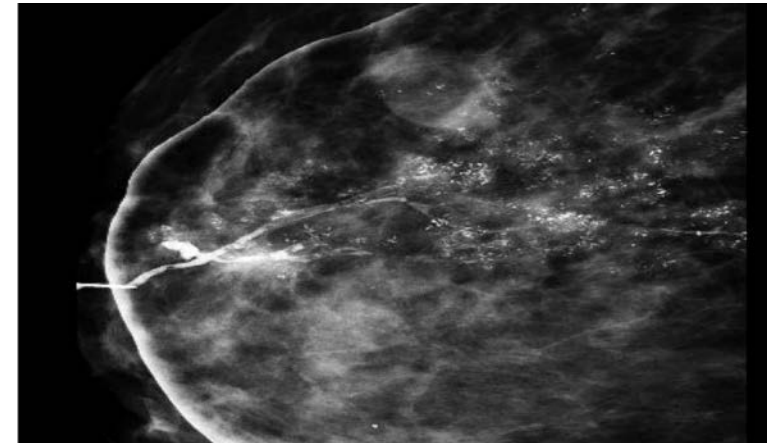
Could we Prevent Breast Cancer with Intraductal Therapy: Prevention in the Her-2/neu Transgenic Mouse Sukumar et al

Prevention Experiment-1, 2, 3, 4 (pooled data)

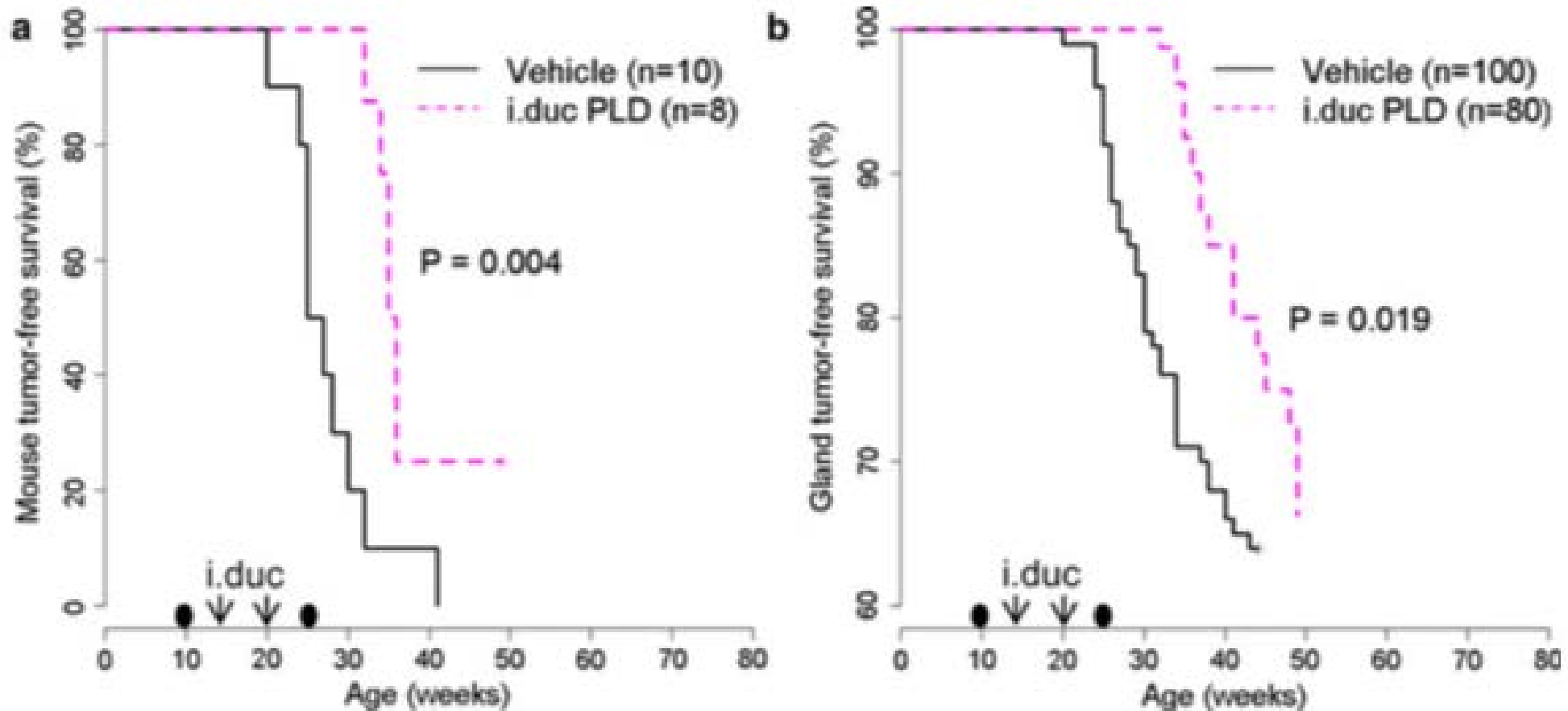


Intraductal Therapy in Women

- CBCRP: Love and Mahoney, Arcata California, April 8th 2005
- JHU: prior to mastectomy
- China study:
 - Premastectomy
 - Multiple ducts
 - Safe
- Eureka, California
 - DCIS patients prior to surgery
 - Duct identified and chemotherapy instilled
 - Patients tolerated well
 - Pathology showed necrosis and inflammation

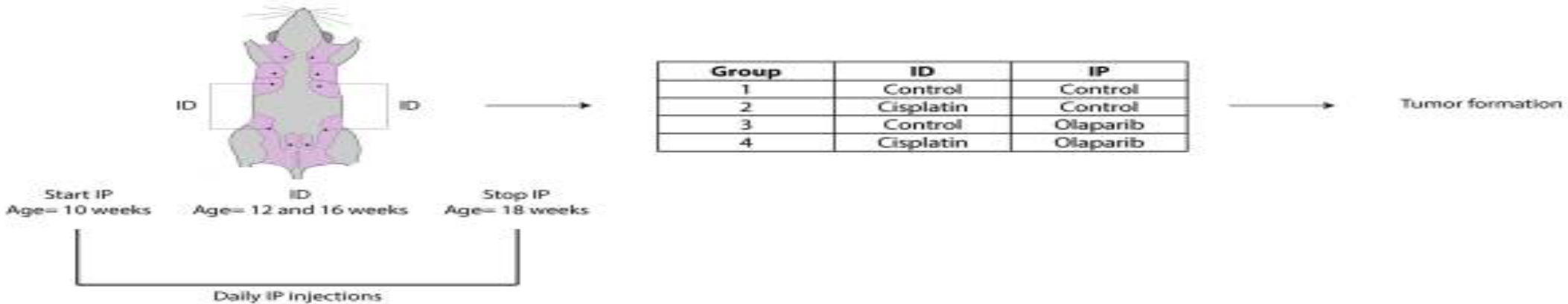


PLD Intraductally in Rats Delayed Cancer Development but did Not Eliminate It

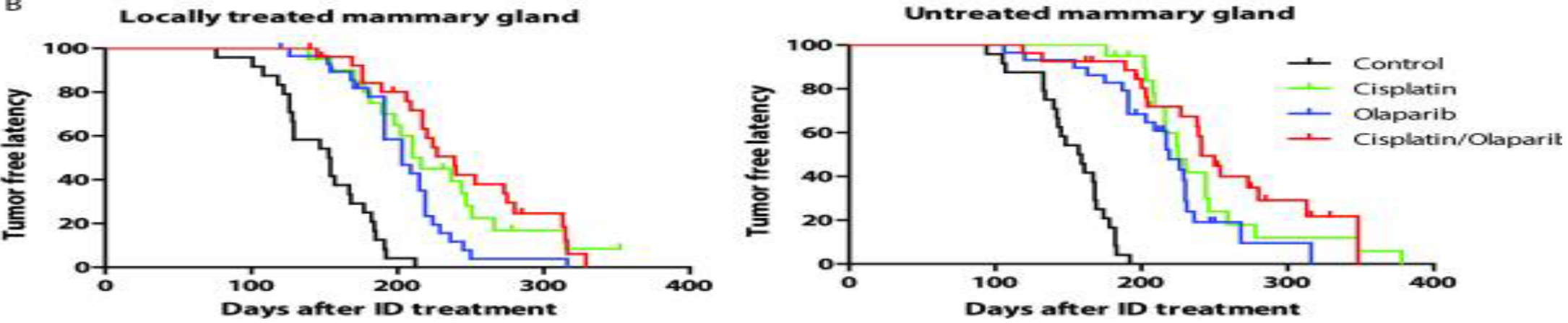


CisPlatin and PARP 1 inhibition intraductally in BRCA mice delayed tumors (153–239 days) but did not eliminate them DeGroot et al

A



B



Do We Also Need to Change the Neighborhood?

Combine:

Intraductal ablation:

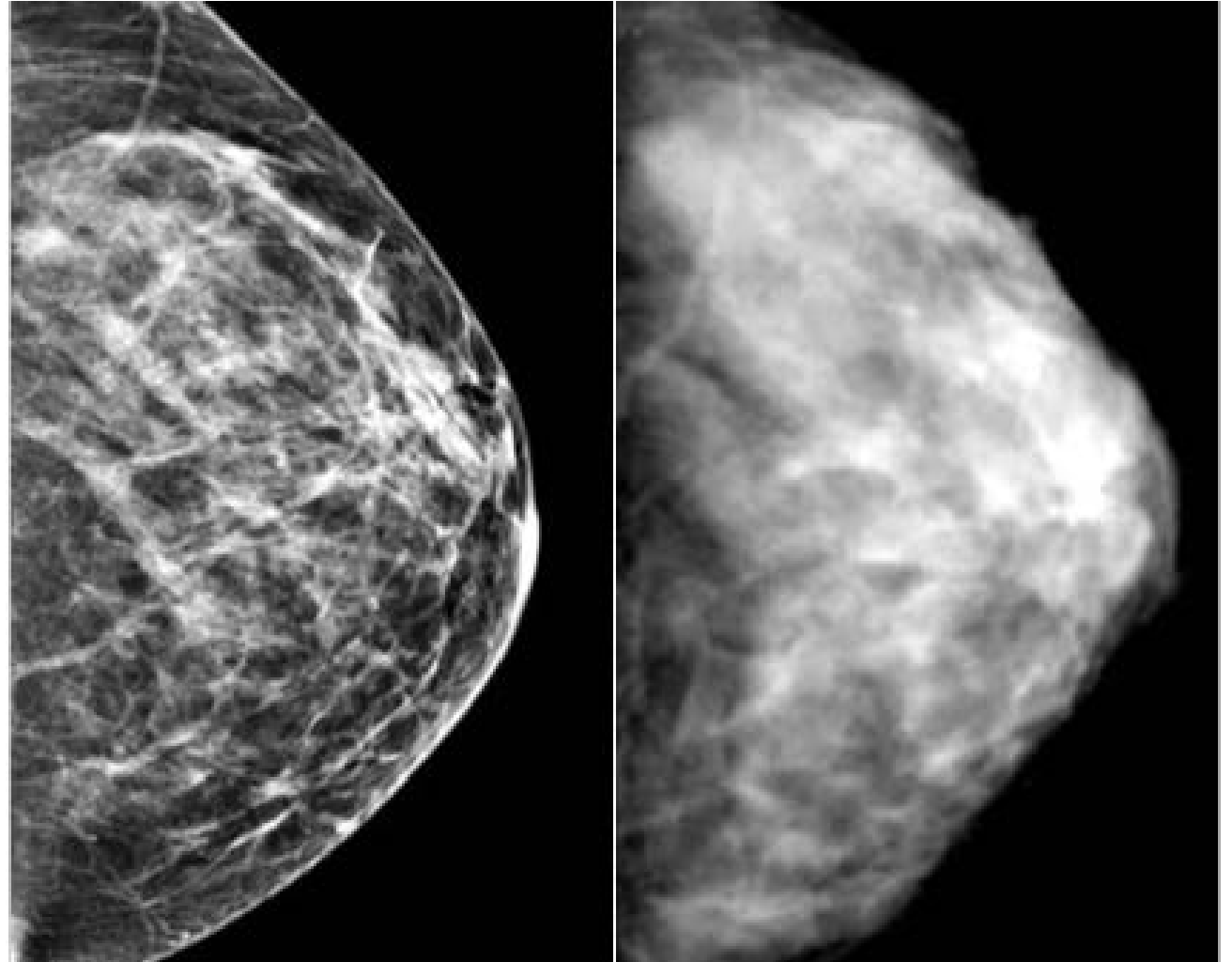
- chemical

- microwave

- immunotherapy

Chemoprevention

- transdermal hormonal change



If We are Going to Understand How to Prevent
and Treat Breast Cancer in Humans We Need to
Study Humans!

Everyone can play a role in ending breast cancer!

JOIN



- **SUPPORT Innovative Research**
 - Give Online: www.DrSusanLoveResearch.org
 - Text "DRSUSANLOVE" to 91999

For more information on our Funding priorities, please contact: Danese Bardot, JD, CFRE, Director of Development at dbardot@DrSusanLoveResearch.org

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