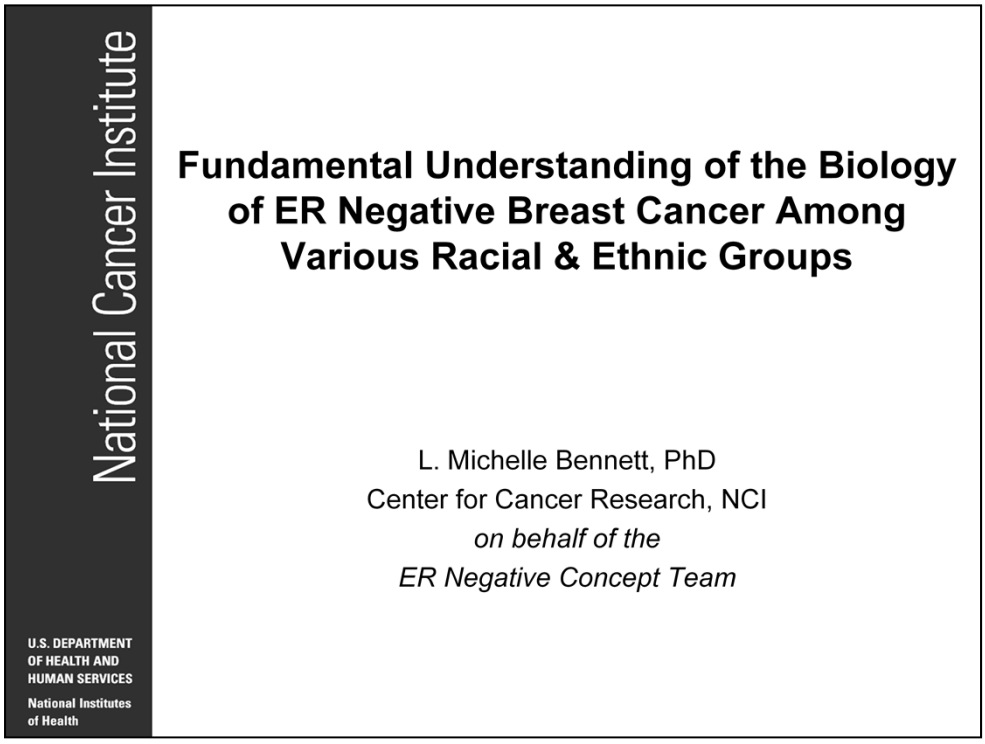


It is a privilege to present the important concept to you today entitled….

Acknowledgements

- **Division of Cancer Biology:** Neeraja Sathyamoorthy
- **Division of Cancer Prevention:** Worta McCaskill-Stevens, Karen Johnson, Barbara Dunn, Doris Browne
- **Center to Reduce Cancer Health Disparities**: Ken Chu, Nelson Aguila, Sanya Springfield
- **Division of Cancer Control & Population Sciences**: Tanya Agurs-Collins, Sheri Schully
- **Division of Cancer Epidemiology & Genetics**: Montserrat Garcia-Closas, Louise Brinton
- **Division of Cancer Treatment & Diagnosis**: JoAnne Zujewski
- **Division of Extramural Activities**: Irina Gordienko, Sonya Roberson
- **Office of Director**: Julia Redmond
- **Office of Science Policy & Analysis**: Anna Levy

The development of this concept was a trans-NCI activity and represents the input of experts from across the NCI

Outline

- ER^- breast cancer background
- Biology of ER^- breast cancer
- NCI Think Tank
- Fundamental questions in ER^- breast cancer biology
- RFA Mechanism and Evaluation

This slide give you a quick glance of what what we will be moving through in the next 10 minutes

And we will start with some background

Comparison of Breast Cancer Types in African-American and Non-Hispanic White Women

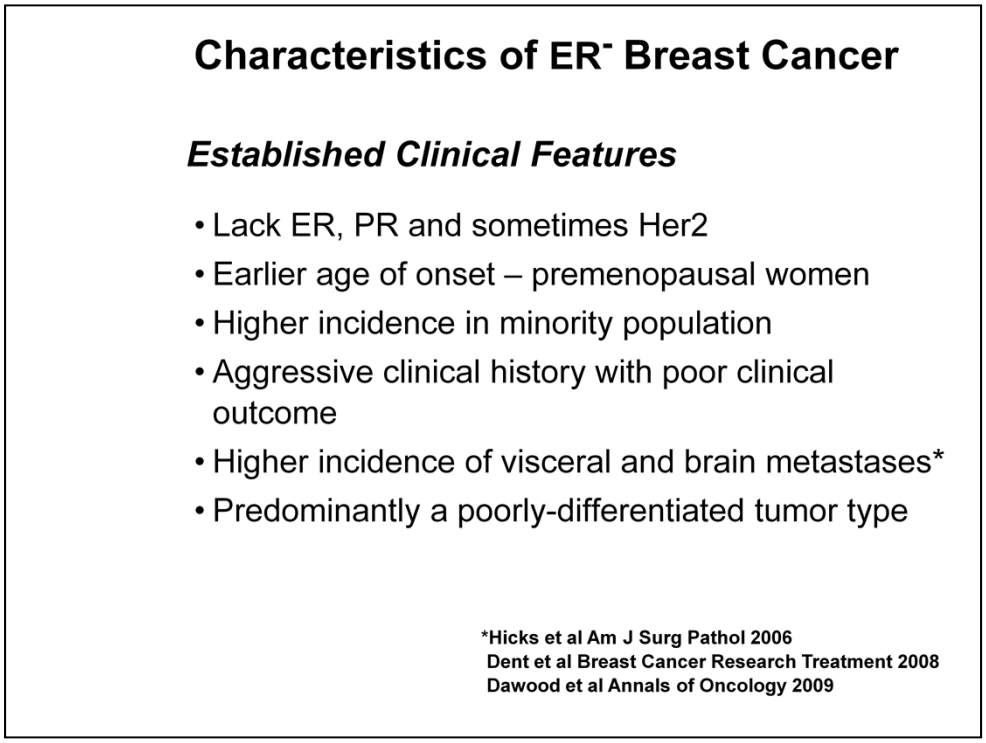
	White	African American
Premenopausal		
ER+	**27.96**	**19.02**
ER-	**11.95 (30%)**	**16.48 (45%)**
Postmenopausal		
ER+	**276.3**	**172.9**
ER-	**62.66 (18%)**	**81.37 (31%)**

Incidence Rates: per 100,000 woman-years
(Data released Nov 2006)
SEER Program (www.seer.cancer.gov)

ER- breast cancer is more common among younger women

And as you can clearly see from the SEER data on this slide, AA women are more likely to be diagnosed with ER- br cancer than whites – the percentage of AA women getting ER- cancer is greater for than for White women – the percentages of permenopausal women getting ER- br ca is also greater than for postmenopausal

Please not that data suggest there in increasing incidence of ER- disease in Lantina women



What else do we know about ER- disease?

Perhaps obviously – ER- disease is characterized by ER-/PR-and sometimes HER2-

Early age

Minority disparity

Aggressive and poor outcome

Incrase mets

In general, poorly differentiated

Human Breast Cancer Subtype Classification

Subtype	Characteristics
Estrogen Receptor Positive	
luminal A	ER^+ and/or $PR^+/Her2^-$
luminal B	ER^+ and/or $PR^+/Her2^+$
Estrogen Receptor Negative	
$Her2^+/ER^-$	$ER^-/PR^-/Her2^+$
basal-like	$ER^-/PR^-/Her2^-$ ($CK5/6^+$ and/or $Her1^+$)
Unclassified (5- marker negative)	$ER^-/PR^-/Her2^-$ ($CK5/6^-/Her1^-$)

Carey et al JAMA 2006

Work from UNC with Chuck Perou and Bob Milliken have shown 5 distinct breast cancer subtypes by expression profile analysis:

2 ER+ and 3 ER- sub-types –

The ER+ subtypes are distinguished by the presence of Her2 expression (luminal A better prognosis than luminal B)

The ER- subtypes include Her2/neu+ disease

And also what has been termed basal-like (also “triple negative”) – for which there is evidence of racial disparity

And 5-maker negative (which is currently not being distinguished from basal-like in clinical trials) – currently it does not appear that there is racial disparity for this sub-type

Challenge

- Targeted therapies do not exist for $ER^-/Her2^-$ breast cancer
  - ER^+ : Tamoxifen, Aromatase Inhibitors
  - $Her2^+$: Herceptin and Lapatinib

There is a paucity of data regarding molecular characteristics & associated signaling for ER^- breast cancer

Currently targeted therapy does not exist for ER- breast cancer

ER+ and Her2+ disease both have targeted options

There is not enough molecular data and knowledge about the signaling pathways for ER- disease

Outline

- ER^- breast cancer background
- Biology of ER^- breast cancer
- NCI Think Tank
- Fundamental questions in ER^- breast cancer biology
- RFA Mechanism and Evaluation

What fundamental biology is known about ER- breast cancer?

Studies are being published and abstracts presented at meetings that are beginning to help define the biology of er- breast cancer

Defining the biology of ER$^-$ breast cancer

- Aberrant expression of transcription factors and growth factor receptors has been correlated with basal-like (triple negative) subtype of breast cancer
- Mechanisms of ER loss can vary:
  - ER promoter methylation in ER$^-$ breast cancer (25%)
  - Src activated ER degradation
- Breast cancer cells lines recapitulate primary breast cancers
- ER$^-$ tumors share similarities with BRCA-1 associated breast cancer
  - Clinical & pathological features
  - Gene profiling data

1. Aberrant Expression – FOXC2 – Weinberg lab AND EGFR overexpression (Zhou et all 2008) AND c-KIT overexpression (imantinib targets cKIT and PDGFR tks)

2. ER methylation – Davidson lab AND Src activated ER degradation -- Slingerland Lab JCI 2007

3. Joe Gray with collaborators examined the genomic and transcriptional characteristics of about 50 breast cancer cell lines and compared them to primary tumors and showed that the cell lines mirror most of the genomic properties of primary breast tumors;

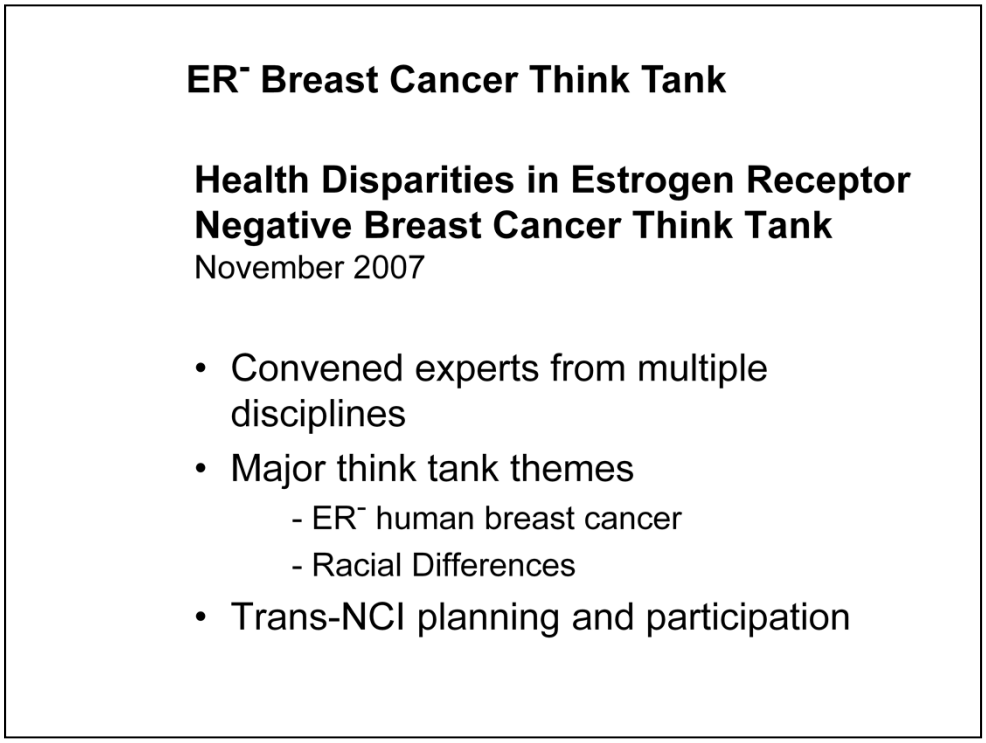
And while fundamental biology is beginning to emerge about this disease – we really do not know enough to move toward developing science-based interventions based on molecular pathways

Outline

- ER^- breast cancer background
- Biology of ER^- breast cancer
- NCI Think Tank
- Fundamental questions in ER^- breast cancer biology
- RFA Mechanism and Evaluation

And while fundamental biology is beginning to emerge about this disease – we really do not know enough to move toward developing science-based interventions based on molecular pathways

To this end, the NCI convened a panel of experts….



At an NCI sponsored think tank in Nov 2007

Experts included epi, nutrition, basic science, animal models, hormone exerts, clinicians, etc…

Roster and summary can be found in the appendix section

Think Tank Recommendations

- *Encourage the systematic study of the biology of ER^- human breast cancer*
- *Emphasize the use of human breast cancer samples supplemented with relevant experimental models*
- *Identify tumor- and stroma-specific biologic differences among racial groups that can be used to improve early detection, diagnosis, and the development of interventions*

a number of recommendations resulted from the meeting…

Among them were:

The true strength of these recommendations was that they encouraged leveraging existing resources to advance our fundamental understanding of the biology of er- breast cancer among racial and ethnic groups

Leverage Existing Resources

Availability of…

- clinically annotated breast cancer tissue samples from cohort studies
- animal models relevant to human disease
- ER^+ and ER^- cell lines
- heterotypic 3D cultures
- enabling technologies
  - imaging modalities
  - high-throughput molecular profiling

There are a number of robust resources available including…

For bullet 1 – OBBR has extensive list of resources for human tissue

For bullet 2 – most mouse models that have been developed for breast cancer are ER- -- MMHCC provides robust resource for animal models

For bullet #3 -Joe Gray with a number of collaborators including Donna Albertson, Fred Waldman Steve Ethier, Adi Gazdar has examined the genomic and transcriptional characteristics of about 50 breast cancer cell lines and compared them to primary tumors and show that the cell lines mirror most of the genomic properties of primary breast tumors; -- NCI has developed kits of these cell lines and makes them available to researchers

For bullet 4 – NCI makes available 3D culture reagents to scientific community

Outline

- ER^- breast cancer background
- Biology of ER^- breast cancer
- NCI Think Tank
- Fundamental questions in ER^- breast cancer biology
- RFA Mechanism and Evaluation

The next slides have a list of research opportunities that when answered will enhance our fundamental understanding of disease

Questions that can be addressed because of existing resources

- Molecular characteristics distinguish ER^+ and ER^- tumors
- Identify key genes that are altered in ER^+ and ER^- tumors
  - genetic and/or epigenetic alterations
- Elucidate signaling pathways unique to ER^- tumors
- Characterize the ER^- subtypes

Questions that can be addressed because of existing resources (cont.)

- Identify progenitor cells that give rise to ER^- tumors
- Determine whether ER^- phenotype is evident in pre-malignant lesions
- Define role of tumor-associated stroma in contributing to ER^- tumor progression
- Understand the molecular basis for racial and ethnic differences

…including stem cell (and early pre-malignant) cells comparisons among racial and ethnic groups

Outline

- ER^- breast cancer background
- Biology of ER^- breast cancer
- NCI Think Tank
- Fundamental questions in ER^- breast cancer biology
- RFA Mechanism and Evaluation

The last section of the talk addresses the RFA mechanism and evaluation

Purpose of the RFA

To stimulate research on the basic biology of ER^- breast cancer among various racial and ethnic groups by leveraging existing resources and encouraging multi-disciplinary collaboration

Funding Mechanism

- Award Mechanism
  - U01 (cooperative agreement)
  - three or four awards at $400K-500K (t.c.)
- Award Date
  - 2010
- Funding Source
  - Breast Cancer Stamp Act Fund
- Cost for Project Period
  - $6M
- Duration
  - five years

I want to emphaize that Breast Cancer Stamp Funds are NOT from RPG pool

Rationale for RFA

- Promotes multi-disciplinary, and potentially multi-institutional, collaboration
- This kind of research is initially discovery-based and hypothesis-generating
  - Such applications fare poorly in CSR-based review
- NCI portfolio review:
  - two currently funded grants focus on the biology of ER^- breast cancer and would fall within scope of this RFA ($663K)
  - ten related projects (GWAS, risk factors, prevention, treatment)

We expect this initiative to lead to an interdisciplinary collaborative effort to study the biology of ER – hu Breast cancer

Only two basic biology grants

Only ten related projects in the ER- research area

Two funded applications:

Dorray El-0Ashry (U of M – Ann Arbor): Mechanisms underlying ER-Negative Breast Cancer

Focus: dissect the mechanisms underlying MAPK induced loss of ER-alpha expression at both protein and RNA levels

-Can ER “positivity” be restored to make tumors TAM sensitive?

Victoria Seewaldt (Duke Univ): ECM-Mediated apoptosis in p53(-) HMECs

Testing hypothesis that loss of CBP (CREB binding protein) is associated with/promotes ER- breast cancer

Desired goal: understand early events of ER- breast cancer and identify targets for prevention

Criteria for portfolio review to say something is “responsive”:

Basic Biology applied to human ER- breast cancer

50% human

-Not only animal models

-Not only cell lines

50% ER- breast cancer

Cooperative Agreement (UO1)

Principal Investigators:

- Primarily responsible for planning & directing research programs

NCI Staff:

- Programmatic involvement – technical assistance, advice and coordination beyond normal stewardship of grants
- Organize and facilitate annual meeting of funded investigators
- Enable the dissemination of resulting scientific information & reagents
- Foster multidisciplinary collaborations

Roles and responsibilities of funded PIs and NCI staff

RFA Evaluation Criteria: *Did the RFA Stimulate Research in the Basic Biology of ER$^-$ Breast Cancer?*

Measures of research progress on the fundamental biology of ER$^-$ disease includes understanding:

- Molecular characteristics of ER$^+$ vs ER$^-$ tumors
- Identifying novel subtypes in ER$^-$ breast cancer
- Identification of unique markers for ER$^-$ breast cancer
- Identification of signaling pathways unique to ER$^-$ tumors for the identification of targets
- Identification of early stromal changes specific for ER$^-$ tumors
- Improved understanding of molecular basis for racial differences

Evaluation Criteria from Concept Text

•Has the research led to a better understanding of the biology of ER-negative breast cancer?

•Has there been progress in identifying novel subtypes in ER-negative breast cancer, if they exist? Have the molecular profiles of these been characterized?

•Has there been progress in identifying unique markers that are associated with ER-negative breast tumors as compared to ER positive tumors?

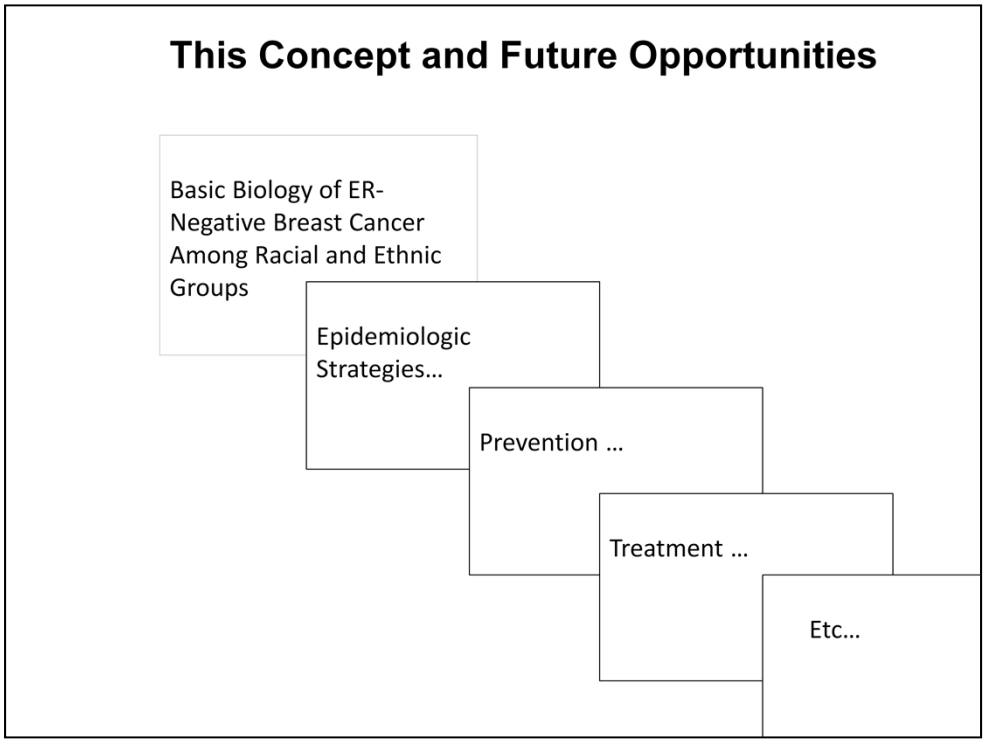
•Has the research identified unique signaling pathways that could serve as markers of ER-negative breast cancer(s)?

•Has the research identified unique signaling pathways that can be potentially exploited therapeutically?

•Have the progenitor cells that give rise to the ER-negative breast cancers been identified and characterized?

•How has the research improved the mechanistic understanding of ER-negative breast cancer biology among racial and ethnic groups?

NCI will know it has successfully stimulated research in the basic biology of ER- breast cancer if we understand….



There is great potential for a cascade of related next concepts….

National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Fundamental Understanding of the Biology of ER Negative Breast Cancer Among Various Racial & Ethnic Groups

RFA Concept
March 2, 2009
BSA Meeting

Breast Cancer Stamp Funds

- The Breast Cancer Research semipostal was issued on July 29, 1998. It was the first semipostal in U.S. history. In Dec 2007 the act was reauthorized through 2011.
- To date, the stamp has raised over $54M for breast cancer research.
- By law, 70% of the net amount raised is given to the NIH and 30% is given to the Medical Research Program at the DOD.

- NIH and DOD are accountable, annually, to both Congress and to the GAO for reporting on:
  - Use of funds
  - Research advances and accomplishments

Animal Models

Mouse Models

- MMTV/WAP driven erbB2 transgenics (ER^-)
- MMTV-Wnt (Heterogenous ER status)
- Mammary-specific $BRCA^{-/-}$ with $p53^{+/-}$ (basal type)
- $BRCA1^{-/-}/p53^{-/-}$ (ER^+ initially -> ER^-)
- Estrogen-induced ACI rat model (ER^+)

- Inducible and conditionally genetically engineered mouse models
- Xenograft models

ER Negative Cell Lines

- Neve *et al* present a number of ER^- cell lines in their paper. Among the more common are:
  - MDA MB 231 (W/51)
  - SKBR3 (W/43)
  - MDA MB 157 (B/44)

Neve RM et al Cancer Cell 2006

Tissue Resources:

NCI Biospecimen Locator
http://pluto3.nci.nih.gov/tissue/default.htm

NCI Office of Biorepositories and Biospecimen Research
http://biospecimens.cancer.gov/default.asp

- Cooperative Human Tissue Network (CHTN)
- Cooperative Breast Cancer Tissue Resource (CBCTR)
- Clinical Trial Cooperative Group Human Tissue Resources
- The Cancer Family Registries (CFRs)
- The Breast Cancer Intergroup of North America (TBCI) Specimen Resource
- OBBR Specimen Resource Locator

ER-Negative Tumors share Similarities with BRCA-1 associated Breast Cancer

- Clinical & pathological features such as
  - High grade, poorly differentiated
  - Visceral metastases
  - High Ki-67 staining
  - CK 5/6 expression
  - P53 commonly mutated
- Gene profiling data

…suggesting dysfunction in BRCA1 or related pathways

Hypotheses:

(i) basal-like precursors may be more tolerant of BRCA1 loss

(ii) if BRCA1 is involved in differentiation then inactivation may result in basal-like phenotype

(iii) BRCA1 loss could directly drive tumor development with basal-like phenotype