This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike License. Your use of this material constitutes acceptance of that license and the conditions of use of materials on this site.

Copyright 2006, The Johns Hopkins University and Nancy E. Davidson. All rights reserved. Use of these materials permitted only in accordance with license rights granted. Materials provided “AS IS”; no representations or warranties provided. User assumes all responsibility for use, and all liability related thereto, and must independently review all materials for accuracy and efficacy. May contain materials owned by others. User is responsible for obtaining permissions for use from third parties as needed.
Breast Cancer

Nancy E. Davidson, MD
Johns Hopkins University
Module 5—breast and prostate cancer

- Most prominent human cancers
- Led by Drs. Nancy Davidson and Terry Brown
Breast Cancer: U.S. Statistics 2005

- 213,000 new cases
- 40,000 deaths
- Lead cancer diagnosis in women
- Second leading cause of cancer death in women
Potential Applications for Breast Cancer: Biology

- Predict risk of cancer development
- Estimate prognosis for established cancer
- Predict response to therapy
- Identify therapeutic targets
Section A

Risk of Cancer
Breast Cancer Risk Factors: Demographics

- Gender
  - Male: female
    - 1:100

- Age
  - 1 in 50 by age 50
  - 1 in 8 over lifetime
Breast Cancer Risk Factors: Reproductive

- Early menarche
- Late menopause
- Nulliparity or late first pregnancy
- ? Lactation
Breast Cancer Risk Factors: Environmental

- Radiation—yes
- Pesticides—no
- Electromagnetic fields—no
Breast Cancer Risk Factors: Lifestyle

- Diet
- Alcohol
- Physical activity
- Tobacco
Breast Cancer Risk Factors: Endogenous Hormones

- High hormone levels
- Post menopausal obesity
- Increased bone density
Breast Cancer Risk Factors: Exogenous Hormones

- Hormone replacement therapy—yes
- Estrogen replacement therapy—no?
- Oral contraceptives—no
Breast Cancer Risk Factors: Pathology

- Atypical ductal or lobular hyperplasia
- Lobular carcinoma in situ
Breast Cancer Risk Factors: Inherited Susceptibility

- Family history
- Major inherited susceptibility
- DNA repair defects
How Much Breast and Ovarian Cancer Is Hereditary?

Breast Cancer

- Sporadic
- Hereditary 5-10%
- Family clusters 15-20%

Ovarian Cancer

- Sporadic
- Hereditary 5-10%
## Causes of Hereditary Susceptibility to Breast Cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Contribution to Hereditary Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>20–40%</td>
</tr>
<tr>
<td>BRCA2</td>
<td>10–30%</td>
</tr>
<tr>
<td>TP53</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>PTEN</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Undiscovered genes</td>
<td>30–70%</td>
</tr>
</tbody>
</table>
- Tumor suppressor gene on chromosome 17
- Autosomal dominant transmission
- Protein has role in genomic stability
- ~500 different mutations reported
Increased Likelihood of BRCA Mutations

- Features that indicate increased likelihood of having BRCA mutations
  - Multiple cases of early onset breast cancer
  - Ovarian cancer (with family history of breast or ovarian cancer)
  - Breast and ovarian cancer in the same woman
  - Bilateral breast cancer
  - Ashkenazi Jewish heritage
  - Male breast cancer
Possible increased risk of other cancers (e.g., prostate, colon)

- Breast cancer (often early age at onset): 50-85%
- Second primary breast cancer: 40-60%
- Ovarian cancer: 15-45%
BRCA2-Associated Cancers: Lifetime Risk

- Increased risk of prostate, laryngeal, and pancreatic cancers (magnitude unknown)

Breast cancer: 50-85%
Ovarian cancer: 10-20%
Male breast cancer: 6%
BRCA2-Linked Hereditary Breast Cancer

- Breast, dx 44
  - d. 48
- Ovary, dx 65
  - d. 68
- Prostate, dx 64
- Breast, dx 33
  - 42
Breast Cancer Risk Estimates in BRCA Mutation Carriers

Options for Carriers of BRCA-1 or BRCA-2 Mutations

- Surveillance
- Chemoprevention
- Prophylactic surgery
Section B

Prognosis for Established Cancer and Response to Therapy
Established at the NIH Consensus Conference 2003

- Axillary lymph nodes
- Tumor size
- Histological grade
- Histological tumor type
- Steroid receptor states
- Age
Prognostic Markers for Breast Cancer

- Established at the NIH Consensus Conference 2003
  - Axillary lymph nodes
  - Tumor size
  - Histological grade
  - Histological tumor type
  - Steroid receptor states
  - Age
Potential Applications for Breast Cancer Biology

- Predict risk of cancer development
- Estimate prognosis for established cancer
- Predict response to therapy
- Identify therapeutic targets
The Estrogen Receptors

Structure and Functional Domains of ERα and ERβ

ER α

1

A/B

C

D

E

F

595

AF-1

AF-2

95%

53%

1

530

ER β

### Steroid Receptors in Breast Cancer

<table>
<thead>
<tr>
<th>Tumor phenotype</th>
<th>Phenotype frequency</th>
<th>Response to hormonal therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/PR+</td>
<td>41%</td>
<td>75–80%</td>
</tr>
<tr>
<td>ER+/PR-</td>
<td>30%</td>
<td>20–30%</td>
</tr>
<tr>
<td>ER-/PR+</td>
<td>2%</td>
<td>40–45%</td>
</tr>
<tr>
<td>ER-/PR-</td>
<td>27%</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

Source: McGuire (1978)
Endocrine Therapy for Breast Cancer

- Ovarian ablation—surgery, radiation, LHRH agonists
- SERMs—tamoxifen, toremifene, fulvestrant
- Aromatase inhibitors—anastrozole, letrozole, exemestane
- Additive—progestins, estrogens, androgens
Some Possible Mechanisms of Hormone Resistance

- Loss of ER expression
  - Mutation or deletion
  - Promoter methylation
  - Altered transcriptional factors
- Altered coactivators or corepressors
- Alternative growth factor pathways
- Drug delivery
The EGFR (ErbB) Family and Ligands

EGF
TGFα
Amphiregulin
β-cellulin
HB-EGF
Epiregulin

Heregulins
NRG2
NRG3
Heregulins
β-cellulin

Cysteine-rich domains

C-terminus

Tyrosine kinase domain

ErbB-1
Her1
EGFR

ErbB-2
Her2
neu

ErbB-3
Her3

ErbB-4
Her4

100
44
36
48

100
82
59
79

100
33
24
28
**HER 2 As a Predictive Marker for Trastuzumab**

<table>
<thead>
<tr>
<th>HER 2 Status</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC 3+</td>
<td>35%</td>
</tr>
<tr>
<td>IHC 2+</td>
<td>0</td>
</tr>
<tr>
<td>FISH positive</td>
<td>34%</td>
</tr>
<tr>
<td>FISH negative</td>
<td>7%</td>
</tr>
</tbody>
</table>

Source: Vogel (2002), JCO
HER 2 as a Predictive Marker

- Resistance to tamoxifen
- Response to anthracycline
Section C

New Therapeutic Targets for Breast Cancer
The EGFR (ErbB) Family and Ligands

- EGF
- TGFα
- Amphiregulin
- β-cellulin
- HB-EGF
- Epiregulin

Heregulins
- NRG2
- NRG3
- Hereregulins
- β-cellulin

Tyrosine kinase domain

ErbB-1 Her1 EGFR
ErbB-2 Her2 neu
ErbB-3 Her3
ErbB-4 Her4

Cysteine-rich domains

C-terminus

36
EGFR Signal Transduction in Tumor Cells
Available Forms of Anti-EGFR Therapy

- Antibody-based
  - Cetuximab
- Small molecule TKI
  - Gefitinib
  - OSI774
Therapy Against Other Targets

- Anti-angiogenic
  - anti–VEGF
    - bevacizumab
- Matrix metalloproteinase inhibitors
- Bisphosphonates
Bevacizumab for Breast Cancer

- Twenty percent clinical benefit in advanced breast cancer

Advanced breast cancer

Paclitaxel

Paclitaxel

Bevacizumab
No effect on time to progression

CR, PR, or SD after chemo

Marimastat

Placebo
Application of Arrays

- Different profile of sporadic versus hereditary breast cancer
  - Heldenfalk et al. (2001), NEJM
- Identify subset of young women with poor prognosis early breast cancer
  - van’t Veer et al. (2002), Nature
- Lack of profile for response to doxorubicin
  - Perou et al. (2000), Nature
Onco
type DX Breast Cancer Assay

- Now available—$3400
- Should we use it?
- For whom?
- How?
Candidate Gene Selection

- Candidate gene selection from ~40,000 genes

Example Papers:
- Van 't Veer et al. (2002). Nature; 415:530;
- Ramaswamy, et al. (2003). Nature Genetics; 33:4;
### Three Breast Cancer Studies Used to Select

#### Sixteen Cancer and Five Reference Genes

<table>
<thead>
<tr>
<th>PROLIFERATION</th>
<th>INVASION</th>
<th>HER2</th>
<th>ESTROGEN</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>Stromelysin 3</td>
<td>GRB7</td>
<td>ER</td>
<td>Beta-actin</td>
</tr>
<tr>
<td>STK15</td>
<td>Cathepsin L2</td>
<td>HER2</td>
<td>PGR</td>
<td>GAPDH</td>
</tr>
<tr>
<td>Survivin</td>
<td></td>
<td>GSTM1</td>
<td>Bcl2</td>
<td>RPLPO</td>
</tr>
<tr>
<td>Cyclin B1</td>
<td></td>
<td>CD68</td>
<td>SCUBE2</td>
<td>GUS</td>
</tr>
<tr>
<td>MYBL2</td>
<td></td>
<td>BAG1</td>
<td></td>
<td>TFRC</td>
</tr>
</tbody>
</table>

*Best RT-PCR performance and most robust predictors*

Three Breast Cancer Studies Used to Develop Recurrence Score (RS) Algorithm

<table>
<thead>
<tr>
<th>RS =</th>
<th>+0.47 x HER2 Group Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.34 x ER Group Score</td>
</tr>
<tr>
<td></td>
<td>+1.04 x Proliferation Group Score</td>
</tr>
<tr>
<td></td>
<td>+0.10 x Invasion Group Score</td>
</tr>
<tr>
<td></td>
<td>+0.05 x CD68</td>
</tr>
<tr>
<td></td>
<td>-0.08 x GSTM1</td>
</tr>
<tr>
<td></td>
<td>-0.07 x BAG1</td>
</tr>
</tbody>
</table>

Continued
### Three Studies Develop Recurrence Score (RS) Algorithm

<table>
<thead>
<tr>
<th>Recurrence Category</th>
<th>RS (0–100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>&lt;18</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>18–30</td>
</tr>
<tr>
<td>High risk</td>
<td>• 31</td>
</tr>
</tbody>
</table>
**Objective**
- Validate Recurrence Score as predictor of distant recurrence in N-, ER+, tamoxifen-treated patients

**Design**
- Randomized
  - Placebo—Not Eligible
  - Tamoxifen—Eligible
- Registered
  - Tamoxifen—Eligible
- Pre-specified 21 gene assay, algorithm, endpoints, analysis plan
- Blinded laboratory analysis of three 10 µ sections
B14 Clinical Results

DRFS—All 668 Patients

10 year DRFS = 85%

Source: Adapted by CTLT from Paik et al. (Dec. 30, 2004), N Engl J Med.
Study Design

- B-14 Results
  - First Primary Objective
    - Validate that 10 year DRFS in the low-risk group (RS < 18) is significantly higher than 10 year DRFS in the high risk group (RS ≥ 31)
    - Assuming: binomial test for differences in proportions = 0; α = 0.05; 600 evaluable patients—240 low-risk patients with DRFS 0.90 and 150 high-risk patients with DRFS 0.70; then power >95%

Source: Adapted by CTLT from Paik et al. (Dec. 30, 2004), N Engl J Med.
## B-14 Results

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Percentage of Patients</th>
<th>10-year Rate Recurrence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (RS &lt; 18)</td>
<td>51%</td>
<td>6.8%</td>
<td>4.0%, 9.6%</td>
</tr>
<tr>
<td>Intermediate (RS 18–30)</td>
<td>22%</td>
<td>14.3%</td>
<td>8.3%, 20.3%</td>
</tr>
<tr>
<td>High (RS • 31)</td>
<td>27%</td>
<td>30.5%</td>
<td>23.6%, 37.4%</td>
</tr>
</tbody>
</table>

*Test for the 10-year DRFS comparison between the low- and high-risk groups: p<0.0001*

Source: Adapted by CTLT from Paik et al. (Dec. 30, 2004), N Engl J Med.
DRFS—Low-, Intermediate-, High-RS Groups

Source: Adapted by CTLT from Paik et al. (Dec. 30, 2004), N Engl J Med.
Recurrence Score As a Continuous Predictor

Source: Adapted by CTLT from Paik et al. (Dec. 30, 2004), N Engl J Med.
Potential Applications for Breast Cancer Biology

- Predict risk of cancer development
- Estimate prognosis for established cancer
- Predict response to therapy
- Identify therapeutic targets