Hormonal Therapy (HT) of Breast Cancer

- Majority of breast cancers (75%) are estrogen receptor (ER) α-positive
- HT (tamoxifen or aromatase inhibitors) are effective in about half
- Resistance can develop more than 5-10 years after diagnosis
- Late recurrences may represent slow growth, tumor dormancy, outgrowth of subpopulations, and/or development of acquired resistance

HT for First-Line Treatment of ER+ Breast Cancer

- Aromatase inhibitors
- Tamoxifen
  - De novo resistance
  - Response
- Switch HT
- Fulvestrant
  - Acquired resistance
  - Response
- Genomics/Proteomics
Outgrowth of Subclones: "Life History of an ER+ BC"

- 188-fold depth sequencing
- >70,000 base changes
- Driver (trisomy 1q) earliest change
- Subclonal evolution (n=4)

Loss of ER\textsubscript{α} Expression is Not Major Mechanism of Acquired Resistance

- The majority of resistant tumors are ER\textsubscript{α}+ when resistance develops
- Discordance rates for primary vs recurrence are 10-18%
- Mechanisms:
  - Promoter methylation (HDAC inhibitors)
  - MAPK signaling (MEK inhibitors)
  - FOXO3
  - PAX2
  - SNAIL
  - TWIST

Acquired Hormone Resistance (HR)

- Complex adaptive process due in part to selective pressure of drugs (de novo vs acquired)
- Inappropriate activation of the ER pathway
- "Escape" proliferation and survival stimuli
- Dormancy
- Mechanisms:
  - Drug metabolism
  - Redundant survival pathways (PI3K, AKT, MAPK)
  - Growth factor crosstalk with ER\textsubscript{α} (EGFR, HER2, IGF-IR)
Dormancy: It's Not Just Low Proliferation

Table 2. Dormancy scores of ER positive and ER negative tumors.

<table>
<thead>
<tr>
<th>Study</th>
<th>P value*</th>
<th>P value from stratified test²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van der Velden et al 1999</td>
<td>0.0001</td>
<td>F = 0.0000091 (Z = 0.2)</td>
</tr>
<tr>
<td>Wang et al. 1999</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>Liu et al. 1998</td>
<td>0.0012</td>
<td></td>
</tr>
</tbody>
</table>

- Luminal B tumors have a 2.6-fold decrease in probability of metastasizing if LOW dormancy score

Kim, Plos ONE, 2012

Acquired Resistance: Clinical Targets

IGF-1R, EGFR, HER2

Brodie and Sabnis, Clin Can Res, 2011

EGFR Inhibitor Gefitinib

Preclinical Tumor Xenograft Data:
HT Naïve Cell Line

<table>
<thead>
<tr>
<th>Volume (mm3)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td>400</td>
<td>20</td>
</tr>
<tr>
<td>600</td>
<td>30</td>
</tr>
<tr>
<td>800</td>
<td>40</td>
</tr>
</tbody>
</table>

Clinical Data in Patients:
AI vs. AI + G
HR 0.55, median PFS 14.7 vs. 8.4 mo.
Post hoc subset: more pronounced benefit in HT naïve

Cristofanilli, Clin Can Res, 2001

Learned Lessons:
Test agents in biomarker-confirmed patients
Heterogeneity and metastasis is more complex than expected

Slide courtesy of Kent Osborne

Arpino, JNCI, 2007
What About ERα in HER2+ BC?

HER2-Enriched ≠ Luminal B

PI3K/Akt Pathway: Integration of RNA/DNA/Protein

It's Not Just Mutation Status, But Pathway Activation (Phospho Protein)
But what we need is a way to identify the dominant metastatic mechanisms activated as a consequence of HT.
**Tumor Heterogeneity and Metastasis During HT**

**Examples of whole genome sequencing in Neoadjuvant AI**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>preKi67</th>
<th>postKi67</th>
<th>Mutation Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low</td>
<td>High</td>
<td>273 (de novo resistant)</td>
</tr>
<tr>
<td>2</td>
<td>High</td>
<td>Low</td>
<td>0</td>
</tr>
</tbody>
</table>

Implications:
- Multiple tumor subclones with different metastatic repertoires
- Mutations in post HT biopsy may be one way to identify “driver targets”
- Should tumor biopsy samples be made mandatory for future translation?

*Ellis EJ. JCO 2011
Ellis, SABCS, 2011*

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**“Super Pathways” – generating information on mechanisms of response**

- Merge all small pathways using an exhaustive breadth-first search of the current database of pathways

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**Pathway Based Aromatase Inhibitor Signature of Response (Ki67-Proliferation in Residual Disease)**

Suggests that response predictors may not be the same as the tumor initiating/driving mutational events

*Ellis MJ, Nature, 2012*
Whole Genome Sequencing of Tamoxifen-Resistant Breast Cancer Cells

Model for HER2's Role in Resistance: Activation of pER

HER2 Overexpression Increases Phosphorylation of S118 in ERα
**EGFR Inhibitor (Erlotinib) Inhibits pS118 ERα**

Presurgical Window

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**Mechanism of Hormone Resistance:**

**Phosphorylation of ERα at S305**

Growth Factor Receptors (IGF-1R)

\[
\text{PI-3K} \rightarrow \text{Rho-GTP} \rightarrow \text{Rho GDI}_\alpha
\]

\[
\text{ER}^\alpha \rightarrow \text{PAR-2} \rightarrow \text{Restores Hormone Sensitivity} \rightarrow \text{Metastatic}
\]

Guri, Can Res, 2004; Barone, Can Res, 2006; Barone, JNCI 2011

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**ERα S305 Phosphorylation With PKA Activation**

Implications:

It's not just the signaling, but whether it activates ERα

Kok, BCRT, 2011
ERα Phosphorylation Underlies Clinical Diversity of BC?

Held, Mol Cancer Res, 2012

Summary and Future Insights Into Hormone Resistance

- Translational discovery in residual and metastatic tumors after HT is required to identify the next generation of clinical targets to treat resistance
- Predict requires combination therapy of resistance PLUS metastatic pathways (e.g., RHO/MTA2, SRC, IGF-IR, MET)
- Novel approaches to also block ERα activation (e.g., phosphorylation) are needed for complete HT blockade

Thank-you

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