Resistance to Endocrine Therapy in ER+ Breast Cancer: Insights from Bedside to Bench Studies

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Disclosures

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Breast Cancer Clinical Subtypes

- **HER2 (ERBB2)**: 20%
- **Triple Negative**: 15%
- **ER**: 75%

**Targeted Therapy**

- **Endocrine Therapy** (tamoxifen, aromatase inhibitors, fulvestrant)
- **Anti-HER2 Therapy** (trastuzumab, lapatinib, pertuzumab, T-DM1)
- **Chemotherapy**
Antiestrogen Therapies

- Reduction of estradiol levels in host and tumor
  - Aromatase inhibitors (anastrozole, letrozole, exemestane) and surgical or medical ovariectomy (LHRH superagonists)
- Selective ER modulators (SERMs) with agonistic and antagonistic activity
  - Tamoxifen, raloxifene
- Pure antagonistic activity (partial downregulation of ER)
  - Fulvestrant, other investigational drugs
ER+ breast cancers exhibit a long-term risk of recurrence

By gene expression, ER+ breast cancers can be Luminal A or Luminal B (B is not that good)

Luminal B
Lower ER levels
PR negative
Higher mitotic rate
Higher tumor grade
TP53 mutations (?)
PAM50 score
Mammaprint

Sorlie et al. PNAS 100:8418-23, 2003
CANCER

Genomic profiling of ER+ breast cancers after short-term estrogen suppression reveals alterations associated with endocrine resistance

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Short term estrogen deprivation with letrozole inhibits tumor cell proliferation (Ki67) in ER+ breast cancers.

10-25 ROIs per tumor
>10,000 nuclei counted
Around 20% of ER+ cancers retain high proliferation (Ki67) after short term estrogen suppression and harbor somatic alterations associated with drug resistance.

2-wk Ki67

**Sensitive (54%)**
Ln ≤1.0 (≤2.7%)

**Intermediate (24%)**
Ln 1.1-1.9 (2.8-7.3%)

**Resistant (22%)**
Ln ≥2.0 (≥7.4%)
Most frequent recurrent somatic alterations associated with resistance to estrogen deprivation (letrozole)

**ESR1**
- Y537S
- D538G
- V442del
Overexpression of FGFR1 attenuates response to estrogen deprivation and to fulvestrant.

*** p<0.001
ER+/FGFR1-amplified patient-derived xenografts are potently inhibited by fulvestrant and FGFR TKI lucitanib

Comparison of mutational frequency between ER+ metastatic vs. primary tumor (in TCGA) suggest cancers change over time

Fisher's exact test

Enrichment

Mutation Type:
- SNV
- High amplification
- Biallelic inactivation
- TSGs

Significance

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MBC N=149
TCGA N=739

Courtesy of Nikhil Wagle, MD (presented at SABCS 2015)
### Mechanisms of endocrine resistance

- **HER2 amplification**
- Ligand-independent ER, **CCND1** amplification
- **PIK3CA** mutations
- **FGFR1** amplification
- **ESR1** mutations, amplification, fusions
- **HER2** mutations

### Targeted therapies

- Trastuzumab, lapatinib, T-DM1
- CDK4/6 inhibitors (palbociclib)
- TORC1 inhibitors (everolimus), pan-PI3K and PI3Kα inhibitors
- FGFR inhibitors
- Fulvestrant (?), novel ER antagonists/degraders
- Neratinib
Extraordinary response of patient with breast cancer to HER2 (ERBB2) tyrosine kinase inhibitor neratinib

ERBB2 mutant (L755_E757delinsS) ER+/HER2– breast carcinoma

Baseline 8 weeks 16 weeks

Confirmed PR: 70% reduction by RECIST following neratinib monotherapy
ERBB2 (HER2) amplifications and mutations across cancer types
HER2 mutations confer resistance to estrogen deprivation (aromatase inhibitors) and to fulvestrant.

**Growth in Estrogen Deprivation**

![Bar graph showing cell count in estrogen deprivation](image)

**Long Term Estrogen Deprivation**

![Images showing cell morphology](image)

**Growth in 1 μM Fulvestrant**

![Bar graph showing cell viability in fulvestrant](image)

**ERE Reporter Assay**

![Bar graph showing fold change in ERE reporter assay](image)
Estrogen rescues ER+/-HER2 mutant cells: Combined blockade of HER2 and ER is required

Croessman S, ….., Arteaga CL. Unpublished
Exceptional response to dual blockade of mutant HER2 and ER in a patient with ER+/HER2 lobular breast cancer
SUMMIT Trial: CR following neratinib + fulvestrant

*ERBB2* mutant (S310F) ER+/HER2– lobular breast cancer

Complete response by RECIST and PET response criteria after 8 weeks

Patient had progressed on prior fulvestrant therapy
# Targeted therapies against mechanisms of endocrine resistance

## Mechanisms of endocrine resistance

- **HER2 amplification**
- **Ligand-independent ER, *CCND1* amplification**
- **PIK3CA mutations**
- **FGFR1 amplification**
- **ESR1 mutations, amplification, fusions**
- **HER2 mutations**

## Targeted therapies

- **Trastuzumab, lapatinib, T-DM1**
- **CDK4/6 inhibitors (palbociclib)**
- **TORC1 inhibitors (everolimus), pan-PI3K and PI3Kα inhibitors**
- **FGFR inhibitors**
- **Fulvestrant (?), novel ER antagonists/degraders**
- **Neratinib**
TCGA: PI3K pathway mutations are the most common in breast cancer, particularly in ER+ (luminal) tumors.
PIK3CA (p110α) mutations are gain-of-function oncogenes

Chakrabarty et al. Oncogene 2010
PIK3CA hot spot mutations enhance natural activation of p110α

Burke et al. PNAS 109:15259, 2012
Combined inhibition of ER and PI3K induces complete regression of ER+/PIK3CA-mutant MCF7 LTED xenografts

Some reasons why PI3K inhibitors are not inducing exceptional responses (as many expected them to do)

- Insulin production is increased upon inhibition of PI3K
- Therapeutic inhibition of PI3K is followed by compensatory upregulation of several RTKs (ERBB receptors, Ins/IGF-IR, FGFRs), ERα, BCL2
- PI3Kα inhibitors are not mutant specific so they also inhibit the wild type enzyme
- ‘Dialing up’ inhibition of PI3K causes severe rash and hyperglycemia, thus inhibition of PI3K is suboptimal and transient
Combination of PI3Kα inhibitor alpelisib and letrozole is active against breast cancers with mutant PIK3CA.
PIK3CA hot spot mutations enhance natural activation of p110α

Burke et al. PNAS 109:15259, 2012
**PIK3CA C2 domain deletions disrupt binding of p110α to p85**

- **V448 : Y467** (backbone to sidechain H-bond)
- **H450 : Y467** (backbone to sidechain H-bond)
- **H450 : Y463** (sidechain to sidechain aromatic)
- **H450 : Y467** (sidechain to sidechain aromatic)
- **D454 : R348** (sidechain to sidechain salt bridge)
- **D453 : R348** (backbone to sidechain H-bond)

**Average Binding Energy of Top 10 Computational Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Average Binding Energy dG</th>
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<tbody>
<tr>
<td>PIK3CA WT</td>
<td>-80.0</td>
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<tr>
<td>447 del</td>
<td>-70.0</td>
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<tr>
<td>450 del</td>
<td>-75.0</td>
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</tbody>
</table>

**Western Blot**

- IP: V5
- IB: GAPDH
- Croessmann S, et al. In review
PIK3CA C2 deletions are activating and respond to PI3Kα inhibitors
Implications

- There are \textit{PIK3CA} mutations outside the known gene ‘hot spots’ that also associate with tumor PI3K dependence and clinical benefit to PI3K inhibitors that can be easily missed by assays that do not sequence the whole \textit{PIK3CA} gene.

- Mutations in \textit{PIK3R1} (p85\textsubscript{\alpha}), the regulatory subunit of PI3K, that disrupt the association with \textit{PIK3CA} (p110\textsubscript{\alpha}) should also respond to PI3K inhibitors.
CDK4/6 Is Required for Estrogen-Independent Growth of ER+ Breast Cancer Cells

Dual blockade of the ER pathway with ER and CDK4 inhibitors
CDK4 inhibitor palbociclib and fulvestrant synergize against ER+ breast cancer xenografts
First-Line, No Prior Endocrine Therapy

**PALOMA2**

- Number of Events: 194 (44%) for PAL + LET (N = 444) vs. 137 (62%) for PCB + LET (N = 222).
- Median (95% CI) PFS: 24.8 (22.1-NR) for PAL + LET vs. 14.5 (12.9-17.1) for PCB + LET.
- HR (95% CI); 1-sided P value: 0.58 (0.46-0.72); \( P < .000001 \).

**MONALEESA2**

- Probability of Progression-free Survival:
  - Ribociclib group: Hazard ratio, 0.56 (95% CI, 0.43-0.72) with \( P = 3.29 \times 10^{-6} \) for superiority.
  - Placebo group:

<table>
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<tr>
<th>Month</th>
<th>Ribociclib</th>
<th>Placebo</th>
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<tr>
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<td>334</td>
<td>334</td>
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<tr>
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<td>294</td>
<td>279</td>
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<td>4</td>
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<tr>
<td>24</td>
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</table>


ORF kinome screen in MCF7 cells treated with fulvestrant ± CDK4/6 inhibitor ribociclib

Formisano L, ….., Arteaga CL. Unpublished
FGFR1 overexpression drives resistance to fulvestrant and palbociclib.
FGFR inhibitor lucitanib enhances action of fulvestrant and palbociclib against MCF-7$^{FGFR1}$ xenografts
Combined inhibition of ER, CDK4/6 and FGFR1 potently inhibits growth of ER$_{\alpha}$/FGFR1-amplified breast cancers PDXs
FGFR pathway aberrations correlate with progression on therapy with antiestrogens plus CDK4/6 inhibitors

Guardant 360 (plasma tumor DNA): 14/34 (41%) FGFR pathway alterations:
- 9/34 FGFR1 amplification
- 1/34 FGFR2 amplification
- 1/34 FGFR1 mutation (N546K)
- 2/34 FGFR2 mutation (N549K)

ER+/HER2- (n=34) → Progression → Palbociclib + Endocrine therapy → Guardant 360

<table>
<thead>
<tr>
<th>Legend</th>
<th>Group</th>
<th>N</th>
<th>Events</th>
<th>Median PFS (95% CI)</th>
<th>HR (95% CI)</th>
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<tr>
<td></td>
<td>PBO+Let Wt</td>
<td>205</td>
<td>139</td>
<td>14.59 (12.85 - 16.43)</td>
<td>0.47 (0.36 - 0.62)</td>
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<tr>
<td></td>
<td>LEE+Let Wt</td>
<td>202</td>
<td>88</td>
<td>24.84 (22.21 - 30.26)</td>
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<tr>
<td></td>
<td>PBO+Let Alt</td>
<td>10</td>
<td>9</td>
<td>11.43 (5.45 - 19.15)</td>
<td>0.73 (0.23 - 2.29)</td>
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<tr>
<td></td>
<td>LEE+Let Alt</td>
<td>10</td>
<td>6</td>
<td>10.61 (2.69 - NA)</td>
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</tbody>
</table>
Breast Cancer: 25 pts with amplification (22 with FGFR1, with FGFR2) 3/25 PRs (Investigator’s Brochure)

<table>
<thead>
<tr>
<th></th>
<th>JNJ-42756493 IC₅₀ (nM)</th>
<th>BGJ398 IC₅₀ (nM)</th>
<th>AZD4547 IC₅₀ (nM)</th>
<th>Lucitanib IC₅₀ (nM)</th>
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<tr>
<td>FGFR1</td>
<td>&lt; 1</td>
<td>4.55</td>
<td>&lt; 1</td>
<td>21</td>
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<tr>
<td>FGFR2</td>
<td>&lt; 1</td>
<td>28.1</td>
<td>&lt; 1</td>
<td>41</td>
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<tr>
<td>FGFR3</td>
<td>1.05</td>
<td>19.5</td>
<td>2.52</td>
<td>51</td>
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<tr>
<td>FGFR4</td>
<td>&lt; 1</td>
<td>376</td>
<td>40.6</td>
<td>---------</td>
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<tr>
<td>FGFR3 (G697C)</td>
<td>1.90</td>
<td>28.8</td>
<td>5.25</td>
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<tr>
<td>VEGFR1</td>
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<td>1</td>
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<tr>
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<td>1.1</td>
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<td>VEGFR3</td>
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<td>7.1</td>
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<tr>
<td>PDGFRα</td>
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<td>---</td>
<td>---</td>
<td>0.43</td>
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<tr>
<td>PDGFRβ</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.26</td>
</tr>
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</table>

Tabernero et al. JCO 33:3401-3408, 2015
Phase Ib/II trial of fulvestrant + a CDK4/6 inhibitor + pan-FGFR inhibitor in ER+/FGFR(1-4) amplified metastatic breast cancer

Postmenopausal women with FGFR-altered*/ER+/HER2– locally advanced or metastatic breast cancer that progressed on/after AI therapy
N ~ 100 (phase Ib/II)

Randomization (2:1)
(after determination of MTD/ RP2D for FGFR inhibitor + CDK4/6 inhibitor + fulvestrant combination)

Primary Endpoints
- Safety, pharmacokinetics (phase Ib)
- Clinical Benefit Rate for 6 months (phase II)

Secondary Endpoints
- Progression Free Survival
- Overall response rate

Exploratory Endpoints
- Concordance of NGS and ctDNA (focusing on FGFR1, FGFR2, FGFR3, FGFR4, CCND1, CCND2, CDK4, CDK6, RB1 and ESR1 point mutations, CDK4, CDK6, FGFR1, FGFR2 amplifications)
- FGFR1 FISH
- PD Assessments (serum phosphate, sVGFR1, sVGFR2, FGF23, sFGFR2, sFGFR3 and sFGFR4)

* FGFR alteration = FGFR1-4
## Targeted therapies against mechanisms of endocrine resistance

### Mechanisms of endocrine resistance

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<td>Neratinib</td>
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