S3-05
Patient-derived xenograft study reveals endocrine therapy resistance of ER+ breast cancer caused by distinct ESR1 gene aberrations

- **Dr. Shao:** Nothing to disclose.
- **Dr. Li:** Nothing to disclose.
- **Dr. Crowder:** Nothing to disclose.
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Dr. Mardis: Nothing to disclose.

Dr. Ellis: Ownership Interest, Holds equity interests and leader positions in Biodassifier and University Genomics. He is also listed as an inventor on patent applications pertaining to the PAM50 gene signature.
S3–05. Patient-Derived Xenograft Study Reveals Endocrine Therapy Resistance of ER+ Breast Cancer Caused by Distinct ESR1 Gene Aberrations


Siteman Cancer Center Breast Cancer Program and The Genome Institute, Washington University in St Louis and *Lineberger Cancer Center, University of North Carolina, Chapel Hill

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Patient-Derived Xenograft (PDX) models

Adapted from the HAMET (Human and Mouse Linked Evaluation of Tumors) core website

Key advantages:
1. Genomic integrity and pharmacological characteristics are preserved upon transplantation.
2. Allows genomic events to be studied in an authentic context.

Shunqiang Li, Ph.D.

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Endocrine-Therapy-Resistant ESR1 Variants Revealed by Genomic Characterization of Breast-Cancer-Derived Xenografts

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Gain-of-function genomic events in the ESR1 gene observed in PDX models from endocrine therapy resistant ER+ breast cancer

- Point mutation
- Gene translocation
- Gene amplification

Li et al. Cell Reports, 2013 Sep 25;4(6):1116-30

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Case 1 (ESR1 Y537S)

- 36 year old Stage 3 ER+/HER2- disease
- Mastectomy
- Adjuvant chemotherapy and radiation
- Tamoxifen
- Recurrence at 26 months post diagnosis
- Letrozole 20 months
- Fulvestrant 4 months
- Grafted sample PDX (WHIM20)
- Temsirolimus one month
- Exemestane one month
- Palliative chemotherapy
- Died after 65 months

Y537N: an activating ESR1 mutation described in 1997 by Zhang et al.

Li et al. Cell Reports, 2013 Sep 26;4(6):1116-20

Ligand-binding domain mutations are frequent in aromatase inhibitor-resistant breast cancer

ESR1

<table>
<thead>
<tr>
<th>AF1</th>
<th>DBD</th>
<th>Hinge</th>
<th>AF2/LBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154</td>
<td>250</td>
<td>350</td>
</tr>
</tbody>
</table>

Metastatic samples (22%):
- 6 of 11 (55%) by Robinson et al, 2013
- 9 of 36 (25%) by Toy et al, 2013
- 5 of 44 (11%) in BOLERO Trial, 2013

Primary Samples (<1%):
- 6 of 183 (3%) in BOLERO Trial
- 0 of 46 (0%) by Ellis et al., 2012
- 0 of >500 (0%) in TCGA
Key structural and pharmacological properties of ESR1 (Y537S or D538G) mutants

1. The agonist ESR1 conformation is conferred by aberrant hydrogen bonding between mutant residues in helix-12 and the D351 residue in helix-3.

2. LBD mutations of ESR1 are therefore aromatase inhibitor-resistant and less sensitive to standard anti-estrogens (e.g. tamoxifen and fulvestrant).

Robinson et al., Nature Genetics 2013, Dec, 45(12):1446-51

PDX tumor harboring ESR1(Y537S) responds to high doses of fulvestrant in vitro

Implication: higher doses of fulvestrant or alternative ER down-regulators could be effective in suppressing ESR1 mutants.

Unpublished (Ellis laboratory), not for reproduction

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Gain-of-function genomic events in the ESR1 gene observed in PDX models from endocrine therapy resistant ER+ breast cancer

- Point mutation
- Gene translocation
- Gene amplification

Case 2 (ESR1/YAP1 fusion)

- 57 year old ER+/HER2-
- Stage 4
- Letrozole 9 months
- Exemestane 3 months
- fulvestrant 1 month
- Sample for PDX (WHIM18)
  - Palliative chemotherapy
  - Died after 31 months

Li et al. Cell Reports, 2013 Sep 26;4(6):1116-30

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ESR1/YAP1 fusion

Li et al. Cell Reports, 2013 Sep 26;4(6):1116-30

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ESR1/YAP1 is constitutively active

ERE Transcription Assay    Hormone-deprived T47D cells

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ESR1/YAP1 associates with estradiol-independent and fulvestrant-resistant tumor growth

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ESR1 fusions observed in TCGA data

POLH: polymerase (DNA directed), eta
AKAP12: A Kinase (PRKA) Anchor Protein 12

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Two ESR1 fusions observed in TCGA RNA-seq.

ERE Luc Assay

POLH: polymerase (DNA directed), eta
AKAP12: A Kinase (PRKA) Anchor Protein 12

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Diverse ESR1 gene alterations captured in our ER+ endocrine resistant PDX models

- Point mutation
- Gene translocation
- Gene amplification

Li et al. Cell Reports, 2013 Sep 25;4(6):1116-30

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Case 3 (ESR1 gene amplification)

- 63 year old, Stage 3A, ER+/HER2-
- Adjuvant chemotherapy, surgery and radiation
- Exemestane 2 years
- Fulvestrant 4 month
- Capecitabine 8 months
- Estradiol 4 months
- Sample for PDX (WHIM16)
- Palliative chemotherapy
- Died after 68 months

Li et al. Cell Reports, 2013 Sep 26;4(6):1116-30

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ESR1 gene amplification causes high-level ESR1 protein expression

Q-PCR on genomic DNA

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ESR1 gene amplification is associated with the paradoxical antitumor effect of estradiol

**Implications:**

1. ESR1 gene amplification may underlie “Haddow’s paradox”: the antitumor effect of estrogenic compounds.
2. ESR1 gene amplification may be an acquired resistance mechanism in response to long-term hormone deprivation.
3. Both estradiol and anti-estrogens may be effective in treating tumors harboring ESR1 gene amplification.

**Lower-Dose vs High-Dose Oral Estradiol Therapy of Hormone Receptor–Positive, Aromatase Inhibitor–Resistant Advanced Breast Cancer A Phase 2 Randomized Study**

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Ellis et al., JAMA 2009, Aug 19;302(7):774-80.

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Conclusions and Future Directions

- Low estradiol **PDX models** captured distinct ESR1 gene aberrations causing endocrine therapy resistance of ER+ breast cancer.

- Each class of ESR1 gene alteration has a different therapeutic implication.
  - **Ligand-binding domain mutations** may be treatable with higher doses of fulvestrant or alternative anti-estrogens with higher potencies but not estrogen deprivation (aromatase inhibitors).
  - **Gene translocations** cannot be treated with classic endocrine therapies and will require alternative therapies.
  - **Gene amplification** could be treatable with both estradiol and anti-estrogens but not estrogen deprivation (aromatase inhibitors).

- Further study of **endocrine therapy refractory** breast cancer is required to understand the incidence and mechanism of ESR1 gene alterations and to develop effective therapies.

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