Morphologic and genomic characterization of circulating tumor cells in patients with ERBB2 mutant HER2 non-amplified metastatic breast cancer treated with neratinib

- Eliminating the unknown of cancer
- Giving confidence in cancer care

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ERBB2 mutant HER2 non-amplified breast cancer

- **ERBB2 mutations** → aggressive breast cancer phenotypes
  - Extremely rare in the absence of gene amplification (2-4% of all cases).
  - Patients do not respond to HER2+ targeting standard of care treatment plans.

- **Neratinib**: an irreversible HER2/EGFR tyrosine kinase inhibitor
  - Clinical activity shown on heavily pre-treated ERBB2 mutant breast cancer patients.
  - Combinational treatment of Neratinib with chemotherapy has shown a response rate of 55% for HER2+ breast cancer patients.

- Using the **HD-SCA workflow** we have evaluated the clinical response to Neratinib/Fulvestrant combinational treatment in relation to enumeration and characterization of circulating tumor cells (CTCs), as well as genomic analysis of the cfDNA.
  - 5 postmenopausal patients with metastatic ERBB2 mutant/non-amplified breast cancer
    - Average of 5.4 lines of therapy prior to enrollment
    - Peripheral blood samples collected at multiple time points
    - Single cell genomic analysis by copy number variation (CNV) profiling and ERBB2 targeted mutational analysis
    - cfDNA genomic analysis by CNV and targeted mutational profiling using the OncoMine panel
The HD-SCA workflow

Primary Tumor → Whole Blood → Thaw and process → Prepare/Store → Scanning

High quality imaging

Single cell genomic analysis

Metastatic Tumor → Bone Marrow Aspirate

cfDNA genomic analysis

- CNV profiling
- Oncomine cfDNA Assay on Ion Torrent

- CNV profiling
- ERBB2 mutational analysis
ERBB2 mutation and clinical timelines

A  Excellent Responder

B  Average Responder

C  Non-Responder

D  Average Responder

E  

Pt1
S310F
Pt3
S653C
Pt4
L755S,V777L
Pt5
L755S
Pt2
P780_Y781ins
HD-CTC morphometrics per patient

A-E) 40x images of HD-CTCs detected per patient.
F) Distribution of HD-CTC morphometric parameters by patient.

- Pt 4 and 5 had minimal number of cells detected limiting our observations.
- Pt 1 and 2 have a highly heterogeneous population of cells.
- Pt 3 had a morphologically distinct population of HD-CTCs
  - More circular nuclei with higher CK expression
Single cell genomic analysis

• Excellent Responder: Pt1

- Tumor Pathology Report: ERBB2 S310F, ARID1A Q479*, CDH1 I581FS*1, NOTCH2 (SEC22B-NOTCH fusion)

- CNV analysis of 54 HD-CTCs, 24 cells identified to have genomic alterations.
  - Subclones persist from draw 4 to 10.
  - 2 unique subclones identified in draw 5.
  - Deep alterations may be due to homologous recombination, leading to amplification or deletion of one arm of the chromosome.

- ERBB2 SNV for S310F: 13/13 WT
Single cell genomic analysis

- **Average Responders:**

  **Pt2**
  - Tumor Pathology Report: **ERBB2 (P780_Y781ins)**, ARID1A (Q1172*), CDH1 (L585fs*4), NUP93 (E14K)
  - 9/21 HD-CTCs have genomic alterations
  - Lack of clonality
  - ERBB2 SNV for P780_781ins: 8/8 WT

  **Pt4**
  - Tumor Pathology Report: **ERBB2 (L755S, V777L)**, PIK3CA (H1047A)
  - Subclonal population of cells
  - Lack of genomic alterations
  - ERBB2 SNV for V777L: 1/1 WT

  **Pt5**
  - Tumor Pathology Report: **ERBB2 (L755S)**
  - Subclonal population of cells
  - Lack of genomic alterations
  - ERBB2 SNV for L755S: 1/1 WT
Non-Responder: Pt3

Tumor Pathology Report: ERBB2 (S653C), PIK3CA (E545K), PTPN11 (E76K)

Genomic analysis of 22 HD-CTCs isolated from Patient 3

Clonal population of cells.

The cell cycle appears to be completely unconstrained.
  - FGFR1 amplified
  - Cyclin-D amplified
  - Heterozygous loss of chromosome 11q containing CHK1 and ATM
  - Loss of P53
  - Loss on chromosome 22 containing CHK2.

ERBB2 SNV for S653C: 9/9 WT
cfDNA genomic analysis

- CNV profile for luminal A tumors from The Cancer Genome Atlas (TCGA).

- The cfDNA CNV profiles match the classic Luminal profile

- The same clonal architecture identified in the HD-CTCs was also detectable in the cfDNA, with a greater tumor fraction present in follow up sampling for Patients 1, 2, 3, and 5.

- The cfDNA from Patient 4 samples had a low to nondetectable tumor fraction, suggesting the tumor DNA was washed out by normal cellular DNA.
cfDNA OncoMine

**Excellent Responder: Pt1**

<table>
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<tr>
<th>Gene</th>
<th>Draw8</th>
<th>Draw10</th>
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<tr>
<td>ERBB2</td>
<td>-</td>
<td>S310F</td>
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<tr>
<td>TP53</td>
<td>R213* (0.47%)</td>
<td>R213* (4.5%)</td>
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- Increase in the fraction of detectable TP53 mutation during treatment
- Identification of ERBB2 mutation from tumor pathology report

**Average Responder: Pt2**

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<tr>
<th>Gene</th>
<th>Draw6</th>
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<tbody>
<tr>
<td>ERBB2</td>
<td>G776V</td>
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**Average Responder: Pt4**

<table>
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<tr>
<th>Gene</th>
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<th>Draw4</th>
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<tr>
<td>ERBB2</td>
<td>L755S, V777L</td>
<td>L755S, V777L</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>H1047R</td>
<td>H1047R</td>
</tr>
<tr>
<td>TP53</td>
<td>H365fs</td>
<td>-</td>
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**Average Responder: Pt5**

<table>
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<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>ERBB2</td>
<td>L755S</td>
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- New ERBB2 mutation identified in Pt2
- Analysis confirms the ERBB2 mutation from the tumor pathology report for Pt 4 and 5

**Non-Responder: Pt3**

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Draw2</th>
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<tbody>
<tr>
<td>ERBB2</td>
<td>-</td>
<td>L755S</td>
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<tr>
<td>ESR1</td>
<td>E380Q, Y537N</td>
<td>E380Q</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>E545K (2.7%)</td>
<td>E545K (30%), E726K</td>
</tr>
<tr>
<td>TP53</td>
<td>R248Q</td>
<td>R248Q, G245D</td>
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</table>

- Increase in the fraction of detectable mutations during treatment
- Identification of new mutations in ERBB2, PIK3CA, and TP53
- Oncomine panel does not detect mutations in transmembrane domain of ERBB2, potentially explaining the lack of the S653C mutation identified in the tumor.
Summary

• The HD-SCA workflow provides a comprehensive view of the complete liquid biopsy from metastatic breast cancer patients with ERBB2 mutations.

• The HD-SCA workflow may be used as a prognostic tool for therapeutic response to directly impact treatment decisions.
  ▪ Stratify patients into treatment arms
    • Patients with concurrent aberrations in cell cycle checkpoints driven by TP53 mutations are associated with a lack of clinical benefit.
    • Monitor tumor heterogeneity by identifying the frequency of mutations at a single cell level
  ▪ Monitor treatment response
    • Patients with stable disease had detectable CTCs that are morphologically and genomically heterogeneous
    • cfDNA analysis provides a general overview of the cellular population.

• Identified genomic aberrations in single CTCs and the cfDNA that may contribute to disease progression on Neratinib/Fulvestrant therapy.
We thank our patients who consented to this study. We also thank the clinical research staff who contributed to the study at USC Norris Comprehensive Cancer Center. We are grateful to past and current technical staff at the Kuhn Laboratory for processing of the blood samples.

This work was supported by the National Cancer Institute (NCI) and Leidos Biomedical Research, Inc. (contract agreements 12XS527 and 15X003) and of the NCI’s USC Norris Comprehensive Cancer Center (CORE) Support (grant # 5P30CA014089-40). The content of this presentation is solely the responsibility of the authors and does not necessarily represent the official views of the NCI or the National Institutes of Health.