Mechanisms of resistance to anti-HER2 agents

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Madrid, February 2015
HER2-Targeted Therapies

Advances in Metastatic Breast Cancer: Trastuzumab + Pertuzumab Historic Landmarks

[CANCER RESEARCH 64, 2343–2346, April 1, 2004]

Advances in Brief

The HER-2-Targeting Antibodies Trastuzumab and Pertuzumab Synergistically Inhibit the Survival of Breast Cancer Cells

Rita Nahta,1 Mien-Chie Hung,2 and Francisco J. Esteve3,2
1Department of Breast Medical Oncology, and 2Molecular and Cellular Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Trastuzumab and Pertuzumab:
Synergy in HER2+ BC cells


HER1, 3

HER2

Trastuzumab

Pertuzumab

Advances in Metastatic Breast Cancer: Trastuzumab + Pertuzumab Historic Landmarks

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Trastuzumab and Pertuzumab: Synergy in HER2+ BC cells1


Trastuzumab + Pertuzumab: Synergy in vivo, HER2+ xenografts2

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Advances in Metastatic Breast Cancer: Trastuzumab + Pertuzumab Historic Landmarks

Trastuzumab and Pertuzumab: Synergy in HER2+ BC cells¹


Trastuzumab + Pertuzumab: Approved for HER2+ MBC³

Trastuzumab + Pertuzumab: Synergy in vivo, HER2+ xenografts²

Trastuzumab + Pertuzumab: Approved for HER2+ ESBC (neoadjuvant)⁴

Improvements in Overall Survival Rates in HER2+ Metastatic Breast Cancer

First-line setting (CLEOPATRA n=808)  Trastuzumab+ pertuzumab+ docetaxel

Second-line setting (EMILIA n=991)  Trastuz + docetaxel  T-DM1  Lapatinib+ capecitabine

Third-/fourth-line (EGF104900 n=291)  Lapatinib+ trastuzumab

Median OS 56.5 months (vs. 40.8 mo)  Gain of 5.8 months  Gain of 4.5 months despite crossover in 52%

Most patients with metastatic breast cancer die because of progressive disease
What are the mechanisms of resistance?
Hallmarks of Cancer => Potential Mechanisms of Resistance to anti-HER2 Therapy

- Upregulation of signal transduction pathways
- Somatic mutations in HER2
- Epigenetics
- Altered Immune Response

Hanahan D and Weinberg RA. Cell 2011;144(5):646-674
The PI3K pathway and Breast Cancer

- Constitutive activation of the PI3K pathway is frequent.
- PI3K pathway activation conveys malignant transformation, cell growth and invasion, tumor neoangiogenesis and resistance towards anti-cancer treatments.
- Known mechanisms of PI3K pathway activation include activating mutations of RTKs, gain-of-function mutation of the PIK3CA gene, and loss-of-function mutations of PTEN.
PTEN-loss/PIK3CA mutation status and patients’ clinical outcomes after Trastuzumab-based therapy

Combining PTEN-loss with PIK3CA mutation status has a better prediction power for Trastuzumab resistance

Phase I/II Study of Trastuzumab in Combination With Everolimus (RAD001) in Patients With HER2-Overexpressing Metastatic Breast Cancer Who Progressed on Trastuzumab-Based Therapy

Phuong Khanh Morrow, Gerburg M. Wulf, Joe Ensor, Daniel J. Booser, Julia A. Moore, Peter R. Flores, Yan Xiong, Siyuan Zhang, Ian E. Krop, Eric P. Winer, David W. Kindelberger, Jeanna Coviello, Aysegul A. Sahin, Rodolfo Nuñez, Gabriel N. Hortobagyi, Dihua Yu, and Francisco J. Esteva

**Diagram:**
- Trastuzumab
- HER2
- PI3K
- PTEN
- src
- Everolimus
- TOR

**Best Response**

<table>
<thead>
<tr>
<th>N (%)</th>
<th>N = 47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (CR)</td>
<td>-</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Stable disease ≥ 24 weeks (SD)</td>
<td>9 (19%)</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Clinical benefit rate</td>
<td>16 (34%)</td>
</tr>
<tr>
<td>Median time to progression</td>
<td>3.4 months (Range 1-14)</td>
</tr>
<tr>
<td>Most frequent grade 3 / 4 adverse events (&gt; 10%)</td>
<td>Lymphopenia, hyperglycemia, mucositis</td>
</tr>
</tbody>
</table>
Everolimus in HER2+ Metastatic Breast Cancer: Randomized Phase III Trials

**Bolero-3**
O'Regan R., et al.
ASCO 2013

**Median PFS**
- Everolimus: 7.00 months
- Placebo: 5.78 months

**Vinorelbine/Trastuzumab**
2nd/3rd Line MBC

**Bolero-1**
Hurvitz S, et al.
SABCS 2014

**Paclitaxel/Trastuzumab**
1st Line MBC
## Effect of PTEN Levels on Treatment Benefit From the Addition of Everolimus

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Therapy</th>
<th>N (# of Events)</th>
<th>Median PFS, wks (95% CI)</th>
<th>HR (95% CI)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroups defined by low or normal PTEN level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-score ≥ 50</td>
<td>EVE</td>
<td>100 (72)</td>
<td>30.1 (24.3, 35.6)</td>
<td>0.97</td>
<td>(0.71, 1.33)</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>108 (85)</td>
<td>30.0 (24.0, 35.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-score &lt; 50</td>
<td>EVE</td>
<td>15 (11)</td>
<td>41.4 (17.3, 66.9)</td>
<td>0.52</td>
<td>(0.21, 1.26)</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>14 (11)</td>
<td>23.7 (10.6, 25.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroups defined by optimal cut-point of PTEN level (20th %ile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-score ≥ 20th %ile</td>
<td>EVE</td>
<td>89 (67)</td>
<td>30.1 (24.0, 35.3)</td>
<td>1.05</td>
<td>(0.75, 1.45)</td>
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<tr>
<td></td>
<td>PBO</td>
<td>100 (78)</td>
<td>30.1 (24.0, 36.0)</td>
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<tr>
<td>H-score &lt; 20th %ile</td>
<td>EVE</td>
<td>26 (16)</td>
<td>41.9 (24.0, 53.1)</td>
<td>0.41</td>
<td>(0.20, 0.82)</td>
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</tbody>
</table>

Median PFS gain is 18-19 weeks for the low PTEN subgroup

* Treatment-biomarker interaction.
PTEN optimal cut-point selected as ≥ and < 20th percentile. Histo-score = 100.
Patients With Low PTEN May Derive More Benefit From Everolimus

- Marker-treatment interaction ($P = .01$)

Patients With Low PTEN May Derive More Benefit From Everolimus

- Marker-treatment interaction ($P = .01$)

BOLERO-3: PIK3CA Mutations Did Not Significantly Affect Everolimus Treatment Benefit

* Not evaluable due to small sample size/event number.
Biomarkers of Response/Resistance to Trastuzumab + Pertuzumab

- TRYPHAENA, NeoSphere, CLEOPATRA trials showed HER2 is the only relevant biomarker for selecting patients for HER2-targeted therapy
- CLEOPATRA:
  - mutations in PIK3CA not associated with resistance to pertuzumab
  - PIK3CA mutational status may identify patients with worse prognosis
- NeoSphere: pCR following anti-HER2 antibody-based therapy was associated with high expression of one or more of the following: interferon gamma, STAT1, MHC2, CD8A and/or programmed cell death-1 (PD1)
- Neoaltto: PIK3CA mutations associated with resistance to trastuzumab + lapatinib (lower pCR in mutant vs. wild type)

## Selected Phase I/II Trials of PI3K/MTOR Inhibitors in HER2+ Metastatic Breast Cancer

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Phase</th>
<th>Sponsors</th>
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</thead>
<tbody>
<tr>
<td>Akt inhibitor MK2206</td>
<td>AKT</td>
<td>II</td>
<td>Albert Einstein of Yeshiva University; NCI</td>
</tr>
<tr>
<td>Akt inhibitor MK2206</td>
<td>lapatinib</td>
<td>AKT</td>
<td>I</td>
</tr>
<tr>
<td>Akt inhibitor MK2206</td>
<td>lapatinib</td>
<td>AKT</td>
<td>I</td>
</tr>
<tr>
<td>BEZ235</td>
<td>PI3K, mTOR</td>
<td>I</td>
<td>Novartis</td>
</tr>
<tr>
<td>BEZ235 and BKM120 in Combination With Paclitaxel With or Without Trastuzumab</td>
<td>PI3K, mTOR</td>
<td>I</td>
<td>Novartis</td>
</tr>
<tr>
<td>BKM120</td>
<td>PI3K</td>
<td>I/II</td>
<td>Novartis</td>
</tr>
<tr>
<td>Lapatinib and RAD001</td>
<td>mTOR</td>
<td>II</td>
<td>University of Kansas</td>
</tr>
<tr>
<td>XL147 plus trastuzumab or paclitaxel and trastuzumab</td>
<td>PI3K, mTOR</td>
<td>I/II</td>
<td>Exelisis</td>
</tr>
</tbody>
</table>

[http://www.clinicaltrials.gov](http://www.clinicaltrials.gov)
The Cancer Genome Atlas

Expression profiling (mRNA, miRNA)

Tumor phenotype

Tumor cells
Stroma cells
Lymphocytes

Tumor genotyping
(mutations, sequencing, amplifications, deletions)

Epigenetic
(Methylation of DNA)

INTEGRATIVE ANALYSIS

Clinical Outcomes

Functional Proteomics
Distribution of the intrinsic molecular subtypes of breast cancer in clinical HER2 status (cHER2)-negative and cHER2+ disease in the combined the cancer genome atlas and molecular taxonomy of breast cancer international consortium dataset. cHER2 = clinical HER2 status.

Prat A et al. JNCI J Natl Cancer Inst 2014;106:dju152
Kaplan-Meier 10-year breast cancer-specific survival analyses in the molecular taxonomy of breast cancer international consortium dataset (all patients).

Prat A et al. JNCI J Natl Cancer Inst 2014;106:dju152
10-year breast cancer-specific survival in the molecular taxonomy of breast cancer international consortium

Prat A et al. JNCI J Natl Cancer Inst 2014;106:dju152
B31 Trial: PAM50 and PIK3CA Status are not predictive of benefit from adjuvant trastuzumab

PTEN expression and outcomes (BCIRG-006)

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*

825 tumors; >400 tumors per platform (sequencing, mRNA, miRNA, methylation, RPPA)

Koboldt DC, et al. Nature 2012;000:1-10
HER2 somatic mutations in “HER2 negative” breast cancer

A

HER2 gene amplification negative stage IV breast cancer

Sequence tumor DNA for HER2 mutations

HER2 mutations present

Study therapy
Neratinib 240 mg p.o. daily

Restage every 8 weeks
Continue therapy until disease progression, or unacceptable adverse events

HER2 mutations absent

Not eligible for study therapy
Save tumor DNA for exploratory sequencing

B

ECD  TM JM  Kinase  Tall


HER mutations in “HER2 positive” breast cancer

Boulbes D, et al. Mol Oncol 2014 [Epub ahead of print]
L726F mutation confers lapatinib resistance in vitro

**Graphs**

- **MDA-MB-175**
- **SKBR3**
- **BT474-M1**

**Bar Graph**

Colony formation in soft-agar treated with Lapatinib 0.1uM

**Legend**

- pLVX
- WT
- L726F

**Western Blot**

- Lapatinib (μM)
- p-Erk1/2 wt
- p-Erk1/2 L726F
- p-308Akt wt
- p-308Akt L726F

**Results**

- L726F mutation confers lapatinib resistance in vitro
L726F mutation confers lapatinib resistance in vivo
12 tumor types

- Leukemia (LAML)
- Lung adenocarcinoma (LUAD)
- Lung squamous (LUSC)
- Kidney (KIRC)
- Bladder (BLCA)
- Endometrial (UCEC)
- Glioblastoma (GBM)
- Head and neck (HNSC)
- Breast (BRCA)
- Ovarian (OV)
- Colon (COAD)
- Rectum (READ)

Omics characterizations

- Mutation
- Copy number
- Gene expression
- DNA methylation
- MicroRNA
- RPPA
- Clinical data

Platforms

- Samples
- Genes/loci

Thematic pathways

Significantly recurrent focal somatic copy number alterations across solid tumors

NCI MATCH Trial

Currently >20 “arms”

EGFR, HER2, MET, BRAF, NF1, GNAQ, GNA11, TSC1/2, PTEN, Patch, NF2, ALK, ROS, FGFR
Epigenetics and HER2 Resistance

- PTEN loss
  - PTEN methylation
  - Upregulation of microRNAs, notably miR-21

- Role of IncRNAs? (unknown)

IncRNA Expression in Breast Cancer (TCGA)

TCGA cohort (n=869)

Filtering of non-infiltrating ductal carcinoma

Infiltrating ductal (n=690)

Filtering of breast cancer classified as normal-like

Infiltrating ductal (n=658)

13,159 IncRNA

Filtering of non-expressed IncRNA

1,623 IncRNA expressed

Unsupervised clustering

I

II

III

IV

GREAT, GSEA, correlation IncRNA-mRNA and chromatin marks
Molecular Portrait of IncRNA in Breast Cancer (TCGA)

Immune Response and Resistance to anti-HER2 Agents

- FcγRIIIa polymorphism is less effective at inducing antibody-dependent cell-mediated cytotoxicity upon trastuzumab binding to HER2
- CD40 gene set enriched in tumors from patients who achieved pCR after neoadjuvant trastuzumab-based chemotherapy
- High PDL1 and CTLA4 correlated with residual disease after neoadjuvant trastuzumab-based chemotherapy on the Neosphere trial

Immunotherapy and HER2 Targeted Therapy

Summary and Conclusions

• The mechanisms of resistance to HER2-targeted therapies in the adjuvant setting are not well understood
  • PTEN loss and PIK3CA mutations are not predictive

• Overcoming drug resistance remains a challenge in patients with metastatic HER2+ breast cancer
  • Biopsy and molecular profiling of tumors recurring after targeted therapies are critical to discover mechanisms of drug resistance

• The goal is to match molecular alterations in tumor with the right drug combination for individual patients to improve survival
## Acknowledgements

<table>
<thead>
<tr>
<th>New York University</th>
<th>MD Anderson Cancer Center</th>
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<tr>
<td>• Christina Adaniel</td>
<td>• Delphine Boulbes</td>
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<tr>
<td>• Adriana Heguy</td>
<td>• Gaurav Chauhan</td>
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<td>• Komal Jhaveri</td>
<td>• Xiaoping Su</td>
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<tr>
<td>• Alyssa Iwano</td>
<td>• Cindy Chen</td>
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<td>• Kyung Chu</td>
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Breast Medical Oncology, Surgical Oncology, Pathology collaborators

Breast cancer patients

Breast Cancer Research Foundation
Thank You!

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