PIK3CA Mutations in HER2-Positive Breast Cancer

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Contents

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  – TCGA data
  – HER2 signaling pathway & PIK3CA

• Preclinical data for PIK3CAmt

• Clinical data from mBC
• Clinical data from Neoadjuvant Trials
• Clinical data from Adjuvant Trials
• Summary
PIK3CA mutations and AKT activation by phosphorylation (pAKT) are often detected in many cancers and especially at high frequencies in breast cancer.
Integrated analysis of the PI(3)K, TP53 and RB1 pathways.

- PI(3)K pathway (390 tumours with mRNA/mutation/protein data)
  - PROCA mutation, PTEN, IGF1R, EGFR, ERBB2, PIK3CA, PIK3R1, PTEN, AKT1, MAP3K1, AKT3, MAP2K4, MAP3K1, PAK1, IKKα/β, CCND1

- TP53 pathway (506 tumours with mRNA/mutation data)
  - TP53 copy, CTNNB1 copy, CDKN1A mRNA, CDKN2A mRNA, CDK4 mRNA, GADD45A mRNA, MDM2 mRNA

- RB pathway (506 tumours with mRNA/mutation data)
  - RB1 mutation, RB1 LOH, RB1 copy, CDKN1A mRNA, CDKN2A mRNA, CDK4 mRNA, CHEK2, BRCA1, BRCA2

Module diagram:
- Inactivating
- Activating
- Not-basal
- Basal

Fingerprint:
- Somatic mutation
- Germline mutation
- Downregulation
- Upregulation
- Homozygous deletion
- High-level amplification
- Hyper-methylation

DC Koboldt et al. Nature 1-10, 2012
The HER2 signalling pathway

HER ligands
(AREG, EGF, TGFα)

IGF1R  EGFR  HER2  HER3

Adapted from:
The phosphorylated signaling tail at the C-terminus of HER3 then binds the PI3K p85 regulatory subunit, which in turn is bound to the catalytic subunit p110α. The p110α kinase catalyzes phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3; reversed by the PTEN lipid phosphatase), which binds AKT and recruits it to the membrane.

Shom Goel, and Ian E. Krop JCO 2015;33:1407-1409
Preclinical Data

HER2/PIK3CA\(^{H1047R}\) tumors are resistant to dual blockade of HER2

Hanker et al., PNAS 110: 14372, 2013
Clinical Implication of PIK3CA mt in mBC
CLEOPATRA: Study design¹,²

Patients with HER2-positive MBC
Centrally confirmed (N = 808)

- Primary endpoint: Independently-assessed progression-free survival (PFS)
- Collection of tissue and serum samples was mandatory
- Study dosing q3w:
  - Pertuzumab/placebo: 840 mg loading dose, 420 mg maintenance
  - Trastuzumab: 8 mg/kg loading dose, 6 mg/kg maintenance
  - Docetaxel: 75 mg/m², escalating to 100 mg/m² if tolerated

* < 6 cycles allowed for unacceptable toxicity or PD; > 6 cycles allowed at investigator discretion

HER2, human epidermal growth factor receptor 2; PD, progressive disease

1. Baselga J, et al. SABCS 2011 (Abstract S5-5);
Shorter median PFS with mutated PIK3CA

*PIK3CA* (wild-type vs mutant: 8 mutations – 420R, 542K, 545K, 545A, 545G, 1047R, 1047L, and 1047Y at four hotspots in exons 7, 9, and 20)

Shorter median PFS with mutated *PIK3CA*

![Graph showing shorter median PFS with mutated PIK3CA](image)

**n at risk**

<table>
<thead>
<tr>
<th></th>
<th>Pla+T+D WT</th>
<th>Pla+T+D Mut</th>
<th>Ptz+T+D WT</th>
<th>Ptz+T+D Mut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pla+T+D WT</td>
<td>191</td>
<td>164</td>
<td>136</td>
<td>114</td>
</tr>
<tr>
<td>Pla+T+D Mut</td>
<td>90</td>
<td>76</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>Ptz+T+D WT</td>
<td>190</td>
<td>179</td>
<td>159</td>
<td>137</td>
</tr>
<tr>
<td>Ptz+T+D Mut</td>
<td>86</td>
<td>71</td>
<td>61</td>
<td>44</td>
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</tbody>
</table>

Analyses of hotspots showed no difference between individual mutations.

Dual blockade works in both cohorts… but larger magnitude of benefit in wild type cohort.

**Shorter median PFS with mutated PIK3CA**

<table>
<thead>
<tr>
<th>PIK3CA Status</th>
<th>Placebo+T+D</th>
<th>Purtuzumab+T+D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mut</td>
<td>Patients, n</td>
<td>Events</td>
</tr>
<tr>
<td>Mut</td>
<td>90</td>
<td>63</td>
</tr>
<tr>
<td>WT</td>
<td>191</td>
<td>101</td>
</tr>
</tbody>
</table>

Trastuzumab Emtansine (T-DM1): Mechanism of Action

Trastuzumab-specific MOAs
• Antibody-dependent cellular cytotoxicity (ADCC)
• Inhibition of HER2 signaling
• Inhibition of HER2 shedding

DM1-specific MOAs

- Emtansine release
- Inhibition of microtubule polymerization
- Cell death

Cytotoxic Activity of T-DM1 in HER2-Positive Breast Cancer Cell Lines With PIK3CA Mutations

SK-BR-3: PIK3CA wild type

KPL-4: PIK3CA H1047R

MCF7-neo/HER2: PIK3CA E545K

EFM-192A: PIK3CA C420R

T-DM1 Activity in PIK3CA Mutant Breast Cancer Xenografts

KPL-4 xenograft

MCF7-neo/HER2 xenograft

EMILIA Phase 3 Study Design

Primary endpoints:
- PFS
- OS

HER2-positive LABC or MBC (N=991)
- Prior taxane and trastuzumab
- Progression on metastatic treatment or within 6 months of adjuvant treatment

1:1

T-DM1
3.6 mg/kg q3w IV

Lapatinib
1250 mg/day PO qd
+ Capecitabine
1000 mg/m² PO bid, days 1–14, q3w

**EMILIA Biomarker Methods**

- Formalin-fixed, paraffin-embedded tumor tissue collected for central HER2 testing in EMILIA was used for qRT-PCR analysis of EGFR, HER2, and HER3 mRNA levels (Roche Molecular Diagnostics).

- Tumor tissue samples analyzed for:
  - PI3KCA mutation status (cobas® PIK3CA mutation test, Roche Molecular Diagnostics)
    - Exon 1: R88Q
    - Exon 4: N345K
    - Exon 7: C420R
    - Exon 9: E542K, E545X, and Q546X
    - Exon 20: M1043I, H1047X and G1049R
  - Cytoplasmic PTEN expression in comparison to adjacent normal tissue (by IHC, antibody 138G6, Cell Signaling Technology®)

- Statistical analyses:
  - PFS and OS were analyzed for each biomarker subgroup using Kaplan-Meier methods and a Cox regression model

*Baselga J et al. Clin Cancer Res Online Feb 26, 2016*
EMILIA Biomarker Analysis:
PFS by PIK3CA Mutation Status and Treatment Arm

<table>
<thead>
<tr>
<th>PIK3CA mutation status</th>
<th>Lap + Cap</th>
<th>T-DM1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Median</td>
<td>n Median</td>
</tr>
<tr>
<td>Mutated</td>
<td>39  4.3</td>
<td>40  10.9</td>
</tr>
<tr>
<td>Wild type</td>
<td>87  6.4</td>
<td>93  9.8</td>
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</tbody>
</table>

T-DM1 works in both cohorts…but larger magnitude of benefit in mutated cohort

No. at risk:

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<tr>
<th>Treatment Arm</th>
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<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
<th>24</th>
<th>26</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lap + Cap (Mutated)</td>
<td>39</td>
<td>28</td>
<td>22</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lap + Cap (Wild type)</td>
<td>87</td>
<td>69</td>
<td>55</td>
<td>31</td>
<td>21</td>
<td>16</td>
<td>13</td>
<td>10</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>T-DM1 (Mutated)</td>
<td>40</td>
<td>31</td>
<td>26</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T-DM1 (Wild type)</td>
<td>93</td>
<td>82</td>
<td>66</td>
<td>42</td>
<td>36</td>
<td>24</td>
<td>19</td>
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<td>12</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

aHazard ratios are based on unstratified analyses.

EMILIA Biomarker Analysis:
OS by PIK3CA Mutation Status and Treatment Arm

<table>
<thead>
<tr>
<th>PIK3CA mutation status</th>
<th>Lap + Cap</th>
<th>T-DM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Median (months)</td>
<td>Median (months)</td>
</tr>
<tr>
<td>Mutated</td>
<td>39</td>
<td>17.3</td>
</tr>
<tr>
<td>Wild type</td>
<td>87</td>
<td>27.8</td>
</tr>
</tbody>
</table>

\(a\)Hazard ratios are based on unstratified analyses.
NE, not estimable.

T-DM1 May Be Able to Bypass a Common Resistance Pathway in HER2-Positive Cancer Cells

- PTEN
- AKT
- mTOR
- PI3K
- HER3
- HER2
- Internalization
- Emtansine release
- Inhibition of microtubule polymerization
- Cell Survival
- Cell Death
Clinical Implication of PIK3CA mt for mBC

• Mutated *PIK3CA* associated with lapatinib resistance\(^1,^2\) and poor prognosis after trastuzumab therapy\(^3\), but TDM-1 may be able to bypass a common resistance pathway\(^2\).

• *1st line HER2 directed therapy for mBC*
  - *PIK3CA* mutational status identify patients with poor prognoses and unmet medical needs, despite deriving a benefit from pertuzumab treatment in CLEOPATRA trial\(^4\).
  - Clinical trials of HER2-targeted molecules in combination with PI3K pathway-targeted agents may be justified for *1st line mBC*.

• *2nd line HER2 directed therapy for mBC*
  - Patients in the lapatinib + capecitabine treatment with *PIK3CA* mutations appeared to have worse clinical outcomes than those with wild type *PIK3CA* in EMILIA trial \(^2\).
  - Patients in the T-DM1 treatment arm, PFS and OS may be unaffected by *PIK3CA* mutation status \(^2\).

Lower pCR rates in PIK3CA mt. patients, especially in HR+ patients in GeparTrials (pCR according to PIK3CA mutation status overall and in all three studies separately)
PIK3CA mutated tumors derive less benefit (lower pCR) from dual blockade using Lapatinib+Trastuzumab

pCR according to the PIK3CA mutation in GEPAR & NeoALTTO

Sibylle Loibl et al. JCO 2014;32:3212-3220

Ian J. Majewski et al. JCO 2015;33:1334-1339
Combined Analysis of 3 trials (N=967): GeparTrials, NeoALTTO, CHERLOB

GeparQuattro/
GeparQuinto: Her-2 positive (Setting III)

Untch et al. JCO 2010 and Lancet Oncol 2012

Neo-ALTTO

Baselga et al. Lancet 2010

CHERLOB


Presented By Sibylle Loibl at 2015 ASCO Annual Meeting
PIK3CA Mutation Analysis (exon 9 & 20) in the Overall Study Cohort (n=967)

PIK3CA mutation rate
21.7%

Presented By Sibylle Loibl at 2015 ASCO Annual Meeting
**PIK3CA Mutations by Trial and exon**

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Exon 9</th>
<th>Exon 20</th>
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</thead>
<tbody>
<tr>
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<td>21.7%</td>
<td>7.2%</td>
<td>14.5%</td>
</tr>
<tr>
<td>GEPARTrials n=504</td>
<td>21.4%</td>
<td>8.3%</td>
<td>13.1%</td>
</tr>
<tr>
<td>Neo-ALTTO n=355</td>
<td>22.5%</td>
<td>6.2%</td>
<td>16.3%</td>
</tr>
<tr>
<td>CHERLOB n=108</td>
<td>20.4%</td>
<td>5.6%</td>
<td>14.8%</td>
</tr>
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PIK3CA Mutations by Trial and HR status

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>HR+ve</th>
<th>HR-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>21.7%</td>
<td>21.7%</td>
<td>21.7%</td>
</tr>
<tr>
<td>GEPARstudies</td>
<td>21.4%</td>
<td>21.3%</td>
<td>21.6%</td>
</tr>
<tr>
<td>n=504</td>
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</tr>
<tr>
<td>Neo-ALTTO</td>
<td>22.5%</td>
<td>22.7%</td>
<td>22.4%</td>
</tr>
<tr>
<td>n=355</td>
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<tr>
<td>CHERLOB</td>
<td>20.4%</td>
<td>20.9%</td>
<td>19.5%</td>
</tr>
<tr>
<td>n=108</td>
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Combined Analysis of 3 trials (N=967): GeparTrials, NeoALTTO, CHERLOB
pCR Rates According to PIK3CA Mutation Status Overall and by HR Status

Presented By Sibylle Loibl at 2015 ASCO Annual Meeting
pCR Rates According to PIK3CA Mutation Status
Overall and by exon

- wt: N=757, 29.6%
- all mut: N=210, 16.2%
- exon 9: N=70, 15.7%
- exon 20: N=140, 16.4%
pCR rate According to PIK3CA Mutation Status
Overall and by anti-HER2 Treatment

\[ P_{interaction} = 0.189 \]

- Overall: 16.2% (PIK3CA mut), 29.6% (PIK3CA wt), \( P < 0.001 \)
- Trastuzumab: 20.3% (PIK3CA mut), 27.1% (PIK3CA wt), \( P = 0.343 \)
- Lapatinib: 11.3% (PIK3CA mut), 16.9% (PIK3CA wt), \( P = 0.389 \)
- T+L: 16.7% (PIK3CA mut), 39.1% (PIK3CA wt), \( P < 0.001 \)
PIK3CA mutation and anti-HER2 treatment

PIK3CA mutated status is a predictor of pCR for double blockade with Trast + Lap.

Presented by Sibylle Loibl at 2015 ASCO Annual Meeting.
## Multivariate Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio</th>
<th>p-Value</th>
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<tbody>
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<td><strong>PIK3CA</strong></td>
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<td></td>
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<tr>
<td>mut vs wt</td>
<td>.455</td>
<td>&lt; .001</td>
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<td><strong>Age, years</strong></td>
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<tr>
<td>50+ vs &lt;50</td>
<td>.944</td>
<td>.718</td>
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<td><strong>cT</strong></td>
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<tr>
<td>cT3-4 vs cT1-2</td>
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<td><strong>cN</strong></td>
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<td>cN+ vs cN0</td>
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<td><strong>Histological tumour type</strong></td>
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<td>lobular/other vs ductal</td>
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<td>.524</td>
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<td><strong>Grading</strong></td>
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<td>G1-2 vs G3</td>
<td>.828</td>
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<tr>
<td>ER and/or PgR positive vs ER and PgR negative</td>
<td>.519</td>
<td>&lt; .001</td>
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<tr>
<td><strong>Anti-HER2 treatment</strong></td>
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</tr>
<tr>
<td>Lapatinib alone vs Trastuzumab alone</td>
<td>.537</td>
<td>.007</td>
</tr>
<tr>
<td>Trastuzumab and lapatinib vs Trastuzumab alone</td>
<td>1.51</td>
<td>.021</td>
</tr>
</tbody>
</table>

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**Presented By Sibylle Loibl at 2015 ASCO Annual Meeting**
Disease Free Survival

- Censored
- Logrank p=0.6373
- HR mut to wt = 1.09, 95%CI (0.77, 1.53), p=0.6374
- wt 140/756 events
- mut 43/210 events
Disease Free Survival by HR Status

PIK3CA does not predict DFS, but the role seems to be reversed in HR- & HR+

P interaction p=0.0259

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Clinical Implication of PIK3CA mt in Neoadjuvant setting

• PIK3CA mt occur in ~ 22% of HER2+ BC
• pCR rate is significantly lower in PIK3CAmt
• PIK3CAmt predicts lower pCR rate in HER2+/HR+ cohort
• Difference in pCR rate between mutant and wil-type is largest in patients receiving trastuzumab & lapatinib
• Patients with HER2+/HR+ receiving double HER2 blockade (Tra+Lap) achieve only a pCR rate of 5.5% (mt) vs 33.9% (wt)
• DFS is not different between wild type and mutant cohort

Sibylle Loibl at 2015 ASCO Annual Meeting
Clinical Implication of PIK3CA mt in Adjuvant setting

- PIK3CA was not a predictive biomarker for adjuvant trastuzumab in NSABP B-31

Katherine L. Pogue-Geile et al. JCO 2015;33:1340-1347
Summary of Clinical Implication of PIK3CAmt in HER2+Breast Cancer

- PIK3CAmt occur in ~ 22% of HER2+ BC

- Mutated PIK3CA associated with lapatinib resistance and poor prognosis after trastuzumab therapy in metastatic HER2+ breast cancer.

- PIK3CAmt patients also get a benefit of adding pertuzumab and TDM-1 may be able to bypass a common resistance pathway (PFS and OS may be unaffected by PIK3CA mutation status who received TDM-1)

- In Neoadjuvant setting, pCR rate is significantly lower in PIK3CAmt, especially in HER2+/HR+ cohort

- Difference in pCR rate between mutant and wild-type is largest in patients receiving trastuzumab & lapatinib

- DFS is not different between wild type and mutant cohort

- PIK3CA was not a predictive biomarker for adjuvant trastuzumab in NSABP B-31