Strategies to Overcome Resistance to Trastuzumab

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Director, Stanford Breast Oncology Program
Co-Director, Molecular Therapeutics Program
Previously postulated mechanisms of resistance

- Shedding of ERBB2
  (Scalitriti et al, J Nat Cancer Inst, 2007)

- Hyperactivation of PI3K/Akt by loss of PTEN and PIK3CA mutation
  (Eichhorn et al, Cancer Res, 2009)

- Overexpression of MUC4 causing steric hindrance of trastuzumab binding
  (Nagy et al. Cancer Res. 2005)

- Increase in p-ERBB3
  (Sergina et al, Nature, 2007)

- Cyclin E amplification/overexpression
  (Scaltriti et al. PNAS 2011)

- Activation of EPO receptor by rHuEPO
  (Liang et al. Cancer Cell 2010)

- Expression of ER
  (Xia et al, PNAS, 2006)

- Upregulation of IGF-IR receptor
  (Gail Phillips, AACR, 2009)

- Activation of AXL
  (Liu et al, Cancer Res, 2009)

- Upregulation of MET receptor
  (Shattuck et al, Cancer Res, 2008)
Neoadjuvant pertuzumab (P) and trastuzumab (H): Biomarker analyses of a 4-arm randomized Phase II study (NeoSphere) in patients (pts) with HER2-positive breast cancer (BC)

L Gianni, G Bianchini, A Kiermaier, G Bianchi, Y.-H Im, T Pienkowski, L Roman, M-C Liu, L-M Tseng, J Ratnayake, T Szado, G Ross, P Valagussa

on behalf of the ‘NeoSphere’ study investigators
NeoSphere: Study design and objectives

- Phase II design
- Primary endpoint: Comparison of pCR rates
  - TH vs THP
  - TH vs HP
  - THP vs TP
- Secondary endpoints:
  - Clinical response
  - DFS
  - Breast conservation rate
  - Biomarker evaluation

Patients with operable or locally advanced /inflammatory* HER2-positive BC

Chemo-naïve & primary tumors >2cm (N=417)

Study dosing: q3w x 4

TH (n=107)
- docetaxel (75→100 mg/m²)
- trastuzumab (8→6 mg/kg)

THP (n=107)
- docetaxel (75→100 mg/m²)
- trastuzumab (8→6 mg/kg)
- pertuzumab (840→420 mg)

HP (n=107)
- trastuzumab (8→6 mg/kg)
- pertuzumab (840→420 mg)

TP (n=96)
- docetaxel (75→100 mg/m²)
- pertuzumab (840→420 mg)

NeoSphere: Primary endpoint – pathologic complete response (ITT population)

H, trastuzumab; P, pertuzumab; T, docetaxel

* p values from Cochran-Mantel-Haenszel test and adjusted for multiplicity

The HER2 signalling pathway
Selection of biomarkers

HER1, HER2, HER3, IGF1R

p95HER2

ER

PTEN
mTOR

p27
Cyclin D1, E

Nucleus

HER ligands

NK cell

FcGR

HER2

Raf

Sos
Shc

Grb2

Grb2

PI3K

Akt

GSK3
BAD

Cell survival

Cell-cycle progression

Nucleus

c-myc

Cell proliferation

mTOR

Ras

MEK 1/2

MAPK

San Antonio Breast Cancer Symposium – Cancer Therapy and Research Center at UT Health Science Center – December 6–10, 2011

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## Biomarker analyses on overall population

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<thead>
<tr>
<th>Assay method</th>
<th>Biomarker</th>
<th>Sample Size</th>
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<tbody>
<tr>
<td>IHC</td>
<td>HER2 mem H-score</td>
<td>416</td>
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<tr>
<td></td>
<td>HER3 mem H-score</td>
<td>377</td>
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<tr>
<td></td>
<td>IGF1R mem H-score</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>PTEN cyt H-score</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>PTEN nuc H-score</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>pAKT cyt H-score</td>
<td>299</td>
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<tr>
<td></td>
<td>pAKT nuc H-score</td>
<td>299</td>
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<tr>
<td>qRT-PCR</td>
<td>HER2/HER3-CR</td>
<td>384</td>
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<td>HER3-CR</td>
<td>384</td>
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<td></td>
<td>EGFR-CR</td>
<td>377</td>
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<tr>
<td>FISH</td>
<td>c-myc</td>
<td>275</td>
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<td>ELISA (serum)</td>
<td>sHER2 (ng/mL)</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>Amphiregulin (pg/mL)</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>TGF-alpha (pg/mL)</td>
<td>384</td>
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<tr>
<td></td>
<td>EGF (pg/mL)</td>
<td>384</td>
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<td>Mutational analyses</td>
<td>PI3K mutation</td>
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<td>PI3K mutation</td>
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The cut-off for analyses of correlation with treatment and sensitivity was defined as the median for each biomarker [except for c-myc (ratio ≥ 2.0) and PI3K status (WT versus Mut)].
HER2 membrane H-score linked to pertuzumab effects

The cut-off associated with prediction of response upon addition of pertuzumab [THP regimen] is not a clinically meaningful discriminator.

H, trastuzumab; P, pertuzumab; T, docetaxel (study dosing: q3w x 4 cycles)
Determination of fragments of HER2: The HER2 ECD/ICD ratio

Chromogenic double staining of HER2 ICD (Ventana 4B5) and ECD (Roche proprietary monoclonal antibody F2)

Single stainings “pure” spectra

ECD/ICD ratio <1 indicates presence of truncated forms of HER2

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Analysis of fragments of HER2 per HER2 ECD/ICD ratio: No association with efficacy

H, trastuzumab; P, pertuzumab; T, docetaxel (study dosing: q3w x 4 cycles)
Results of PI3K mutational analyses per exon in pooled arms

<table>
<thead>
<tr>
<th>Mutation</th>
<th>non-pCR</th>
<th>pCR</th>
<th>pCR/non-pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 7</td>
<td>4</td>
<td>0</td>
<td>0/4</td>
</tr>
<tr>
<td>Exon 9</td>
<td>26</td>
<td>2</td>
<td>2/28 (7.1%)</td>
</tr>
<tr>
<td>Exon 20</td>
<td>47</td>
<td>19</td>
<td>19/66 (28.7%)</td>
</tr>
</tbody>
</table>

Exon 9 mutation: of 28 mutation detected in the 4 arms, 26 were in cases who did not achieve a pCR
Hormone receptor status and pCR in NeoSphere

ER, estrogen receptor; PR, progesterone receptor
H, trastuzumab; P, pertuzumab; T, docetaxel (study dosing: q3w x 4 cycles)

## OS According to Subgroups

### ACTH vs. ACT (reference group)

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of Events</th>
<th>ACT</th>
<th>ACTH</th>
<th>HR</th>
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<tr>
<td><strong>Age</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;40 years</td>
<td>654</td>
<td>65</td>
<td>45</td>
<td>0.67</td>
</tr>
<tr>
<td>40-49</td>
<td>1373</td>
<td>121</td>
<td>87</td>
<td>0.65</td>
</tr>
<tr>
<td>50-59</td>
<td>1336</td>
<td>129</td>
<td>90</td>
<td>0.68</td>
</tr>
<tr>
<td>60+ years</td>
<td>683</td>
<td>103</td>
<td>64</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Hormone Receptor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER- and PR-</td>
<td>1828</td>
<td>212</td>
<td>149</td>
<td>0.65</td>
</tr>
<tr>
<td>ER+ or PR+</td>
<td>2215</td>
<td>206</td>
<td>137</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Tumor Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2cm</td>
<td>1598</td>
<td>129</td>
<td>67</td>
<td>0.51</td>
</tr>
<tr>
<td>2.1-5.0cm</td>
<td>2096</td>
<td>239</td>
<td>176</td>
<td>0.68</td>
</tr>
<tr>
<td>5.1cm+</td>
<td>345</td>
<td>50</td>
<td>42</td>
<td>0.58</td>
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<tr>
<td><strong>Nodal Status</strong></td>
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<tr>
<td>LN 0</td>
<td>282</td>
<td>11</td>
<td>9</td>
<td>0.94</td>
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<tr>
<td>LN 1-3</td>
<td>2144</td>
<td>161</td>
<td>104</td>
<td>0.59</td>
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<tr>
<td>LN 4-9</td>
<td>1084</td>
<td>133</td>
<td>103</td>
<td>0.72</td>
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<tr>
<td>LN 10+</td>
<td>536</td>
<td>113</td>
<td>70</td>
<td>0.56</td>
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<tr>
<td><strong>Histologic Grade</strong></td>
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<tr>
<td>Good</td>
<td>76</td>
<td>8</td>
<td>1</td>
<td>0.11</td>
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<tr>
<td>Intermediate</td>
<td>1123</td>
<td>108</td>
<td>59</td>
<td>0.52</td>
</tr>
<tr>
<td>Poor</td>
<td>2801</td>
<td>299</td>
<td>219</td>
<td>0.67</td>
</tr>
</tbody>
</table>

**Romond, et al., SABCS 2012**
Conclusions from NeoSphere biomarker analyses

- HER2 expression (H-score) associated with sensitivity to pertuzumab
- PI3K mutations in exon 9 linked to lack of sensitivity to HER2-directed MAb’s
- Intrinsic differences between HER2+ tumors based on hormone receptor status
- No predictive role for truncated forms of the HER2 receptor including p95\(^{\text{HER2}}\)
- So far none of the analyses provided clinically useful assays for patient and/or regimen selection in addition or alternative to the conventional assessment of HER2 by IHC or FISH
Candidate markers 
(8 year DFS NSABP B-31)
The trastuzumab HER2 Complex Is a Potent Mediator of ADCC

FcγRIIIa - 158V/F Polymorphism (RFLP Analysis)

Fc γRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc γRIIIa, independently of the Fc γRIIIa-48L/R/H phenotype. Koene, et al.
Fig 2. Progression-free survival (PFS) by immunoglobulin G (IgG) fragment C receptor IIIa (Fc(\gamma)RIIIa) 158 valine (V)/phenylalanine (F) and Fc(\gamma)RIIa 131 histidine (H)/arginine (R) polymorphisms

Fc-Engineered Antibodies Maximize Effector Cell Activity

Retain Anti-Proliferative Effects of Trastuzumab

Superior Engagement of Immune Effector Cells

Significantly Improved Cell Killing
MGAH22 Significantly Outperforms Trastuzumab

Activity in Resistant Breast Cancer Lines (JIMT-1, Her2 2+)

No Response with Wild Type mAb (Trastuzumab)

MGAH22 Controls Tumor Growth

* First time when tumor size is significantly less than control (P<0.05)
# First time when tumor size is significantly less for MGAH22 than RES120 (P<0.05)

Note: these models incorporate MacroGenics’ proprietary transgenic models: mCD16-/ hCD16A+ Mice
Genetic landscape of gene re-arrangements, gene copy number changes and SNVs in lapatinib sensitive and resistant SKBR3 cells

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Vichien Srimuninnimit – Siriraj Hospital, Bangkok, Thailand
Patrapim Sunpaweravong – Prince of Songkla University Hospital, Thailand

Central laboratories
TARGOS Molecular Pathology GmbH
Roche TRS DNA lab
Roche protein lab
Roche TRS DNA lab

Michelangelo Foundation
Roche Product Development
2 flavors of FcγR influence ADCC

- **FcγRIIIa (activating)** - activates ADCC effector cells
  - immunoreceptor tyrosine activation motif (ITAM)

- **FcγRIIb (inhibitory)** - abrogates effector cell activity
  - immunoreceptor tyrosine-based inhibitory motif (ITIM)