ER+PR- Breast Cancer: A Unique Subtype of Breast Cancer Driven by Growth Factor Signaling, Molecular Response to EGFR Targeted Therapies

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Epidermal Growth Factor Receptor and Gefitinib

- EGFR 170 kD tyrosine kinase receptor
- Aberrant expression and activation of EGFR has been implicated in multiple epithelial malignancies including breast cancer
- Gefitinib (IRESSA, AstraZeneca) is an oral small molecule tyrosine kinase inhibitor of the EGFR-TK
- At the time of study initiation, studies had not shown significant clinical activity of gefitinib in women with breast cancer
- BCIRG 103 was a pre-surgical study to evaluate the molecular changes that occur in primary breast cancer tissue after short term exposure to gefitinib
**Study Plan**

**Diagnosis**
Invasive breast adenocarcinoma (T>2cm; N0 or N1)

250mg gefitinib daily, 14 – 45 days, minimum 10 consecutive days prior to surgery

**Pre-treatment Biopsies**

Four core biopsies

Transcript Profile of >18,000 well-characterized genes

**Comparison of pre- and post-treatment signals reveals molecular effects after gefitinib exposure**

**Surgery**

**Post-treatment Tumour**

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**Patients assigned to ‘response’ groups on basis of surrogate markers:**
- Ki67 mRNA change
- Cell cycle gene changes
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Provide baseline molecular subtype of breast cancers in study

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Post-treatment Tumour

Transcript Profile of >18,000 well-characterized genes

Patients assigned to ‘response’ groups on basis of surrogate markers:
- Ki67 mRNA change
- Cell cycle gene changes

Opportunity to define baseline markers of drug ‘response’

Transcript Profile of >18,000 well-characterized genes

Provide baseline molecular subtype of breast cancers in study

Four core biopsies
# Patients Characteristics

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>N = 59</strong></td>
<td></td>
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<tr>
<td><strong>Median Age</strong></td>
<td>61(27-79)</td>
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<tr>
<td><strong>Median KPS</strong></td>
<td>100% (80-100)</td>
</tr>
<tr>
<td><strong>Pre-menopausal</strong></td>
<td>27%</td>
</tr>
<tr>
<td><strong>Post-menopausal</strong></td>
<td>73%</td>
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<tr>
<td><strong>Average Length of Study Treatment</strong></td>
<td>17 (12-35 days)</td>
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Methods
OCT Embedded Tumor Tissue

Dry ice
H&E of BCIRG103 Samples
High Quality Tumor Pairs

<table>
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<tr>
<th>PRE</th>
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<tr>
<td>3004C D</td>
<td>3004</td>
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<tr>
<td>21.8 ug</td>
<td>67.7 ug</td>
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<tr>
<td>RIN 10</td>
<td>RIN 9.8</td>
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<tr>
<td>CD</td>
<td>1048</td>
</tr>
<tr>
<td>28 ug</td>
<td>11 ug</td>
</tr>
<tr>
<td>RIN 10</td>
<td>RIN 9.4</td>
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<tr>
<td>049CA</td>
<td>1049</td>
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<tr>
<td>20.6 ug</td>
<td>1</td>
</tr>
<tr>
<td>RIN 9.4</td>
<td>RIN 9.5</td>
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<tr>
<td>050CA</td>
<td>1050</td>
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<tr>
<td>14.2 ug</td>
<td>40.8 ug</td>
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<tr>
<td>RIN 8.9</td>
<td>RIN 9.1</td>
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<tbody>
<tr>
<td>051CC</td>
<td>1051</td>
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<tr>
<td>7.5 ug</td>
<td>1b</td>
</tr>
<tr>
<td>RIN 9.7</td>
<td>RIN 10</td>
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<tbody>
<tr>
<td>052CA</td>
<td>1052</td>
</tr>
<tr>
<td>9.1 ug</td>
<td>17.0 ug</td>
</tr>
<tr>
<td>RIN 8.8</td>
<td>RIN 9.1</td>
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AGILENT MICROARRAY PLATFORM

1. Pre treatment core vs tumor reference
2. Pre- vs post
3. Post-vs pre-

Fluor reversal

22,000 spots each containing a 60mer representing a known gene. The two probes hybridize to the spots in proportion to concentration of the specific RNA in each probe.
Assigning Molecular Response

• No clinical measurement of response

• Molecular endpoint defined by changes in mRNA expression between pre- and post-treatment specimens
  – Ki67 mRNA change
  – Cell cycle gene sets mRNA change

• Cell line studies with gefitinib validated these markers as correlating with response in vitro
Ki67 mRNA favored over Ki67 IHC: Reasons

- A high proportion of study cases had pre- and post-treatment samples at or below the lower limit of reliable quantitation by IHC (~5%)

- High intra-tumor variation for many samples made assessment of response less reliable by IHC

- Tumors may be misclassified based on any one single measure of response (Tau Y et al. 2003)

- mRNA data is objective and quantitative
Cell Cycle Gene Sets

• Set I – 52 Genes – *In Vitro* Treatment Cell Cycle Cluster
  – Selected from hierarchical unsupervised clustering of 17 breast cell line gefitinib treatment experiments

• Set II – 80 Genes – BCIRG 103 Treatment Experiment Cell Cycle Cluster
  – defined by hierarchical clustering of the subset of BCIRG 103 treatment experiments with a significant change in Ki67 mRNA (n=30) and then selecting the cluster of genes where expression correlated with known cell cycle markers

31/52 (59%) genes from the *in vitro* Cell Cycle Cluster are in the BCIRG 103 Treatment Set
Ki67 and Cell Cycle Gene Set (I): *in vitro*
Ki67 and Cell Cycle Gene Set (I): *patient samples*
Ki67 and Cell Cycle Gene Set (II): *patient samples*

Genes in red are found in the *in vitro* cell cycle set (I)
Ki67 and Cell Cycle Gene Set (II): \textit{in vitro}

Gene names

Most Sensitive Cell Lines IC50< 1 \textmu M
Incorporates:

1. Tissue quality
2. RNA integrity (RIN)
3. Labeling efficiency (FOI)
4. Correlation of profiles (fluor reversal)
5. Molecular response
### Assignment of Patients to Response Groups

<table>
<thead>
<tr>
<th>‘Response’ Group</th>
<th>Ki67 mRNA</th>
<th>Predominant Change in Cell Cycle Cluster (mRNA)</th>
<th>No. Patients</th>
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<tbody>
<tr>
<td><strong>Inhibition</strong></td>
<td>↓</td>
<td>↓</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>↓</td>
<td>1</td>
</tr>
<tr>
<td>(minor change)</td>
<td></td>
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<td>11</td>
</tr>
<tr>
<td><strong>Proliferation</strong></td>
<td>↑</td>
<td>↑</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>↔</td>
<td>↑</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
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</tbody>
</table>
Results
EGFR mRNA Expression in Baseline Experiments

- Proliferation
- Inhibition
- No change
Subtype Classification from Baseline Experiments

Sample ID

ESR1 Log(ratio) mRNA

Strong ER
Moderate ER
Weak ER
Negative ER
HER-2

"triple negative"
Subtype Classification from Baseline Experiments and Molecular Response

Sample ID

Proliferation
Inhibition
No change

ESR1 Log(ratio) mRNA

Strong
ER

Moderate
ER

Weak
ER

Negative
ER

“triple negative”

HER-2
PGR mRNA expression in Luminal Breast Tumors
Coded by Response

PGR mRNA log(ratio)

Sample

Proliferation
Inhibition
No change
PR IHC 'H' Score for Luminal Samples with Paraffin Blocks Available Coded by Response to Gefitinib

- **H Score > 150**
  - 5 proliferating
  - 1 inhibited
  - 2 no change

- **150 > H Score ≥ 50**
  - 0 proliferating
  - 3 inhibited
  - 9 no change

- **H Score < 50**
  - 0 proliferating
  - 4 inhibited
  - 7 no change

**Protein/ mRNA mismatch**

- Proliferation
- Inhibition
- No change
PR protein changes with exposure to gefitinib

Changes in PR IHC Score with Treatment for Luminal Samples

- Proliferation Inhibition
- No change
Does the literature support the hypothesis that ER+ PR weak/negative breast cancer is associated with peptide growth factor signaling?
ER Positive - PR weak/ negative
Breast Cancer & Growth Factors

• Clinical reports suggest high growth factor signaling may be associated with decreased PR levels in breast cancer (Konecny 2003)

• Large retrospective study shows ER+ PR- tumors have more aggressive phenotype, higher levels of HER1 and HER2, and higher recurrence rate with tamoxifen (Breast Center at Baylor Study, Arpino 2005)

• Randomized study of pre-surgical gefitinib or gefitinib with anastrazole in ER+, EGFR+ primary breast cancers with decrease in Ki67 and size by ultrasound in both arms, though favoring combination (Polychronis 2005)

• Phase II study of gefitinib and docetaxel in first-line metastatic breast cancer shows response rates of 70% in ER+ tumors versus 20% in ER- tumors (Ciardiello 2006)
What is the incidence of this subtype?
Proportion of ER+/PR weak/ negative Tumors

- **Baylor Breast Cancer Study (Arpino 2005) - 25% (by protein)**
  - ER Positive at least 3 fmol; PR Positive at least 5 fmol
  - measured by ligand binding at two central labs
  - Stage I – IIIA
  - N = 54,865

- **Cohort A (Konecny 2003) - 10.57% (by protein)**
  - ER Positive at least 3 fmol; PR Positive at least 5 fmol protein
  - measured by Eliza at University of Munich
  - Stage I – IV
  - N = 664

- **Rosetta/NKI NEJM Study – 20% (by RNA)**
  - Define mRNA thresholds for ER+ and PR+
  - Stage I & II
  - N = 295
Conclusions from BCIRG 103 (I)

• High quality tissue and nucleic acid can be obtained in a prospective study for pharmacodynamic/genomic studies

• In an unselected population of women, all molecular subgroups of breast cancer were represented (Luminal-ER+, HER2+ and Basal- ER-PR-HER2-) in 43 patients evaluable for molecular alterations

• Three molecular response groups were identified based on mRNA changes in Ki67 and Cell Cycle Gene Sets (1) Molecular proliferation, (2) Molecular inhibition, and (3) No significant change
Conclusions from BCIRG 103 (II)

- When considering luminal breast cancers, a relationship between PR status and molecular response was seen both at the RNA and Protein (IHC) level
  - Molecular proliferation was seen only in ER+ PR strong subgroup
  - Molecular inhibition was seen in the HER2+ patients and in ER+ disease when accompanied by weak/low PR levels with few exceptions

- ER+ PR weak/negative breast cancer defines a unique subtype of the disease that is driven by peptide growth factor signaling and may be more likely to respond to EGFR targeted therapies

- The hypothesis is consistent with literature that lower PR levels in ER+ disease is a marker for peptide growth factor dependence (as opposed to steroid hormone dependence)
Acknowledgements

The 59 women that enrolled in this study

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Kai-Ming Chang

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