Molecular Diversity of Human Breast Cancers: Biologic and Therapeutic Implications
HER-2/neu Program at UCLA

Clinical Material (Tumor Specimens)

Clinical Trials (Current & Past Studies)

Molecular Studies (DNA, RNA, Protein Analyses)

Clinical Data (Patient Information)

Therapeutic Model (Cell Line and Animal Data)

Basic Science Hypothesis Testing (Cell Line and Animal Data)
HER-2/neu Gene is Amplified in Human Breast Cancers

The HER2 Alteration

Southern
Northern
Western
IHC

Slamon et al. *Science* 1989
HER-2 Oncogene Amplification

Breast Cancer

HER-2 Oncoprotein Overexpression

Shortened Survival

Median Survival from First Diagnosis

HER-2 overexpressing 3 yrs
HER-2 normal 6 - 7 yrs

Slamon et al, 1987
Median survival in the HER2 normal (non-amplified) cohort = 6.8 years

Median survival in the HER2 amplified cohort = <3 years

HER2 amplification was an independent prognostic variable in multi-variate analyses using all standard prognostic variables.
CONTROVERSIES

♦ It is NOT amplified at a rate of ~25% but much less frequently (~10-15%)

♦ There is no association between amplification and clinical outcome
HER-2/neu Program at UCLA

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Breast Cancer Subtypes are associated with disease outcome

Fig. 5. Kaplan–Meier analysis of disease outcome in two patient cohorts. (A) Time to development of distant metastasis in the 97 sporadic cases from van’t Veer et al. Patients were stratified according to the subtypes as shown in Fig. 2B. (B) Overall survival for 72 patients with locally advanced breast cancer in the Norway cohort. The normal-like tumor subgroups were omitted from both data sets in this analysis.
HER-2/neu Program at UCLA

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Target Validation - A
Human Breast Cancer Cells

MCF-7
Single copy Low Expressor

Transfect
HER-2/neu

MCF-7*
Multiple copy High Expressor

Human Ovarian Cancer Cells

CaOv-3
Single copy Low Expressor

Transfect
HER-2/neu

CaOv-3*
Multiple copy High Expressor

*Consistent results in 9 additional Breast & Ovarian Cancer Cell Lines
Immunohistochemistry

MCF 7

+ Control

+ HER-2/neu

CaOV 3
Engineered HER-2 Over-expression in MCF-7 cells
Increased Proliferation and Decreased Contact Inhibition

Anchorage-Independent Growth

Growth on Plastic

MCF-7 CN
MCF-7 H2

Number of cells x 10^3

2  4  6  8  9

days

MCF-7 CN
MCF-7 H2
Biologic Effects of HER-2/neu Overexpression in Human Breast Cancer Cells

HER2- Breast Cancer Cell Lines → HER-2 Transfection → HER2+ Breast Cancer Cell Lines

↑ DNA Synthesis
↑ Cell Growth
↑ Growth in Soft Agar
↑ Tumorigenicity
↑ Metastatic Potential
↓ E2 Response, ↑ Tam Resist.
HER-2/neu Program at UCLA

Clinical Material
(Tumor Specimens)

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(Current & Past Studies)

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Basic Science
Hypothesis Testing
(Cell Line and Animal Data)
Target Validation - B
Dose-dependent anti-proliferative effects of 4D5 against HER2-overexpressing breast carcinoma cells *in vitro*


Pegram M, Slamon D
Preclinical Impact of Trastuzumab on Tumor Growth

Effect of Trastuzumab Treatment on HER2+ Breast Cancer Xenografts

51 Human Breast Cell Lines

25 Luminal
- 10 ER positive, normal HER-2
- 9 ER positive, HER-2 amplified
- 6 ER negative, HER-2 amplified

26 Non-luminal
- 4 Non-malignant
- 13 Basal/Progenitor
- 9 Mesenchymal

- 1 HER-2 Amplified
- 1 HER-2 Amplified
HER-2/neu Program at UCLA

Clinical Material (Tumor Specimens) → Clinical Trials (Current & Past Studies)

Clinical Trials (Current & Past Studies) → Molecular Studies (DNA, RNA, Protein Analyses)

Molecular Studies (DNA, RNA, Protein Analyses) → Clinical Data (Patient Information)

Clinical Data (Patient Information) → Basic Science Hypothesis Testing (Cell Line and Animal Data)

Basic Science Hypothesis Testing (Cell Line and Animal Data) → Therapeutic Model (Cell Line and Animal Data)
CALGB 9741
Interim Analyses

**Disease-Free Survival**

85 vs 81% (P=0.0072)

**Overall Survival**

92 vs 90% (P=0.014)

N = 1973; Median F/U = 36 mos
Overall Survival (ITT)

Cumulative Probability

Survival Time (months)

Overall Survival (ITT)

N Events HR P-value
Stratified Log-Rank

TAC 745 91 0.70 .0080
FAC 746 130

TAC 87%
FAC 81%
The HER2 Alteration

Southern

Northern

Western

IHC

Slamon et al. *Science* 1989
Lessons from the HER2 Story

1.) Target Identification
2.) Target Validation
3.) Preclinical Confirmation
4.) Determination of Potential Usage Preclinically
5.) Clinical Translation - Proof of Concept
6.) Clinical Optimization
Clinical Significance of HER2 Testing of Primary Breast Cancers

Why test for HER2?
- HER2 is recognized as an important predictive and prognostic factor\(^1\)\(^{-3}\)
- HER2 overexpression continues throughout the course of the disease and drives tumor growth\(^4\)
- HER2 positivity is required for consideration of HER2-targeted Herceptin\(^\circledR\) (trastuzumab) and Lapatinib (Tykerb) therapy\(^5\)

5. Herceptin\(^\circledR\) (trastuzumab) PI, February 2005.
Testing Issues

- Integrity of the macromolecule being analyzed - degradation of DNA, RNA, or protein
- Accuracy of the reagent - variability of the antibodies
- Stability of the target, e.g. fixation artifacts in proteins - altering antigenic sites and recognition that the pre-analytic phase cannot be controlled
- Accuracy of the testing method
- Heterogeneity of the sample being tested
HER-2 Gene Amplification is Responsible for “Pathologic/Pathogenic” Overexpression
Molecularly Characterized Cohort

- A cohort of 189 snap-frozen breast cancer specimens of sufficient size to allow the simultaneous extraction of DNA, RNA and protein - all from the same specimen

- **Confirmed intact integrity** of the DNA, RNA and protein - i.e. no degradation of the macromolecules PRIOR to commencing analyses

- Formalin-fixed/paraffin-embedded tissue available from the exact same specimens

- **Serves as the “REFERENCE COHORT”** for all of our subsequent studies
Evaluation of Diagnostic Methods

HER-2 Molecularly Characterized Samples “REFERENCE COHORT” assembled in Multi-Tumor Blocks

Amplification Level:

- > 10
- 5 - 10
- 2 - 5
- 1

Southern

Northern

Western

Frozen IHC

Multi-Tumor Paraffin-Embedded Tissue Block
Testing Issues

- Integrity of the macromolecule being analyzed - degradation of DNA, RNA, or protein

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Percent of Breast Cancers in Various Expression Categories Identified by Immunostaining with 28 Different Antibodies.
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Fixation and Paraffin Embedding Result in Decreased Antigenicity

2 to 5-fold Amplified and Overexpressed

Slamon et al., *Science* 244: 707-712, 1989

*Immunohistochemistry in Archival Tissue Samples*
Enter - “Antigen Retrieval”
Schematic Summary of HER-2 Assay Results: Concordance with Known HER2-Positive Status “REFERENCE COHORT”

- **IHC: HER2 in Frozen Tissue**: 99%
- **IHC: HER2 in Paraffin Tissue**: 84%
- **IHC: HER2 in Paraffin Tissue with Ag Retrieval**: 97%
- **FISH: Paraffin Tissue**:

*Estimated Concordance or Accuracy*

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Comparison of Six Different HER-2 Assays in Molecularly Characterized “REFERENCE COHORT” Breast Cancers Specimens

Comparison of FISH vs. IHC
Concordance Study: Two things to note

Results
1:1 population

<table>
<thead>
<tr>
<th></th>
<th>FISH 0</th>
<th>FISH 1+</th>
<th>FISH 2+</th>
<th>FISH 3+</th>
<th>CTA-IHC 0</th>
<th>CTA-IHC 1+</th>
<th>CTA-IHC 2+</th>
<th>CTA-IHC 3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH-</td>
<td>207</td>
<td>28</td>
<td>67</td>
<td>21</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>FISH+</td>
<td>7</td>
<td>2</td>
<td>21</td>
<td>176</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amplification rate

- 3%
- 7%
- 24%
- 89%

Overall concordance between FISH and IHC results was 82% (95% CI; 78–85%) (p < 0.0004).

Breast Cancer Research and Treatment 93: 3-11, 2005.
BCIRG Central Laboratory Concordance Study

### Results

<table>
<thead>
<tr>
<th>Central FISH</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Overall Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>538</td>
<td>230</td>
<td>67</td>
<td>90</td>
<td>73%</td>
</tr>
<tr>
<td>+</td>
<td>20</td>
<td>15</td>
<td>33</td>
<td>316</td>
<td>23%</td>
</tr>
</tbody>
</table>

Amplification rate

- 4%
- 6%
- 17%
- 78%

4.3% N = 1407

Overall concordance between FISH and IHC results was 79% (95% CI; 77–81%).

Arguments Against Screening with IHC and Reflex Testing with FISH

♦ Between 9 - 17% of women with HER-2/neu alteration are IHC-negative (0/1+) : definite false negatives.
♦ Between 8 and 22% of women with IHC 3+ do not have the HER-2/neu alteration (gene amplification by FISH) : ? false positives.
♦ Trastuzumab (Herceptin) and lapatinib are expensive therapeutics; errors in testing are costly.
♦ Women deserve the most accurate testing methods.
Response Rates in the Genentech H0649 Pivotal Clinical Trial of Trastuzumab

<table>
<thead>
<tr>
<th>FISH Ratio</th>
<th>Non-Resp (n)</th>
<th>Responder (n)</th>
<th>Rate (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.0</td>
<td>36</td>
<td>0</td>
<td>0%*</td>
<td>0%, 10%</td>
</tr>
<tr>
<td>2.0 - 6.0</td>
<td>75</td>
<td>11</td>
<td>13%**#</td>
<td>7%, 22%</td>
</tr>
<tr>
<td>&gt;6.0</td>
<td>65</td>
<td>22</td>
<td>25% #</td>
<td>17%, 36%</td>
</tr>
</tbody>
</table>

FISH results obtained for 209 of the 222 (94%) women entered in trial.

Fisher’s exact test, overall p=0.0005; *p= 0.033, # p= 0.052.
Response Rates in the Genentech H0650 Clinical Trial of Trastuzumab

<table>
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<th>Responder (n)</th>
<th>Rate (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.0</td>
<td>28</td>
<td>1</td>
<td>3%*</td>
<td>0.1%, 18%</td>
</tr>
<tr>
<td>2.0 - 6.0</td>
<td>24</td>
<td>10</td>
<td>29%**#</td>
<td>15%, 47%</td>
</tr>
<tr>
<td>&gt;6.0</td>
<td>31</td>
<td>18</td>
<td>37% #</td>
<td>23%, 52%</td>
</tr>
</tbody>
</table>

FISH results obtained for 112 of the 114 (98%) women entered in trial.
Fisher’s exact test: overall p-value = 0.002; *p=0.008, #p=0.64.
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Correlation of HER-2 Gene Amplification with Overexpression

Amplification Level:
- > 10
- 5 - 10
- 2 - 5
- 1

HER2 Biology

4.4 kb - 12.5 kb - p185

Southern
Northern
Western

Frozen
IHC

27%
63%
% Women

10%

Slamon et al., Science 244: 707-712, 1989
"Single Copy" Overexpression

HER-2 Gene Assessment by FISH

< 2.0 Not Amplified (FISH-)

≥ 2.0 Amplified (FISH+)
Results

Gene Amplification by Southern or Dot blot Hybridization

<table>
<thead>
<tr>
<th></th>
<th>Ampl</th>
<th>Not Ampl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos (+)</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Neg (-)</td>
<td>1</td>
<td>90</td>
</tr>
</tbody>
</table>

N = 140

Sensitivity = 98%, Specificity = 100%.

New guidelines - A case is indeterminate i.e. may be called amplified, normal or equivocal if the ratio is between 1.8 -2.2 instead of the FDA approved definition of >2.0 = amplified.

HAVE THEY DISCOVERED NEW FUNDAMENTAL BIOLOGY SINCE WATSON & CRICK OR THE KNOWN FIDELITY OF DNA REPLICATION DURING THE CELL CYCLE ???

The consequence of this change has not been to make things easier. Instead, non-amplified cases are now sometimes called amplified. Conversely amplified cases may now be called non-amplified and hence either not receive the drug or incorrectly be classified as negative cases which benefit from trastuzumab or lapatinib.
FISH Ratios Plotted from Lowest to Highest in the BCIRG Trials
Use of Fixed/Paraffin Embedded Tissues for m-RNA Expression Levels
Testing Issues

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♦ Accuracy of the testing method

♦ Heterogeneity of the sample being tested
Acknowledgements (con’t)

♦ Genentech:
  Axel Ullrich
  H. Michael Shepard,
  Hank Fuchs,
  Bob Mass,
  Mark Sliwkowski

♦ Amgen:
  Frank Calzone
  Elena Cajulis


♦ USC:
  Michael Press

♦ Revlon Foundation:
  Ronald Perlman
  James Conroy
  Lilly Tartikoff

♦ Herceptin Clinical Investigators Network & BCIRG

♦ Community-based/UCLA Clinical Research Network

♦ The Group of 20