NEW THERAPEUTIC DEVELOPMENT APPROACHES TO HUMAN CANCERS:

Target ID - Target Validation in Pathogenesis - Evaluation of Therapeutic Approaches and Combinations - Clinical Application

Dennis J Slamon, MD, PhD
University of California at Los Angeles
THE PAST
The “One-Size-Fits-All” Approach to Cancer
Traditional Clinical Approaches to Initial Malignancy

♦ SURGERY - Traditional excisional approaches with clean margins i.e. “we got it all”. Newer approaches include cryosurgery, hyperthermic surgery, radiofrequency ablative surgery, etc.

♦ RADIATION THERAPY - Traditional external beam, IMRT, brachytherapy (implants)

♦ SYSTEMIC THERAPY - Cytotoxics (chemotherapy), hormonal therapy, biologic therapy
We Need a Paradigm Shift - A New Approach Based on the Biology of the Disease

♦ Premise #1 - Cancer is not a single disease.

♦ Premise #2 - Cancer is not a single disease even within a given histology. The only thing ALL breast cancers share in common is that they arise in the organ that defines us as a species - the breast.

♦ Premise #3 - A need to develop new therapeutic approaches that take into account #1 and #2
Self-sufficiency in growth signals

Evading apoptosis

Insensitivity to anti-growth signals

Sustained angiogenesis

Tissue invasion & metastasis

Limitless replicative potential

Hanahan and Weinberg Cell 2000
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
Target Identification
The HER2 Alteration

Southern
Northern
Western
IHC

Slamon et al. Science 1989
HER-2/neu Program at UCLA

Clinical Material (Tumor Specimens) → Molecular Studies (DNA, RNA, Protein Analyses)

Clinical Trial (Current Studies) → Clinical Data (Patient Information)

Therapeutic Model (Cell Line and Animal Data) → Basic Science Hypothesis Testing (Cell Line and Animal Data)
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
HER-2/neu Program at UCLA

Clinical Material
(Tumor Specimens)

Clinical Trial
(Current 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)
Human Breast Cancer Cells

- MCF-7
  - Single copy
  - Low Expressor
  - Transfect HER-2/neu
  - MCF-7*
  - Multiple copy
  - High Expressor

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

Human Ovarian Cancer Cells

- CaOv-3
  - Single copy
  - Low Expressor
  - Transfect HER-2/neu
  - CaOv-3*
  - Multiple copy
  - High Expressor
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

MCF-7 → HER-2 Transfection → MCF-7/HER-2

- ↑ DNA Synthesis
- ↑ Cell Growth
- ↑ Growth in Soft Agar
- ↑ Tumorigenicity
- ↑ Metastatic Potential
- ↓ E2 Response, ↑↑ Tam Resist.
Target Validation
In Vivo Growth Inhibition Assay

MCF 7/HER-2

Days Post Injection

Days: 7-52

Y-axis: 0-2,000

- + 4D5
- + IgG

Graph shows the growth inhibition of MCF 7/HER-2 cells over time with different treatments.
Clinical Translation
HER-2/neu Program at UCLA

Clinical Material
(Tumor Specimens)

Clinical Trial
(Current Studies)

Molecular Studies
(DNA, RNA,
Protein Analyses)

Clinical Data
(Patient Information)

Basic Science
Hypothesis Testing
(Cell Line and Animal Data)

Therapeutic Model
(Cell Line and Animal Data)
## Phase I Clinical Trials of Anti-HER-2 MAbs

<table>
<thead>
<tr>
<th>Phase I</th>
<th>N</th>
<th>Study Design</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuMAb 4D5</td>
<td>20</td>
<td>Single dose (0.12 - 500 mg)</td>
<td>UCLA</td>
</tr>
<tr>
<td>H0453g</td>
<td>15</td>
<td>CDDP 100 mg/m² x 3 + rhuMAb HER-2 (10 - 500 mg x 9)</td>
<td>UCLA</td>
</tr>
<tr>
<td>H0452g</td>
<td>17</td>
<td>Multi-dose (10 - 500 mg)</td>
<td>UCLA, MSKCC, UCSF</td>
</tr>
<tr>
<td>H0407g</td>
<td>16</td>
<td>Single dose (10 - 500 mg)</td>
<td>UCLA, MSKCC</td>
</tr>
</tbody>
</table>
Herceptin in Combination with Chemotherapy

Objective - Combination Compared to Chemotherapy Alone

- Primary
  - Time to disease progression (REC)
  - Safety

- Secondary
  - Overall response rates
  - Durations of response
  - Time to treatment failure
  - 1-year survival
  - Quality of life
Herceptin in Combination with Chemotherapy

Design - Stratification to Chemotherapy

No prior anthracyclines

\[ \text{AC} = \text{doxorubicin (60 mg/m}^2) \text{ or epirubicin (75 mg/m}^2) + \text{ cyclophosphamide (600 mg/m}^2) \text{ q 3 wks x 6 cycles} \]

Prior anthracyclines

\[ \text{T} = \text{paclitaxel (175 mg/m}^2 \text{ x 3 hr) q 3 wks x 6 cycles} \]
## Herceptin in Combination with Chemotherapy

### Enrollment

<table>
<thead>
<tr>
<th>Total enrolled</th>
<th>469</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomization</td>
<td>H + CT</td>
</tr>
<tr>
<td></td>
<td>235</td>
</tr>
<tr>
<td>Subgroups</td>
<td>H + AC</td>
</tr>
<tr>
<td></td>
<td>143</td>
</tr>
</tbody>
</table>
## Summary: Phase III Clinical Trial Comparing Best Available Chemotherapy to Same Therapy + Herceptin

<table>
<thead>
<tr>
<th></th>
<th>Enrolled</th>
<th>R.R. (%)</th>
<th>Dur. Res.</th>
<th>T.T.P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H + CT</td>
<td>235</td>
<td>49 (53%↑)</td>
<td>9.3M (58%↑)</td>
<td>7.6M (65%↑)</td>
</tr>
<tr>
<td>CT</td>
<td>234</td>
<td>32</td>
<td>5.9M</td>
<td>4.6M</td>
</tr>
<tr>
<td>H + AC</td>
<td>138</td>
<td>52 (20%↑)</td>
<td>9.1M (40%↑)</td>
<td>8.1M (33%↑)</td>
</tr>
<tr>
<td>AC</td>
<td>145</td>
<td>43</td>
<td>6.5M</td>
<td>6.1M</td>
</tr>
<tr>
<td>H + T</td>
<td>92</td>
<td>42 (163%↑)</td>
<td>11.0M (150%↑)</td>
<td>6.9M (130%↑)</td>
</tr>
<tr>
<td>T</td>
<td>96</td>
<td>16</td>
<td>4.4M</td>
<td>3.0M</td>
</tr>
</tbody>
</table>
Herceptin in Combination with Chemotherapy

Survival Time

- Overall Herceptin impact on survival uncertain
  - Limited duration of follow-up ($\geq$12 months)
  - CT alone patients allowed to enter Herceptin extension protocol

- Preliminary analysis - improved 1-yr survival
  - $H + CT = 78\%$ alive
  - CT alone = $67\%$ alive
Clinical Safety

Summary of Herceptin Safety

- Herceptin is generally well tolerated
  - Single agent
  - Combined with chemotherapy
- Most adverse events mild to moderate in severity
  - Infusion associated symptoms, including fever and chills primarily with first dose
- Serious adverse events infrequent
- Increased incidence of cardiac dysfunction, particularly when administered with anthracycline based therapy
## Herceptin in Combination with Chemotherapy

### Cardiac Dysfunction Outcomes (CREC)

<table>
<thead>
<tr>
<th></th>
<th>H + AC</th>
<th>AC</th>
<th>H + T</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac Dysfunction Events (#)</strong></td>
<td>39 (27%)</td>
<td>9 (7%)</td>
<td>11 (12%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td><strong>Herceptin Rx Post Event (#)</strong></td>
<td>14</td>
<td>5*</td>
<td>6</td>
<td>1*</td>
</tr>
<tr>
<td><strong>Deaths (#)</strong></td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MBC</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cardiac</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Herceptin extension protocol
Conclusion

♦ The results of this study indicate that Herceptin™ (Trastuzumab) in combination with chemotherapy is well-tolerated and provides substantial clinical benefit in first-line treatment of HER-2 overexpressing metastatic breast cancer. Drug approved in Sept. 1998 as the first proto-oncogene kinase targeted therapeutic.

♦ Future studies of Herceptin will be important
  – Adjuvant breast cancer - preclinical data show earlier rx better
  – Other combinations
Adjuvant use of Herceptin must be evaluated in a randomized-controlled trial.
BCI RG 006
Adjuvant Breast Cancer
Node Positive and High Risk Node Negative

HER2 +
FISH

N=3150

4 x AC
60/600 mg/m²

4 x Docetaxel
100 mg/m²

4 x AC

4 x Docetaxel

AC→T

AC→TH

6 x Docetaxel and Platinum salts
75 mg/m²
75 mg/m² or AUC 6

TCH

1 Year Trastuzumab

1 Year Trastuzumab
The “One-Size-Fits-All” Approach to Breast Cancer
CALGB 9344: Overall Survival

CALGB 9741
Interim Analyses

Disease-Free Survival

Overall Survival

85 vs 81% (P=0.0072)

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

Cumulative Probability

Survival Time (months)

N | Events | HR | \(p\)-value
---|---|---|---
TAC | 745 | 91 | 0.70 | .0080
FAC | 746 | 130 | 

Stratified Log-Rank

TAC 87%  FAC 81%
Can We Do Better?

The Hope - Clinical Translation of Biologically Relevant Molecular Information Should Lead to More Effective and Less Toxic Therapeutic Approaches
The HER2 Alteration

Southern
Northern
Western
IHC

Slamon et al. Science 1989
Disease-Free Survival

**B-31**

- AC\(\rightarrow\)TH
- AC\(\rightarrow\)T

\(N\) Events

- AC\(\rightarrow\)TH: 864, 83 events
- AC\(\rightarrow\)T: 872, 171 events

HR=0.45, 2P=1x10\(^{-9}\)

**N9831**

- AC\(\rightarrow\)TH
- AC\(\rightarrow\)T

\(N\) Events

- AC\(\rightarrow\)TH: 807, 90 events
- AC\(\rightarrow\)T: 808, 51 events

HR=0.55, 2P=0.0005

Years From Randomization
BCIRG 006
Adjuvant Breast Cancer
Node Positive and High Risk Node Negative

HER2 +

FISH

N=3150

4 x AC
60/600 mg/m²

4 x Docetaxel
100 mg/m²

6 x Docetaxel and Platinum salts
75 mg/m² 75 mg/m² or AUC 6

1 Year Trastuzumab

1 Year Trastuzumab

AC → T

AC → TH

TCH
<table>
<thead>
<tr>
<th>LVEF Declines by NYHA Class</th>
<th>AC-T</th>
<th>AC-TH</th>
<th>TCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10%, &lt;LLN</td>
<td>9</td>
<td>34</td>
<td>7</td>
</tr>
<tr>
<td>&gt;15%, &lt;LLN</td>
<td>6</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Grade 3/4 CHF</td>
<td>2</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>
THE FUTURE
Can We Do Even Better?

The Hope - Clinical Translation of Biologically Relevant Molecular Information Should Lead to Even More Effective and Less Toxic Therapeutic Approaches
Can we recognize molecular signaling pathway activation in cancer?

- Is activation ligand dependent?
- Are the initiating receptors interchangeable?
- What are the downstream effector genes?
- How do we determine if a cancer cell is dependent on a particular pathway or receptor?
- Can we identify gene signatures that predict response to molecularly targeted therapies?
Pathway Analysis
Biologic Effects of HER-2/neu Overexpression in Human Breast Cancer Cells

- Increased DNA Synthesis
- Increased Cell Growth
- Increased Growth in Soft Agar
- Increased Tumorigenicity
- Increased Metastatic Potential
- Decreased E2 Response, Increased Tam Resistant
How Does an Alteration in This One Gene Result in So Many Changes in Biologic Behavior?

♦ While it is an important “inciting” event, amplification of HER2/neu does not cause it’s associated clinical phenotype in isolation.

♦ What other genes and/or pathways need to be engaged to bring about this profound clinical picture?

♦ A better understanding of those genes and/or pathways directly associated with the HER2/neu alteration will lead to more effective therapeutic approaches
Global gene expression profiling

Confirmation of expression

Possible Biologic Relevance

Confirmation of Functional Relevance
cDNA Microarrays
Synteni/Incyte Double Fluorescence Method
GEMS 1-4, V (representing 40,000 elements)

Self RNA test
MCF-7/H2 v.s. CN

490 elements Δ > 2.5 fold
Data Analysis

♦ Clustering:
  – gene expression relatedness

♦ Pathway construction:
  – biologically biased hierarchical ordering
Summary: cDNA Microarray

<table>
<thead>
<tr>
<th>Category</th>
<th>aMCF-7 Down</th>
<th>aMCF-7 Up</th>
<th>bMCF-7 Down</th>
<th>bMCF-7 Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptors</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Growth factors, cytokines</td>
<td>8</td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>GF induced proteins</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Cell cycle related</td>
<td>1</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Apolipoprotein related</td>
<td>26</td>
<td>31</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Cell adhesion-cytoskeleton</td>
<td>19</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Oncogenes/transcription factors</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Proteases and protease inhibitors</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>DNA/chromosome maintenance</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Drug resistance</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Complement related</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Housekeeping/chaperone proteins</td>
<td>29</td>
<td>13</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Nucleotide exchange factors</td>
<td>20</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tRNA synthetases</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enzymes/metabolism</td>
<td>20</td>
<td>12</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Misc. surface antigens</td>
<td>0</td>
<td>0</td>
<td>103</td>
<td>47</td>
</tr>
<tr>
<td>Uncatagorized known genes</td>
<td>29</td>
<td>13</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Unknown genes</td>
<td>20</td>
<td>7</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>EST with homology</td>
<td>24</td>
<td>15</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>EST without homology</td>
<td>103</td>
<td>47</td>
<td>103</td>
<td>47</td>
</tr>
<tr>
<td>Total changes greater than 2.5 fold</td>
<td>302</td>
<td>188</td>
<td>302</td>
<td>188</td>
</tr>
</tbody>
</table>
Selection Criteria for Analysis of Differentially Expressed Genes

♦ Genes falling into identifiable pathways

♦ Genes effected in multiple cell lines

♦ Changes most likely to directly contribute to the HER-2/neu phenotype

♦ Expression changes reversed by Herceptin
## Angiogenic Pathways

<table>
<thead>
<tr>
<th>Gene name</th>
<th>MCF-7 con vs H2</th>
<th>ZR-75 con vs H2</th>
<th>LnCap con vs H2</th>
<th>SKBR3 W/Hcpt</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>1.64 (f)</td>
<td>4.5 (f)</td>
<td>2.2 (f)</td>
<td>-</td>
</tr>
<tr>
<td>Angiopoietin-1</td>
<td>4.2 (f)</td>
<td>-</td>
<td>-</td>
<td>1.9 (f)</td>
</tr>
<tr>
<td>FGFR 4</td>
<td>2.8 (f)</td>
<td>2.3 (f)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Global gene expression profiling

Confirmation of expression

Possible Biologic Relevance

Confirmation of Functional Relevance
Cell Line RNA Northern: VEGF Probe

- HMEC Neo
- HMEC HER-2
- HBL-100 Neo
- HBL-100 HER-2
- BT20 Neo
- BT20 HER-2
- MCF-7 Neo
- MCF-7 HER-2
- MDA-231 Neo
- MDA-231 HER-2
- MDA-435 HER-2
- BT474
- SKBr3

Kb
4.4
3.7
Does activation of HER-2/neu result in increased VEGF production?
Concentration of VEGF in Conditioned Media of MCF-7 Neo and MCF-7 HER-2/neu Cells

VEGF (ng/cell)

Treatment

MCF-7 Neo

MCF-7 HER-2/neu

1 nm Control

1 nm HRG

10 nm Control

10 nm HRG
♦ Global gene expression profiling

♦ Confirmation of expression

♦ Possible Biologic Relevance

♦ Confirmation of Functional Relevance
Are the increased VEGF levels in HER-2/neu transfectants associated with increased angiogenesis \textit{in vivo}?
Angiogenesis in MCF-7 Spheroids:

Day 0

**MCF-7 Neo:**

1 x mag.
913 µm x 789 µm

**MCF-7 HER-2/neu:**

1 x mag.
876 µm x 857 µm
Angiogenesis in MCF-7 Spheroids: Day 3

**MCF-7 Neo:**
- 1 x mag.
- Vessel buds starting to form
- Vessels dilated

**MCF-7 HER-2/neu:**
- 1 x mag.
- Increased # of vessels
  - Vessels dilated
  - Vessels tortuous
Angiogenesis in MCF-7 Spheroids: Day 7

**MCF-7 Neo:**
- 1 x mag.
- Small capillaries and a few buds present
- 10 x mag.
- Vessels hemorrhaging

**MCF-7 HER-2/neu:**
- 3.5 x mag.
- Huge vessel network
- Large amount of vessel budding
Angiogenesis in MCF-7 Spheroids:

Day 14

**MCF-7 Neo:**
- 3.5 x mag.
- Mature vasculature
- No vessel buds
- Development stopped

**MCF-7 HER-2/neu:**
- 10 x mag.
- High number mature vessels
- Vessel buds in center of tumor
- Vasculature still growing
Does Herceptin decrease the levels of VEGF production in tumor cells?
Levels of VEGF in MCF-7 Cells after Herceptin Treatment

**MCF-7 HER-2/neu Cells**

- **Control**
- **Herceptin**

**MCF-7 Neo Cells**

- **Control**
- **Herceptin**

<table>
<thead>
<tr>
<th>VEGF (ng/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00E+00</td>
</tr>
<tr>
<td>2.50E-05</td>
</tr>
<tr>
<td>2.25E-05</td>
</tr>
<tr>
<td>2.00E-05</td>
</tr>
<tr>
<td>1.75E-05</td>
</tr>
<tr>
<td>1.50E-05</td>
</tr>
<tr>
<td>1.25E-05</td>
</tr>
<tr>
<td>1.00E-05</td>
</tr>
<tr>
<td>7.50E-06</td>
</tr>
<tr>
<td>5.00E-06</td>
</tr>
<tr>
<td>2.50E-06</td>
</tr>
<tr>
<td>0.00E+00</td>
</tr>
</tbody>
</table>

Days: 0 4 5 7 10 11 12 13

<table>
<thead>
<tr>
<th>VEGF (ng/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00E+00</td>
</tr>
<tr>
<td>1.00E-05</td>
</tr>
<tr>
<td>9.00E-06</td>
</tr>
<tr>
<td>8.00E-06</td>
</tr>
<tr>
<td>7.00E-06</td>
</tr>
<tr>
<td>6.00E-06</td>
</tr>
<tr>
<td>5.00E-06</td>
</tr>
<tr>
<td>4.00E-06</td>
</tr>
<tr>
<td>3.00E-06</td>
</tr>
<tr>
<td>2.00E-06</td>
</tr>
</tbody>
</table>

Days: 0 4 10 11 12 13
Do the Preclinical Data Translate to Findings in Clinical Specimens?
### Patient and disease characteristics in node-negative and -positive primary breast cancer patients (n=611)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>58 years</td>
<td>611</td>
</tr>
<tr>
<td><strong>Tumor size</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;2 cm)</td>
<td>231</td>
<td>38.2</td>
</tr>
<tr>
<td>(2-4.9 cm)</td>
<td>310</td>
<td>51.2</td>
</tr>
<tr>
<td>(&gt;5 cm)</td>
<td>64</td>
<td>10.6</td>
</tr>
<tr>
<td><strong>Number of positive nodes</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>290</td>
<td>48.7</td>
</tr>
<tr>
<td>1-3</td>
<td>183</td>
<td>30.7</td>
</tr>
<tr>
<td>4-9</td>
<td>61</td>
<td>10.3</td>
</tr>
<tr>
<td>≥10</td>
<td>61</td>
<td>10.3</td>
</tr>
<tr>
<td><strong>Lymph node status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>290</td>
<td>48.3</td>
</tr>
<tr>
<td>Positive</td>
<td>310</td>
<td>51.7</td>
</tr>
<tr>
<td><strong>Nuclear grade</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>368</td>
<td>60.4</td>
</tr>
<tr>
<td>3-4</td>
<td>241</td>
<td>39.6</td>
</tr>
<tr>
<td><strong>Hormone receptor status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>137</td>
<td>22.4</td>
</tr>
<tr>
<td>Positive</td>
<td>474</td>
<td>77.6</td>
</tr>
<tr>
<td><strong>HER-2/neu status</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>497</td>
<td>81.3</td>
</tr>
<tr>
<td>Positive</td>
<td>114</td>
<td>18.7</td>
</tr>
<tr>
<td><strong>VEGF_{121} status</strong>****</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>252</td>
<td>41.2</td>
</tr>
<tr>
<td>Positive</td>
<td>359</td>
<td>58.8</td>
</tr>
<tr>
<td><strong>VEGF_{165} status</strong>****</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>158</td>
<td>25.9</td>
</tr>
<tr>
<td>Positive</td>
<td>453</td>
<td>74.1</td>
</tr>
</tbody>
</table>
Prognostic Significance of Detectable VEGF$_{165}$ and VEGF$_{121}$ Expression for Survival in Primary Breast Cancer

A biological concentration-effect relationship between VEGF expression and survival

Log-Rank p = 0.0097
Correlation between HER2 and VEGF$_{121}$ in Primary Breast Cancer

<table>
<thead>
<tr>
<th></th>
<th>VEGF$_{121}$ negative</th>
<th>VEGF$_{121}$ positive*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 negative</td>
<td>226 (45.5%)</td>
<td>271 (54.5%)</td>
<td>480 (100%)</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>26 (22.8%)</td>
<td>88 (77.2%)</td>
<td>108 (100%)</td>
</tr>
</tbody>
</table>

Chi-Square Test: $p < 0.001$

* VEGF$_{121}$-positive - detectable VEGF$_{121}$ levels above the lower assay sensitivity of 16 pg/ml

Combined effects of HER2 and VEGF$_{165}$ expression on survival

♦ Global gene expression profiling

♦ Confirmation of expression

♦ Possible Biologic Relevance

♦ Confirmation of Functional Relevance
What is the effect of Herceptin and the VEGF antibody on tumor growth \textit{in vivo}?
Effect of Herceptin, rhuMAb VEGF, and the Combination against HER2-overexpressing xenografts.

Do the Preclinical Therapeutic Data Translate into the Clinic?
Phase I/II clinical trial of Herceptin and Avastin in breast cancer

**Hypothesis:** upregulation of VEGF in HER2+ MBC contributes to the aggressive phenotype of HER2+ MBC. The ‘angiogenic switch’ modulated by Herceptin can be exploited in the clinic by combined blockade of these two “linked” pathways.

**Study Endpoints**
1. Clinical Safety
2. Pharmacokinetics
3. Efficacy

**Inclusion Criteria:**
- LABC or MBC
- HER2+ by FISH
- ECOG 0-1
- Age >18 Y
- LVEF WNL

**Treatment Schedule:**
- Herceptin 4mg/kg → 2mg/kg qw
- Avastin dose escalation (n=24)
  - A 3mg/kg → 5mg/kg → 10mg/kg
  - IV d7 then q14d

Herceptin 4mg/kg → 2mg/kg qw
+ Avastin q14d
Pharmacokinetics:

Mean $t_{1/2}$ bevacizumab = 19.3d
Mean $t_{1/2}$ trastuzumab = 22.2d

Trastuzumab + Bevacizumab, Phase I
PK/Toxicity/Efficacy Data in 9 pts

- No change in the PK of either antibody when used as combo
- No untoward toxicity induced by combo - 1 pt with mild $^\text{bp}$ treated with diazide
- 2 CR’s
- 3 PR’s
- 2 SD’s > 7 months
- 2 PD’s
Challenges to combined use of targeted therapeutics

♦ Identifying the appropriate patient population

♦ Do we simply integrate new targeted therapies with established regimens? Advantages/Problems

♦ Is broader target specificity better than more narrow targeting?

♦ What are the most rational targeted combinations to test clinically?

♦ Can we determine the best likely combinations preclinically before going into the clinic?
Acknowledgements - UCLA

♦ Jane Arboleda
♦ Raul Ayala
♦ Gina Bernardo
♦ Jenny Chen
♦ Amy Cook
♦ Judy Dering
♦ Melinda Epstein
♦ Robert Ferdman
♦ Richard Finn
♦ Chuck Ginther
♦ Padraic Glaspy
♦ Fairooz Kabbinavar
♦ Gottfried Konecny
♦ Mark Pegram
♦ Richard Pietras
♦ Lillian Ramos
♦ David Reese
♦ Hong Mei Rong
♦ Nishan Tchekmedyian
♦ Cindy Wilson
♦ Steve Wong
Acknowledgements (con’t)

♦ Industry Partners:
  ♦ Amgen, Genentech,

♦ Amgen:
  Frank Calzone
  Elaina Cajulis


♦ Revlon Foundation:
  Ronald Perlman
  Jim Conroy

♦ Community-based/UCLA Clinical Research Network --- TORI
  Nancy Ryba, Polly Candella
  David Reese, Fairooz Kabbinavar