Herceptin®
(Trastuzumab)

Oncologic Drugs Advisory Committee Meeting

December 5, 2001

Genentech, Incorporated
Introduction

Marianne Armstrong, Ph.D.
Sr. Director, Regulatory Affairs
Genentech, Incorporated
Purpose

To seek approval of Genentech’s sBLA that requests inclusion of fluorescence *in situ* hybridization (FISH) testing using the PathVysion™ HER2 DNA Probe Kit (Vysis, Inc.) in the current Herceptin label as a diagnostic method to select patients for Herceptin therapy.
Herceptin Profile

- Herceptin is a recombinant DNA-derived humanized monoclonal antibody that targets HER2, the protein product of \(c-erbB-2\).
- More than 60,000 women worldwide have received Herceptin since market introduction.
Regulatory History

- Herceptin was approved in September 1998 for:
  - First line treatment in combination with paclitaxel in MBC patients whose tumors overexpress HER2.
  - Second- or third-line, single agent therapy in MBC patients whose tumors overexpress HER2.
The only FDA-approved diagnostic method to aid in the selection of patients for Herceptin therapy is immunohistochemistry (IHC).

The two FDA-approved HER2 IHC diagnostic kits include the HercepTest® (DAKO, Inc.) and Pathway™ (Ventana, Inc.).

Only the HercepTest is included in the Herceptin package insert.
Today we will:

- Present data that demonstrate PathVysion, a HER2 FISH kit, is an appropriate method to aid in the selection of patients for Herceptin therapy.

This data will include:

- HER2 biology and the scientific rationale
- Concordance data from the Herceptin clinical trials database
- Exploratory clinical outcomes analysis from the Herceptin clinical trials database
Agenda

Marianne Armstrong, Ph.D.
Sr. Director, Regulatory Affairs
Genentech, Inc.

Michael Press, M.D., Ph.D
Professor, Dept. of Pathology
Harold E. Lee Chair for Cancer Research
Norris Comprehensive Cancer Center
University of Southern California

Robert Mass, M.D
Assoc. Director, Medical Affairs
Genentech, Inc.

Introduction
HER2 Biology and Methods of Assessment
Concordance & Clinical Outcome Analyses
Conclusions
Our goal today is to demonstrate that PathVysion is an appropriate method to aid in the selection of patients for Herceptin therapy.
HER2 Biology and Methods of Assessment

Michael Press, M.D., Ph.D.
Professor
Harold E. Lee Chair for Cancer Research
Department of Pathology
Norris Comprehensive Cancer Center
University of Southern California
HER2 Biology and Methods of Assessment

- HER2 biology
- Immunohistochemistry (IHC)
- Fluorescence *In Situ* Hybridization (FISH)
- Clinical significance
Clinical Implications of HER2/neu Amplification

Node-positive patients with no amplification vs node-positive patients with greater than 5 copies of HER2/neu

Slamon et al., Science 235:177-182, 1987
Localization of HER2/neu Gene on Chromosome 17

Normal interphase nucleus and metaphase spread

HER-2/neu Chromosome 17 centromere
Epidermal Growth Factor Receptor Family

HER2 Biology

EGF
TGF-α
Amphiregulin
Betacellulin
HB-EGF

ErbB1
EGFR

ErbB2
HER2
neu

Heregulins

ErbB3
HER3

ErbB4
HER4

NRG2
NRG3
Heregulins
Betacellulin

Cysteine Rich Domains

Tyrosine Kinase Domain
HER2 Biology
Correlation of HER2/neu Gene Amplification with Overexpression

Amplification Level:
- 12.5 kb
- 4.4 kb
- p185

Frozen IHC:
- 27%

10% 63% % Women

Slamon et al., Science 244: 707-712, 1989
HER2 Biology

Single Copy Overexpression

HER2 Biology

HER-2/neu Gene Amplification is Responsible for Overexpression

Diagram illustrating gene amplification leading to HER-2/neu overexpression.
Fixation and Paraffin Embedding Result in Decreased Antigenicity

2 to 5 fold Amplified Frozen IHC

2 to 5 fold Amplified/Fixed, Paraffin IHC

Slamon et al., Science 244:707-712, 1989
Immunohistochemistry: Clinical Trial Assay

Key Features:

- Primary antibody - two different monoclonals
  - 4D5 and CB11
- Procedure - indirect avidin-biotin for each antibody
- Antigen retrieval
  - 4D5 - protease digestion
  - CB11 - microwave
HER2 Overexpression Detection by Immunohistochemistry

Negative or, 0+

1+

2+

3+
Detection of HER2 Protein by Immunohistochemistry

Pros
- Widely available
- Rapid procedure
- Light microscope based
- HercepTest™ and Pathway™ FDA-approved assays for Herceptin eligibility selection

Cons
- Variable antibody sensitivity and specificity
  - Highly impacted by tissue processing variables
  - Affected by antigen retrieval and reagent variability
- Non-FDA-approved assays in routine use
- Subjective scoring criteria
  - Low pathologist concordance and high interlaboratory variability
Fluorescence *in situ* Hybridization: PathVysion

**Key Features:**
- **Probes**
  - Direct labeled
  - HER2 sequence
  - Chromosome 17 centromere
- **Interpretation**
  - Signal enumeration
  - Ratio of HER2:Chr 17 signals
HER2/neu Gene Assessment by FISH

< 2.0 Not Amplified (FISH-)

≥ 2.0 Amplified (FISH+)
Population Distribution of FISH Scores
Use of FISH to Measure HER2 Gene Copy Number

**Pros**
- DNA is a stable target
- Standardized threshold for positivity
- Built-in internal control
- Low interlaboratory variability
- High accuracy (sensitivity and specificity)

**Cons**
- Fluorescence microscope equipped with correct filter sets is required
- Certain fixatives interfere with assay (non-informative result)
- Limited community experience with tissue-based FISH
**Clinical Significance**

HER2/neu Gene Amplification Associated with Poor Outcome in Node-negative Breast Cancer

HER-2/neu Gene Amplification by FISH is Predictive of Response to “High-Dose” Adriamycin Chemotherapy

Overall survival

**Clinical Significance**

Comparison of Overall Survival in FISH+ / IHC+ versus FISH- / IHC+ Breast Cancer

**Pauletti et al, J Clin Onc, 21:3651-3664, 2000**
Conclusions

- Direct correlation exists between gene amplification and overexpression
- FISH is a robust method for detecting gene amplification
- Amplification, as determined by FISH, is a clinically meaningful measure associated with poor prognosis and predictive of therapeutic response
Robert D. Mass, MD

Associate Director, Oncology
Genentech, Inc.
Fundamental biologic link between HER2 amplification and protein overexpression

PathVysion has the ability to provide both prognostic and predictive information in human breast cancer

IHC, the only FDA approved methodology to select patients for Herceptin therapy, appears to have significant accuracy issues when applied to formalin fixed clinical material
Introduction

Survival
Chemotherapy +/- Herceptin, 1st line MBC

PathVysion will provide an alternative, non-IHC assay method to accurately identify patients for Herceptin therapy.
Introduction

Goal: to provide data supporting the addition of PathVysion (FISH) to the Herceptin label to identify patients for Herceptin therapy
Introduction

Two studies support the label supplement

- **Concordance Study**
  - Concordance between PathVysion and the Herceptin Clinical Trials Assay (CTA)

- **Clinical Outcomes Study**
  - Clinical outcomes analysis assessing FISH status in the pivotal Herceptin trials
  - Interlaboratory validation assessment
Introduction

Source of Tissue Specimens

- The Herceptin pivotal trials represent the only large database available to correlate HER2 diagnostics with treatment outcome

- Both studies utilized archived tissue sections that had been stored for 2 to 5 years
Concordance Study

Objective: To establish the concordance between the CTA and FISH (PathVysion)

Methodology

- Prospectively defined study utilizing clinical trials samples that were retrospectively tested with PathVysion
- Single blinded
  - Analysis plan identical to the HercepTest concordance protocol used for FDA approval
    - 1:1 positive:negative sample ratio
      - positive (CTA 2+/3+)
      - negative (CTA 0/1+)
    - Provides maximal statistical power to assess concordance
- FISH positive defined as HER2:CEP17 ratio ≥ 2
Statistics

- **Primary endpoint**
  - Concordance in 1:1 population

- **Secondary endpoints**
  - Concordance extrapolated to the clinical trials population
  - Kappa statistic

- **Assumptions**
  - Concordance \( \leq 75\% \) was pre-specified as ‘unacceptable’
  - 90% power to detect 5% improvement over that ‘unacceptable’ level (\( \leq 75\% \))
  - 1-sided test on proportion

- **Sample Size:** \( \sim 600 \) total specimens
Concordance Study

Specimen Identification

5998 patients with CTA results

5271 patients with \( \geq 2 \) unstained tissue sections archived at LabCorp

623 randomly selected approximate 1:1 ratio

317 CTA (+) 2+/3+

306 CTA (-) 0/1+

529 FISH Results (85%)
### Concordance Study

**Results**

1:1 population

Concordance = 82% (95% CI of 78%, 85%)

κ statistic = 0.64 (95% CI of 0.58, 0.70)

<table>
<thead>
<tr>
<th></th>
<th>CTA -</th>
<th>CTA +</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH -</td>
<td>235</td>
<td>88</td>
</tr>
<tr>
<td>FISH +</td>
<td>9</td>
<td>197</td>
</tr>
<tr>
<td>TOTAL</td>
<td>244</td>
<td>285</td>
</tr>
</tbody>
</table>
## Concordance Study

### Results

**1:1 population**

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

| Amplification rate | 3% | 7% | 24% | 89% |

**Amplification rate**
Extrapolation to the Population Screened for Herceptin Trials

Concordance Study

Clinical Trials

<table>
<thead>
<tr>
<th>CTA</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>

% of population

- FISH +
- FISH -

(0) 58%
(1+) 9%
(2+) 10%
(3+) 23%
## Concordance Study

### Results

Extrapolated to Clinical Trials Population

Concordance = 88%  (95% CI of 85%, 91%)

<table>
<thead>
<tr>
<th></th>
<th>CTA -</th>
<th>CTA +</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH -</td>
<td>342</td>
<td>53</td>
</tr>
<tr>
<td>FISH +</td>
<td>12</td>
<td>122</td>
</tr>
<tr>
<td>TOTAL</td>
<td>354</td>
<td>175</td>
</tr>
</tbody>
</table>
### Concordance Study

**PathVysion versus HercepTest**

<table>
<thead>
<tr>
<th></th>
<th>1:1 concordance</th>
<th>Extrapolated concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PathVysion/CTA</td>
<td>82%</td>
<td>88%</td>
</tr>
<tr>
<td>HercepTest/CTA</td>
<td>79%</td>
<td>83%</td>
</tr>
</tbody>
</table>
Conclusions

- The concordance between PathVysion and the CTA in a 1:1 population is 82%
- This exceeded the pre-specified level of acceptability (p < 0.0001)
- The level of concordance between PathVysion and the CTA is consistent with HercepTest
- PathVysion will provide similar performance, compared to HercepTest, when used as a surrogate for the CTA to select patients for Herceptin therapy
Clinical Outcomes Analysis
Rationale

- Post-approval commitment to the FDA to explore other HER2 diagnostics in the context of Herceptin clinical trials
- Provide clinical outcomes data, in addition to concordance, to support FISH as an appropriate method to select patients for Herceptin therapy
Explore the relationship between FISH status (FISH+ versus FISH-) and Herceptin clinical benefit as assessed by a retrospective analysis of:

- Response rate
- Time to disease progression
- Survival

In 3 Herceptin clinical trials (n=799 patients)

- Chemotherapy +/- Herceptin, 1st line MBC
- Herceptin monotherapy, 2nd & 3rd line MBC
- Herceptin monotherapy, 1st line MBC
Study Population

- The Herceptin pivotal trials represent the only large database available to correlate HER2 diagnostics with treatment outcome
  
  - Tissue database was not designed for subsequent validation of alternative diagnostic assays
  
  - Tumor blocks or tissue sections submitted: *only* tissue sections archived
  
  - Clinical outcomes data available only for the CTA 2+/3+ subset who enrolled into Herceptin trials
**Specimen Identification**

- 799 patients enrolled
- 15 enrolled based on Non-CTA results
- 784 with archived sections

**Clinical Outcomes Analysis**

**LabCorp**
- 618 unused tissue sections
- 540 FISH results from LabCorp

**USC**
- 244 immunostained tissue sections
- 225 FISH results from USC

765/799 (96%) FISH Results “Primary Analysis Dataset”
Eligible Patients (n = 222)

- Metastatic breast cancer
- HER2 overexpression (2+/3+)
- 1 or 2 prior CT for MBC anthracycline and taxane

**Study Design**

*Herceptin monotherapy, 2\(^{nd}\) & 3\(^{rd}\) line MBC*

- **Eligible Patients (n = 222)**

- **Herceptin**
  - 4 mg/kg loading dose
  - 2 mg/kg/wk maintenance

**Primary Endpoint:** Response Rate

**Secondary Endpoints:** Time to Progression, Survival
## Clinical Outcomes Analysis

### Response Rate

*Herceptin monotherapy, 2\textsuperscript{nd} & 3\textsuperscript{rd} line MBC*

<table>
<thead>
<tr>
<th>FISH</th>
<th>n</th>
<th>Herceptin monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH +</td>
<td>33/163</td>
<td>20% (14.4, 27.2)</td>
</tr>
<tr>
<td>FISH -</td>
<td>0/46</td>
<td>0% (0.0, 7.7)</td>
</tr>
</tbody>
</table>

2+/3+ 15%
Eligible Patients (n = 469)
- Metastatic breast cancer
- HER2 overexpression (2+/3+)
- No prior CT for MBC

Study Design
Chemotherapy +/- Herceptin, 1st line MBC

Primary Endpoint: Time to Progression
Secondary Endpoints: Response Rate, Survival
### Clinical Outcomes Analysis

#### Response Rate

**Chemotherapy +/- Herceptin, 1st line MBC**

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy alone</th>
<th>Chemotherapy + Herceptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH + n=325</td>
<td>30%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td><em>p &lt; 0.0001</em></td>
<td></td>
</tr>
<tr>
<td>FISH - n=126</td>
<td>38%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td><em>p = 0.7452</em></td>
<td></td>
</tr>
</tbody>
</table>

2+/3+ 32→50%
Clinical Outcomes Analysis

Time to Disease Progression
Chemotherapy +/- Herceptin, 1st line MBC

FISH+
- Herceptin + Chemo (n = 164)
- Chemotherapy Alone (n = 161)
  Risk ratio = 0.44
  95% CI = 0.24, 0.57

FISH -
- Herceptin + Chemo (n = 62)
- Chemotherapy Alone (n = 64)
  Risk ratio = 0.66
  95% CI = 0.44, 0.99
Survival
Chemotherapy +/- Herceptin, 1st line MBC

Clinical Outcomes Analysis

FISH+
- Herceptin + Chemo (n = 164)
- Chemo Alone (n = 161)

Risk ratio = 0.69
95% CI = 0.53, 0.91

FISH -
- Herceptin + Chemo (n = 62)
- Chemo Alone (n = 64)

Risk ratio = 1.07
95% CI = 0.70, 1.63
Within both pivotal trials FISH+ status appears to consistently identify a population which benefits from Herceptin therapy.

<table>
<thead>
<tr>
<th></th>
<th>FISH (+)</th>
<th>FISH (-)</th>
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</thead>
<tbody>
<tr>
<td><strong>Monotherapy trial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response rate</td>
<td>20%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Combination trial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response rate</td>
<td>30 → 54%</td>
<td>38 → 40%</td>
</tr>
<tr>
<td>Time to progression, risk ratio</td>
<td>0.44</td>
<td>0.66</td>
</tr>
<tr>
<td>Survival, risk ratio</td>
<td>0.69</td>
<td>1.07</td>
</tr>
</tbody>
</table>
Clinical Outcomes Analysis

Inter-laboratory Validation Assessment

- **Objective**
  - To ensure that assay methodology differences between the laboratories would not influence the interpretation of the clinical outcome results

- **Methods**
  - Previously immunostained tissue sections from 248 patients with known FISH results at LabCorp were sent to USC for repeat FISH testing in two stages
  - All patients with a FISH- result at LabCorp were retested at USC
  - Results obtained in 221/248 (89%)
Results

- Overall agreement 82%
- LabCorp FISH+ agreement 98% (79/81)
- LabCorp FISH- agreement 74% (103/140)
  - 84% of the 37 discordant results were CTA 3+
  - Indicative of underscoring at LabCorp

Exploration of laboratory differences suggests

- Different condition of the specimens
- Different methodology for protease digestion step
Clinical Outcomes Analysis

Exploratory Secondary Analysis

- Re-analysis of the clinical data using USC results, when available
- No impact on the results of clinical outcomes analysis

<table>
<thead>
<tr>
<th></th>
<th>FISH (+)</th>
<th></th>
<th>FISH (-)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>primary</td>
<td>secondary</td>
<td>primary</td>
<td>secondary</td>
</tr>
<tr>
<td>Monotherapy trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response rate</td>
<td>20%</td>
<td>19%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Combination trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response rate</td>
<td>30→54%</td>
<td>31→54%</td>
<td>38→40%</td>
<td>38→38%</td>
</tr>
<tr>
<td>Time to progression</td>
<td>0.44</td>
<td>0.45</td>
<td>0.66</td>
<td>0.68</td>
</tr>
<tr>
<td>risk ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival risk ratio</td>
<td>0.69</td>
<td>0.70</td>
<td>1.07</td>
<td>1.13</td>
</tr>
</tbody>
</table>

§ Re-analysis of the clinical data using USC results, when available
§ No impact on the results of clinical outcomes analysis
Unanswered Questions

- Do FISH+ / IHC (0,1+, 2+) benefit to the same extent as FISH+ / IHC 3+?
- Do FISH- / IHC 3+ benefit to the same extent as FISH+ / IHC 3+?
- What can be concluded regarding these subsets from retrospective analyses of the Herceptin pivotal trials?
- Are prospective clinical trials feasible?
Clinical Outcomes Analysis

Do FISH+ / IHC (0,1+, 2+) benefit to the same extent as FISH+ / IHC 3+?

<table>
<thead>
<tr>
<th>CTA</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>56%</td>
<td>8%</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>+</td>
<td>2%</td>
<td>1%</td>
<td>2%</td>
<td>21%</td>
</tr>
</tbody>
</table>

Expected Distribution in Clinical Trials Population
Prospective Confirmatory Trials

- Metastatic Breast Cancer
  - Assumptions:
    - Comparison is 3+/FISH+ versus <3+/FISH+
    - Non-inferiority design

<table>
<thead>
<tr>
<th>Required Number of Screened Patients</th>
<th>Required Sample Size</th>
</tr>
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<tbody>
<tr>
<td>~30,000</td>
<td>3300</td>
</tr>
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</table>
Summary

- The concordance analysis demonstrates that PathVysion will provide similar performance, compared to HercepTest, when used as a surrogate for the CTA to select patients for Herceptin therapy.

- The clinical outcomes analysis provides additional data supporting FISH as an appropriate method to select patients for Herceptin therapy.
Final Conclusion

The Herceptin package insert should be modified to include PathVysion as an appropriate method to aid in the selection of patients for Herceptin therapy.