Monoclonal Antibodies in Cancer

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Genentech, Inc.
Disclaimer

- I had nothing to do with Herceptin
- Using lessons learned in new antibody projects
The HER2/ErbB Signaling Network

Why Target **Human EGF Receptor 2**

**HER2 Oncogene Amplification**

**HER2 Oncoprotein Overexpression**

**Shortened Median Survival**
- HER2 overexpressing: 3 yrs
- HER2 normal: 6 - 7 yrs

Slamon et al, 1987
**Normal Cell**

In normal breast tissue cells, the HER2 gene produces a protein receptor on the cell surface. These growth factor-like receptors are thought to play a role in normal cell growth by signaling the cell to divide and multiply.

**Herceptin® (Trastuzumab)**

Herceptin (a HER2 antibody) binds to numerous HER2 receptor sites found on the cell surface, blocking the receptor sites and possibly preventing further growth by interrupting the growth signal. As a result, the HER2 antibody may slow progression of the disease.

**HER2 Overexpressing Cancer Cell**

Cancerous breast tissue cells that overexpress (or overproduce) the HER2 gene produce extra protein receptors on the cell surface, which triggers the cell to divide and multiply at an accelerated rate, thus contributing to tumor growth.
Herceptin™ confers survival benefit in metastatic breast cancer

Slamon et al (2001) NEJM 344, 783-792
<table>
<thead>
<tr>
<th>Discovery</th>
<th>Development</th>
<th>Marketing and Line Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idea for new target</td>
<td>IND-enabling safety and manufacturing</td>
<td>Post marketing studies</td>
</tr>
<tr>
<td>Development of antibody with appropriate properties</td>
<td>Diagnostic test(s)</td>
<td>New clinical indications pursued</td>
</tr>
<tr>
<td>Testing for activity in vitro in vivo</td>
<td>IND filed</td>
<td>New dosage forms and formulations developed</td>
</tr>
<tr>
<td>Humanization</td>
<td>Clinical studies initiated</td>
<td>Safety surveillance</td>
</tr>
<tr>
<td></td>
<td>NDA prepared and submitted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NDA approved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drug launched</td>
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</tbody>
</table>
Production of Monoclonal Antibodies
Hybridoma Technology

Monoclonal antibodies are purified

Desired clones are cultured and frozen

Clones are tested for desired antibody

Monoclonal antibodies are purified

Hybridoma tumors are kept alive in mice

Hybridoma grow in culture

Cells fuse to make hybridoma

Cancerous plasma cells

Antibody-producing plasma cells

Cells fuse to make hybridoma

Hybridoma tumors are kept alive in mice
4D5 becomes Herceptin

- In vitro proliferation assays
- Screening of many antibodies for activity
- Murine hybridoma “4D5” was selected out of a panel of anti-HER2 antibodies because it selectively inhibits growth of breast cancer cells overexpress HER2 but not cells with normal levels of HER2.
4D5 only inhibits cells with high HER2

(Gail Phillips)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Relative HER2 expression</th>
<th>Proliferation (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMEC</td>
<td>1.0</td>
<td>4D5 116, 3H4 114, 2C4 109, 7F3 116, 7C2 117, 6E9 103</td>
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<tr>
<td>HBL-100</td>
<td>1.0</td>
<td>4D5 104, 3H4 102, 2C4 103, 7F3 96, 7C2 104, 6E9 105</td>
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<tr>
<td>MCF7</td>
<td>1.2</td>
<td>4D5 101, 3H4 113, 2C4 100, 7F3 111, 7C2 112, 6E9 105</td>
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<tr>
<td>MDA-MD-231</td>
<td>1.2</td>
<td>4D5 91, 3H4 100, 2C4 93, 7F3 98, 7C2 104, 6E9 103</td>
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<tr>
<td>ZR-75-1</td>
<td>3.3</td>
<td>4D5 102, 3H4 105, 2C4 99, 7F3 97, 7C2 108, 6E9 97</td>
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<tr>
<td>MDA-MB-436</td>
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<td>4D5 97, 3H4 91, 2C4 98, 7F3 93, 7C2 92, 6E9 101</td>
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<tr>
<td>MDA-MB-175</td>
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<td>MDA-MB-453</td>
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<td>4D5 61, 3H4 65, 2C4 88, 7F3 80, 7C2 70, 6E9 101</td>
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<tr>
<td>MDA-MB-361</td>
<td>16.7</td>
<td>4D5 63, 3H4 67, 2C4 64, 7F3 76, 7C2 105, 6E9 99</td>
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<td>BT-474</td>
<td>25.0</td>
<td>4D5 27, 3H4 29, 2C4 60, 7F3 21, 7C2 78, 6E9 91</td>
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<tr>
<td>SK-Br-3</td>
<td>33.0</td>
<td>4D5 33, 3H4 40, 2C4 73, 7F3 51, 7C2 82, 6E9 89</td>
</tr>
</tbody>
</table>
Herceptin Inhibition of Anchorage-Independent Growth Is Related to HER2 Expression Level

Gail Phillips
Testing Preclinical Efficacy In Vivo

• Antibodies have potential to incorporate immune system effector functions for activity.

• Preclinical testing typically done in nude mouse xenografts
  – Need human cells as target
  – Need immunodeficient host to grow tumor
  – Minimal immune effector function
Antibody-Dependent Cellular Cytotoxicity in Herceptin Mechanism

FcR\textgreek{\textgamma}^{-/-} nude mice

D265A-Herceptin Mutant

Producing clinical grade antibody

- Humanization
- Antigenicity
  - HAMA vs HAHA
- Glycosylation
  - QC
  - quantity
Humanization

Monoclonal Antibody
- Human

Monoclonal Antibody
- Mouse

Humanized Antibody
Potential Antigenicity

Minimal (Human Anti-Mouse Antibody)

HAMA

HAHA (Human Anti-Human Antibody)

Human Antibody  Mouse Antibody  Humanized Antibody
To glycosylate or not to glycosylate?

- Required for effector functions
- CHO cells do it
- e. coli don’t

Herceptin®
Manufacturing Process and Product Packaging

Large Scale Fermentation (1,000 L)

Purification

Packaging
Safety Assessment

- Helps determine starting dose in clinical trials
- Material should be as close as possible to clinical product.
  - Avoid false positive safety signals.
  - Determine manufacturing standards.
- Often need to be done in primates because of limited species cross-reactivity.
  - Monkey anti-human IgG immune response may limit duration.
Clinical Trials

- Identifying patients
- Phase I, II, III paradigm
- Recruiting patients
- Difficulty of adjuvant trials
- Initial trials often in relapsing patients for whom other therapies have failed.
Identifying patients to treat

- Herceptin only active against HER2 3+
  - Need biopsy
- Only ~30% of breast cancer patients are HER2 3+
  - need to “see” 3 patients for every one treated
- HercepTest
  - Immunohistochemistry
- FISH
  - Fluorescent In Situ Hybridization
Immunohistochemistry (IHC): Performance Issues (HercepTest®)

- Pre-analytical tissue processing
- Reagent variability
- Antigen retrieval
- Scoring

0

1+

2+

3+
Herceptin™ confers survival benefit in metastatic breast cancer

Slamon et al (2001) NEJM 344, 783-792

enrollment based on HercepTest
Fluorescence *in situ* Hybridization (FISH): PathVysion™

- **Key features:**
  - Probes
    - Direct labeled
    - HER2 sequence
    - Chromosome 17 centromere
  - Interpretation
    - Signal enumeration
    - Ratio of HER2:Chr 17 signals
**HER2 Diagnostics: Fluorescence In Situ Hybridization**

- Measures the level of HER2 gene amplification
- PathVysion™ may be preferable due to internal control
- Issue: not performed in-house at all hospitals

<2.0 not amplified (FISH–)

≥2.0 amplified (FISH+)
Herceptin® Combination Pivotal Trial: Overall Survival

FISH+

<table>
<thead>
<tr>
<th>Probability of survival</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herceptin + CT (n=176)</td>
<td>26.2 mo</td>
</tr>
<tr>
<td>CT (n=169)</td>
<td>20.0 mo</td>
</tr>
</tbody>
</table>

RR=0.71
P=0.007

FISH–

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<thead>
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<th>Probability of survival</th>
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<tr>
<td>Herceptin + CT (n=50)</td>
<td>24.0 mo</td>
</tr>
<tr>
<td>CT (n=56)</td>
<td>19.8 mo</td>
</tr>
</tbody>
</table>

RR=1.11
P=NS

Phase I = “First in Human” studies

- n = 10s
- Patients or healthy volunteers
- Start with single dose, escalate to multi-dose
- Purpose
  - Determine safety and tolerability
  - Determine pharmacokinetics
  - Determine dose for later phases
Phase II = “Small Population” studies

- n = 10s-100s
- Determine safety and efficacy in relevant populations
  - eg, HER2 3+ (FISH+)
- Subgroup analysis
  - eg, prior therapies
- Additional dose-ranging
- Randomized, placebo-controlled
  - nobody wants to be in placebo group
- Drug interactions
Phase III = “Pivotal” studies

- n = 100s - 1000s
- Confirm efficacy with good statistics
- Determine true clinical benefit and its magnitude
  - Increased survival
    - Not always predictable from response rate
  - Quality of life??
- Typically similar to phase II
Phase IV = “Post marketing” studies

- $n = 1000s - millions$
- Extend safety, dose-schedule
- Test new indications not covered in phase II, III
  - eg Herceptin in early breast cancer
Delivery of Therapeutic Antibodies

- Long half-life
  - can be dosed weekly, or every 2-3 weeks.

- Must be delivered IV, requires clinic visit, iv access.
  - Subcutaneous dosing would be ideal, but difficult
    - 5 mg/kg = 350 mg/patient
      - ~3.5 ml at 100 mg/ml
Recruiting patients

• Competition between new therapies for patients

• <15% of eligible patients participate in clinical trials
  – Stigma that there’s nothing else
  – Reimbursement
  – Hassle of coming to clinic every week

• New drugs often tested in combination with current standard of care.
Recruiting patients (cont’d)

• Cross over design (example)

  Taxol + Antibody X

  Taxol + Placebo \(\xrightarrow{\text{progression}}\) Taxol + Antibody X

• Good for recruiting patients
• Bad for interpreting results
  – May make your drug look less effective
Measuring response

- Response rate = tumor shrinkage
  - PR = Partial Response = >50% decrease
  - CR = Complete Remission
- Time to progression vs survival
- Avastin in breast cancer increased response rate to chemotherapy but failed to improve survival.
- Approvable endpoints vs pharmacodynamic markers of response
  - Iressa rash
Adjuvant Clinical Trials

- “Adjuvant” therapy is given to patients in whom detectable disease has been surgically removed but there is chance that tumor cells have spread to other sites
  - local lymph nodes positive
- Purpose: to minimize/prevent relapse of disease later in life.
- Most likely scenario for antibody therapies to be efficacious
  - low bulk
  - minimal selection
Difficulties with Adjuvant Clinical Trials

• Only fraction of patients will develop disease later
  – can’t predict who
    - need to treat more patients

• Relapse often takes years
  – long clinical trials.

• need to know drug has activity before exposing patients to this.
Large Scale Fermentation (12,000 L) | Purification | Packaging
Meeting the market

• Advocates help encourage clinical trial participation

• Time between end of trial and approval difficult
Breast Cancer Subpopulations

Herceptin

Breast Cancers
The HER Family Presents Several Targets for Biologic Therapies

HERCEPTIN

HER2 homodimer

HER2/EGFR heterodimer

EGFR homodimer

Extracellular

Intracellular

HER2

HER2

EGFR

EGFR

RTK

RTK

Site of phosphorylation – necessary for activation of the tyrosine kinase pathway

RTK = receptor tyrosine kinase
Issues Surmounted by Herceptin

- Identification
- Production
- Preclinical Efficacy
- Safety Assessment
- Clinical Trials
Acknowledgements

• Virginia Paton
• Gail Phillips
• Mark Sliwkowski
• Genentech Herceptin team
• Graphics
Issues in developing monoclonal antibodies as cancer therapeutics

- Identification of antibody
- Preclinical Efficacy
- Production on appropriate version of antibody
- Safety Assessment
- Clinical Trials
Targeting HER2: Scientific Rationale

HER2 gene amplification

HER2 protein overexpression

Shortened median survival*

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<tr>
<th>HER2 status</th>
<th>Survival</th>
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<tr>
<td>HER2 positive</td>
<td>3 years</td>
</tr>
<tr>
<td>HER2 normal</td>
<td>6–7 years</td>
</tr>
</tbody>
</table>

*Combined metastatic and adjuvant patients.