Breast & Colon Cancer: Molecular Alterations & Therapeutic Targets
Part 1

Nancy Hynes  
April 8, 2005
Molecular targets for cancer therapy

- In the past 25 yrs cancer research has generated a large body of knowledge on molecular mechanisms controlling all aspects of normal cellular physiology/signaling pathways.

- These studies have also provided knowledge on molecules & mechanisms underlying cancer development.

- **Goal** - to design rational/molecularly targeted therapeutics for cancer treatment.

Major goal of lectures

- Present studies from the area of breast & colon cancer to show how molecular analyses can be used to achieve the ultimate goal of providing better therapies for patients.
Molecular targets for cancer therapy

- **Breast cancer**
  1 in 8 women develop the cancer
  30% will die of metastatic disease
  
  therapeutics targeting proteins involved in breast cancer are in clinical use

- **Colon cancer**
  3rd most common cancer
  
  therapeutics targeting proteins involved in colon cancer are in preclinical testing; clinical data on drugs targeting angiogenesis have recently been published
The original description of pathological stages of colorectal cancer as defined by Duke

The colorectal adenoma-carcinoma sequence

- A well defined molecular/pathological sequence of colorectal cancer development has emerged over the past 15 yrs based on the pioneering work of Vogelstein & colleagues.

Pathological/Histological Classification of Breast Cancer

- Presence or absence of invasion
  - In situ or infiltrating cancer

- Histological appearance of cells - ductul or lobular
  - Ductal carcinoma in situ
  - Lobular carcinoma in situ
  - Infiltrating ductal carcinoma - very common

- Histology, combined with other clinical features, e.g., tumor size, cancer cells in lymph nodes, is routinely used to choose treatment & to predict clinical outcome of the patient.
Gene Expression Patterns & Breast Cancer

- A well defined molecular/pathological sequence of breast cancer is not available.

- Gene expression profiling is a broad method for molecularly defining tumor types.

- Tumor sub-types with distinct gene expression patterns have been described.
  - prognosis
  - diagnosis
  - new therapeutic targets
**Gene expression profiling & breast cancer**

- Breast cancers fall into 5 clinically-relevant subtypes*

> Basal-like  ErbB2+  Normal  Luminal Type B  Luminal Type A

*cDNA microarrays on a core set of 8100 genes were carried out, then 427 unique genes formed a basis for classification based upon significantly greater variation in expression between different tumors than between paired samples from same tumor.

T. Sorlie et al PNAS 2001 98:10869; ibid 2003 100:8413
**Gene expression profiling & breast cancer**

- Breast cancers fall into 5 clinically-relevant subtypes:
  - Luminal have best prognosis
  - Basal have worst prognosis

*Diagram showing differences in gene expression profiles among different subtypes of breast cancer.*

- Overall survival for different subtypes
- Disease-free survival for different subtypes
What influences Breast Cancer development?

- Hormones
- Genetic Alterations
What influences breast cancer development?

Many of these factors are related to estrogen exposure.
Estrogen & Breast Cancer

- Estrogens play an essential role in development of the normal breast & in cancer.

- The life time exposure to estrogens has a strong influence on cancer development.

  Loss of ovarian function prevents breast cancer

  Early onset of menarche & late menopause

  Low incidence of male breast cancer
George Beatson described that removal of the ovaries from women with metastatic breast cancer sometimes led to tumor regression.

"On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases".

Lancet (1896)
**Estrogen & Breast Cancer**

- Jensen & Jacobsen identified the target for estrogen action, the estrogen receptor (ER) in 1962
  
  "Basic guides to the mechanism of estrogen action"
  
  Recent Prog Horm Res, 1962

- Jensen correlated the presence of ER with hormone responsiveness of the breast tumor.

  "Estrogen receptors & breast cancer response to adrenalectomy"

  Natl Cancer Inst Monogr, 1971
**Estrogen Receptor**

- **Member of the steroid/thyroid/retinoid receptor superfamily of transcription factors.**

- **Ligand binding induces DNA binding, recruitment of transcriptional coactivators (NCoAs) & histone acetyltransferases (HATs) leading to transcription of target genes.**
**Classical & novel modes of estrogen action**

- There are rapid responses that occur within minutes after estrogen administration that cannot be accounted for by changes in gene expression.

- Prossnitz & colleagues recently described a non-ER, transmembrane protein that also initiates signaling cascades in response to estrogen *(Revankar et al 2005 Science 307 1625)*
Normal Adult Breast

~ 30% of the epithelial cells in the ducts express estrogen receptor (ER).

**ER+ cells act as sensors in the normal breast**

- The ER+ cells respond to estrogens by releasing peptide factors that stimulate proliferation of neighboring ER- cells.

![Diagram showing estrogen binding to ER+ cells releasing peptide factors like EGF or TGF\(\alpha\) which stimulates ER- cells to divide]

- Epithelial cells proliferate in response to cyclical variation of ovarian hormones during the menstrual cycle.

- The normal increase in cell number is balanced by normal cell death.
Structure of the normal and malignant breast

Tumors have many ER+ cells

In breast cancer ER$^+$ tumor cells proliferate

- ER$^+$ tumor cells proliferate in response to estrogen.

- The normal balance between proliferation & apoptosis is lost in the tumor.
Estrogen Receptor Expression in Breast Cancer

- ER expression in invasive breast cancers is variable.
- Panels a-f are all invasive breast cancers - ER expression ranges from 0% (a) to ~100% (f).


Why is this important?
Prognosis & treatment
Gene expression profiling & breast cancer

Breast cancer patients whose tumors show high ER expression have the best prognosis.

T. Sorlie et al PNAS 2001 98:10869; ibid 2003 100:8413
The estrogen receptor as target for therapy

- ER$^+$ tumors depend upon estrogen for proliferation.

- In 1971 Jensen correlated the presence of ER with hormone responsiveness of the breast tumor.

- ER antagonists were some of the first targeted, rational therapeutics. (proposed in 1936 by Lacassagne)
**The estrogen receptor as a therapeutic target**

- ER antagonists bind the receptor but prevent transcription by interfering with coactivator binding.
The estrogen receptor as a therapeutic target

The non-steroidal, anti-estrogen tamoxifen was the 1st agent approved for clinical use.

1973 UK; 1977 USA
Estrogen Receptor & Tamoxifen

- Two distinct domains of ER (AF1 & 2) mediate transcriptional activation.
- Tamoxifen interferes with AF2 causing block in transcription of genes involved with cancer cell proliferation.

Nature Reviews | Cancer
The Anti-Estrogen* Tamoxifen

- Tamoxifen was approved for treatment of advanced breast cancer in 1973.
  Clinical trial - 22% response rate

- Tamoxifen has made a substantial contribution to the reduced mortality rate that has been reported in several countries since 1990.

- George Beatson described that removal of the ovaries from women with metastatic breast cancer sometimes led to tumor regression.

- Ovarian ablation removes the major estrogen source, thus blocking tumor cell proliferation.

* SERM = selective estrogen receptor modifiers
Many tumors are ER+. Relapse suggests that tumor cells grow in an estrogen/ER independent manner. Some patients respond to other drugs targeting estrogen/ER.

Unfortunately after a few years most breast cancer patients relapse & show metastases during treatment with tamoxifen.
Aromatase inhibitors & breast cancer

- In postmenopausal women, adrenal & ovarian androgens are converted into estrogens, in different peripheral tissues: muscle, skin, normal breast & in breast cancer cells, by the enzyme aromatase.
Aromatase inhibitors & breast cancer

- Various aromatase inhibitors are in clinical trials.
- Some have shown efficacy in patients who relapsed on tamoxifen therapy.

Review on aromatase inhibitors - IA Smith, 2004, The Breast 13, S3-S9
Aromatase inhibitors & breast cancer

- Various aromatase inhibitors are in clinical trials.

- Some have shown efficacy in patients who relapsed on tamoxifen therapy.

- These results suggest that tumors still require estrogen/ER for proliferation; lowering the level of estrogen in the tumor & adipose tissue has an impact on disease.

- These results are very important for breast cancer patients:
  1. after relapse on tamoxifen there's another therapeutic option with proven efficacy
  2. anti-estrogen therapy is relatively non-toxic
Mechanisms of action of therapeutic agents used in endocrine therapy

Major mechanisms - antagonize ER (SERMs) or lower estrogen levels (aromatase inhibitors)
**Tamoxifen for breast cancer prevention**

- Analysis of ~10,000 breast cancer patients treated with tamoxifen as an adjuvant therapy for breast cancer revealed that the frequency of new breast cancer in the tamoxifen-treated group was lower than in the control group.
Tamoxifen for breast cancer prevention

Frequency of new breast cancer (contralateral breast) in clinical trials of tamoxifen therapy.

<table>
<thead>
<tr>
<th>8 Clinical Trials</th>
<th>Tam-treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. patients</td>
<td>No. cancers</td>
</tr>
<tr>
<td>NATO</td>
<td>564</td>
<td>15</td>
</tr>
<tr>
<td>Scottish NSABP</td>
<td>661</td>
<td>9</td>
</tr>
<tr>
<td>etc</td>
<td>1419</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4975</strong></td>
<td><strong>79</strong></td>
</tr>
</tbody>
</table>

1.6% 2.4%

Significant reduction in breast cancer in the Tam-treated group

Effects of estrogen & tamoxifen on human tissues

Tamoxifen is an ER antagonist in the breast

Tamoxifen increases frequency of uterine cancer

<table>
<thead>
<tr>
<th>Tam treated patients (n=4028)</th>
<th>Controls (n=4006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cancers</td>
<td>Frequency</td>
</tr>
<tr>
<td>uterus</td>
<td>19</td>
</tr>
<tr>
<td>endometrial</td>
<td>6</td>
</tr>
</tbody>
</table>

Endometrial cancer is significantly higher in the Tam-treated group

Effects of estrogen & tamoxifen on human tissues

Tamoxifen is an ER agonist in the uterus

Estrogen Receptor & Tamoxifen

- Two distinct activation domains (AF1 & 2) mediate transcriptional activation.
- Tamoxifen blocks AF2 - the main activator in the breast.
- In the uterus, AF1 is more important for ER activity - thus tam acts as an agonist in this organ.
Tamoxifen & breast cancer prevention

- Despite the partial ER agonistic activity in some organs, the decrease in contralateral breast cancer incidence observed in women treated with tamoxifen for breast cancer therapy, led to the proposal that the drug might be useful for breast cancer prevention.

- In 1992 a randomized clinical trial was begun “Does tamoxifen prevent breast cancer?”
Tamoxifen & breast cancer prevention

- 13,388 women were enrolled

- At an increased risk were considered to be those:
  - >60 yrs of age
  - 35-59 yrs of age with, e.g., family history
  - with a history of lobular carcinoma in situ

- Results published 1998 (Fisher et al JNCI 90: 1371)

  “Tamoxifen administration reduced the risk of invasive & noninvasive breast cancers by ~50% in all age groups.”
The role of estrogens/ER in breast cancer

- The ER is a perfect example of a rational therapeutic target.

- Ironically, tamoxifen was developed before the age of molecular biology, which set the stage for molecular target identification in cancers.

- Anti-estrogens might generally become important in breast cancer prevention.
Important questions for the future

- Why are some tumors dependent upon estrogen for Proliferation & survival? Stem cell?

- Why do patients initially respond to anti-estrogen therapy then relapse?

- Why do patients who failed one type of anti-estrogen therapy respond to another one? tam vs. aromatase inhibitors

Despite the open questions, anti-estrogen strategies have been extremely important for treatment of breast cancer patients.
Breast cancer, stem cells & the ER

Some models* propose that transformation of different sub-sets of stem cells leads to the observed diversity in breast cancer phenotypes - including ER status.

*G. Dontu et al 2004 Trends Endocrinology & Metabolism 15: 193-197
Important questions for the future

Why are some tumors dependent upon estrogen for proliferation? Stem cell?

Why do patients initially respond to anti-estrogen therapy then relapse?

Why do patients who failed one type of anti-estrogen therapy respond to another one? tam vs. aromatase inhibitors

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What influences breast cancer development?

- Hormones
- Genetic Alterations

Specific Molecular Alterations & Therapies

- Development of targeted therapeutics based upon characterizing specific molecular alterations in tumors. e.g., ErbB2 gene amplification
Tumors develop as a result of multiple genetic alterations

- loss of tumor suppressor genes
- “activation” of dominant oncogenes
  - mutation
  - altered expression
    - gene amplification
    - chromosomal rearrangement
    - increased transcription
Why is it important to characterize molecular alterations in tumors?

- Provides hints about how cancer develops.
- Identify candidates for rational targeted therapeutics.
- Provide new cancer diagnostic tools.
**Tumor suppressor genes**

- Deletion or mutation of one allele is either inherited or occurs during the development of the cancer.

  When the second WT allele is lost in a somatic cell, visualized by loss of heterozygosity (LOH), these cells have a growth advantage.
Tumor suppressor genes & breast cancer

**BRCA 1 & 2**

Existence of genes responsible for inherited predisposition to breast & ovarian cancer was suggested more than 100 yrs ago.

Due to foresight of certain human geneticists, G. Lenior & MC King, who collected material from “cancer families”, linkage analyses were used initially to determine chromosomal location of the genes, this was followed by their positional cloning.
Family Pedigrees for Breast Cancer

Hereditary: Breast cancer in all generations.

~ 5% of all breast cancers fall in this group; the majority have mutations in BRCA genes.
What characterizes a family with hereditary breast cancer?

Features That Indicate Increased Likelihood of Having BRCA Mutations

- Multiple cases of early onset breast cancer
- Ovarian cancer (with family history of breast or ovarian cancer)
- Breast and ovarian cancer in the same woman
- Bilateral breast cancer
- Ashkenazi Jewish heritage
- Male breast cancer

Women with germline heterozygous mutations in BRCA1 or BRCA2 are at increased risk of developing breast, ovarian & other cancers.
BRCA1 & BRCA2 mutations predispose to breast cancer

BRCA1 mapped in 1990
Cloned in 1994

BRCA2 mapped in 1994
Cloned in 1995

As predicted for a tumor suppressor, the second WT BRCA allele is found mutated in the breast tumor.
Domain structure of BRCA1 & BRCA2

The BRCA proteins have been implicated in many different processes including DNA repair & recombination, cell cycle control & transcription.
BRCA proteins & DNA Repair

- Cells with gross chromosomal rearrangements (GCR) have defects in DNA repair or recombination pathways.

- GCRs in BRCA-deficient cells result from inappropriate DSB repair during the S & G2 phases of the cell cycle.

- The BRCA proteins are essential for preserving chromosome structure.
Mouse cells deficient in BRCA2 show spontaneous aberrations in chromosome structure.

Aberrations in the usual U-shaped mouse chromosomes are visible & enlarged on the right.

Venkitaraman 2002 Cell 108: 171
BRCA proteins & DNA Repair

- GCRs in BRCA-deficient cells result from inappropriate DSB repair during S & G2.

Venkitaraman 2002 Cell 108: 171

The preferred error-free HR pathway does not function in cells lacking BRCA proteins.
The two BRCA proteins have different functions in this process.

BRCA proteins & DNA Repair

HR = homologous recombination

Turner et al 2004 Nature Reviews Cancer vol 4
BRCA proteins & DNA repair complexes

BRCA1 has a broad range of functions; e.g., transcription*

AN Monteiro et al 1996 PNAS 93:13595
T Ouchi et al 2000 PNAS 97:5208

BRCA2 appears to have a more restricted function - RAD51 dependent DNA repair.

*BRCA1 & transcription:
Turner et al 2004 Nature Reviews Cancer vol 4
**Tumors with BRCA mutations have distinctive features**

In order for a cell with unrepaired DNA damage to escape apoptosis, cell-cycle check-points need to be lost.

This might explain the higher rate of p53 mutations & MYC amplifications.

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**Table 1: Features differentiating familial BRCA from sporadic breast cancers**

<table>
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<tr>
<th>Phenotypic feature</th>
<th>Familial-BRCA1 samples (%)</th>
<th>Control sporadic breast cancer samples (%)</th>
<th>Familial-BRCA2 samples (%)</th>
</tr>
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<tr>
<td>High-grade*</td>
<td>66(^*)</td>
<td>36</td>
<td>41(^*)</td>
</tr>
<tr>
<td>Oestrogen-receptor (ER)-negative</td>
<td>90(^*)</td>
<td>35</td>
<td>34 (NS)</td>
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<td>Continuous pushing margins(^5)</td>
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Turner et al 2004 Nature Reviews Cancer vol 4
Tumors with BRCA1 mutations have a distinctive phenotype

A high % of ER negative tumors

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Turner et al 2004 Nature Reviews Cancer vol 4
**BRCA1 vs. BRCA2 phenotype**

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<th>BRCA2 phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>? No specific subtype</td>
</tr>
<tr>
<td>ER-negative</td>
<td>ER-positive in similar proportion to sporadic cancer</td>
</tr>
<tr>
<td>EGFR expression</td>
<td>–</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td>–</td>
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<td>? c-MYC amplification</td>
</tr>
<tr>
<td>TP53 mutations</td>
<td>TP53 mutations</td>
</tr>
<tr>
<td>Loss of RAD51-focus formation</td>
<td>Loss of RAD51-focus formation</td>
</tr>
<tr>
<td>Extreme genomic instability</td>
<td>Extreme genomic instability</td>
</tr>
<tr>
<td>Sensitivity to DNA-crosslinking agents</td>
<td>Sensitivity to DNA-crosslinking agents</td>
</tr>
</tbody>
</table>

Features specific to pathway of tumour development and particular BRCA defect

Features that reflect underlying DNA-repair defect

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Turner et al 2004 Nature Reviews Cancer vol 4
Gene expression profiling & breast cancer

- BRCA1 tumors have a basal cancer “expression signature”

![Gene expression profiling & breast cancer diagram]

- Basal
- ErbB2+
- Normal
- Luminal B
- Luminal A

- Overall survival
- Disease-free survival

Disease-free survival

Overall survival
BRCA1 & BRCA2 have not been implicated in sporadic breast cancer

In contrast to other inherited tumor suppressor genes, sporadic breast cancers do not carry mutations in BRCA genes.
Hereditary Colon Cancer

- ~15% of colorectal cancers (CRC) is due to germ line transmission of mutated genes.

- Familial Adenomatous Polyposis (FAP)
  - APC

- In 1991 the APC gene was cloned; germ line & sporadic mutations were described.
  
Epigenetic mechanisms of BRCA1 inactivation

- BRCA1 transcription is silenced by promoter methylation in sporadic breast & ovarian cancers.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Somatic BRCA mutations</th>
<th>BRCA1 methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Very rare(^7,8)</td>
<td>11–14(^%)(^26,28)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>&lt;5(^%)(^29,48,71)</td>
<td>5–31(^%)(^26,28,30)</td>
</tr>
<tr>
<td>HNSCC</td>
<td>ND</td>
<td>0(^%)(^34)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>ND</td>
<td>4(^%)(^34)</td>
</tr>
<tr>
<td>Cervical</td>
<td>ND</td>
<td>6.1(^%)(^35)</td>
</tr>
</tbody>
</table>

Turner et al 2004 Nature Reviews Cancer vol 4

- BRCA1 promoter methylation is infrequently detected in lung (NSCLC) & cervical cancers; it is never observed in other cancers - colon or leukemias.
Inactivation of BRCA interacting proteins

- Other proteins on the BRCA pathway might be mutated in sporadic cancer.
- BRCA proteins are parts of large protein complexes.
How might you use tumor suppressors in cancer therapy?

- Restore WT function which often blocks proliferation.

- This is feasible but technically very challenging for many reasons.
  
  specific introduction into cancer cells
  efficiency
  etc
Tumors develop as a result of multiple genetic alterations

- loss of tumor suppressor genes
- “activation” of dominant oncogenes
  - mutation
  - altered expression
    - gene amplification
    - chromosomal rearrangement
    - increased transcription
Proteins aberrantly expressed or activated in breast cancer

- ErbB2 & EGF receptor tyrosine kinases
- Cyclin D1
- C-myc
There are many examples of proteins that are aberrantly expressed in breast cancer.

Goal of rational cancer therapy has been to use these “altered” proteins as therapeutic targets.

What characterizes a good therapeutic target?

- Enzymatic activity
- Surface expression
- Expressed only in the tumor
- Etc.
The superfamily of receptor tyrosine kinases (RTKs)

There are approx. 60 RTKs - at least 30 of these have been found altered in human tumors.

The ErbB or EGF related family of RTKs

ErbB - avain erythroblastosis virus oncogene B
ErbB RTKs & Breast Cancer

- Gene encoding ErbB2 is amplified in ~ 25% of primary human breast tumors.

- ErbB2 amplification correlates with “poor” prognostic factors
  - Tumor grade
  - Lymph node status – sign of metastases
  - Estrogen receptor expression

- ErbB2 amplification correlates with poor patient survival.

- EGFR is not amplified in breast cancer, but the receptor is activated by autocrine production of ligands in the tumor.
Gene expression profiling & breast cancer

- ErbB2+ group has a bad prognosis

- Overall survival
- Disease-free survival
In a normal cell the ErbB RTKs depend upon ligand binding for activation.
ErbB receptors are constitutively active in tumors

Transformation of Cells by ErbB Receptors

Autocrine Stimulation of ErbB1/EGFR

Ligand-Independent Receptor Activation by Overexpression of ErbB2
Therapeutic approaches to target ErbB receptors

- Antibodies
- Small molecule tyrosine kinase inhibitors
# ErbB targeted recombinant antibodies*

<table>
<thead>
<tr>
<th>Target</th>
<th>Company</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>Genentech/Roche</td>
<td>Approved ErbB2-overexpressing breast cancer; trials in combination with various drugs ongoing.</td>
</tr>
<tr>
<td>(Herceptin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>Genentech</td>
<td>Phase II - ovarian, breast, prostate, NSCLC; inhibits ErbB2 dimerization; trials ongoing in low ErbB2 expressors.</td>
</tr>
<tr>
<td>(Omnitarg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>ImClone/Merck KGaA</td>
<td>Approved CRC; trials in combination with various drugs ongoing- pancreatic, HNSCC &amp; NSCLC.</td>
</tr>
<tr>
<td>(Erbitux)</td>
<td>Bristol-Myers Squibb</td>
<td></td>
</tr>
<tr>
<td>Matuzumab</td>
<td>Merck KGaA</td>
<td>Phase II - NSCLC, gynecologic, pancreatic, Esophageal.</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Abgenix</td>
<td>Pivotal trial ongoing - 3rd line CRC, other indications- RCC and NSCLC.</td>
</tr>
</tbody>
</table>

*humanized or chimeric

Hynes & Lane 2005 Nature Rev Cancer May issue
Recombinant antibody structures

Antibody treatment can induce human anti-mouse antibodies (HAMA).

EGFR targeted C225: chimeric γ heavy chain

ErbB2 targeted Herceptin: humanized γ heavy chain

Nature Reviews | Cancer
P.Carter Vol 1, p118
MAb225 blocks autocrine activated EGFR

Transformation of Cells by ErbB Receptors

Autocrine Stimulation of ErbB1/EGFR

Ligand-Independent Receptor Activation by Overexpression of ErbB2
**MAb225 Shows Efficacy in Preclinical Models**

Xenografts - A431 human cancer cells with active EGFR.

Mice treated with mAb225, Doxorubicin or Cis-platin (CDDP) or cytotoxics in combination with C225.

Mendelsohn & Baselga 2000 Oncogene 19:6550
4D5/Herceptin causes G1 arrest and downregulation of mitogenic pathways.
Herceptin has Anti-Proliferative Activity on ErbB2-Overexpressing Breast Cancer Cells

BT474 Cells Treated with 4D5/Herceptin®
Arrest in G1

<table>
<thead>
<tr>
<th>% Cell Cycle Stage</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>67</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>FRP5</td>
<td>65</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>4D5</td>
<td>96</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
Herceptin/Trastuzumab has Anti-Proliferative Activity on ErbB2-Overexpressing Breast Cancer Cells Growing as Tumors in Nude Mice

Doses:
1-30 mg/kg Herceptin/Trastuzumab twice weekly

BT474 tumor model

Mendelsohn & Baselga 2000 Oncogene 19:6550
Combination Treatment With Herceptin + Standard Chemotherapeutic Agents

0.3 mg/kg: sub-optimal Herceptin dose twice weekly

Sub-optimal doses of both drugs show increased activity in combination with Herceptin.

BT474 tumor model

Mendelsohn & Baselga 2000 Oncogene 19:6550
### ErbB2 as a target for breast cancer

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>gene encoding ErbB2 cloned</td>
</tr>
<tr>
<td>1987/88</td>
<td>gene amplification &amp; protein overexpression</td>
</tr>
<tr>
<td></td>
<td>found in ~ 20-30% primary breast tumors; correlation with poor prognosis</td>
</tr>
<tr>
<td>1989</td>
<td>growth inhibitory ErbB2 specific mAbs described</td>
</tr>
<tr>
<td>1991</td>
<td>Phase I clinical trial with murine mAb 4D5</td>
</tr>
<tr>
<td>1992</td>
<td>Phase I clinical trial with “humanized” 4D5 - Herceptin</td>
</tr>
<tr>
<td>1996</td>
<td>Phase II trial results published</td>
</tr>
<tr>
<td>1997</td>
<td>Phase III trials with published</td>
</tr>
<tr>
<td>1998</td>
<td>Herceptin approval in the USA for treatment of ErbB2++ , metastatic breast cancer</td>
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</tbody>
</table>
Pivotal Phase III Clinical Trial with Trastuzumab/Herceptin in Combination with Chemotherapy

### Table 3

<table>
<thead>
<tr>
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<th>H + AC (n=143)</th>
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<td>Response Rate (%)</td>
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TTP, time to progression; DR, duration of response; AC, doxorubicin + Cyclophosphamide; B, paclitaxel; H, trastuzumab; CT, chemotherapy (Slamon et al. 1998; Norton et al. 1999)

n = 469, metastatic breast cancer patients who had failed previous therapies.
### Pivotal Phase III Clinical Trial with Trastuzumab/Herceptin in Combination with Chemotherapy

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Mendelsohn & Baselga 2000 Oncogene 19:6550
Pivotal Phase III Clinical Trial with Trastuzumab/Herceptin in Combination with Chemotherapy

Table 3  Trastuzumab in combination with chemotherapy

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Mendelsohn & Baselga 2000 Oncogene 19:6550
Pivotal Phase III Clinical Trial with Trastuzumab/Herceptin
in Combination with Chemotherapy

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TTP, time to progression; DR, duration of response; AC, doxorubicin + Cyclophosphamide; P, paclitaxel; H, trastuzumab; CT, chemotherapy (Slamon et al., 1998: Norton et al., 1999)

Based upon these results Herceptin was approved for the treatment of breast cancer patients in 1998.

Mendelsohn & Baselga 2000 Oncogene 19:6550
**Potential Mechanisms Contributing to Herceptin's Anti-Tumor Activity**

- Blocks ErbB2's constitutive kinase activity & downstream signaling pathways.

- Induces loss of ErbB2 from tumor cells, i.e., down-regulation.

- Allows recruitment of immune effector cells to the tumor.
## Antibody Classes

<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>IgD</th>
<th>IgG</th>
<th>IgA</th>
<th>IgE</th>
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<tbody>
<tr>
<td><strong>Heavy Chain</strong></td>
<td>µ</td>
<td>δ</td>
<td>γ</td>
<td>α</td>
<td>ε</td>
</tr>
<tr>
<td><strong>Light Chain</strong></td>
<td></td>
<td></td>
<td>κ or δ</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Activates Complement</strong></td>
<td>++++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Binds macrophages &amp; neutrophils</strong></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

γ heavy chain can recruit immune effector functions
Potential Mechanisms Contributing to Herceptin's Anti-Tumor Activity

1. Fix complement - initiate opsonization, lysis, inflammation.
   Protection from tumor

2. Crosslink FcγRIII. Initiate ADCC and cytokine release.
   Protection from tumor

3. Crosslink FcγRII. Inhibit effector cell.
   No protection from tumor

4. Crosslink antigens on cancer cell. Initiate signals, block growth or survival.
   Protection from tumor

Houghton & Scheinberg 2000 Nat Med 6: 373
Herceptin's Anti-tumor Activity is Partially Dependent on Fc Receptor Activation

WT nude mice

FcRγ −/− nude mice

~40% activity is retained in FcRγ −/− mice

Clynes 2000 Nat Med 6: 443
Potential Mechanisms Contributing to Herceptin's Anti-tumor Activity

mAb binding directly affects ErbB2 activity

Houghton & Scheinberg 2000 Nat Med 6: 373
**Therapeutic antibodies targeting ErbB2**

- Pertuzumab & Trastuzumab block ErbB2 function in tumors overexpressing the receptor.

Is Herceptin a “Perfect” Drug?

- Patients treated with Herceptin in combination with AC (dox + cyclophosphamide) had an increased rate of cardiac toxicity.

- Ablation of ErbB2 in mice by gene KO technology, revealed that this receptor was essential for normal cardiac development. (KO embryos died at E 10.5).
Specific deletion of ErbB2 in cardiomyocytes reveals a role for the receptor in stress conditions.

Cardiomyocytes from ErbB2 KO mice are more sensitive to adriamycin/dox-induced cell death.
**Herceptin is a Rationally Designed Drug**

Development of Herceptin is one of the best examples of a rationally designed therapeutic strategy.

- Biology of the target protein.
- Potential of ErbB2 to cause transformation.
- Animal models were used to show that inhibiting ErbB2 would block tumor growth. *(Proof of Concept)*
- Clinical data suggested that high levels of ErbB2 in breast cancer correlated with bad prognosis.
Herceptin is a Rationally Designed Drug

However:

- Not all patients whose tumors overexpress ErbB2 respond to Herceptin.

- We need to understand more about how Herceptin works in order to better predict who will respond to treatment.
Anti-Tumor Approaches using Antibodies

**Direct arming**
- Complement-dependent cytotoxicity
- Point mutations and/or modified glycosylation
- Antibody-dependent cellular cytotoxicity
- Cytokine
- Immunocytokine
- Small molecule or protein toxin

**Enhancing effector functions**
- Biotin-chelator–radionuclide
- Streptavidin
- Prodrug
- scFv–enzyme

**Indirect arming**
- scFv fragment
- Sterically stabilized immunoliposomes
- Radionuclide, toxin or immunological effector cell

**Pre-targeting**
- Radionuclide, toxin or immunological effector cell
Structure of Single Chain Antibody - scFv

FRP5 - murine mAb that binds ErbB2’s extracellular domain

W.Wels
**ErbB2 specific mAb FRP5**

- Binds ErbB2's extracellular domain with high affinity.

- Hybridoma cells producing FRP5 were used to isolate cDNAs encoding the specific heavy and light chain variable domains.
**Chimeric scFv-Toxin Fusion Protein**

**Bifunctional antibody fragments for directed cancer therapy**

- **MAb**
  - Tumor cell-specific antibody
  - Cloning

- **scFv**
  - Recombinant single chain antibody
  - Gene fusion

- **scFv-Effecter**
  - Effector with novel binding properties

W. Wels
Chimeric scFv-Toxin Fusion Protein

Domain Structure of Pseudomonas Exotoxin A

binding

translocation

ADP-Ribosylation

1a

253-364

365-404

405-613

eta 66 kDa

l.a

III

scFv-ETA 67 kDa

VH

linker

VL

II

IB

III

scFv

ETA 252-613

W.Wels
scFv-Toxin Binds ErbB2 & Internalizes into Tumor Cells

Toxin Receptor

α2-macroglobulin receptor

cell-type specific receptor (recombinant toxin)

ErbB2

Internalized ErbB2 enters the endosomes

Inhibition of Protein Synthesis

Trans-Golgi

Cytosol

Internalization

Endosome

pH ↓

Processing

Translocation

KDEL receptor

ETA

EF-2

-ADP-ribose

Golgi

Endoplasmic Reticulum

W. Wels
**scFv-Toxin Is Bacterially Produced & Purified to Homogeneity**

**Bacterial expression and purification of antibody toxins**

Transformation of E. coli with plasmid DNA → Expansion of bacteria → Expression of protein → Purification from bacterial lysate → Purified protein

SDS-PAGE

W. Wels
The scFv-Toxin FRP5-ETA Specifically Kills ErbB2+ Cells

Specificity and cytotoxic activity of scFv(FRP5)-ETA

<table>
<thead>
<tr>
<th>Control</th>
<th>scFv(FRP5)-ETA 1 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB453</td>
<td>SKBR3</td>
</tr>
<tr>
<td>ErbB2+</td>
<td>ErbB2-</td>
</tr>
</tbody>
</table>

W.Wels
Tumor Cells with High ErbB2 are Very Sensitive to FRP5-ETA

Sensitivity of prostate cancer cells to scFv(FRP5)-ETA

Wang et al., 2001

W. Wels
ErbB2-Overexpressing Tumor Xenografts are Sensitive to FRP5-ETA

Treatment of established breast carcinoma with scFv(FRP5)-ETA

MAXF1162 human breast carcinoma xenografts
Intratumoral injection of 8 µg scFv(FRP5)-ETA on days 25, 27, 29, 33, 35 p.i.

W.Wels

scFv-FRP5-ETA is currently in early phases of clinical development.