To improve human health, scientific discoveries must be translated into practical applications.

Such discoveries typically begin at **“the bench”** with basic research — in which scientists study disease at a molecular or cellular level — then progress to the clinical level, or the patient's **“bedside.”**
Definition of Translational Research

- “Translational research transforms scientific discoveries arising from laboratory, clinical or population studies into clinical or population-based applications to improve health by reducing disease incidence, morbidity and mortality”
  - Modified from the NCI translational research working group (2006)
Basic Discovery Today Provides the Foundation for Tomorrow’s Medicine

Basic Research and Technology Development

Translational Research

Clinical Applications

Elias Zerhouni, M.D., Director, NIH
Targeted Therapy in Oncology

Goals

- Identify agents that target tumor-specific molecules, thus sparing normal cells
  - Increased specificity leads to decreased toxicity
- Identify ideal drug target
  - Drives tumor growth
  - Turns on key mechanisms of cancer progression
  - Reversible by inhibition with agent
  - Dispensable in normal cells
  - Target measurable in tumor tissue
Targets in Radiation Oncology

The Reductionist View

A Heterotypic Cell Biology

Hanahan D, Weinberg RA. Cell 2000;100:57-70
Molecular Targeting with Radiotherapy (RT)

- Novel therapeutic agents are not curative.
- RT is extremely efficient in the eradication of clonogenic cells.
- RT, in contrast to chemotherapy, can be modified in terms of dose, time AND space.
- Recurrence can occur from single surviving cell.
“The Hallmarks of Cancer”

1. Evading apoptosis
2. Self-sufficiency in growth signals
3. Insensitivity to anti-growth signals
4. Tissue invasion & metastasis
5. Limitless replication potential
6. Sustained angiogenesis
Understanding the Molecular Basis of Cancer

- **Initiation**
- **Promotion**
- **Angiogenesis**
- **Invasion**
- **Metastasis**

Increasing genetic change

- Early tumour
- Enlarging tumour with developing blood and lymphatic vessels
- Lymph duct

- Tumour cells squeeze into blood and lymphatic vessels

- Tumour cells
- Metastasis in lymph node
- Lymph node

- Early metastasis
- Liver cells

- Tumour cells adhere to blood vessel walls and squeeze through to form distant metastasis

- Metastasis in lymph node
- Liver cells

- Early metastasis
- Tumour cells adhere to blood vessel walls and squeeze through to form distant metastasis
Many Potential Therapeutic Targets in the Tumour and the Host

Tumour targets – biological processes in the tumour cell itself

- **Growth factor stimuli**
  - Exogenous growth factors

- **Cell-cycle control**
  - Mediators of cell division

**Intracellular signalling**
- Downstream transduction enzymes

Host targets – biological processes in the body that facilitate the growth and spread of the tumour

- **Angiogenesis**
  - The growth of new blood vessels

- **Vascular permeability**
  - Restriction of tumour blood flow

- **Invasion/metastasis**
  - The movement and dissemination of tumour cells in host tissues
Example: Cell proliferation and self-sustained growth

How do cells grow and divide?
How do cells learn self-sustained growth?

What causes a cell to divide?
- Start with synthesis of the EGF Receptor protein
  - Central Dogma
- Localization to the cell membrane
  - Trans-membrane protein
- EGF and EGF receptor binding → signal into the cell
  - EGF-EGFR crystal structure
- Cascade leads to a protein called RAS
  - Molecular Switch
    - "ON" → cell proliferation

What is different about cancer cells?
- e.g. cancer cells require little growth factors
A simple view of the pathway

1. EGF binds to EGFR
2. 2 EGFRs activate a series of proteins that lead to activation of RAS
3. RAS is activated to the “on” position
4. Cell Division
5. RAS is turned “off”

Cell enters irreversibly into “S” phase
Receptor locations

- **Cytosolic or Nuclear**
  - Lipophilic ligand enters cell
  - Often activates gene
  - Slower response
- **Cell membrane**
  - Lipophobic ligand can't enter cell
  - Outer surface receptor
  - Fast response

Figure 6-4: Target cell receptors
Membrane Receptor Classes

- Ligand-gated channel
- Receptor enzymes
- G-protein-coupled
- Integrin
Membrane Receptor Classes

Figure 6-5: Four classes of membrane receptors

- Ligand binding opens or closes the channel.
- Ligand binding to a receptor-enzyme activates an intracellular enzyme.
- Ligand binding to a G protein-coupled receptor opens an ion channel or alters enzyme activity.
- Ligand binding to integrin receptors alters the cytoskeleton.
Signal Transduction

- Transforms signal energy
- Protein kinase
- Second messenger
- Activate proteins
  - Phosphorylation
  - Bind calcium
- Cell response

Figure 6-8: Biological signal transduction
Signal Amplification

- Small signal produces large cell response
- Amplification enzyme
- Cascade

Figure 6-7: Signal amplification
Receptor Enzymes

- Transduction
- Activation cytoplasmic
  - Side enzyme
- Example: Tyrosine kinase

Figure 6-10: Tyrosine kinase, an example of a receptor-enzyme
G-Protein-coupled Receptors

- Hundreds of types

- Main signal transducers
  - Activate enzymes
  - Open ion channels
  - Amplify:
    - adenyl cyclase-cAMP
  - Activates synthesis
G-Protein-coupled Receptors

Figure 6-11: The G protein-coupled adenylyl cyclase-cAMP system

1. Signal molecule binds to G protein-linked receptor, which activates the G protein.
2. G protein turns on adenylyl cyclase, an amplifier enzyme.
3. Adenylyl cyclase converts ATP to cyclic AMP.
4. cAMP activates protein kinase A.
5. Protein kinase A phosphorylates other proteins, leading ultimately to a cellular response.
Signal Transduction Pathways & Molecular Targets in Oncology
Proliferation

Growth Factor Receptor

Receptor TKIs
IRESSA, ZD6474

Downstream Signal Inhibitors
AZD3409

Cell Cycle Inhibition
AZD1152, AZD5438

Nucleus
Signal Transduction and Kinase Pathways

Phosphorylation: primary mechanism for information transfer.
Growth Factors & Cell Cycle

Gene Transcription

Growth Factors

Receptors

Priming

G₁

S

Cell Cycle

M

G₂
Activation of STAT Pathway

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION

STATs 1, 3, and 5 implicated in cancer

cyclin D1, c-Myc, Bcl-xL, Mcl-1, survivin, VEGF

Gene Transcription
Activation of MAPK Pathway

SHC

GRB2

SOS

RAS

RAF

MEK

ERK

TF’s

Gene Transcription
Activation of PI3K Pathway

Gene Transcription
Activation of PI3K Pathway

Translation
Metabolism
Apoptosis

Gene Transcription
Growth Factor Receptor Signaling

- STAT
- SHC
- GRB2
- SOS
- RAS
- RAF
- MEK
- ERK
- PI3K
- PDK
- AKT
- PTEN

Translation
Metabolism
Apoptosis

Gene Transcription
Monoclonal Antibodies - Hybridoma Technology: Evolution

Mouse
100% mouse protein

Chimeric
34% mouse protein

Humanized
10% mouse protein

Fully Human
100% human protein
Introduction of molecular-targeted agents: an important paradigm-shift in our approach to the treatment of cancer

1. Growth factors and growth-factor receptors
   HER family, VEGF/R, c-kit/SCFR

2. Signal-transduction pathways
   Ras, raf, MAPK, MEK, ERK, protein kinase C, PI3K

3. Tumor-associated antigens/markers
   Gangliosides, CEA, MAGE, CD20, CD22

4. Proteasome

5. Cell-survival pathways
   Cyclin-dependent kinases, mTOR, cGMP, COX-2, p53, Bcl-2

6. Extracellular matrix/angiogenic pathways
   MMPs, VEGF, integrins

Ullrich A. Oncology 2002;63(Suppl. 1):1–5
Biological Agents for Solid Tumors

Signal Transduction/Cell-Cycle Inhibitors
- Farnesyl transferase
- Flavopiridol
- Retinoids
- UCN-101

Gene Therapy
- GM-CSF
- Wild-type p53
- Antisense
  - c-myc
  - PKC

Vaccines
- Tumor cells
- Peptides
- Dendritic cells
- Viral vaccines

Angiogenesis Inhibitors
- SU5416/SU6668
- Anti-VEGF antibodies
- Interferon-a/b
- Marimastat
- ZD6474
- LY317615
- TNP-470
- Endostatin/angiostatin

Receptor-Targeted Therapy
- Trastuzumab
- Anti-EGFR
  - Gefitinib
  - Erlotinib
  - Cetuximab
  - Panitumumab
HER (erbB) & VEGF Family

• HER & erbB Family
  – EGFR Inhibitors
    • Monoclonal Antibodies- Cetuximab, Panitumumab
    • Tyrosine Kinase Inhibitors- Gefitinib, Erlotinib
  – HER 2 Inhibitors
    • Monoclonal Antibodies- Trastuzumab, Pertuzumab
    • Tyrosine Kinase Inhibitors

• VEGF Family
  – VEGF Inhibitors
    • Monoclonal Antibodies- Bevacizumab
    • Tyrosine Kinase Inhibitors
Multi Targeted Therapies

• **Dual kinase Inhibitor**
  - EGFR/VEGF Inhibitors
    • Tyrosine Kinase Inhibitors- Vandatanib (ZD6474)
  - EGFR/HER 2 Inhibitors
    • Tyrosine Kinase Inhibitors- Lapatinib

• **Multi kinase Inhibitor**
  - VEGFR, PDGFR, KIT and FLT3R
    • Tyrosine Kinase inhibitor- Sunitinib
  - VEGFR2 and VEGFR3, FLT-3, PDGFR, c-KIT
    • Tyrosine Kinase Inhibitor- Sorafenib
      – **Sorafenib is an oral inhibitor of RAF**
Targeted Therapies

- Kinase Inhibitors
  - PDGFR Inhibitors
    - Tyrosine Kinase Inhibitors - Imatinib
  - Proteosome Inhibitors - Apoptosis
    - Tyrosine Kinase Inhibitors - Bortezomib
Epidermal Growth Factor Receptor (EGFR)
Some Landmarks in EGFR Signalling

Stanley Cohen

- EGF in mice (1960’s)
- Human EGF (1970’s)
- Isolation and cloning of EGFR (1980’s). Link between EGFR and malignant transformation of cells demonstrated

Mendelsohn et al.,

- Blocking EGFR signalling to treat cancer
- Murine monoclonal antibodies targeting EGFR-TK → Human: murine chimeric version

More than 20 anti-EGFR agents in development
Consequence of proliferation of EGFR receptors

Mutation

Normal Cell

Up Regulation

Cancerous Cell
Human Epidermal Growth Factor Receptor Family

EGF, TGFα, b Cellulin
Amphiregulin, HB-EGF

No specific ligands - often acts as dimer partner

Heregulins
NRG2
NRG3
Hereregulins
β-cellulin

EGF, TGFα, b Cellulin
Amphiregulin, HB-EGF

No specific ligands - often acts as dimer partner

Heregulins
NRG2
NRG3
Hereregulins
β-cellulin

erbB1
HER1
EGFR

erbB2
HER2

erbB3
HER3

erbB4
HER4
Mechanisms of increased EGFR activation

1. Increased expression of EGFR protein
2. Ligand / autocrine loop
3. Heterodimerization and cross-talk
4. Phosphatase
5. Mutant EGFR

Arteaga 2002
EGFR Homo Dimerisation

erbB1
HER1
EGFR

erbB2
HER2
neu

erbB3
HER3

erbB4
HER4
Hetero Dimerisation

erbB1
HER1
EGFR

erbB2
HER2
neu

erbB3
HER3

erbB4
HER4

↑ Risk for cancer

Risk for cancer
EGFR signal transduction in tumour cells

Gene transcription

- Survival (anti-apoptosis)
- Metastasis
- Angiogenesis

Proliferation/maturation
Chemotherapy/radiotherapy resistance

TK → PI3-K → PTEN → AKT → STAT3 → MAPK → G1

TK → RAS → RAF → MEK → G2
Other mechanisms of EGFR stimulation

- MMP
- HB-EGF
- Ca++
- Pyk2
- Src
- Ras
- MAPK
- Transcription

Steroid hormone

Other mechanisms of EGFR stimulation

erbB Ligand
Gene

Steroid hormone receptor
EGFR variant

ATP

TK

Gene transcription
Cell Cycle Progression

Cell Proliferation

Anti Apoptosis

Metastasis

Cancer
EGFR Expression in Solid Tumors

EGFR is expressed in a variety of solid tumors

<table>
<thead>
<tr>
<th>Tumor Target</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck cancer</td>
<td>95–100</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>72–89</td>
</tr>
<tr>
<td>Pancreas</td>
<td>upto 95 %</td>
</tr>
<tr>
<td>Lung cancer (NSCLC)</td>
<td>40–80</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>14–91</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>35–70</td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>50–90</td>
</tr>
</tbody>
</table>

Tumor EGFR Expression as a Prognostic Factor

- EGFR expression correlates with poor prognosis.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Prognosis</th>
<th>Survival</th>
<th>Risk of metastasis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>Poor</td>
<td>-</td>
<td>Increased</td>
<td>Hemming (1992)</td>
</tr>
<tr>
<td>Lung (NSCLC)</td>
<td>Poor</td>
<td>Decreased OS</td>
<td>-</td>
<td>Ohsaki (2000)</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>-</td>
<td>Increased</td>
<td>Pavelic (1993)</td>
</tr>
<tr>
<td>Head &amp; neck (SCCHN)</td>
<td>Poor</td>
<td>Decreased DFS</td>
<td>-</td>
<td>Grandis (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased OS</td>
<td></td>
<td>Maurizi (1996)</td>
</tr>
</tbody>
</table>

- EGFR expression also linked to reduced response, and/or increased resistance to chemotherapy

DFS = Disease-free survival;
OS = overall survival
Common Approaches to Targeting HER1/EGFR

Tyrosine Kinase EGFR Inhibitor - Gefitinib, Erlotinib

- HER1/EGFR extracellular ligand-binding domain

- HER1/EGFR TK

- Small-molecule inhibitor of HER1/EGFR TK
- Chemical class: quinazoline
- Previously known as CP-385,774 and OSI-774
- A joint global development program with Roche, OSI Pharmaceuticals Inc. and Genentech Inc.
Anti-EGFR Monoclonal Antibodies

Extracellular

Intracellular

PI3-K

Pd-K

Akt

mTOR

S6K

Gα2

S6K-1

Erk

Raf

MEK

MEKK-1

JNK

ERK

Ras

mTOR

Grb2

AKT

Sos-1
ERBITUX + RT improves locoregional control over RT alone in locally advanced SCCHN

- 9.5-month increase in duration of locoregional control (24.4 vs 14.9 months)
- 32% reduction in the risk of locoregional progression

Hazard ratio = 0.68 (95% CI: 0.52 to 0.89) Log rank p=0.005

ERBITUX + RT prolongs survival over RT alone in locally advanced SCCHN

Hazard ratio = 0.74 (95% CI: 0.57 to 0.97)
Log rank p=0.03

- Almost 20 months increase in overall survival (49 vs 29.3 months).
- 26% reduction in Risk of death

CETUXIMAB + RT prolongs survival over RT alone in locally advanced SCCHN (1)

<table>
<thead>
<tr>
<th></th>
<th>RT (n=213)</th>
<th>Cetuximab + RT (n=211)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median overall survival</td>
<td>29.3 months</td>
<td>49.0 months</td>
<td>0.03</td>
</tr>
<tr>
<td>Survival rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 year</td>
<td>45%</td>
<td>55%</td>
<td>0.05</td>
</tr>
</tbody>
</table>

- Addition of Cetuximab to RT:
  - Increased median survival by nearly 20 months (49.0 vs 29.3 months)

CETUXIMAB does not increase acute RT-induced toxicity in locally advanced SCCHN

Relevant Grade 3–5 Side Effects

<table>
<thead>
<tr>
<th>Side effect</th>
<th>RT  (n=212)</th>
<th>ERBITUX + RT</th>
<th>p-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucositis/stomatitis</td>
<td>52%</td>
<td>56%</td>
<td>0.44</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>30%</td>
<td>26%</td>
<td>0.45</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>3%</td>
<td>5%</td>
<td>0.32</td>
</tr>
<tr>
<td>Fatigue/malaise</td>
<td>5%</td>
<td>4%</td>
<td>0.64</td>
</tr>
<tr>
<td>Acne-like rash</td>
<td>1%</td>
<td>17%**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infusion-related reactions(^b)</td>
<td>0%</td>
<td>3%*</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\)Fisher’s exact test
\(^b\)Listed for its relationship to ERBITUX

ERBITUX Quality of life

ERBITUX plus RT increased overall survival without adversely affecting quality of life

- No significantly difference in Quality of life scores between patients treated with ERBITUX plus radiotherapy and radiotherapy alone

Comparison ERBITUX compliance

Compliance to the treatment is higher when using ERBITUX plus RT instead of CRT

- 90% of the patients treated by ERBITUX + RT receive the full treatment
- 26% to 35% patient will not receive the full treatment of chemoradiotherapy

\[\text{% patient receiving the full treatment} \]

- ERBITUX + RT: 90%
- Cisplatin + 5FU + RT: 74%
- Carboplatin + 5FU + RT: 65%

CETUXIMAB in locally advanced SCCHN: Summary

- CETUXIMAB + high-dose RT demonstrated significant efficacy benefits over high-dose RT alone

- 32% reduction in locoregional relapse
- Extends survival by nearly 20 months
- No increase in acute RT-related side effects
- 26% reduction in Risk of death

Normal HER2 expression

Normal Epithelial cell has 2 copies of HER 2 gene & 20000 to 50000 HER 2 receptors on cell surface
Molecular pathway for activation of HER2/NEU

EGF or NEU differentiation factor activates receptor

Tyrosine kinase receptor ie, HER2 (CERBB2/NEU)

Induces phosphorylation cascade in cytoplasmic kinases

Increased transcription of nuclear protein

Increased cell proliferation

Nucleus
Mutations affecting HER 2 gene results in gene amplification thereby Causing 100 fold increase in no. of HER 2 receptors on cell surface.
HER2 overexpression $\rightarrow$ tumour proliferation

Excess amounts of HER 2 leads to Tumor Proliferation

Prof. Tim Cooke at Special EONS symposia, ECCO 10, Austria
HER2 Receptor Provides an Extra-Cellular Therapeutic Target

- Tyrosine kinase activity
- Binding site
- Signal transduction to nucleus
- Nucleus
- Cytoplasm
- Plasma membrane
- Gene activation

CELL DIVISION
Molecular Targeting of Breast Cancer

**Molecular Forecasting**
- **How it’s done**
  With microarrays, scientists can study patterns of gene activity using strands of cancer DNA and predict which tumours are likely to spread. The technique may someday be used to design customised treatments.

- **Availability**
  Clinical trials for breast cancer are starting this year; treatment may be widely available within the decade.

**Smart Drugs**
- **How it’s done**
  As scientists come to understand at the molecular level precisely how tumours form, they are designing a new generation of smart drugs that bind to specific receptors or block particular proteins.

- **Availability**
  Herceptin, the first of these smart drugs for breast cancer is available for certain advanced cancers.
Binding of Trastuzumab to HER2
Lapatinib: Targeting EGFR and HER2

- Lapatinib oral tyrosine kinase inhibitor of ErbB1 and ErbB2
  - Blocks signaling through EGFR and HER2 homodimers and heterodimers
  - May also prevent signaling between ErbB1/ErbB2 and other ErbB family members

Lapatinib—A Dual Receptor Tyrosine Kinase Inhibitor

- Potent, oral, reversible dual tyrosine kinase inhibitor
- Binds to ATP site of erbB-1 and erbB-2 receptor kinases, blocking kinase activity and downstream signaling
Heat shock protein-90 (HSP-90) is a chaperone protein for a variety of oncogenic proteins, including HER2, ER/PR, AKT, MET, and Raf kinase.

17-AAG (KOS-953), an inhibitor of HSP-90, suppresses tumor growth in mouse xenograft models of HER2+ human breast cancers.

Angiogenesis
VEGF
Tumor Angiogenesis by Vascular Sprouting

Tumor angiogenesis

1. Secretion of angiogenic factors
2. Proteolytic destruction of ECM
3. Endothelial cell proliferation and migration
4. Appearance of new tumor vasculature
5. Intravasation
Angiogenesis is involved throughout tumour formation, growth & metastasis.

Stages at which angiogenesis plays a role in tumour progression:

- Premalignant stage: (Avascular tumour)
- Malignant tumour: (Angiogenic switch)
- Tumour growth: (Vascularised tumour)
- Vascular invasion: (Tumour cell intravasation)
- Dormant micrometastasis: (Seeding in distant organs)
- Overt metastasis: (Secondary angiogenesis)

The angiogenic switch in tumour development

Small tumour (1–2mm)
- avascular
- dormant

Larger tumour
- vascular
- metastatic potential

Angiogenic switch
Results in overexpression of pro-angiogenic signals, such as VEGF

VEGF: A Key Mediator of Angiogenesis

Increased VEGF levels

Environmental factors (hypoxia, pH)

Growth factors, hormones (EGF, bFGF, PDGF, IGF-1, IL-1α, IL-6, estrogen)

Genes implicated in tumorigenesis (p53, p73, src, ras, vHL, bcr-abl)

bFGF, basic fibroblast growth factors; EGF, epidermal growth factor; IGF, insulin-like growth factor; IL, interleukin; PDGF, platelet-derived growth factor; VEGFR, VEGF receptor.
The VEGF Family and Its Receptors

PIGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D

VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), VEGFR-3 (Flt-4)

Angiogenesis, Lymphangiogenesis
VEGF: A Key Mediator of Angiogenesis

VEGF

VEGFR binding and activation

Endothelial cell activation

PLC
PI3-K
FAK
Ras
PKC
IP3
AKT
Paxillin
MAPK

Survival
Proliferation
Migration

ANGIOGENESIS
Agents targeting the VEGF pathway

- Anti-VEGFR antibodies
  - IMC-IC11

- Anti-VEGF antibodies
  - Avastin™

- Soluble VEGF receptors
  - VEGF-TRAP

- Small-molecule VEGFR inhibitors (TKIs)
  - PTK-787

- Anti-VEGF antibodies

- Permeability

- Cation channel

- Calcium release

- PLA

- MAP

- SAPK/JNK

- AKT

- Protein kinase C

- Raf-1

- MAP

- SAPK/JNK

- AKT
Multi-Targeted Inhibitors

Sunitinib

VEGFR

SHC

GRB2

SOS

RAS

SOS

GRB2

SHC

Endothelial Cells

Tumor Cells

KIT

CSF1R

PDGFR

Sunitinib

RAF

MEK

ERK

Gene Transcription
Multi-Targeted Inhibitors

VEGFR ← Sorafenib ← CSF1R

Endothelial Cells

? Tumor Cells

GRB2 SOS RAS GRB2 SHC

Tumor Cells

Pericytes

KIT PDGFR

Gene Transcription
Targeting VEGF: The Bevacizumab Story

VEGF

Bevacizumab
Targeting VEGF: The Bevacizumab Story

VEGF Activation BLOCKED
Bevacizumab - MOA

- Regression of existing microvasculature
- Normalisation of existing vasculature
- Inhibition of tumour vessel growth
Tumour characteristics and environment promote VEGF expression

- Hypoxia
- PDGF
- EGF
- IGF-1
- IL-8
- bFGF
- COX-2
- Nitric oxide
- Oncogenes

Increased expression (MMP, tPA, uPA, uPAR, eNOS, etc.)

Binding and activation of VEGF receptor

- P
- P

Survival
Proliferation
Migration
Permeability

ANGIOGENESIS

IGF = insulin-like growth factor; PDGF = platelet-derived growth factor
VEGF Trap
VEGF Trap
VEGF Trap

$F_c$ portion
VEGF Trap

VEGF Activation BLOCKED
Tyrosine Kinase Inhibition and VEGF

VEGF

TKI (Tyrosine Kinase Inhibitor)
Tyrosine Kinase Inhibition and VEGF

Downstream phosphorylation BLOCKED
Pericyte

**PDGF-B** → **PDGF-Rβ**

**Ras** → **Raf** → **MEK** → **ERK** → **p38MAPK**

**PI3K** → **Akt** → **Caspase-9** → **eNOS**

**CELL ACTIVATION**

**VESSEL MATURATION**
A Specific Endothelin-A Receptor Antagonist

Potential actions of an ET<sub>A</sub> antagonist:

• Inhibition of prostate tumour cell proliferation
• Promotion of tumour cell apoptosis
• Inhibition of osteoblast proliferation
• Reduction in pain

ZD4054 is in clinical development
Role of Aurora kinases in mitosis and cell division

- **Aurora A**
  - localized at the spindle poles
  - role in spindle assembly

- **Aurora B**
  - initially localized at the centromeres
  - role in chromatid alignment and segregation, and in cytokinesis
  - moves to the midzone during cytokinesis
Aurora kinase inhibition: A new anticancer approach

- Aurora A and B are overexpressed in cancer
- Aurora A is oncogenic when overexpressed
- Selectively inhibiting Aurora kinase activity leads to chromosome segregation errors and deregulation of the spindle checkpoint
  - mitosis occurs in a highly disordered manner
  - cytokinesis fails
  - proliferating tumor cells apoptose
Core Components of the Apoptotic Pathway

Death Signals

Death Regulators
- Bcl-2 family
- IAPs and anti-IAPs
- Usurpins
- Phosphorylation

Caspases

Neurodegeneration
Cancer

Apoptotic “Victims”
Extrinsic and Intrinsic Cell Death Pathways
IAP Antagonism During Apoptosis

Mitochondrion

ATP
APAF-1
C9
C3/C7

SMAC/Diablo
HtrA2/Omi

IAPs

Apoptosis
DNA damage Replicative Stress

Cell Cycle Arrest Apoptosis

Sensors Effectors

Signals Effects Alterations

ATM ATR p53 chk1 chk2

p21 gadd45 mdm2 c-abl bax CD95 14-3-3 Noxa ....

HR NHEJ NER MMR BER

Apoptosis Cell Cycle S Arrest G2 DNA Repair

MRE11 BRCA1 NSB1 Ku70/Ku86 DNA-PK PARP cdc25

Effects

Vigano et al. EMBO J. 2006;25:5105.
Rossi et al. PNAS. 2006;103:12753

p73 Promoter, Proteins, and Translational Targets

Signals
- Trascriptional inducers
  - p73
  - Interacting Proteins
    - Regulatory Proteins
    - Posttranslational modifications
    - Degradation pathways

Effects
- Transcriptional targets

Regulators of p73 promoter
- Oncogenes: E2F1, c-Myc, Ras
- Viral proteins: E1A, Tax
- Drugs: retinoids

p73 interacting proteins
- Oncogenes: c-Myc, c-Abl, Hipk2, WT1
- Viral proteins: E1A, E4orf6, Tax
- Cell cycle genes: p300, MM1, HMBG1
- E3 ligases: MDM2, MDMX, Sumo1, PIAS1
- Family members: p53, p63, p73

Evolution

p73 transcriptional targets
- Cell cycle genes: p21WAF1, gadd45, cyclin, kip2
- Apoptosis: Bax, CD95, PUMA, perp, Noxa, p53AIP1
- Signaling: 14-3-3, Pig13, RTP, ADTC, p53R2, IGFBP2, jun-B, IKKa family members: DeltaN-p73
- Differentiation: AGP3, VEGF (neg), loricrin, involucrin, NCAM
A Model for ITCH-Mediated Regulation of p73 Function

Tumor cell

N4BP1

Ub, degradation

DNA damage

Apoptosis
Caspase Activation Cascades

(1) Extrinsic
Death receptors

(2) Intrinsic
BH3-only proteins

(3) Granzyme B
CTL/NK cells
BH3-Only Proteins: Pathway-Specific Sensors of Stress and Damage
The TRAIL–TRAIL-R System

TRAIL-R1 (DR4)/TRAIL-R2 (DR5) → TRAIL (Apo2L) → Apoptosis-inhibitory receptors?

TRAIL-R3 (DcR1) → TRAIL-R4 (DcR2)

- Death domain
- Death effector domain
- Truncated death domain

Apoptosis
Agonistic mAbs
TRAIL-R1/TRAIL-R2
TRAIL Receptor Agonists Currently in Clinical Trials
Double Hit on Tumor Cells

TRAIL receptor agonists  Chemotherapy radiotherapy

- High crosslinking
- Decoy receptor expression
- High cFLIP expression
- High XIAP expression

DISC  Active Caspase-8  Bid  tBid  Bax/Bak  Apoptosome  Cytochrome c

(Pro-) Caspase-3  Caspase-9  XIAP  Smac/DIABLO  HtrA2/Omi

Activated BH3-only proteins
The Insulin and IGF-1R Signaling Axis

<table>
<thead>
<tr>
<th>Adult Serum Concentration, nM</th>
<th>Insulin</th>
<th>IGF-2</th>
<th>IGFBP</th>
<th>IGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.1-1.5</td>
<td></td>
<td></td>
<td>60-85</td>
</tr>
<tr>
<td>Human</td>
<td>0.1-1.5</td>
<td>80-130</td>
<td></td>
<td>9-35</td>
</tr>
</tbody>
</table>

Unpublished data.
IGF-1R Signaling Pathways

IGF-1 or IGF-2 → IGF-1R

PIP2 → PIP3 → AKT (PKB) → GSK3, TSC2, FKHR, BAD, p53, NFκB

PTEN

Glycogen synthesis, Protein synthesis, Apoptosis

Ras-GDP → Ras-GTP → Raf → MEK → ERK → EIK

Cycle entry, differentiation

PI3K, PI3K

mTOR

NFκB

BAD

MDM2

CASP9

FKHRL

IKK

SHC, SOS1

GRB2
Plasma IGF-1 Suppresses GH Release Through IGF-1R Signaling in Pituitary
Src

Cell-cell adhesion

LPA
endothelin
thrombin

GPCR

Steroid hormone receptor

Steroid hormone

LPA

EGFR

Growth factors

Growth hormone

Cytokine

Integrin-ECM

Integrin-ECM

Migration

Survival

Angiogenesis

Mitogenesis

Upregulation of gene transcription

c-fos

c-jun

c-myc

Cyclin D

uPA

Target genes

VEGF

Y845

c-Src

FAK

Pax

c-Src

c-Src

c-Src

c-Src

c-Src

c-Src

c-Src

c-Src

c-Src

Src
Src kinase activity is essential for focal adhesion turnover.

Focal adhesion complex

Tumour invasion

Cell-cell adhesion weakening

Increased Src activity

Pre-invasive tumour cell growth

Cadherins

Src kinase activity is essential for focal adhesion turnover.
• Invasive growth of primary tumors and metastases
  Potential role in early and advanced disease

• Src
  dependent bone resorption

• Pivotal role in key survival, growth, and resistance pathways

• Invasion and metastasis

• Src and Bcr/Abl kinases role in imatinib resistance

• Src kinase activity

• Src kinase

• Leukemia

• Signal transduction modulation

• Osteoclast
Potential Clinical Application of Src Inhibition

- Antiangiogenesis
- Osteoclast activation
- Invasion
- Proliferation

- Tumor growth
- Bone metastasis
- Metastasis/adjuvant
- Tumor growth
Gene Transcription

TFC
(Transcription Factor Complex)
HDAC and Gene Expression

HDAC

TFC (Transcription Factor Complex)
Breast Epithelial Stem Cells & Cancer Stem Cells

Cancer

Carcinogenic agents/mutations
Drug Development: Cancer Stem Cells

Drugs that kill cancer stem cells

Drugs that kill majority of cancer cells but not cancer stem cells

Cancer cells lose the ability to sustain cell growth

Recurrence of cancer growth

Tumour Death
Cancer patient with suspected relapsed lesion

- Collect tumor sample
- Extract mRNA
- Initiate treatment
- Collect data
- CRF

Analysis
CONCLUSIONS

• Targeted therapies are molecular radiosensitisers
• Can be combined with RT or CT for a better survival benefit
• Preclinical studies have been authenticated by clinical data (Bonner’s trial)
• Hitting the right target with the right drug can produce great results
Special Article


Renita Bhamrah, PhD, Pramod Kumar Julka, MD, Omana Nair, PhD, Rajinder Parshad, MS, Siddhartha Dattagupta, MD, Ranju Ralhan, PhD, Sadanand Dwivedi, PhD, Darpreet Singh Bhamrah, MS, Guresh Kumar, PhD, Sanjay Gupta, M Pharm, Gaurav Dhawan, MBBS, Goura Kishor Rath, MD.

Abstract:

We studied the prognostic impact of EGFR positivity in Indian scenario and its correlation with known prognostic markers such as Estrogen receptor (ER) and HER-2/neu oncogene. 210 women aged less than 70 years with histopathologically proven carcinoma breast and having ambulatory general condition were included in the study. EGFR, Her-2/neu and ER expressions were evaluated immunohistochemically. Of the 210 patients, the EGFR, ER and HER-2/neu expressions were positive in 87 (41%), 139 (66%) and 60 (29%) patients respectively. EGFR had a positive correlation with systemic recurrence and inverse correlation with overall survival of the patient. In multivariate analysis it was observed that EGFR and node positivity were significant factors for overall survival and disease free survival. Our study reveals that expression of EGFR may serve as a prognostic indicator for poor survival in breast cancer patients.
Trastuzumab and Docetaxel for Metastatic Breast Cancer: An Experience from a Cancer Centre in India

P. K. Julka, D. N. Sharma, P. Mukhopadhyay, G. K. Rath

Department of Radiotherapy and Oncology, All India Institute of Medical Sciences, New Delhi, India

ABSTRACT:
Aims: To study the toxicity profile and effectiveness of combination of trastuzumab and docetaxel in women with metastatic breast cancer showing HER-2 overexpression (IHC 3+).
Materials and methods: Sixteen women with metastatic breast cancer were treated with trastuzumab (2 mg/kg every week) and docetaxel (100 mg/m²) as first-line therapy. A loading dose of 4 mg/kg trastuzumab was given on week 1. We planned to give a minimum of six cycles of docetaxel chemotherapy.
Results: A total of 89 cycles of docetaxel chemotherapy was given (median five cycles per patient). Median number of cycles of trastuzumab was 44 with a range of 20–71. Of the 16 patients, seven (44%) had complete response (CR), whereas five patients (31%) had partial response (PR). The overall response rate (CR + PR) was 75%. Two patients died of progressive disease, and the other two died at home, for which the cause of death could not be known. No anaphylaxis, cardio-toxicity or febrile neutropenia was observed in any patient. Overall, the toxicity was within tolerable limits.
Conclusion: The combination of trastuzumab and docetaxel in women with metastatic breast cancer showing HER-2 overexpression (IHC 3+) is a safe and effective regimen. However, further randomised trials are needed to establish its role in metastatic breast cancer. Julka P. et al. (2004). Clinical Oncology 16, 115–118

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Full Paper

A phase II study of sequential neoadjuvant gemcitabine plus doxorubicin followed by gemcitabine plus cisplatin in patients with operable breast cancer: prediction of response using molecular profiling

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CONCLUSION (Cont.)

- Kinase inhibitors are most effective against tumors that are heavily, perhaps solely, dependent on the targeted kinase.
- Monotherapy with TKIs is limited by the development of resistance.

Translational research studies are critical to understanding the results from clinical trials of targeted therapeutics.
Thank You