Protein kinases
a CRASH course

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Institute of Pharmacology
Protein kinases

Outline:
Kinome - multitude of kinases (why, what for?)

Specificity

Regulation

Significance for drug development
Figure 1. Protein Modules and Signal Transduction. This figure of a cell shows how modular protein and lipid interaction domains are used in a variety of cell signaling pathways.
Low-Activity and Doubly Phosphorylated ERK2
Ribbon diagram of (A) ERK2 and (B) ERK2-P2. The N-terminal domain (residues 1–109 and 320–358) is formed largely of β strands (green) and two helices, C (blue) and αL16 (magenta). The C-terminal domain (residues 110–319) is mostly helical (blue), contains the phosphorylation lip (red) and the MAP kinase insertion (magenta, labeled MKI), and is the locus of the P+1 site, the catalytic loop (residues Arg-147–152, labeled C loop). The side chains of Thr-183, Tyr-185, pTyr-183, and pTyr-185 are shown.
Protein kinases:

Genome contains more than 500 kinases (513) = kinome

Why so many?
gene duplication - fine tuning

Why cascades?
signal amplification

Why multiple sites of phosphorylation?
Signal integration
PKA, PKG, PKC, GRK, Akt/PKB

STE-7,11,20-homologs = MAPKKK etc.

Tyrosine kinase-like

Casein kinase

CDK, MAPK, GSK3, CLK
Protein kinases:

Why so many?
gene duplication - fine tuning

Why cascades?
signal amplification

Why multiple sites of phosphorylation?
Signal integration
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Protein kinases:

**Why so many?**
gene duplication - fine tuning

**Why cascades?**
signal amplification - storage of information

**Why multiple sites of phosphorylation?**
Signal integration
Signal amplification

Glycogenolysis

Inhibition of glycogen synthesis
CaMKII isoforms: contain an N-terminal catalytic domain (blue), a central regulatory (REG) domain (pink) and a C-terminal association domain (green).

The regulatory domain contains **auto-inhibitory** and calmodulin (CaM; purple)-binding sequences, as well as **Ca\(^{2+}\)/calmodulin-dependent** (white P in black circle) and **Ca\(^{2+}\)-independent** (black P in white circle) autophosphorylation sites.
The amino acid sequence of the regulatory domain (residues 281–309; pink) contains several residues (maroon or cyan) that are critical for interactions with residues in the catalytic domain (above in blue) and/or in Ca2+/calmodulin (below in purple). The C-terminal end of the autoinhibitory domain occupies the catalytic site (S-site) of the kinase by acting as a pseudosubstrate, whereas residues surrounding Thr286 occupy a distinct hydrophobic pocket in the catalytic domain, termed the T-site (inset above).
A space-filled structure of Ca\textsuperscript{2+}/calmodulin (purple) bound to a ‘ball and stick’ structure of an \( \alpha \)-helical calmodulin-binding peptide from CaMKII (residues 293–310) is also shown. The peptide structure is oriented with the N-terminus on the left, with residues in green making critical interactions with Ca\textsuperscript{2+}/calmodulin. These include Leu\textsuperscript{299} and Leu\textsuperscript{308}, which make initial low-affinity interactions with several amino acids in calmodulin, including Met\textsuperscript{72} and Met\textsuperscript{124} (yellow in space-filled structure).

Ca\textsuperscript{2+}/calmodulin-dependent autophosphorylation at Thr\textsuperscript{286} (white P in black circle) blocks interactions of the autoinhibitory domain with the T-site, generating an autonomously active form of CaMKII. Ca\textsuperscript{2+}-independent autophosphorylation (black P in white circle) at Thr\textsuperscript{305} or Thr\textsuperscript{306} blocks Ca\textsuperscript{2+}/calmodulin binding to CaMKII.
CaMKII holoenzymes appear as a stacked pair of hexameric rings by electron microscopy, with association domains forming the hub and catalytic domains projecting out: a single ring is shown in the diagram for clarity.

Autophosphorylation at Thr$^{286}$ requires simultaneous binding of Ca$^{2+}$/calmodulin to adjacent subunits in the holoenzyme, one of which serves as the catalytic unit and the other as the substrate; thus, in this example, Ca$^{2+}$/calmodulin-bound subunits 4 and 5 will be autophosphorylated (wide arrows), whereas subunits 2 and 6 will not.
Autophosphorylation at Thr$^{305/306}$ is an intra-subunit reaction that occurs only in the absence of Ca$^{2+}$/CaM (e.g. in subunits 1 and 3; narrow arrow). Thr$^{306}$ is the preferred Ca$^{2+}$/CaM-independent site in the absence of prior Thr$^{286}$ autophosphorylation, but is only slowly phosphorylated; CaMKII is inactivated because binding of Ca$^{2+}$/CaM is blocked. In contrast, removal of Ca$^{2+}$/CaM from Thr$^{286}$-autophosphorylated kinase results in rapid autophosphorylation at Thr$^{305}$ or Thr$^{306}$ (not shown), which also blocks Ca$^{2+}$/CaM binding, but the kinase remains active due to the Thr$^{286}$ autophosphorylation. Thus PPs can potently regulate CaMKII activity.
Protein kinases:

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signal amplification - storage of information

Why multiple sites of phosphorylation?
Signal integration
Fig. 1. MAPK phosphorelay systems. GTP, guanosine triphosphate; p90RSK, 90-kD ribosomal protein S6 kinase; Src, an oncogenic tyrosine kinase; MEF2, myocyte enhancer factor 2; IL-1, interleukin 1; TRAF6, tumor necrosis factor receptor-associated factor 6; TAK1, transforming growth factor-activated protein kinase 1; and MNK1, MAPK-interacting kinase 1. The modules shown are representative of pathway connections for the respective MAPK phosphorelay systems. There are multiple component MKKKs, MKKs, and MAPKs for each system. For example, there are three Raf proteins (c-Raf1, B-Raf, A-Raf), two MKKs (MKK1 and MKK2), and two ERKs (ERK1 and ERK2) that can compose MAPK phosphorelay systems responsive to growth factors. The ERK, JNK, and p38 pathways in the STKE Connections Map demonstrate the potential complexity of these systems. Our understanding of the connections within the MAPK systems is incomplete and often controversial and continues to be defined in different cell types.
EGFR/ErbB2 Heterodimer

Sustained MAPK activation:
$G_0/ G_1$ progression, differentiation
Protein kinases:

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signal amplification - storage of information

Why multiple sites of phosphorylation?
Signal integration
Feed-Back regulation
The insulin-stimulated phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB) signalling pathway and its downregulation by phosphorylation of insulin receptor substrate-1 (IRS1):

1) The insulin-induced activation and autophosphorylation of the insulin receptor leads to the recruitment of insulin-receptor substrate-1 (IRS1) to the plasma membrane and its phosphorylation at multiple tyrosine residues by the receptor.

2) The p85 regulatory subunit of Class I PI3Ks binds to specific phosphotyrosine-containing motifs on IRS1, allowing PI3K to convert the membrane lipid phosphatidylinositol (4,5)-bisphosphate (PIP$_2$) to phosphatidylinositol (3,4,5)-trisphosphate (PIP$_3$).

3) PIP$_3$ interacts with both PDK1 and PKB, allowing the former to activate the latter. PKB then regulates many proteins, including glycogen synthase kinase 3 (GSK3) and FKHR (Forkhead in rhabdomyosarcoma).

4) PKB also phosphorylates IRS1 at a serine residue(s), which inhibits tyrosine phosphorylation by the insulin receptor. This could be a feedback control mechanism for downregulating the pathway.

5) A distinct residue on IRS1 (S307) becomes phosphorylated in response to TNF. This phosphorylation, which is possibly catalysed by stress-activated protein kinase-1 (SAPK1, also called JNK), or another MAPK family member, also inhibits the tyrosine phosphorylation of IRS1 and might underlie TNF-induced resistance to insulin.
Multisite phosphorylation of BAD allows it to be controlled by several different agonists. Agonists that switch on the MAPK cascade induce the activation of MAPK-activated protein kinase-1 (MAPKAP-K1, also called RSK), which phosphorylates BAD at S112. Signals that activate the phosphatidyl inositol 3-kinase (PI3K) pathway turn on protein kinase B (PKB), which phosphorylates BAD at S136 in neuronal cells. Cyclic-AMP-elevating agents activate PKA, which phosphorylates BAD at S155. Each of these phosphorylations inhibits the pro-apoptotic activity of BAD.
Protein kinases

How to achieve specificity?
(i) amino acids: serine/threonine; tyrosine; (histidine)

(ii) consensus sites:
  e.g. RRXS = PKA
  SPXR/K = CDK1

(iii) anchoring/scaffolding proteins:
  AKAP; JIP
Fig. Sequence logos of experimentally verified phosphorylation sites of six ser/thr kinases
Protein kinases

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Fig. 1. MAPK phosphorelay systems. GTP, guanosine triphosphate; p90RSK, 90-kD ribosomal protein S6 kinase, Src, an oncogenic tyrosine kinase; MEF2, myocyte enhancer factor 2; IL-1, interleukin 1; TRAF6, tumor necrosis factor receptor-associated factor 6; TAK1, transforming growth factor-activated protein kinase 1; and MNK1, MAPK-interacting kinase 1. The modules shown are representative of pathway connections for the respective MAPK phosphorelay systems. There are multiple component MKKKs, MKKs, and MAPKs for each system. For example, there are three Raf proteins (c-Raf1, B-Raf, A-Raf), two MKKs (MKK1 and MKK2), and two ERKs (ERK1 and ERK2) that can compose MAPK phosphorelay systems responsive to growth factors. The ERK, JNK, and p39 pathways in the STKE Connections Map demonstrate the potential complexity of these systems. Our understanding of the connections within the MAPK systems is incomplete and often controversial and continues to be defined in different cell types.
Kinase road map. Custom-designed scaffolding/adapter proteins route MAPK modules in mammals (top) and yeast (bottom).

Protein kinases
How to achieve regulation?

(i) by diffusible small molecules (second messengers)
cAMP, cGMP, Ca\textsuperscript{2+}, AMP
inactivation = degradation/removal of small molecule

(ii) by membrane-anchored small molecules (second messengers)
DAG, PIP2/PIP3
inactivation = degradation/removal of small molecule

(iii) by upstream phosphorylation (inactivation = dephosphorylation)
Lissandron et al., 2005, J. Mol. Biol.
FRET based cAMP detection in PC12 cells

100 nM CGS 21680
30min at 25°C

wash & recovery
45min at 25°C

100 nM CGS 21680
10min at 25°C

---

basal
0min

CGS21680
2min
30min

wash
45min

CGS21680
10min

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Ingrid Gsandtner & Oliver Kudlacek
Ingrid Gsandtner & Oliver Kudlacek

**NFRET**

- CGS21680
- wash
- CGS21680

**PC12 cells**

- time (min)
  - 0min
  - 2min
  - 30min
  - 45min
  - 10min

**NFRET**

- 0.05
- 0.10
- 0.15
- 0.20
- 0.25
- 0.30
- 0.35
- 0.40

**n=4**
Protein kinases
How to achieve regulation?

(i) by diffusible small molecules (second messengers)
cAMP, cGMP, Ca\(^{2+}\), AMP
inactivation = degradation/removal of small molecule

(ii) by membrane-anchored small molecules (second messengers)
DAG, PIP2/PIP3
inactivation = degradation/removal of small molecule

(iii) by upstream phosphorylation (inactivation = dephosphorylation)
Phosphatidylinositol = PtdIns
PKC isoforms differ with respect to their mechanism of activation.
Protein kinases
How to achieve regulation?

(i) by diffusible small molecules (second messengers)
cAMP, cGMP, Ca^{2+}, AMP
inactivation = degradation/removal of small molecule

(ii) by membrane-anchored small molecules (second messengers)
DAG, PIP2/PIP3
inactivation = degradation/removal of small molecule

(iii) by upstream phosphorylation (inactivation = dephosphorylation)
Growth factor

Receptor tyrosine kinase

- pY
- Grb2

SOS

GDP-Ras

GTP-Ras

RAF (active)

pS217

pS221

M KK1

ERK (inactive) (inactive)

M KK1

ERK (active) (inactive)

pS217

pS221

M KK1 (active)

ERK (active) dimerizes

goes to nucleus

Several phosphatases

pT183

pY185

pT183

pY183

ERK (inactive)
Protein kinases

How to achieve activation?
(i) by relieving pseudosubstrate autoinhibition
(ii) by switching from an inactive to an active conformation
The autoinhibitory domain of the R-subunit contains the sequence Arg-Arg-Gln-Ala-Ile
Protein kinases

How to achieve activation?
(i) by relieving pseudosubstrate autoinhibition
(ii) by switching from an inactive to an active conformation
Figure 2. Ribbon Diagram Showing the Structure of the c-Src Protein-Tyrosine Kinase in Its Inactive State Bound to AMP-PNP
Autoregulation and activation of Src. 

A, space-filling model of Src based on the crystal structure. B, the kinase domain of Src is held in an inactive state through two distinct intramolecular interactions: the binding of the SH2 domain to pTyr\textsuperscript{527} and the binding of the SH3 domain to the SH2-kinase linker, which contains a short polyproline type II helix. As a result of extracellular stimuli, the kinase activity of Src can be enhanced by binding of the SH3 domain to proline-rich sequences, binding of the SH2 domain to phosphotyrosine-containing sequences, or dephosphorylation of pTyr\textsuperscript{527}. Full activation of Src requires autophosphorylation of Tyr\textsuperscript{416} in the A-loop.
Protein kinases

How to achieve activation?
(i) by relieving pseudosubstrate autoinhibition
(ii) by switching from an inactive to an active conformation
Model for stimulation of tyrosine kinase activity by receptor dimerization. The A-loop of RTKs is relatively mobile and probably adopts numerous conformations. A majority of these conformations (red) will interfere with protein substrate binding and perhaps ATP binding. A subset of conformations (green) are compatible with substrate (protein and ATP) binding. In the absence of ligand, the probability of a trans-autophosphorylation event occurring between randomly colliding receptors is low. Binding of ligand to the extracellular domain substantially increases local receptor (substrate/enzyme) concentration, providing sufficient opportunity for trans-autophosphorylation to occur. Autophosphorylation of the A-loop tyrosine(s) shifts the A-loop equilibrium toward the active conformation, which accommodates substrate binding and facilitates the proper positioning of residues involved in MgATP binding. Autophosphorylation occurs on additional tyrosines, which serve as binding sites for downstream signaling proteins.
The Philadelphia Chromosome: t(9;22)
BCR-ABL is a Tyrosine Kinase, which is Inhibited by Imatinib
Binding of Imatinib to the Abl-kinase
Mercedes E. Gorre et al. (2001) Science 293: 876
Mercedes E. Gorre et al. (2001) Science 293: 876
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<td>Immunosuppressant for organ transplantation, Solid tumours, rheumatoid arthritis</td>
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<tr>
<td>CEP-1347</td>
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<td>Artisense</td>
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<td>NSCLC</td>
</tr>
<tr>
<td>LY335531</td>
<td>Eli Lilly</td>
<td>PKCβ</td>
<td>Small molecule</td>
<td>Phase III</td>
<td>Diabetic peripheral neuropathy</td>
</tr>
<tr>
<td>VX-702</td>
<td>Varini Pharmaceuticals</td>
<td>p38</td>
<td>Small molecule</td>
<td>Phase II</td>
<td>Inflammation/heart disease</td>
</tr>
</tbody>
</table>

EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; PKC, protein kinase C; SAP, stress-activated protein; VEGFR, vascular endothelial growth factor receptor.
**Table 2** | **Specificity of selected protein kinase inhibitors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>SU1124</th>
<th>ZD6474</th>
<th>Bay 43-9006</th>
<th>PTK787</th>
<th>Erlotinib</th>
<th>Imatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-Raf</td>
<td></td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EGFR/Her 1</td>
<td>8.9</td>
<td>0.5</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>0.002</td>
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<tr>
<td>erbB2/Her 2</td>
<td>&gt;10</td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Flt-1/VEGFR1</td>
<td></td>
<td></td>
<td></td>
<td>0.077</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>KDR/VEGFR2</td>
<td>0.01</td>
<td>0.04</td>
<td>0.09</td>
<td>0.037</td>
<td>0.6</td>
<td></td>
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<tr>
<td>PDGFRβ</td>
<td>0.01</td>
<td>1.1</td>
<td></td>
<td>0.58</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Kit</td>
<td>0.01</td>
<td>0.07</td>
<td></td>
<td>0.73</td>
<td>0.1</td>
<td></td>
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<tr>
<td>Flt-3</td>
<td>0.25</td>
<td>0.06</td>
<td></td>
<td></td>
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<tr>
<td>FGFR1</td>
<td>0.7</td>
<td>3.6</td>
<td>0.58</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flt-4</td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MEK</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDK2</td>
<td>&gt;10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1R</td>
<td>&gt;200</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In vitro kinase assays, IC_{50} (μM). CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; MEK, mitogen-activated protein kinase/extracellular-signal regulated kinase kinase; IGF-1R, insulin-like growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Trial phase</th>
<th>Adverse events</th>
<th>Incidence of clinical activity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>R115577 (Zamestra)</td>
<td>FTI</td>
<td>III</td>
<td>Myelosuppression</td>
<td>Ineffective against colorectal cancer; ineffective in combination with gemcitabine against pancreatic cancer; most encouraging activity (30–60% objective response rate) observed against leukaemias</td>
<td>Trials are ongoing in patients with multiple myeloma</td>
</tr>
<tr>
<td>SCH66336 (Sarasar)</td>
<td>FTI</td>
<td>II</td>
<td>–</td>
<td>Early reports of increased PR rate against head and neck cancer in Phase I trial; no effect on transitional-cell cancer; not as effective as gemcitabine against pancreatic cancer</td>
<td>–</td>
</tr>
<tr>
<td>BAY 43-9006</td>
<td>RAF, VEGFR, other</td>
<td>III</td>
<td>Fatigue, skin rash, diarrhoea, pancreatitis</td>
<td>2% PR rate and 37% SD rate in Phase I trial; favourable responses seen against renal-cell carcinoma (monotherapy) and against with melanoma (in combination: paclitaxel/carboplatin); in Phase II trial, effective against hepatocellular carcinoma (43% SD and 9% SD)</td>
<td>Multitargeted kinase inhibitor; Phase III monotherapy trials are ongoing in patients with renal-cell carcinoma; a Phase I study in combination with docetaxel against multiple tumour types has been initiated</td>
</tr>
<tr>
<td>CI-1040</td>
<td>MEK</td>
<td>II</td>
<td>Fatigue, skin rash, diarrhoea</td>
<td>One PR in patient with pancreatic cancer in Phase I trial; no responders in Phase II trials; ~25% SD</td>
<td>Non-ATP-competitive; metabolically unstable; clinical trials terminated due to lack of efficacy and exposure</td>
</tr>
<tr>
<td>PD0325901</td>
<td>MEK</td>
<td>I</td>
<td>?</td>
<td>?</td>
<td>Non-ATP-competitive; clinical data pending</td>
</tr>
<tr>
<td>ARRY-142886</td>
<td>MEK</td>
<td>I</td>
<td>?</td>
<td>?</td>
<td>Non-ATP-competitive; clinical data pending</td>
</tr>
</tbody>
</table>

FTI, farnesyl transferase inhibitor; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; PR, partial response; SD, stable disease; VEGFR, vascular endothelial growth factor receptor.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Target</th>
<th>Technology</th>
<th>Status</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib</td>
<td>Genentech/CSI Pharma/Poche</td>
<td>EGFR</td>
<td>Small molecule</td>
<td>FDA decision Jan 05</td>
<td>Phase III NSCLC, Pancreatic cancer</td>
</tr>
<tr>
<td>ABX-EGF</td>
<td>Amgen/Abgenix</td>
<td>EGFR</td>
<td>Monoclonal antibody</td>
<td>Phase III</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>SU11248</td>
<td>Pfizer</td>
<td>VEGFR</td>
<td>Small molecule</td>
<td>Phase III</td>
<td>Renal cell carcinoma, gastrointestinal stromal tumors</td>
</tr>
<tr>
<td>BAY 43-9006</td>
<td>ONX/Bayer</td>
<td>Raf, VEGFR</td>
<td>Small molecule</td>
<td>Phase III</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>PTK787</td>
<td>Novartis</td>
<td>VEGFR</td>
<td>Small molecule</td>
<td>Phase III</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>ZD6474</td>
<td>AstraZeneca</td>
<td>VEGFR</td>
<td>Small molecule</td>
<td>Phase II</td>
<td>Solid tumours</td>
</tr>
<tr>
<td>RAD001</td>
<td>Novartis</td>
<td>mTOR</td>
<td>Small molecule</td>
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EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; PKC, protein kinase C; SAP, stress-activated protein; VEGFR, vascular endothelial growth factor receptor.
Figure 1 | Actual and projected sales for currently marketed oncology protein kinase inhibitors.  *Projected sales.
Gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; Genentech/OSI Pharmaceuticals/Roche) are tyrosine kinase inhibitors that target ATP binding to EGFR. Gefitinib was approved in the United States in 2002 as a monotherapy for the third-line treatment of patients with advanced non-small-cell lung cancer (NSCLC).

The effectiveness of gefitinib is based on objective response rates; there are no controlled trials demonstrating an improvement in disease-related symptoms or increased survival.

About 10% of patients have rapid and strong positive responses to gefitinib. It seems that these patients have heterozygous gain-of-function mutations within the tyrosine-kinase domain of EGFR. Screening for such mutations in lung cancers could identify those patients who are likely to respond to gefitinib.
Even though gefitinib was first to the market, its approval without survival data leave it vulnerable to competition from erlotinib, which is also being developed for NSCLC and is currently under a priority review with the FDA; a decision is expected by January 2005. In a pivotal Phase III clinical trial, erlotinib demonstrated a 42% improvement in median survival, and improved one-year survival of 45%. These results make erlotinib the first and only targeted therapy to demonstrate an improvement in survival for NSCLC patients. [Patients responsive to erlotinib were also noted to have EGFR-activating mutations.]
Comparison of PFS by mutation status within treatment arms

Probability of PFS

- Gefitinib EGFR M+ (n=132)
- Gefitinib EGFR M- (n=91)
- Carboplatin / paclitaxel EGFR M+ (n=129)
- Carboplatin / paclitaxel EGFR M- (n=85)

Gefitinib, HR=0.19,
95% CI 0.13, 0.26, p<0.0001
No. events M+ = 97 (73.5%)
No. events M- = 88 (96.7%)

Carboplatin / paclitaxel, HR=0.78,
95% CI 0.57, 1.06, p=0.1103
No. events M+ = 111 (86.0%)
No. events M- = 70 (82.4%)

Hazard ratio <1 implies a lower risk of progression in the M+ group than in the M- group
M+, mutation positive;  M-, mutation negative
Timeline | The discovery and development of gefitinib

- Gefitinib has a benign safety profile because it is used at its optimal biological dose, unlike chemotherapy, which is used at its maximum tolerated dose.
- Japan is the first country to approve gefitinib for use in patients with inoperable or recurrent NSCLC.
- Gefitinib is the first EGFR-TKI to reach the clinic.
- Food and Drug Administration approves the first EGFR-TKI.
- Understanding more about the molecular mechanism of gefitinib.


- EGFR proposed as an anticancer target by Mendelevich.
- First clinical trial of anti-EGFR mAb confirms mode of action.
- First clinical trials of gefitinib confirm mode of action.
- Phase II trials demonstrate clinically meaningful activity of gefitinib 250 mg/day in non-small-cell lung cancer (NSCLC).
- Gefitinib investigated in combination with chemotherapy in NSCLC.
- Clinical experience with EGFR-TKIs grows.
- Phase III trials demonstrate no added benefit of adding gefitinib to standard first-line chemotherapy in NSCLC.
- Gefitinib is now approved in >30 countries for use in pretreated NSCLC; more than 100,000 patients have been treated worldwide.
- 2004 and beyond: building knowledge to optimize the use of gefitinib in cancer.
THANK YOU for your attention