Targeting the ErbB Family of Receptors

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May 27, 2015

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NCI Cancer 2015 USA

New Cases and Deaths

Cancer Type	Estimated New Cases	Estimated Deaths
Bladder	74,000	16,000
Breast (Female – Male)	231,840 – 2,350	40,290 – 440
Colon and Rectal (Combined)	132,700	49,700
Endometrial	54,870	10,170
Kidney (Renal Cell and Renal Pelvis) Cancer	61,560	14,080
Leukemia (All Types)	54,270	24,450
Lung (Including Bronchus)	221,200	158,040
Melanoma	73,870	9,940
Non-Hodgkin Lymphoma	71,850	19,790
Pancreatic	48,960	40,560
Prostate	220,800	27,540
Thyroid	62,450	1,950

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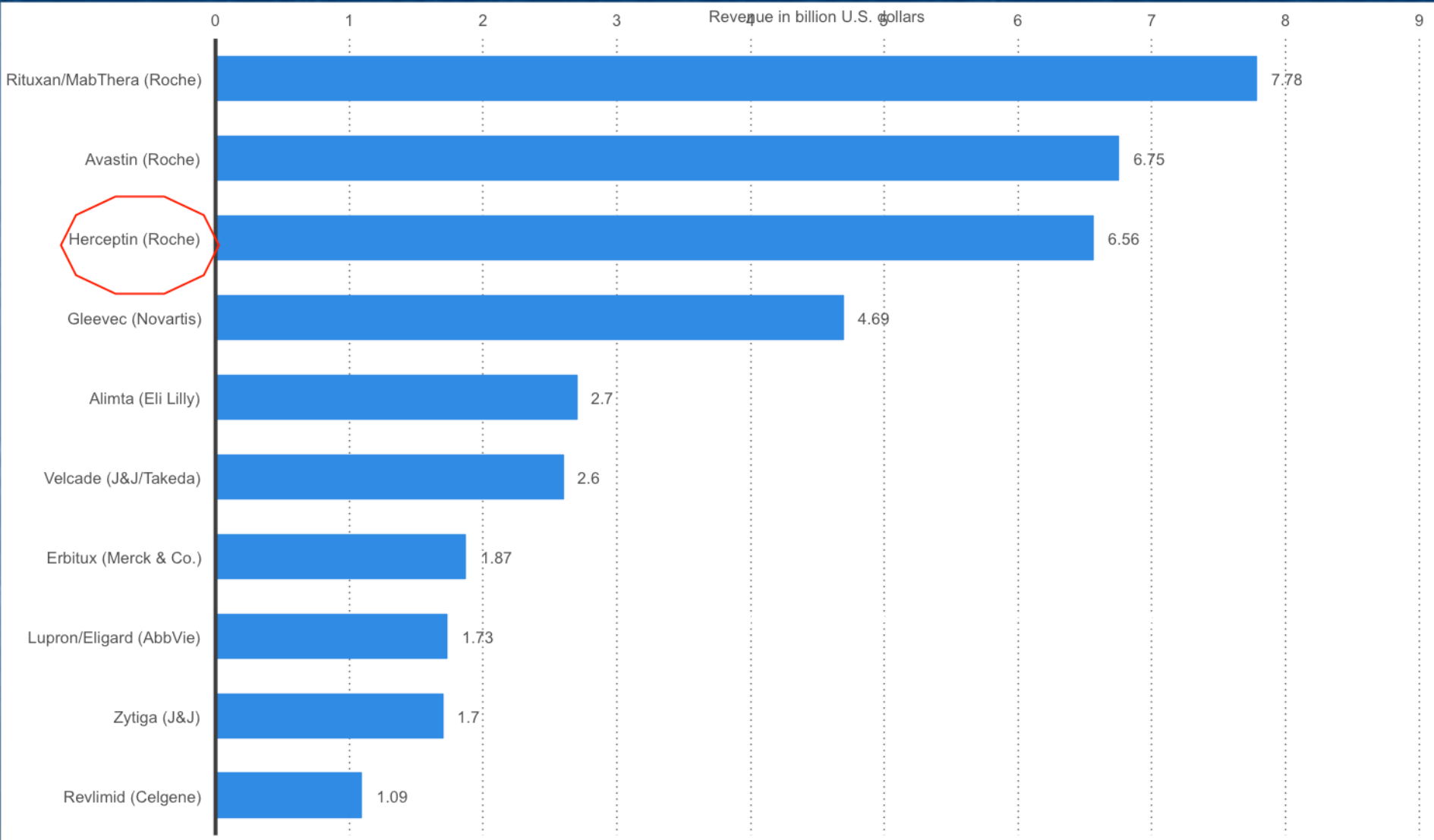
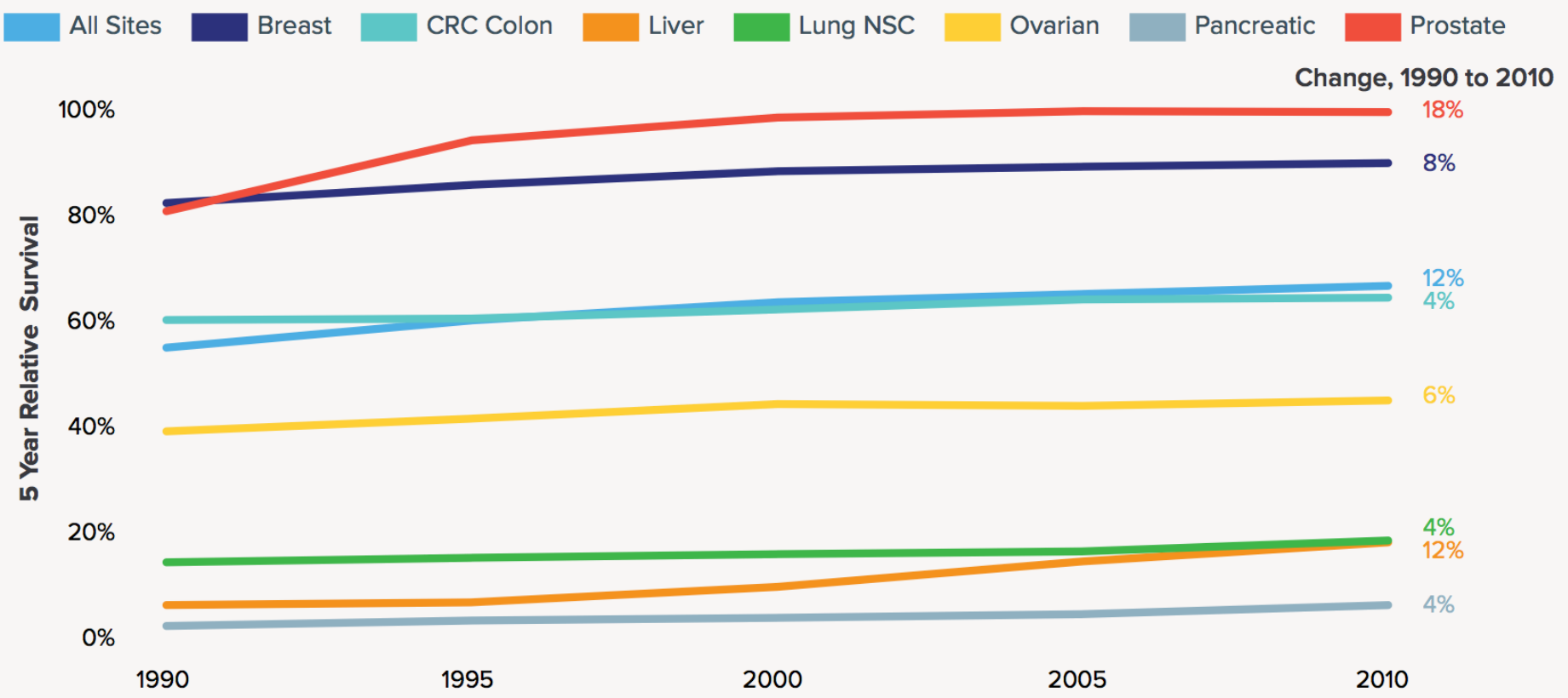
Top cancer drugs based on revenue worldwide 2013



Further information regarding this statistic can be found on page 8.

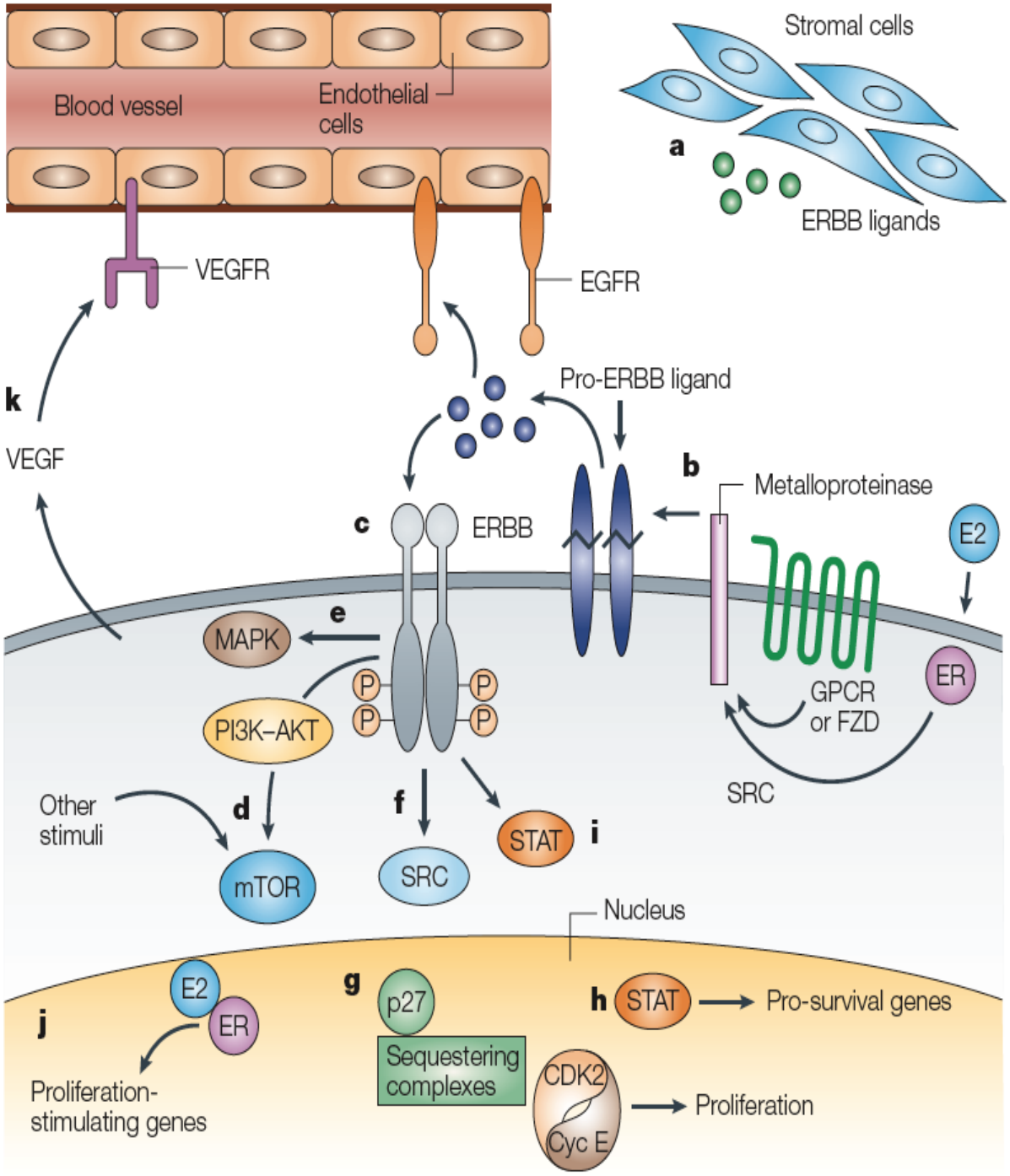
Changes in 5-year Cancer Survival Rates over 20 Years



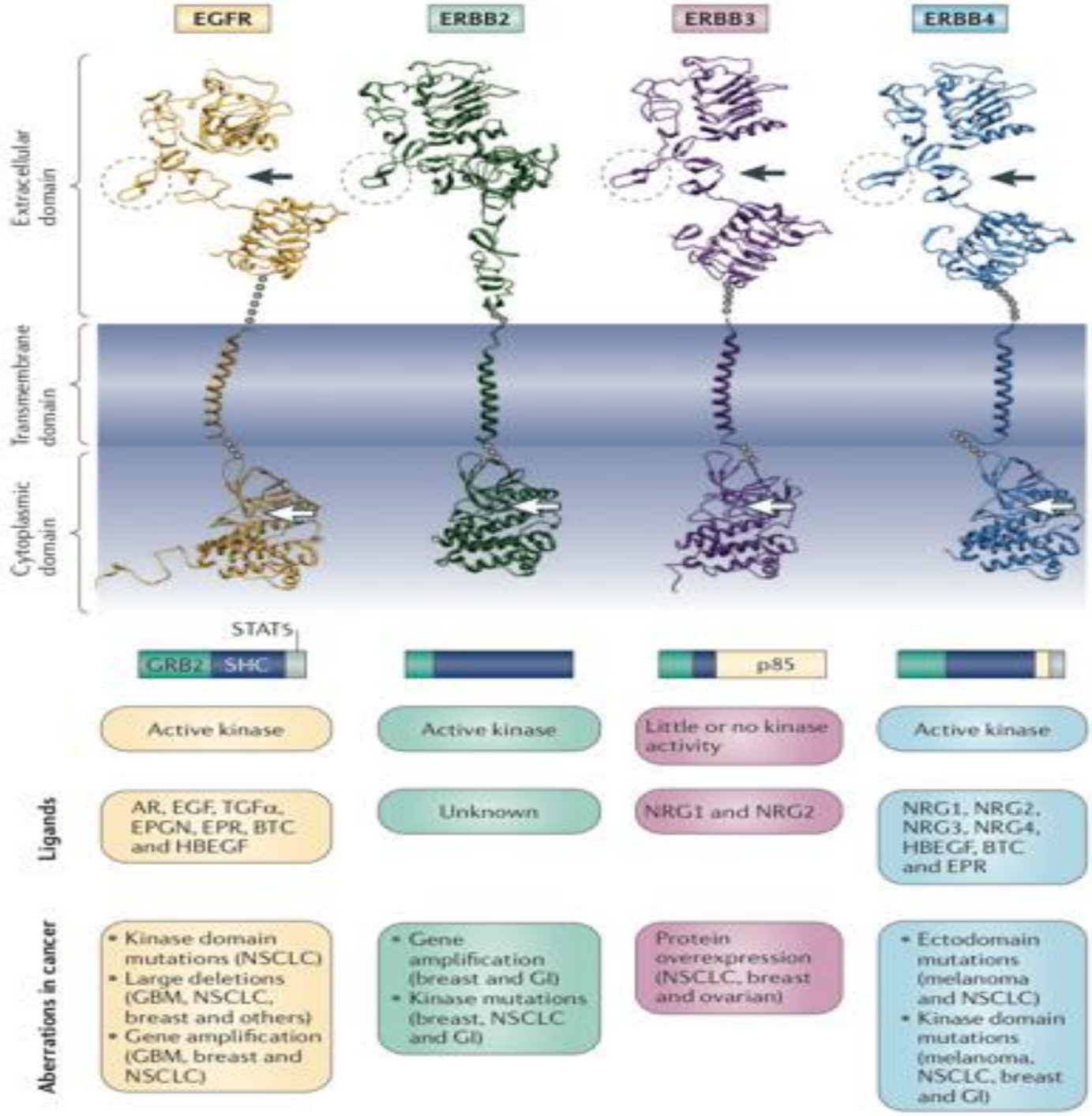
Hopefully, recent progress has dramatically improved survival in the last 5 years?

Paracrine ErbB ligands (green circles) are released from stromal cells. (b) Autocrine ligand (blue circles) production results from the activation of GPCRs, Frizzled (FZD) or estrogen receptor (ER), which causes the cleavage and release of pro-EGF-related ligands. (c) Active ErbB receptors recruit proteins to specific phosphorylated tyrosine residues. (d) The phosphatidylinositol 3-kinase (PI3K)–AKT pathway is stimulated through recruitment of the p85 adaptor subunit of PI3K to the receptor.

(Hynes and Lane, Nat Rev Cancer, 2005)



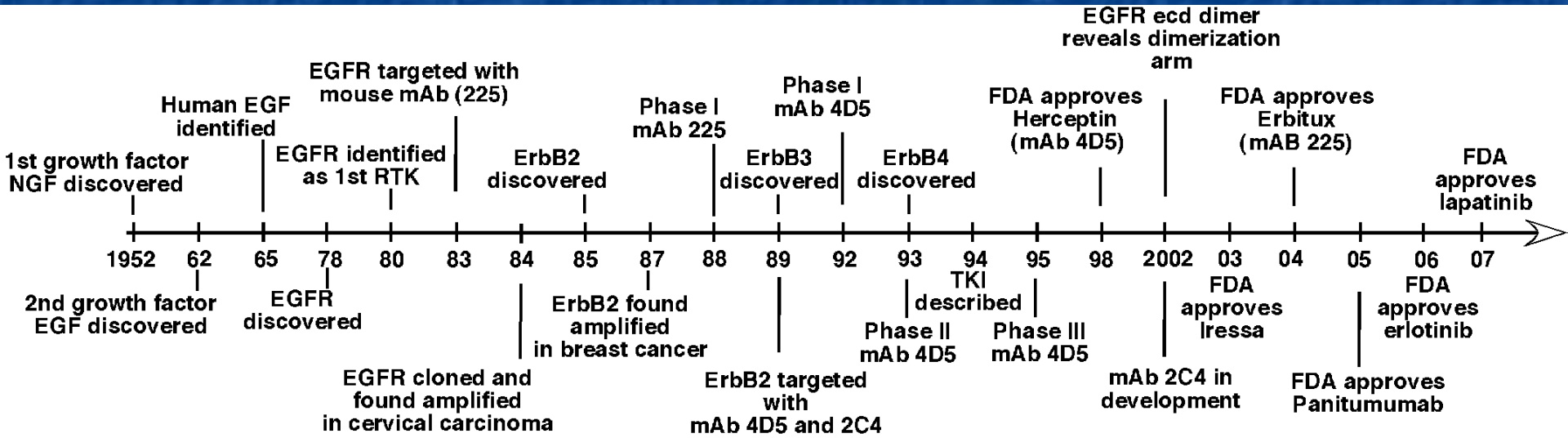
ErbB Family of Receptor Tyrosine Kinases



Yarden, Y. and G. Pines (2012). "The ERBB network: at last, cancer therapy meets systems biology." Nat Rev Cancer 12(8): 553-563.

ErbB Receptors and Cancer

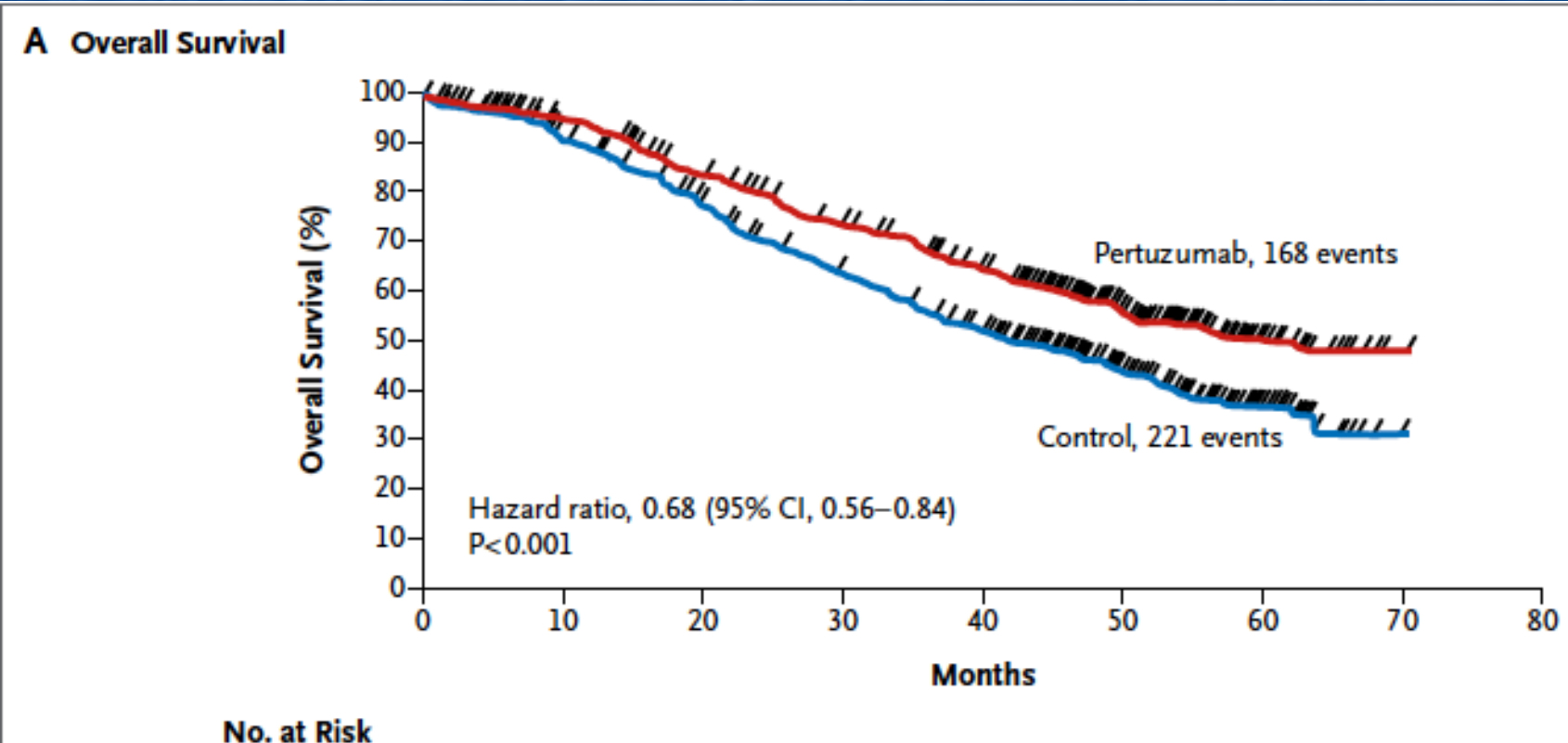
- **Misregulation of ErbBs is strongly correlated with a variety of cancers: breast, head and neck, non-small cell lung cancer, etc. :**
  - **25-30% breast cancer patients overexpress ErbB2.**
  - **20-45% breast cancer patients overexpress EGFR.**
  - **Co-overexpression of EGFR and ErbB2 are associated with aggressive malignancy.**



ErbB Receptors and Cancer

Swain et al. New England J Med, 2015

Addition of Pertuzamab to treat metastatic breast cancer



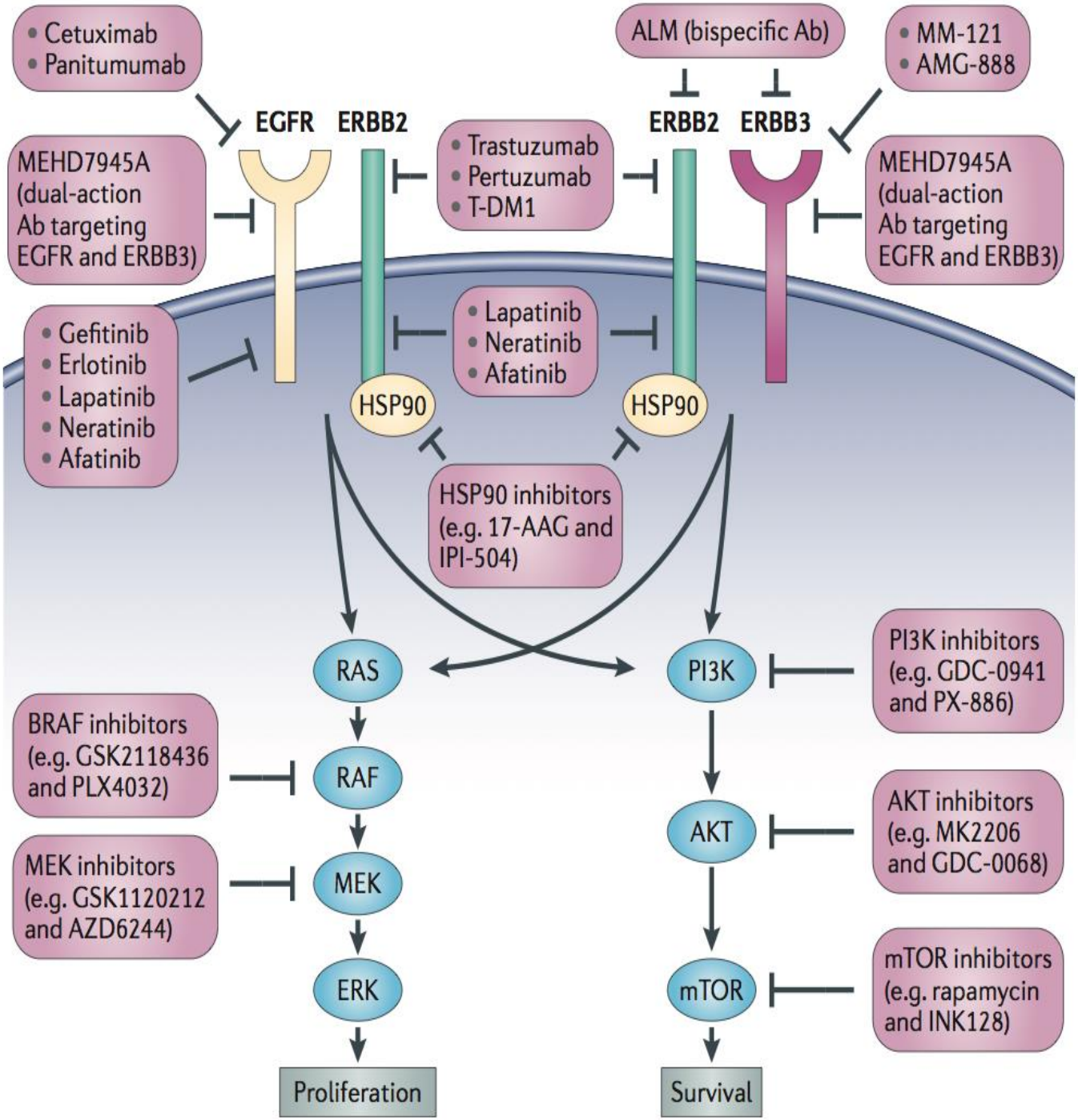
ErbB Receptors and Cancer

Swain et al. New England J Med, 2015

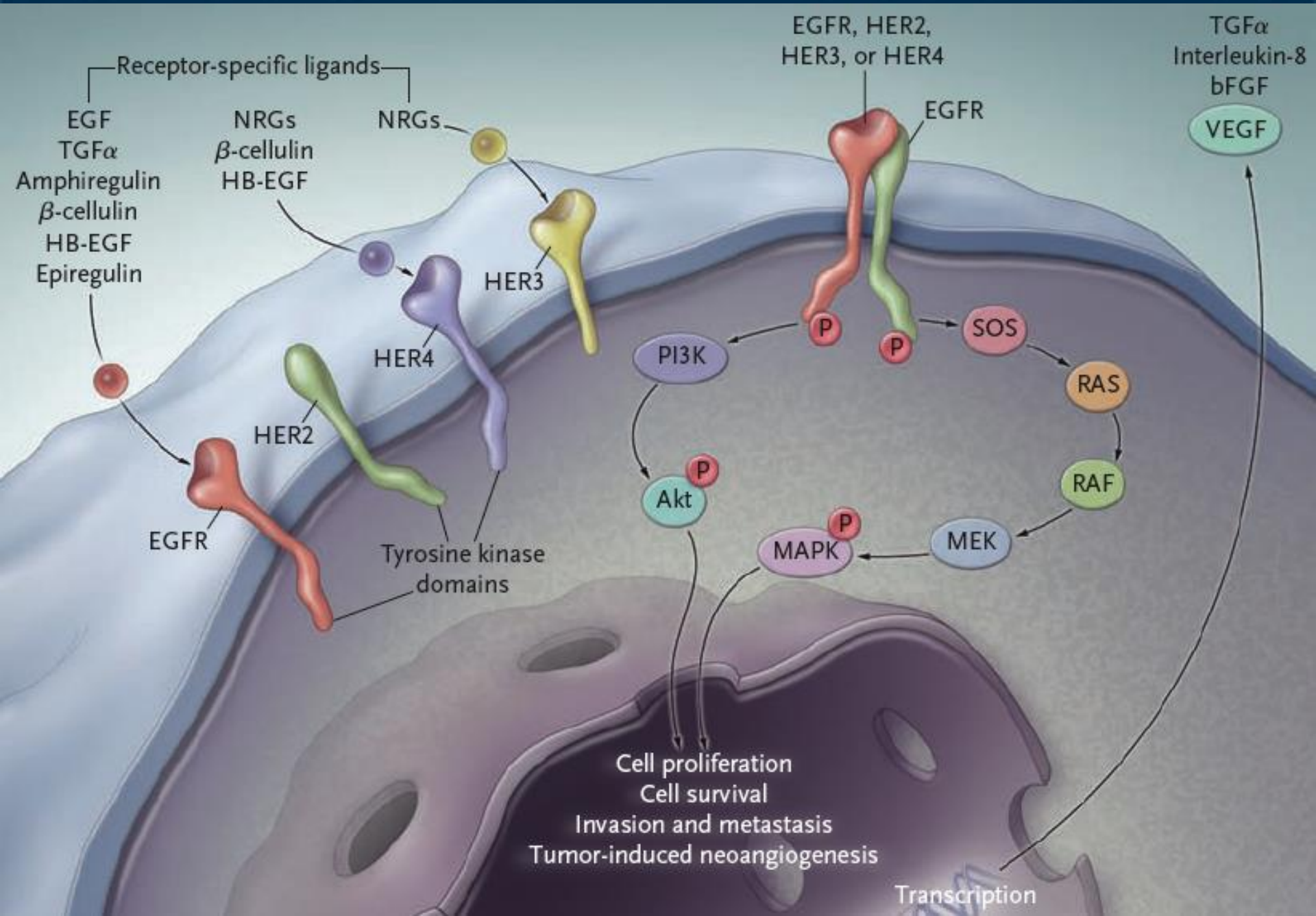
Addition of Pertuzamab to treat metastatic breast cancer

Adverse Event	Control Group (N=261)	Pertuzumab Group (N=306)
Most common events of any grade — no. of patients (%)†		
Alopecia	6 (2.3)	5 (1.6)
Diarrhea‡	37 (14.2)	86 (28.1)
Neutropenia	13 (5.0)	10 (3.3)
Nausea	30 (11.5)	39 (12.7)
Fatigue	25 (9.6)	41 (13.4)
Rash‡	21 (8.0)	56 (18.3)
Asthenia	23 (8.8)	41 (13.4)
Decreased appetite	14 (5.4)	22 (7.2)
Peripheral edema	32 (12.3)	28 (9.2)
Vomiting	17 (6.5)	30 (9.8)
Myalgia	19 (7.3)	25 (8.2)
Mucosal inflammation	4 (1.5)	11 (3.6)
Headache	32 (12.3)	52 (17.0)
Constipation	18 (6.9)	17 (5.6)
Upper respiratory tract infection‡	32 (12.3)	56 (18.3)
Pruritus‡	15 (5.7)	42 (13.7)
Febrile neutropenia	0	0
Dry skin	10 (3.8)	10 (3.3)
Muscle spasm‡	6 (2.3)	24 (7.8)

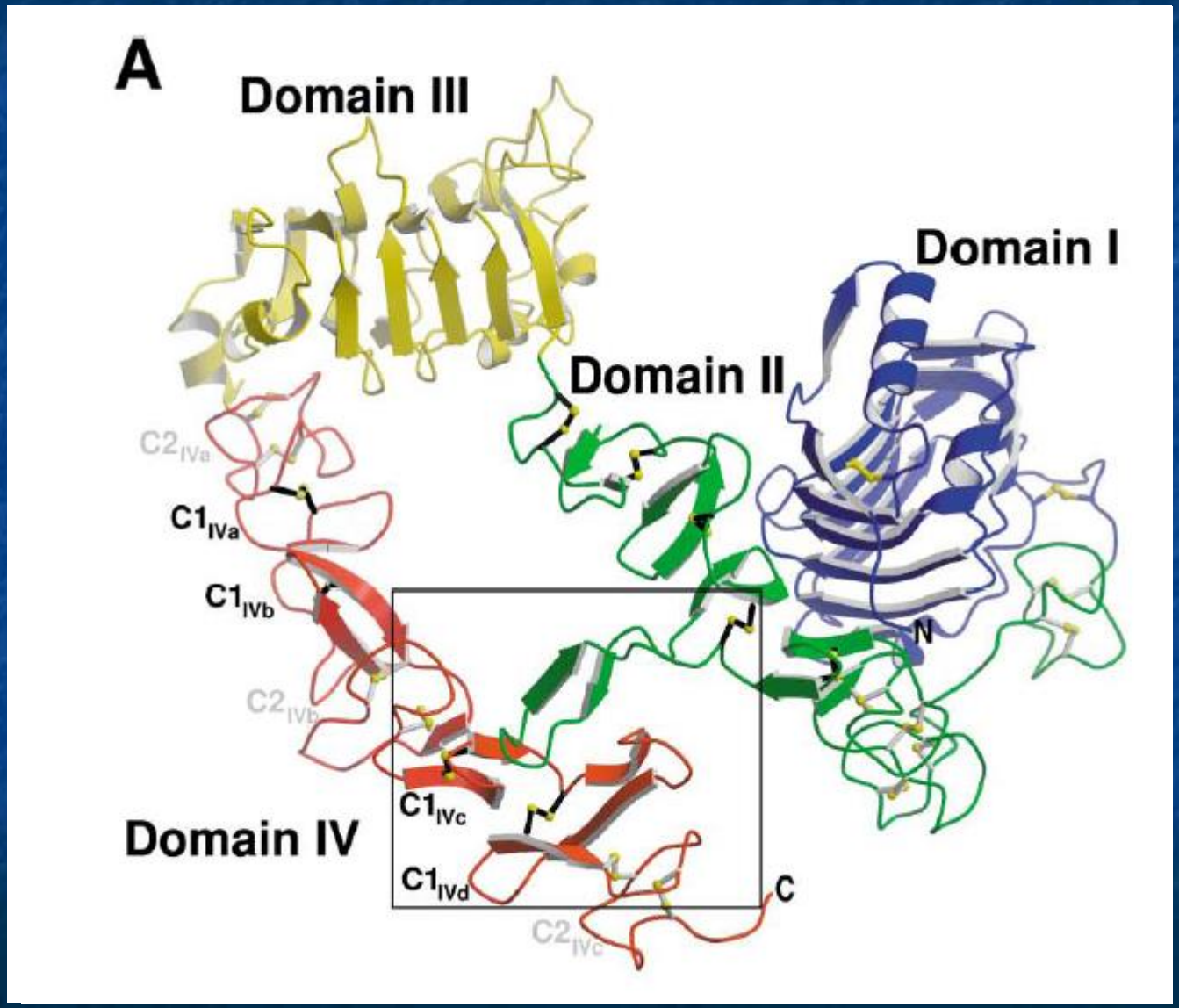
Therapeutics targeting ErbB receptors and their downstream activation partners



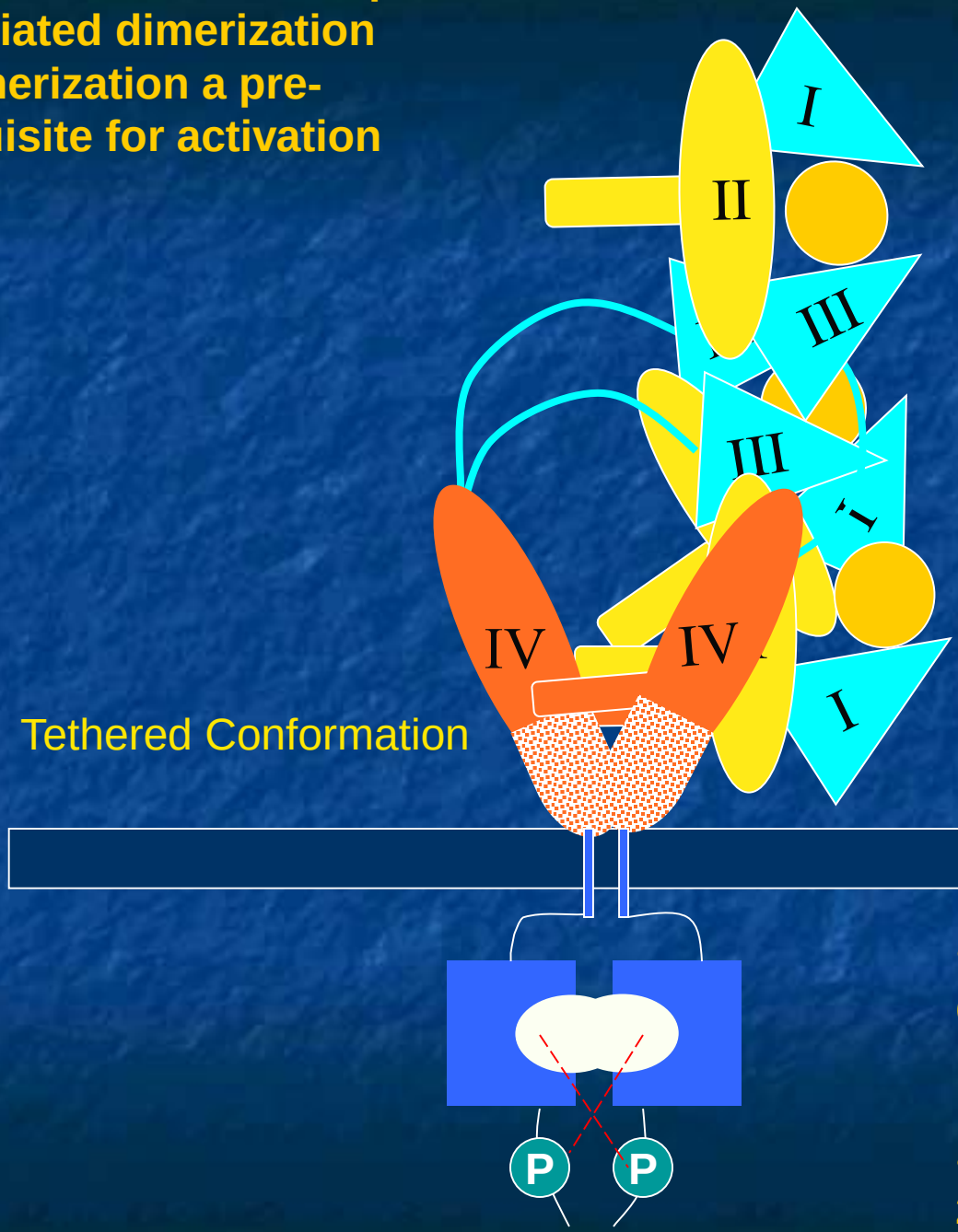
Signal Transduction Pathways Activated by ErbB Receptors



Tethered Conformation of EGFR Extracellular Domain



•Ligand-induced, receptor-mediated dimerization
•Dimerization a pre-requisite for activation



Extended Conformation

Tethered Conformation

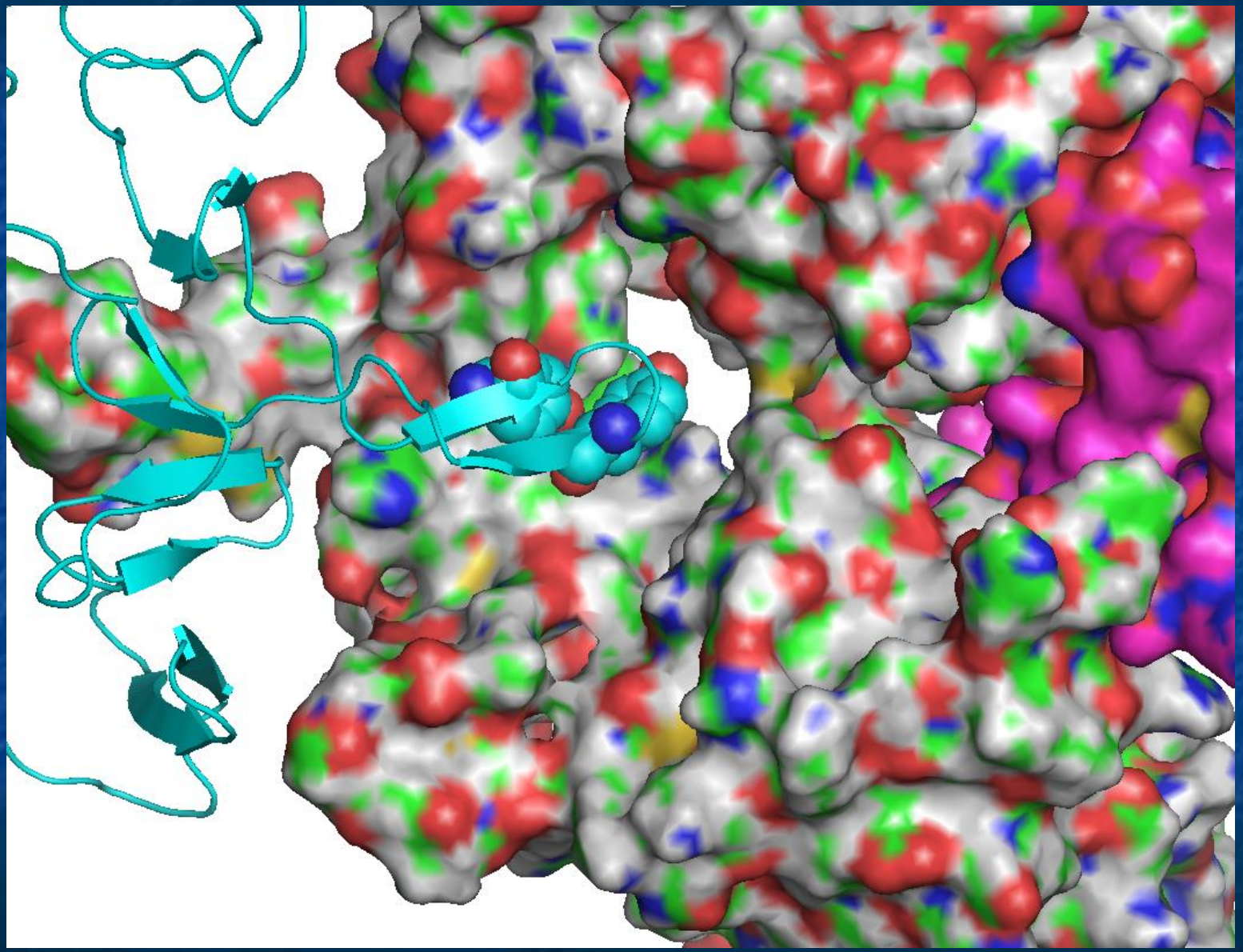
•Mutations to the domain II dimerization arm abolish EGFR homodimerization.

•Dawson, et al. Mol. Cell. Biol. 25, 2005

Current Therapeutics

- Anti-EGFR and Anti-ErbB2 drugs are among the most advanced breast cancer therapeutics.
- mAb-based (monoclonal antibody):
  - Trastuzumab (Herceptin), pertuzumab, cetuximab (Erbitux)
- TKI (tyrosine kinase inhibitors):
  - Gefitinib, erlotinib, lapatinib

EGFR Homodimerization Interface



Y246/Y251 "Hotspot"

Garrett Cell **2002**, *110*, 763-773 (PDB:1MOX)

DEVELOPMENT OF CONSENSUS vHTS PROTOCOL
ROB YANG – PH.D. THESIS

AUTODOCK 4.0 WAS USED TO DOCK COMPOUNDS INTO A DOCKING BOX CENTERED ON ACTIVE SITE.

DOCKING POSES WERE SCORED BY EIGHT INDEPENDENT SCORING FUNCTION AND THE CONSENSUS USED.

The Larmackian genetic algorithm with Solis and Wets local search was used to generate 100 docking poses per compound. All poses were subsequently scored using: HP, HM, HS (implemented in X-score 1.2.189), D-score, PMF, G-score, Chem-score (implemented in Sybyl 7.3 CSCORE module), and Dfire90. A consensus score for each pose was calculated by summing the rankings given by each of the 8 scoring functions.

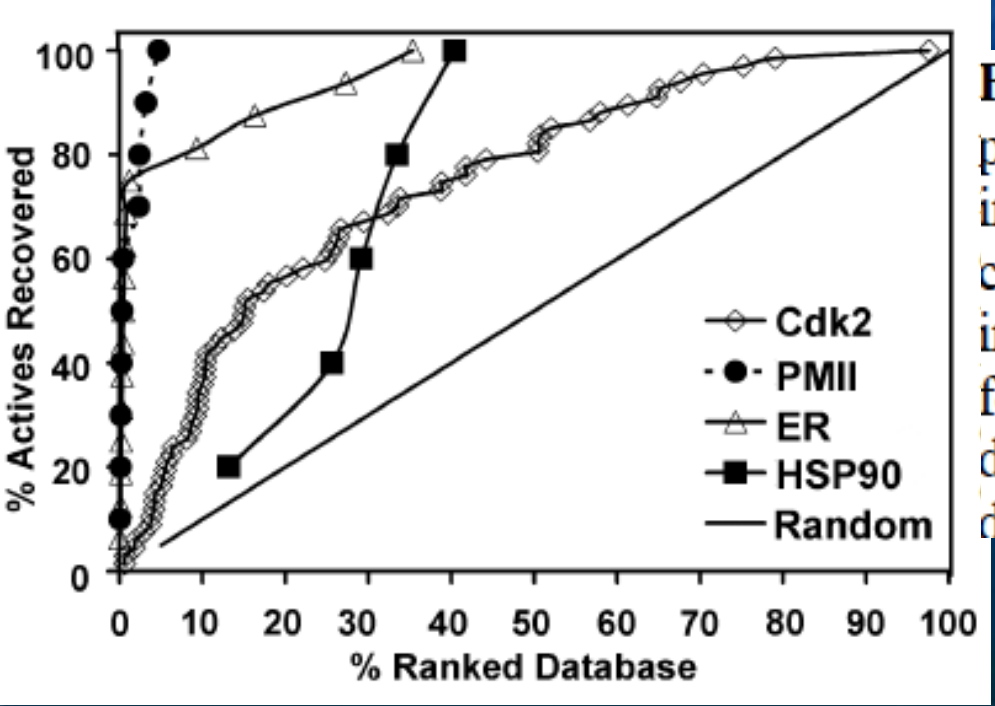


Figure 2.1. Evaluation of the vHTS protocol against four testing cases shown in an enrichment curve analysis. In each case, multiple known ligands were mixed in with ~2000 random compounds to form the screening library. The black diagonal line represents the random distribution of active molecules.

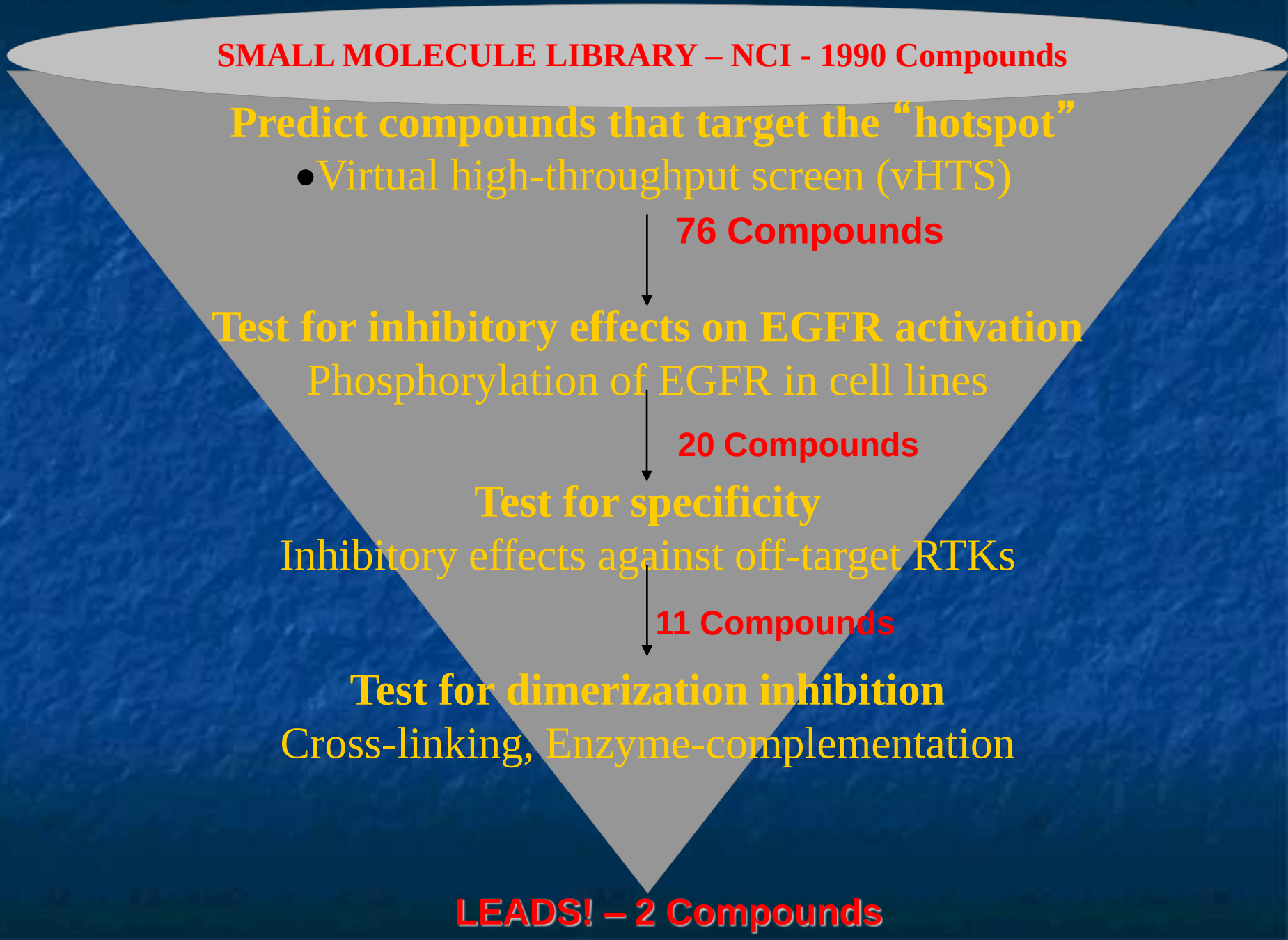
VALIDATION OF vHTS PROTOCOL WITH VARIETY OF TARGETS
ROB YANG – PH.D. THESIS

Table 2.1: Efficacy and robustness of the vHTS protocol.

Targets	$Coverage_{1\%}$[1]	$Coverage_{15\%}$	$Coverage_{30\%}$	$Coverage_{50\%}$	Best[2]
Cdk2	3%	49%	67%	79%	0.05%
PMII	60%	100%	100%	100%	0.65%
ER	69%	81%	94%	100%	0.05%
HSP90	0%	20%	60%	100%	13.21%
Avg	33%	63%	80%	100%	3.5%

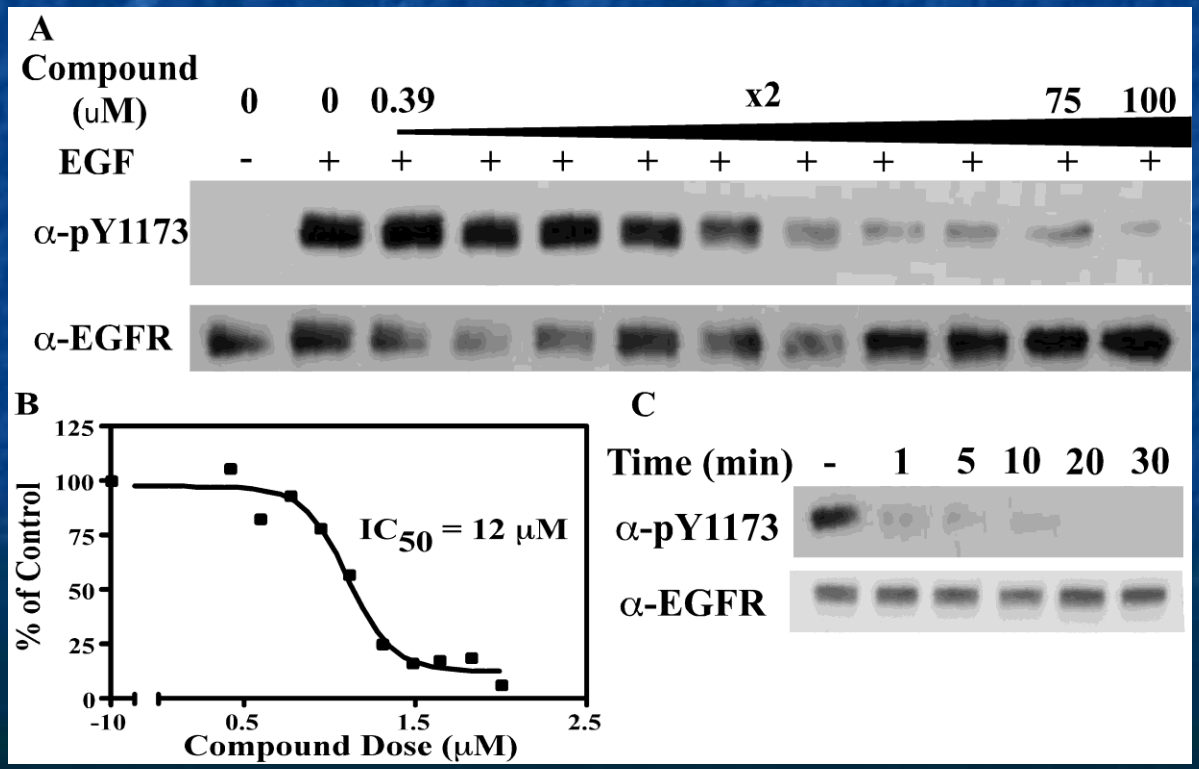
[1] $Coverage_{fraction}$ = Number of known actives recovered within the given fraction of the database / Total number of actives present in the database x 100%

[2] Best = ranking of the best predicted active / database size x 100


SMALL MOLECULE LIBRARY – NCI - 1990 Compounds
Predict compounds that target the "hotspot"
•Virtual high-throughput screen (vHTS)
76 Compounds
Test for inhibitory effects on EGFR activation
Phosphorylation of EGFR in cell lines
20 Compounds
Test for specificity
Inhibitory effects against off-target RTKs
11 Compounds
Test for dimerization inhibition
Cross-linking, Enzyme-complementation
LEADS! – 2 Compounds

Inhibition of EGFR Activation Assay

- Screened the NCI-diversity database:
  - 1990 compounds that is a subset of 140,000 compounds.
- Tested top 80 compounds
- 20/80 inhibited EGFR activation

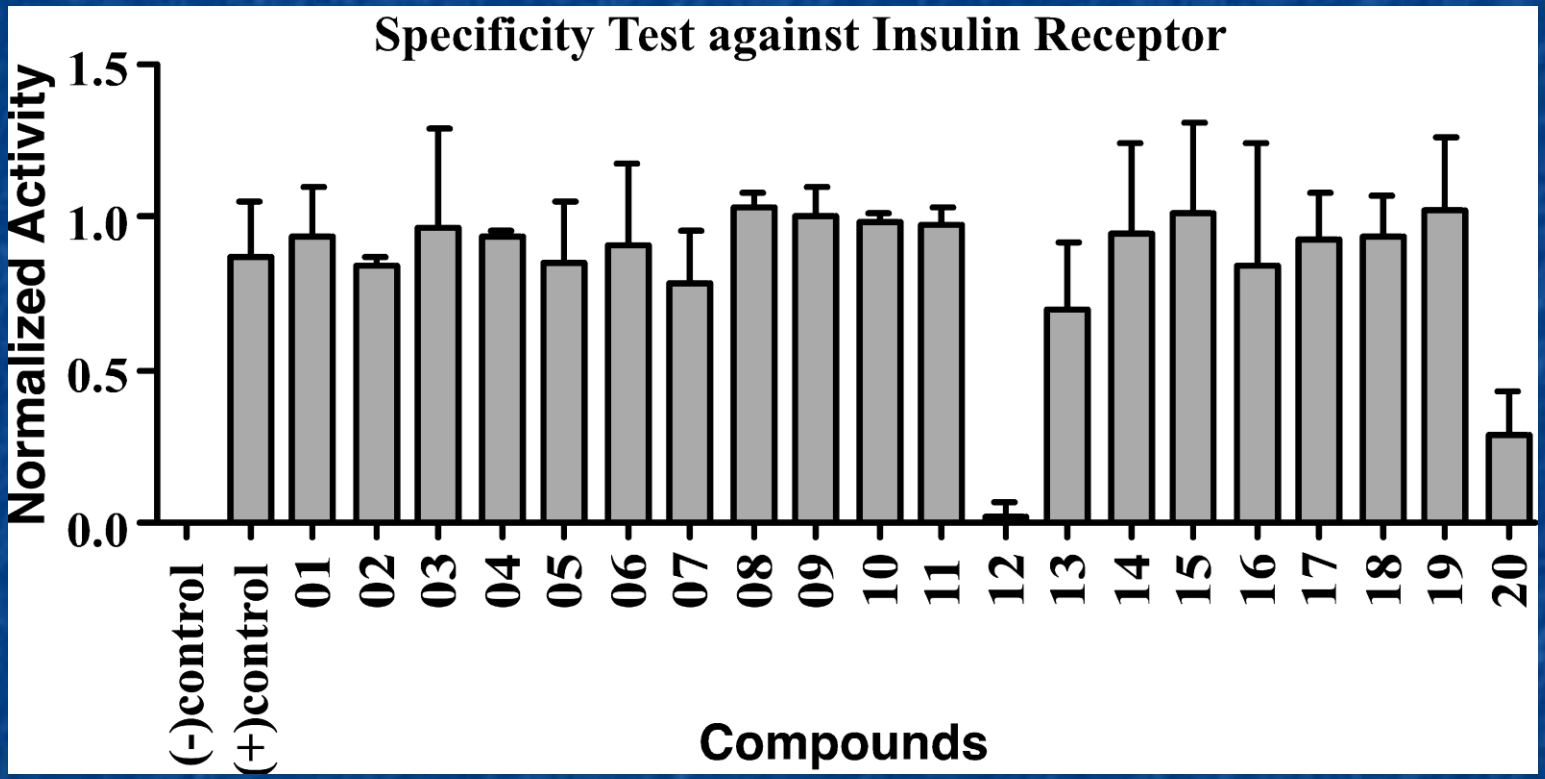


Specificity: Other RTKs

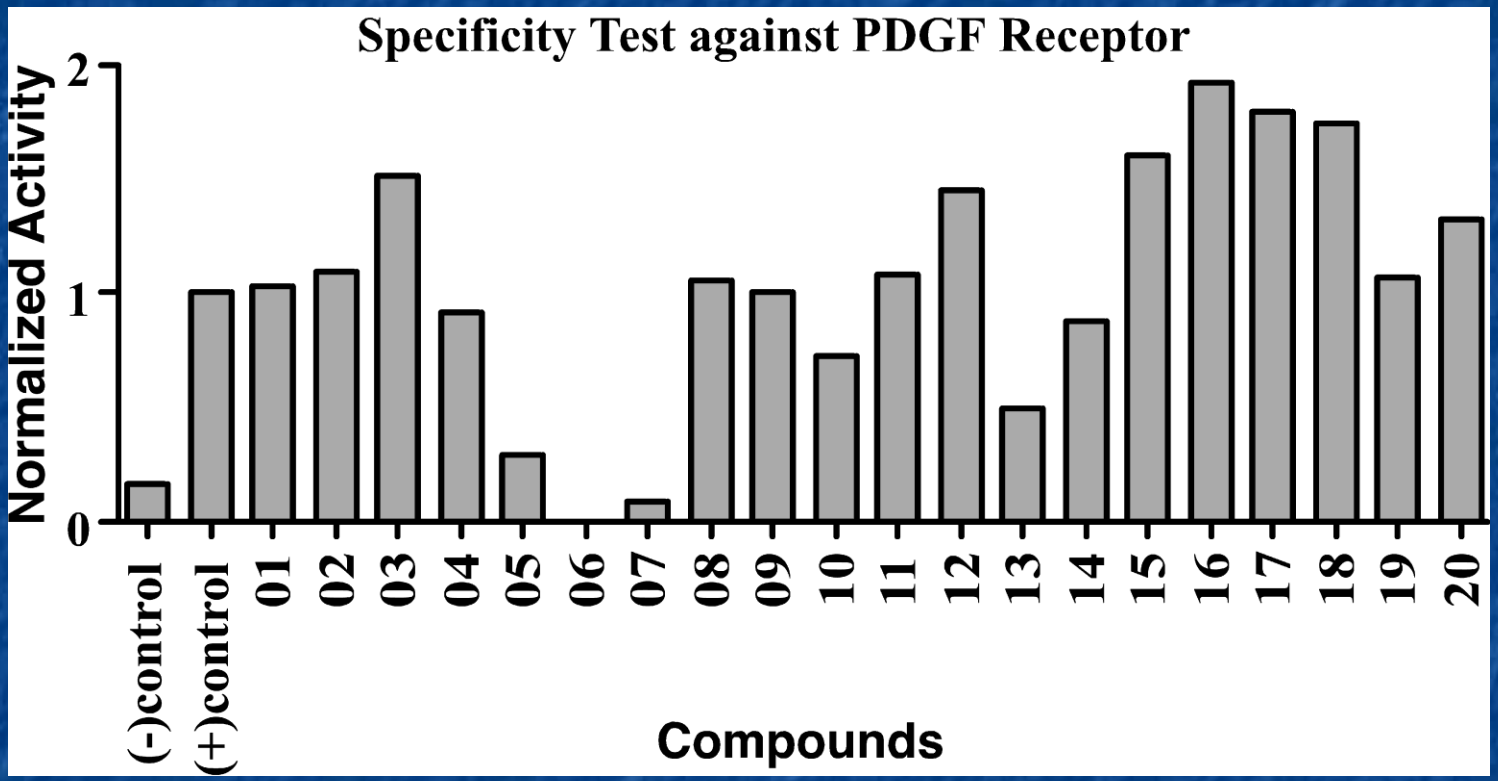
RTKs	Activating ligands	Cell line
Insulin Receptor (IRS-1)	Insulin	3T3-L1
Platelet-derived growth factor receptors (PDGFR)	PDGF	NIH-3T3

- Stimuation with activating ligands → quantify receptor phosphorylation using antibody pY20 → record compound effects.

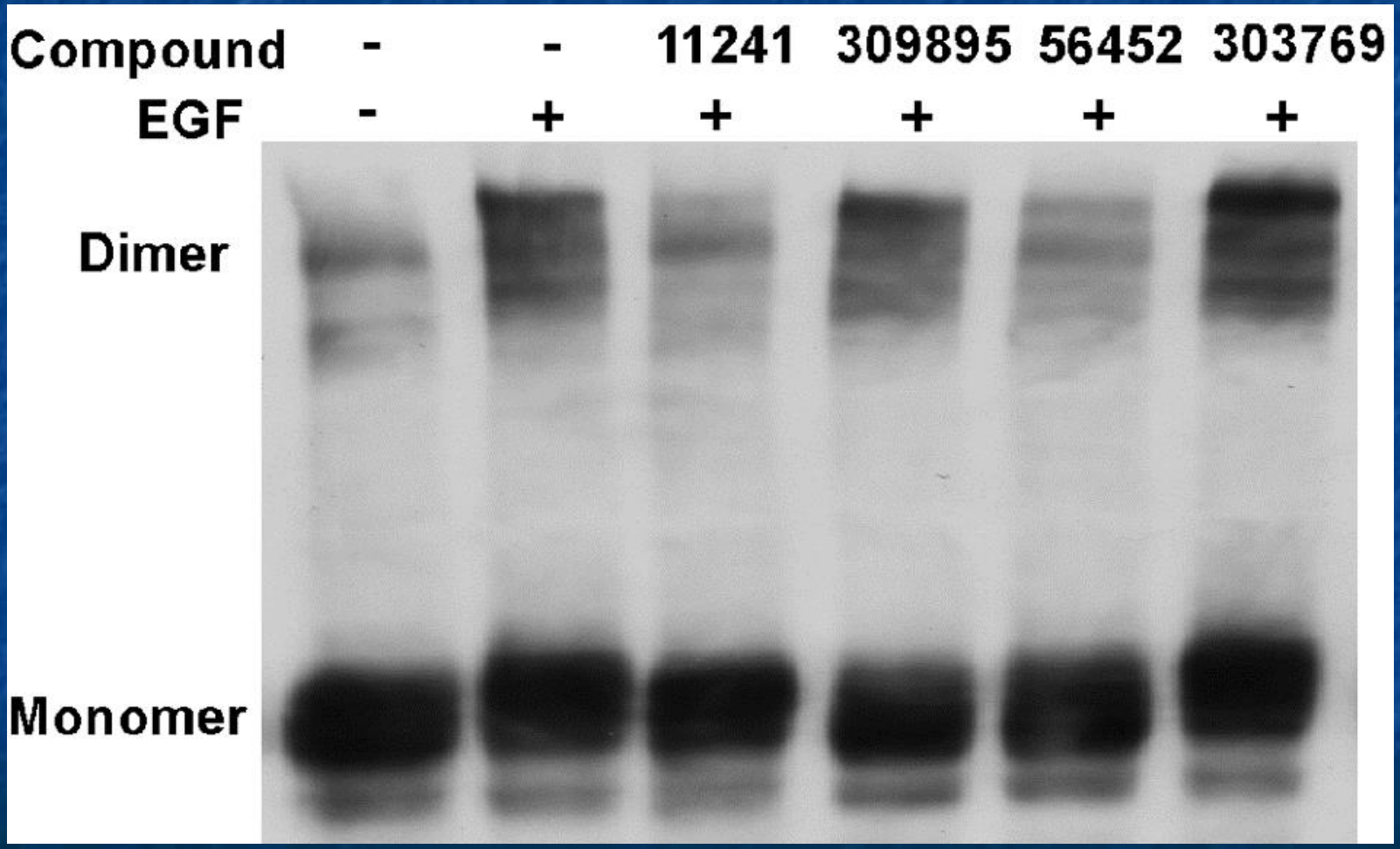
Two compounds inhibited insulin-receptor stimulation



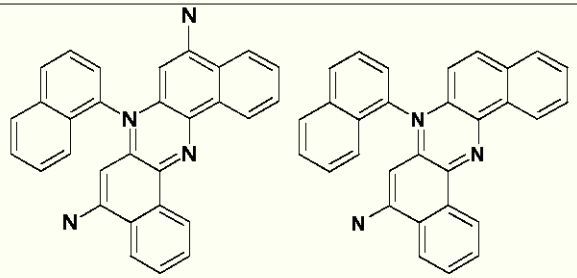
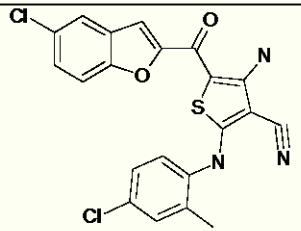
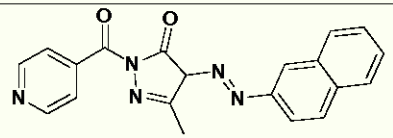
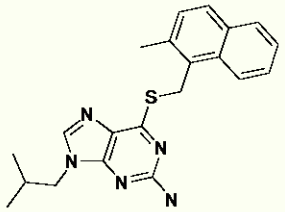
Four compounds inhibited PDGF-receptor stimulation



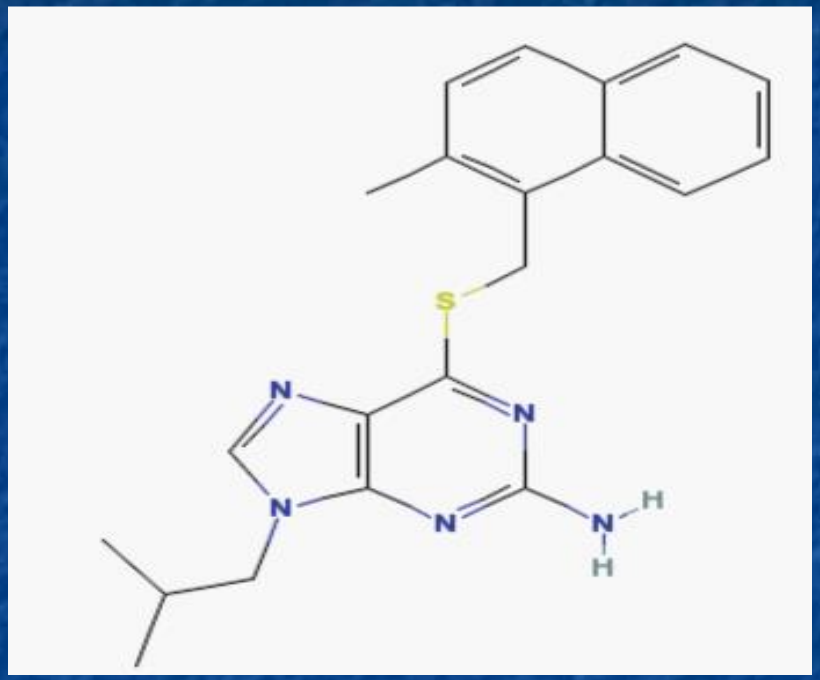
Inhibition of Dimer Cross-Linking

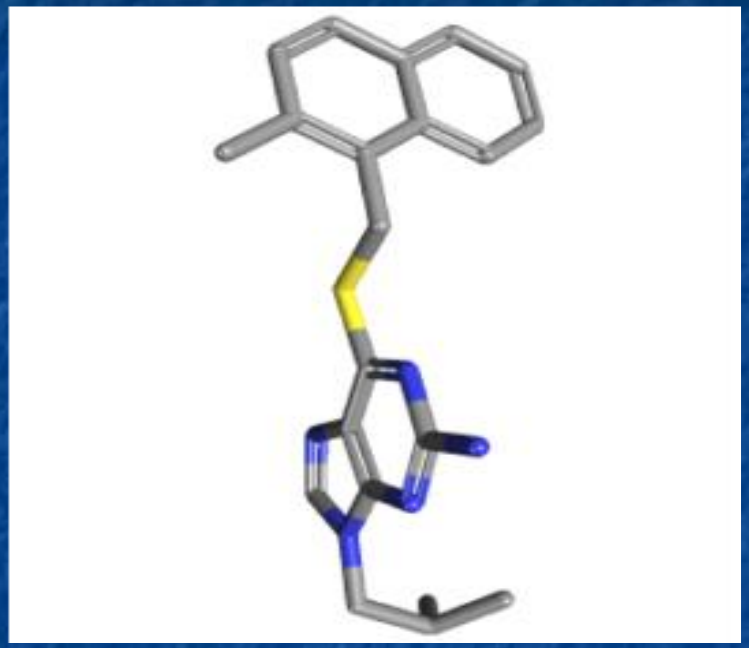


FOUR LEADS FROM NCI SCREENING

Inhibitor No.	IC_{50} (μ M)	Dimer inhibition	Chemical Structure
11241	12.8	++	
309895	24.4	+	
303769	3.97	+	
56452	0.39	+	

Best Lead from NCI Screening

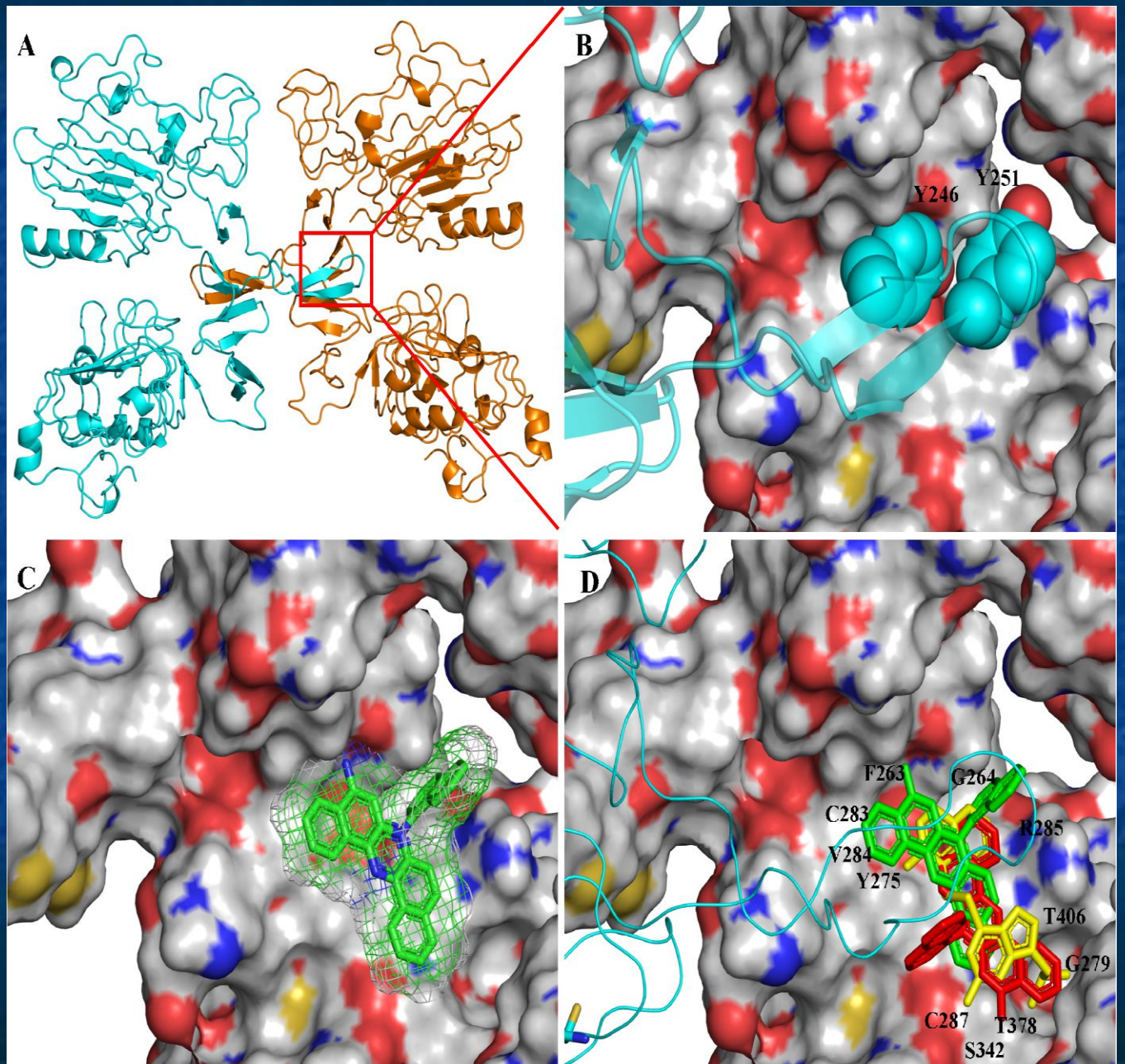




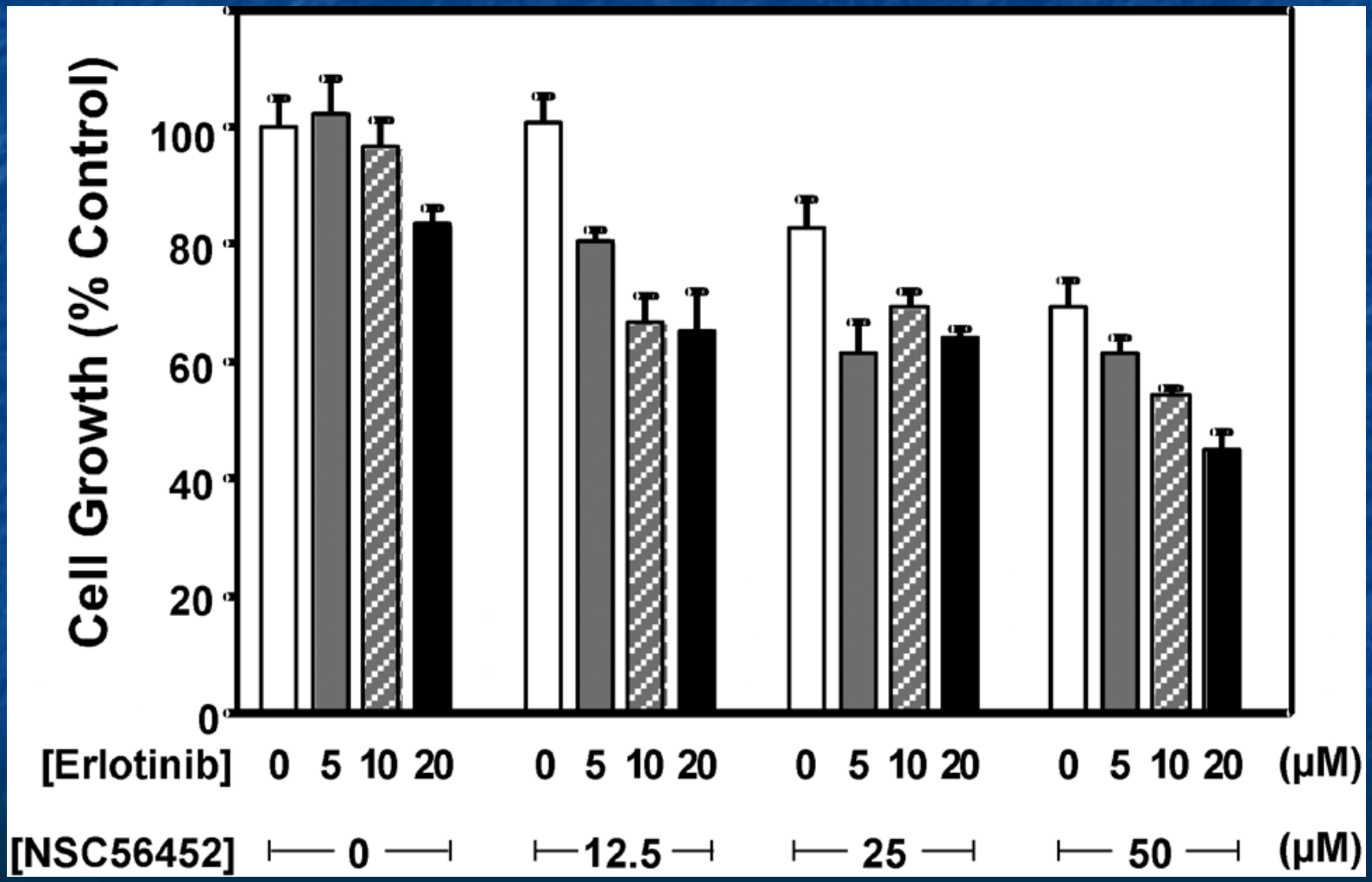
Initial lead NSCS56452 -
6-[(2-methylnaphthalen-1-yl)methylsulfanyl]-9-(2-methylpropyl)purin-2-amine

MW = 3.78, LogP = 4.9, H-bond donor =1, H-bond acceptor = 4, rotatable bonds = 5 Achiral!

Brief Summary



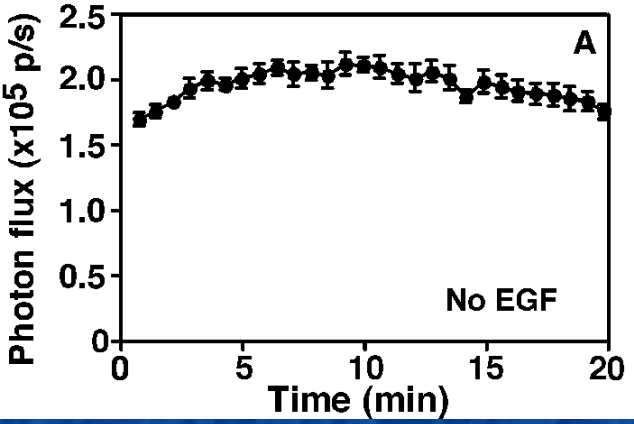
Apparent Synergy between Erlotinib and Dimerization Inhibition – Inhibition of HeLa Cell Growth

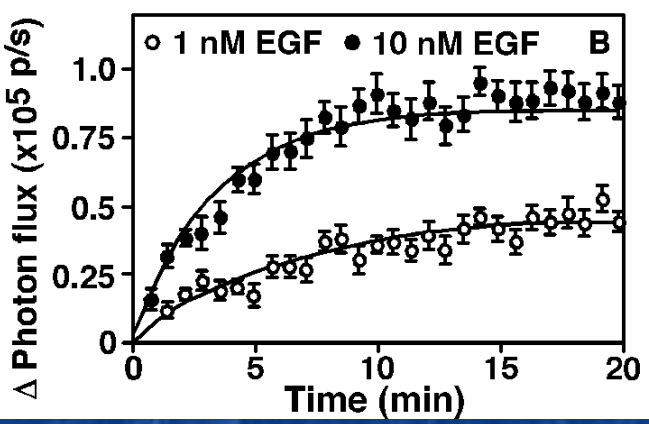


Split-Luciferase EGFR Dimerization Assay
Yang et al. JBC 284:7474-7482, 2009.

EGFR has tyrosine-kinase domain omitted; impact of compounds cannot be due to RTK inhibition

Figure 1





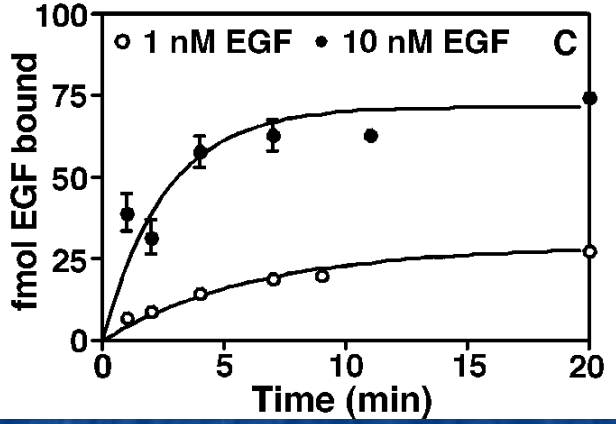
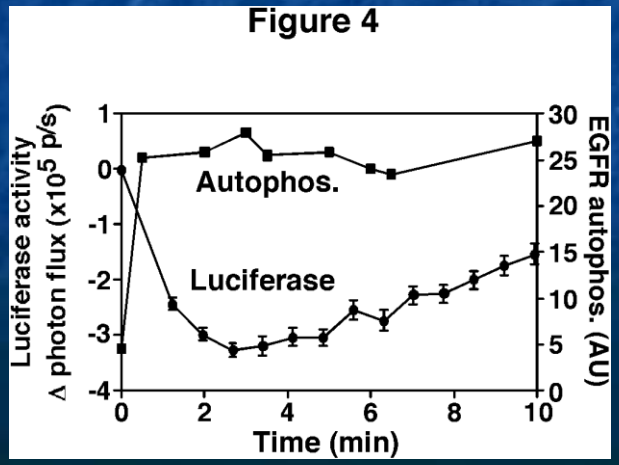
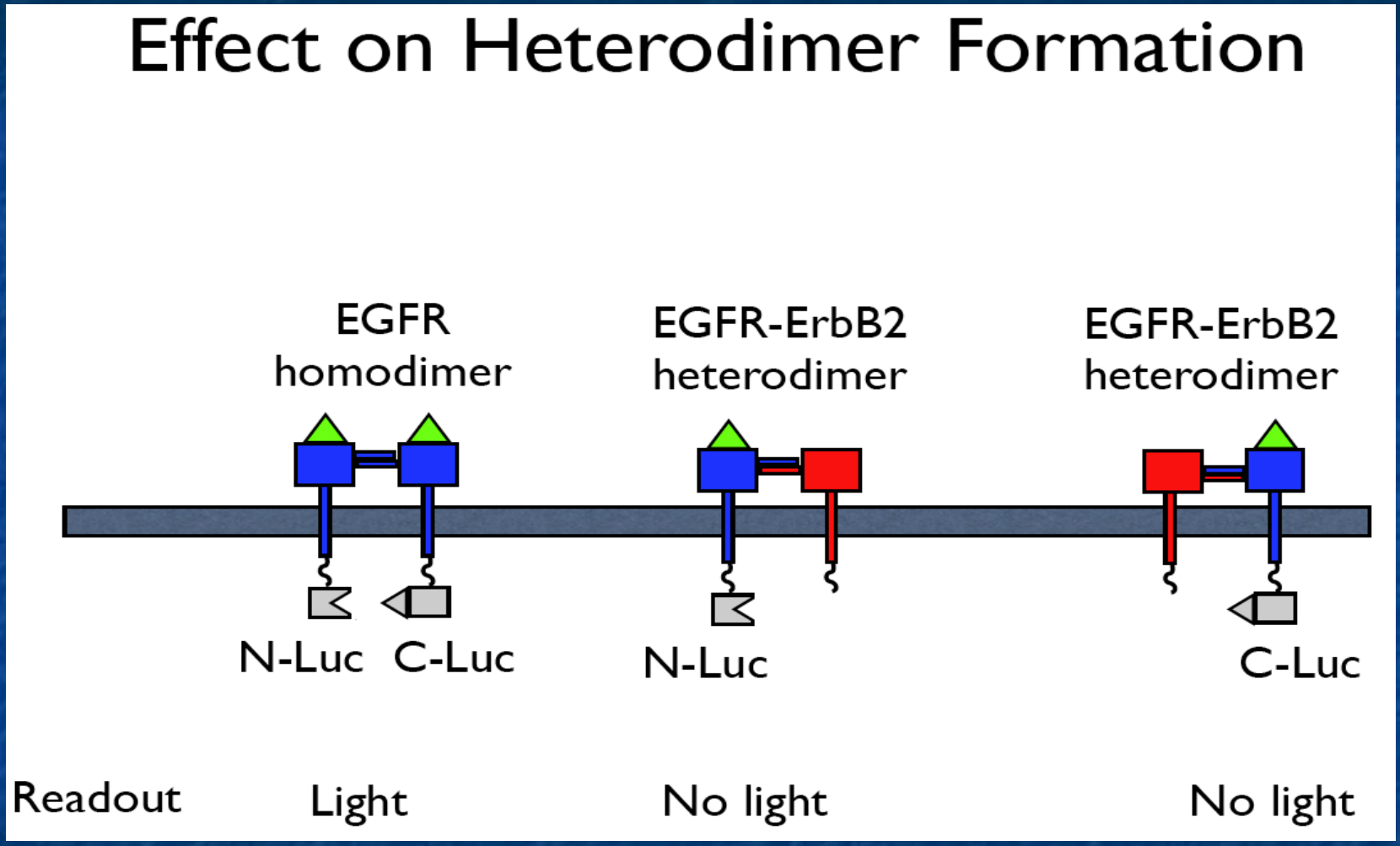


Figure 4

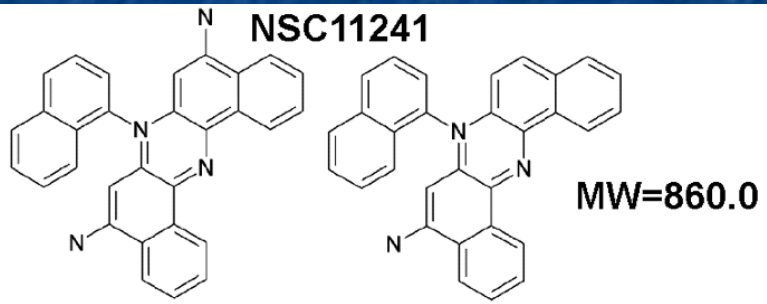


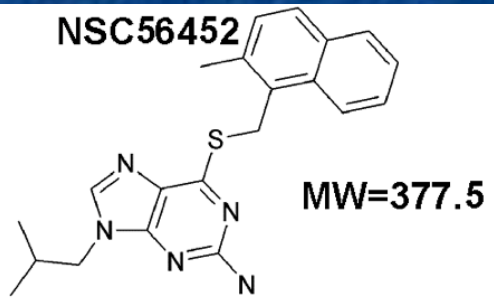
Characterization of Leads by EFGR Split-Luciferase Assay

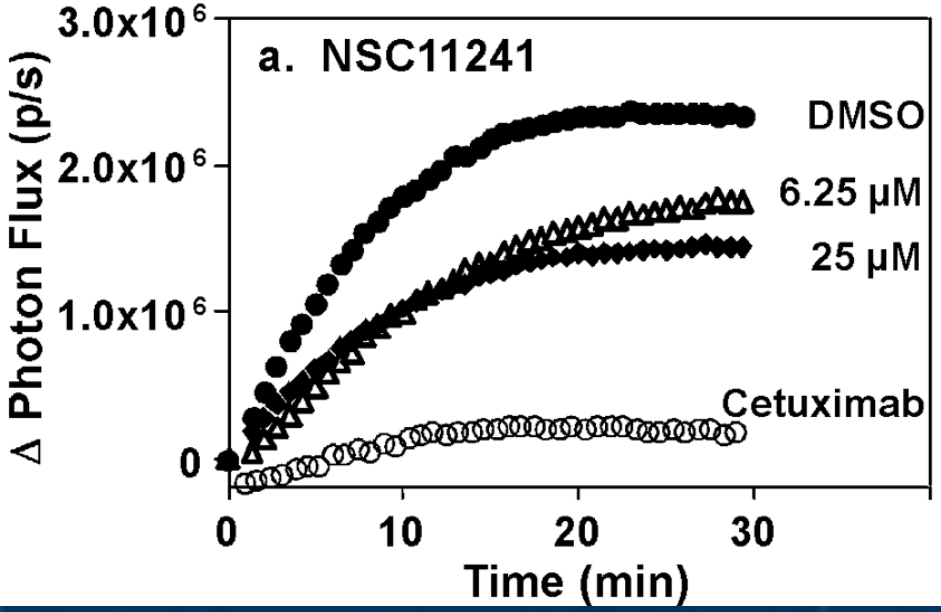


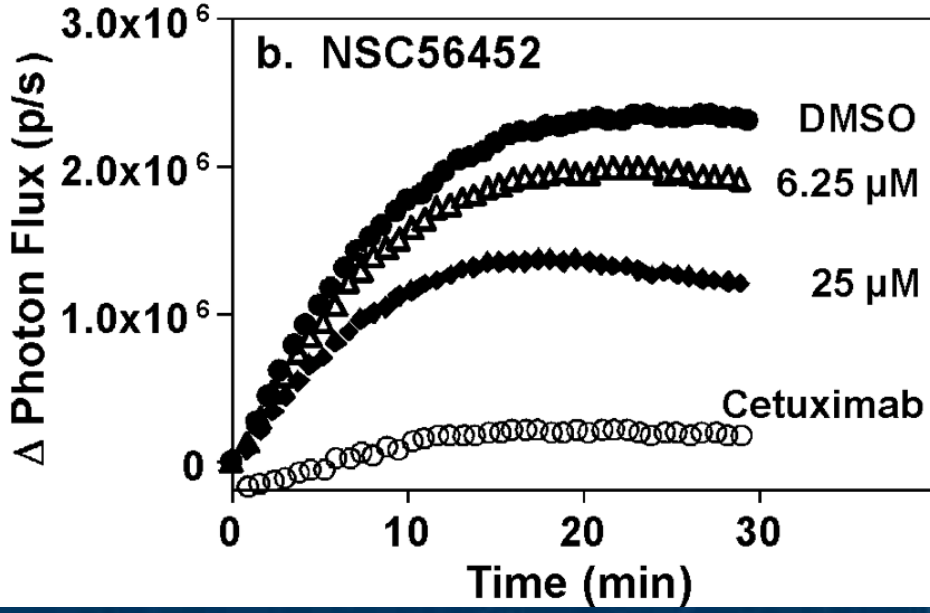
Only homodimerization of EGFR leads to signal despite presence of other ErbB receptors

Characterization of Leads by EFGR Split-Luciferase Assay









Fishing for Leads with NSC56452

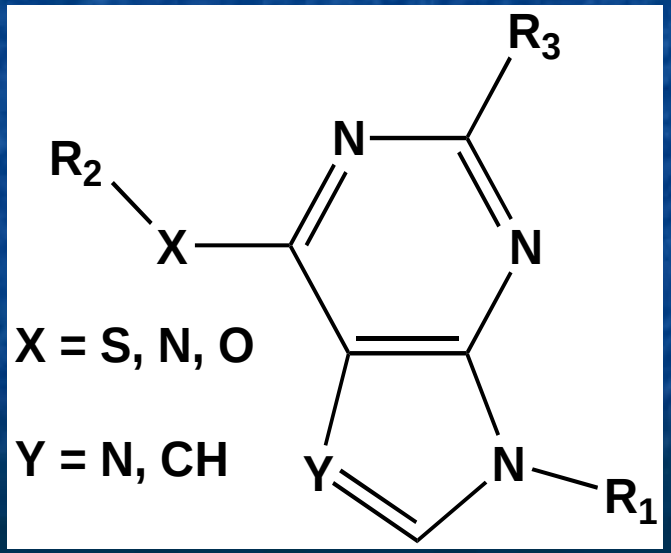
Compounds that shared at least 90% Tanimoto similarity in structure and composition with NSC56452.

66 compounds were screened with the split-luciferase assay – one NSC59485 had an IC_{50} = 144 nM

In addition, sixty 6-substituted purine derivatives prepared by Laufer et al. (J Med Chem, 2005) as potential ATP-competitive kinase inhibitors that structurally resembled NSC56452 were screened with the most potent DH199 having an IC_{50} = 4 μM. These compounds had been profiled against a panel of kinases.

Fishing for Leads with NSC56452

In addition, sixty 6-substituted purine derivatives prepared by Laufer et al. (J Med Chem, 2005) as potential ATP-competitive kinase inhibitors that structurally resembled NSC56452 were screened with the most potent DH199 having an IC_{50} = 4 μM. These compounds had been profiled against a panel of kinases with minimal activity.



Screening for Inhibition of EGFR Dimerization of Structurally Similar Compounds with Split-Luciferase Assay

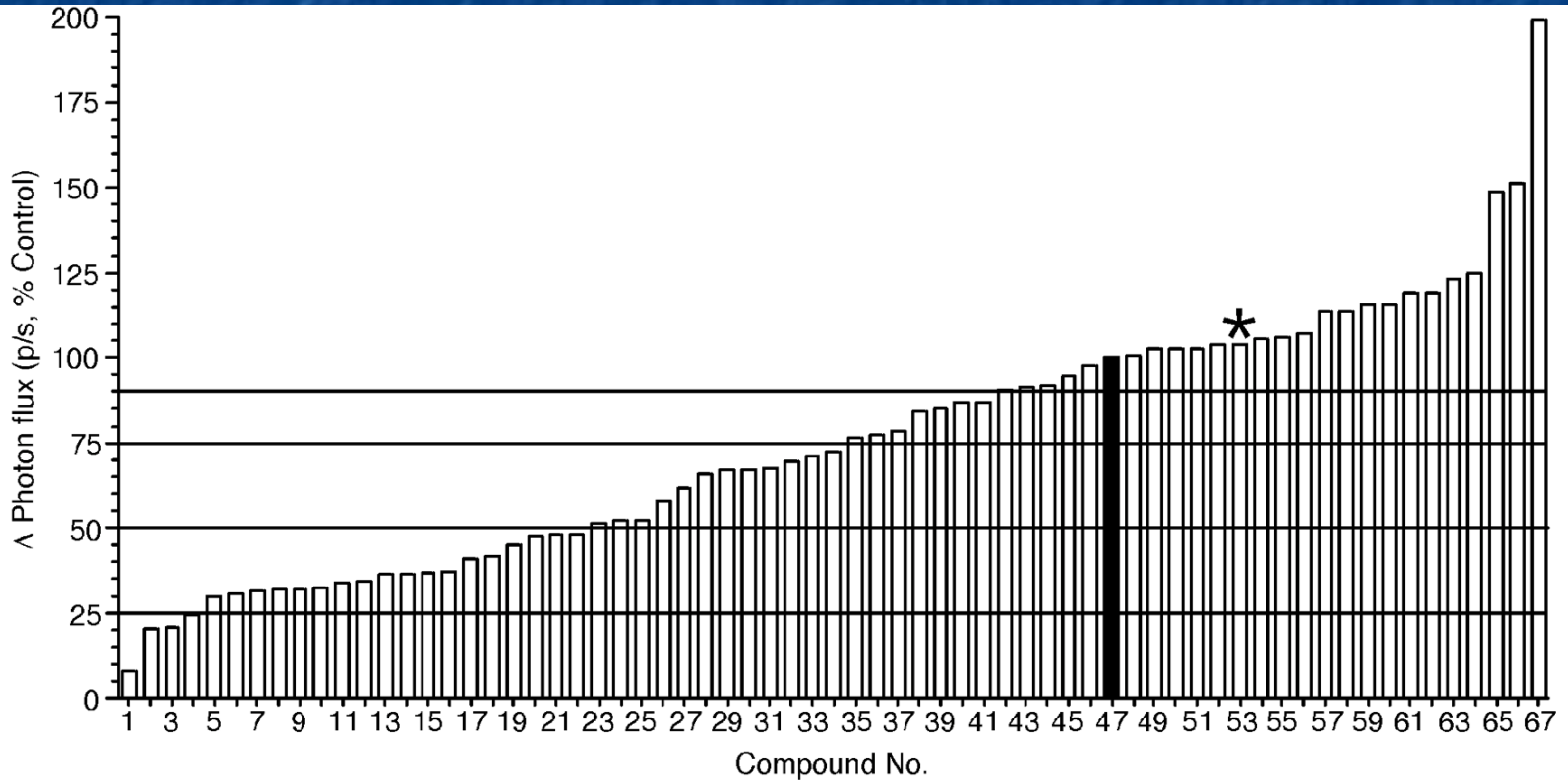
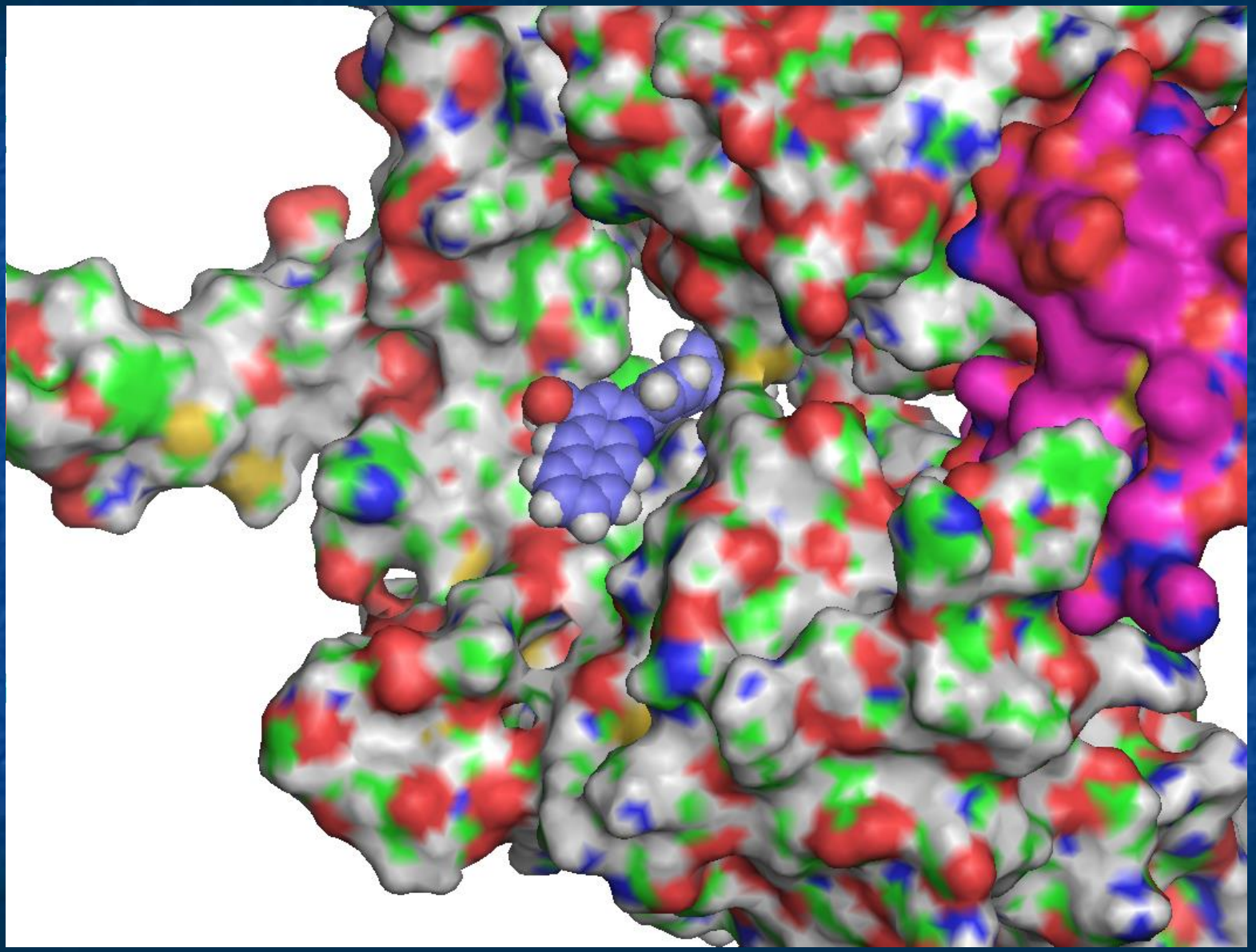


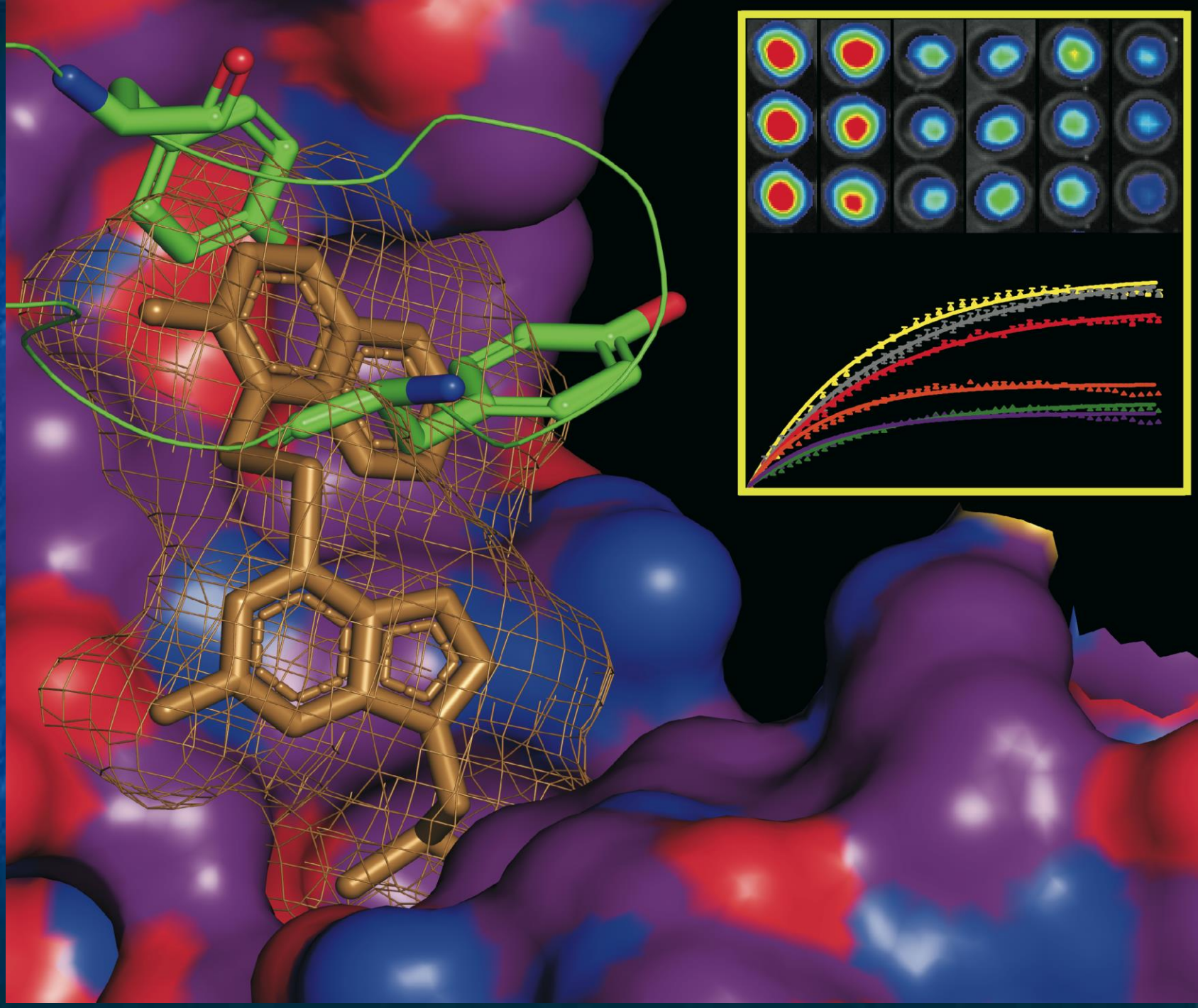
Figure 5.1. Graphical summary of the screening results. Compound 47 is the control with DMSO; compound 53 (*) is the tyrosine kinase inhibitor erlotinib. Compounds showing effect below 25% was denoted as (+++) in Table 5.1, 50% denoted as (++), 75% denoted as (+). Experiment was done in triplicate of 3 wells at concentration of 25 μM compound.

NSC56542 Docked into Dimerization-Arm Recognition Site



Garrett Cell 2002, *110*, 763-773 (PDB:1MOX)

Cover: Yang et al. CBDD, 76, 2010



Hypothesis: Treatment with dimerization inhibitors will be less likely to lead to recurrance of drug-resistant cancer

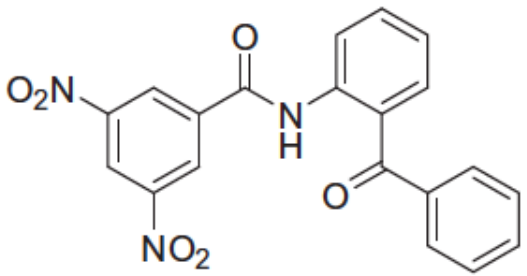
Rationale: Mutation in dimerization-arm recognition domain (“armpit”) to block drug will require a compensatory mutation in dimerization arm to maintain function

QED: Blocking dimerization exploits an Achille’s heel of ErbB receptors

Other dimerization inhibitors >10 µM found by virtual screening of 100,000 compounds

Petch et al. Bioorg Med Chem, 2012, 5901-5914

The Yang et al. paper is quoted, but very indirectly!



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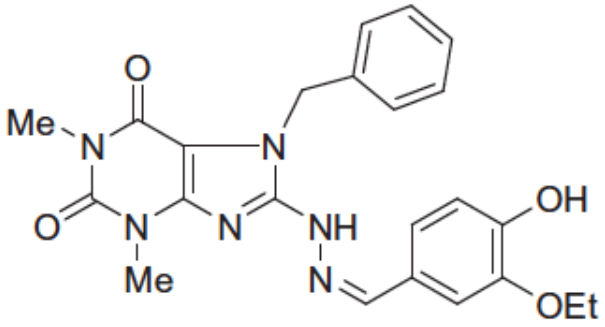

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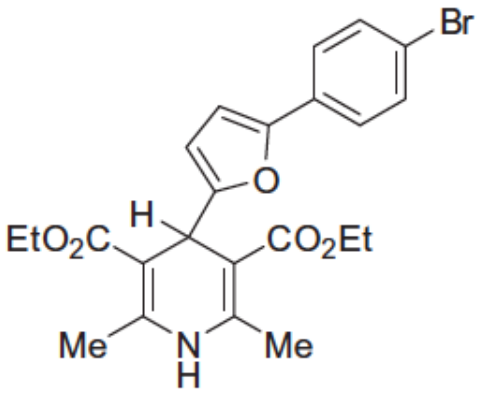

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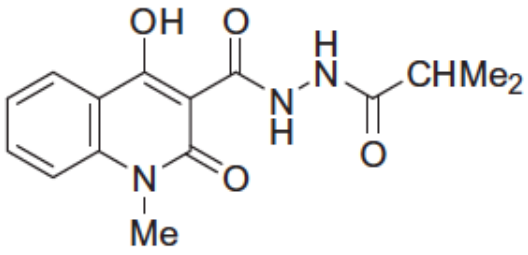

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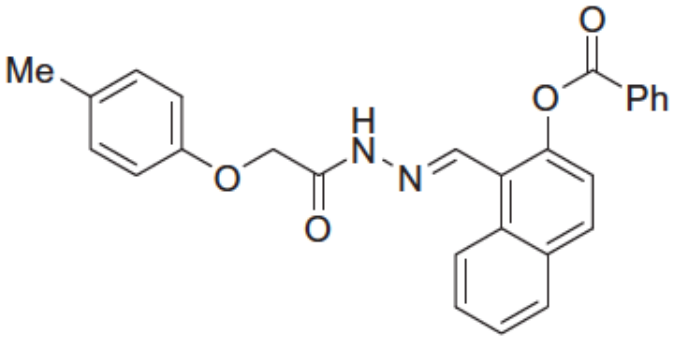
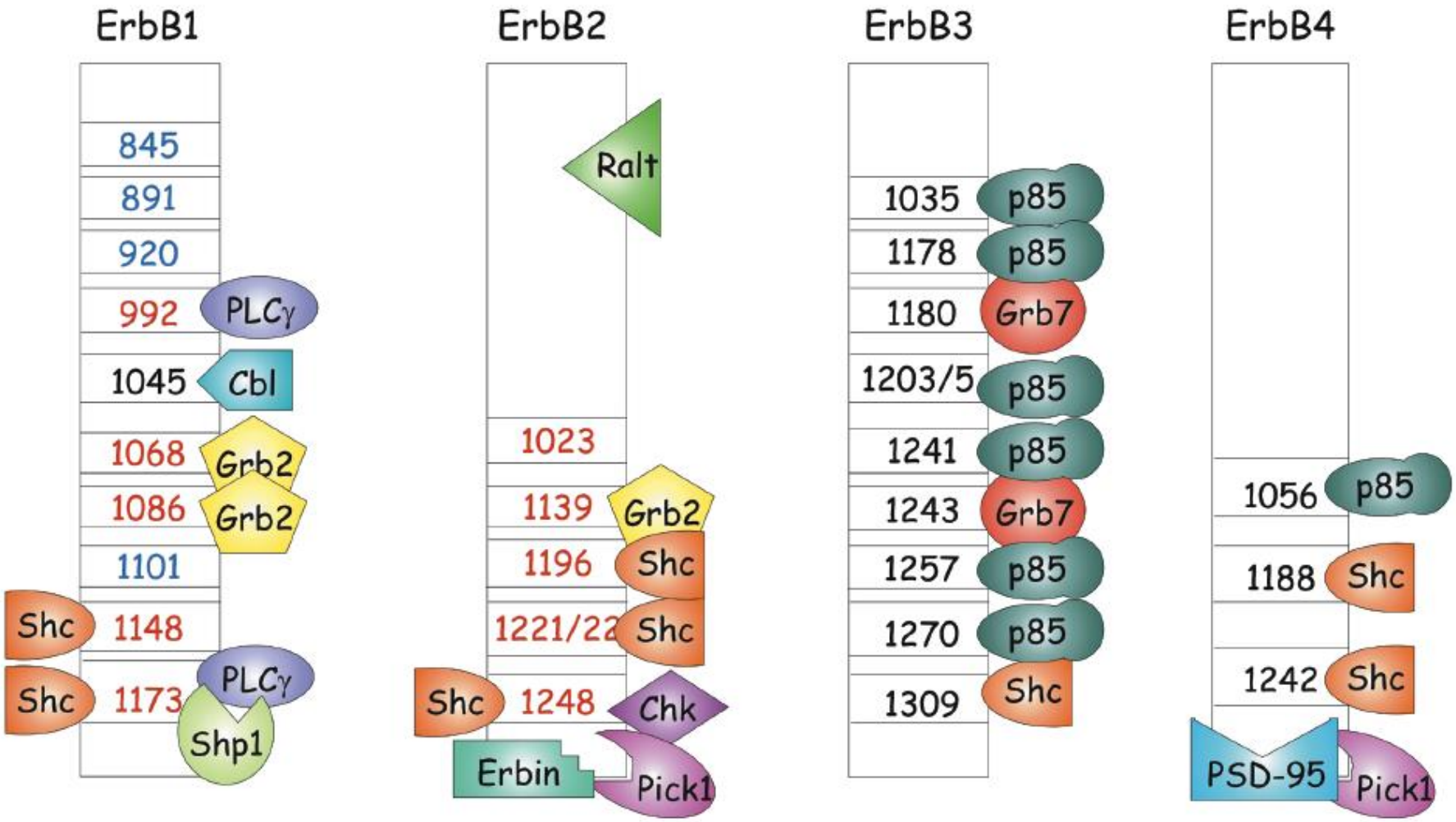


5

Intracellular Messengers Recruited by ErbB Family Members

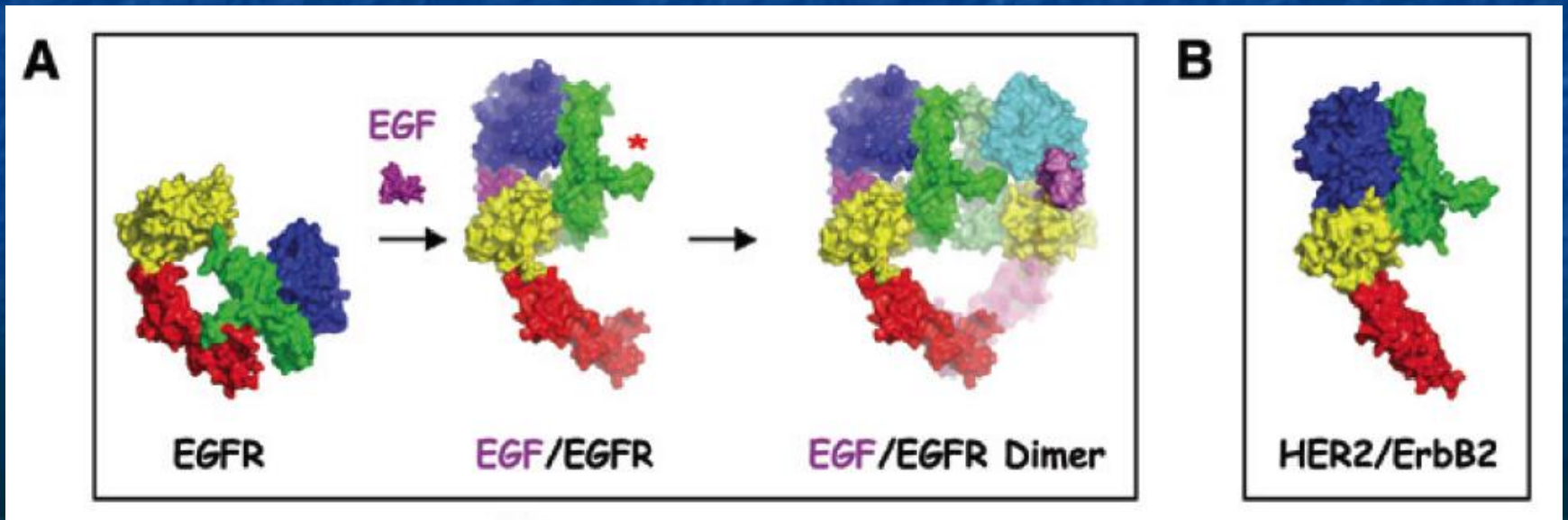


ErbB receptors and their cytoplasmic partners. The interaction of various proteins containing Src homology 2 and phosphotyrosine binding domains has been mapped to specific ErbB carboxy-terminal tyrosines. Autophosphorylation sites are shown in red, interaction sites demonstrated by phosphopeptide competition analyses are in black, and sites identified as Src phosphorylation sites are in blue. The receptor-associated late transducer (Ralt) and the PDZ proteins PSD-95, Erbin and Pick1 interact with the receptors in a phosphorylation-independent manner.

(Olayioye, *Breast Cancer Res* 2001, **3**:385-389)

Why do receptors heterodimerize?

- ErbB2 (HER2) does not homodimerize - Why?
- ErbB2 preferentially heterodimerizes with EGFR, ErbB3 and ErbB4 receptors - Why?
- ErbB2 does not require ligand activation - Why?
- Are there differences in structure of extracellular domains?



Synthetic Transmemebrane Segments of ErbB Receptors Used to Estimate Dimerization Affinities of TM Segments

Table 1: Hierarchy of Dimerization Affinities[a]

dimeric species	K_{dapp} (μM)	ΔG° (kJ/mol)	$\Delta\Delta G^\circ$ (kJ/mol)
EGFRtm−2tm	0.20 ± 0.05	38.3	0
HER2tm−3tm	0.85 ± 0.25	34.6	3.6
EGFRtm−3tm	2.0 ± 0.9	32.4	5.9
HER2tm−2tm	2.2 ± 0.5	32.3	6
EGFRtm−EGFRtm	2.3 ± 0.8	32.1	6.1
HER2tm−4tm	2.6 ± 0.5	31.9	6.4
EGFRtm−4tm	2.9 ± 0.7	31.6	6.6
HER3tm−3tm	4.0 ± 0.5	30.8	7.5
HER3tm−4tm	6.2 ± 1.6	29.7	8.6
HER4tm−4tm	70 ± 20	23.7	14.6

Duneau, J. P.; Vegh, A. P.; Sturgis, J. N. A dimerization hierarchy in the transmembrane domains of the HER receptor family. *Biochemistry* 2007, *46*, 2010-9.

US008404838B2

(12) **United States Patent**
Marshall et al.

(10) **Patent No.: US 8,404,838 B2**
(45) **Date of Patent: Mar. 26, 2013**

(54) **INHIBITORS OF TYROSINE KINASE RECEPTOR DIMERIZATION**

(75) Inventors: **Garland R. Marshall**, Clayton, MO (US); **Linda J. Pike**, Clayton, MO (US); **Robert Yang**, Medford, MA (US)

(73) Assignee: **Washington University**, Saint Louis, MO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/157,472**

(22) Filed: **Jun. 10, 2011**

(65) **Prior Publication Data**

US 2011/0312919 A1 Dec. 22, 2011

Related U.S. Application Data

(62) Division of application No. 12/384,511, filed on Apr. 6, 2009, now abandoned.

(60) Provisional application No. 61/042,715, filed on Apr. 5, 2008.

(51) **Int. Cl.**
C07D 473/00 (2006.01)

(52) **U.S. Cl.** **544/264**

(58) **Field of Classification Search** 544/264
See application file for complete search history.

(56) **References Cited**

PUBLICATIONS

Brooks, et al. Journal of Chemical Information and Modeling, 47(5), 2007, 1897-1905.*

* cited by examiner

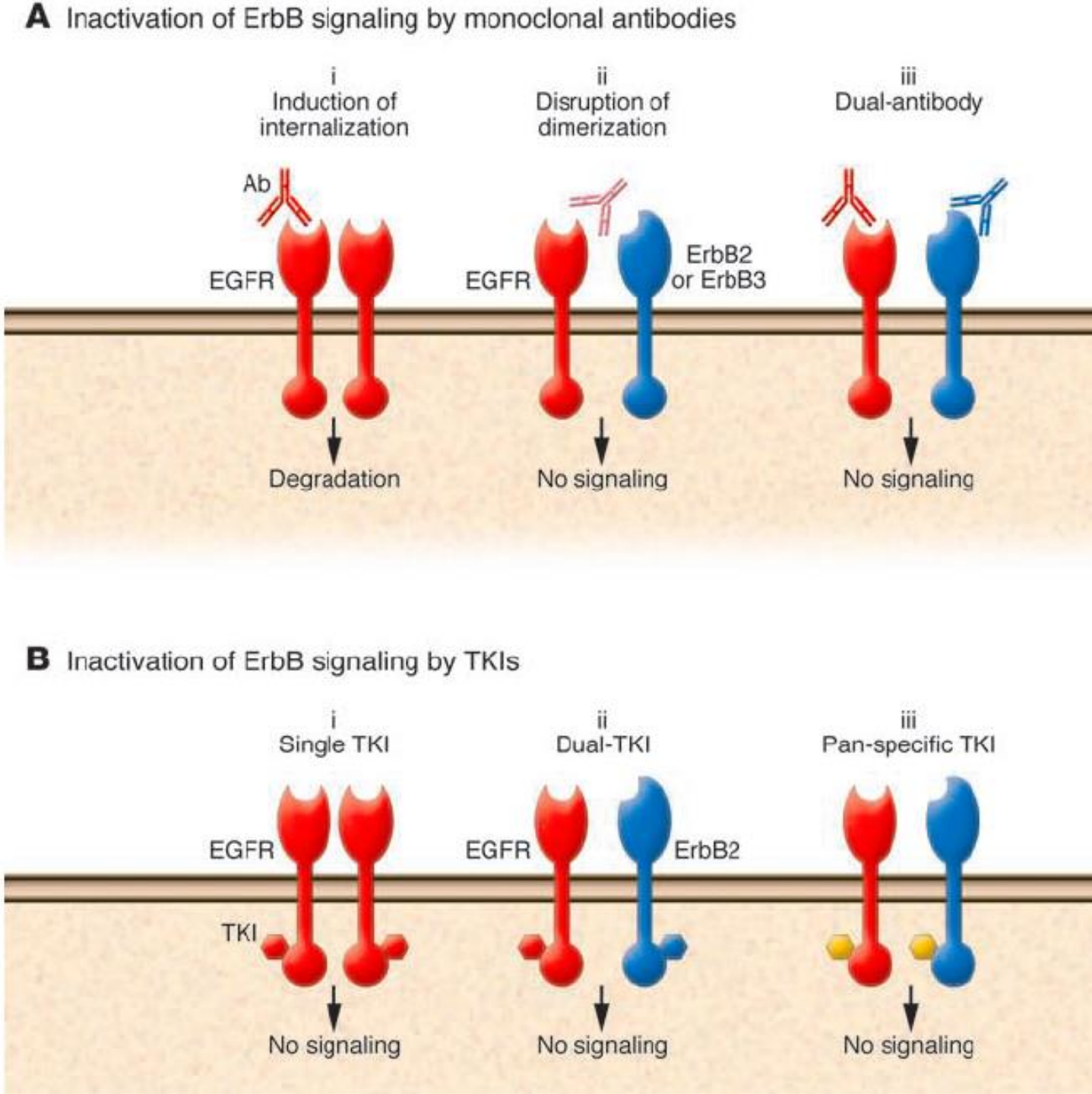
Primary Examiner — Douglas M Willis
(74) *Attorney, Agent, or Firm* — Zackson Law LLC; Saul L. Zackson

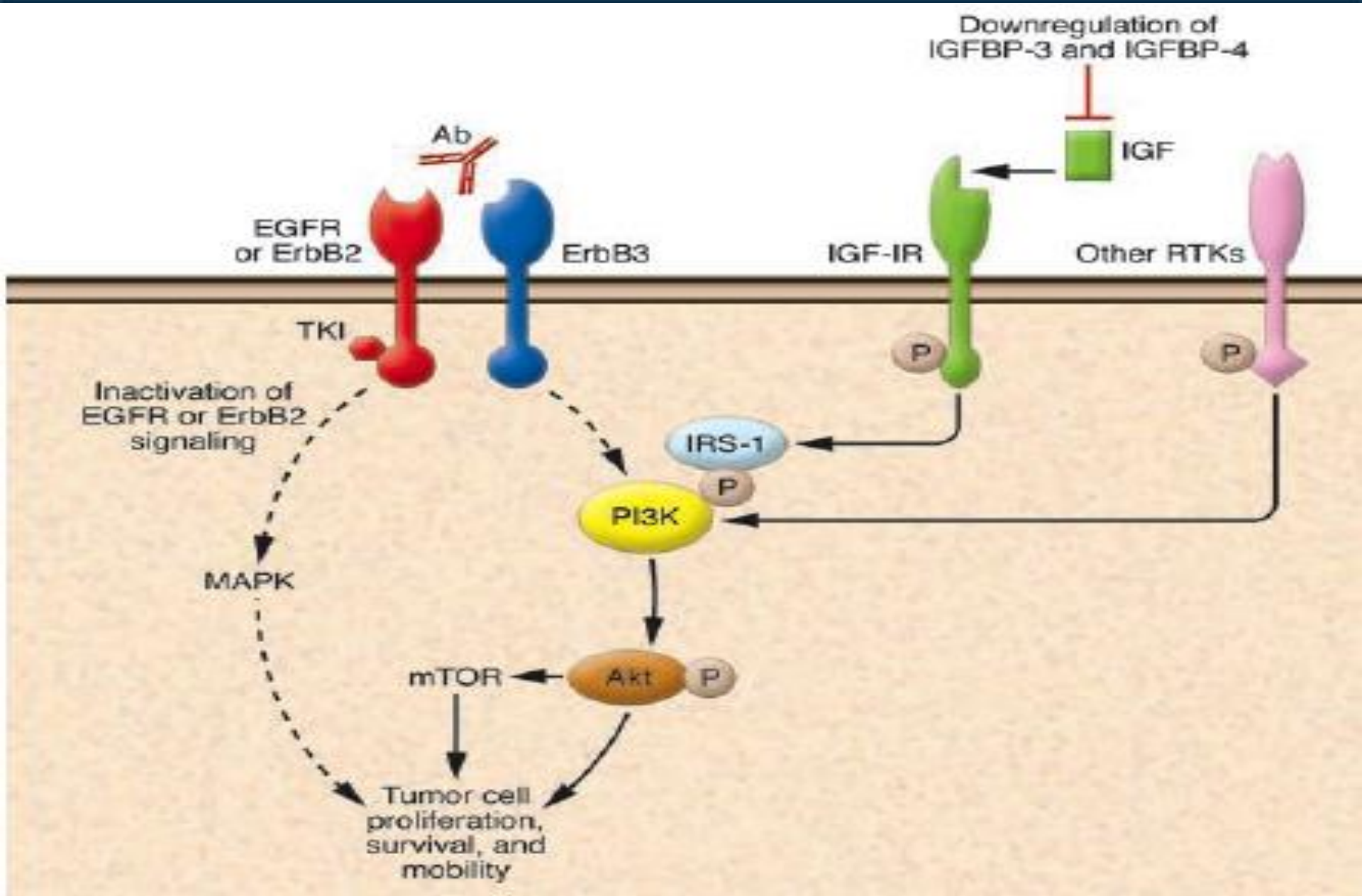
(57) **ABSTRACT**

The teachings relate to methods of identifying inhibitors of dimerization of tyrosine receptor kinases such as EGFR. The methods comprise providing, on a digital computer, a molecular model comprising a complex of extracellular dimerization domains of an RTK, docking a chemical databases to the molecular model, scoring the compounds comprised by the database, and identifying one or more high-scoring compounds. The methods further comprise testing a compound for RTK inhibitory activity in vitro, and testing a compound for specificity as an RTK inhibitor. Also disclosed are compounds selected by the described methods, and methods of treatment using the compounds. Two compounds (NSC11241 and NSC56452) are disclosed that inhibit EGF receptor kinase activation in a dose-dependent manner.

5 Claims, 12 Drawing Sheets

Development of resistance to ErbB-pathway targeted pathways for cancer therapies. (Wang & Greene, J Clin Invest, 2008. 118(7): p. 2389-92)





Development of resistance to ErbB-pathway targeted pathways for cancer therapies. (Wang & Greene, J Clin Invest, 2008. 118(7): p. 2389-92)

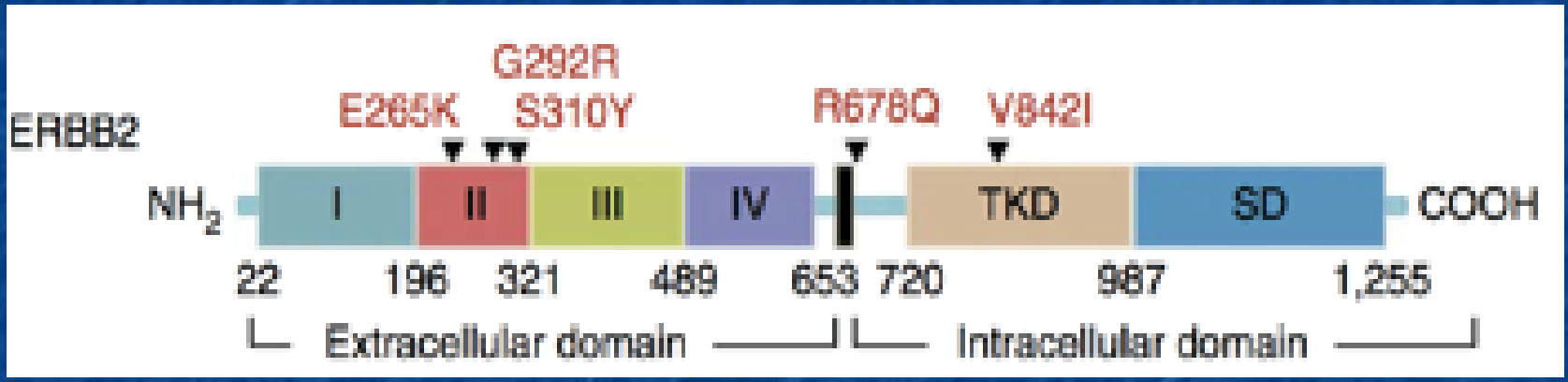
Therapeutic Opportunity?

Overall Hypothesis: Cancers that recur following treatment with mAB therapeutics (trastuzumab, cetuximab, etc.) targeting the ECDs, or tyrosine kinase domains (gefitinib, erlotinib, lapatinib, etc.), of ErbB receptors will respond to treatment with dimerization inhibitors.

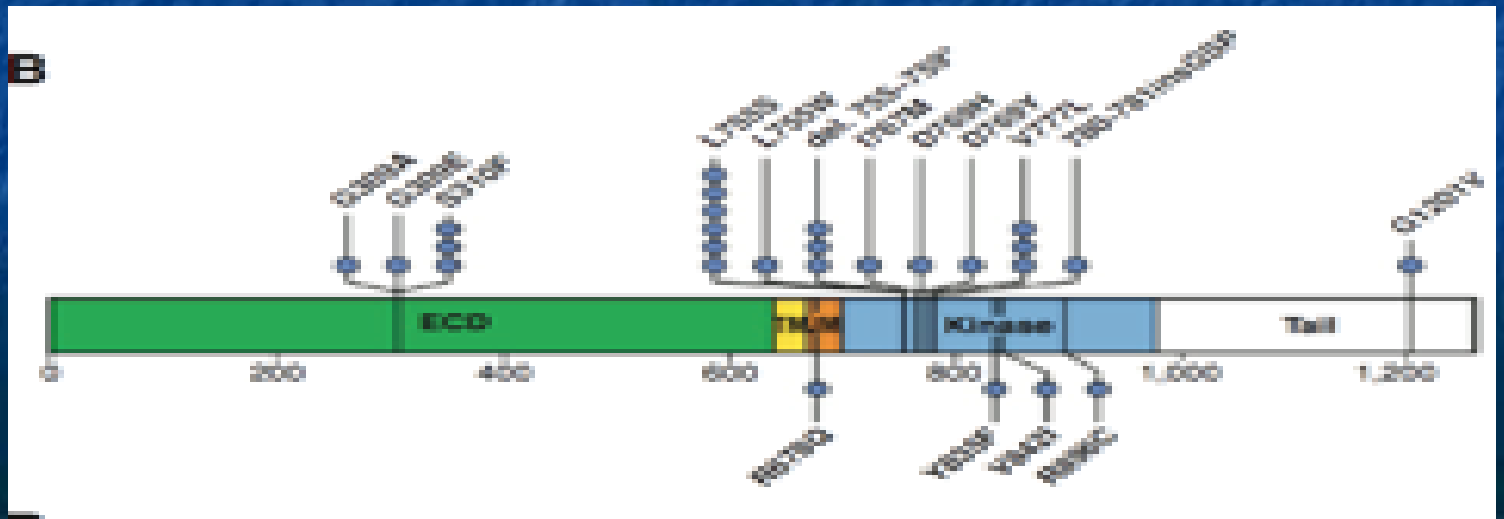
Rationale: Dimerization inhibitors target a unique interface that is not modified by mutations causing resistance to mABs or TKIs.

Oncogenic Mutations in ErbB2 Receptors in Different Cancers

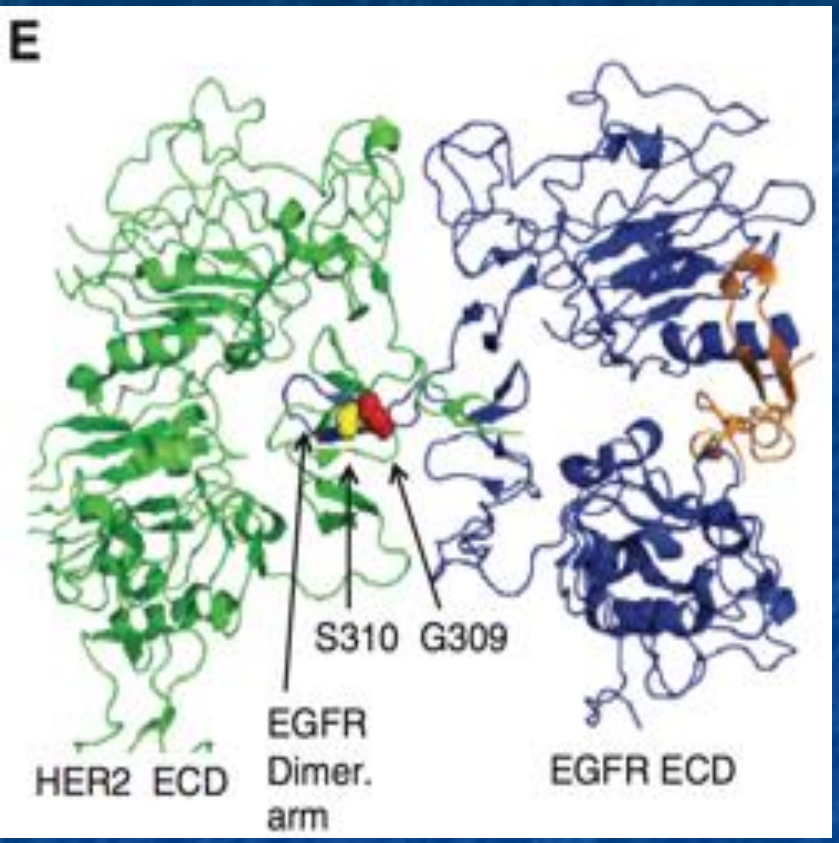
Gallbladder Cancer



Breast Cancer



Oncogenic Mutations in the Extracellular Domain of ErbB2 Receptors in Different Cancers

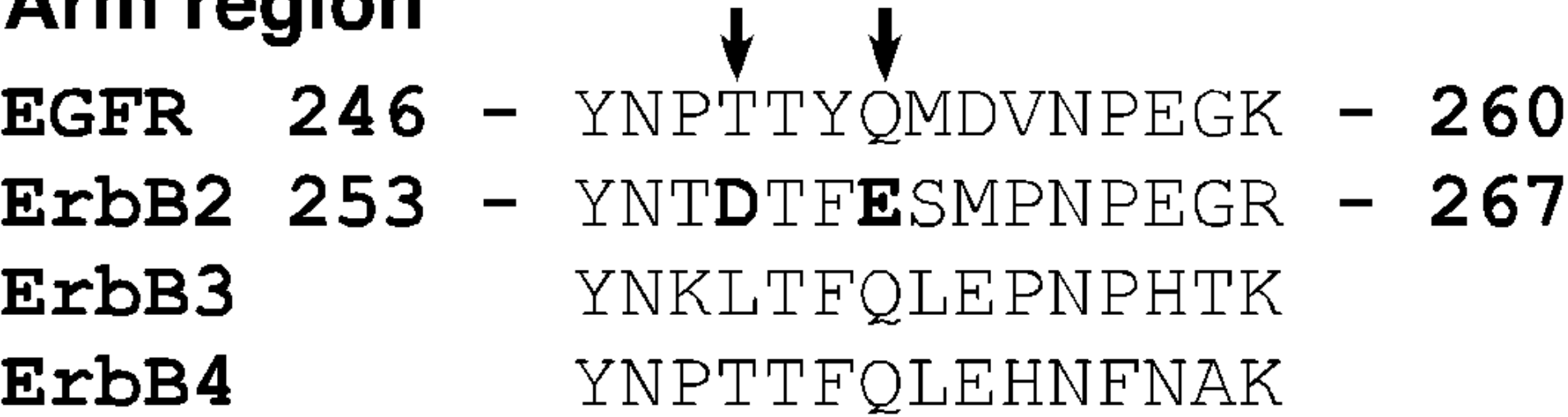


S319F (39)	S319Y (5)
7 Lung	2 Bladder
6 Unknown	1 Kidney
5 Bladder	1 Unknown
3 Bile Duct	1 Salivary Gland
3 CRC	
2 Kidney	
2 Stomach	
2 Small Intestine	
2 Ovary	
1 Gall Bladder	
1 Uterus	
1 Liver	
1 Gastroesophageal	
1 Duodenum	
1 Cervix	
1 Breast	

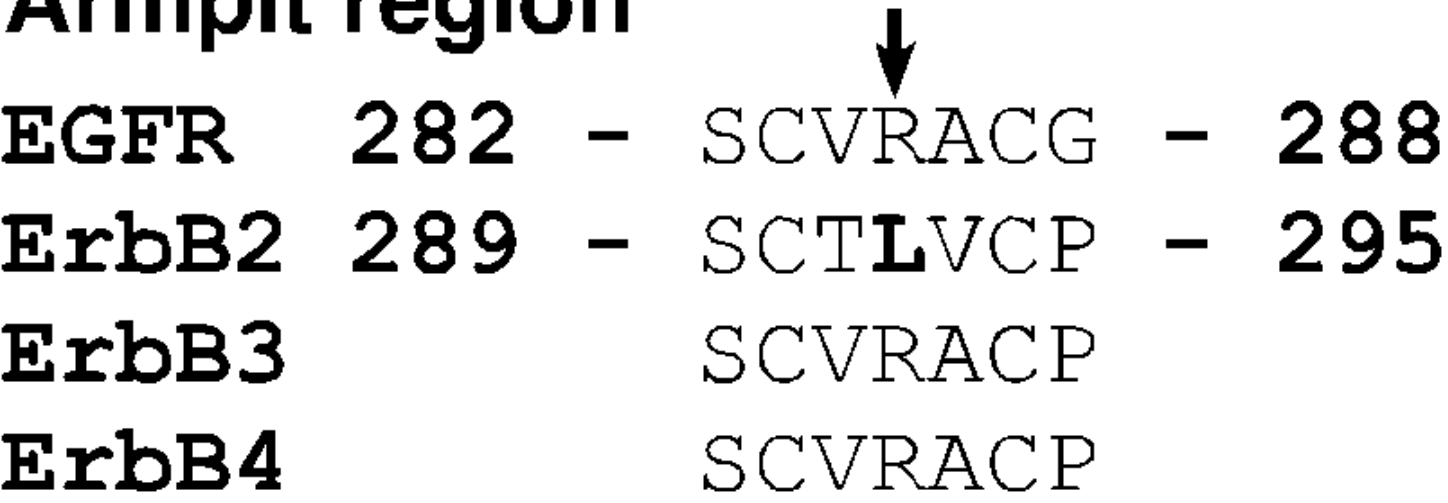
Breast Cancer Associated ECD Mutations

Oncogenic Mutations in the Extracellular Domain of

Arm region

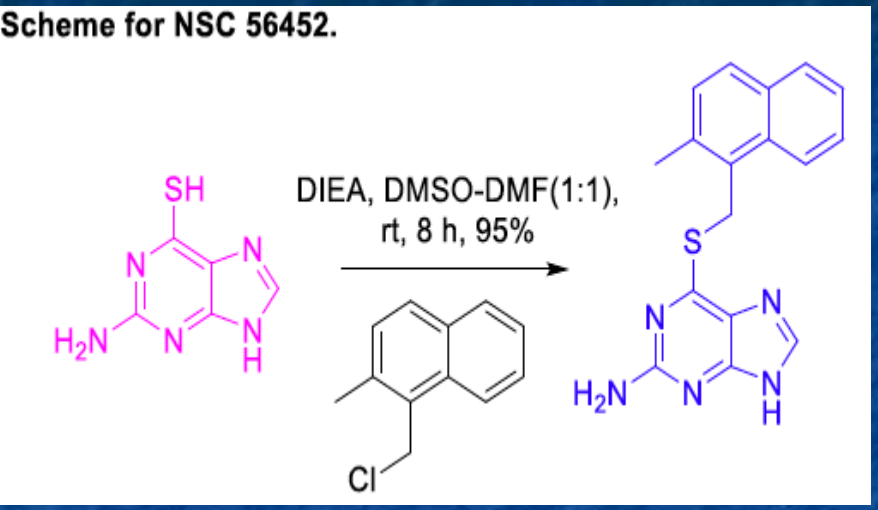


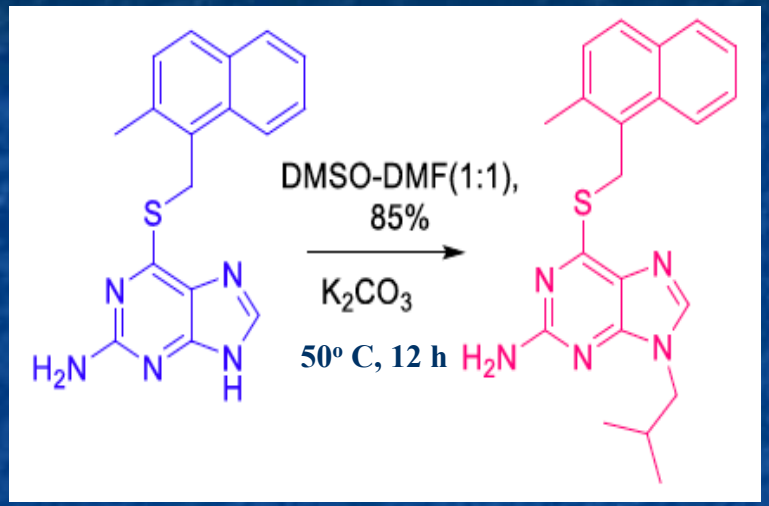
Armpit region



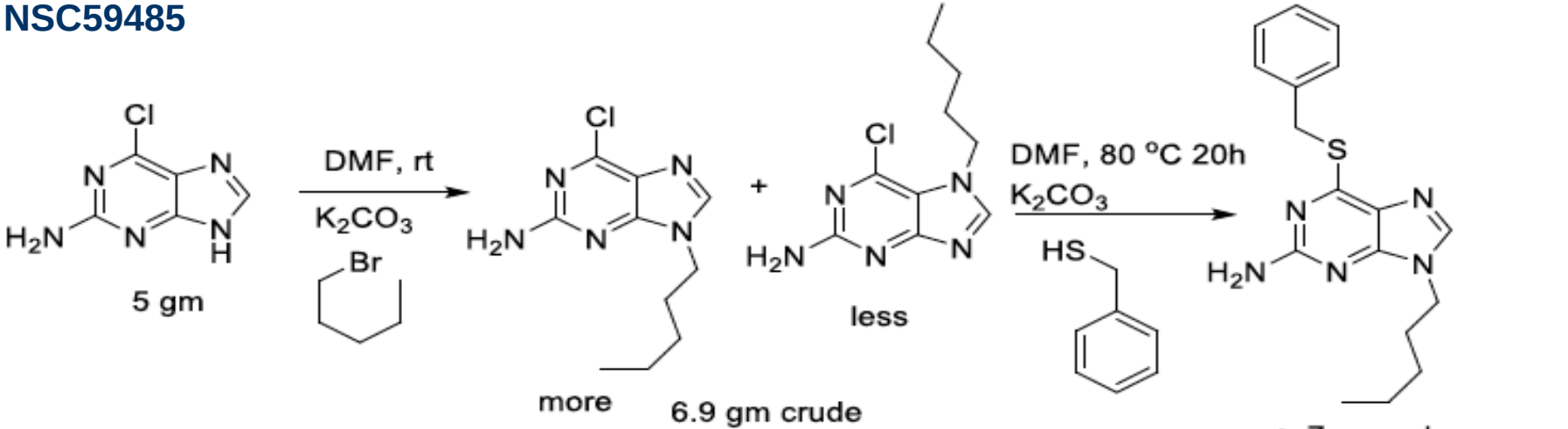
Syntheses of two lead inhibitors of Yang et al.

Scheme for NSC 56452.





NSC59485

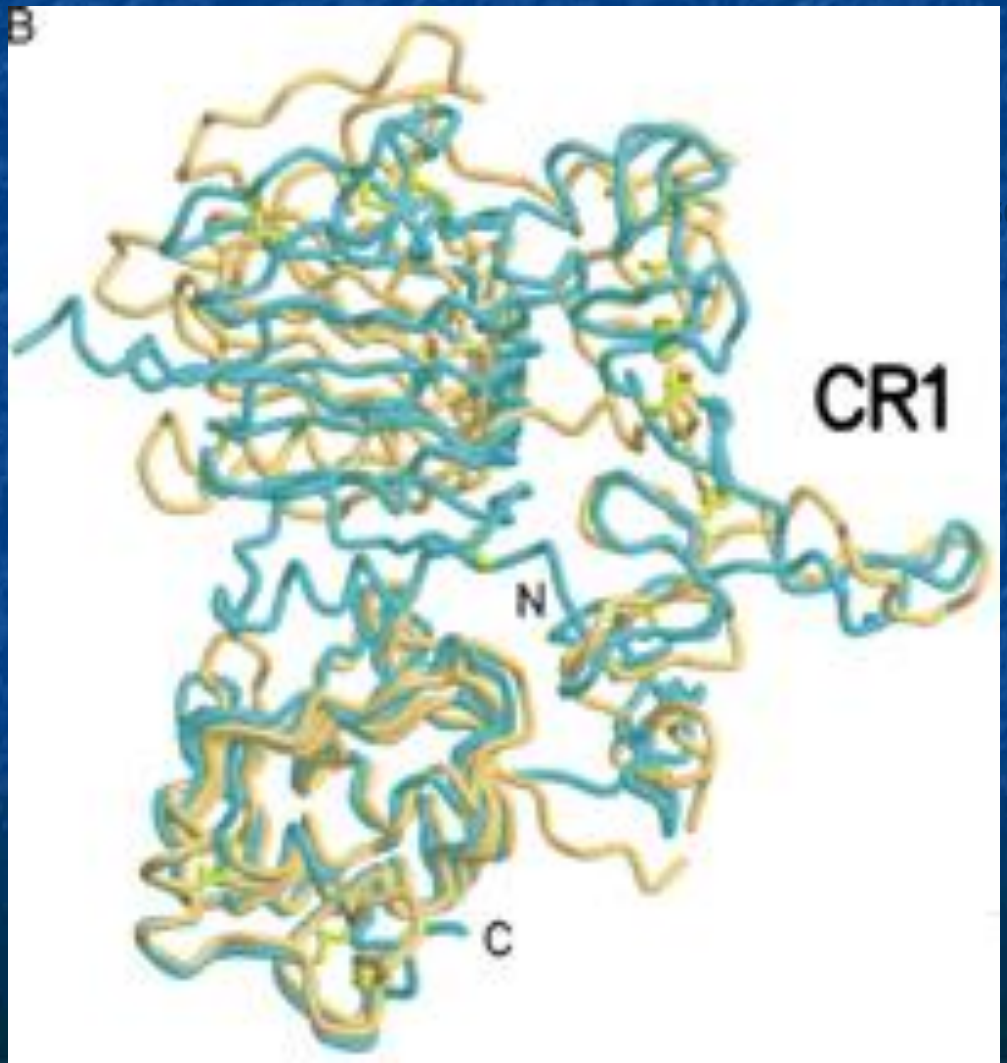


Syntheses of two lead inhibitors of Yang et al.

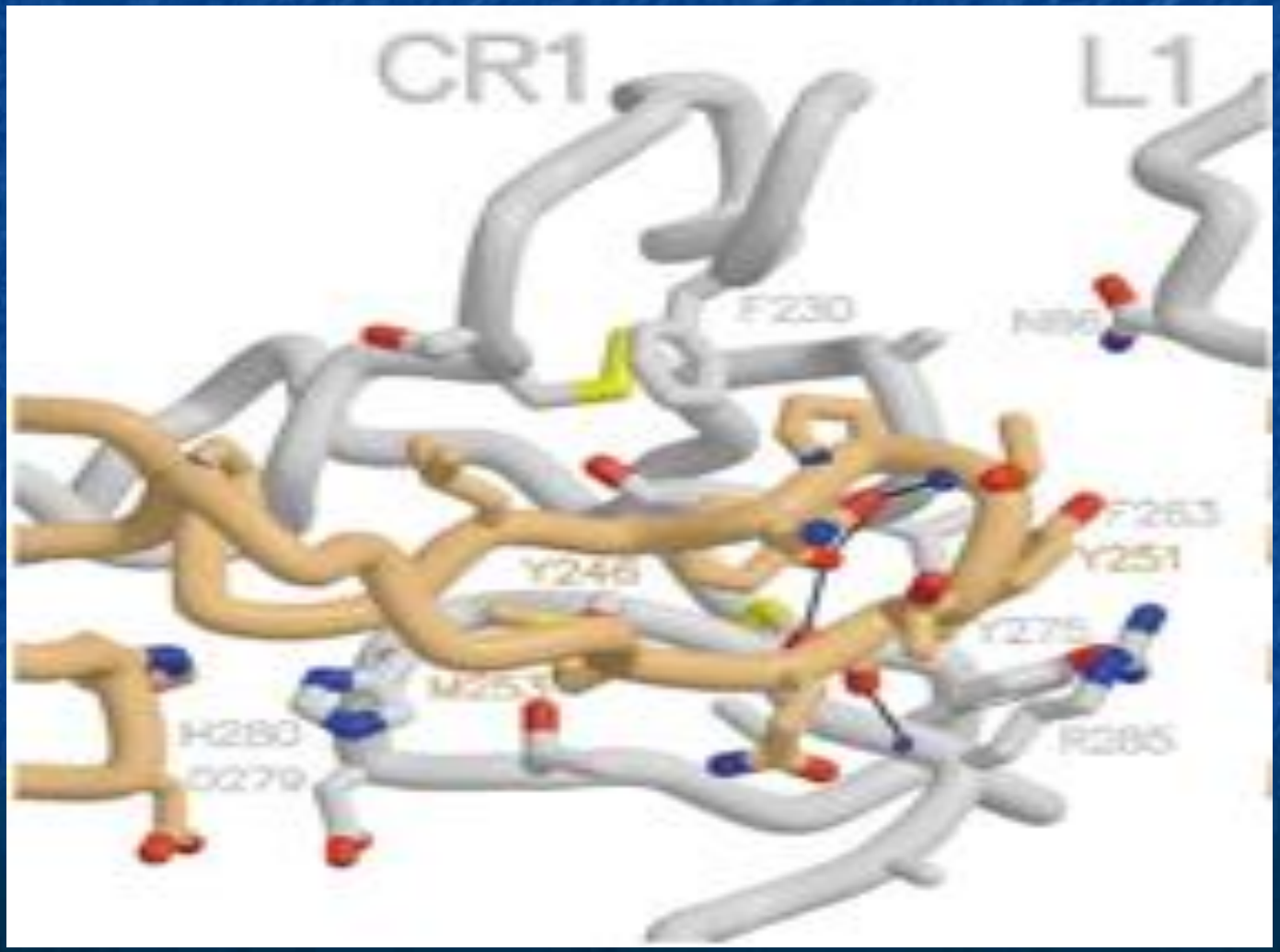
Patient-Derived Xenograft Cancer Models in Mice at WUSM HAMLET Center

PDX ID	ER	HER2	Molecular Subtype (PAM50)	HER2-targeted therapy of tumor prior to engraftment into mice
WHIM8	Neg.	Positive	HER2-E	Herceptin, Lapatinib
WHIM22	Neg.	Positive	HER2-E	Trastuzumab
WHIM35	Positive	Positive	HER2-E	treatment naïve
WHIM38	Positive	Positive	HER2-E	treatment naïve
WHIM49	Neg.	Positive	HER2-E	Herceptin, Lapatinib
WHIM56	Neg.	Positive	HER2-E	treatment naïve

Superposition of Main Chain Traces for ErbB2 (1–509, blue) and activated EGFR (1–501, yellow). Note the overlap of dimerization arms between activated EGFR and unliganded ErbB2.

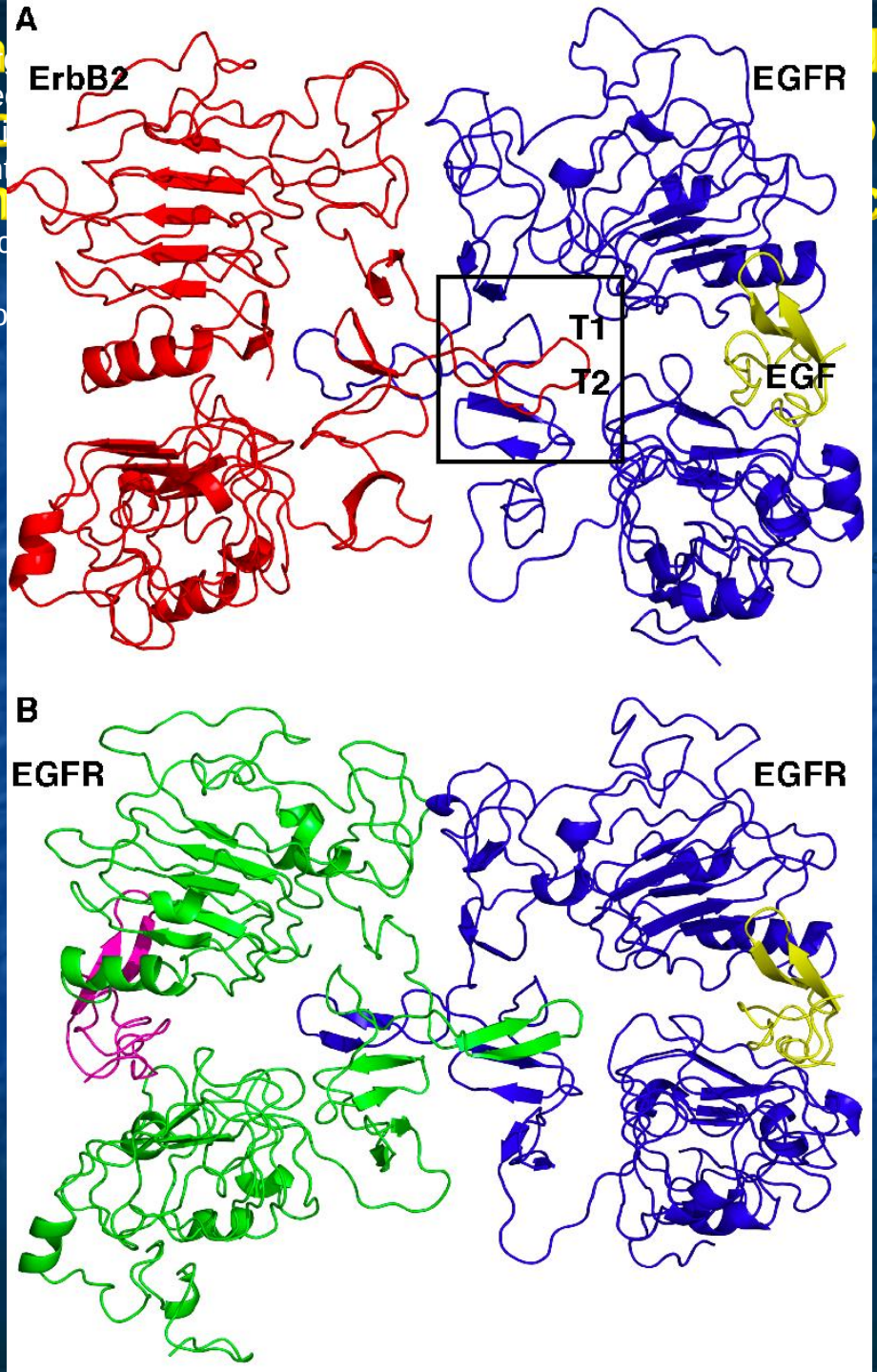


Ribbon diagram of the contacts between the dimerization arm of EGFR molecule A with “armpit” recognition site of EGFR molecule B at the dimer interface



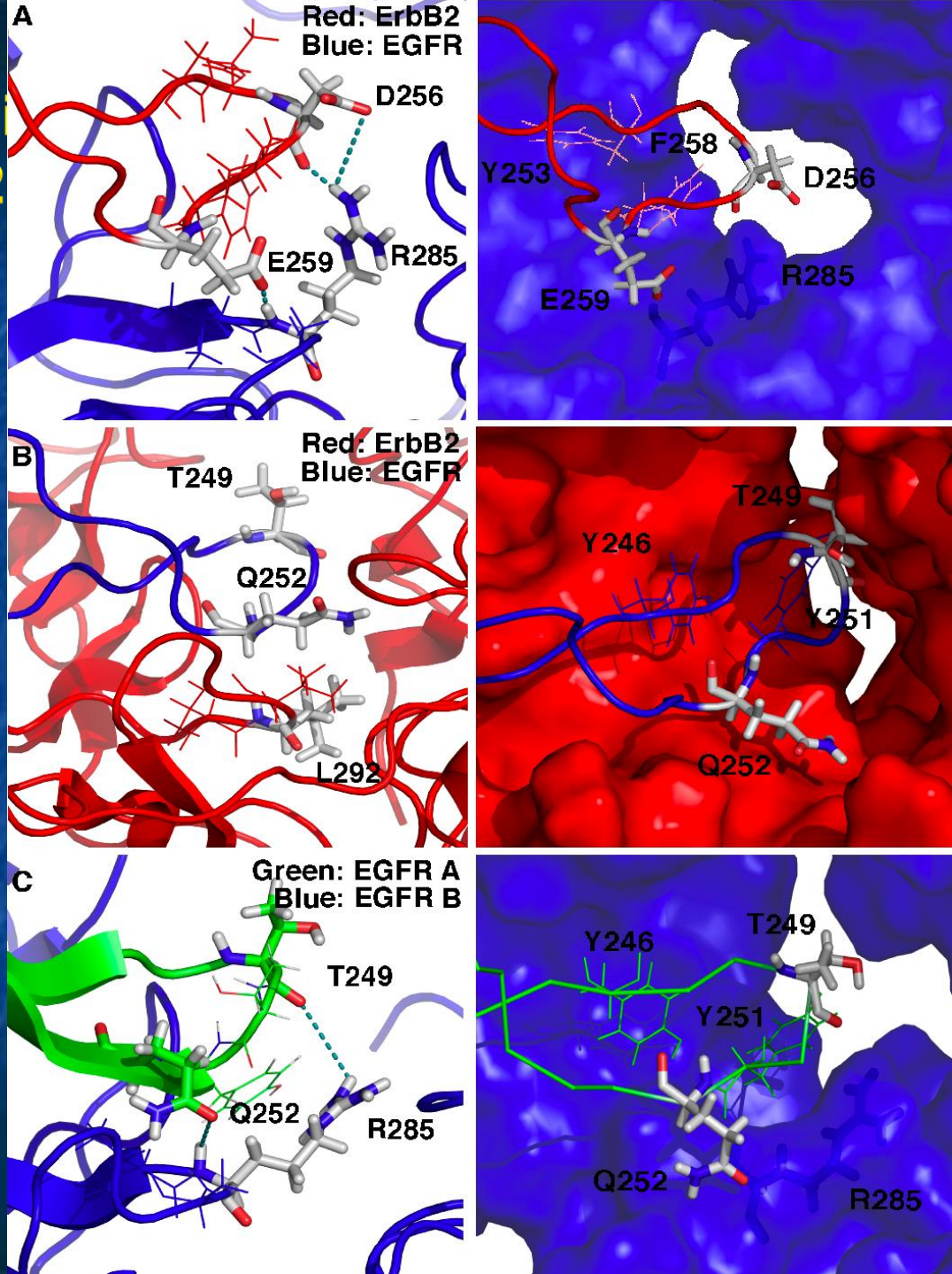
Ribbon diagram … limerization arm of EGFR molec… n site of EGFR …ce

…two negatively…
…computationally pre…
…critical for the stabil…
…the ErbB2-EGFR in…
…residues and the…
…heterodimer interfac…
…development of…
…heterodimer inhibito…



…50° C, 12 h

Representative snapshots of the arm-armpi interface between A) ErbB2 arm-EGFR armpit, B) EGFR-arm/ErbB2-armpit; and C) EGFR arm- EGFR armpit. A and B are from the heterodimer simulation while C comes from the homodimer simulation.



Acknowledgements

"We all know that the real reason universities have students is in order to educate the professors."

John Archibold Wheeler, Physicist (Ph.D. mentor of Richard Feynman and Kip Thorne)

And have I been educated by this crew!

Washington University in St. Louis