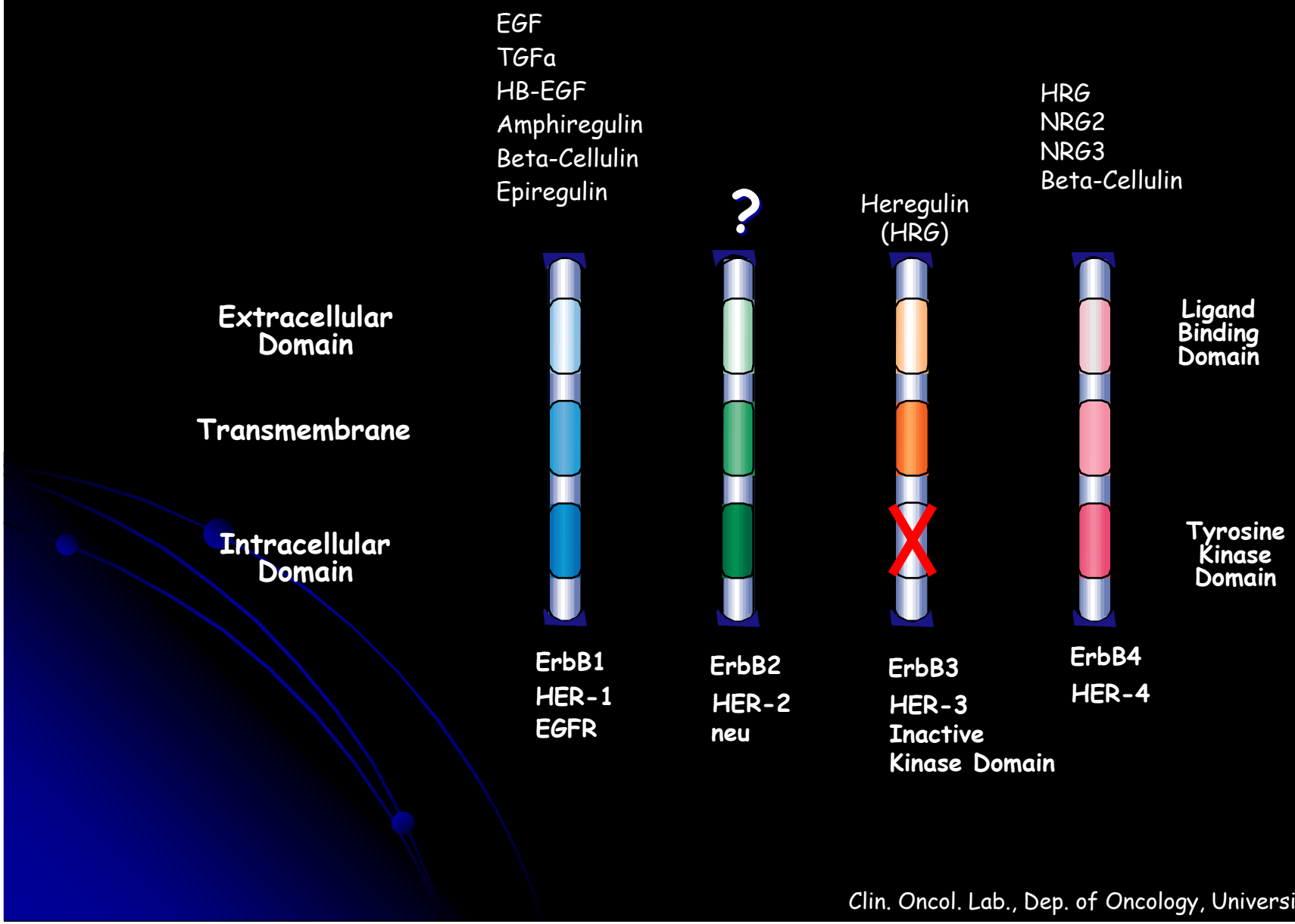
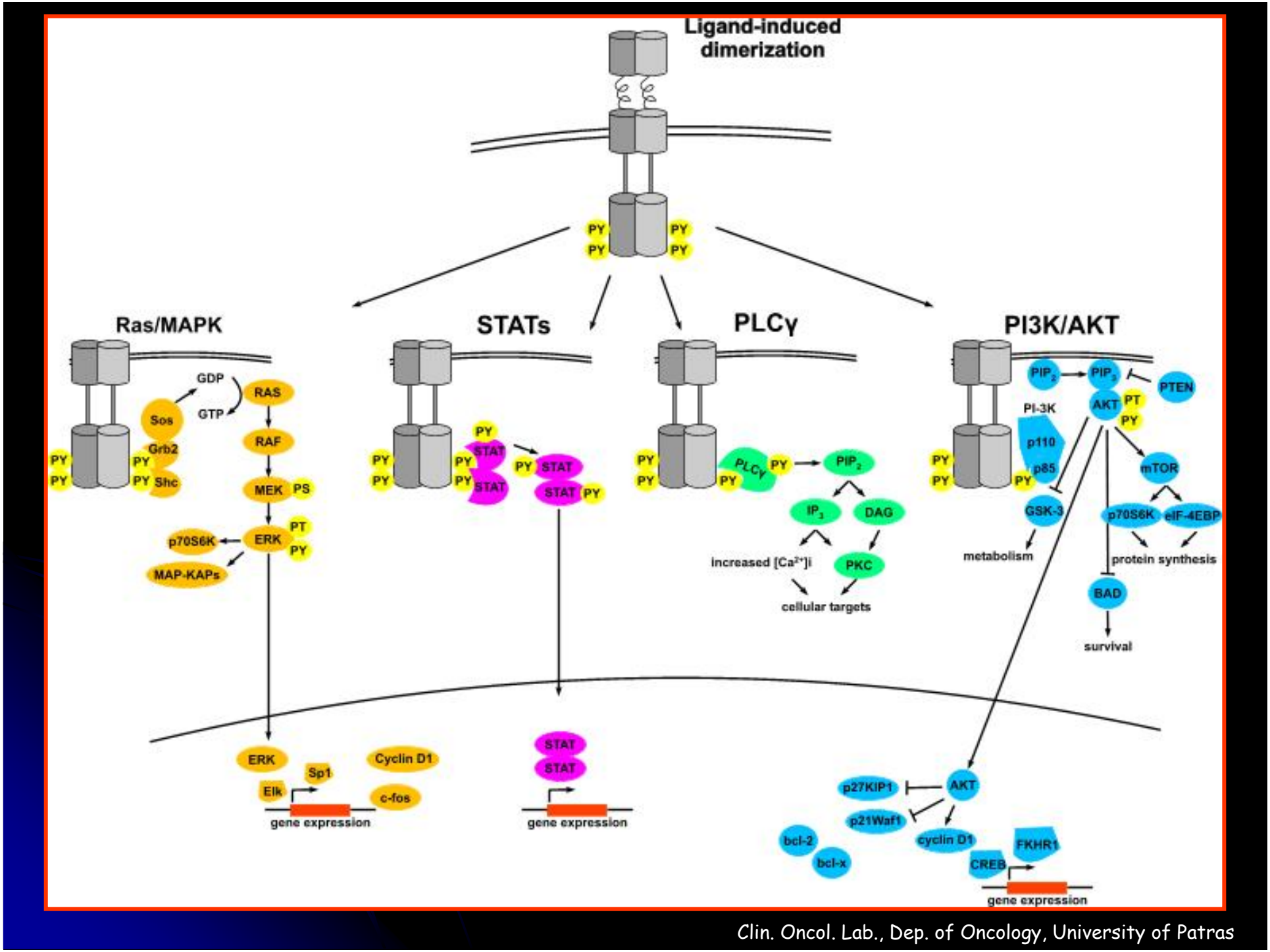
# (Multi-)Targeting of receptors, intracellular kinases and cell death pathways

E. Giannopoulou,
Chemist, MSc., PhD,
Clinical Oncology Laboratory,
Division of Oncology, Department of Medicine,
University Hospital of Patras, Rio, Greece

# The ErbB/HER Family of Receptors




Ligand-induced dimerization
PY
Ras/MAPK
GDP
GTP
RAS
Sos
Grb2
Shc
RAF
MEK
PS
ERK
PT
p70S6K
MAP-KAPs
STATs
STAT
PLCγ
PIP2
IP3
DAG
increased [Ca2+]i
PKC
cellular targets
PI3K/AKT
PIP2
PIP3
PTEN
AKT
PT
PI-3K
p110
p85
mTOR
GSK-3
p70S6K
eIF-4EBP
metabolism
protein synthesis
BAD
survival
ERK
Sp1
Elk
Cyclin D1
c-fos
gene expression
STAT
STAT
gene expression
p27KIP1
AKT
p21Waf1
cyclin D1
bcl-2
bcl-x
FKHR1
CREB
gene expression

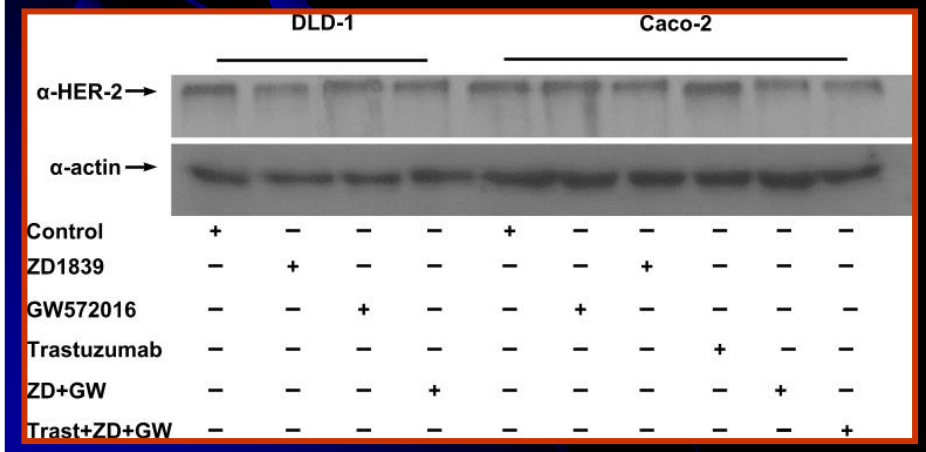
Monoclonal antibodies:
trastuzumab,
cetuximab,
panitumumab,
pertuzumab

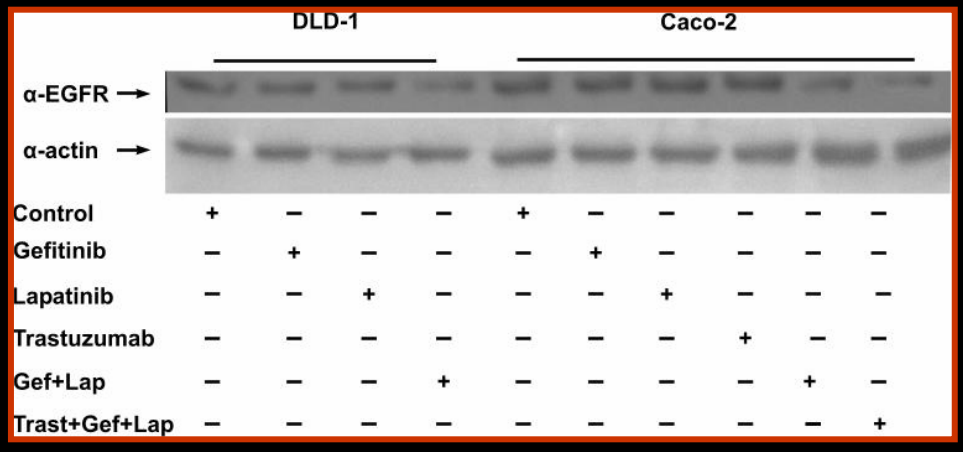
Inhibitors
of tyrosine kinases:
gefitinb,
erlotinib,
lapatinib,
BIBW2992

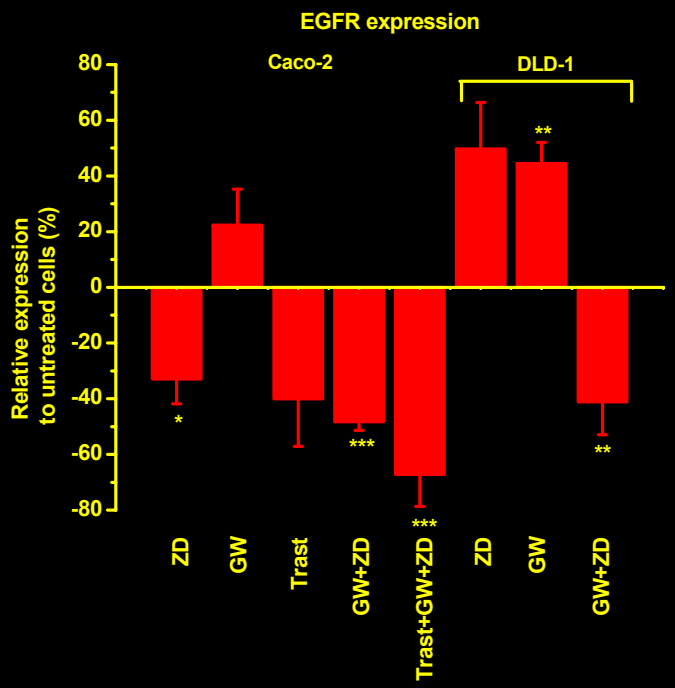
# I.A. Combined targeting of EGFR and HER-2 in colon cancer cells

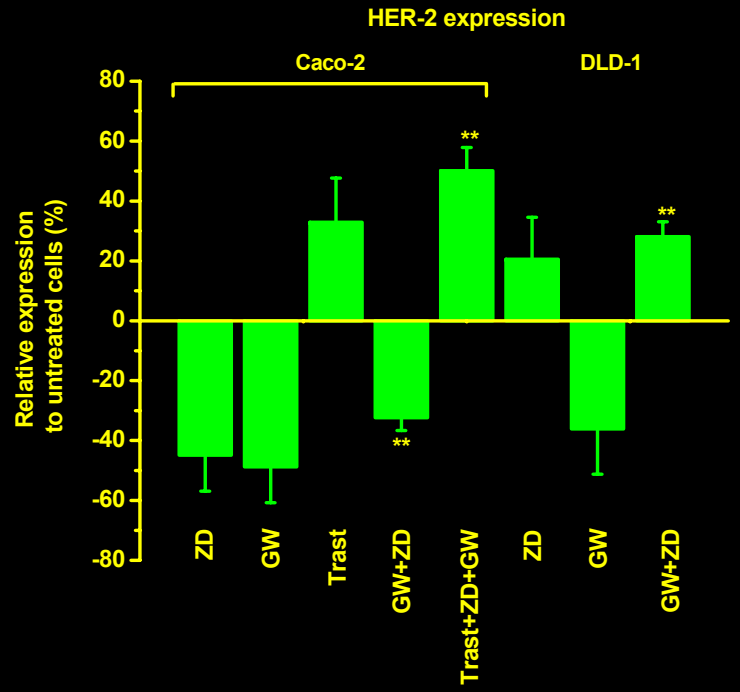
- Gefitinib (Iressa): an oral, EGFR-TK inhibitor approved by FDA and EMEA for locally, advanced or metastatic non-small-cell lung cancer (NSCLC) with EGFR-TK mutations.
- Lapatinib (Tykerb/Tyverb): an oral, dual EGFR and HER-2 TK inhibitor approved by FDA and EMEA for hormone positive and HER-2 positive advanced breast cancer and HER-2 overexpressing breast cancer.
- Trastuzumab (Herceptin): a monoclonal ab against HER-2 approved by FDA and EMEA for HER-2 positive breast cancer.

- Iressa, Lapatinib and Herceptin decreased cell proliferation by increasing apoptosis. The agents' combination exerted a synergy effect only in Caco-2 cells.





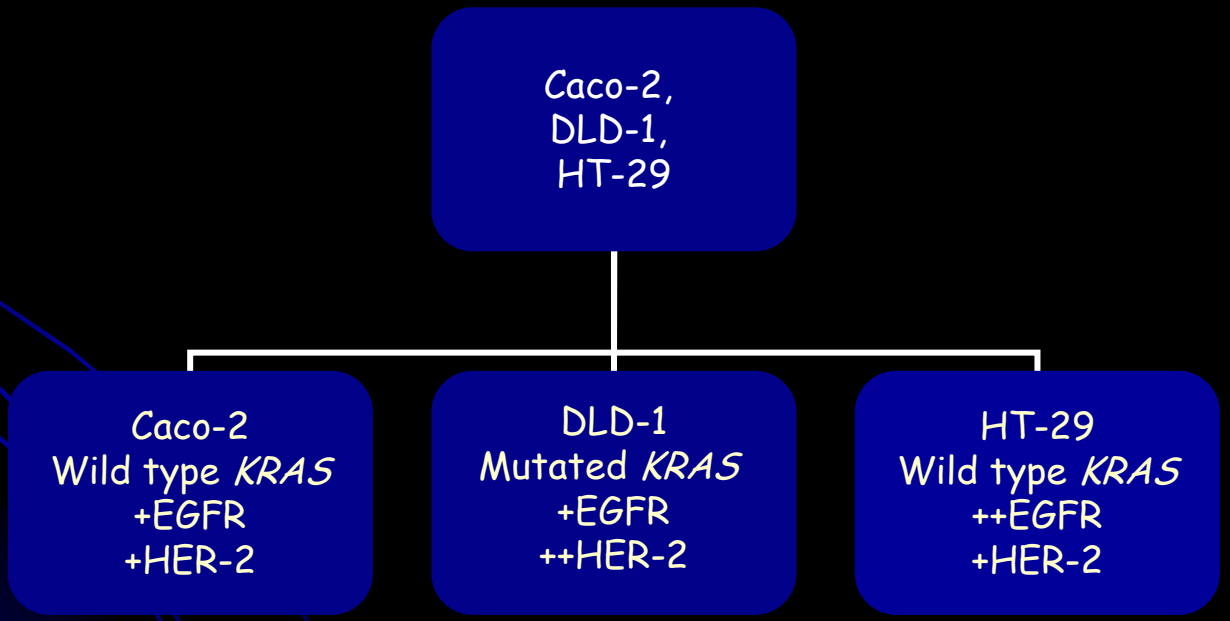



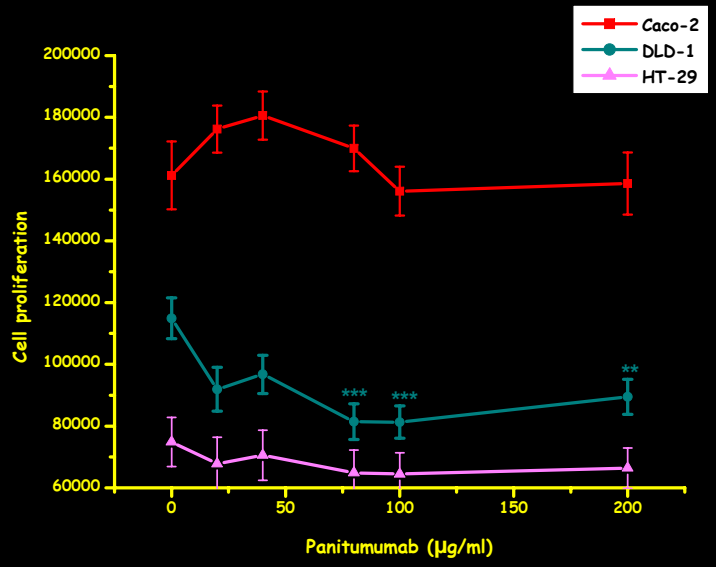

The synergy effect of tested TKIs in Caco-2 cells was in line with a decrease in EGFR and HER-2 gene and protein levels.

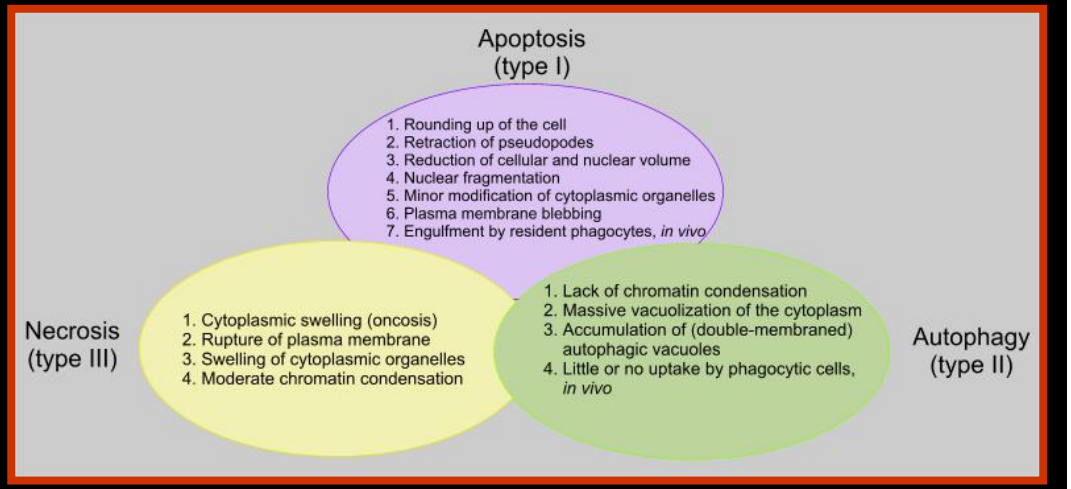
Giannopoulou *et al.*, Dual targeting of EGFR and HER-2 in colon cancer cell lines, 2009.

# I.B. The implication of panitumumab in autophagy and redox status in colon cancer cells

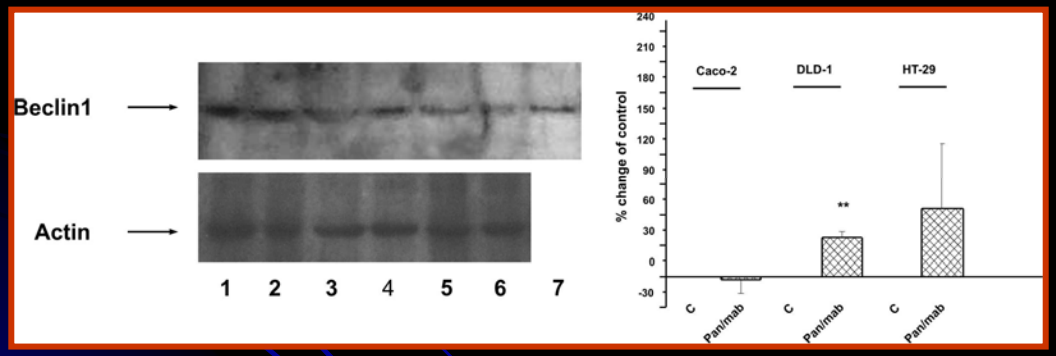
- Panitumumab (Vectibix): a monoclonal ab against EGFR approved by FDA for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irrinotecan-containing chemotherapy regimens (Giusti *et al.*, 2007). EMEA approved panitumumab with the same indications but only for patients with *wild type KRAS* (http://www.emea.europa.eu ).






Kroemer *et al.*, 2009

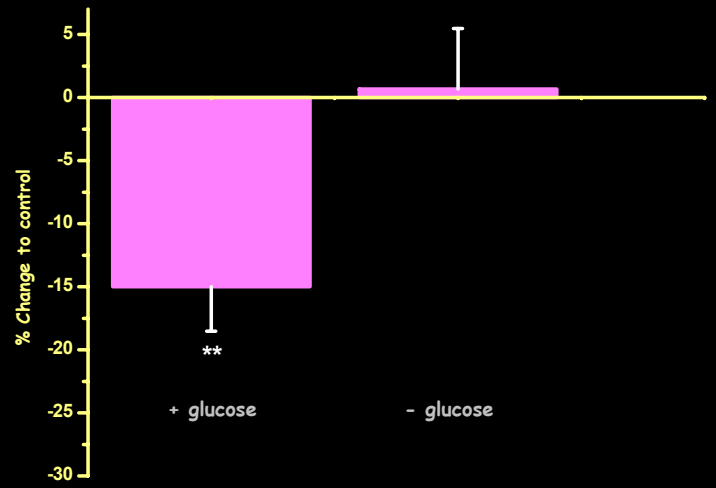



Panitumumab did not affect cell cycle, apoptosis or necrosis

**1:** Caco-2 cells treated with IgG antibody
**2:** Caco-2 cells treated with panitumumab 80 μg/ml
**3:** DLD-1 cells treated with IgG antibody
**4:** DLD-1 cells treated with panitumumab 80 μg/ml
**5:** HT-29 cells treated with IgG antibody
**6:** HT-29 cells treated with panitumumab 80 μg/ml,
**7:** positive control for beclin 1, MCF-7 cells.

Giannopoulou *et al.*, Autophagy: novel action of panitumumab in colon cancer, 2009.

EGFR may interact and stabilize the sodium/glucose co-transporter 1 (SGLT1) maintaining the intracellular glucose levels and preventing autophagic cell death (Weihua *et al.*, 2008).




The glucose deprivation may induce oxidative stress in breast cancer cells (MCF-7)

↓

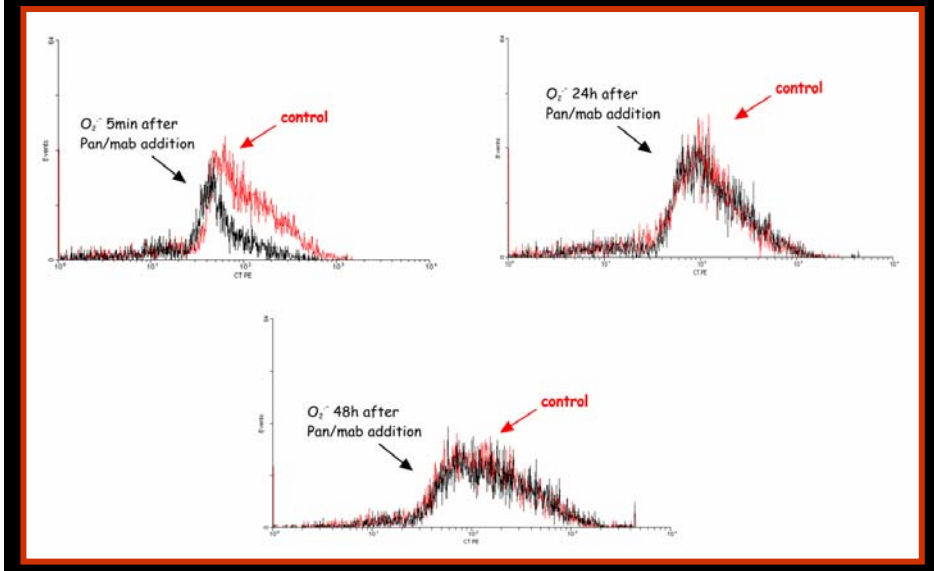
Accumulation of pro-oxidants (superoxide and hydrogen peroxide)

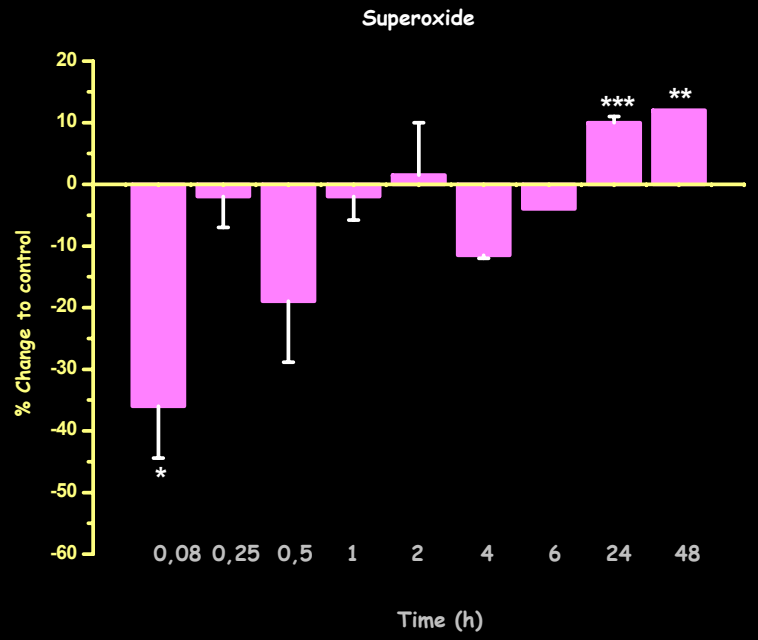
↓

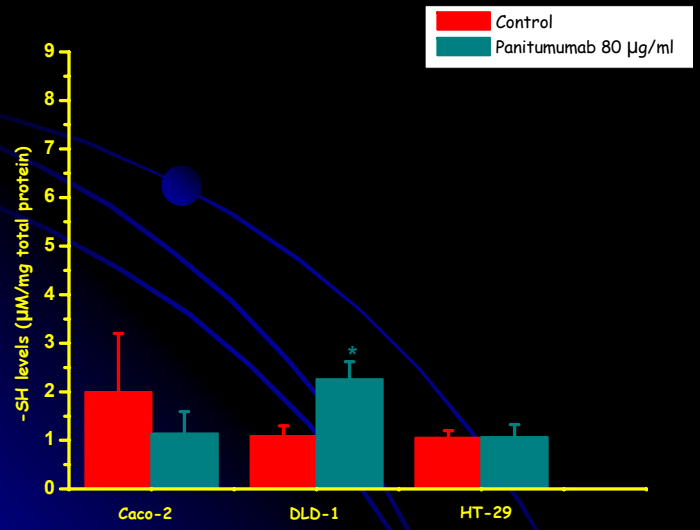
Activation of signal transduction pathways like JNK1, ERK1/ERK2

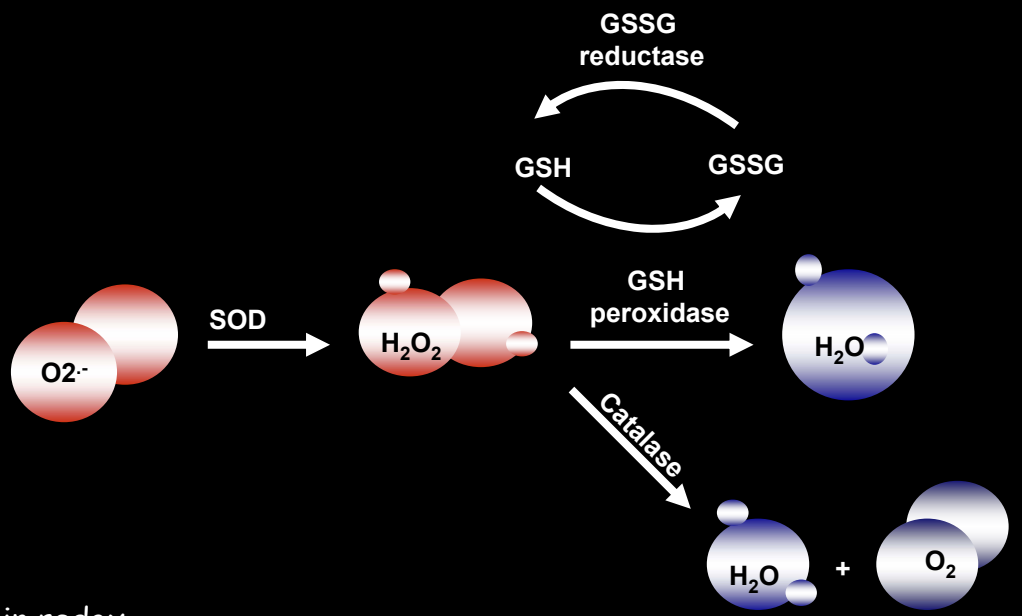
↓

Activation of AP-1 and cell death



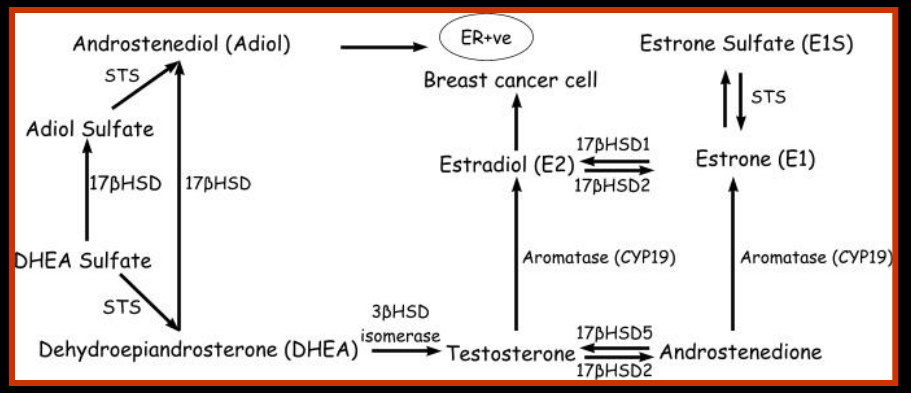

Giannopoulou *et al.*, The implication of Panitumumab in redox status of colon cancer cells, (submitted).

Clin. Oncol. Lab., Dep. of Oncology, University of Patras

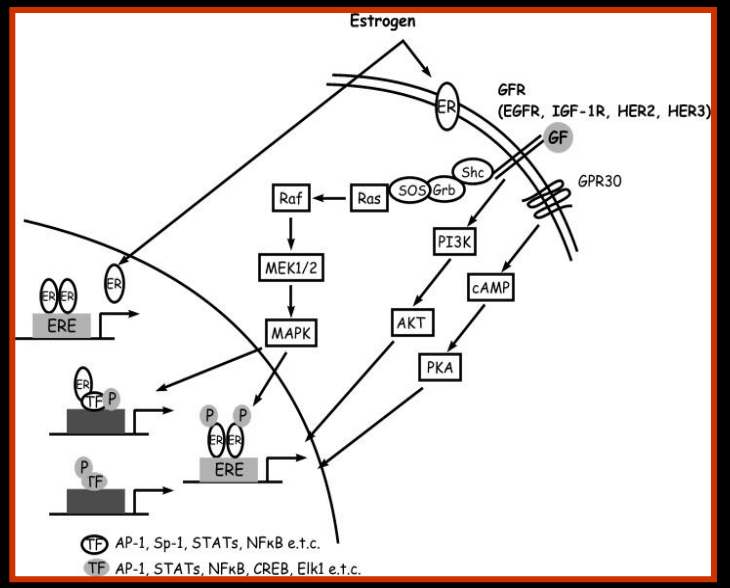
# II.A. Dual targeting of EGFR and aromatase in non small cell lung cancer (NSCLC)

Exemestane: Irreversible steroidal aromatase inhibitor. Approved for postmenopausal women with breast cancer after treatment with tamoxifen.

Erlotinib: Small molecule EGFR inhibitor. Approved for patients with advanced NSCLC resisting to chemotherapy and in combination with gemcitabine for patients with advanced pancreatic cancer without previous treatment.

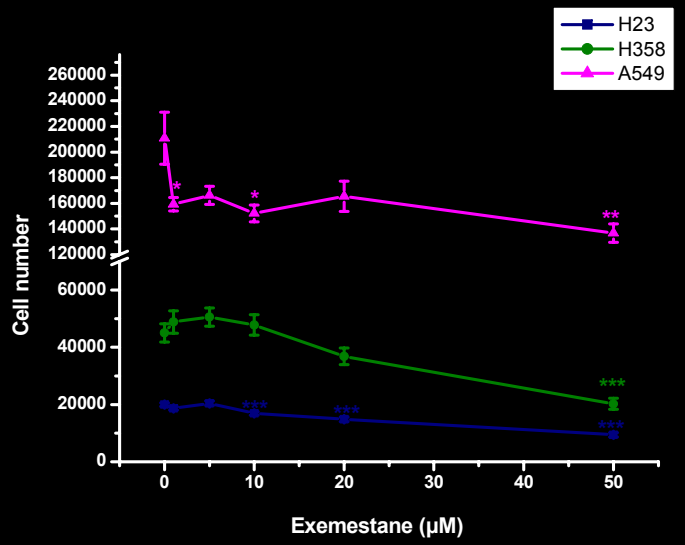


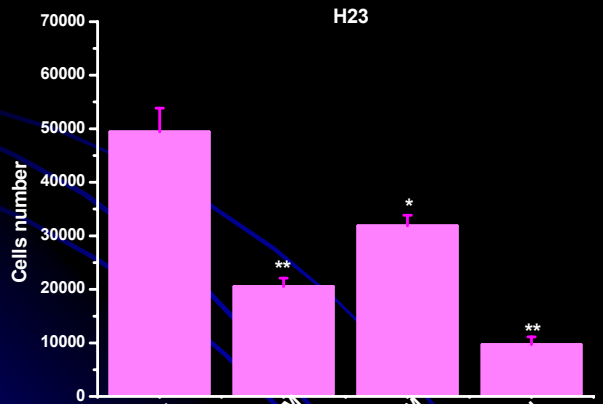

Subramaniam *et al.*, 2007

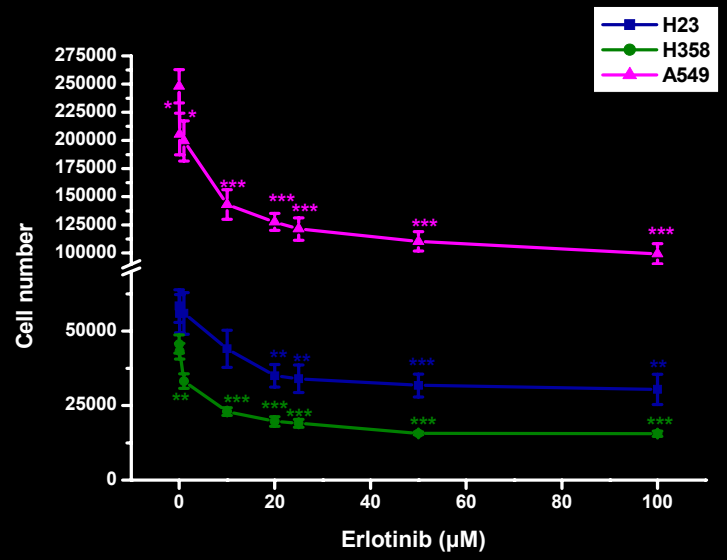



Yamagushi, 2007

| | H23 | A549 | H358 |
|---|---|---|---|
| Aromatase levels | low | high | low |
| EGFR levels | moderate | high | low |
| EGFR mut. | wt | wt | wt |
| KRas | mut. | mut. | mut. |
| PTEN | mut. | wt | wt |

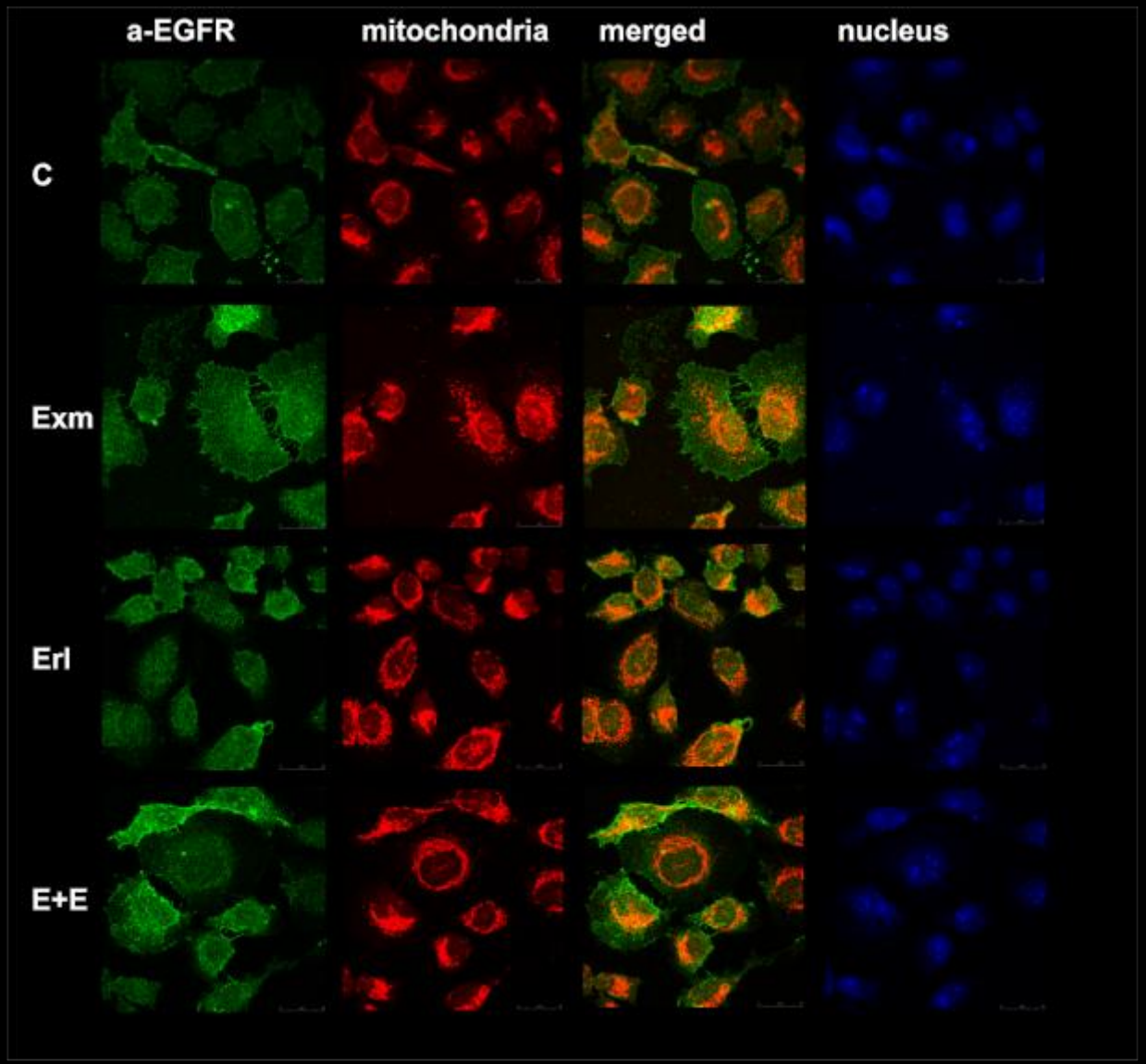



Clin. Oncol. Lab., Dep. of Oncology, University of Patras

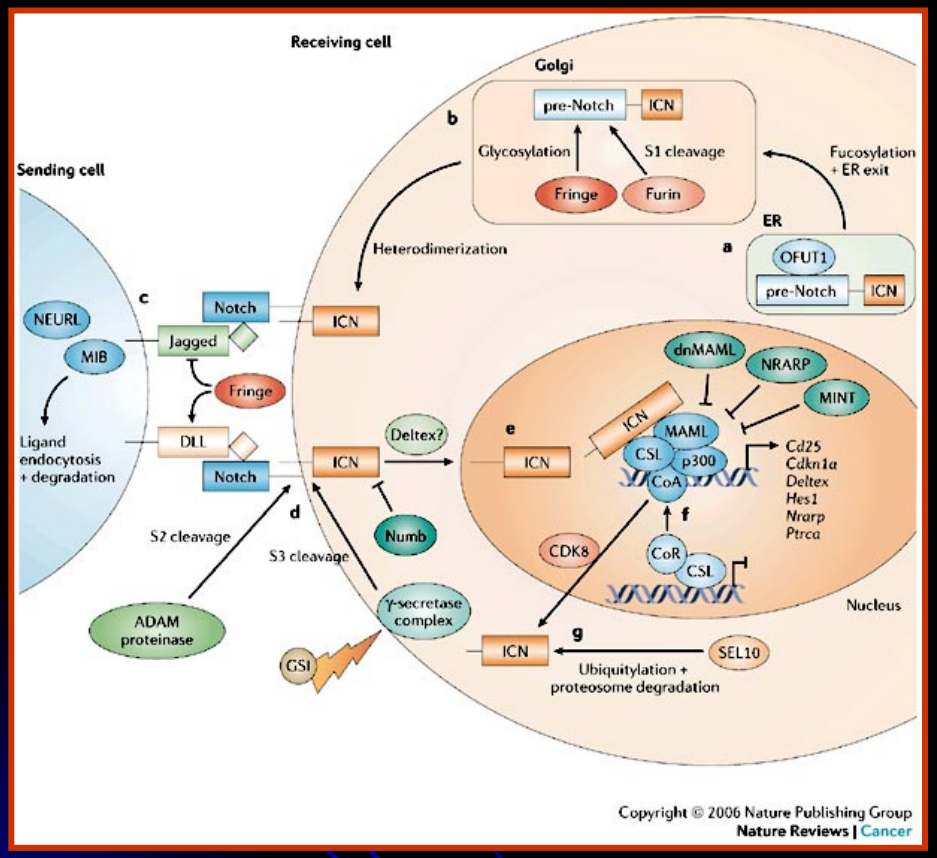
In H23 cells, a translocation of EGFR from membrane to mitochondria was observed after treatment of cells with exemestane and erlotinib. This effect is related to apoptosis and autophagy.




Koutras *et al.*, Antiproliferative effect of exemestane in lung cancer cells, 2009.

Kritikou *et al.*, Combined targeting of Aromatase and Epidermal Growth Factor Receptor in Non-Small-Cell Lung Cancer, (submitted).

# II.B. Dual targeting of EGFR and Notch in non small cell lung cancer (NSCLC)




Grabher *et al* 2006

- Notch may behave as an oncogene or a tumor suppressor gene. Both behaviors for Notch have been described in lung cancer.
- Notch has a growth promoting function in NSCLC, whereas in SCLC exerts an inhibitory effect.

Collins *et al.*, 2004; Westhoff *et al.*, 2009

Cell lines: H23, H661, A549 (wild type EGFR) and HCC827 (mutated EGFR).

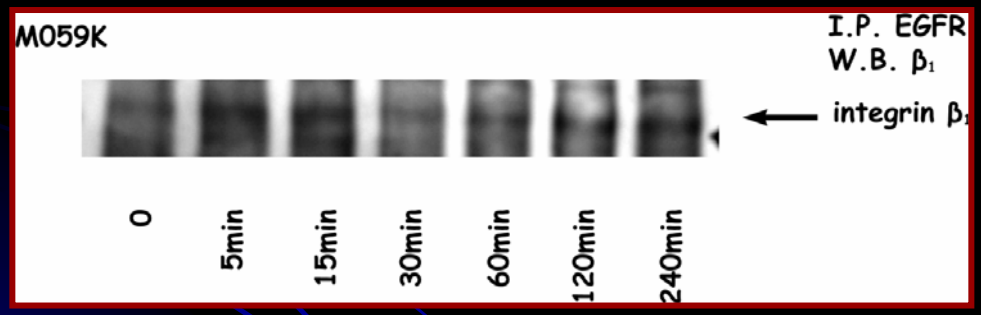
Inhibitors: Erlotinib and DAPT (inhibitor of γ-secretase).

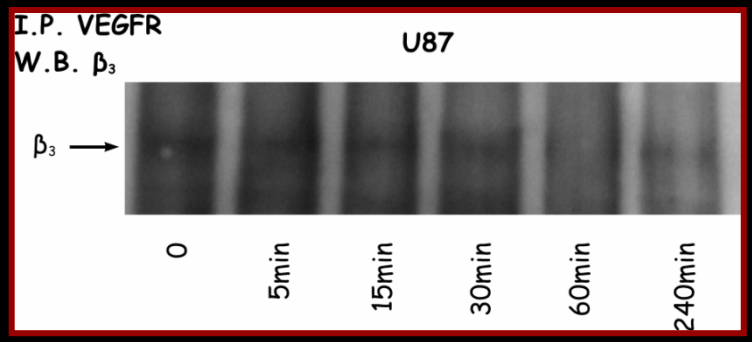
Topics: cell death (apoptosis, necrosis, autophagy, cell cycle arrest) and type of migration.

# III. Combined targeting of HER, VEGFR and PDGFR in glioblastoma cells

Sunitinib (Sutent): an oral, multi-tartgeted TK inhibitor (VEGFR, PDGFR and c-KIT) approved for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors.

- Lapatinib, sunitinib and their combination decreased cell proliferation of U87 and M059K through increasing apoptosis. A synergy effect was observed in both cell lines.
- Both of tested agents as well as their combination decreased cell migration.




Lapatinib and sunitinib affected the migration of glioma cells, through a mechanism implying interruption of growth factor receptor-integrin complexes formation.

Giannopoulou *et al*., An in vitro study, evaluating the effect of sunitinib and/or lapatinib on two glioma cell lines, 2010
Dimitropoulos *et al*., The effects of anti-VEGFR and anti-EGFR agents on glioma cell migration through implication of growth factor with integrins, (accepted).

# Current goals

- Screening of abs and TKIs of HER family for induction of autophagy in order to improve their antitumor effect.
- Study of the effect of tested agents in intracellular molecules.
- The evaluation of Notch and EGFR inhibition in NSCLC treatment.
- How the disruption of GFRs with integrins affects cell migration?
- The verification of *in vitro* results in fresh frozen and paraffin embedded tissues.

• Basil Rigas, MD, PhD,
Professor of Medicine/ Chief,
Division of Gastroenterology/ Chief,
Division of Cancer Prevention,
Department of Medicine,
Stony Brook University, USA

• Laboratory of Anatomy-Histology-Embryology
Dionysios J. Papachristou, MD, PhD
Pathologist Assistant Professor, Dept. of Anatomy-Histology-Embryology Univ. of Patras, School of Medicine, Patras, Greece, Assistant Professor (Adj.), Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA, USA

• Laboratory of Molecular Pharmacology
Evangelia Papadimitriou, PhD
Associate Professor of Molecular Pharmacology,
Dept. of Pharmacy, Univ. of Patras, Patras, Greece

• Laboratory of Biological Chemistry,
Nikos Papanikolaou, PhD,
Associate Professor of Biological Chemistry,
Dept. of Medicine, Univ. Campus
Thessaloniki, Greece,

• Molecular Oncology Laboratory,
Cancer Biobank Center
Evangelos Briassoulis, MD, PhD,
Associate Professor of Oncology,
Dept. of Medicine,
Andreas Tzakos, PhD,
Assistant Professor of Organic Chemistry and Biochemistry, Dept. of Chemistry,
Univ. of Ioannina, Greece

• Biochemistry Laboratory,
Nikos Karamanos, PhD,
Professor of Biochemistry
Dimitrios Vynios, PhD,
Professor of Biochemistry,
Achilleas Theocharis, PhD,
Assistant Professor of Biochemistry,
Dept. of Chemistry,
Univ. of Patras, Greece

• Laboratory of Pharmacokinetic,
Gregory Sivolapenko, PhD,
Assistant Professor of Pharmacokinetic,
Dept. of Pharmacy, Univ. of Patras, Patras, Greece