Small Cell Lung Cancer (SCLC)

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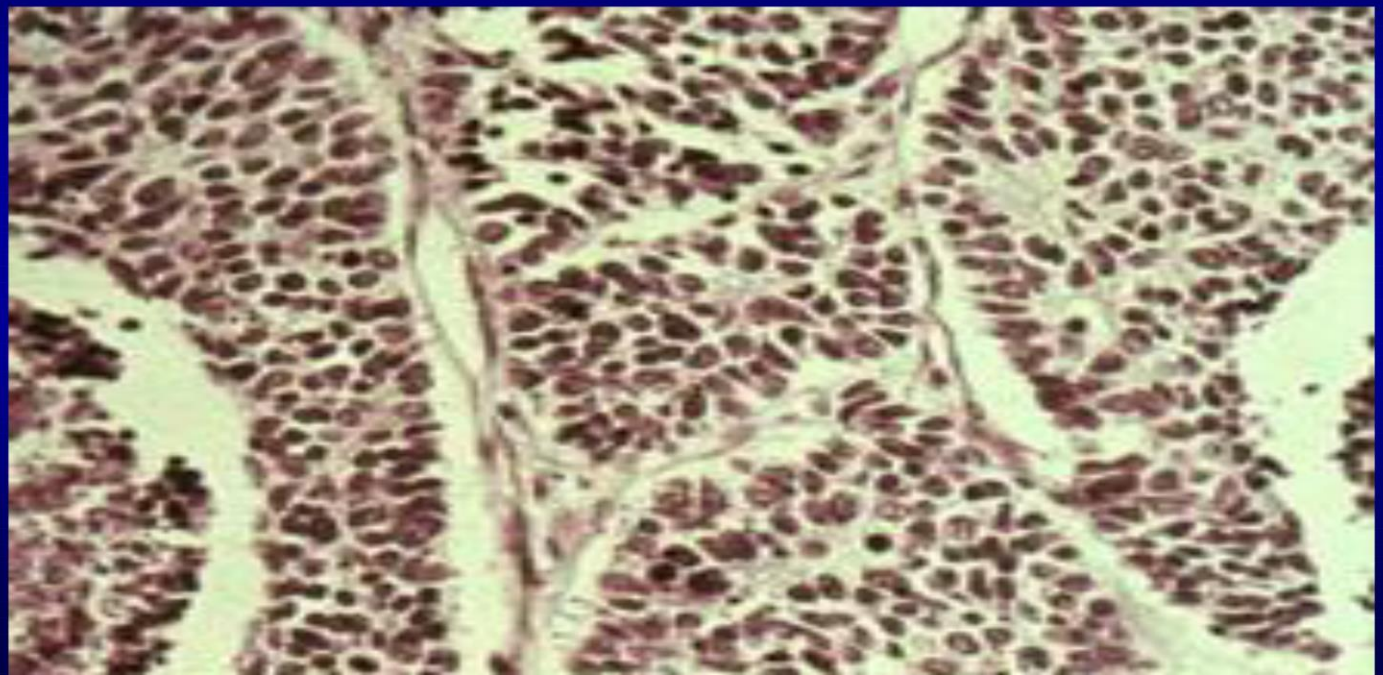
SCLC or oat cell carcinoma

- Kills approximately 25,000 patients in the U.S. annually.
- Is a neuroendocrine tumor.
- Is responsive to chemo- and radiation therapy, but relapse frequently occurs. The median survival time is less than one year.

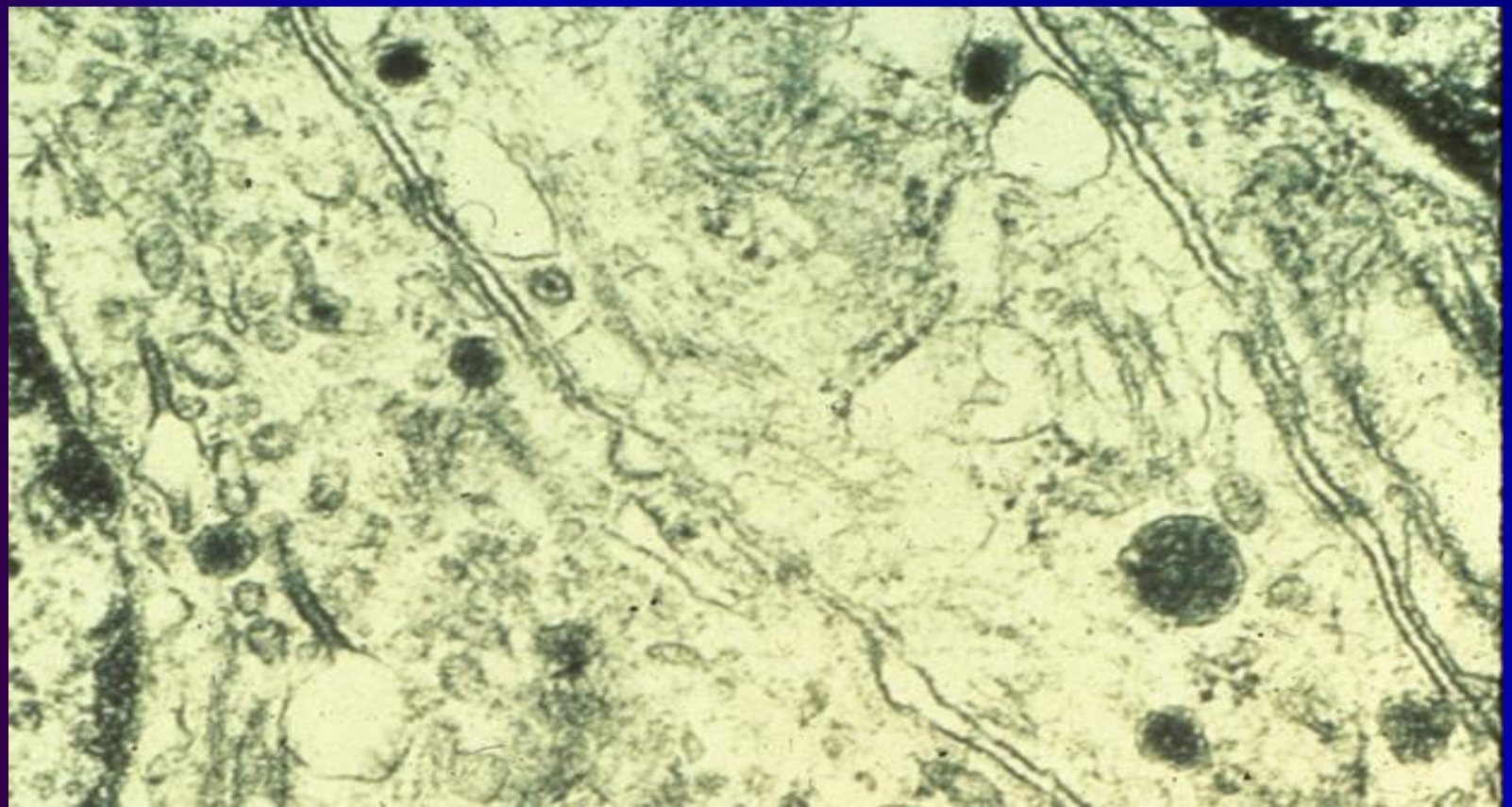
SCLC biopsy specimen

SCLC biopsy specimen

SCLC biopsy specimen



Neural enzymes, peptides and transmitters may be stored in the dense core neurosecretory granules associated with SCLC.



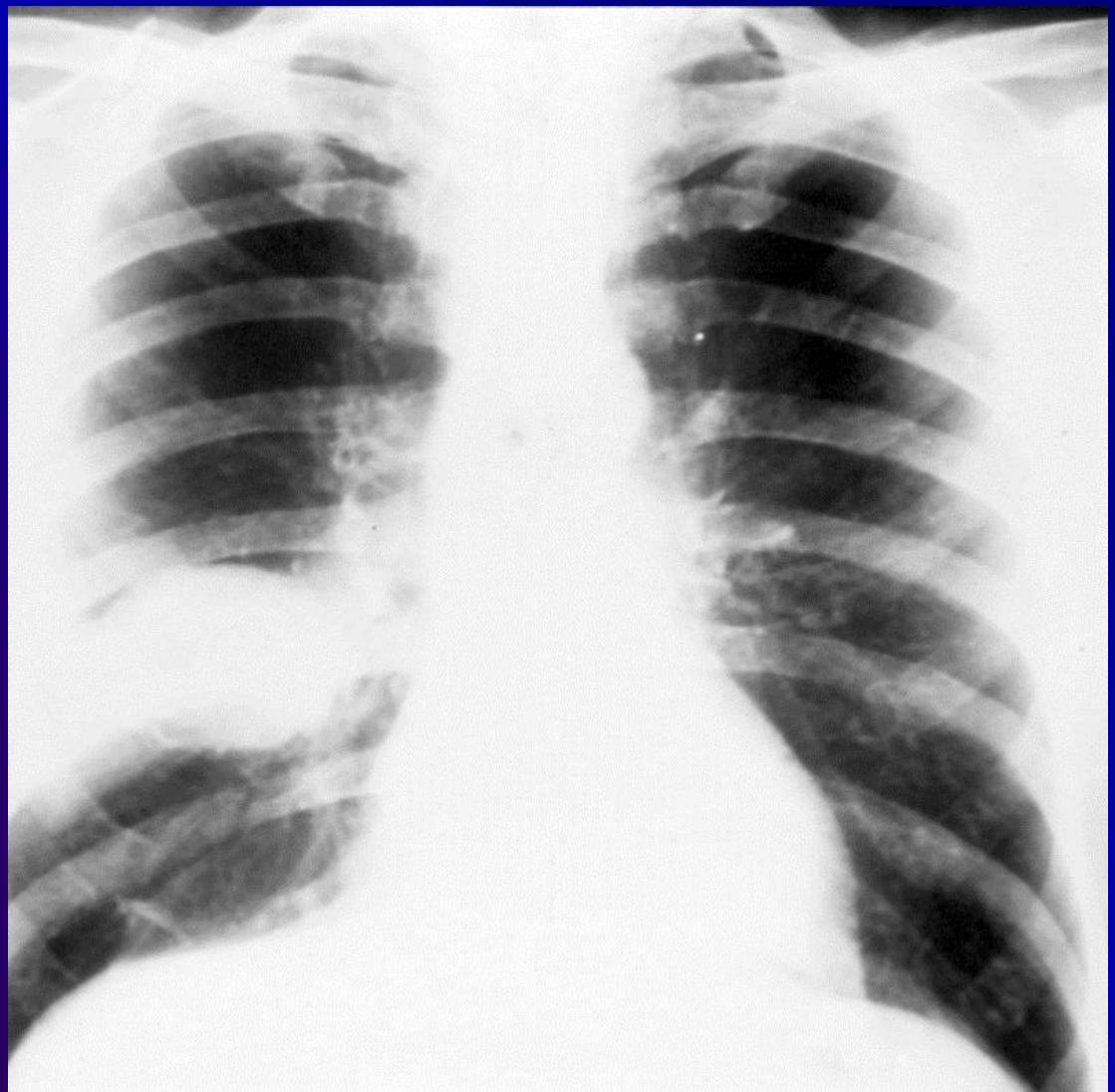
Lung cancer symptoms.

- Cough
- Chest pain
- Shortness of breath
- Pneumonia or bronchitis
- Bloody sputum.

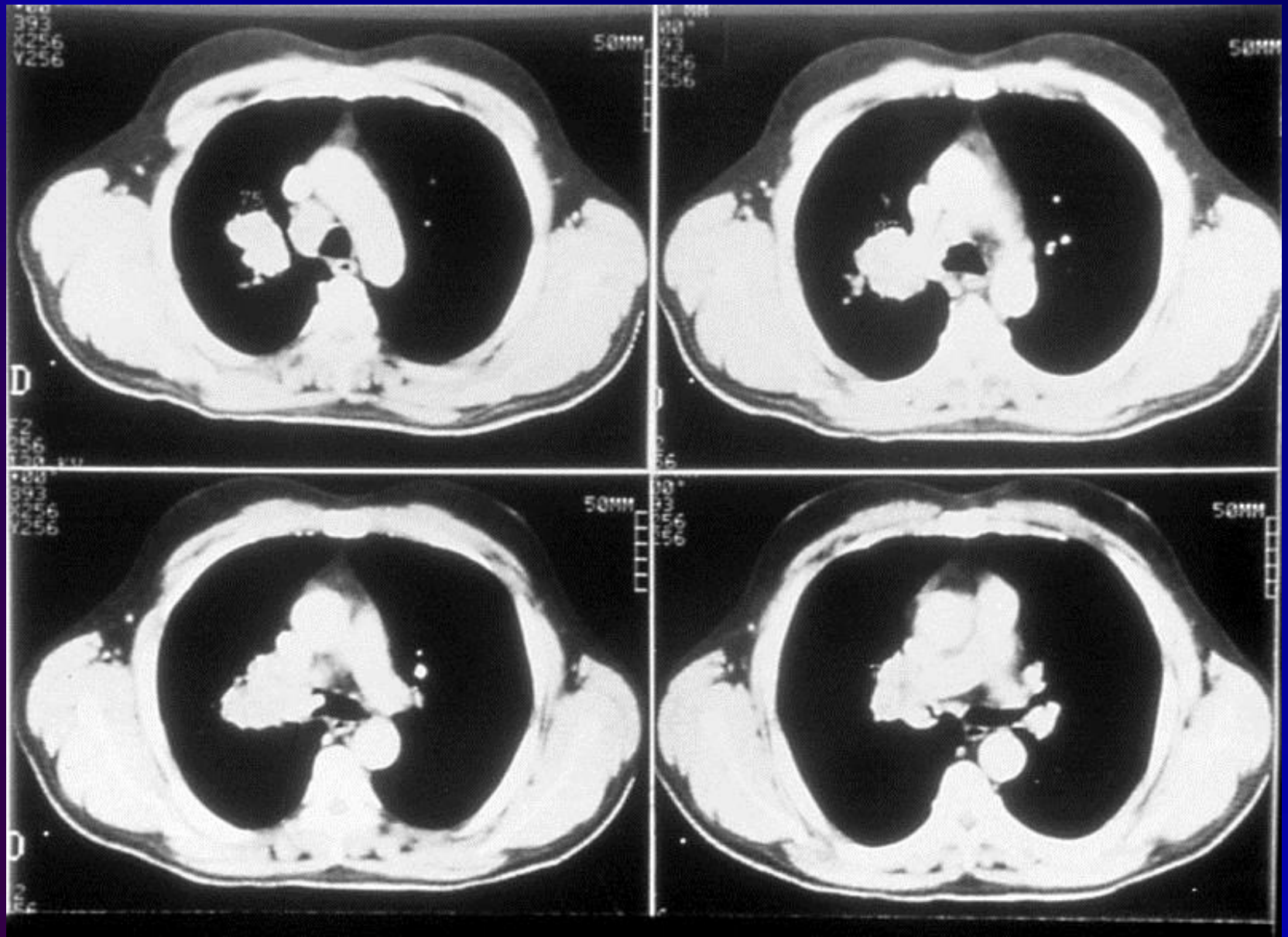
Diagnosing lung cancer.

- Chest x-ray
- Bronchoscopy
- Needle aspiration
- Thoracentesis
- Thoracotomy
- Spiral CT

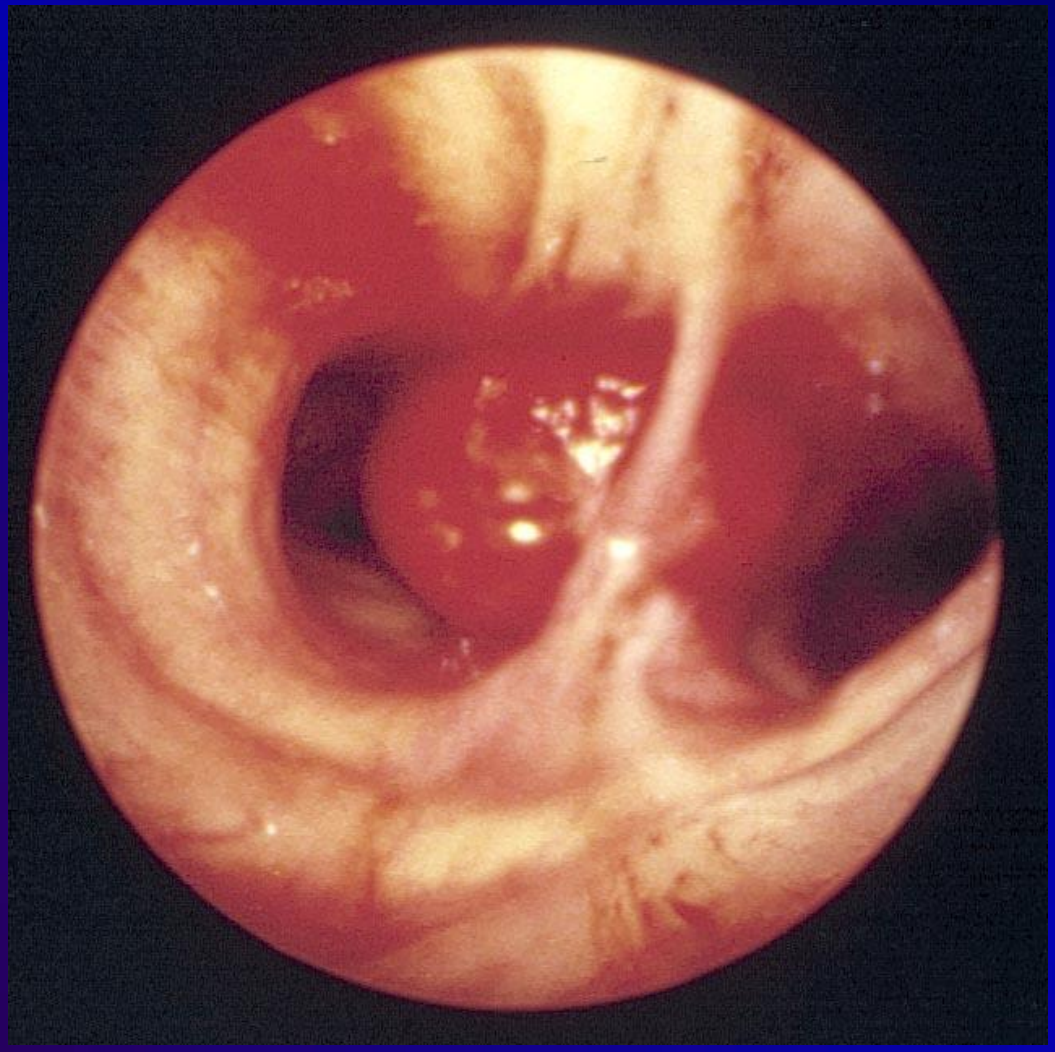
Lung cancer: chest X-ray



Lung cancer: chest CT-scan



Lung cancer: bronchoscopy



Staging lung cancer.

- CT scan
- MRI
- PET scan
- Radionuclide scanning
- Bone scan
- Mediastinoscopy

SCLC patient survival.

Treatment	Survival
Surgery	6.5 months
Radiotherapy	10 months

Murren et al., Cancer: Principles and Practice of Oncology (2001) pp 983-1018

SCLC chemotherapy

Active agents include:

Carboplatin	Cisplatin
Cyclophosphamide	Docetaxel
Doxorubicin	Epirubicin
Gemcitabine	Irinotecan
Ifosfamide	Paclitaxel
Teniposide	Topotecan
Vincristine	Vindesine
Vinorelbine	VP-16

Combination chemotherapy

Active combinations include:

Cyclophosphamide, doxorubicin, VP-16 (CDE)

C, doxorubicin, vincristine (CAV)

E, cisplatin (EP)

VP-16, ifosfamide, P (VIP) and

I, carboplatin, VP-16 (ICE)

Combination chemotherapy plus radiotherapy.

Radiotherapy: 40 Gy/20 F. EP: VP-16, cisplatin

The chemoradiation package increased median survival from 10 to 34 months and 5-year survival from 6% to 30%.

SCLC relapse.

- Initially, SCLC often responds to chemotherapy
- After relapse, chemotherapy is often ineffective
- Field effect

SCLC metastasis

- Liver (27%)
- Bone (41%)
- Adrenals (31%)
- Lymph nodes, mediastinal (80%)
- Brain (14%)

SCLC carcinogenesis.

- Initiated by tobacco smoke carcinogens
- Is SCLC derived from neuroendocrine Kulchitsky cells or stem cells?

Akt activation by nicotine and NNK (4-(methylnitrosamino)-1-(3-pyridyl-1-butanone).

- Nicotine binds to acetylcholine receptors on lung cancer cells causing Akt phosphorylation.
- NNK forms DNA adducts and if cells do not undergo apoptosis, DNA mutations accumulate. NNK causes Akt phosphorylation.

West et al., J. Clin. Invest. 111:81 (2003).

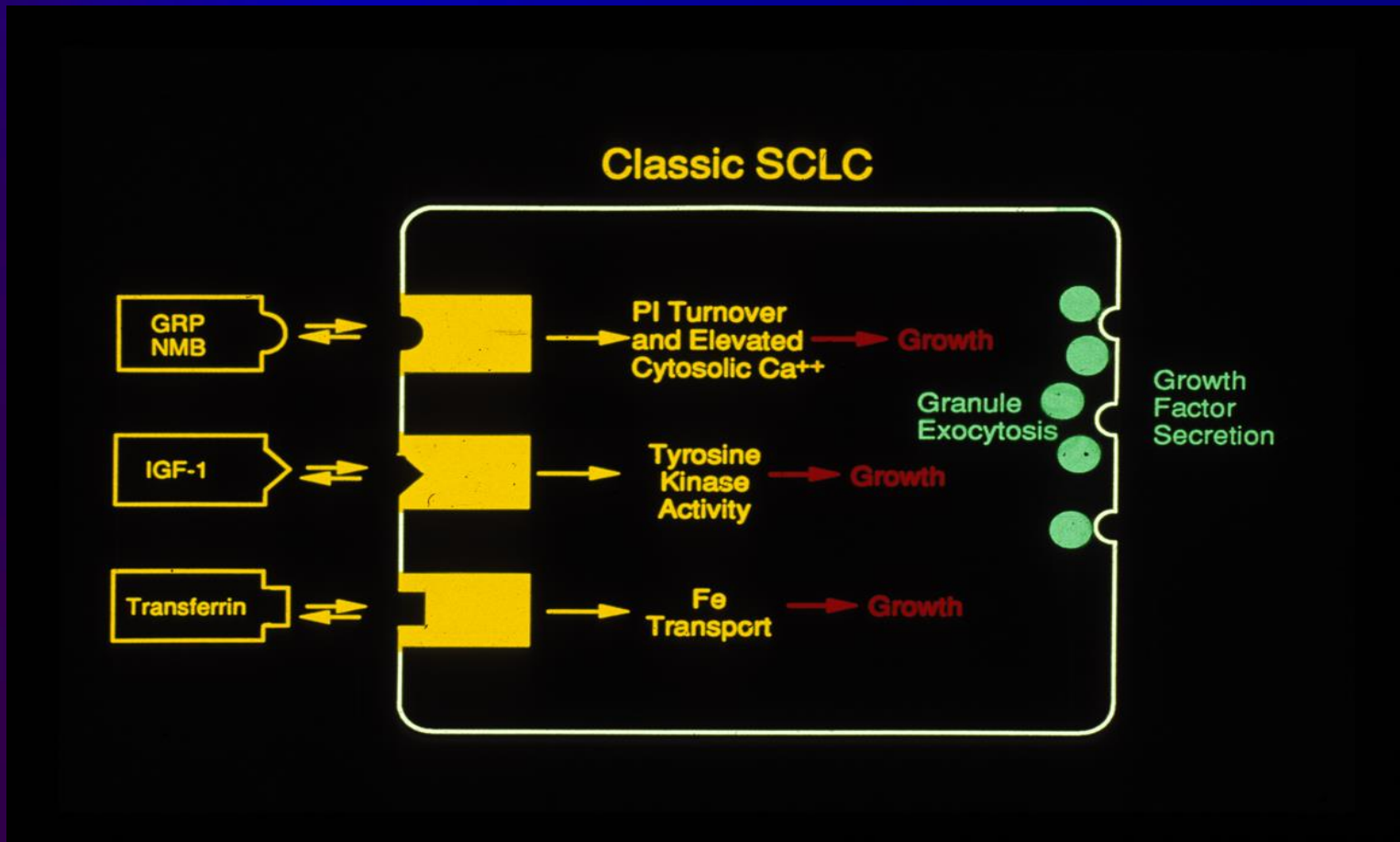
NNK is metabolized to NNAL which is excreted into the urine.

- NNAL is a unique metabolite which can be measured in the urine of patients by gas chromatography. Its presence is indicative of exposure to cigarette smoke.
- NNAL is increased in non-smokers who breathe in cigarette smoke.

SCLC cell lines.

- Bone marrow aspirates were obtained from patients and mononuclear cells collected.
- Lymph node aspirates and other solid tumors were mechaniccaly dissociated and cell suspensions obtained by mincing and passing through 60 gauge steel mesh.
- The cells were cultured in a serum free medium containing selenium, IGF-I and transferrin. SCLC cells grew as suspension cultures in approximately 15% of the cases.

Numerous lung cancer cell lines were isolated from biopsy specimens.



SCLC cell lines.

- SCLC cells survive because they make their own autocrine growth factors.
- From 1982-4, NCI established 31 SCLC cell lines. Subcutaneous injection of each of the 31 SCLC cell lines into nude mice resulted in tumor formation.
- The classic SCLC cell lines had high levels of dopa decarboxylase (DDC: 2-657 units/mg), bombesin (BB: 0.2-22 pmol/mg) and neuron specific enolase (NSE: 1200-18000 ng/mg).

Carney et al., Cancer Res. 45:2913 (1985).

SCLC cell lines.

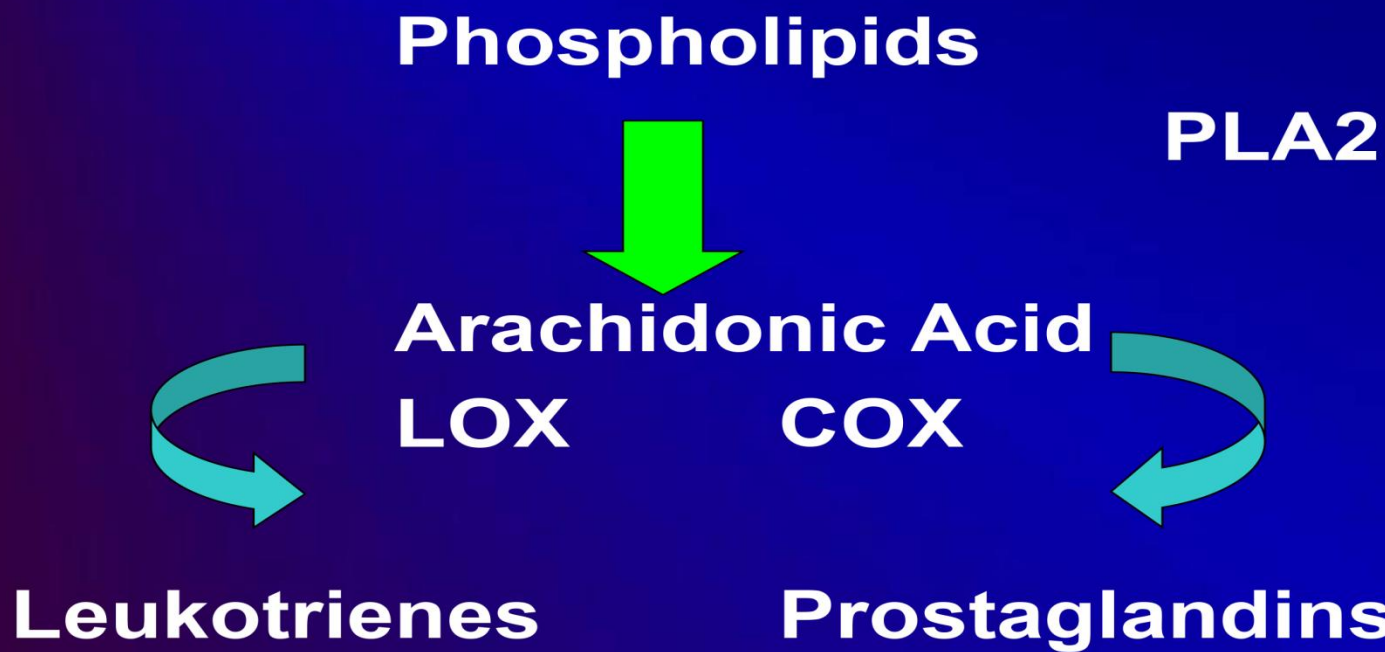
- Over a 20 year perioid, NCI established 113 SCLC cell lines and 110 NSCLC cell lines.
- A subtype of SCLC is the variant phenotype, which has low levels of DDC, BB and NSE.

Phelps et al., J. Cell Bioc. Supp. 14:32(1996).

The A/J mouse is one of the few reliable lung cancer animal models.

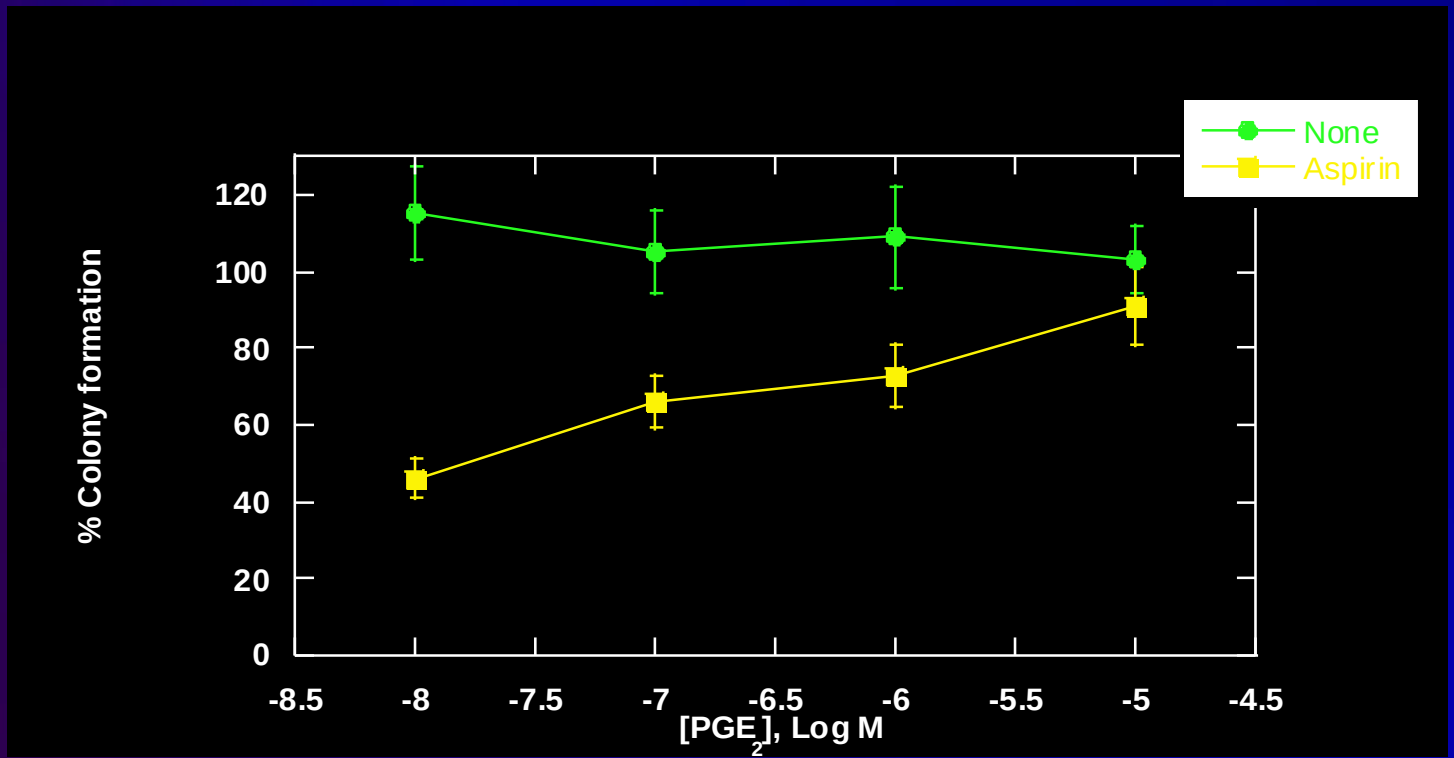
Leukotrienes and Prostaglandins

Lung Cancer cells produce LTs and PGs.



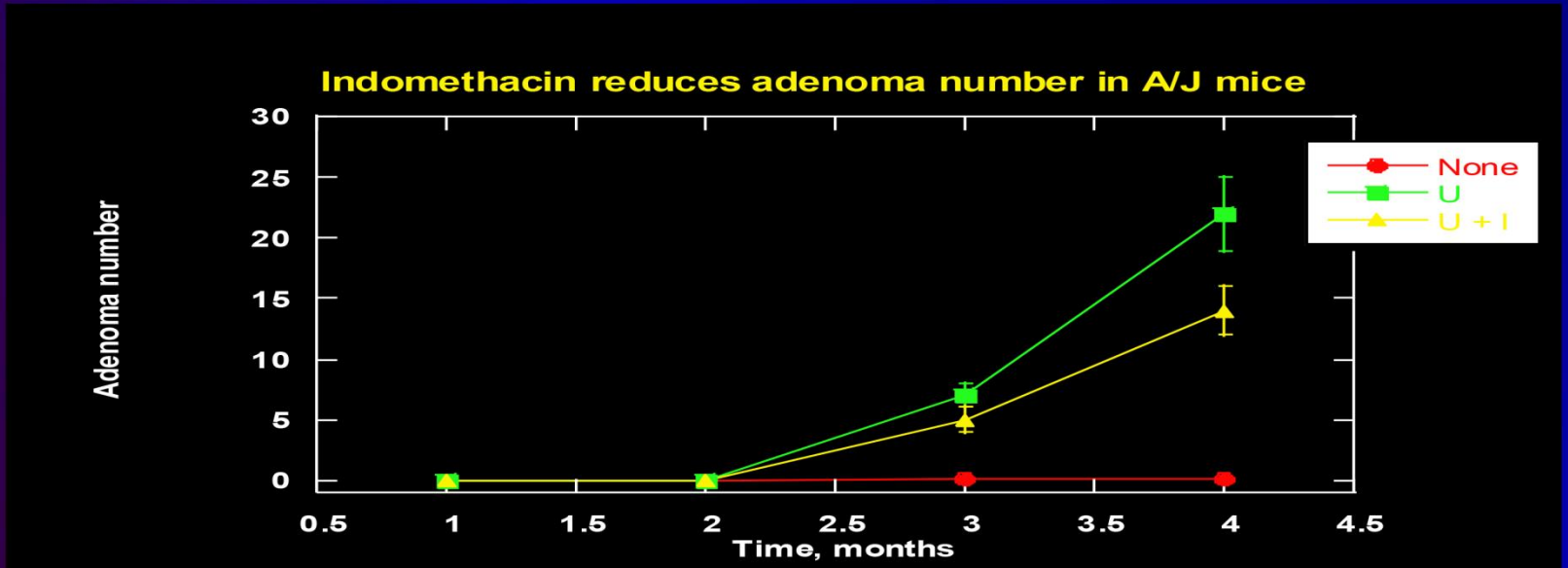
NSAIDS inhibit proliferation

Aspirin and indomethacin, which are non-steroidal anti-inflammatory drugs (NSAIDs), inhibit lung cancer growth and the growth inhibition is reversed by prostaglandin (PG)E_2.



Indomethacin and adenomas

Indomethacin reduces tumor number.
Indomethacin, which blocks COX-1 and COX-2, prevents lung adenoma formation in A/J mice.



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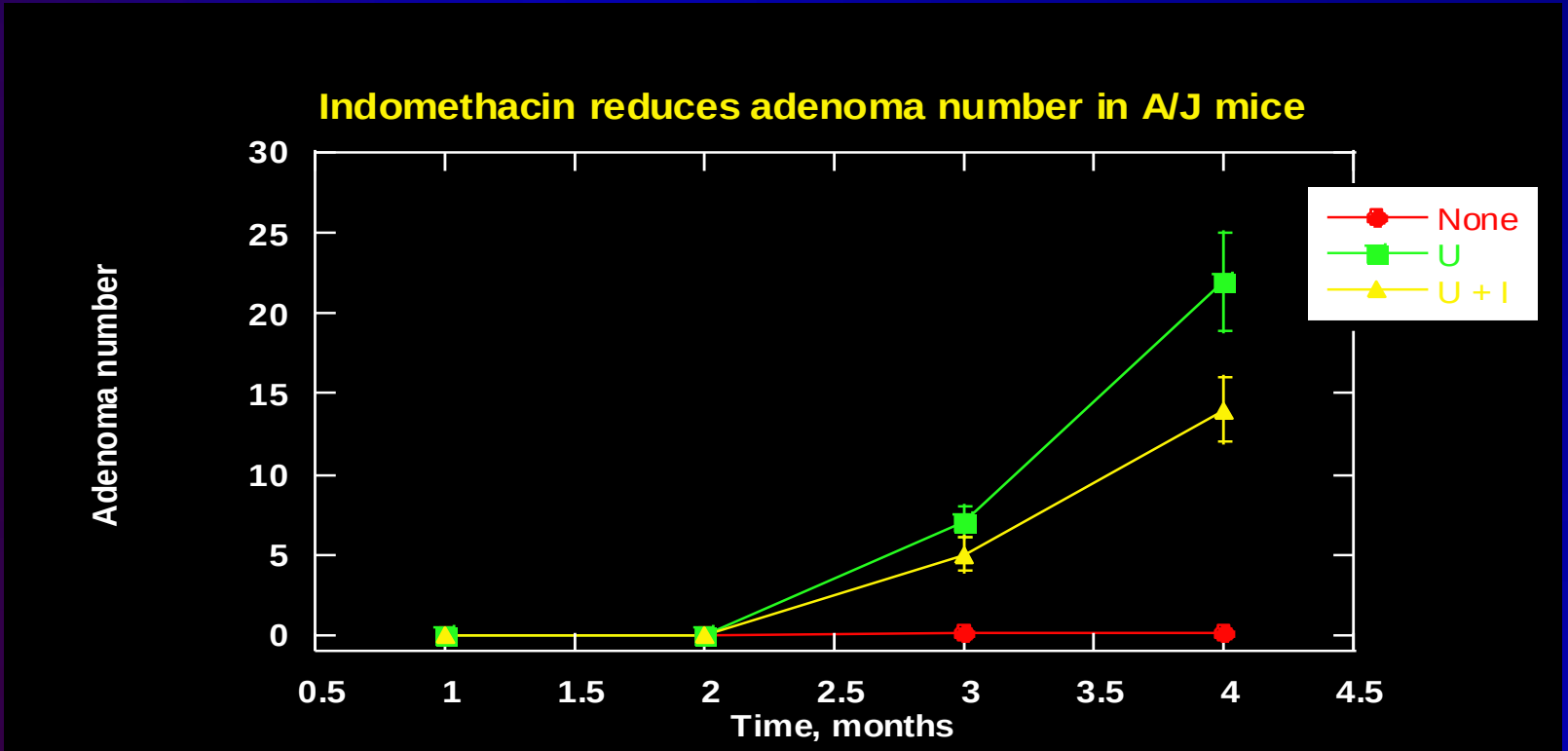
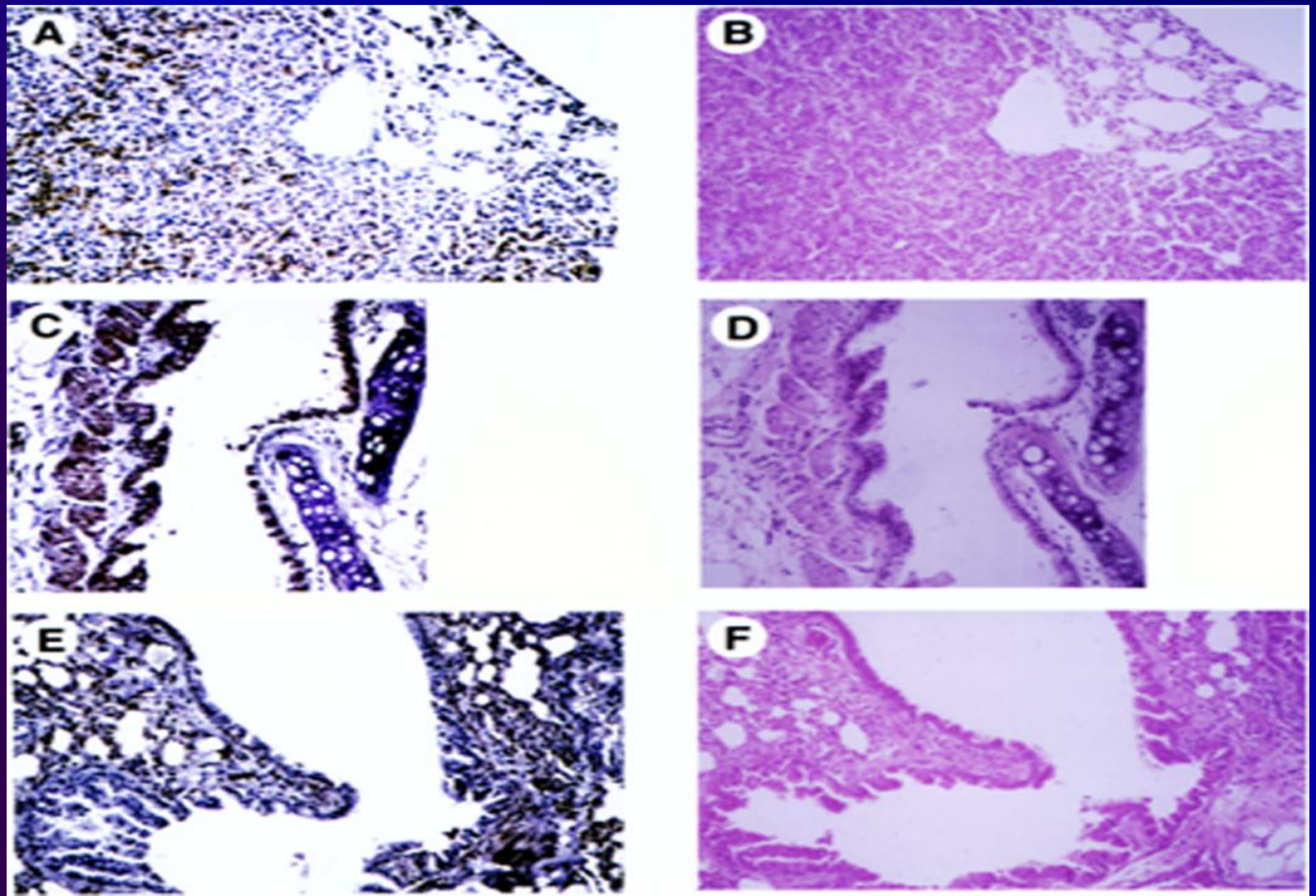


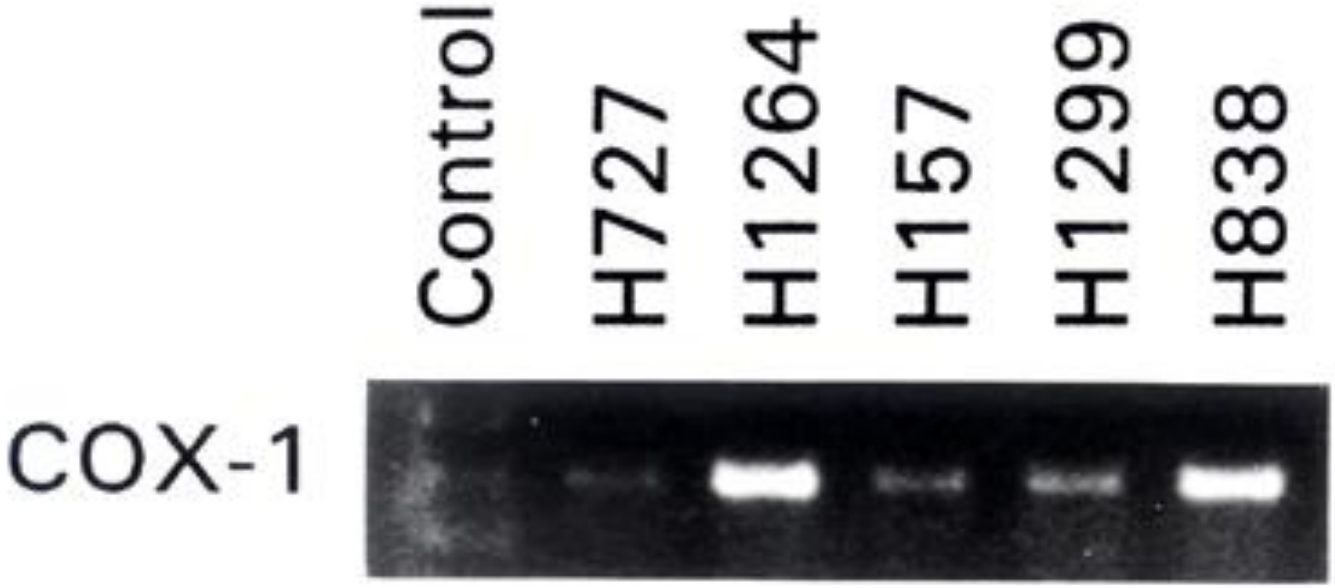
COX-2 immunostaining in the A/J mouse lung.



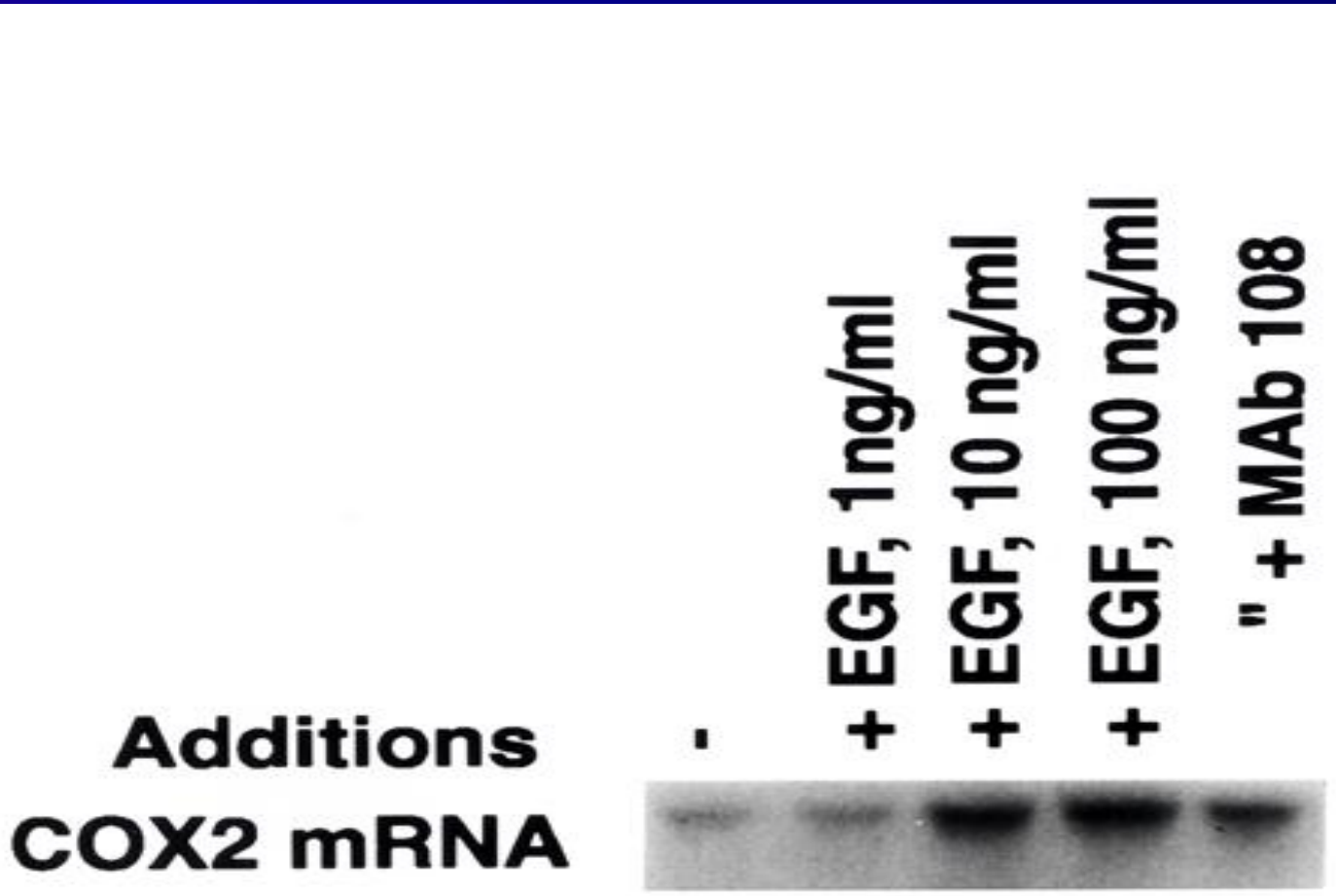
Lung compartments and COX-2.

- Bronchus-epithelial cells show intense staining with moderate staining in the muscle but not cartilage.
- Bronchioles-Moderate staining in epithelial cells.
- Alveoli-Moderate staining in type 2 cells.
- Adenoma-scattered cellular staining.

COX-1 is expressed in lung cancer cells.

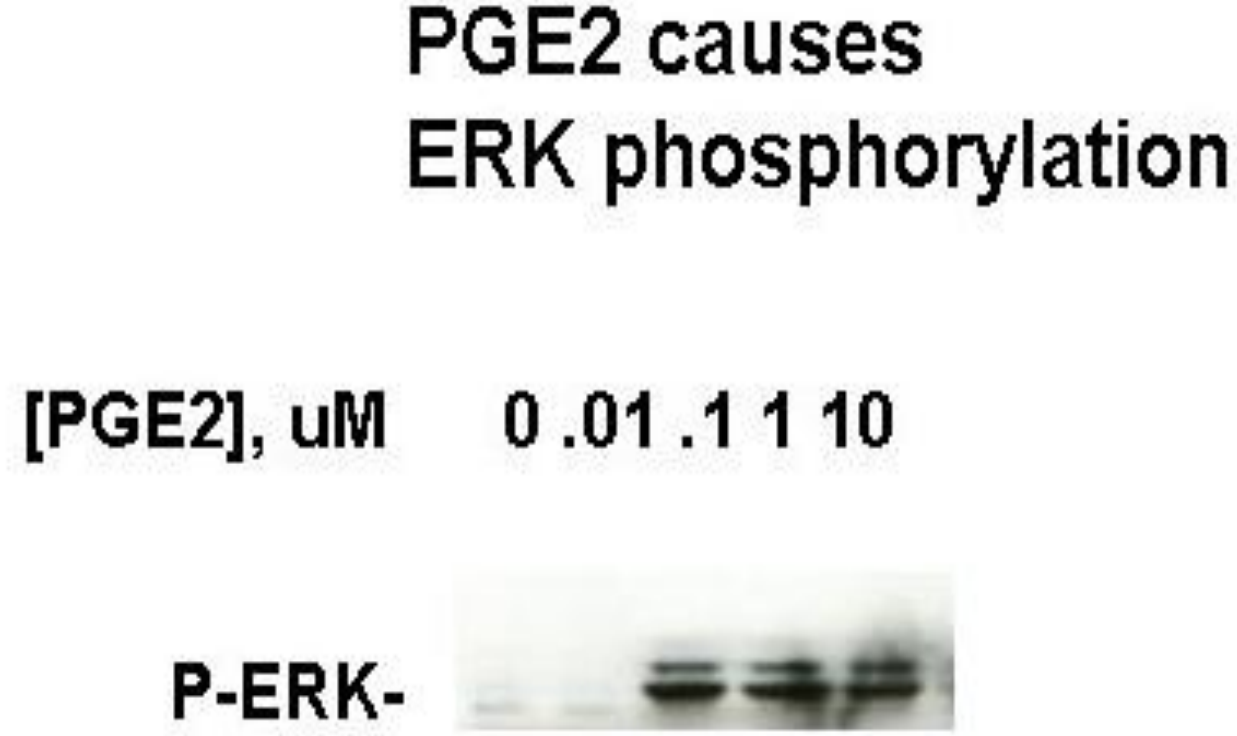


EGF causes increased COX-2 expression.



Transactivation of EGF-R caused by PGE2 is reversed by AH6809.

PGE_2 causes ERK tyrosine phosphorylation.

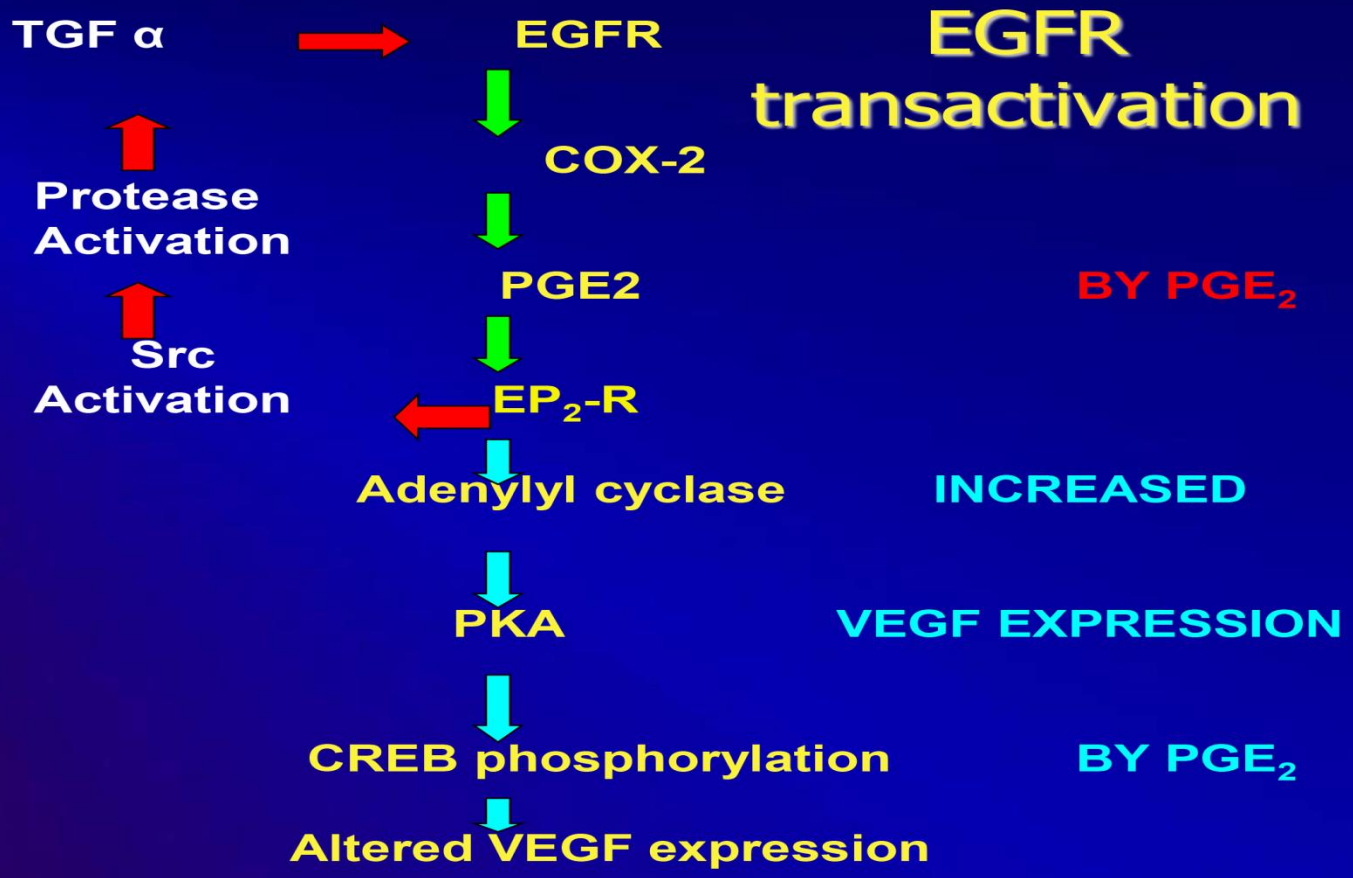


VEGF mRNA is increased by PGE_2 in a PKA-dependent manner

Addition	Relative VEGF mRNA
None	100 ± 5
PGE_2, 1 uM	200 ± 17*
EGF, 0.1 ug/ml	185 ± 16*
H89, 50 uM	104 ± 3
PGE_2 + H89	110 ± 6

The mean value ± S.D. of 4 determinations is indicated; $p < 0.05$, *

PGE2 signal transduction



COX inhibitors.

- NSAIDs inhibit COX-1 and COX-2. COX-1 inhibition can result in side effects e.g. stomach ulcers.
- Celecoxib has selectivity for COX-2. Therefore its use is associated with minimal side effects.

Celecoxib, which is a selective COX-2 inhibitor, is in clinical trials for lung cancer (PDQ®Clinical Trials).

- Phase II randomized pilot chemoprevention study of celecoxib in heavy smokers at high risk or primary or second primary lung cancer.
- Phase II randomized study of preoperative paclitaxel and carboplatin with or without celecoxib in patients with stage III non-small cell lung cancer.

http://cancer.gov

SCLC molecular abnormalities.

- Rb inactivation (90%)
- P53 inactivation (90%)
- FHIT inactivation (75%)
- Bcl2 overexpression (85%)

p53.

- Mediates the G1 to S-phase checkpoint of the cell cycle.
- Drives programmed cell death or apoptosis after DNA damage.

Rb mutations (truncations, deletions, nonsense mutations and splicing abnormalities) occur in many lung cancer patients.

- Usually the wild type allele is lost especially in SCLC. The Rb protein is absent or abnormal in 90% of the SCLC patients.

FHIT (fragile histidine triad)

- The FHIT gene is located on chromosome 3p14.
- The loss of FHIT protein expression is associated with smoking.
- Is FHIT a tumor suppressor gene associated with apoptosis?

BCL2 is overexpressed in approximately 85% of the SCLC tumors.

- BCL2 suppresses apoptosis and inhibits responses to chemotherapy and radiotherapy.
- Antisense-BCL2 therapeutic trials are being conducted (Genasense is an 18-mer phosphothioate oligonucleotide).

SCLC molecular abnormalities.

- Allelic loss (3p, 4p, 4q, 5q, 8p, 9p, 10q, 13q, 17p, 22q)
- Microsatellite instabilities (35%)
- MYC overexpression (30%)
- Stem cell factor, c-kit overexpression (30%)
- Bombesin/gastrin releasing peptide (BB/GRP) overexpression

Chromosome losses in SCLC include:

- 3p deletion is an early event and
- 5q, 13q and 17p deletions occur later.

Microsatellite alterations

- In lung cancer there is a laddering of short-tandem DNA repeat sequences at multiple loci.
- This laddering may result from mutations in DNA mismatch repair enzymes.
- This microsatellite instability may be useful for early diagnosis of lung cancer using sputum, bronchial washings or blood.

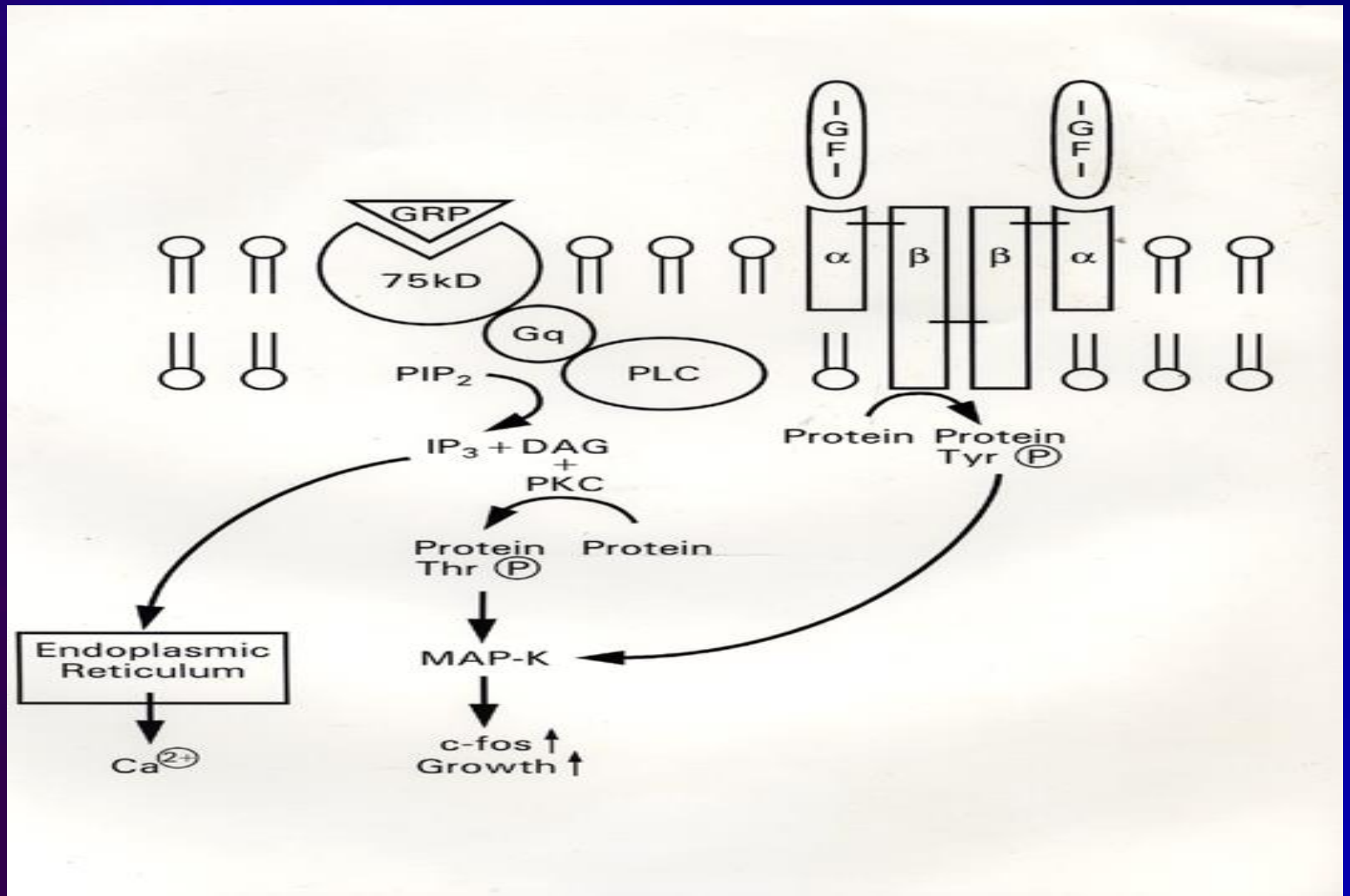
MYC

- N-MYC and L-MYC are are amplified in SCLC
- MYC heterodimerizes with MAX and functions as a transcription factor facilitating cell-cycle progression.

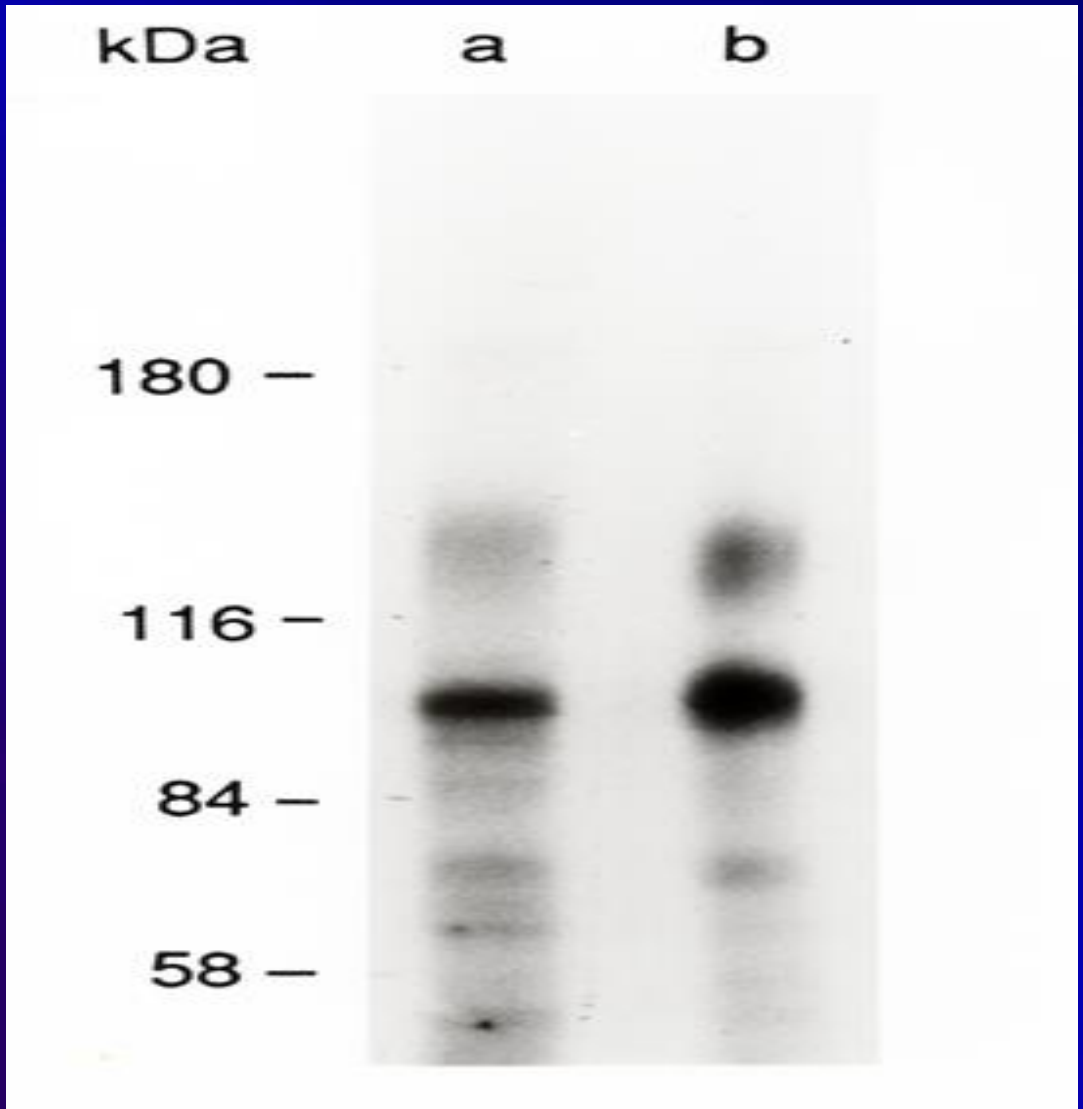
LKB1 inactivation

- **LKB1 is a serine/threonine kinase that is inactivated in approximately 50% of the SCLC patients.**
- **LKB1 causes phosphorylation of AMP activated protein kinase (AMPK) resulting in tumor growth suppression.**

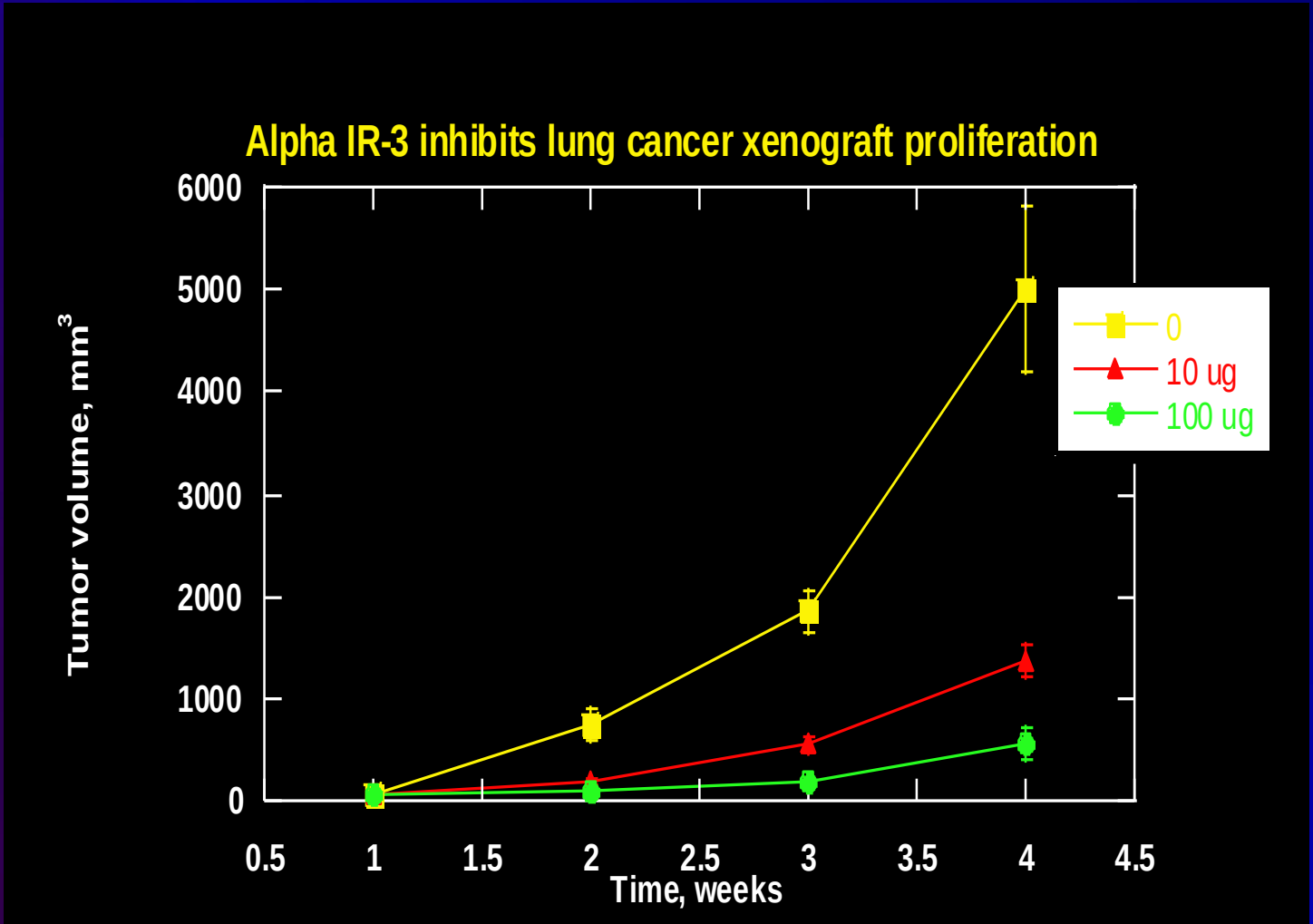
IGF-I binds to a 90 kDal subunit but causes signal transduction through a 130 kDal subunit



MAb αIR-3 recognizes the 90kDal subunit.



αIR-3 inhibits lung cancer xenograft proliferation.



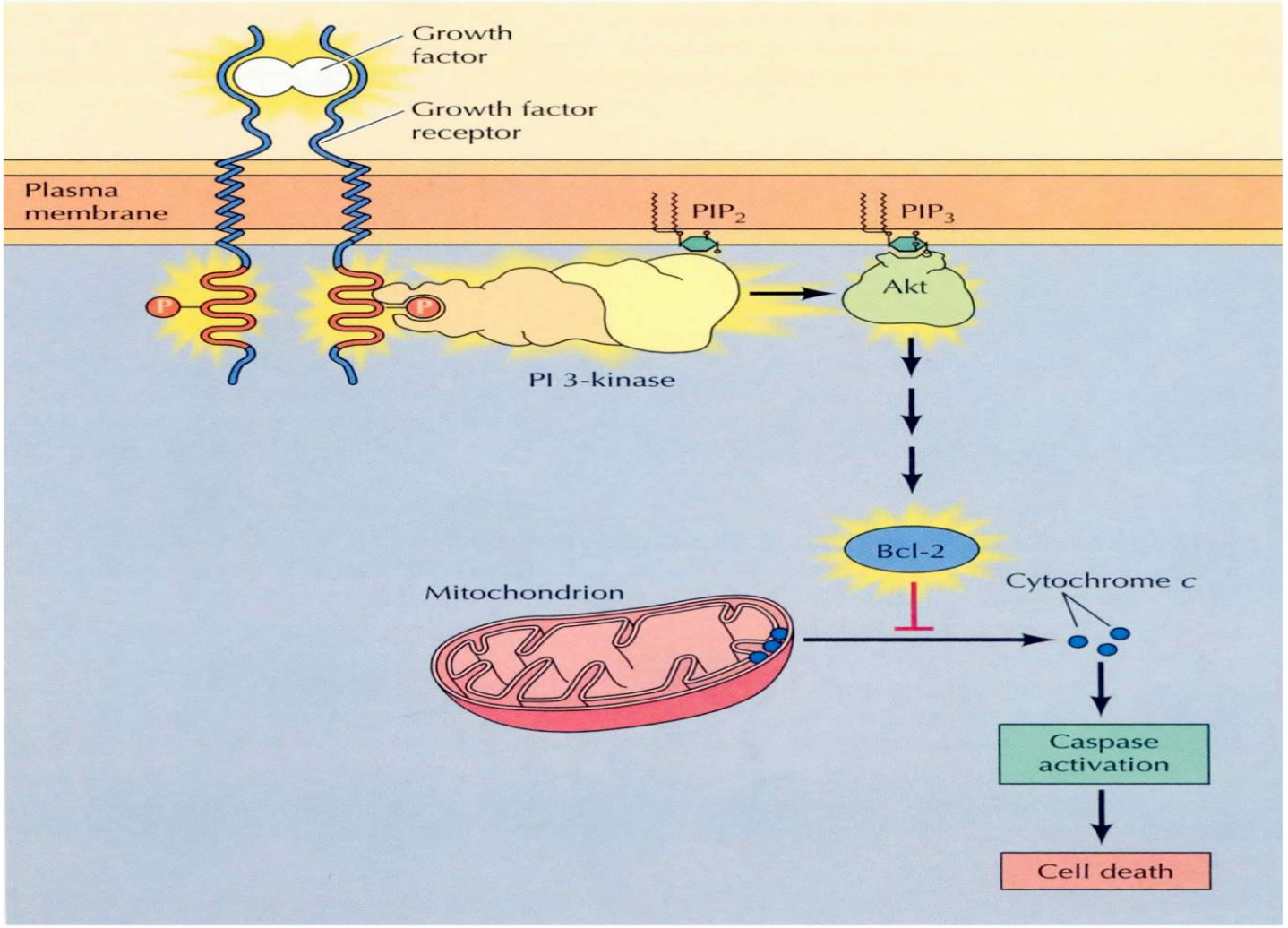
IGF-I enhances survival of SCLC cells.

- IGF-I and stem cell factor (SCF) activate phosphatidylinositol-3-kinase (PI3K)-Akt signaling enhancing cellular survival.
- Ly294002, a PI3K inhibitor, decreases the phosphorylation of AKT caused by IGF-I and SCF.

Krystal et al., Mol. Can. Ther. 1, 912 (2002).

Tyrosine kinase receptors cause increased cell survival.

Molecular biology of the cell; Alberts et al. 2001



The receptor for SCF is c-kit.

- SCF and c-kit are present in approximately 70% of the SCLC cell lines examined.
- The growth of c-kit transfected cell lines is further enhance by the addition of IGF-I or BB.
- C-kit is inhibited by Gleevec.

The c-kit receptor is a 976 amino acid integral membrane protein.

- The 520 amino acid extracellular domain binds SCF with high affinity.
- The 23 amino acid transmembrane domain anchors the receptor into the membrane.
- The 433 amino acid intracellular domain contains tyrosine kinase activity.
- ATP binds to the tyrosine kinase domain and Tyr substrates are subsequently phosphorylated.

SCLC and GRP

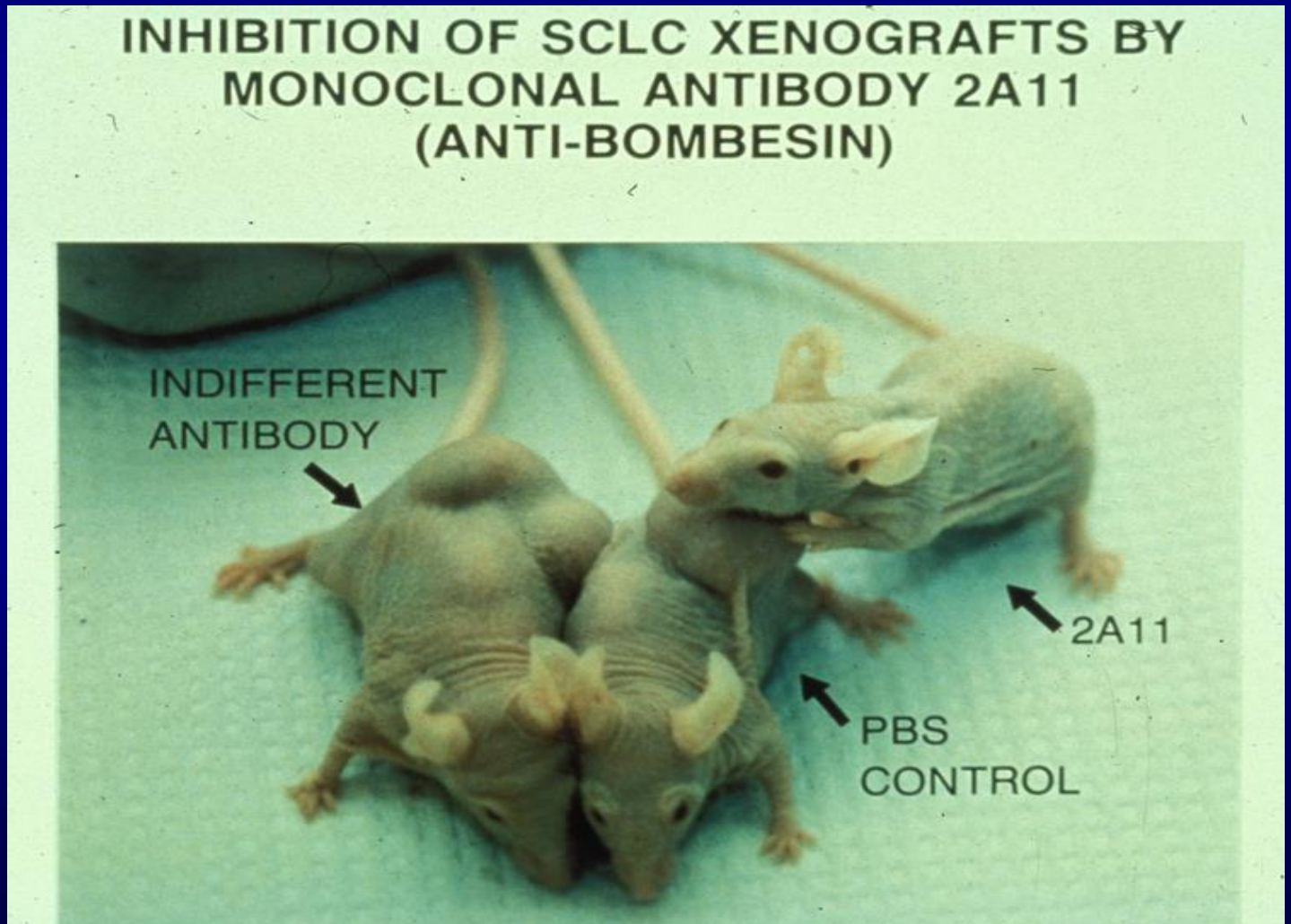
SCLC cells have high levels of GRP

Cell line	Density (pmol/mg)
NCI-H209	18.3
NCI-H345	3.5
NCI-H69	1.7

Moody et al., Science 214:1246 (1981)

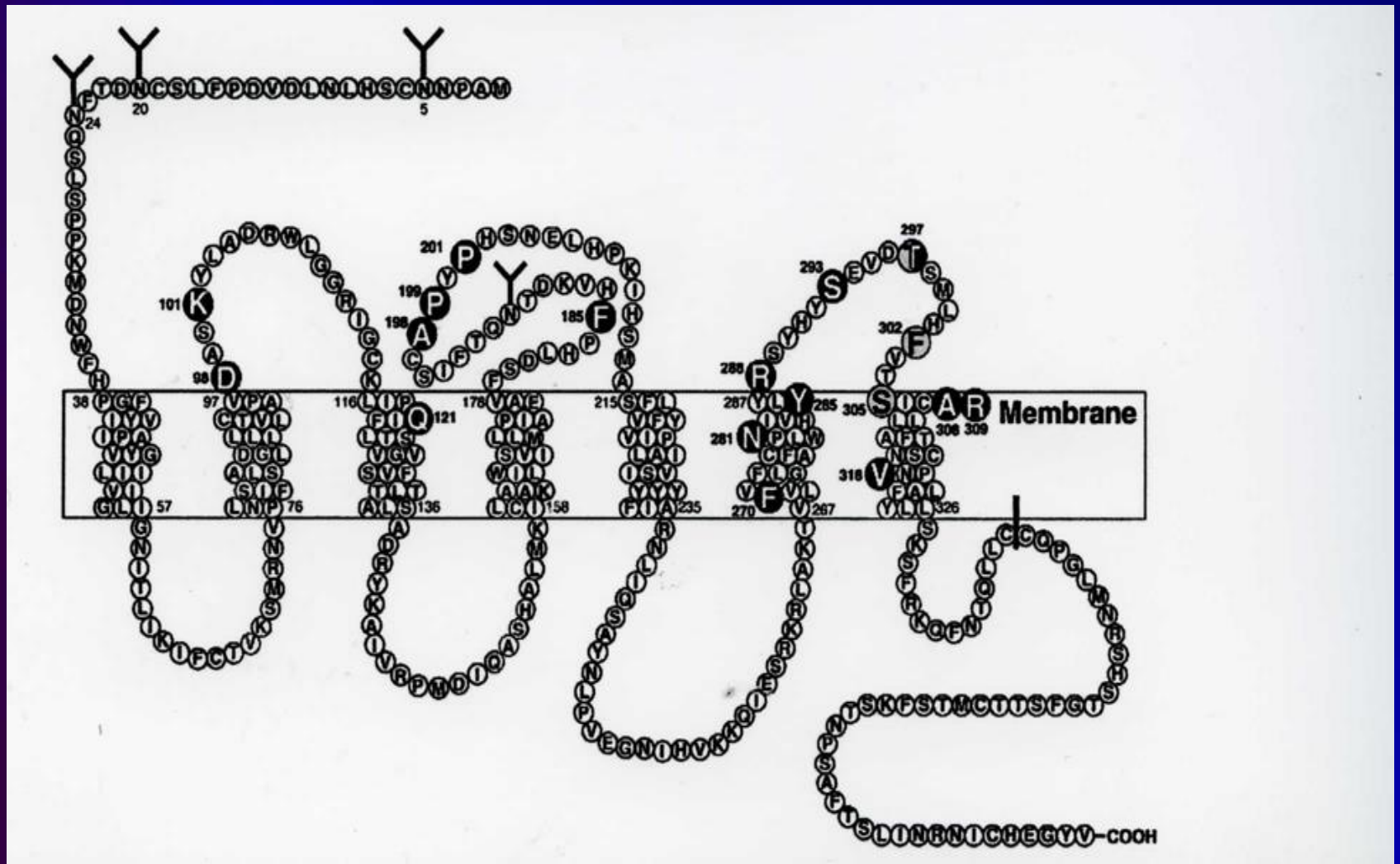
Mab 2A11 inhibits lung cancer xenograft growth in nude mice.

Cuttitta et al., Nature 1985;316:823



R^{288}, Q^{121}, P^{199} and R^{308} are essential for high affinity agonist binding. T^{297}, F^{302} and S^{305} are essential for antagonist binding to the BB_2R.

Jensen et al., 2008, Pharm Rev 61:1-42.

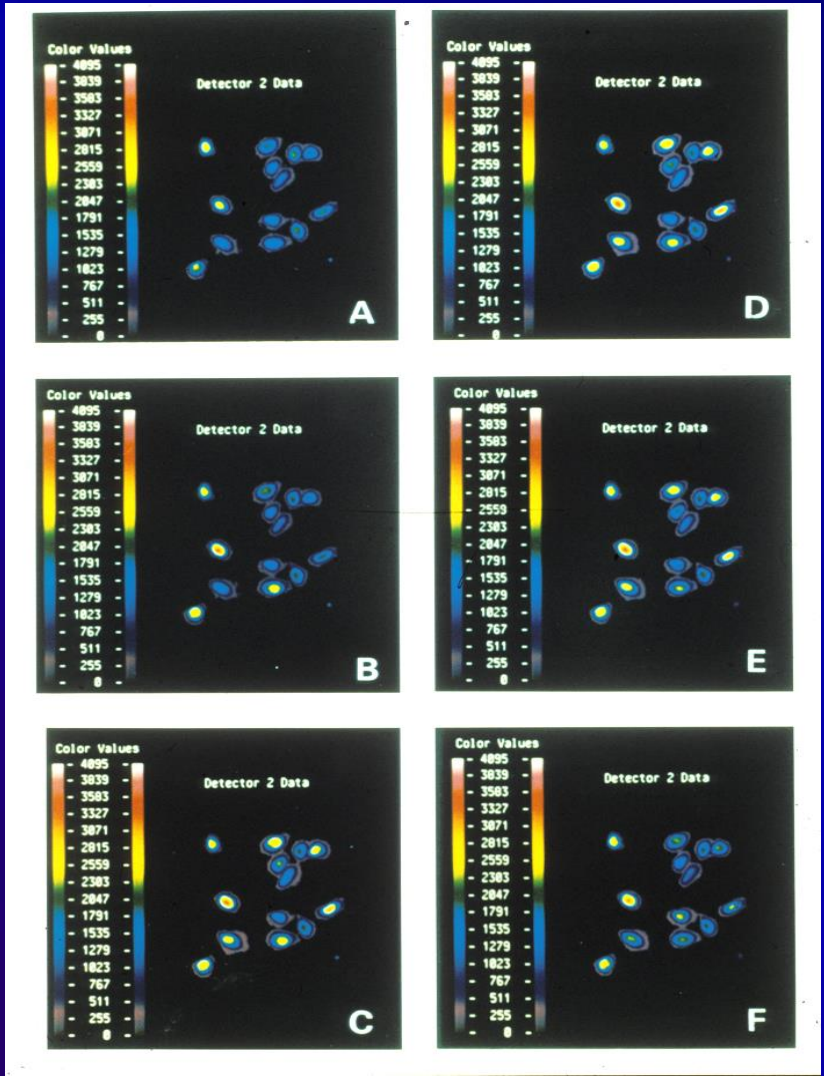


Are females more susceptible to lung cancer?

- Lung cancer in U.S. women is rapidly increasing and it now kills 67,000 women annually.
- Expression of the GRP-R, which is on the x-chromosome, is more abundant in female non-smokers and short-term smokers (1-25 pack years) than males.

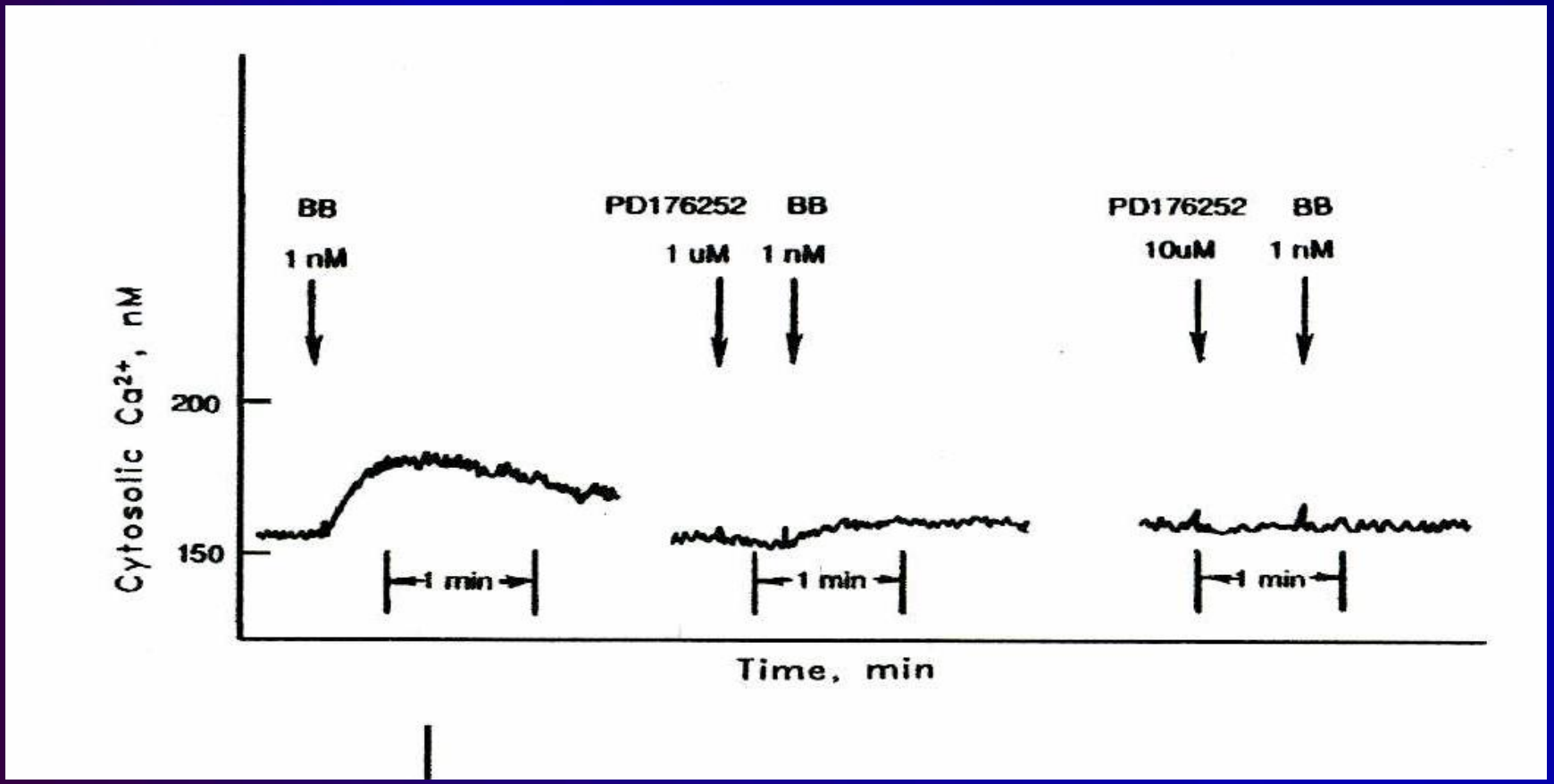
Shriver et al., JNCI 92:24(2000)

Most of the lung cancer cells have elevated cytosolic calcium after addition of BB.

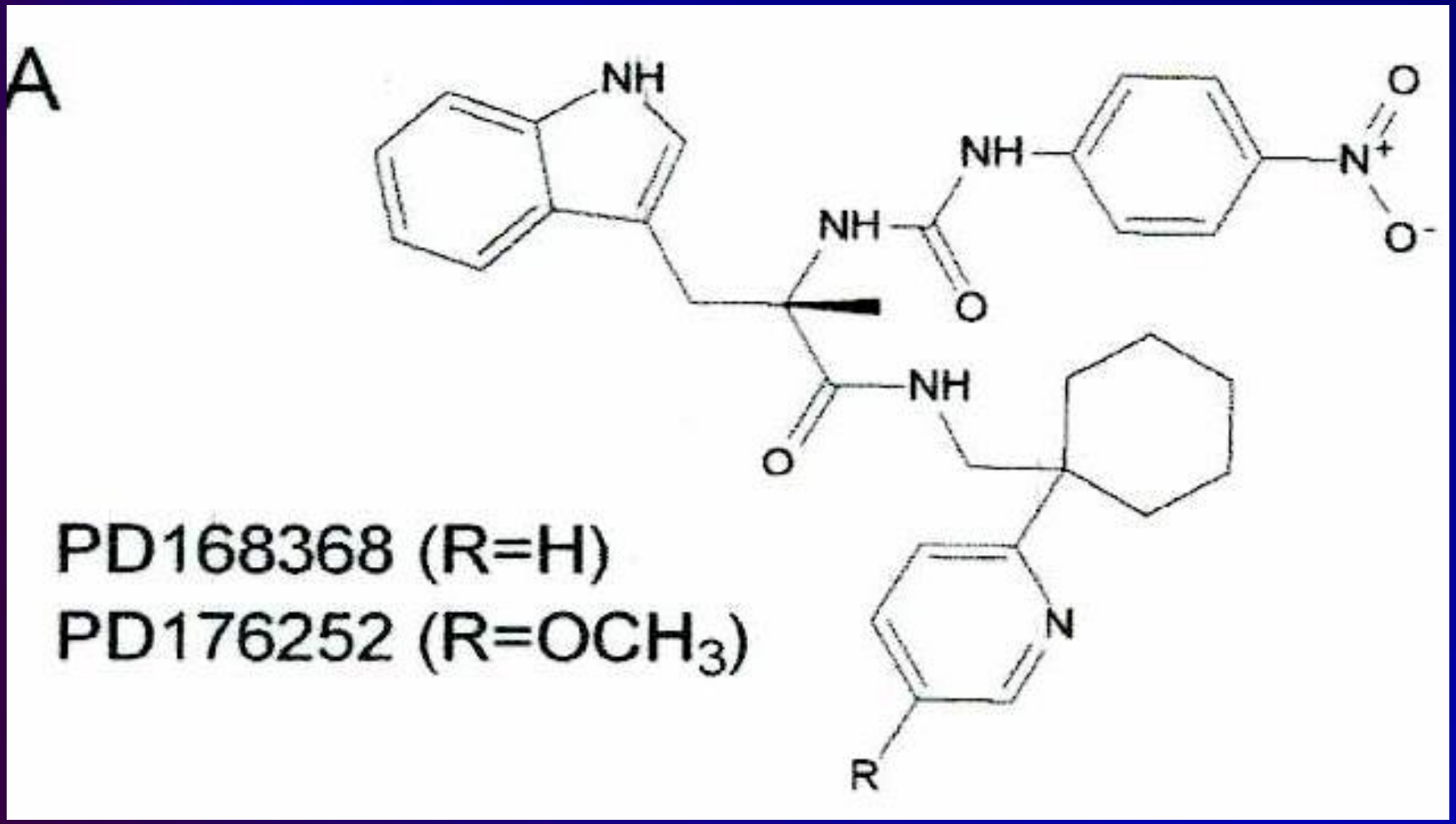


PD176252 antagonizes the ability of BB to elevate cytosolic Ca^{2+}

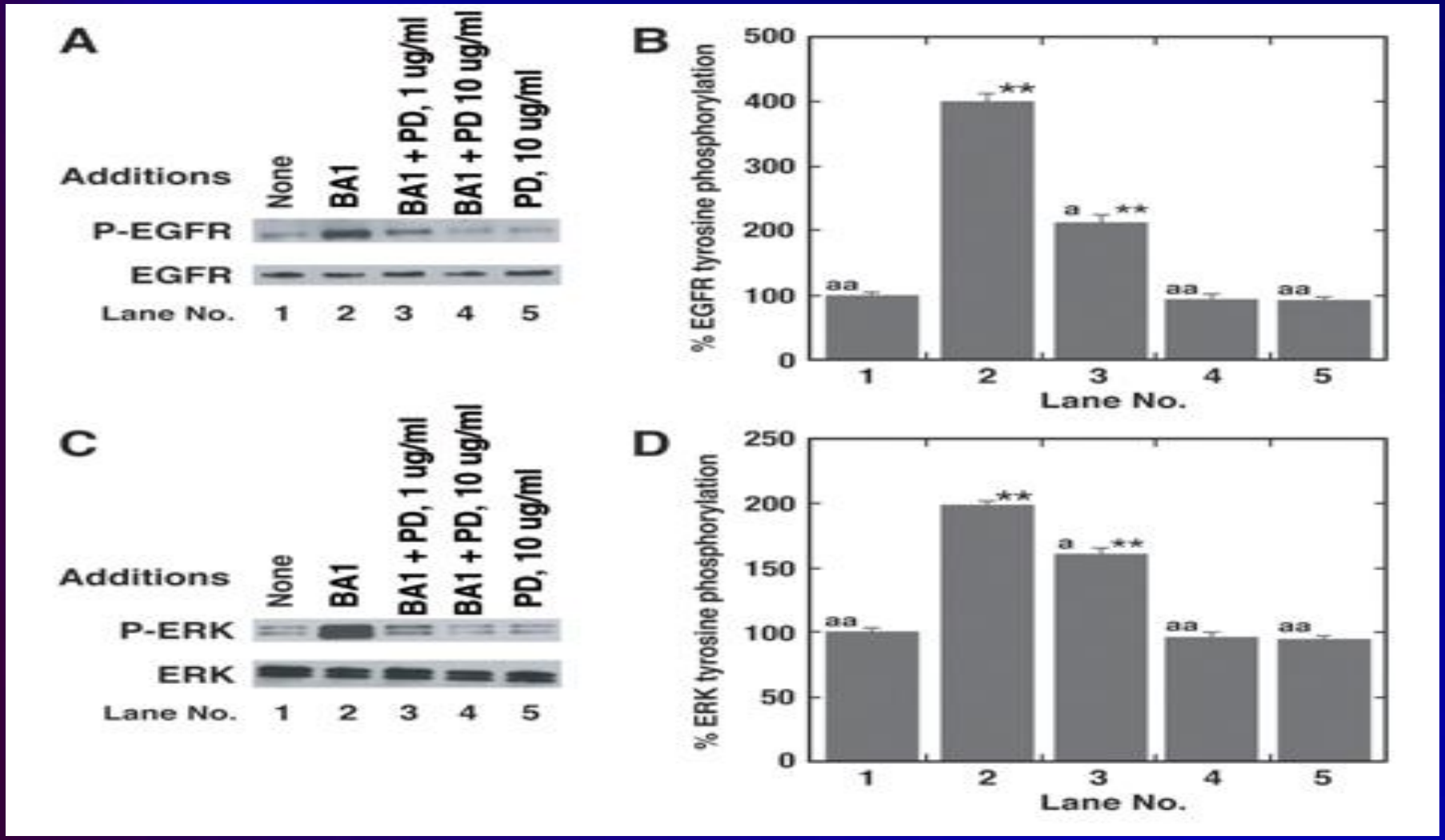
Moody et al. Eur. J. Pharmacol. 2003; 474:21



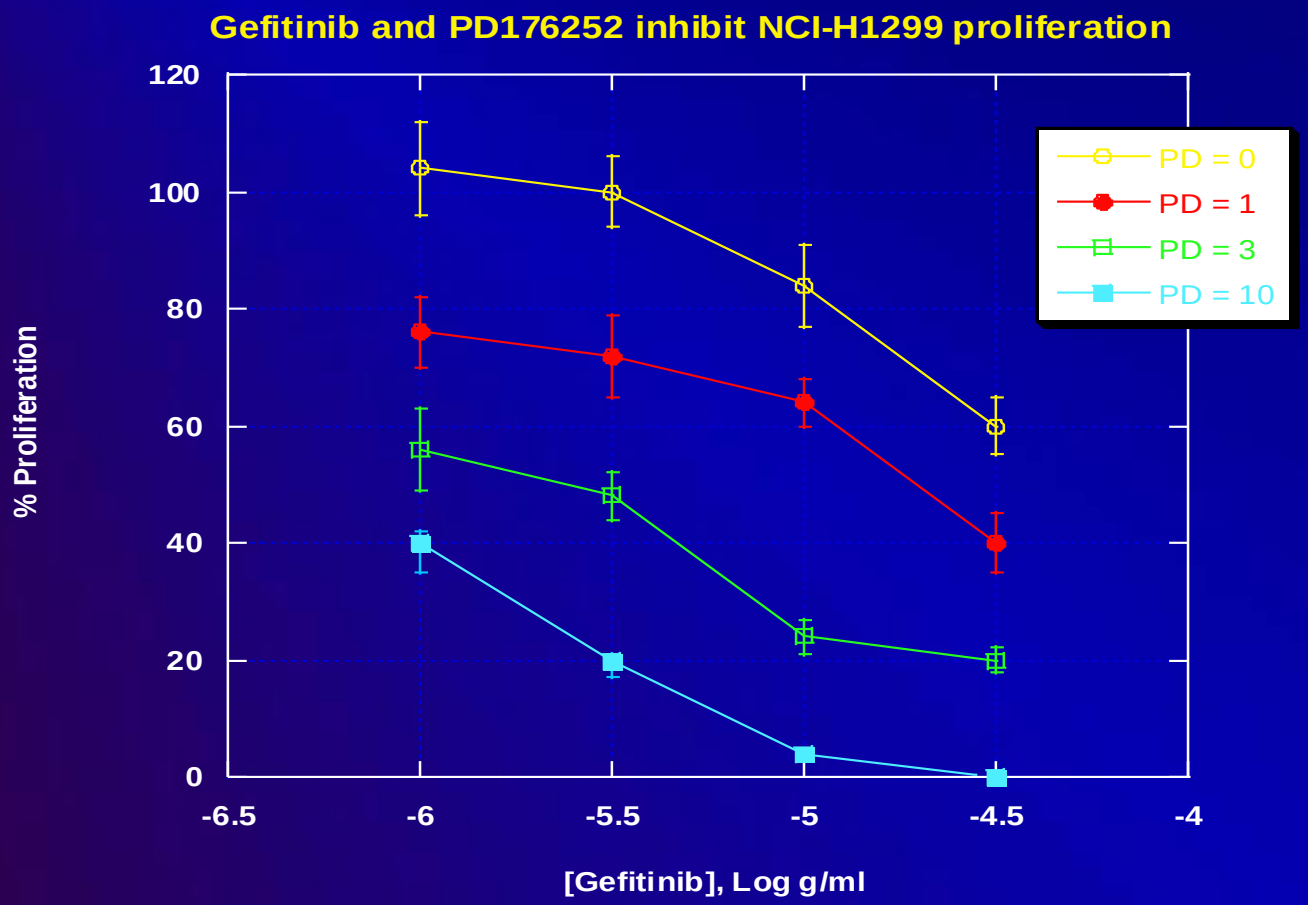
PD176252 is a BB_1R and BB_2R non-peptide antagonist ((S)-N-[[1-(5-methoxy)-2-pyridinyl)cyclohexyl] a-methyl-a[[[-nitrophenyl)amino]carbonyl]amino-1H-indo le-3-propane amide)



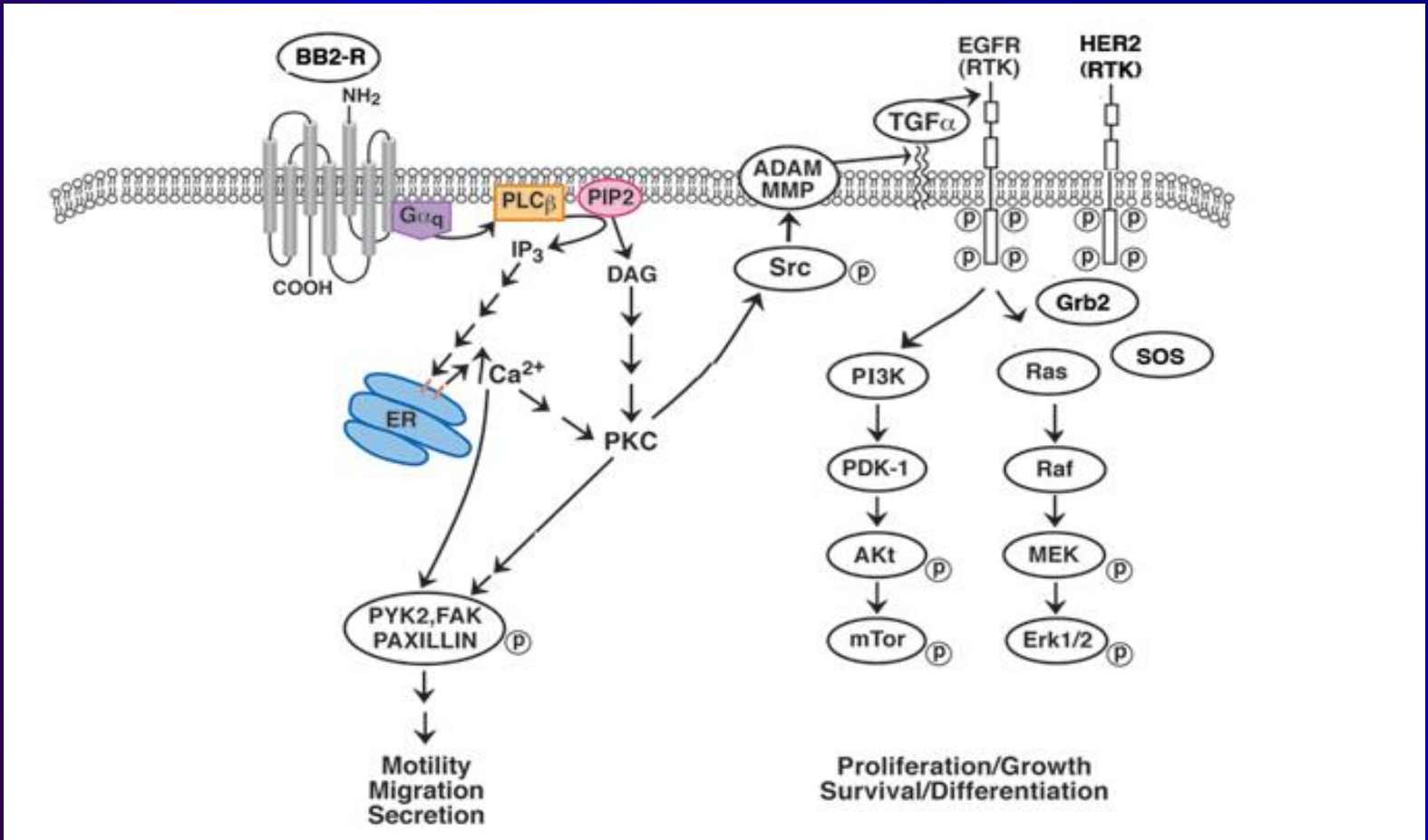
PD176252 antagonizes the ability of BB to cause EGFR and ERK tyrosine phosphorylation



PD176252 increases the potency of gefitinib to inhibit lung cancer proliferation.



The BB_2R regulates EGFR tyrosine phosphoryation leading to increased cancer cell proliferation, survival and metastasis



SUMMARY

- SCLC is a neuroendocrine tumor which initially responds to chemotherapy but subsequently relapse occurs.
- Multiple clinical trials are in progress to improve the treatment of SCLC patients.

Smoking cessation. First line treatments approved by FDA.

- Nicotine replacement therapy (NRT) includes gum (Nicorette), patch (Nicoderm CQ) or inhaler or nasal spray (Nicotrol)
- Pills. Bupropion (antidepressant) or Varenicline tartrate (Chantix) which reduces smoking urge and withdrawal symptoms

Smoking cessation

- Smoking cessation can be achieved with or without assistance from healthcare professions or the use of medication.
- Early “failure” is a normal part of trying to stop smoking.
- Smoking cigarettes leads to nicotine addiction.
- Most smokers quit cold turkey after gradual reduction

Health benefits

- Within 20 min after quitting, blood pressure and heart rate decrease
- Within 12 hours, carbon monoxide levels in blood are normal
- Within 2 days the sense of smell and taste return
- Within 9 months there is a decrease in cough and shortness of breath
- Within 10 years, the risk of stoke is normal and the risk of dying from lung cancer is reduced by 50%

Smoking cessation.

- There are now 45 million ex-smokers at high risk of getting lung cancer.
- There remain 45 million smokers in the U.S., but only 16% will die from lung cancer.
- 1-800-QUITNOW; http://www.smokefree.gov
- http://www.cancer.gov/cancertopics/tobacco/smoking

References:

Y. Sekido, K.M. Fong and J.D. Minna. Cancer of the Lung, In “Cancer:Principles and practice of Oncology.” Edited by V.T. DeVita, S. Hellman and S.A. Rosenberg, Lippincott, Williams & Wilkins, 745-752 (2006).

L.C. Cantley, C.L. Carpenter, W.C. Hahn, M. Meyerson. Cell signaling, growth factors and their receptors, In “Cancer:Principles and practice of Oncology.” Edited by V.T. DeVita, T.S. Lawrence and S.A. Rosenberg, Lippincott, Williams & Wilkins, 75-67 (2011).