# TRAnslational research in Clinical Oncology (TRACO)

# Program Director

**Terry W. Moody, Ph.D.**

**(240)276-7785**

**9609 Medical Ctr. Dr., Rm. 2W340**

**moodyt@mail.nih.gov**

# Organizing Committee

Irwin Arias

Terry Moody

Lyuba Vartikovski

Jonathan Wiest

Farah Zia

# SYLLABUS

| DATE | TOPIC | SPEAKERS |
| --- | --- | --- |
| Sept. 4 | Introduction, Cervical cancer | Moody, Schiller |
| Sept. 13 | Prostate cancer, SCLC | Madan, Chen |
| Sept. 17 | Ovarian cancer, Immune checkpoints | Annunziata, Goff |
| Sept. 27 | Clinical trials, TGF beta | Smith, Jakowlew |
| Oct. 1 | Tumor imaging, Small molecules | Choyke, Simeonov |

# SYLLABUS, continued

| DATE | TOPIC | SPEAKERS |
|---|---|---|
| Oct. 11 | Radiation oncology, CAR T-cell | Nichols, Kochenderfer |
| Oct. 15 | Health disparities, Case reports | Ryan, Olaku |
| Oct. 22 | Breast cancer, Epidemiology | Zia, Caporaso |
| Oct, 29 | Topoisomerase, Precision medicine | Pommier, Harris |
| Nov. 5 | NSCLC, Genomics | Szabo, Wei |

# SYLLABUS, continued

| DATE | TOPIC | SPEAKERS |
| --- | --- | --- |
| Nov. 19 | K-RAS, Chaperone proteins | Luo, Neckers |
| Nov. 26 | Epigenetics, HIV | Verma, Maldarelli |
| Dec. 3 | Pancreatic cancer, Nanotechnology | Hussain, Dobrovolskaia, |

# REGISTRATION

The course is open to all interested personnel without charge. Registration is available at the NCI CCR Web site (http://ccr.cancer.gov/training/trainee-resources/courses-workshops/traco)

# CCR component

Registrants can attend tumor boards, grand rounds, visit technology and/or core facilities. Please contact Dr. Moody, if interested to make appropriate reservations.

# COURSE CERTIFICATION

Registrants can obtain a course certificate upon passing a computer graded final examination.

# Lung, colon, breast and prostate cancer account for half of the U.S. cancer mortalities.

| TYPE | INCIDENCE | (MORTALITY) |
|---|---|---|
| Lung | 171,900 | (157,200) |
| Colon/Rectum | 147,500 | (57,100) |
| Breast | 211,300 | (39,800) |
| Prostate | 220,900 | (28,900) |
| Others | 582,500 | (273,500) |
| Total | 1,334,100 | (556,500) |

*Jemal, Ward and Thun, "Cancer: Principles & Practice of Oncology." Edited by DeVita, Hellman and Rosenberg. (2006), pp. 226-241*

# Cancers which kill 10,000-30,000 U.S. patients annually include:

- Pancreatic cancer
- Non-Hodgkin's Lymphoma
- Leukemia
- Stomach cancer
- Ovarian cancer
- Brain cancer
- Liver cancer
- Bladder cancer
- Esophageal cancer
- Kidney cancer

# Cancer risks include:

- Alcohol
- Asbestos
- Diet
- Familial
- Hormones

# Cancer risks (continued)

- Obesity
- Ion Radiation
- Tobacco
- U.V. Radiation
- Viral

# Lung Cancer kills over 150,000 patients in the U.S. annually.

- There are 45 Million current smokers and 45 Million ex-smokers in the U.S.
- It is difficult to quit smoking due to nicotine addiction.

# Carcinogens which have been identified in cigarette smoke include:

- Polyaeromatic hydrocarbons (PAH),
- aza-arenes,
- 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK),
- 1,3 butadiene,
- ethyl carbamate,
- ethylene oxide,
- nickel, chromium, cadmium,
- polonium, arsenic
- hydrazine

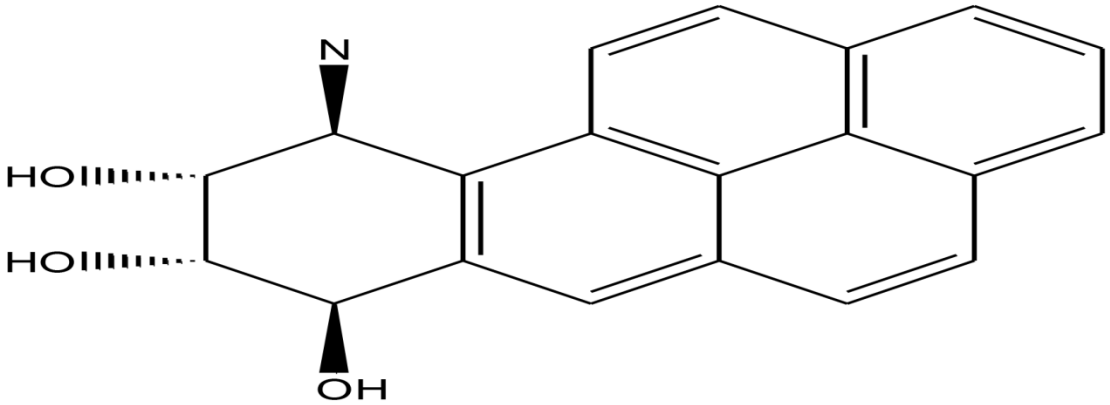
**The process by which unreactive carcinogen converts to a form which binds DNA is known as metabolic activation.**

- **Bay region diol epoxides are the principal PAH metabolites involved in DNA adduct formation. For Benz[a]pyrene (BaP), BaP-7,8-diol-9,10-epoxide (BPDE) forms adducts with DNA leading to G:C>T:A mutations in pulmonary DNA. The genes for p53 and k-ras are frequently mutated.**

# BENZ(a)Pyrene

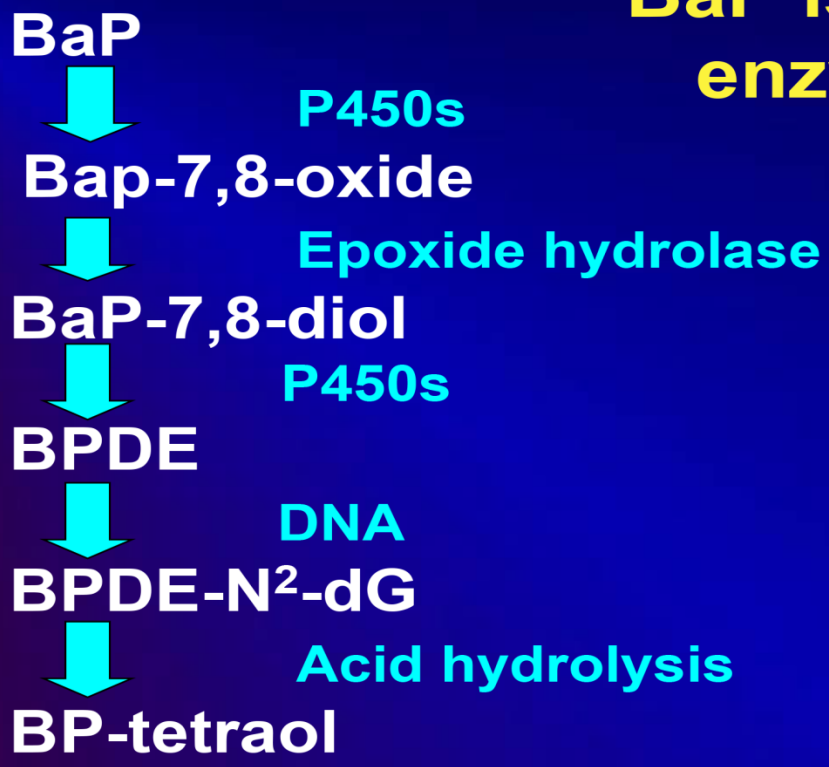
## BENZ(a)Pyrene

- The chemical structure of BaP is shown.



# BaP is metabolized to BPDE

BaP is metabolized by enzymes to BPDE.




*Boysen and Hecht, Mutation Res. 543:17(2003).*

# Carcinogens can be detoxified and excreted prior to DNA damage.

- Cytochrome p450 enzymes catalyze addition of an oxygen to the carcinogen, increasing its water solubility.
- Phase 2 enzymes convert the oxygenated carcinogen to a form that is highly soluble in water, converting it to a form that can be excreted.

# DNA is mutated if the rate of carcinogen activation exceeds the rate of carcinogen detoxification and/or DNA repair.

- DNA adducts as well as intra- and interstrand DNA crosslinks are removed by nucleotide excision repair.

# P53, a tumor suppressor gene:

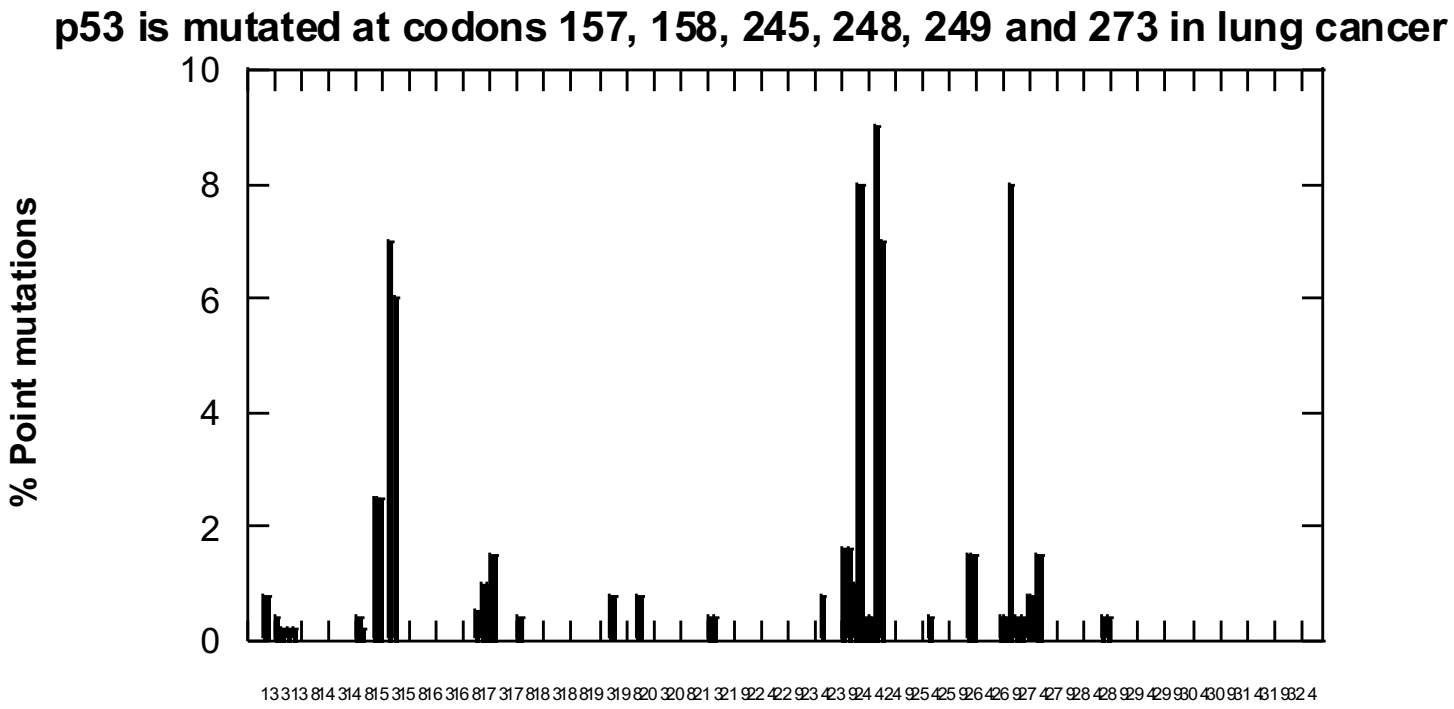
- mediates the G1 to S-phase checkpoint of the cell cycle,
- drives programmed cell death or apoptosis after DNA damage,
- is increased along with p21 (cell cycle checkpoint) after DNA damage.
- Phosphorylated p53 induces expression of BAX (apoptosis), GADD45 (DNA repair) and thrombospondin (angiogenesis)

# P53 mutations are detected in most of the lung cancer patients.

- G to T transversions occur at the CpG rich codons including 153-158 (exon 5), 248 and 249 (exon7) and 273 (exon 8) of the p53 gene. There is an excess of G to T transversions in smokers relative to non-smokers.

# P53 mutations.

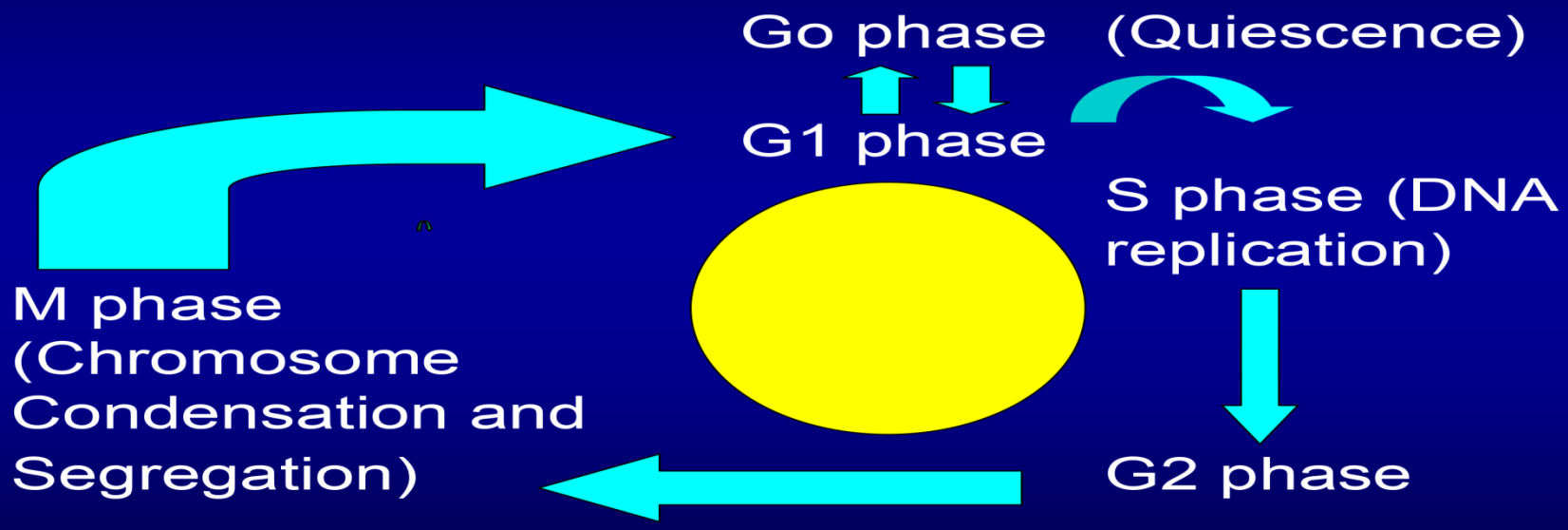
- P 53is mutated at codons 157, 158, 245, 248, 249 and 273 in lung cancer.



# Cell cycle phases

## Cell cycle phases.

- Cell cycle phases include G1, S, G2 and M



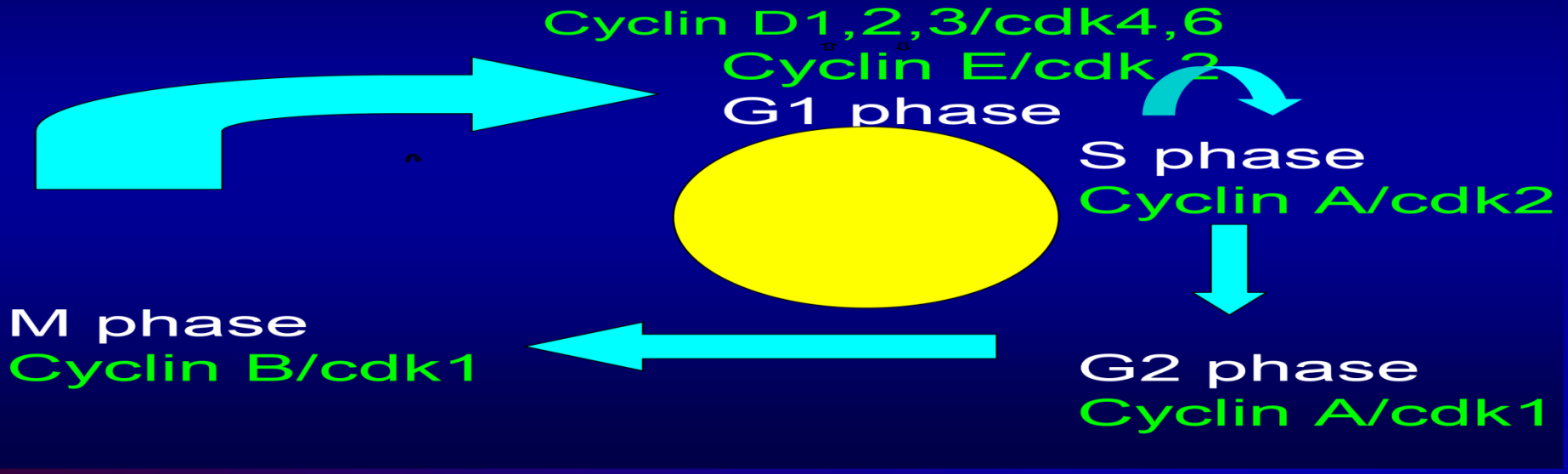
# p53 mediates the $G_1$ to S-phase checkpoint of the cell cycle

- DNA damage increases p21 and p53.
- P53 drives programmed cell death or apoptosis after DNA damage

# Cell cycle enzymes

## Cell cycle enzymes.

- Cyclin D/cdk is inhibited by p21,27,57,15,16,18 and 19.

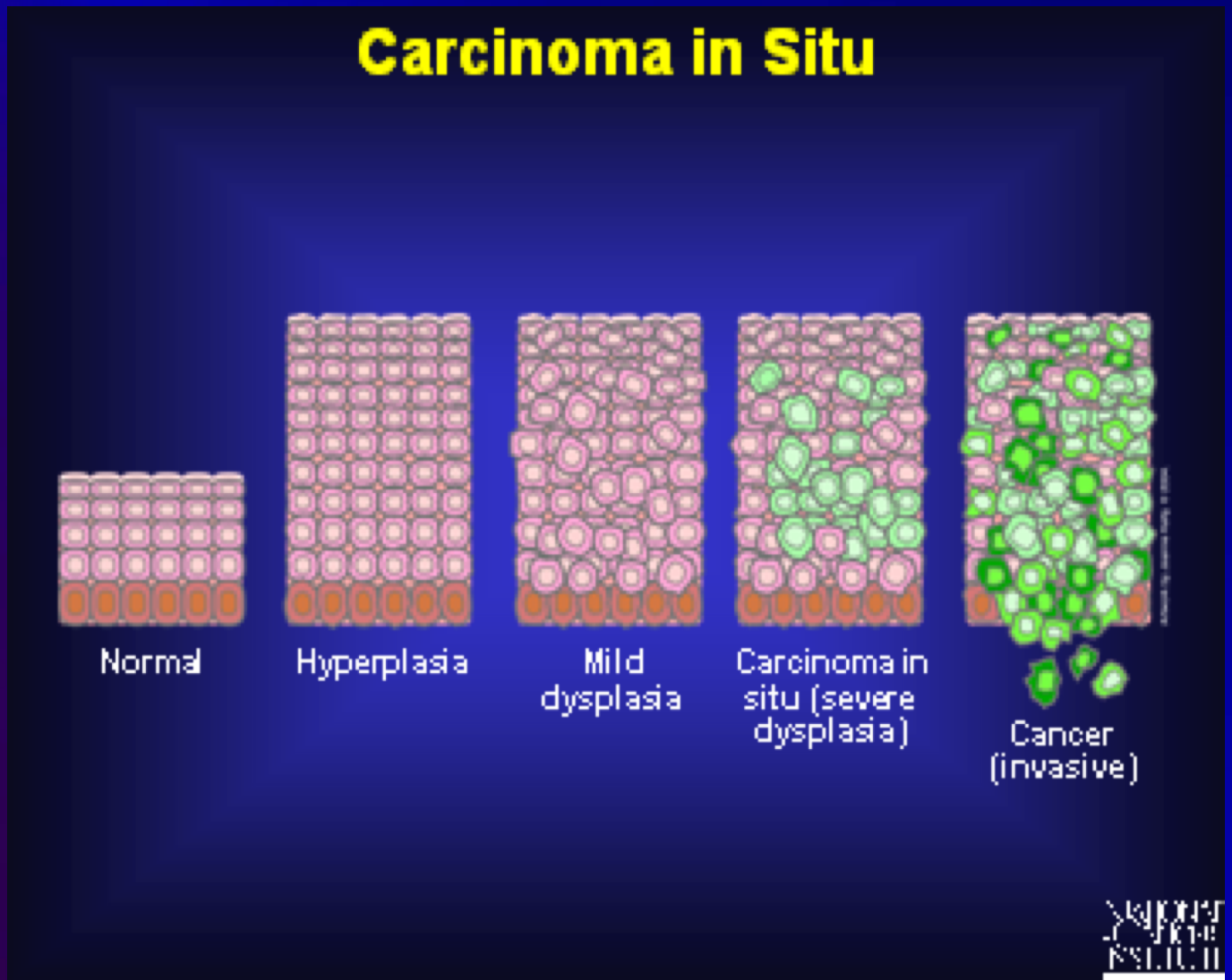


# Genotoxicity of tobacco smoke.

- After 10 years of chronic cigarette smoking, normal lung tissue can undergo hyperplasia and metaplasia.
- After 15 years, dysplasia can result.
- After 20 years, a carcinoma in situ can form.
- After 25 years, a malignant cancer can form.

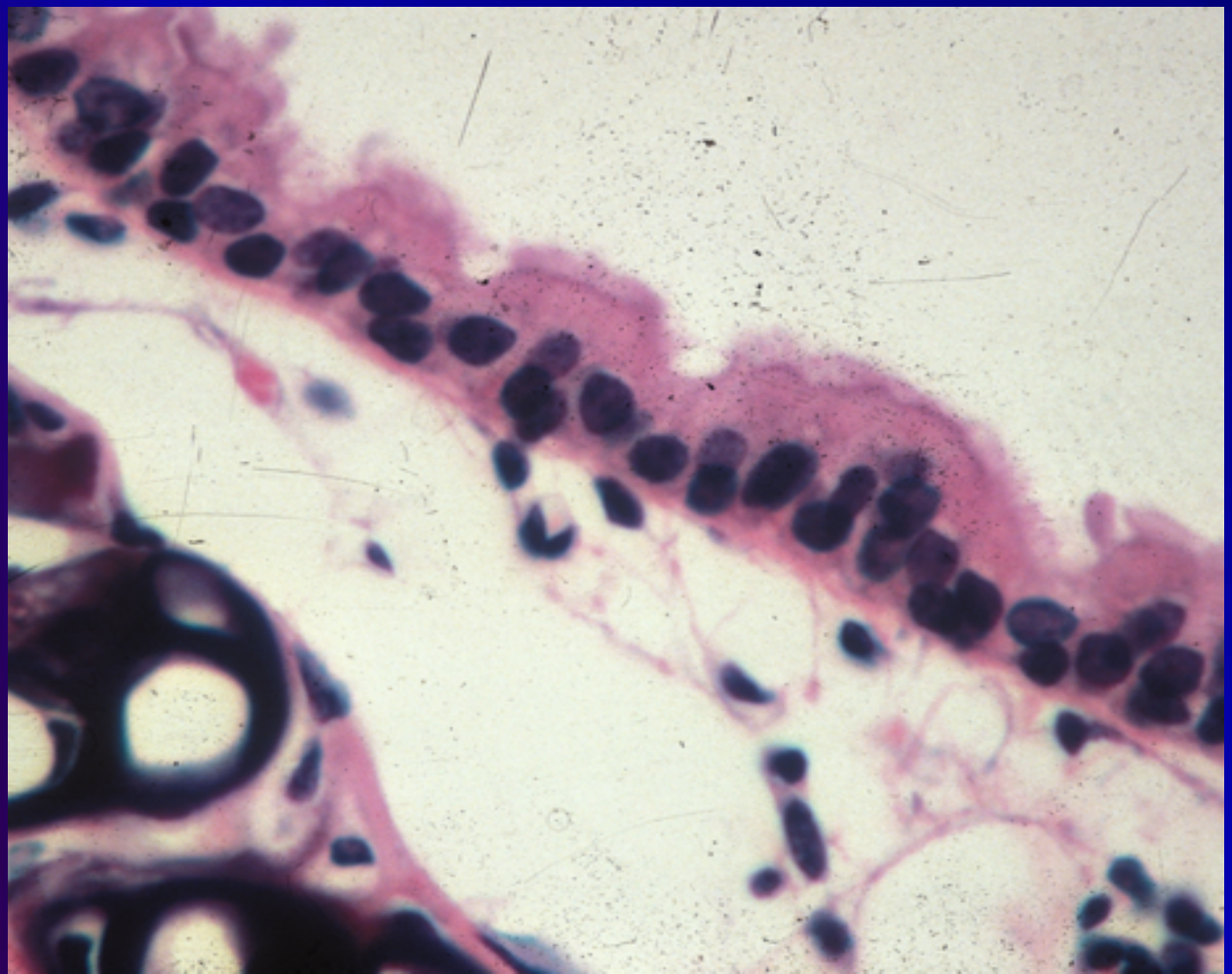
# Carcinogenesis

- Cancer progression occurs over a period of decades.



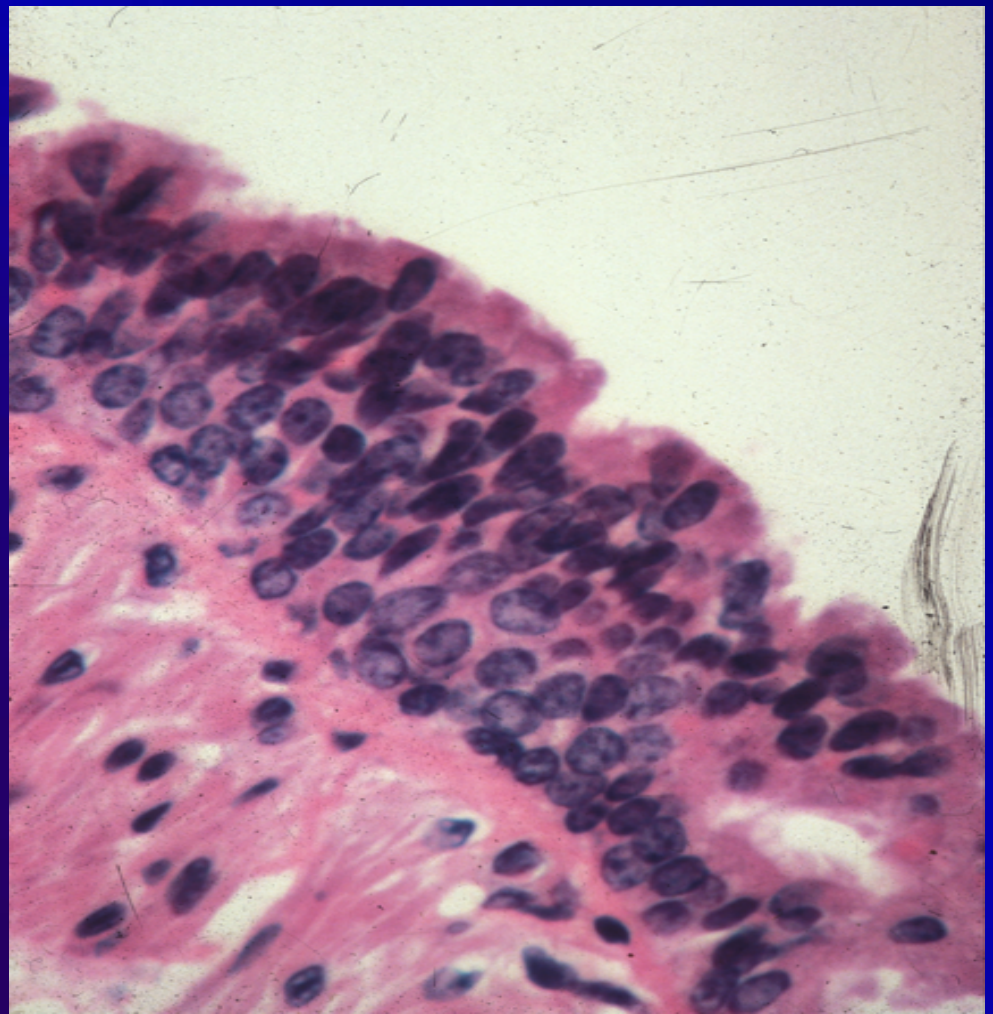
# Normal lung

- Carbon dioxide is exhaled from the lung whereas oxygen is inhaled.



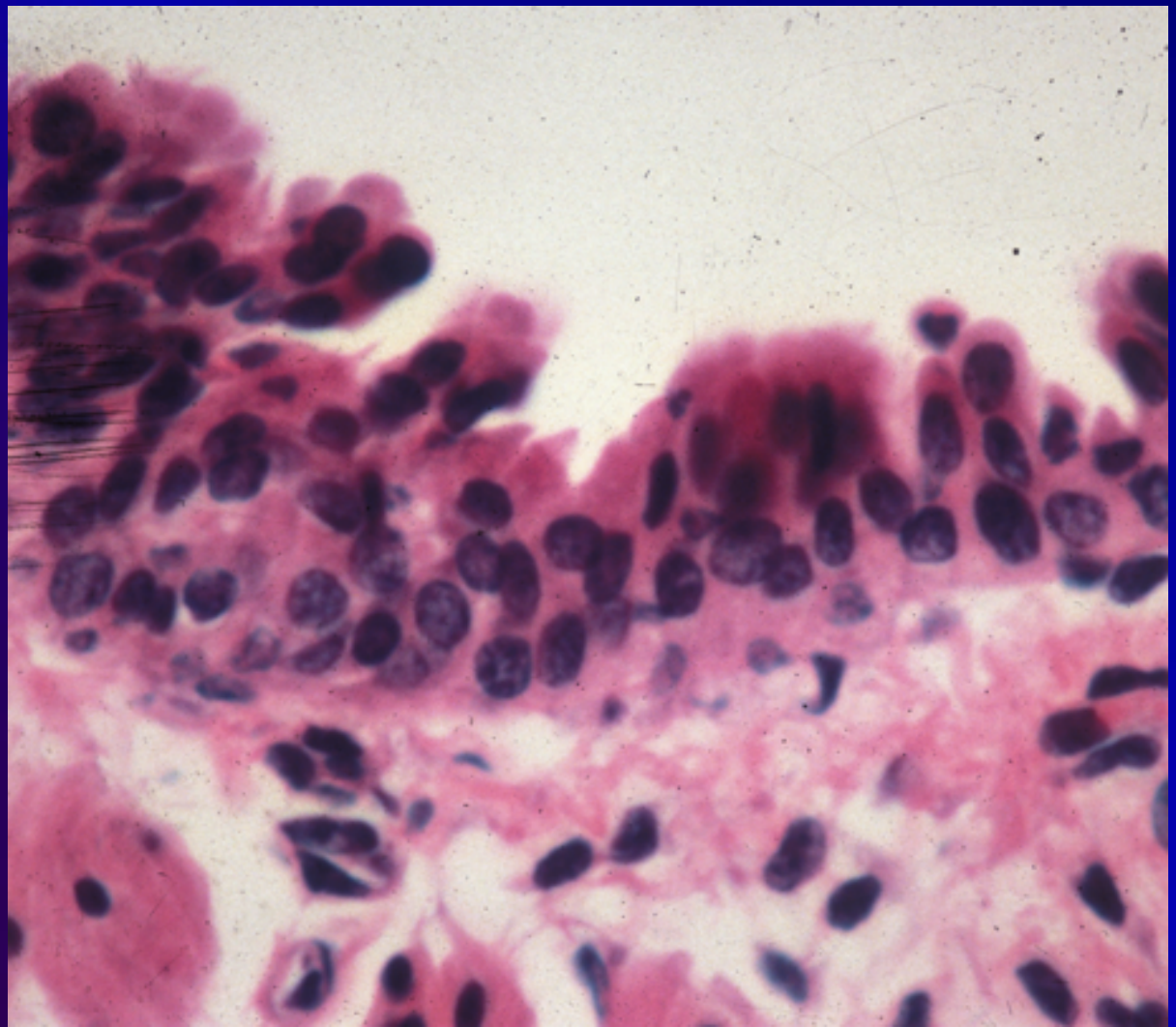
# Hyperplasia

- After exposure to tobacco smoke, hyperplasia can result.



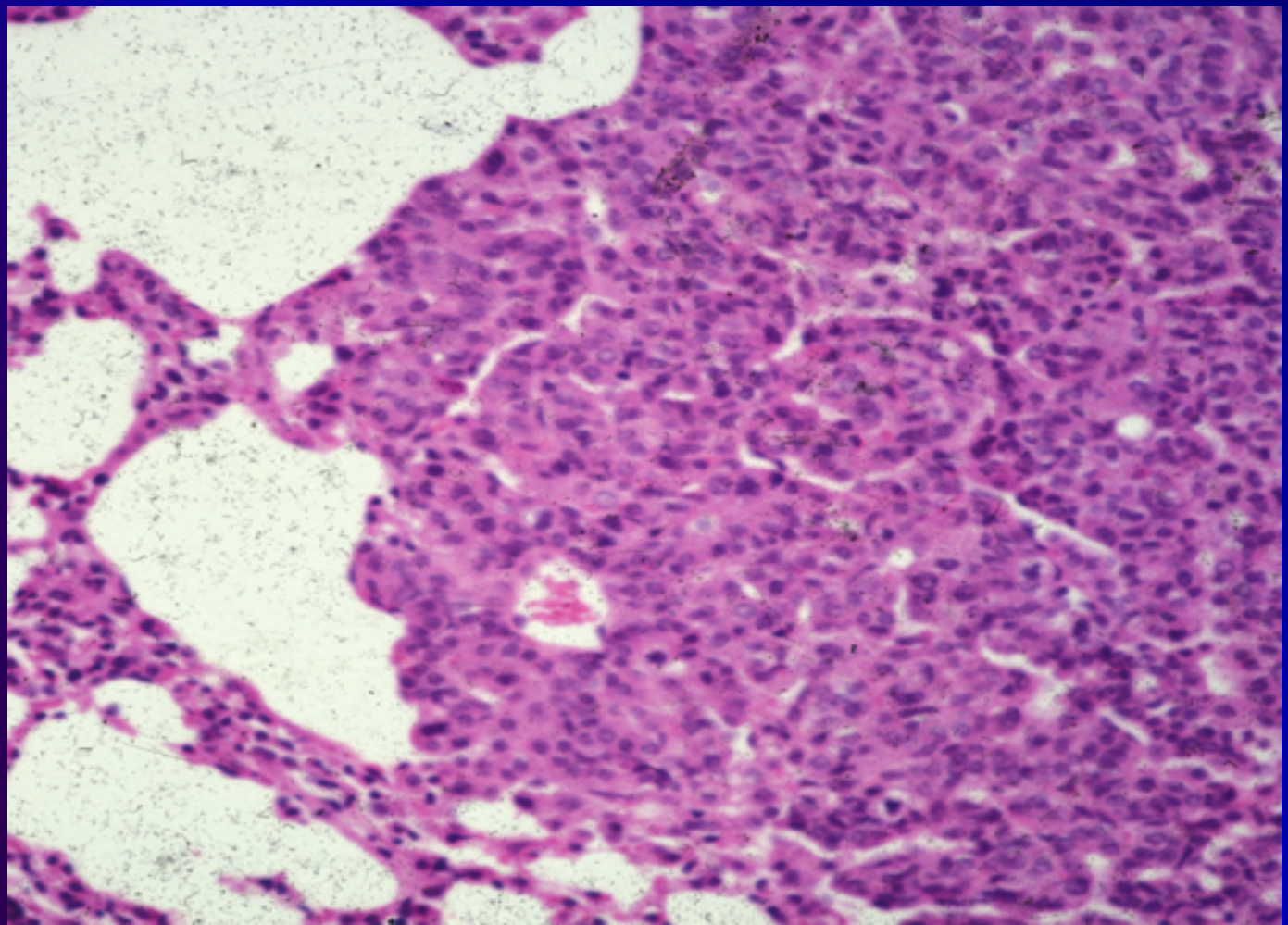
# Dysplasia

**Continued exposure to tobacco smoke leads to dysplasia.**



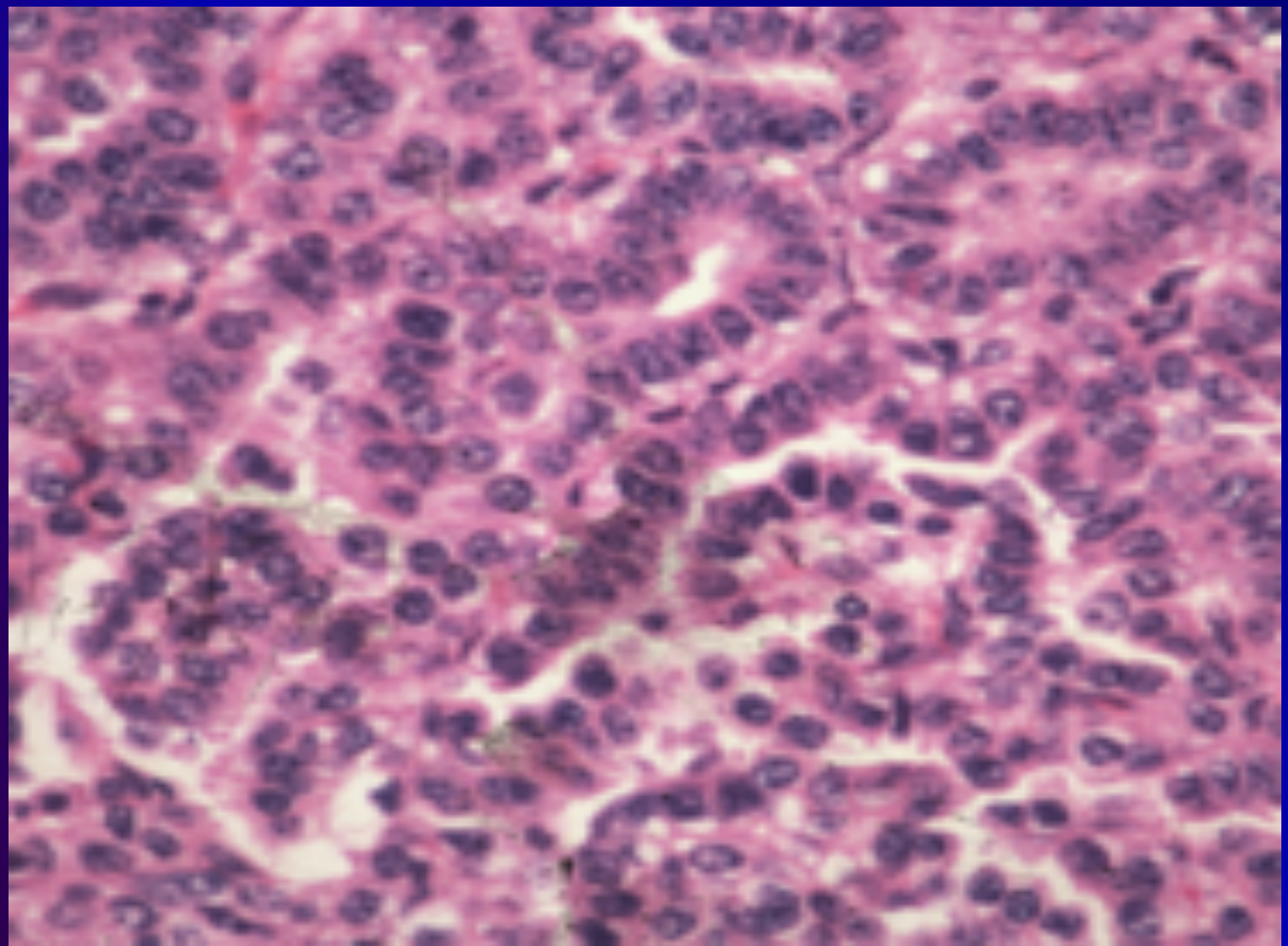
# Adenoma

- Continued exposure to carcinogens leads to benign tumors such as adenomas.



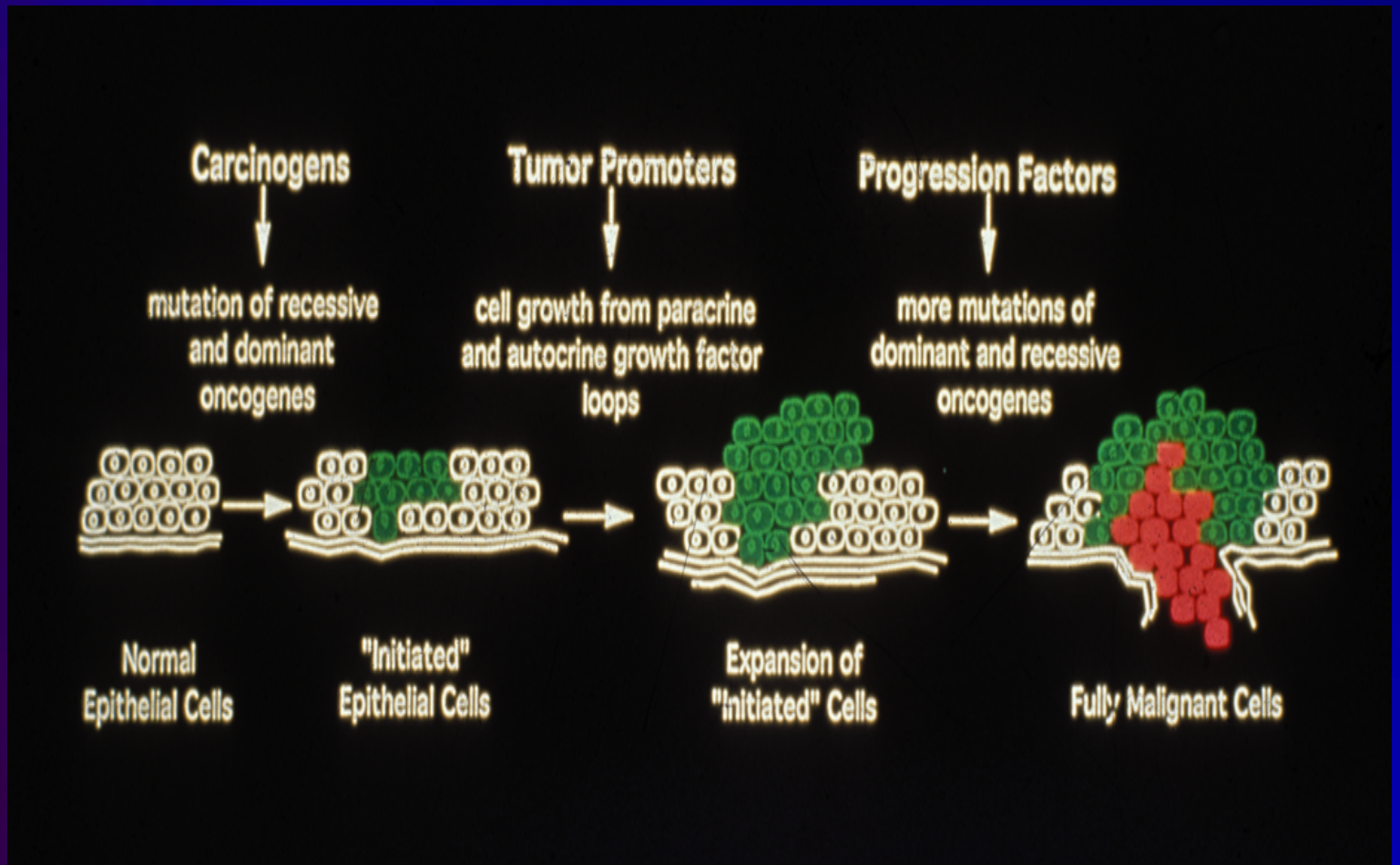
# Adenocarcinoma

- Chronic exposure to tobacco leads to malignant tumors such as adenocarcinoma.



# Tumor formation

- Growth factors promote carcinogenesis. Progression factors lead to malignant tumors.



# Tumor growth

## Tumors

- The primary cancer can undergo metastasis to distant organs.

Carcinoma

Angiogenesis

Migration, Invasion and Metastasis.

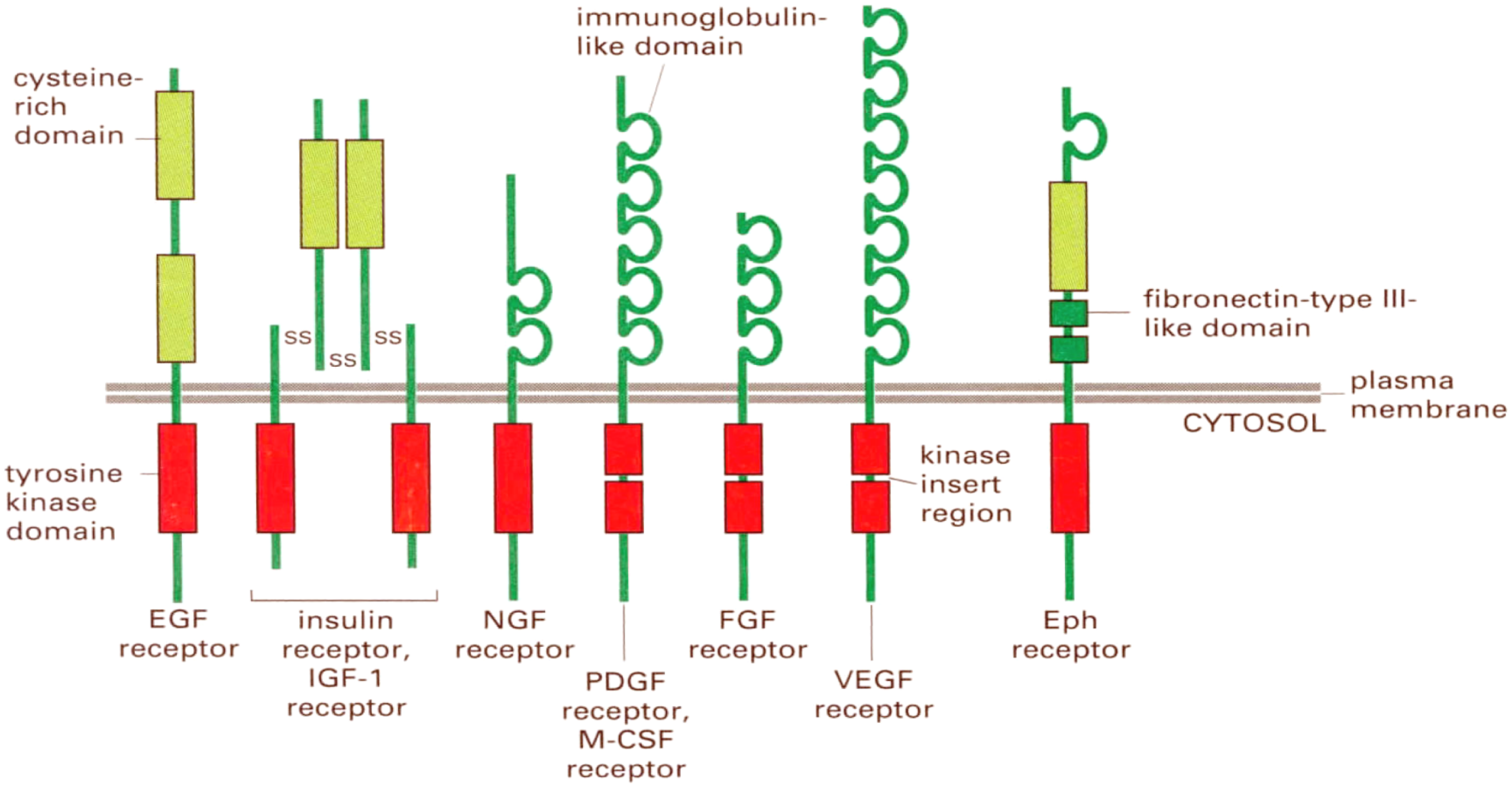
# Genetic abnormalities in lung cancer include:

- Mutation of tumor suppressor genes such as p53
- Silencing of tumor suppressor genes such as p16, Rb
- Amplification of oncogenes such as

  c-myc, cyclin D1, EGF receptor, erbB-2

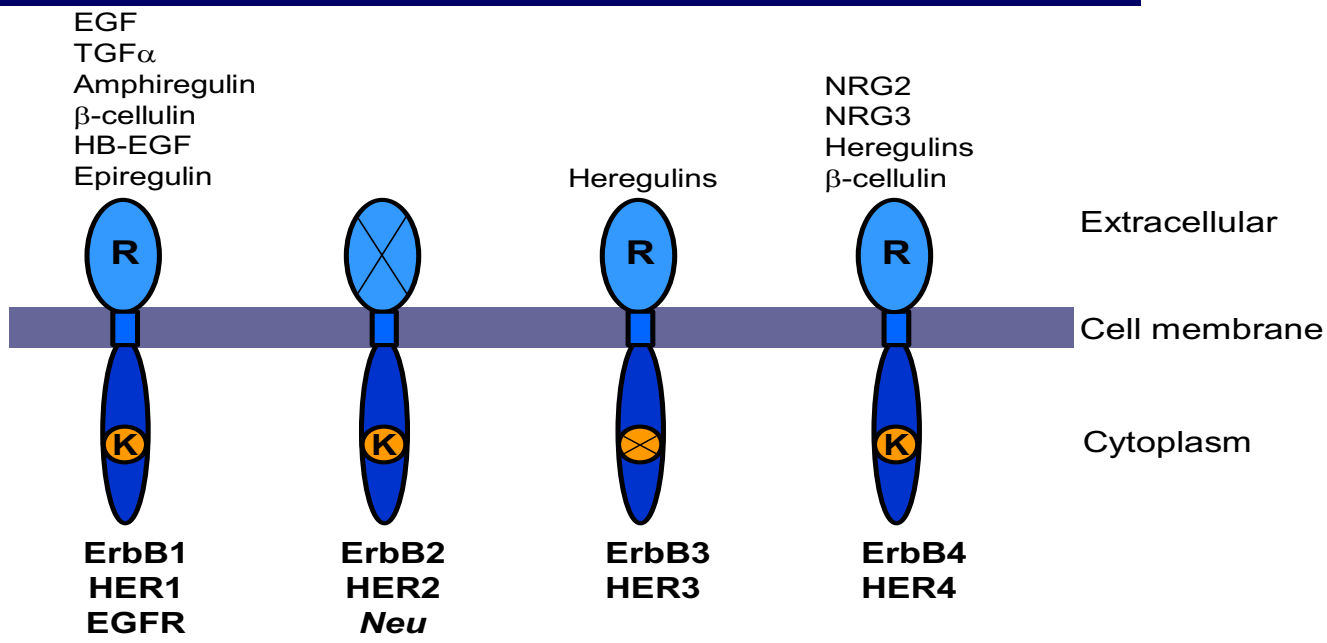
# Tyrosine kinase receptors.

## *Molecular Biology of the Cell, Alberts et al., 2001.*



# Tyrosine kinase receptors and ligands

## ErbB family of receptor tyrosine kinases (RTKs) and ligands



# The EGFR is an 1186 amino acid integral membrane protein.

- The 621 amino acid extracellular domain binds EGF with high affinity. Domains I and III form the EGF binding site whereas domains II and IV are enriched in cysteine amino acids.
- The 24 amino acid transmembrane domain anchors the receptor into the membrane and tranduces signaling.
- The 541 amino acid intracellular domain contains tyrosine kinase activity.
- Lys721 binds ATP and Tyr amino acids are subsequently phosphorylated.
- Tyr1068, 1086, 1148, 1174 are autophosphorylated

# EGF, TGFα and mAb 108 bind with high affinity to lung cancer cells.

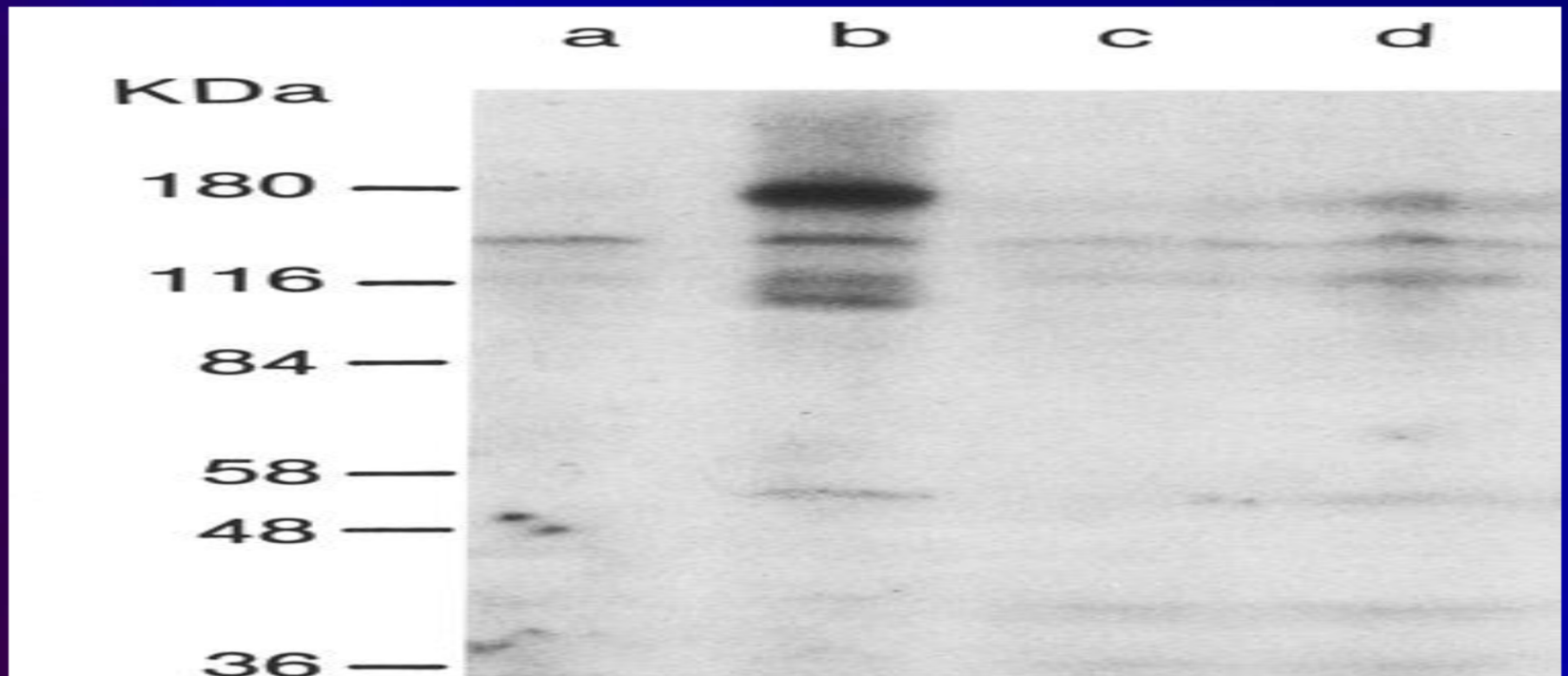
| Agent | $IC_{50}$, ug/ml |
|---|---|
| EGF | .03 |
| TGFα | .8 |
| TGFα-PE38 | .4 |
| mAb 108 | 3 |
| IgG | >10 |

The $IC_{50}$ to inhibit $^{125}$I-EGF specific binding to NCI-H157 cells was determined.

*Draoui et al., Life Sci. 1994; 35:352.*

# EGF tyrosine phosphorylation

**EGF causes tyrosine phosphorylation of the EGFR, PLCγ, and PI-3-K.**

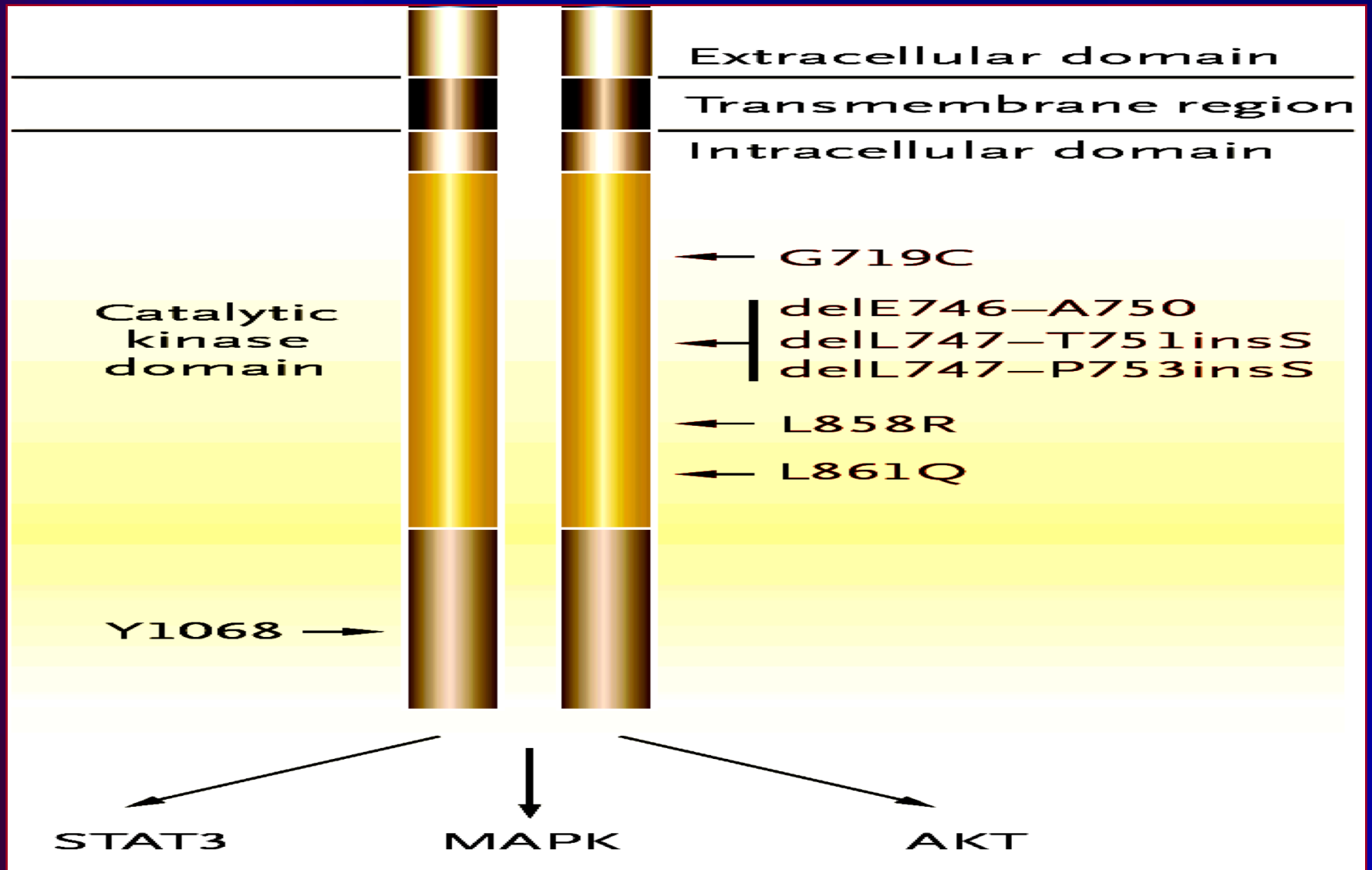


# Tyrosine kinase receptors are mutated in several diseases leading to increased cancer proliferation.

- EGFR mutations occur in the activation loop, especially L858R and G719C.
- Tyrosine kinase inhibitors (gefitinib and erlotinib) have been developed for the EGFR.

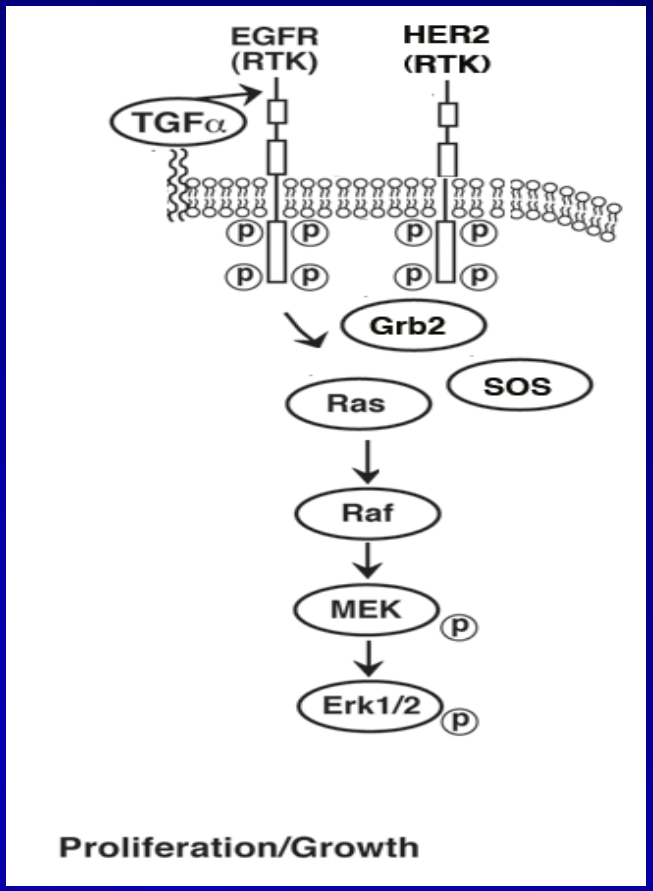
*Paez et al., Science 304:1497 (2004)*

# EGFR mutations



# RAS, RAF, MEK and ERK

- Receptor tyrosine kinases (RTK) stimulate proliferation Through the RAS, RAF, MEK and ERK pathway
- In NSCLC, K-RAS is Mutated in approximately 20% of the patients.



# RAS

- Mutated RAS has reduced GTPase activity resulting in an abundance of biologically active RAS-GTP.
- Most of the RAS mutations are G-to-T transversions in codon 12.
- The Frederick National Lab has a new initiative with RAS as a molecular target.

# RAF

- RAF is a serine threonine kinase which activates MEK.  B-RAF-V600E mutations occur in approximately 60% of melanoma patients leading to an active kinase.
- PLX4032 is a kinase inhibitor which has an 81% response rate in patients with metastatic melanoma.
- RAS and B-RAF are driver mutations in several types of cancer.

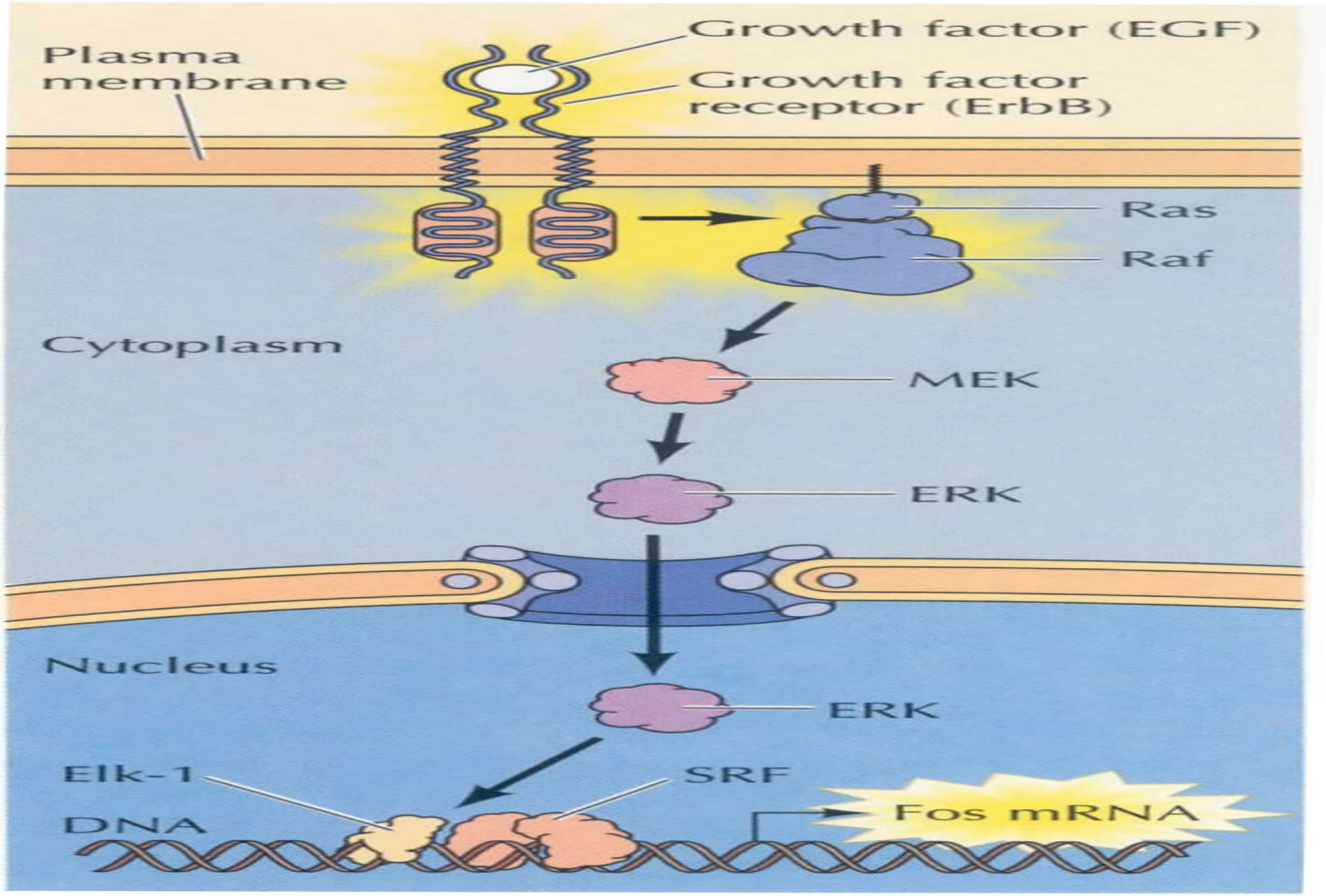
# MEK

- RAF phosphorylates mitogen activated protein kinase kinase (MEK) increasing its activity.
- MEK1 and MEK2 are inhibited by trametinib in B-RAF inhibitor –naïve patients.
- The MEK1/MEK2 inhibitor selumetinib plus docetaxel are being investigated in KRAS-mutant NSCLC patients.

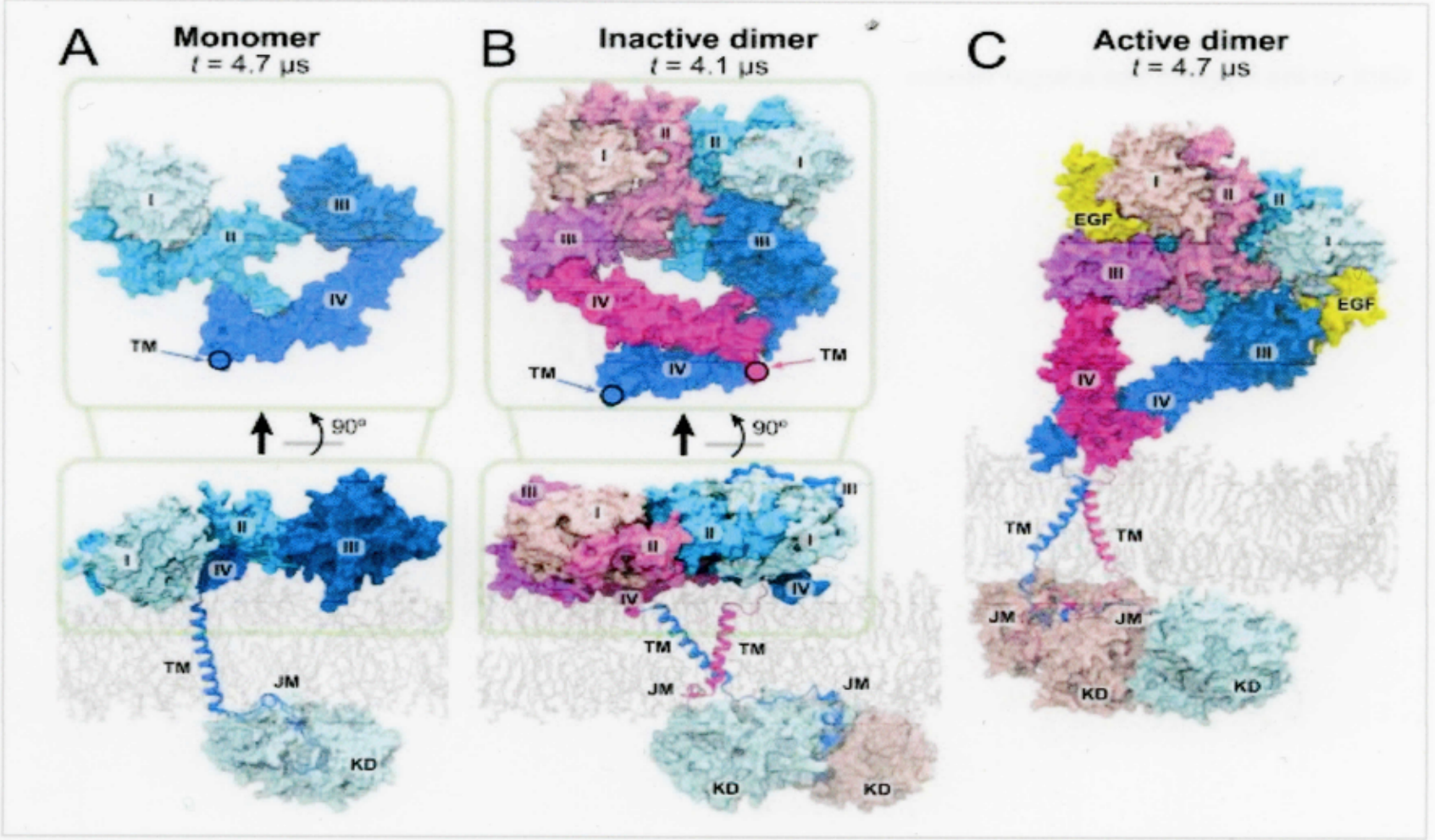
# ERK

- MEK1/MEK2 regulates the phosphorylation of extracellular signal-regulated kinases (ERK) 1 and 2.
- Phosphorylated ERK goes to the nucleus where it regulates expression of transcription factors such as fos, jun or myc.

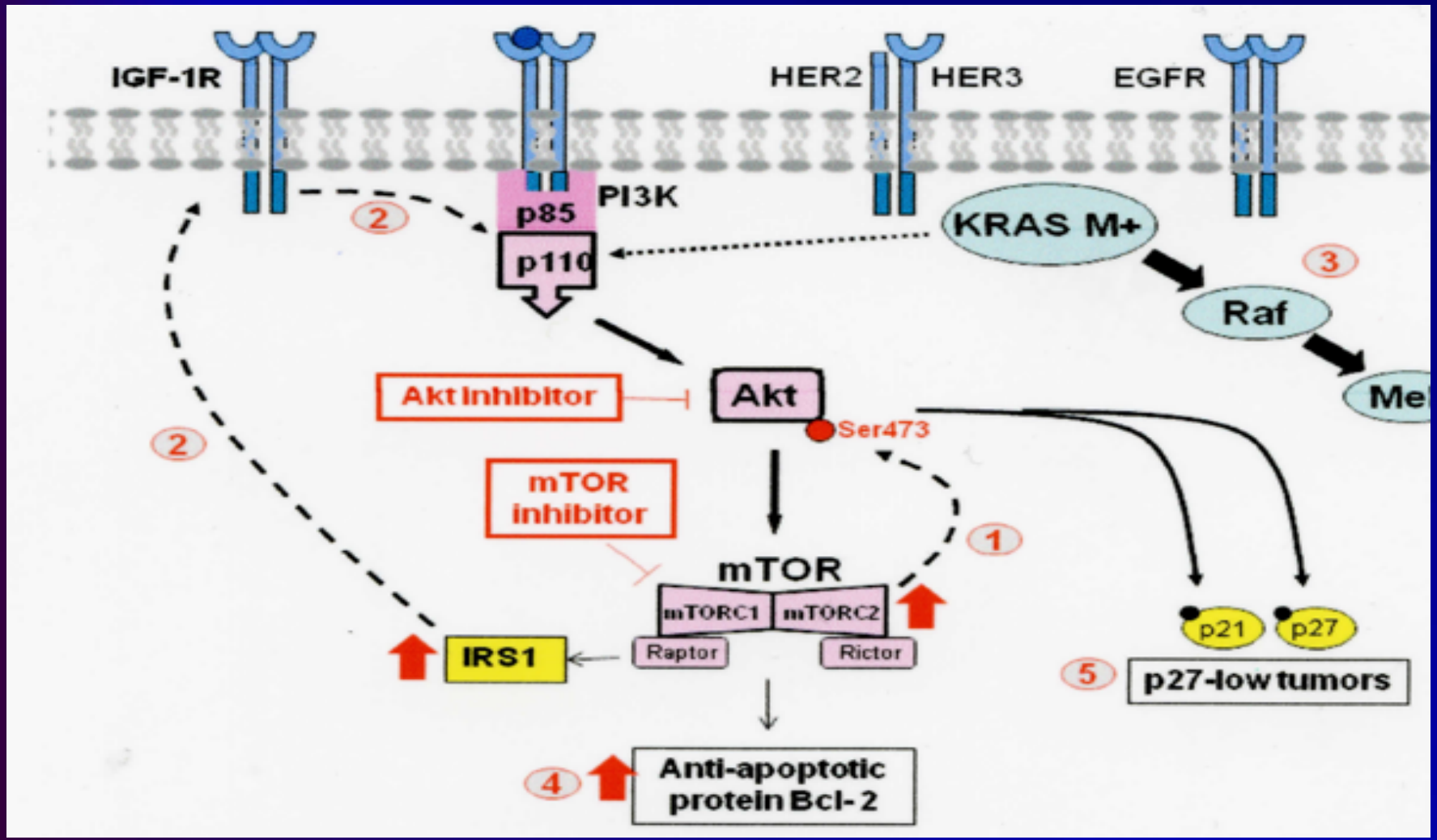
# The EGFR stimulates cancer cell growth. *Molecular Biology of the cell; Alberts et al., 2001.*



# EGFR dimerization



# PI3K, Akt, mTOR pathways stimulate cellular survival.



# PI3K

- The phosphatidylinositol 3 kinase (PI3K) pathway promotes cancer cell survival.
- The catalytic 100 kDa subunit metabolizes $PIP_2$ to $PIP_3$
- PI3K is mutated in breast (25%), brain (27%), colon (30%) and stomach (25%) at E542, E545 or H1047 resulting in a gain of enzymatic activity.

# PTEN

- PI3K mutations involve chromosome 10q, which contains phosphatase and tensin homolog (PTEN).
- PTEN metabolizes $PIP_3$ to $PIP_2$ leading to inhibition of AKT signaling.
- PTEN is mutated in approximately 13% of breast cancer patients but loss of heterozygosity is more common.

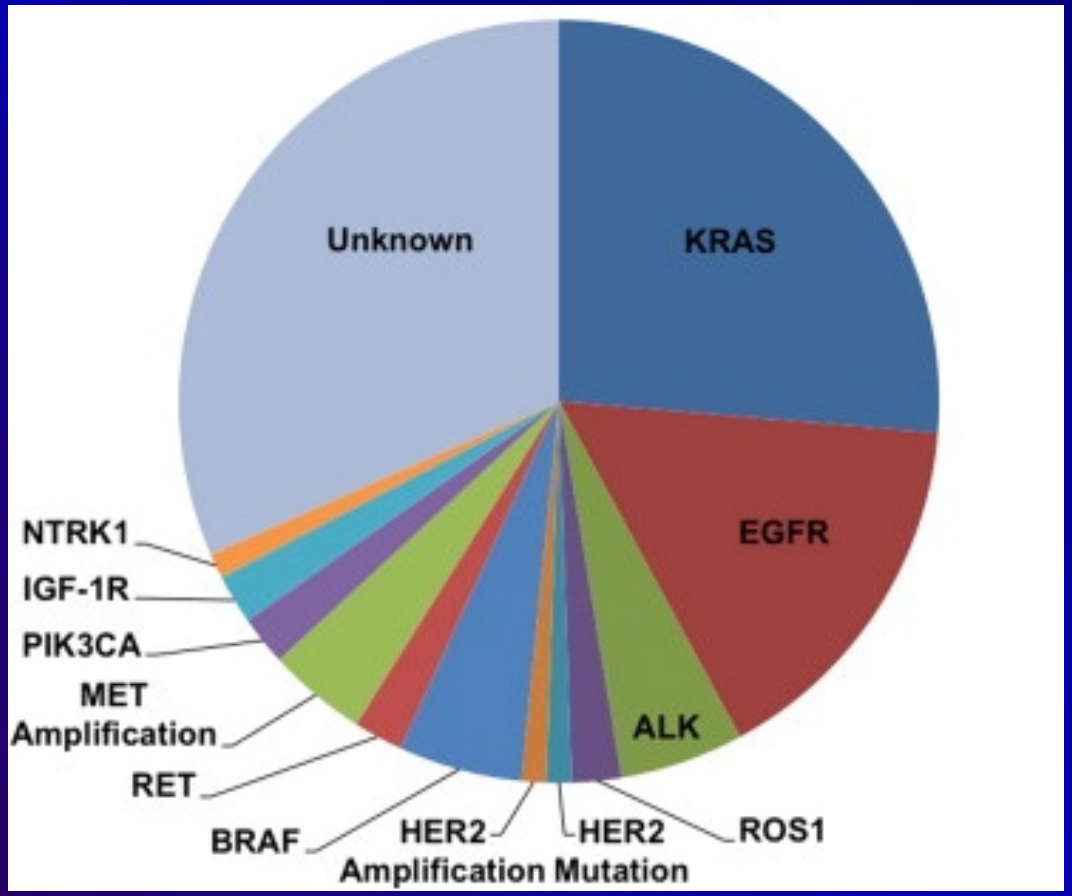
# Akt

- AKT or protein kinase B prevents apoptosis of cells.
- AKT is a serine/threonine kinase which is phosphorylated at Ser473 increasing phosphorylation of mTOR.
- AKT promotes cellular survival by phosphorylating BAD and caspase-9 preventing apoptosis of cancer cells.
- AKT is mutated in breast cancer (5%), colorectal cancer (6%) and ovarian cancer 2%.

# mTOR

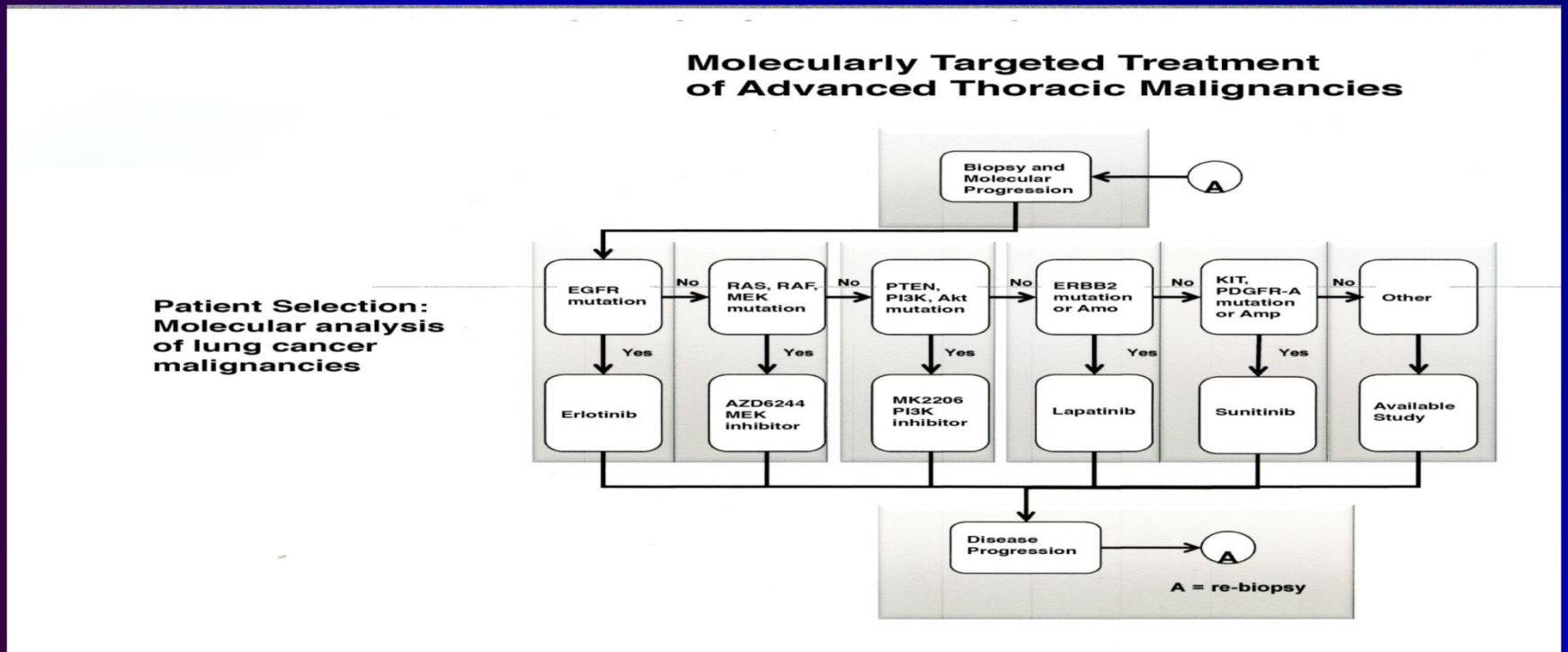
- Mammalian target of rapamycin (mTOR) or FRAP1 is a serine/threonine kinase.
- mTOR activation enhances phosphorylation of p70S6 kinase and 4E-BP1 increasing protein translation and cellular proliferation.
- mTOR activation decreased autophagy, a lysosome-dependent degradation pathway.

# Personalizing Therapy for NSCLC Genetic Abnormalities in Lung Adenocarcinoma

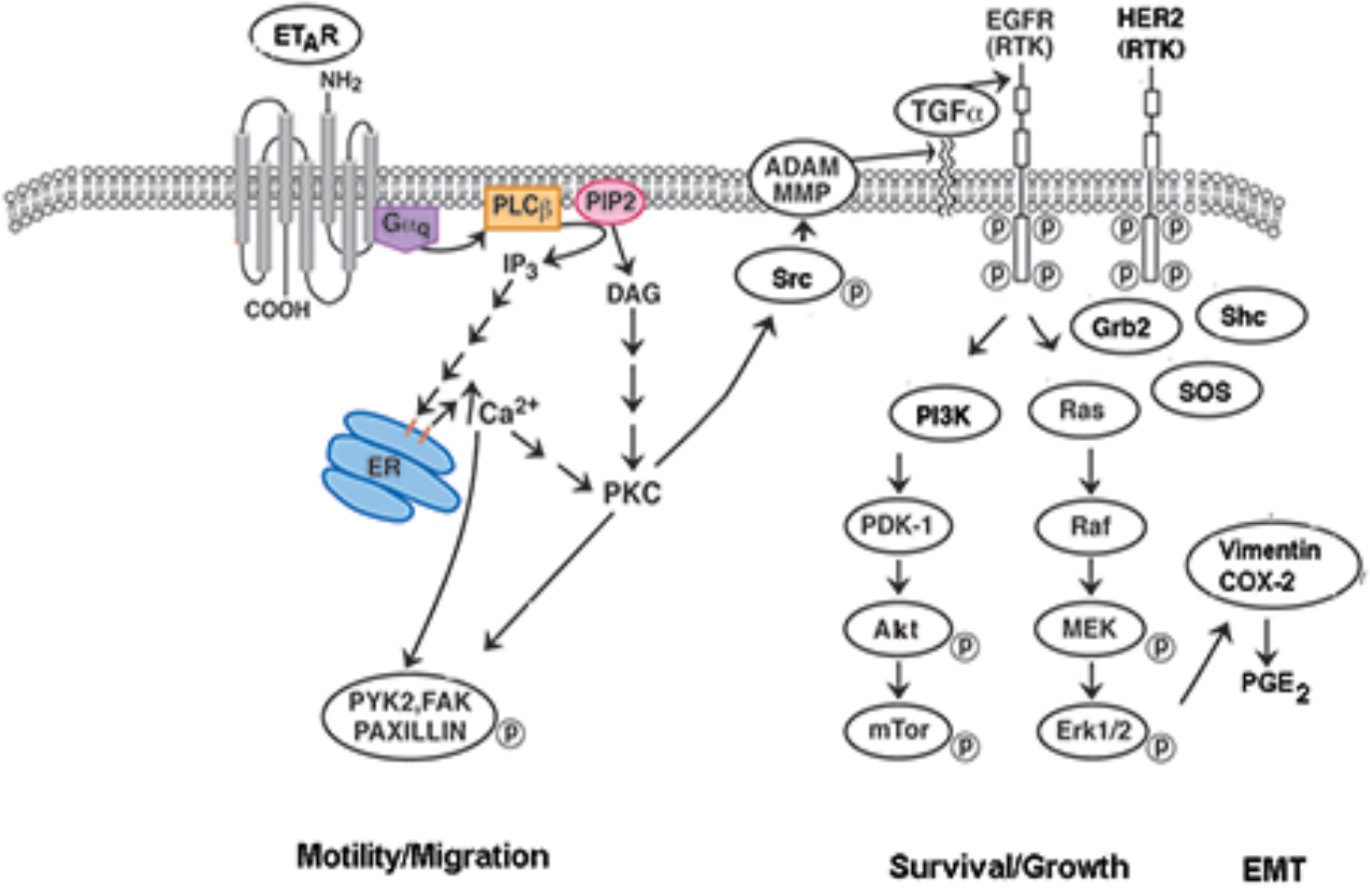


# Molecular medicine

## Molecularly Targeted Treatment of Advanced Thoracic Malignancies



# GPCR transactivate the EGFR and HER2



# Erlotinib/gefitinib resistance

- Approximately 50% of NSCLC patients develop resistance to erlotinib/gefitinib after 1 year due to a secondary mutation in the EGFR (T790M).

# CML patients are sensitive to the small molecule TKI Gleevec.

- This restores blood counts in patients and delays disease progression.

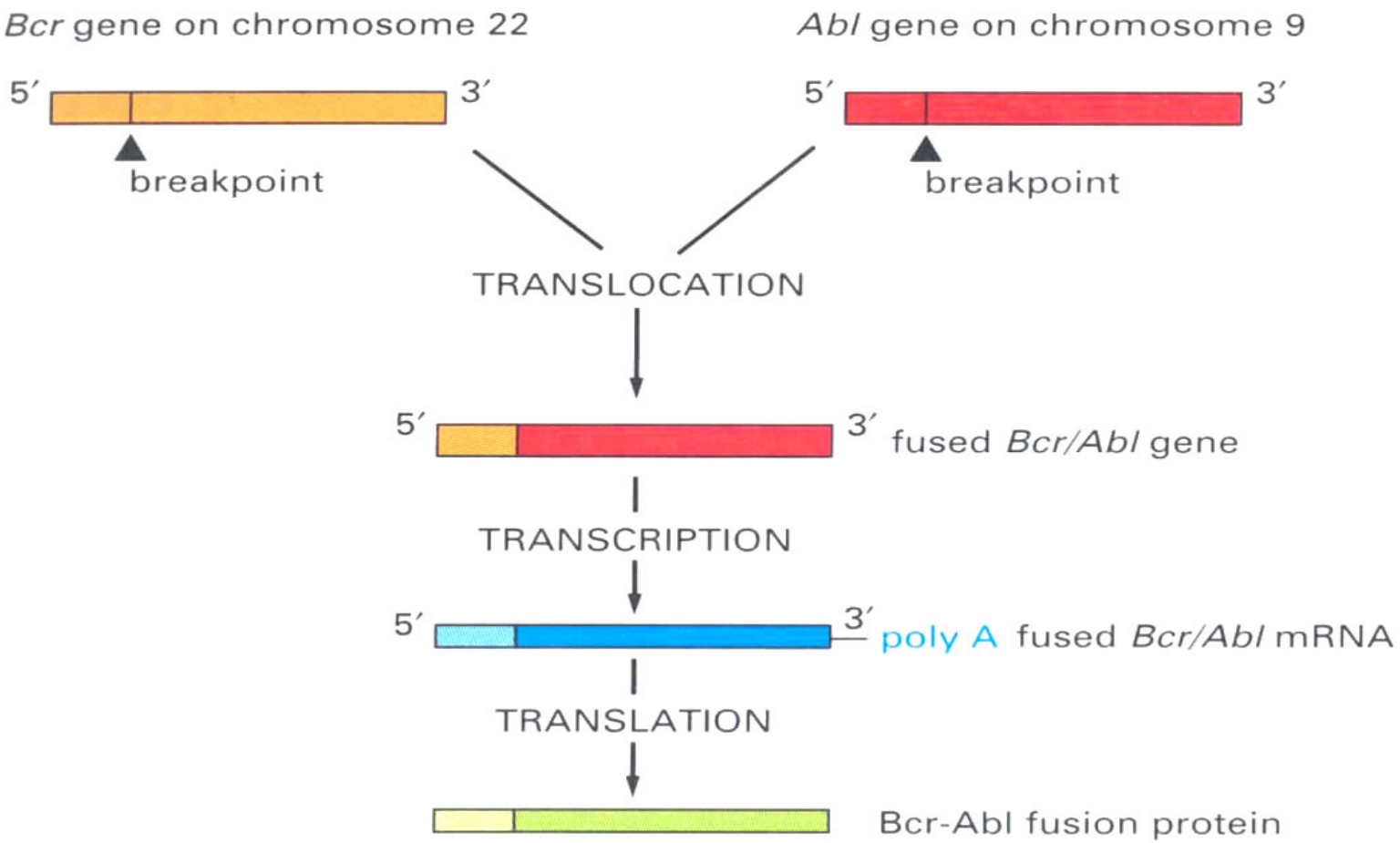
# CML patients

**CML patients have a genetic abnormality on chromosome 22 (Philadelphia chromosome).**

- Segments of chromosome 9 and 22 are fused resulting in the bcr-abl gene.
- The resulting tyrosine kinase is constituitively active.
- Bcr-abl tyrosine kinase actvity is inhibited by Gleevec.

# Translocation of Bcr/Abl.

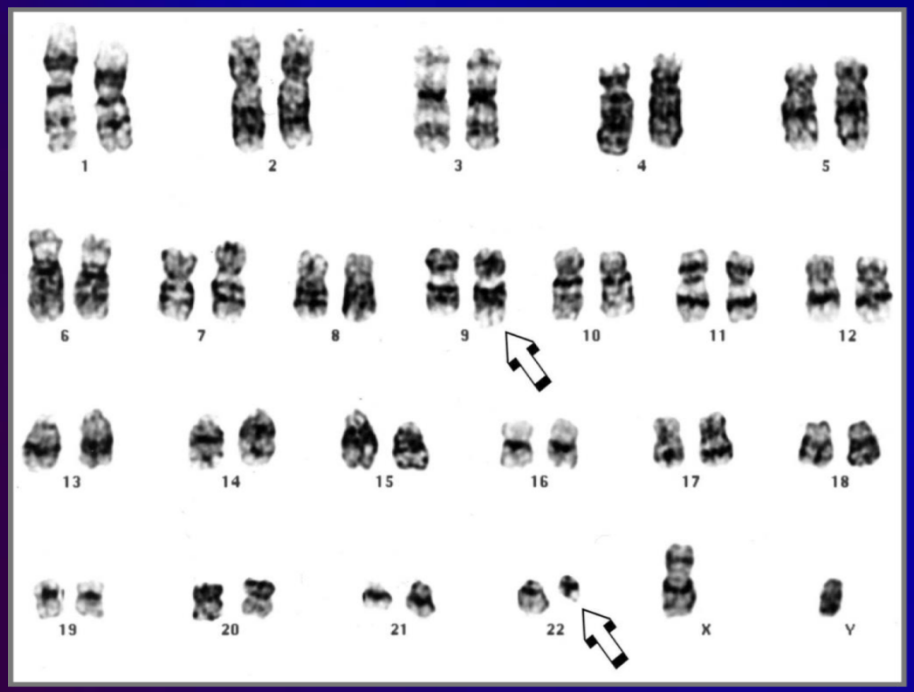
- Chromosome 22 translocates with chromosome 9. Molecular Biology of the Cell; Alberts et al., 2001.



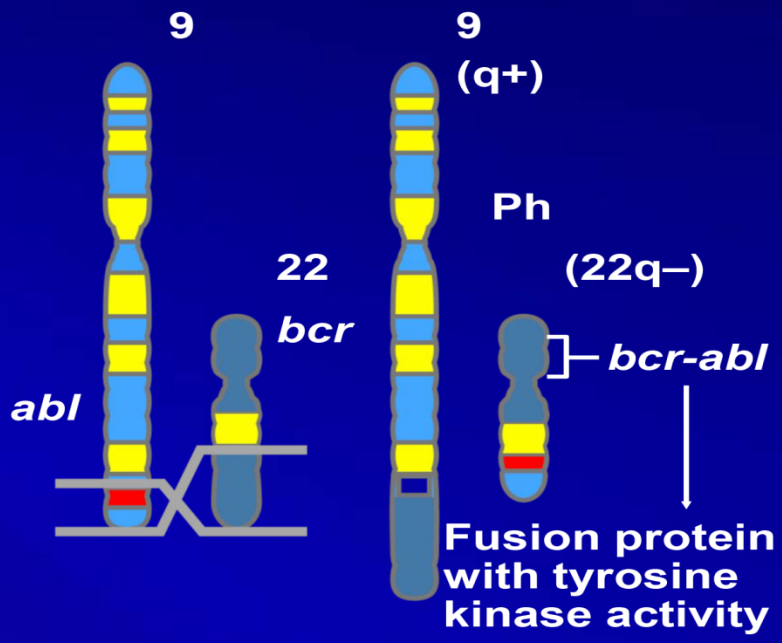
# Bcr-Abl

**Translocation of *Bcr-Abl* Genes**

- **Translocated chromosome 9 appears larger and translocated chromosome 22 appears smaller: Freebies for Teachers"; D. Kerrigan.**




Artwork by Jeanne Kelly. © 2004.



# In a Phase I Clinical Trial, Gleevec™ was effective orally at a daily dose of 300 mg or greater.

- Dose limiting toxicities included nausea, vomiting, edema and rash. (Sawyers and Druker. Cancer J. Sci. Am. 1999;5:63).

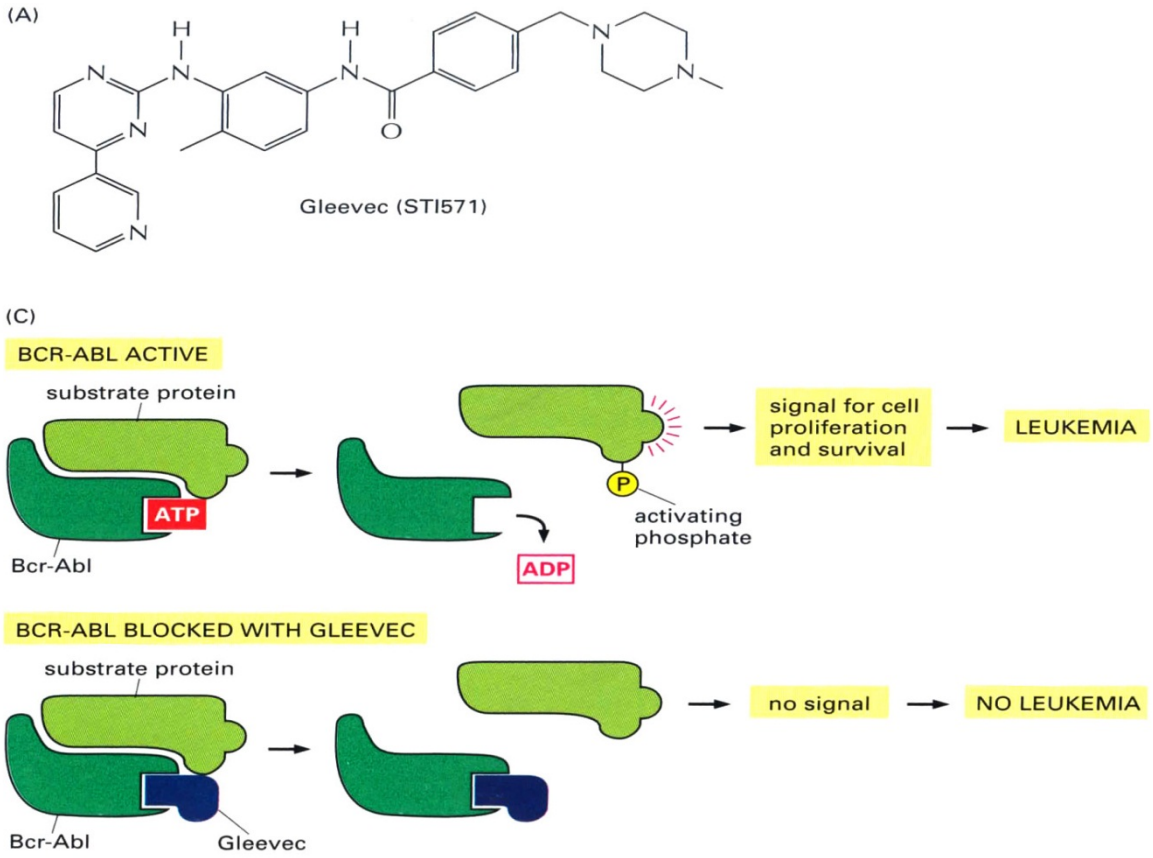
# In a Phase II Clinical Trial, Gleevec™ restored normal blood counts in 53 out of 54 chemotherapy-resistant CML patients.

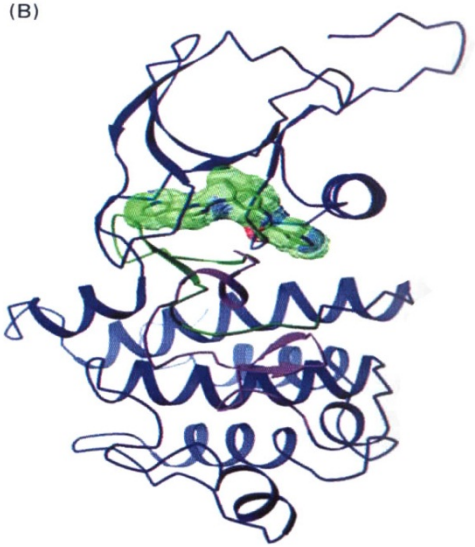
- After a year on Gleevec, 51 of these patients were still doing well. (Druker et al. N. Engl. J. Med. 2001; 344: 1038.).

- Over a 5 year period, 89% of the patients treated with Gleevec had progression-free survival (O'Hare et al., Clin. Cancer Res. 2011; 17: 212).

# Gleevec mechanism of action

- Gleevec blocks the ATP binding site. Molecular biology of the cell; Alberts et al., 2001.



# GLEEVEC RESISTANCE

- Over a 5 year period, 17% of the patients initially sensitive to Gleevec became resistant.
- BCR-ABL point mutations occurred such as T315I near the ATP binding site impairing Gleevec interactions
- New drugs such as ponatinib or DCC-2036 are being developed which bind with high affinity to mutated BCR-ABL

# Tyrosine kinase inhibitors in cancer

| | | |
|---|---|---|
| • CML | Bcr-Abl | Imitanib/dasatanib |
| • Breast cancer | HER2 | Herceptin/lapatanib |
| • Melanoma | B-RAF | PLX4032 |
| • GIST | c-KIT | Imatinib/sunitinib |
| • NSCLC | EGFR | Gefitinib/erlotinib |

# PRACTICAL STEPS TO PREVENT CANCER

- Check your house for radon.
- Check your house for asbestos.
- Take precautions at your workplace.
- Check your community water system.
- Avoid breathing polluted air.
- Protect your skin.
- Don’t breathe smoke.
- Exercise daily.

# Cancer Prevention

## PRACTICAL STEPS TO PREVENT CANCER (continued)

- Avoid pesticides.
- Eat fruits and vegetables.
- Reduce red-meat consumption.
- Eat fish.
- Minimize fried foods.
- Drink alcohol in moderation.
- Avoid unnecessary x-rays.
- Reduce infections.

# REFERENCES


**REFERENCES**

- Hanahan, D. and Weinberg, R.A. Hallmarks of cancer: The next generation. Cell 2011; 144(5): 646-74.
- O'Hare, T., Deininger, M.W.N., Elde, C.A., Clackson, T., and Druker, B.J. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. Clin. Cancer Res. 2011; 17(2):212-21.