TRAnslational research in Clinical Oncology (TRACO)

Program Director

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Organizing Committee

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SYLLABUS

DATE	TOPIC SPEAKERS
Sept. 5	Introduction, Tumor Imaging Moody, Choyke
Sept. 11	Clinical trials, Precision medicine Smith, Harris
Sept. 25	Immune checkpoint, Ovarian cancer Goff, Annunziata
Oct. 2	Prostate cancer, TGF beta Madan, Jakowlew
Oct. 10	Radiation oncology, Topoisomerase Nichols, Pommier

SYLLABUS, continued

DATE	TOPIC	SPEAKERS
Oct. 17	Lymphoma, Small molecules	Dunleavy, Simeonov
Oct. 23	Breast cancer, SCLC	Zia, Chen
Oct. 30	Epidemiology, Cancer health disparities	Caporaso, Ryan
Nov. 6	Cervical cancer, Genomics	Schiller, Wei
Nov. 14	NSCLC, HSP90	Szabo, Neckers

SYLLABUS, continued

DATE	TOPIC	SPEAKERS
Nov. 20	Functional Genomics, HIV	Caplen, Maldarelli
Nov. 27	Epigenetics, Case reports	Verma, Olaku
Dec. 4	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

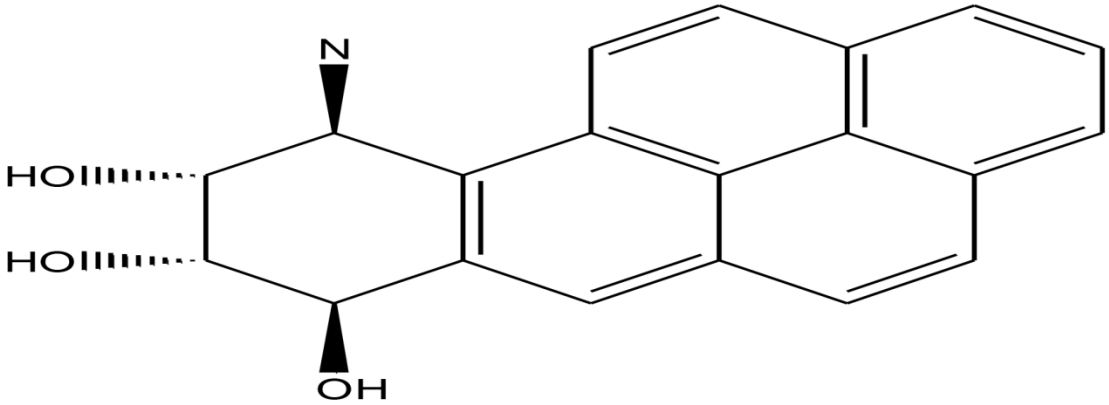
The process by which unreactive carcinogen converts to a form which binds DNA is known as metabolic activation.

- **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

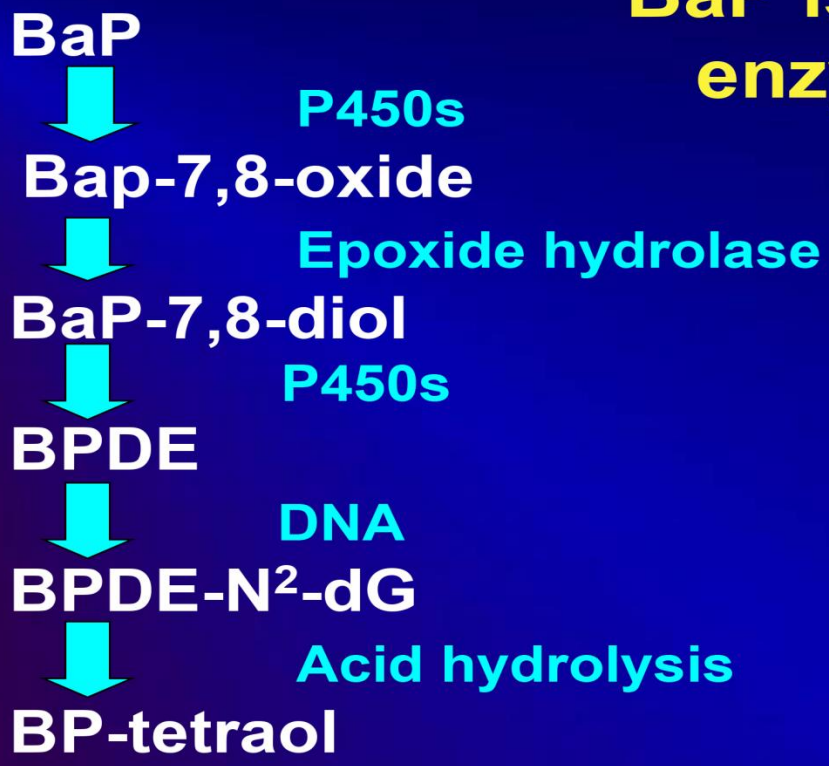
BENZ(a)Pyrene

- 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:

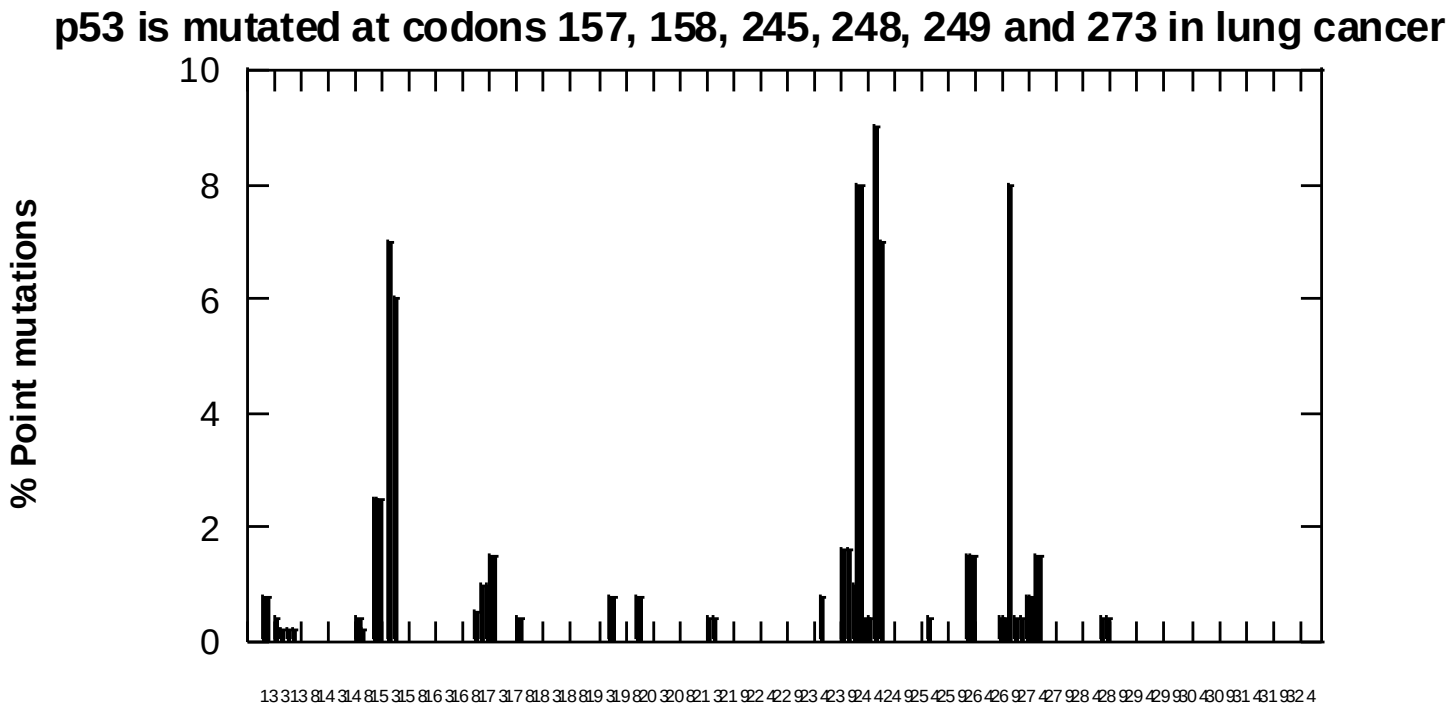
- mediates the G1 to S-phase checkpoint of the cell cycle,
- drives programmed cell death or apoptosis after DNA damage,
- is increased along with p21 (cell cycle checkpoint) after DNA damage.
- Phosphorylated p53 induces expression of BAX (apoptosis), GADD45 (DNA repair) and thrombospondin (angiogenesis)

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.

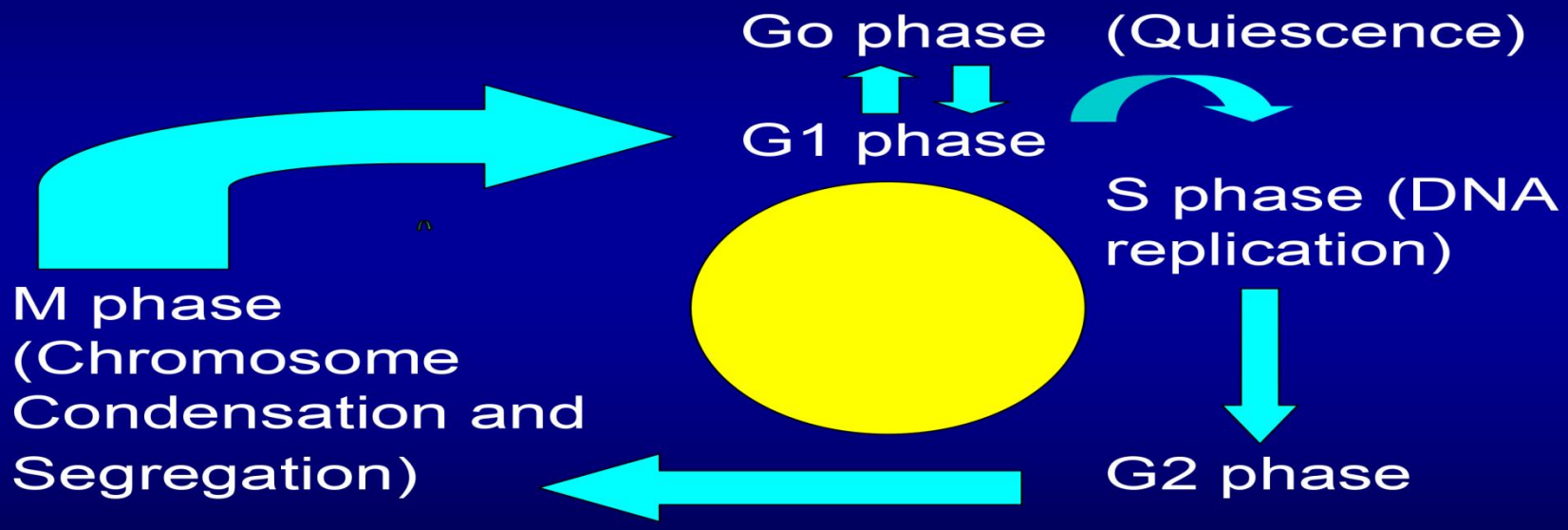
- P 53is mutated at codons 157, 158, 245, 248, 249 and 273 in lung cancer.



Cell cycle phases

Cell cycle phases.

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



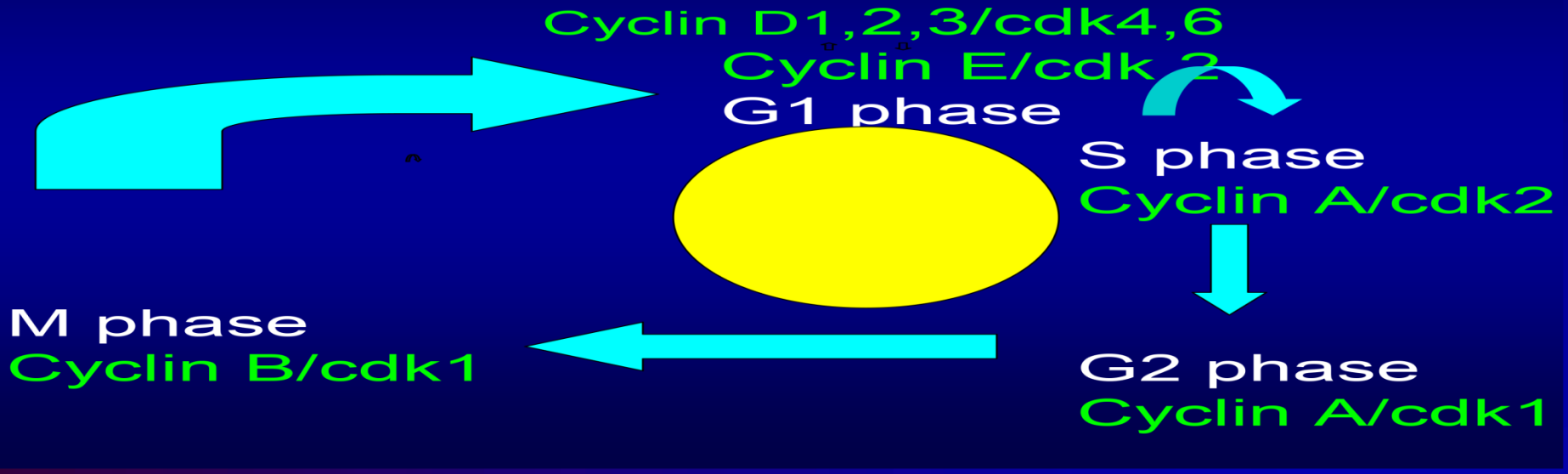
p53 mediates the G_1 to S-phase checkpoint of the cell cycle

- 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.

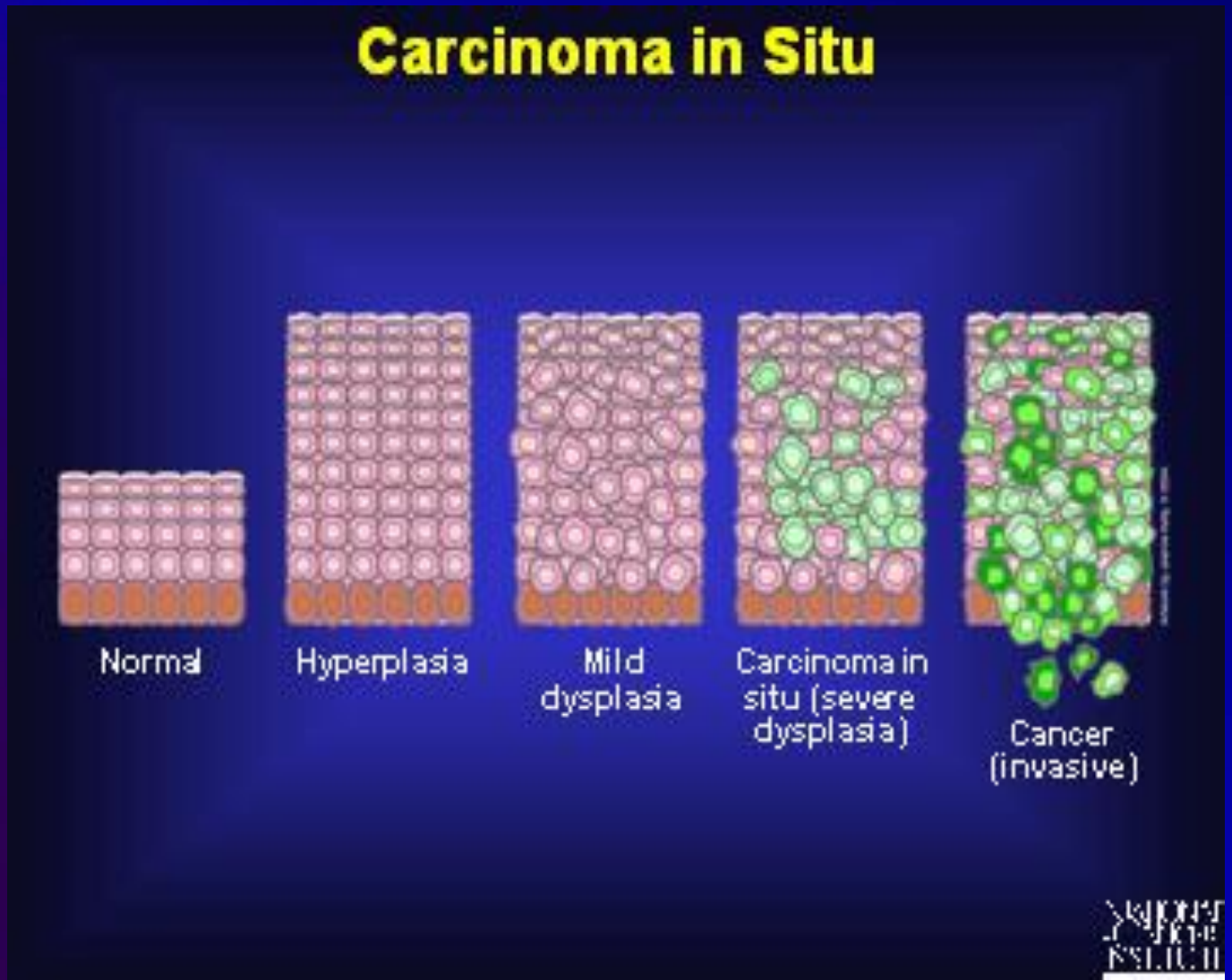


Genotoxicity of tobacco smoke.

- 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.

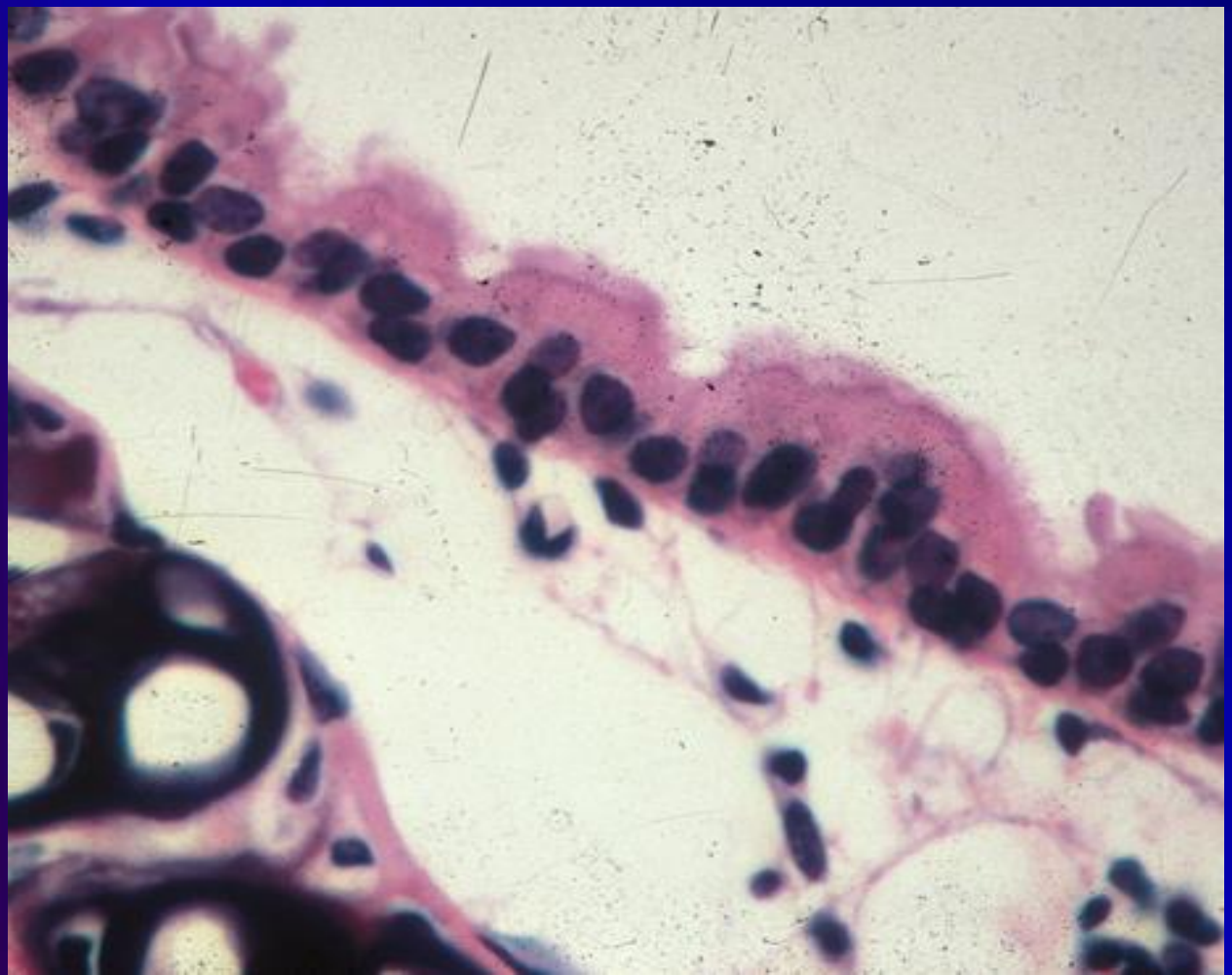
Carcinogenesis

- Cancer progression occurs over a period of decades.



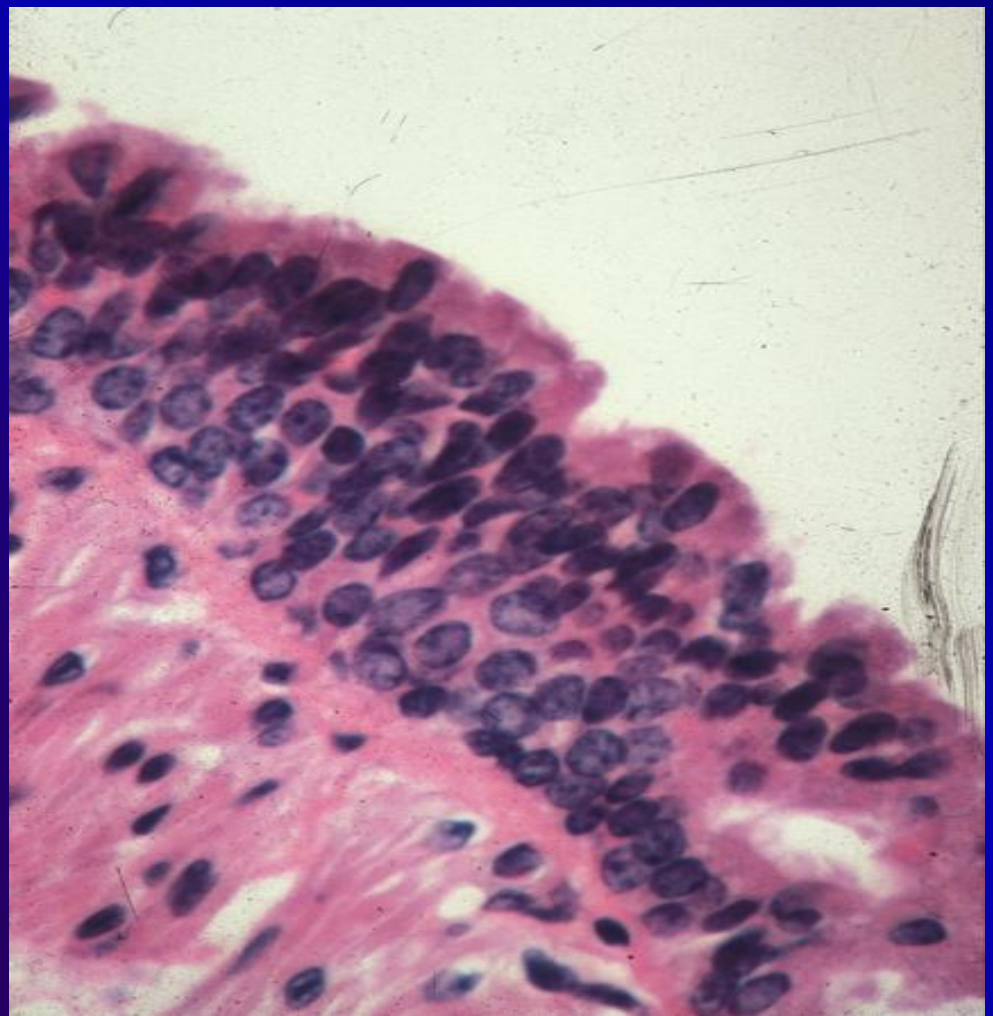
Normal lung

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



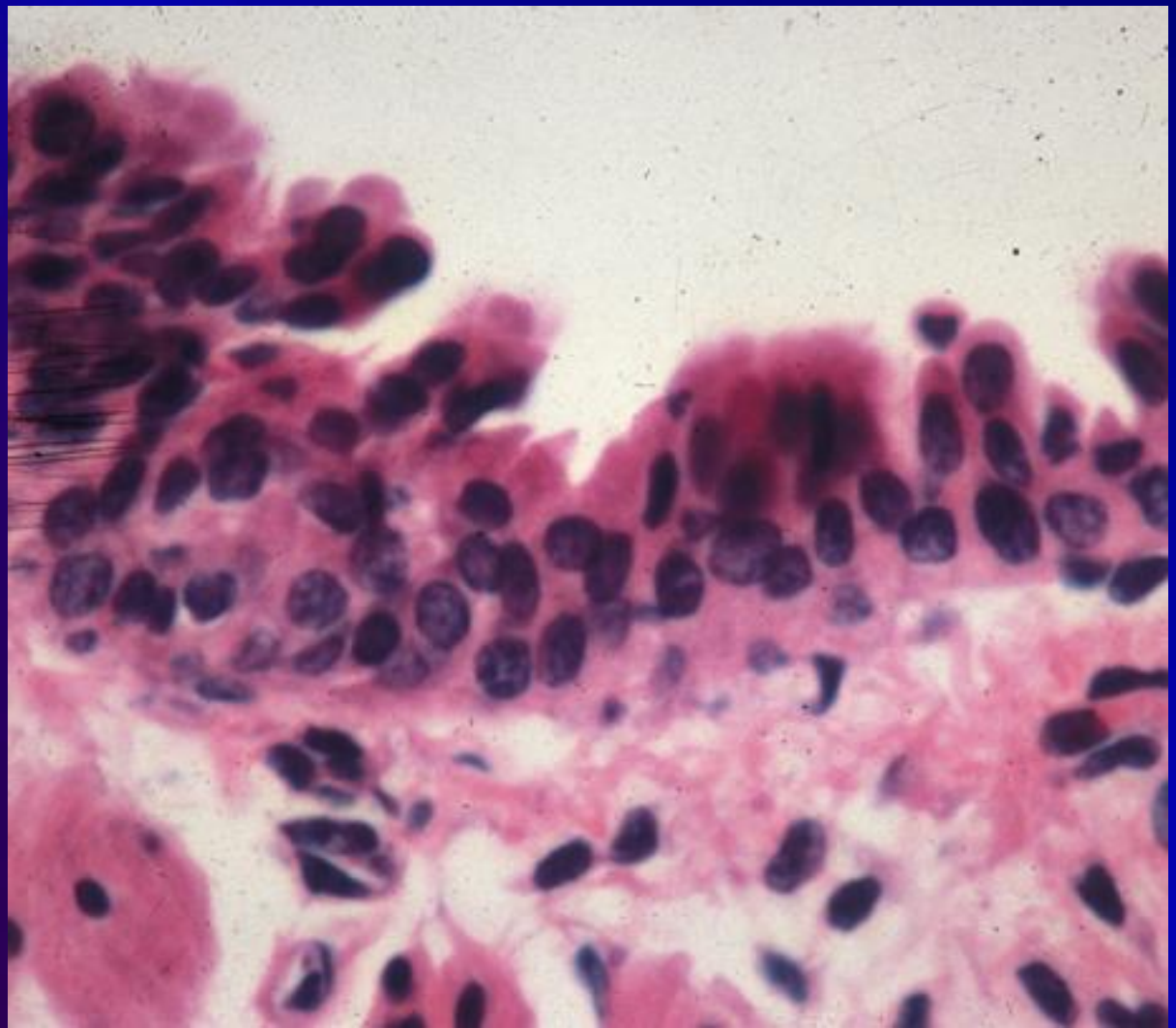
Hyperplasia

- After exposure to tobacco smoke, hyperplasia can result.



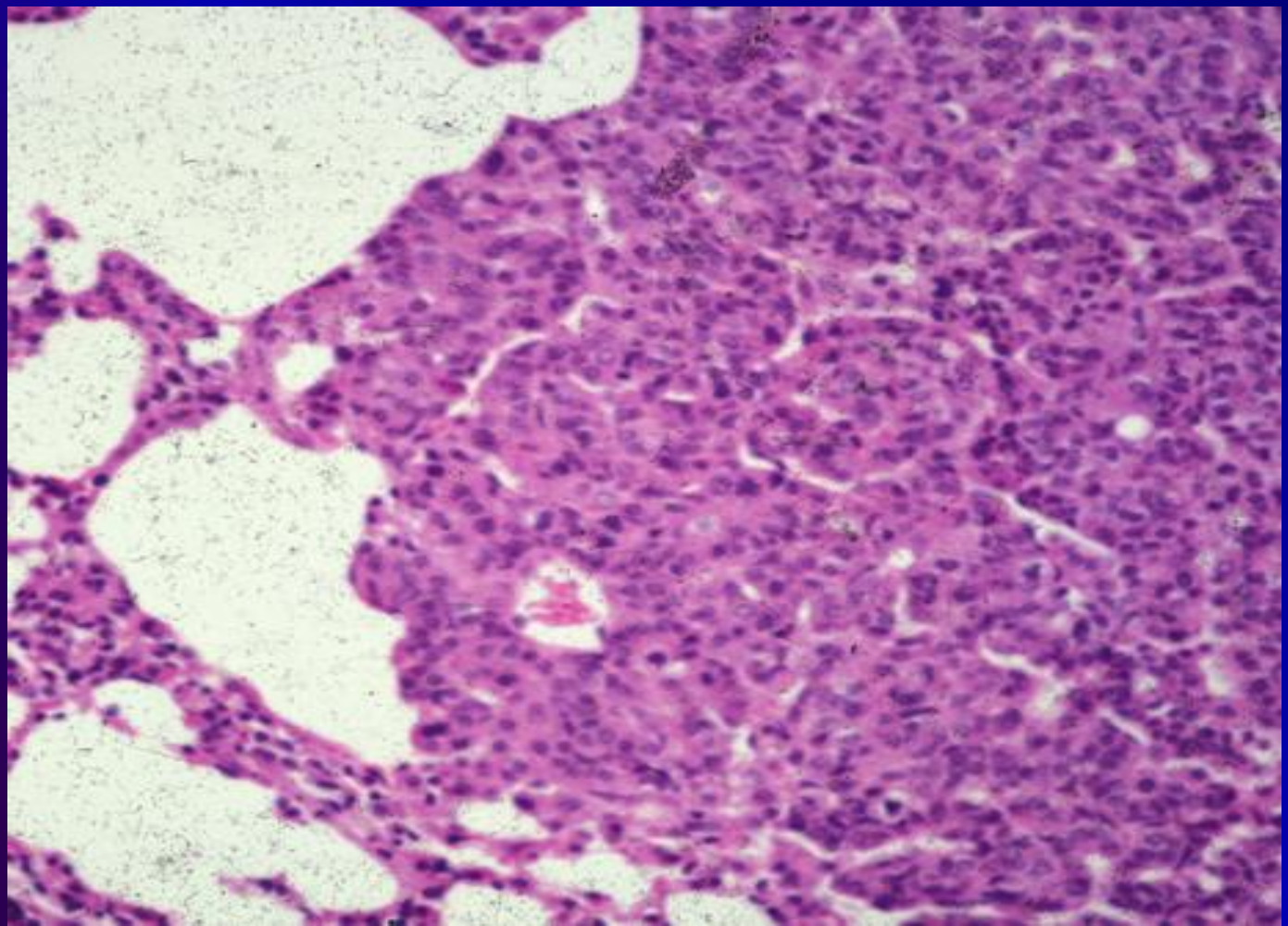
Dysplasia

Continued exposure to tobacco smoke leads to dysplasia.



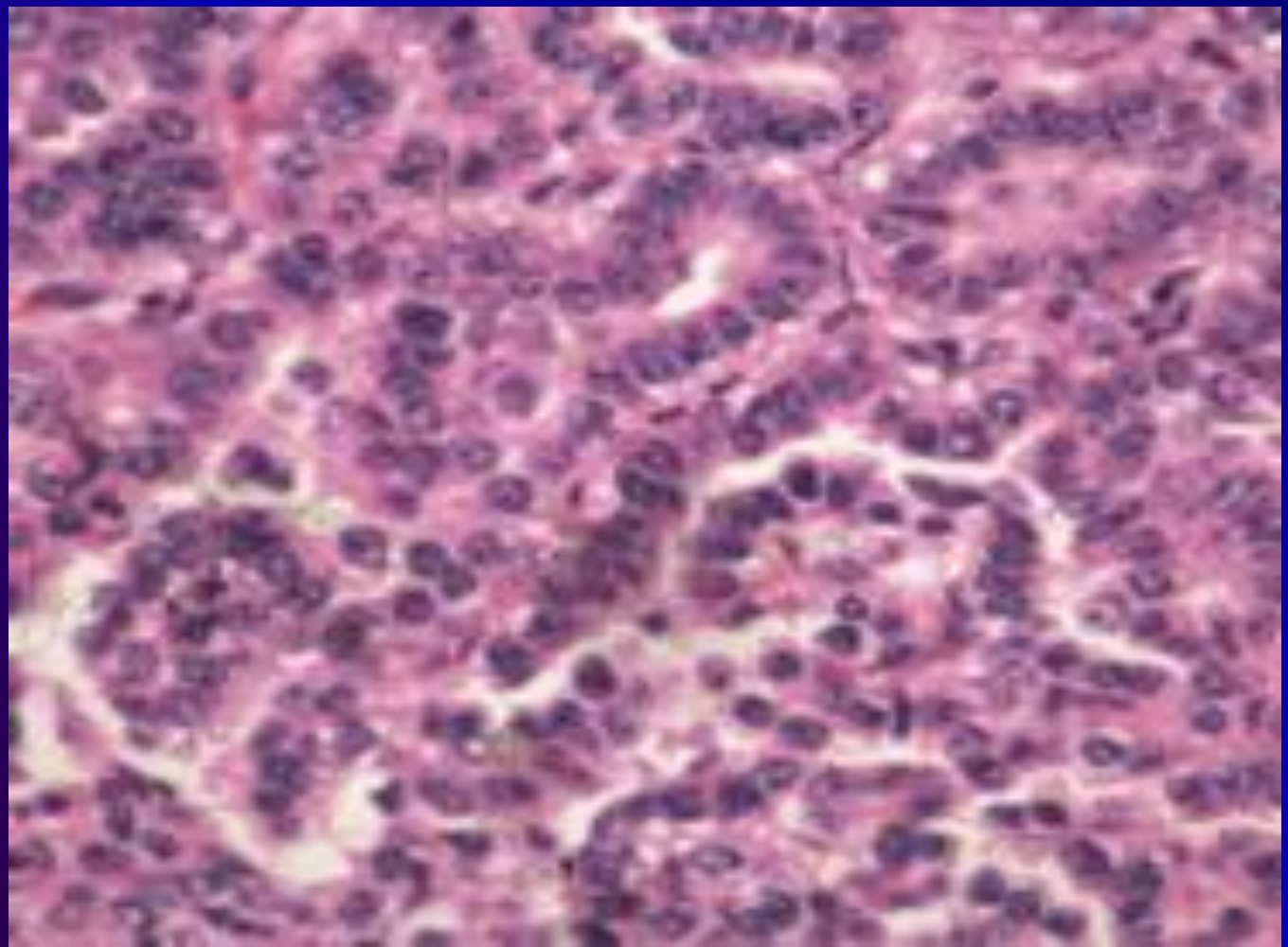
Adenoma

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



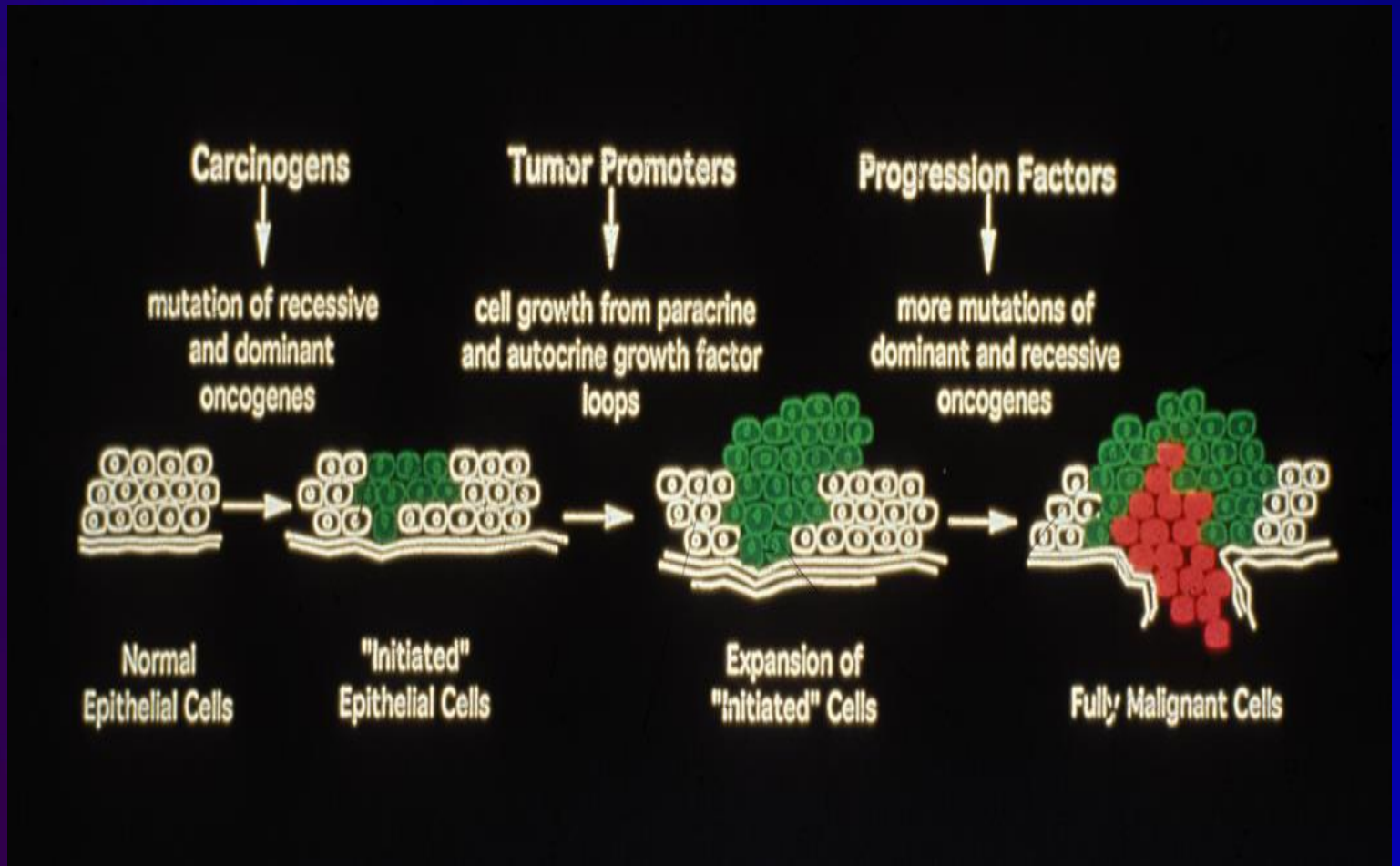
Adenocarcinoma

- 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.

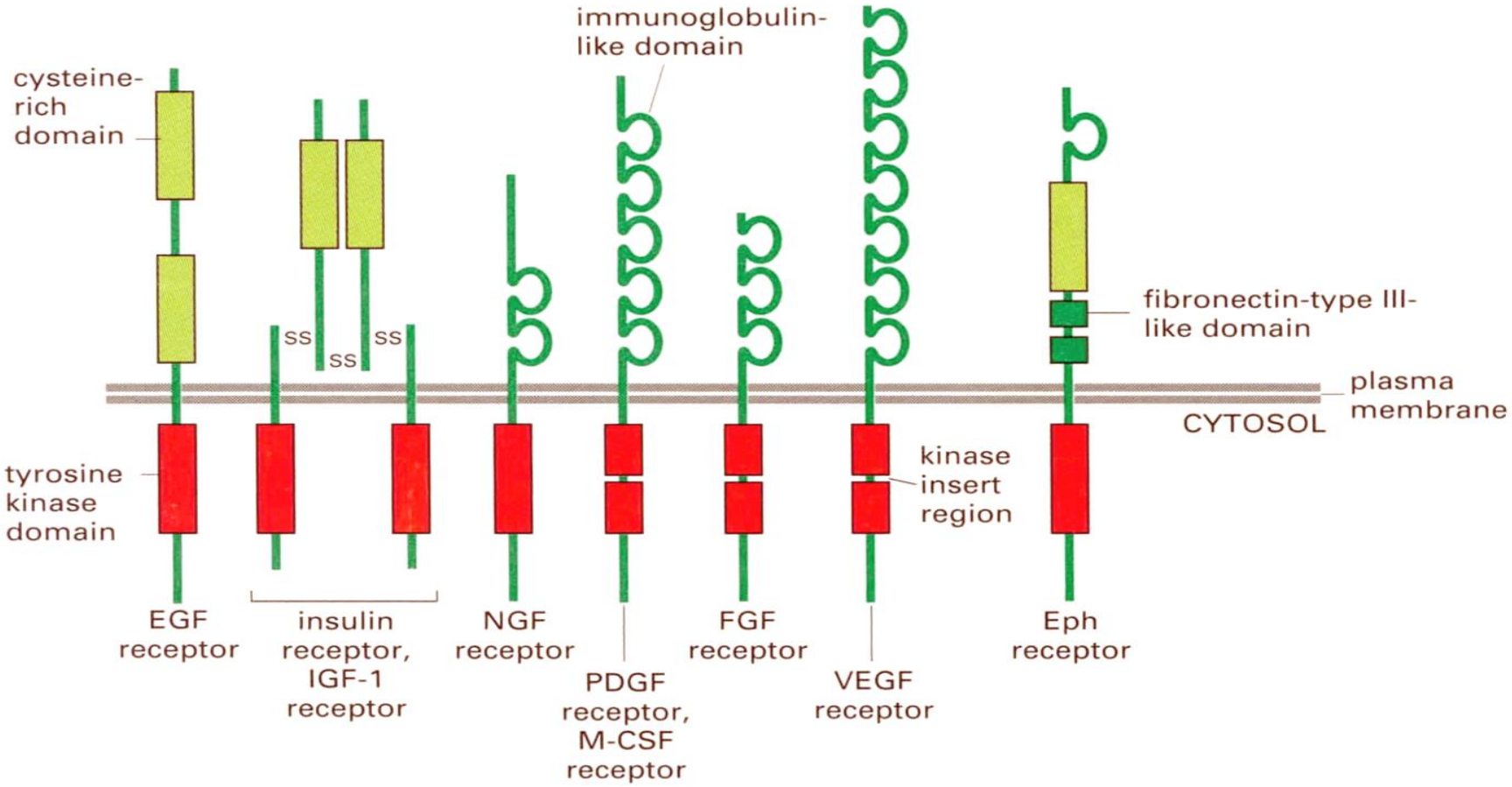
Genetic abnormalities in lung cancer include:

- **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

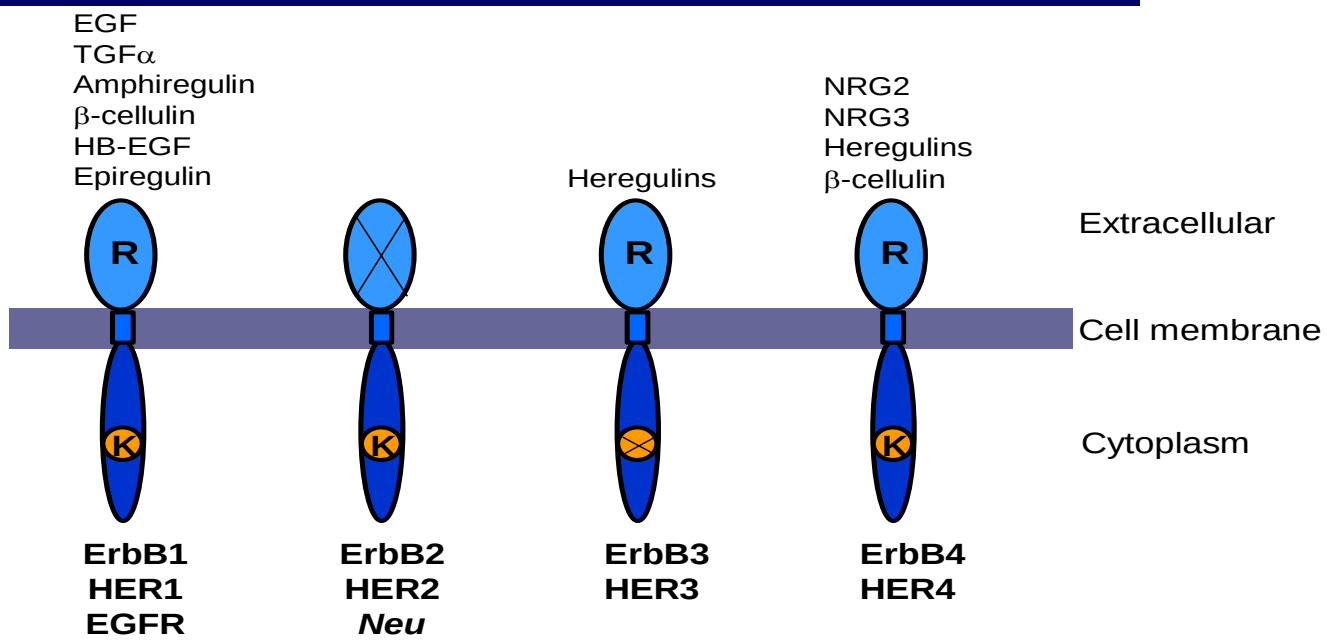
Tyrosine kinase receptors.

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.

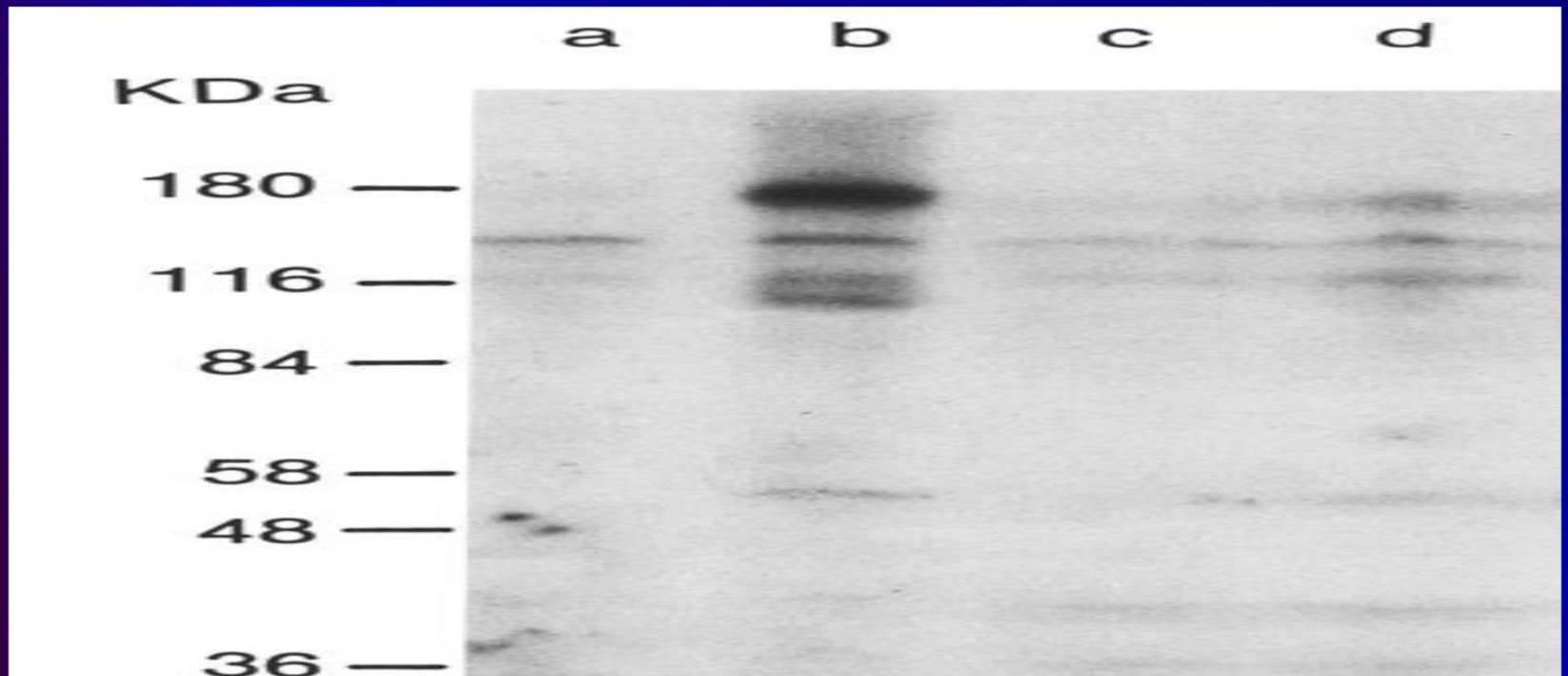
Agent	IC_{50}, ug/ml
EGF	.03
TGFα	.8
TGFα-PE38	.4
mAb 108	3
IgG	>10

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

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

EGFR tyrosine phosphorylation

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

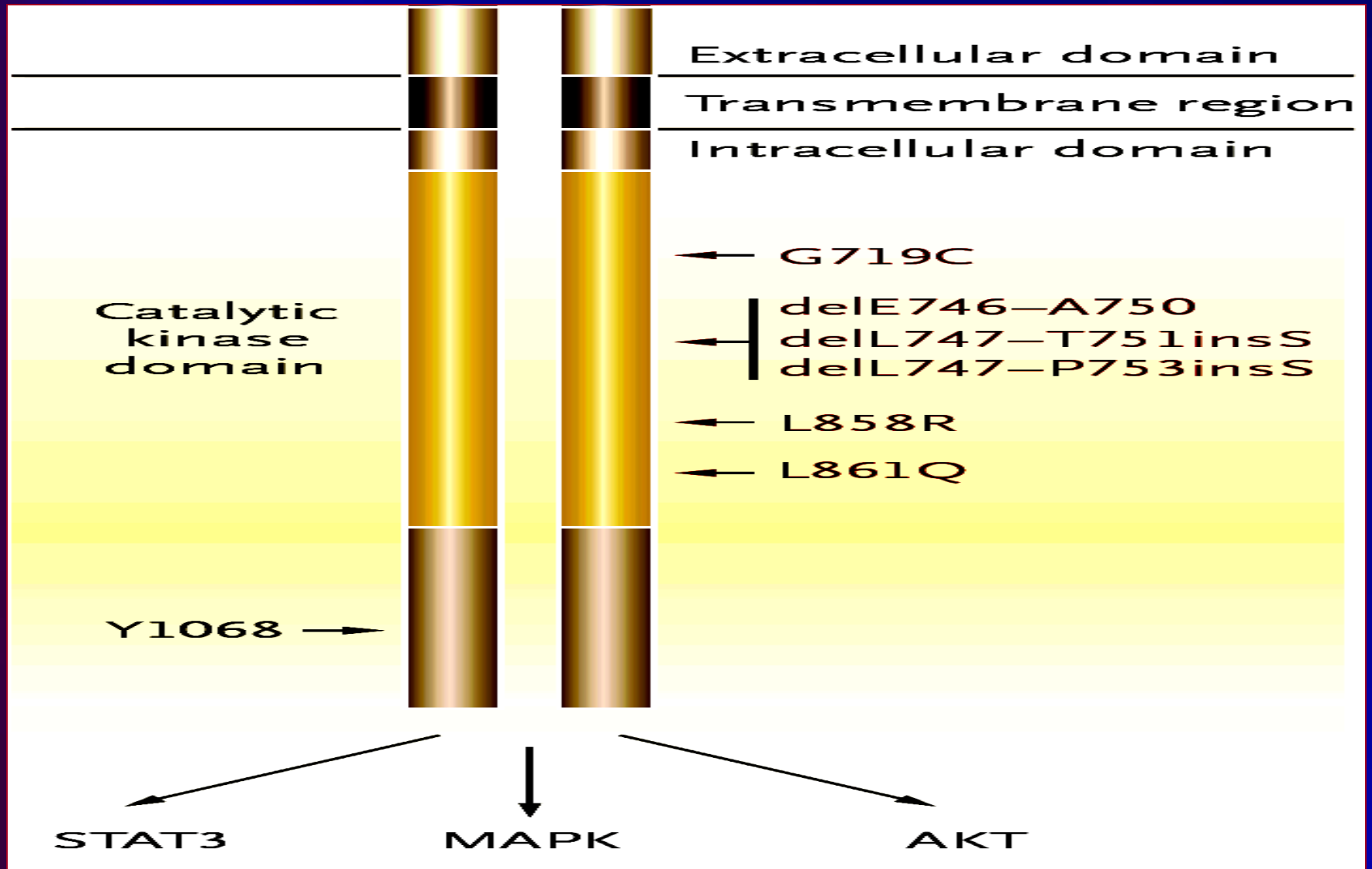


Tyrosine kinase receptors are mutated in several diseases leading to increased cancer proliferation.

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

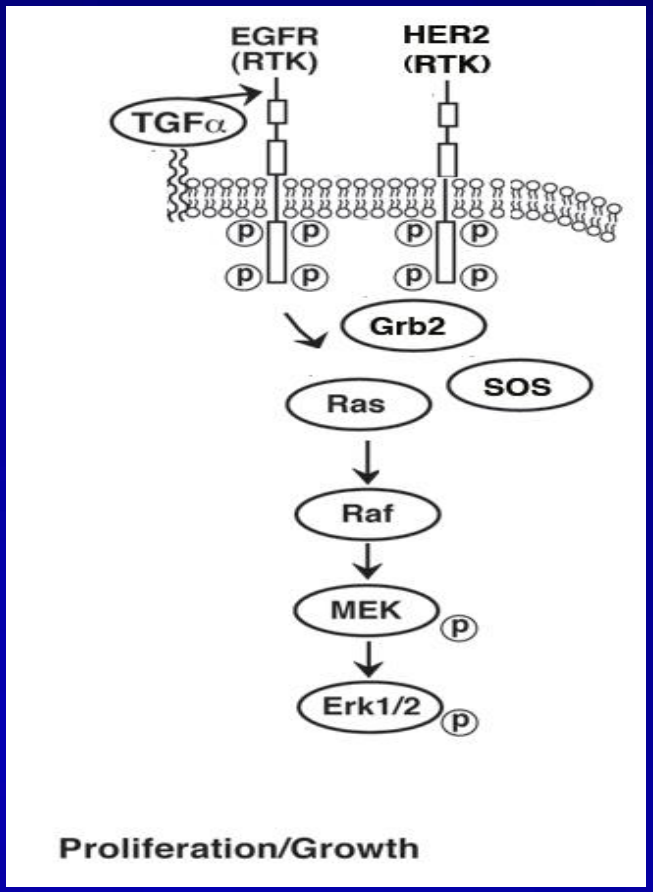
Paez et al., Science 304:1497 (2004)

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 an 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.

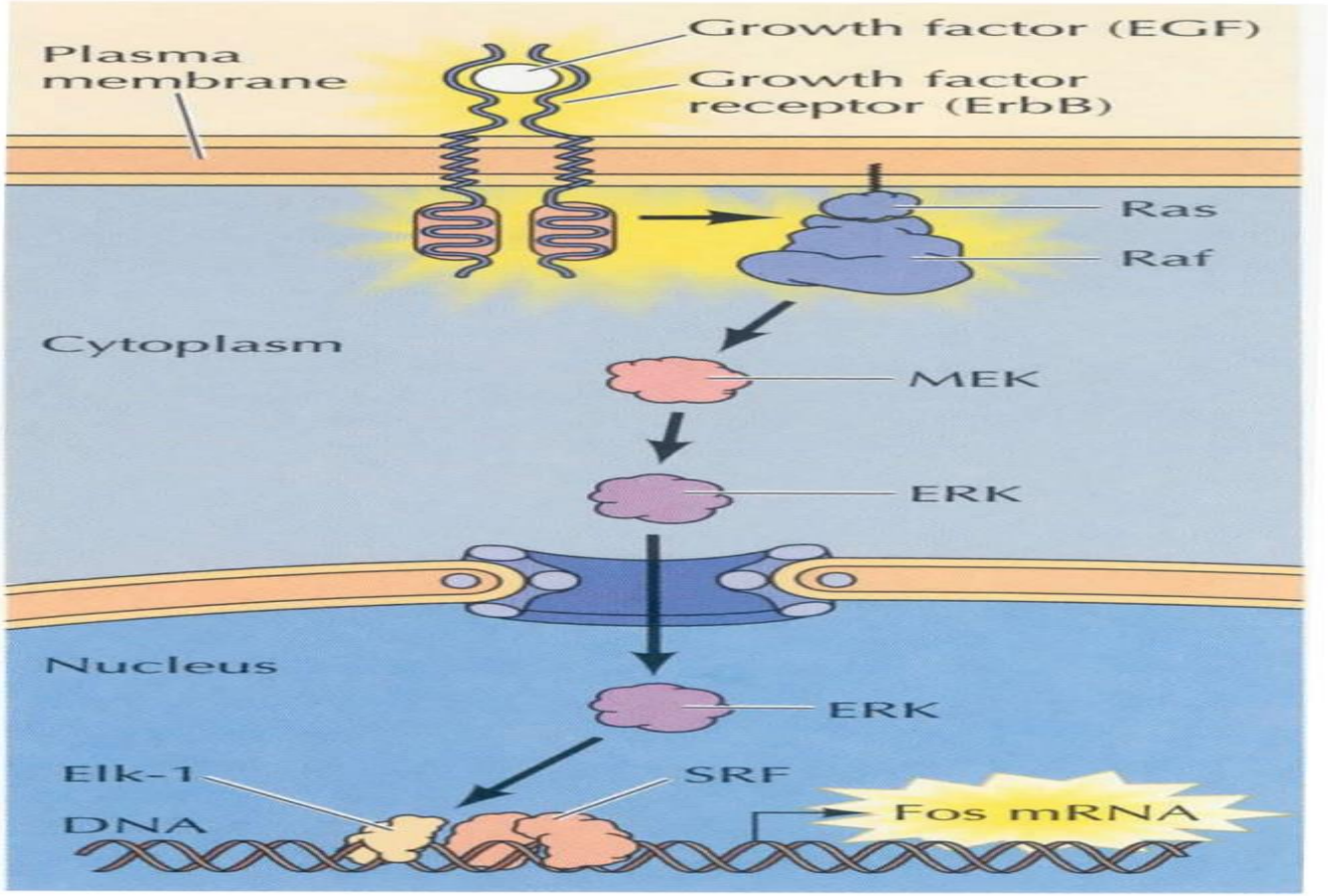
MEK

- 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.

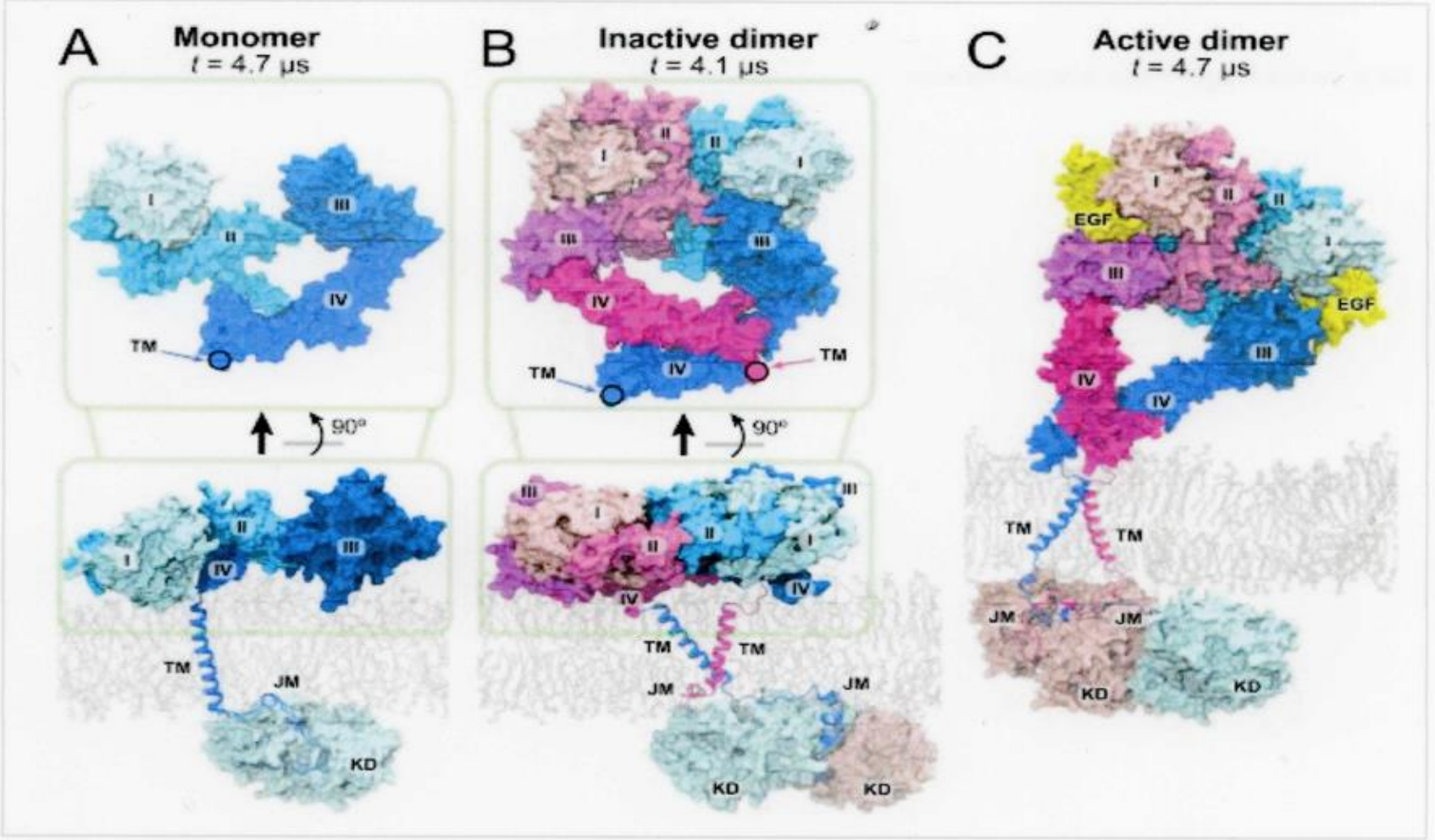
ERK

- 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.

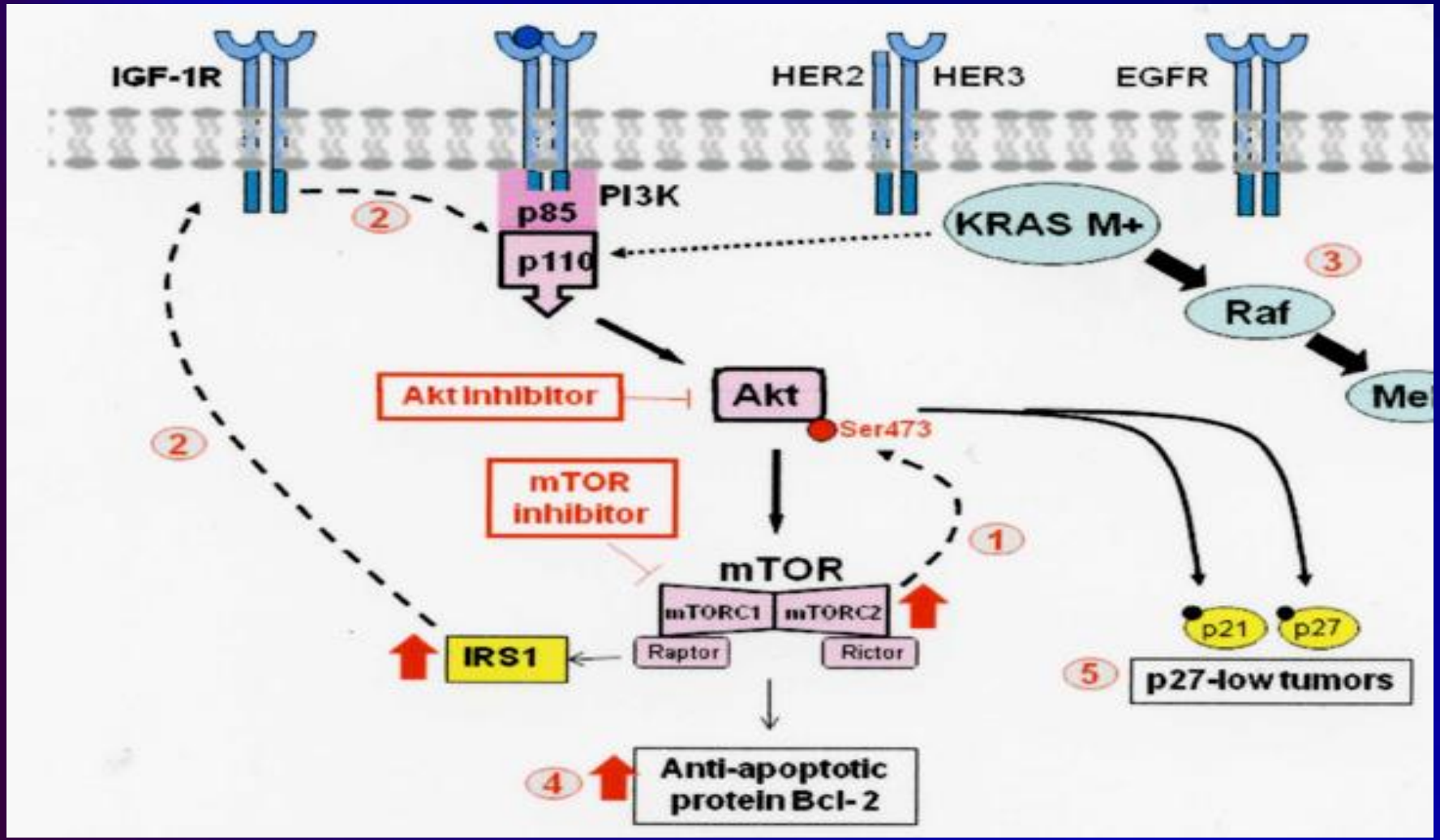
The EGFR stimulates cancer cell growth. *Molecular Biology of the cell; Alberts et al., 2001.*



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.

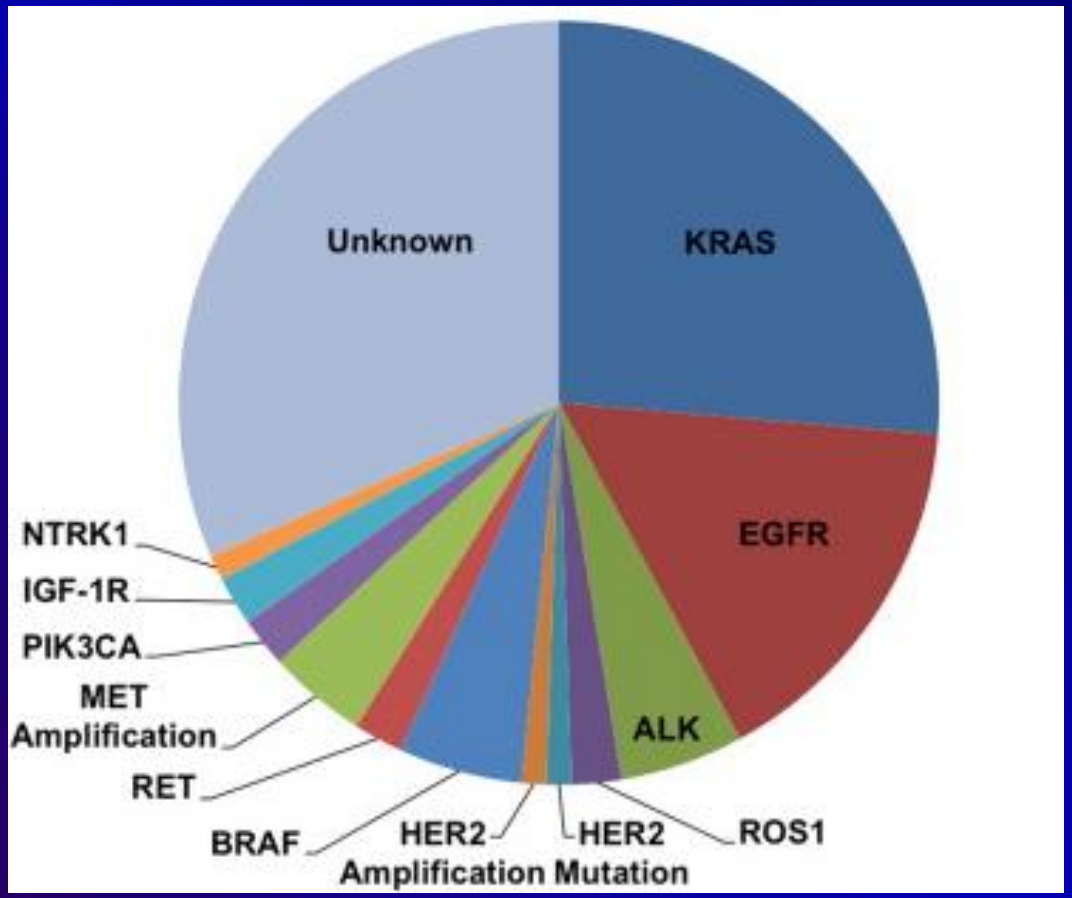
Akt

- 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

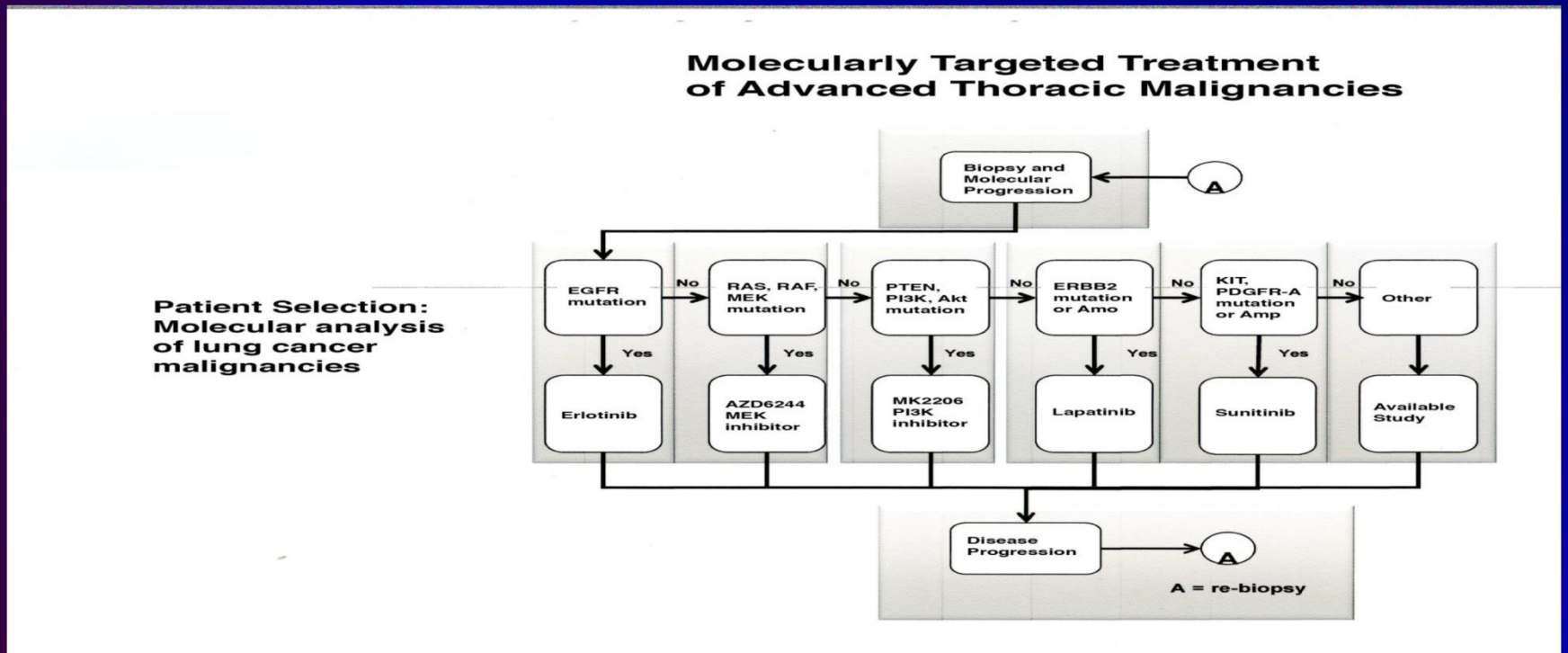
- Mammalian target of rapamycin (mTOR) or FRAP1 is a serine/threonine kinase.
- mTOR activation enhances phosphorylation of p70S6 kinase and 4E-BP1 increasing protein translation and cellular proliferation.
- mTOR activation decreased autophagy, a lysosome-dependent degradation pathway.

Personalizing Therapy for NSCLC Genetic Abnormalities in Lung Adenocarcinoma

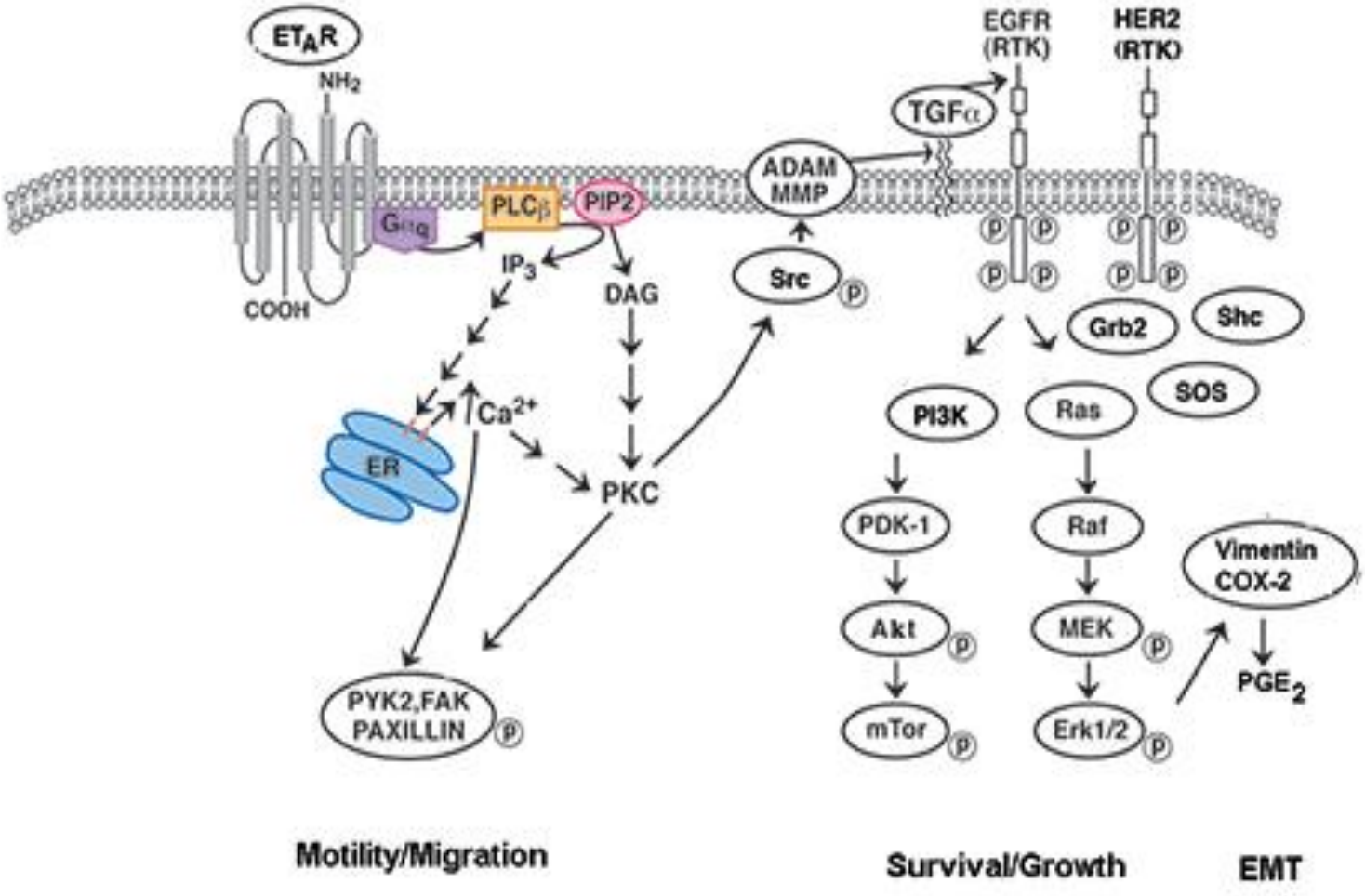


Molecular medicine

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.

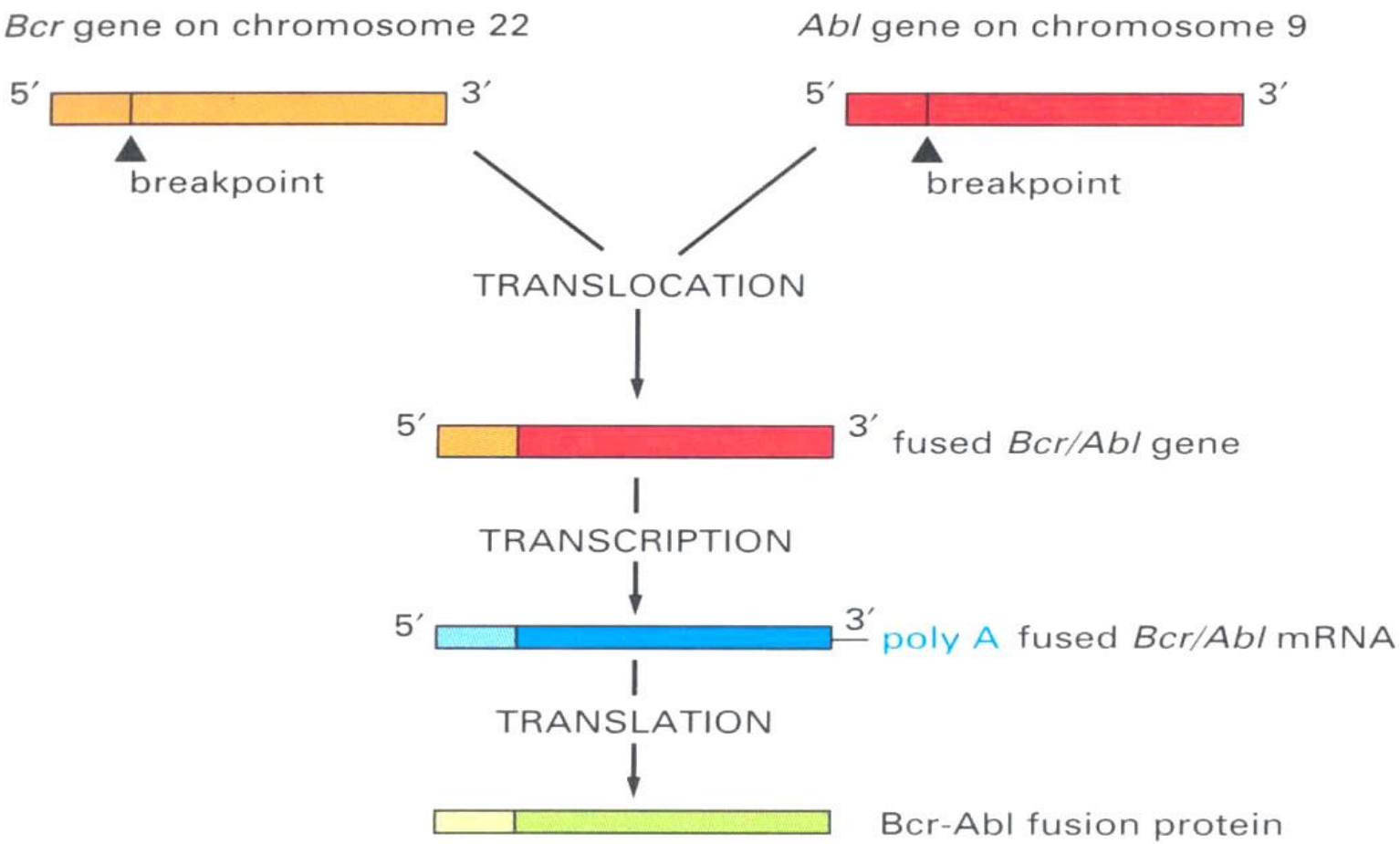
CML patients

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.

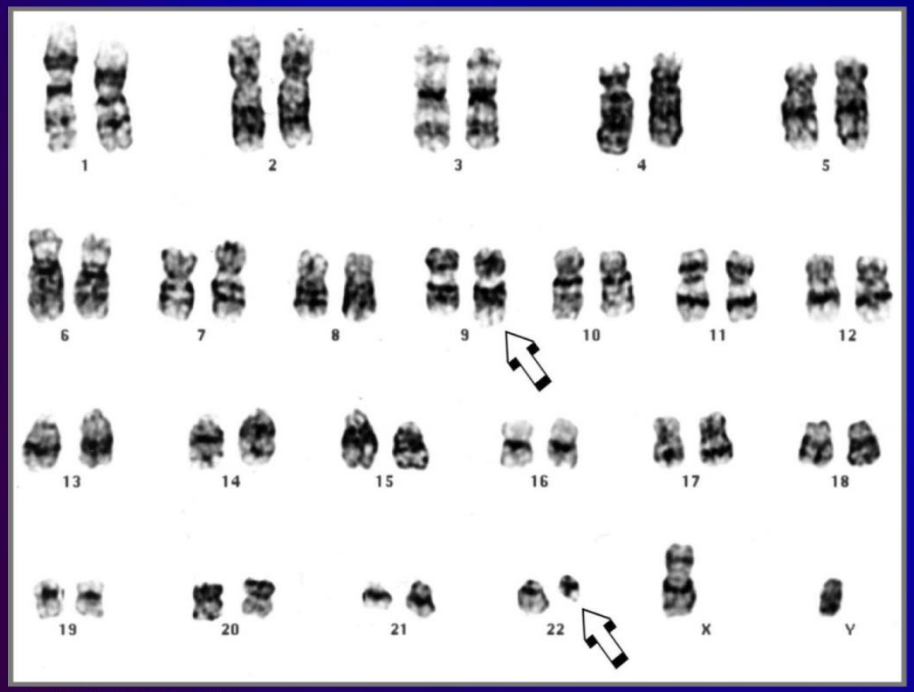
- Chromosome 22 translocates with chromosome 9. Molecular Biology of the Cell; Alberts et al., 2001.



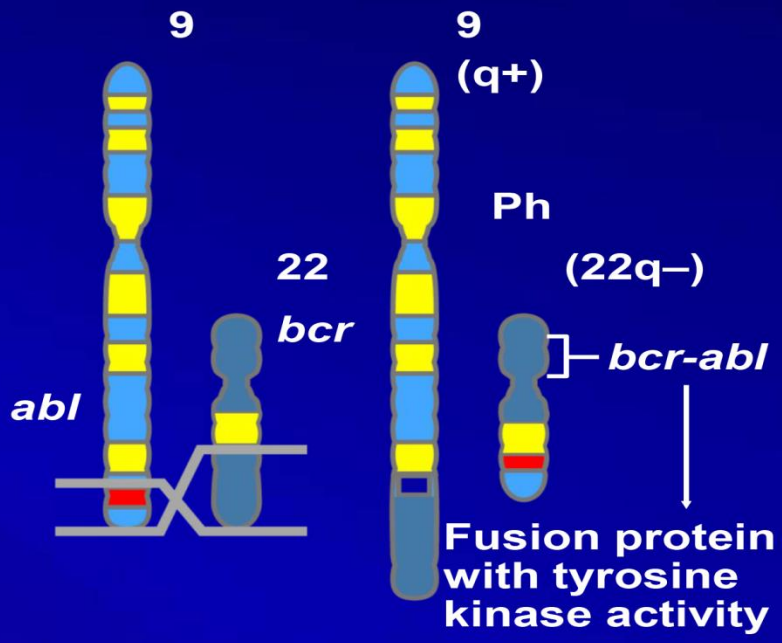
Bcr-Abl

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.

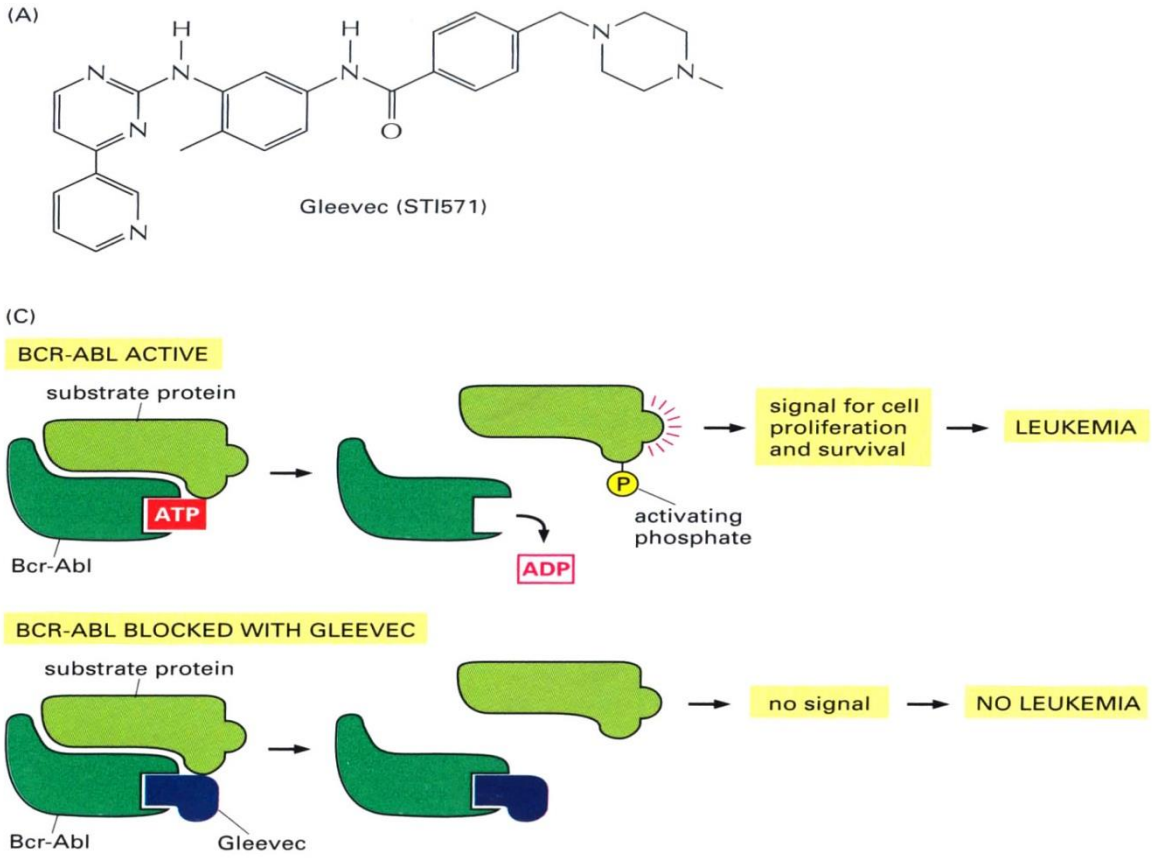
- Dose limiting toxicities included nausea, vomiting, edema and rash. (Sawyers and Druker. Cancer J. Sci. Am. 1999;5:63).

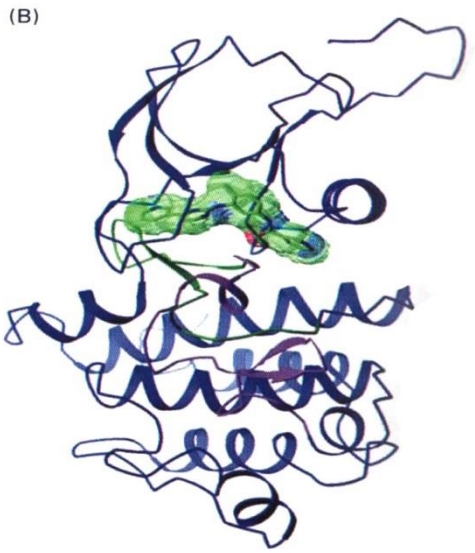
In a Phase II Clinical Trial, Gleevec™ restored normal blood counts in 53 out of 54 chemotherapy-resistant CML patients.

- 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.