TRAnslational research in Clinical Oncology (TRACO)

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SYLLABUS

DATE	TOPIC	SPEAKERS
Sept. 5	Introduction, Cancer imaging	Moody, Choyke
Sept. 11	Cervical Cancer, Cancer disparities	Schiller, Ambs
Sept. 18	Cancer global health, Immune checkpoints	Gopal, Goff
Sept. 25	CAR-T cells, Cancer microbiota	Shah, Greten
Oct. 2	Liquid biopsy, Clinical Trials	Ossandon, Smith

SYLLABUS, continued

DATE	TOPIC	SPEAKERS
Oct. 10	Radiation oncology, Small molecules	Nichols, Simeonov
Oct. 16	Prostate cancer, Ovarian cancer	Karzai, Lee
Oct. 23	Breast cancer, TBA	Zia
Oct. 30	Genomics, Cannabinoids	Wei, Freedman
Nov. 6	KRAS, NSCLC	Luo, Szabo

SYLLABUS, continued

DATE	TOPIC SPEAKERS
Nov. 13	Epigenetics, TBA Verma
Nov. 20	HIV, Case reports Maldarelli, Olaku
Nov. 27	Pancreatic cancer, Topoisomerase Alewine, Pommier
Dec. 4	Precision medicine, Nanotechnology Harris, Dobrovolskaia

REGISTRATION

The course is open to all interested personnel without charge. Registration is available at the NCI Web site (http://www.cancer.gov/grants-training/training/resources-trainees/courses-fellowships/translational-research-clinical-oncology). The lecture PDFs will be posted on the website after they are made 508 compliant. WEBEX chats will be taken at the end of each lecture.

Lecture recordings

- The archived lectures will be on available on WEBEX. The 2 hour lecture for Sept. 5 will be TRACO1. The number will increase each week and the Dec. 4 lecture will be TRACO14.

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)

Thun, Jamal and Ward, "Cancer: Principles & Practice of Oncology." Edited by DeVita, Lawrence and Rosenberg. (2011), pp. 241-260

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

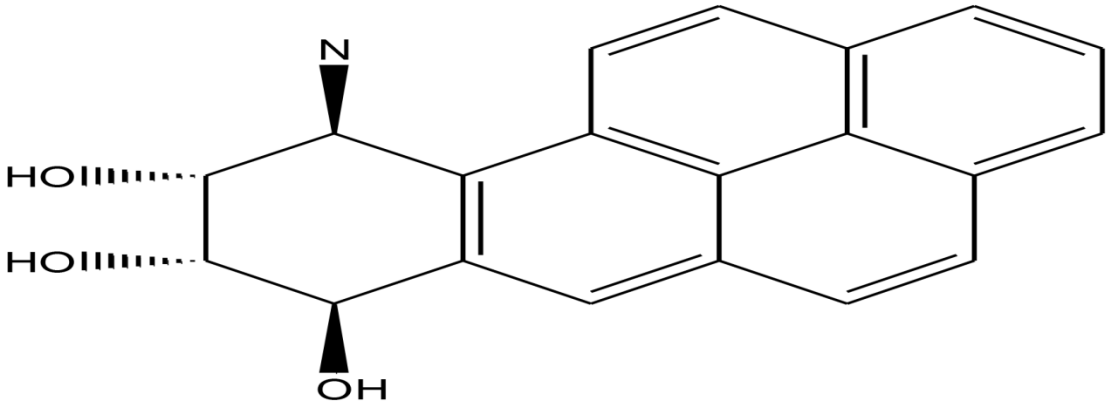
The process by which unreactive carcinogen converts to a form which binds DNA is known as metabolic activation.

- **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

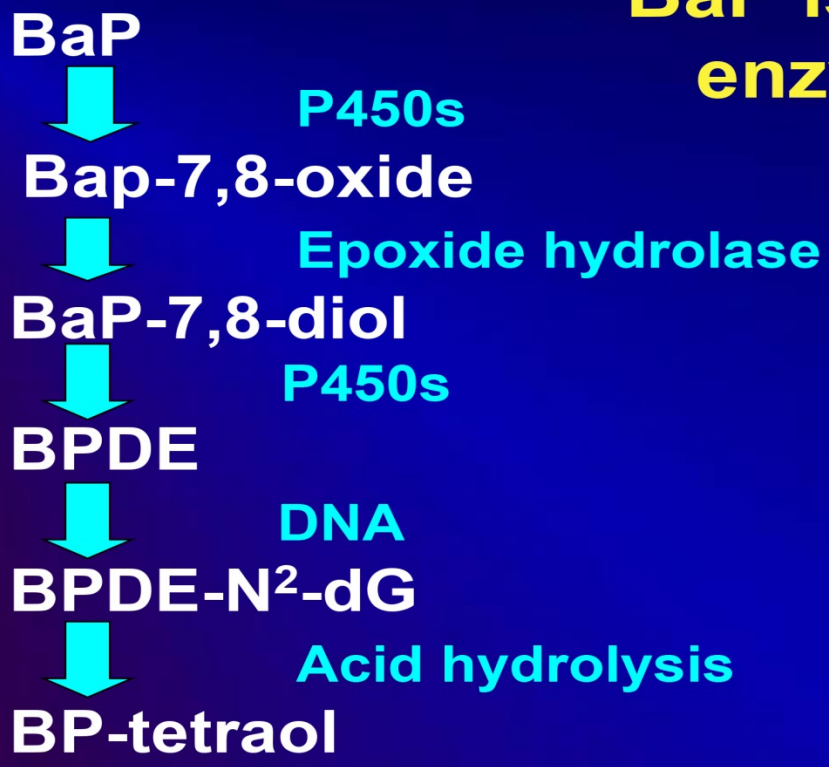
BENZ(a)Pyrene

- 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).

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 inter-strand DNA crosslinks are removed by nucleotide excision repair.

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.

P53, a tumor suppressor gene:

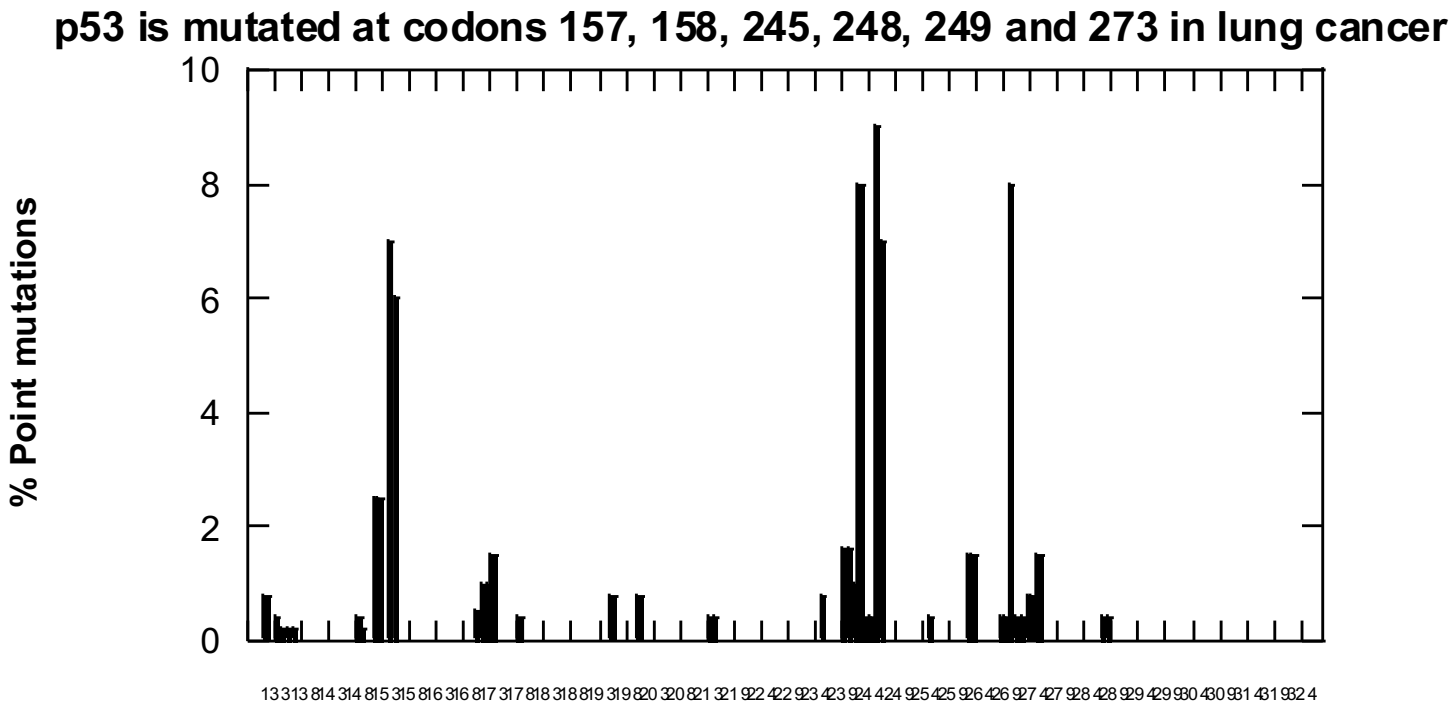
- mediates the G1 to S-phase checkpoint of the cell cycle,
- drives programmed cell death or apoptosis after DNA damage,
- is increased along with p21 (cell cycle checkpoint) after DNA damage.
- Phosphorylated p53 induces expression of BAX (apoptosis), GADD45 (DNA repair) and thrombospondin (angiogenesis)

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.

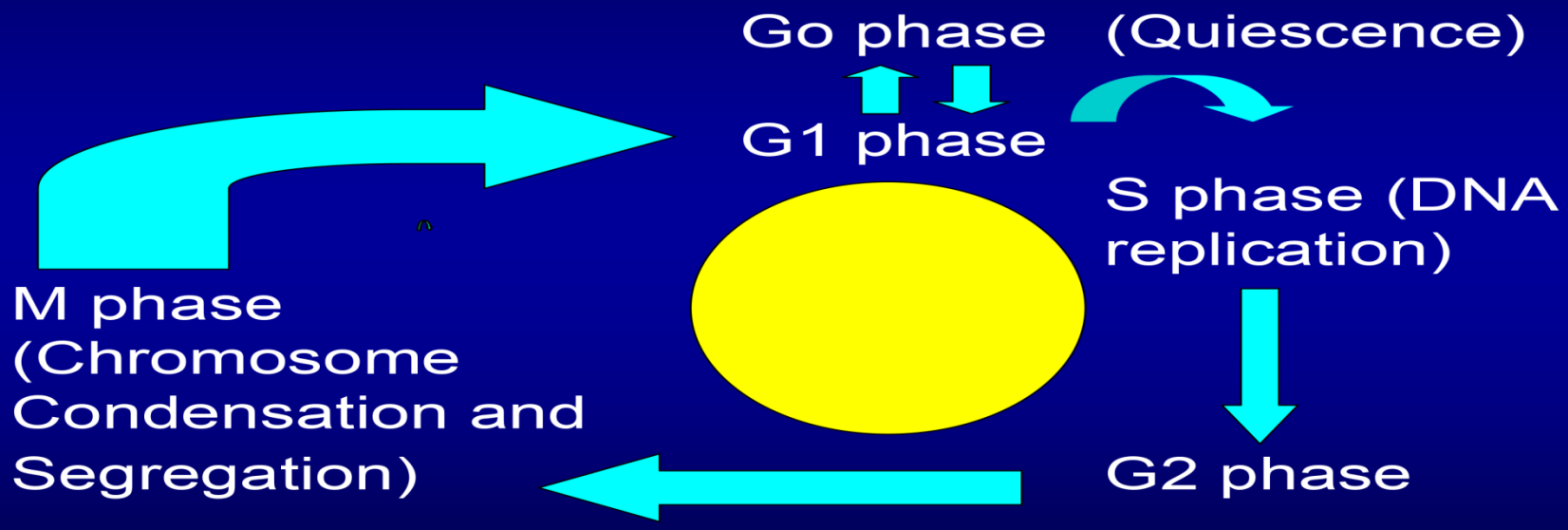
- P53 is mutated at codons 157, 158, 245, 248, 249 and 273 in lung cancer.



Cell cycle phases

Cell cycle phases.

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



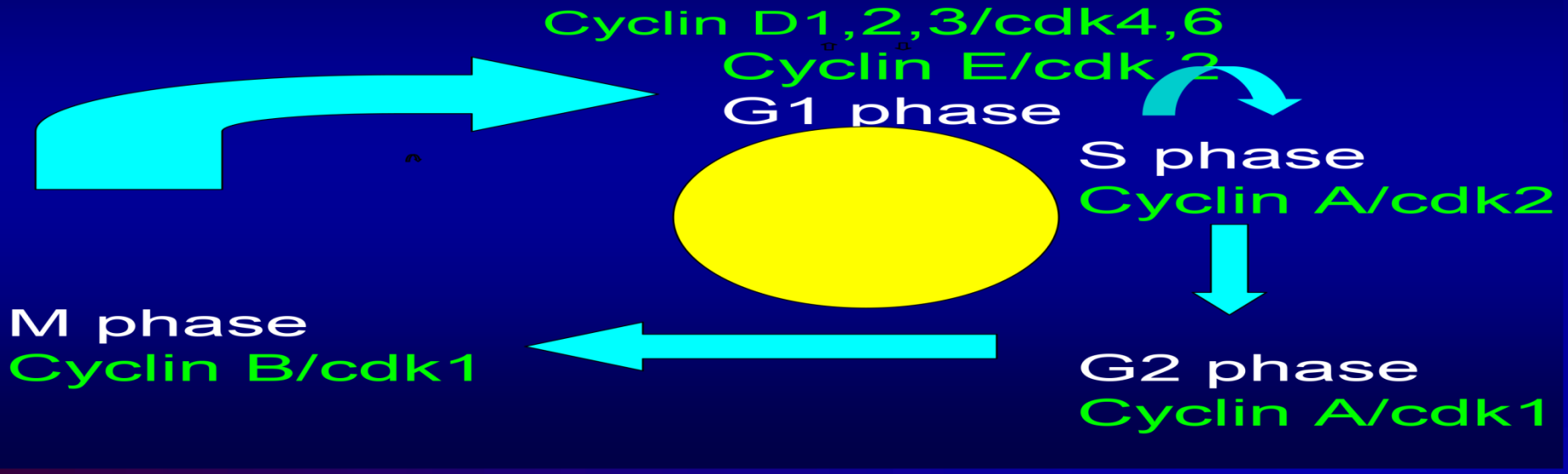
p53 mediates the G_1 to S-phase checkpoint of the cell cycle

- 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.

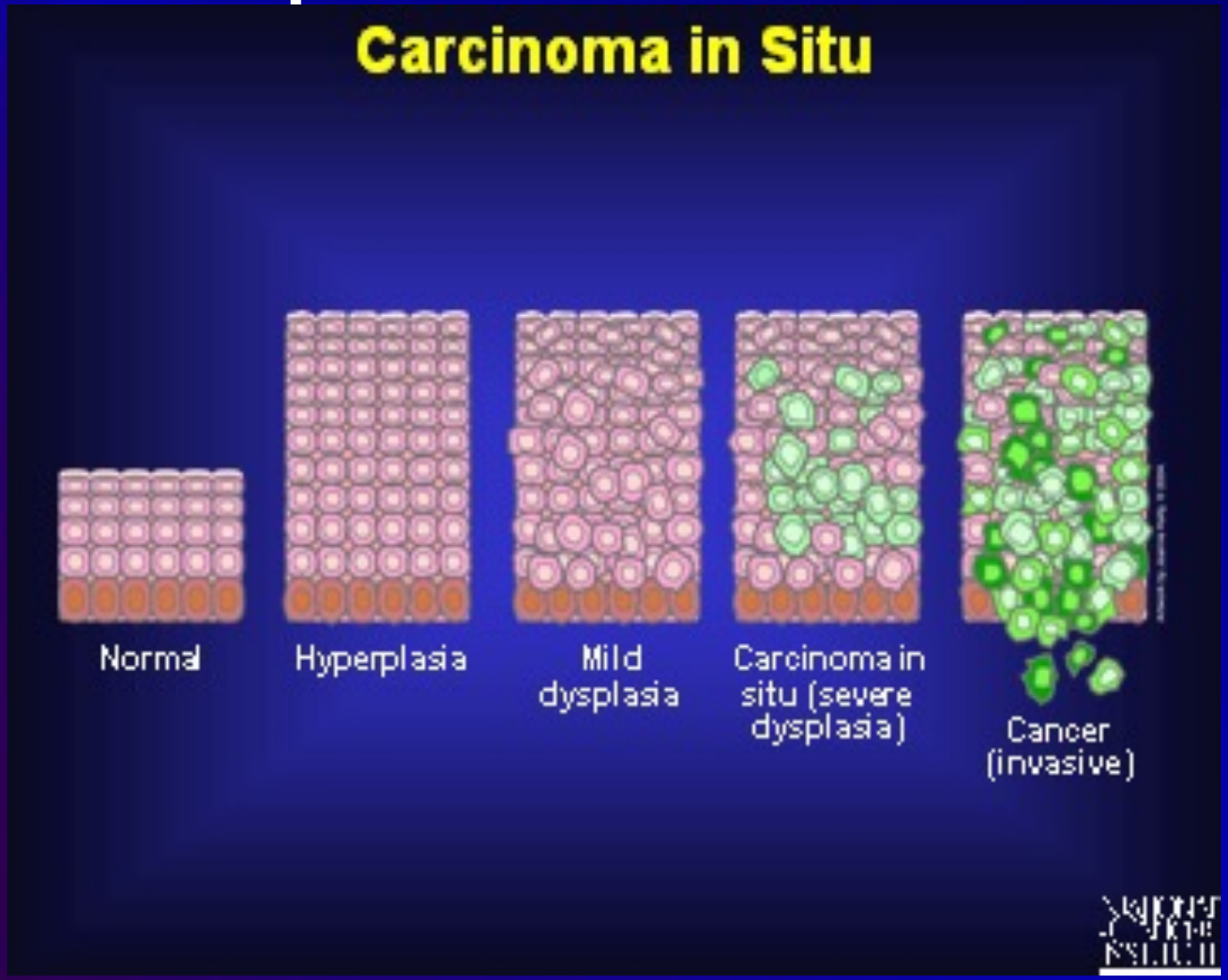


Genotoxicity of tobacco smoke.

- 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.

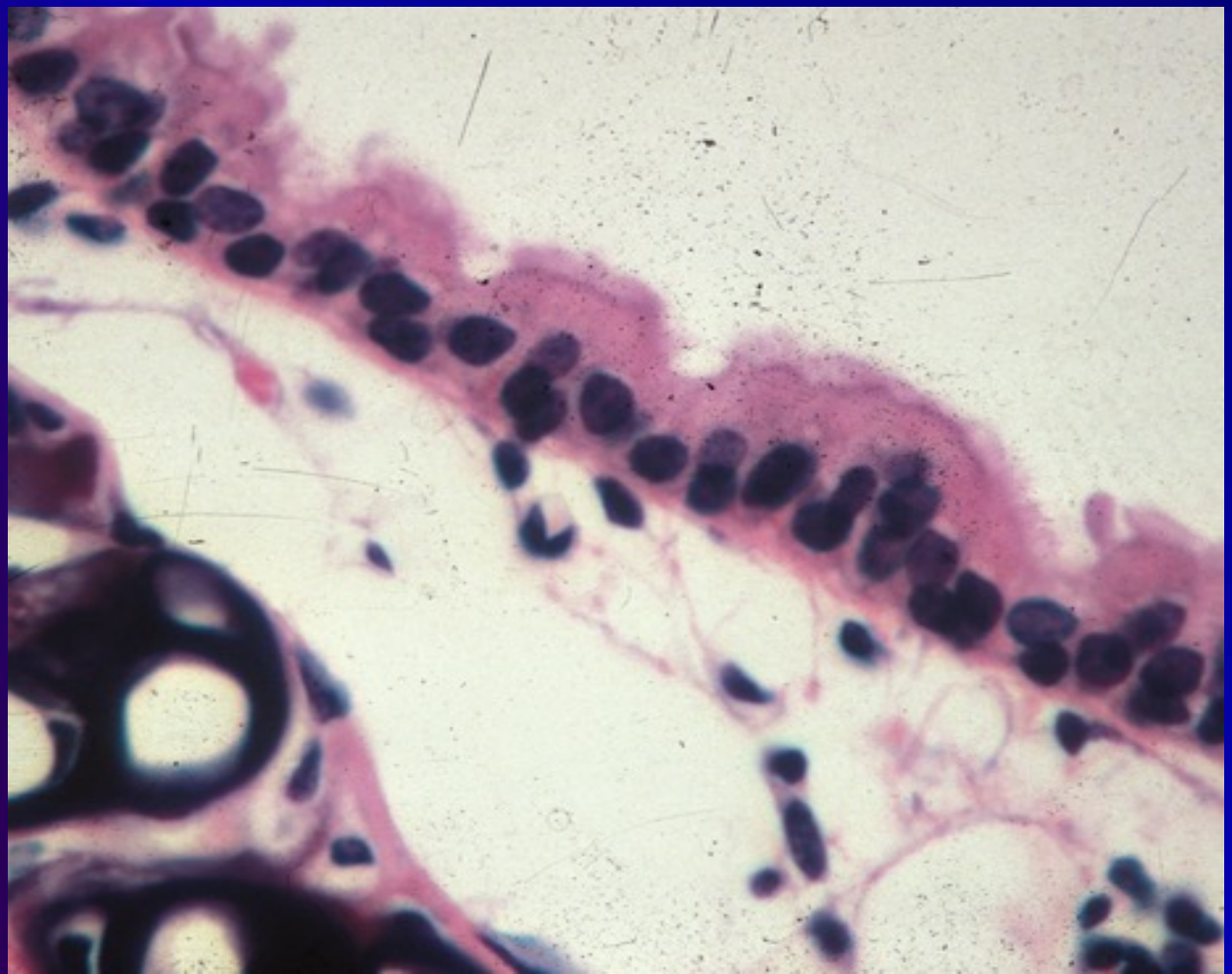
Carcinogenesis

- Cancer progression occurs over a period of decades.



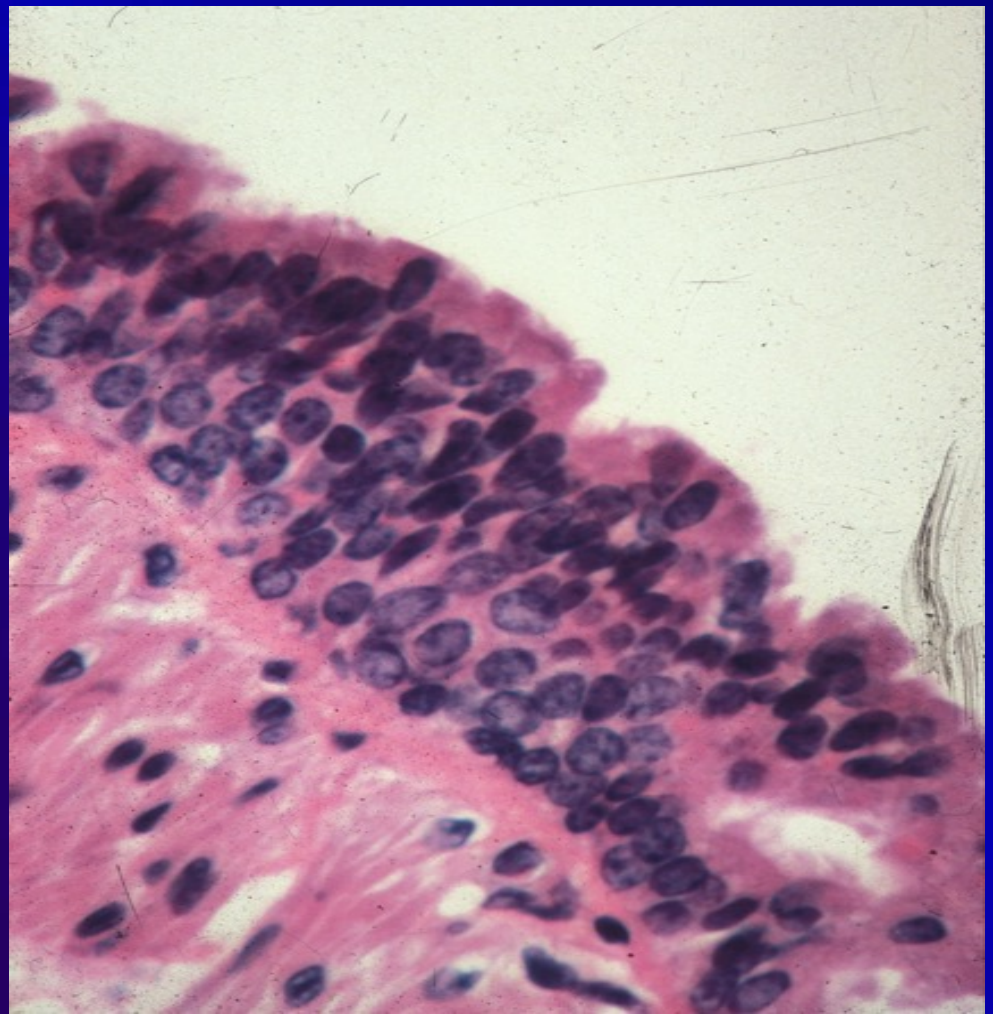
Normal lung

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



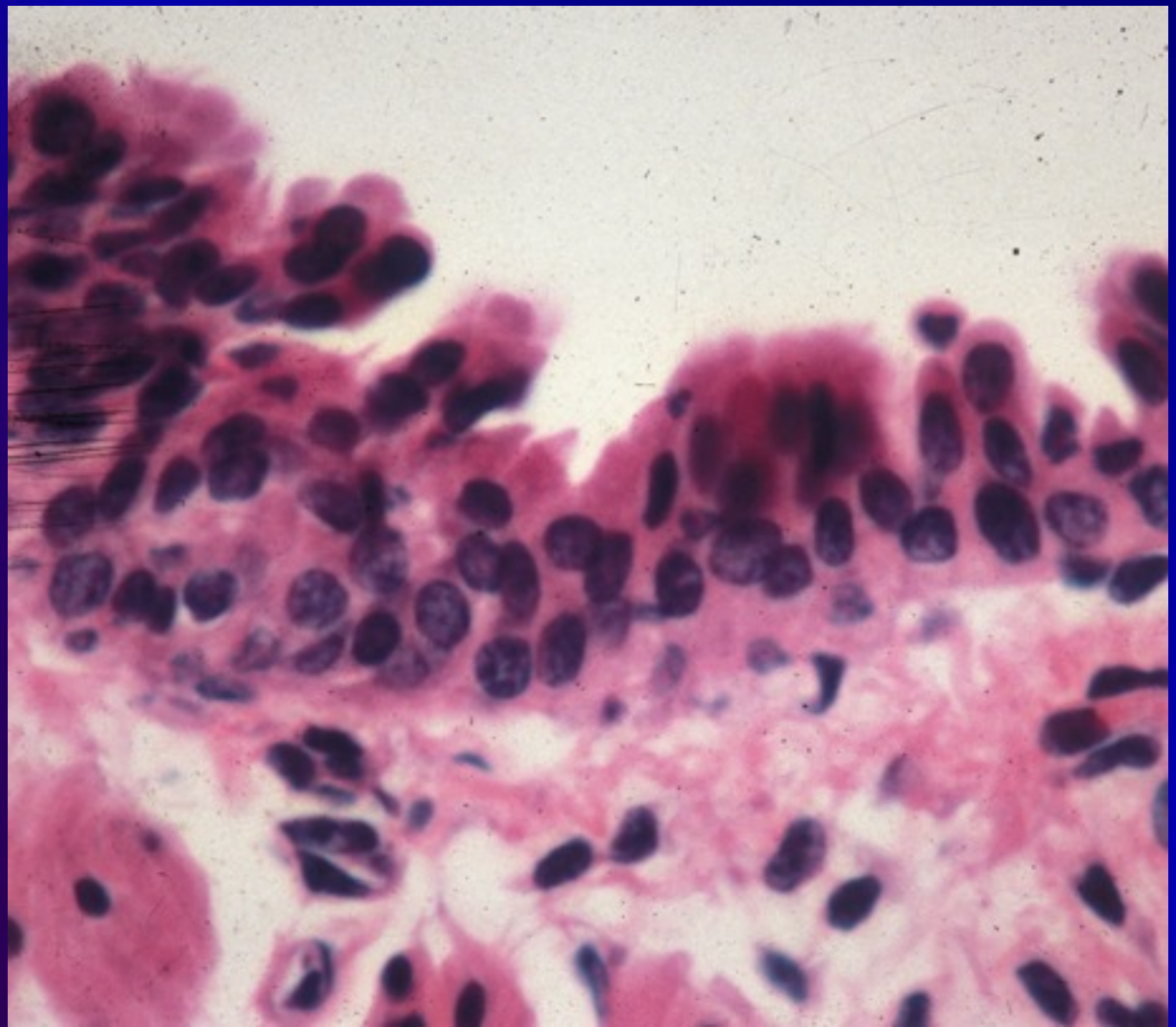
Hyperplasia

- After exposure to tobacco smoke, hyperplasia can result.



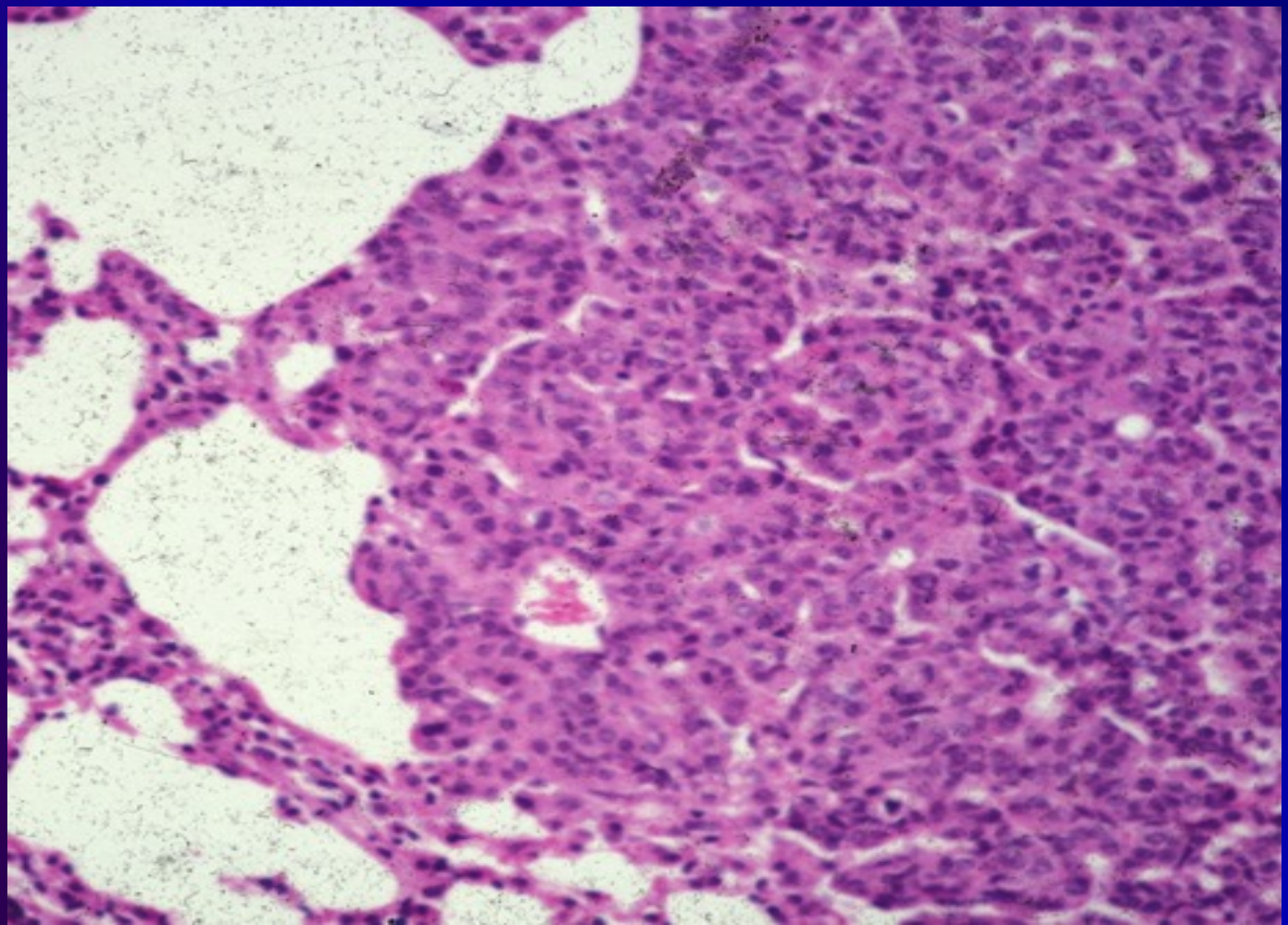
Dysplasia

Continued exposure to tobacco smoke leads to dysplasia.



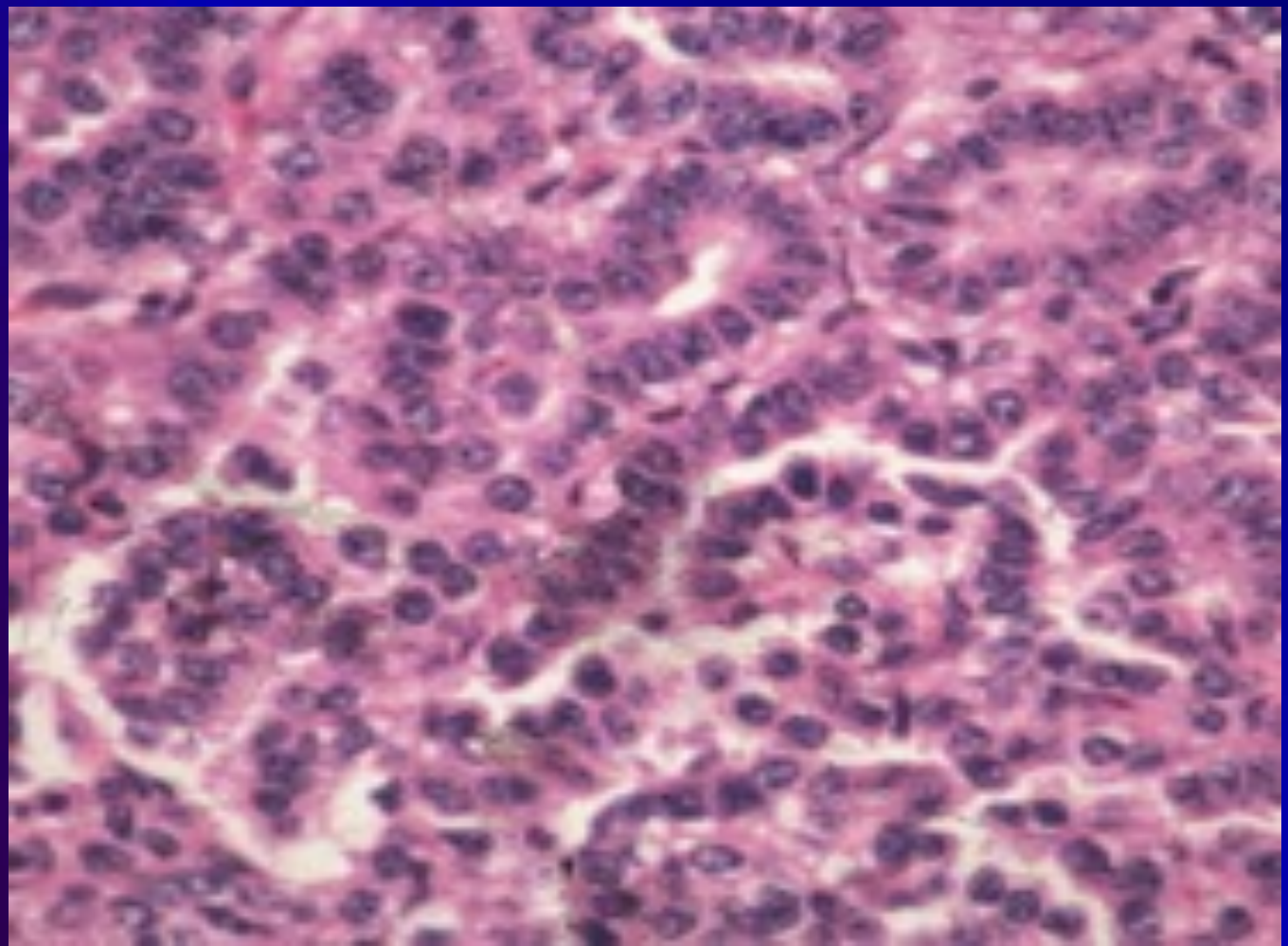
Adenoma

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



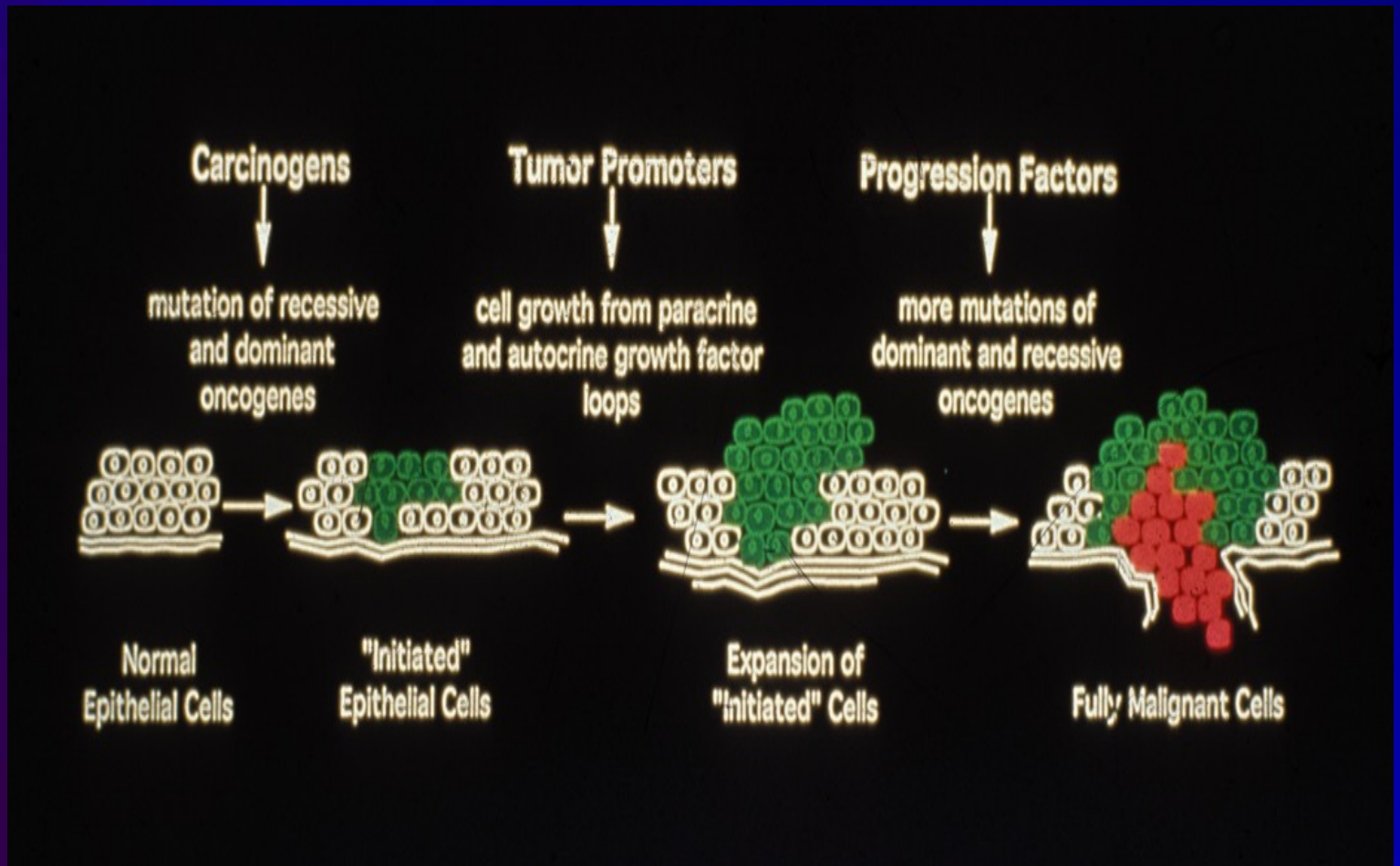
Adenocarcinoma

- 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

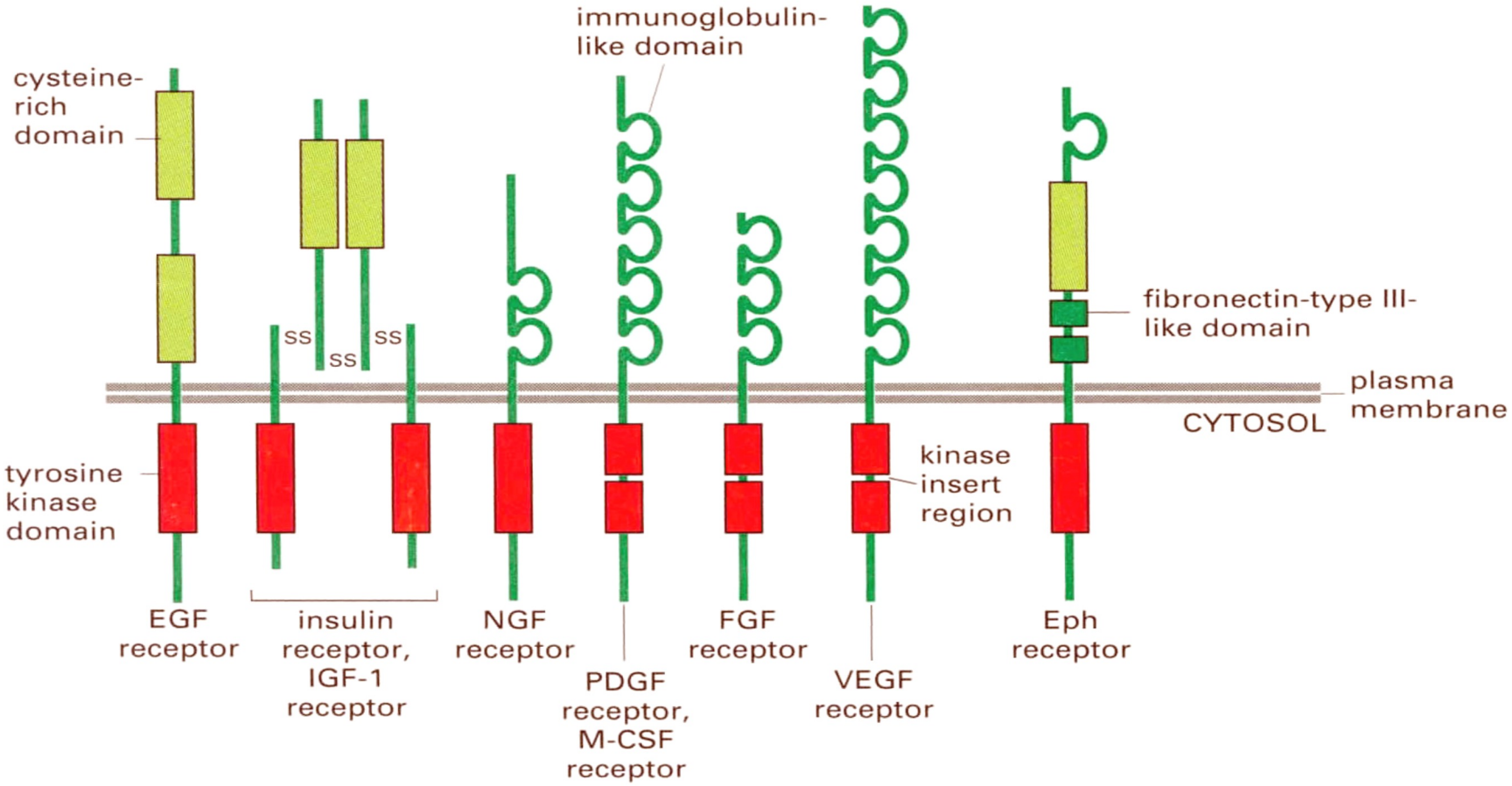
Migration, Invasion and Metastasis.

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, erbB-2
- Mutation of oncogenes such as K-ras, EGFR

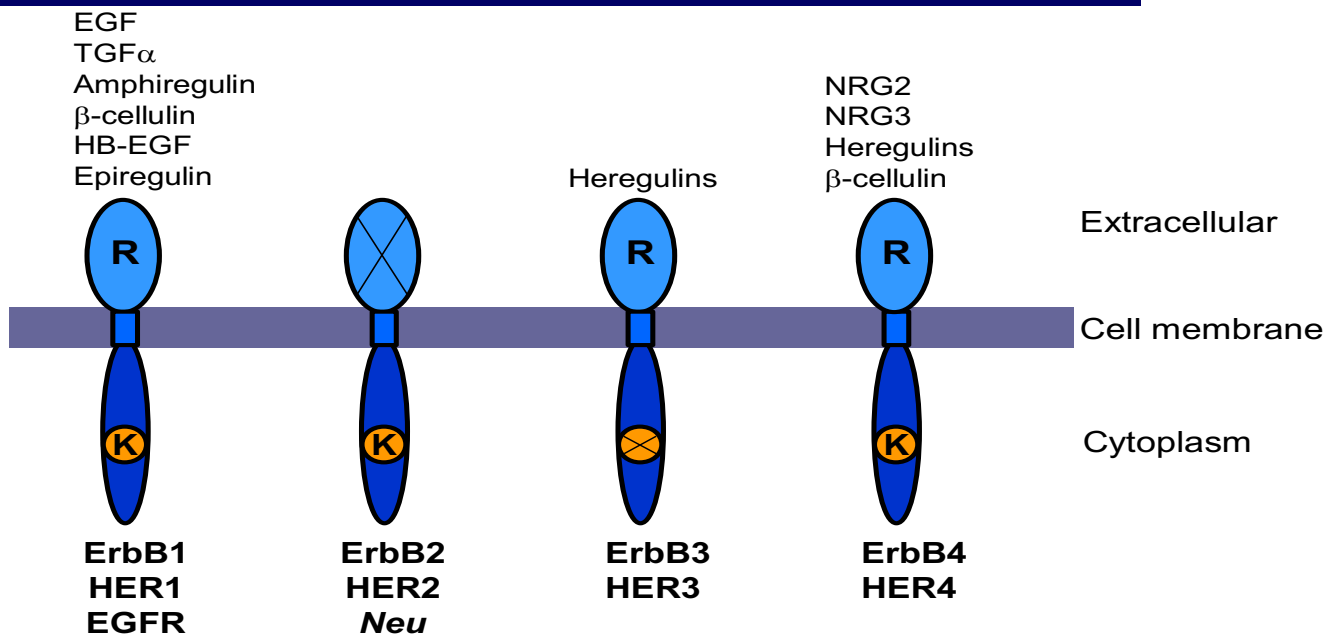
Tyrosine kinase receptors.

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 phosphorylated

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

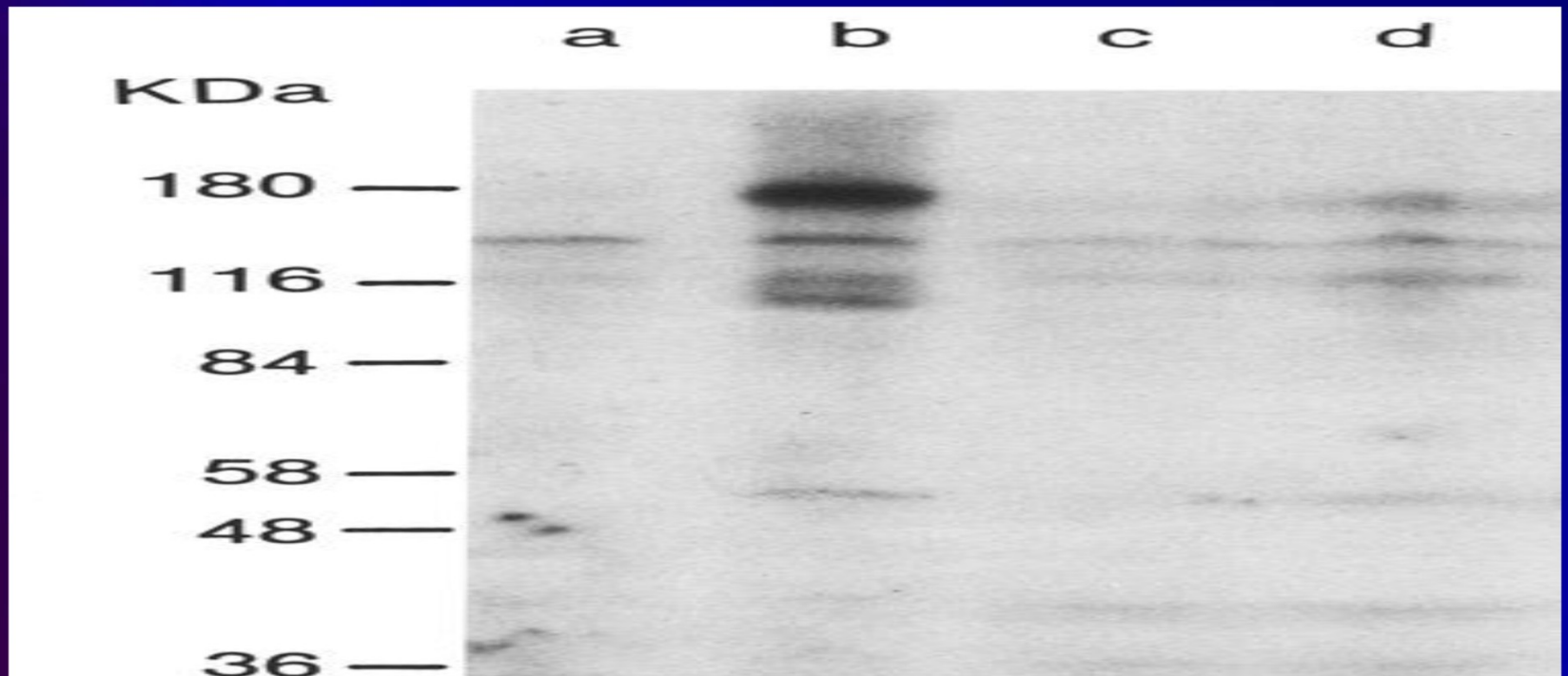
Agent	IC_{50}, ug/ml
EGF	.03
TGFα	.8
TGFα-PE38	.4
mAb 108	3
IgG	>10

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.

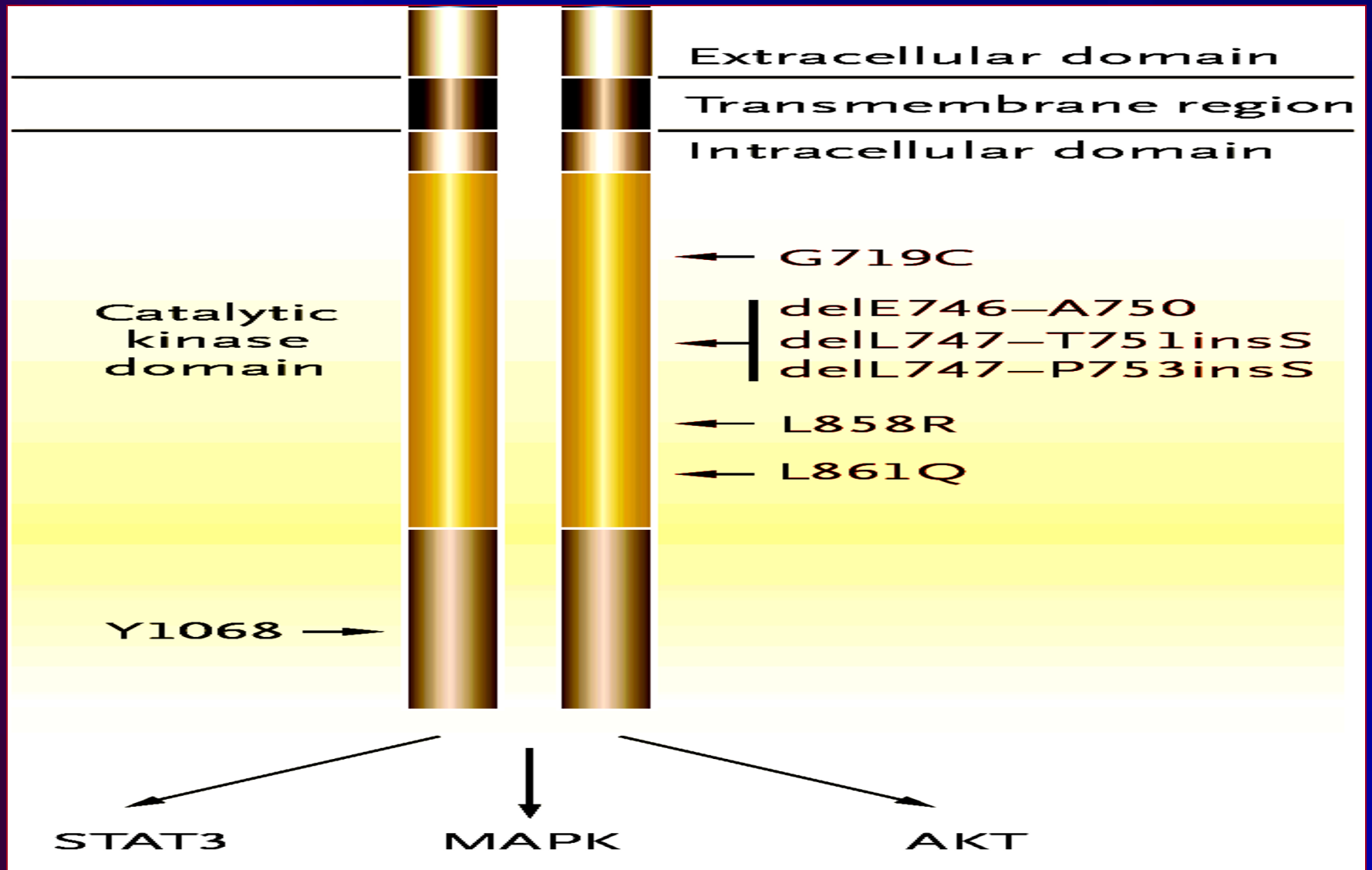


Tyrosine kinase receptors are mutated in several diseases leading to increased cancer proliferation.

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

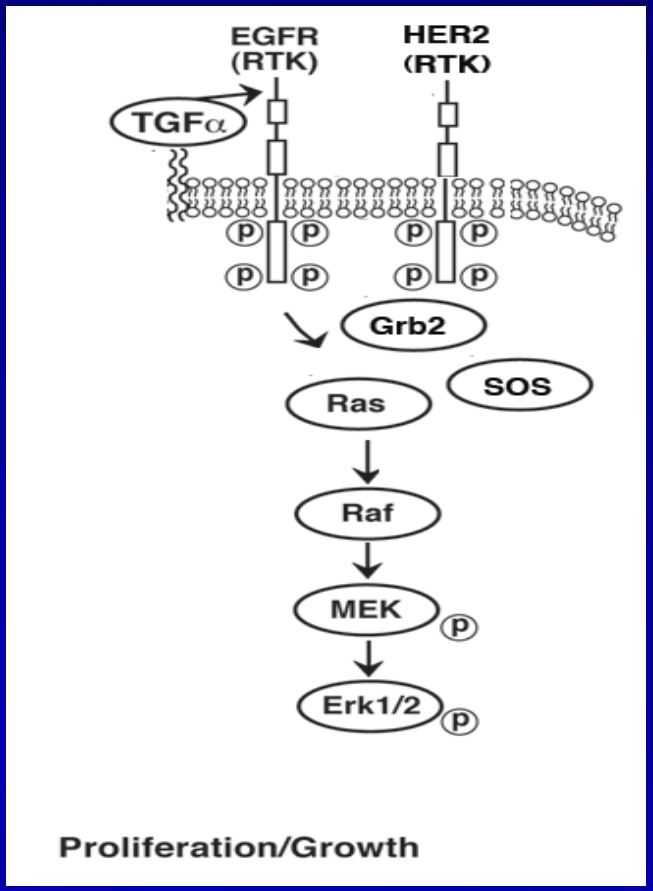
Paez et al., Science 304:1497 (2004)

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 30% 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 FDA recently approved sotorasib (AMG510) for treatment of lung cancer patients with the KRAS G12C mutation.

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.

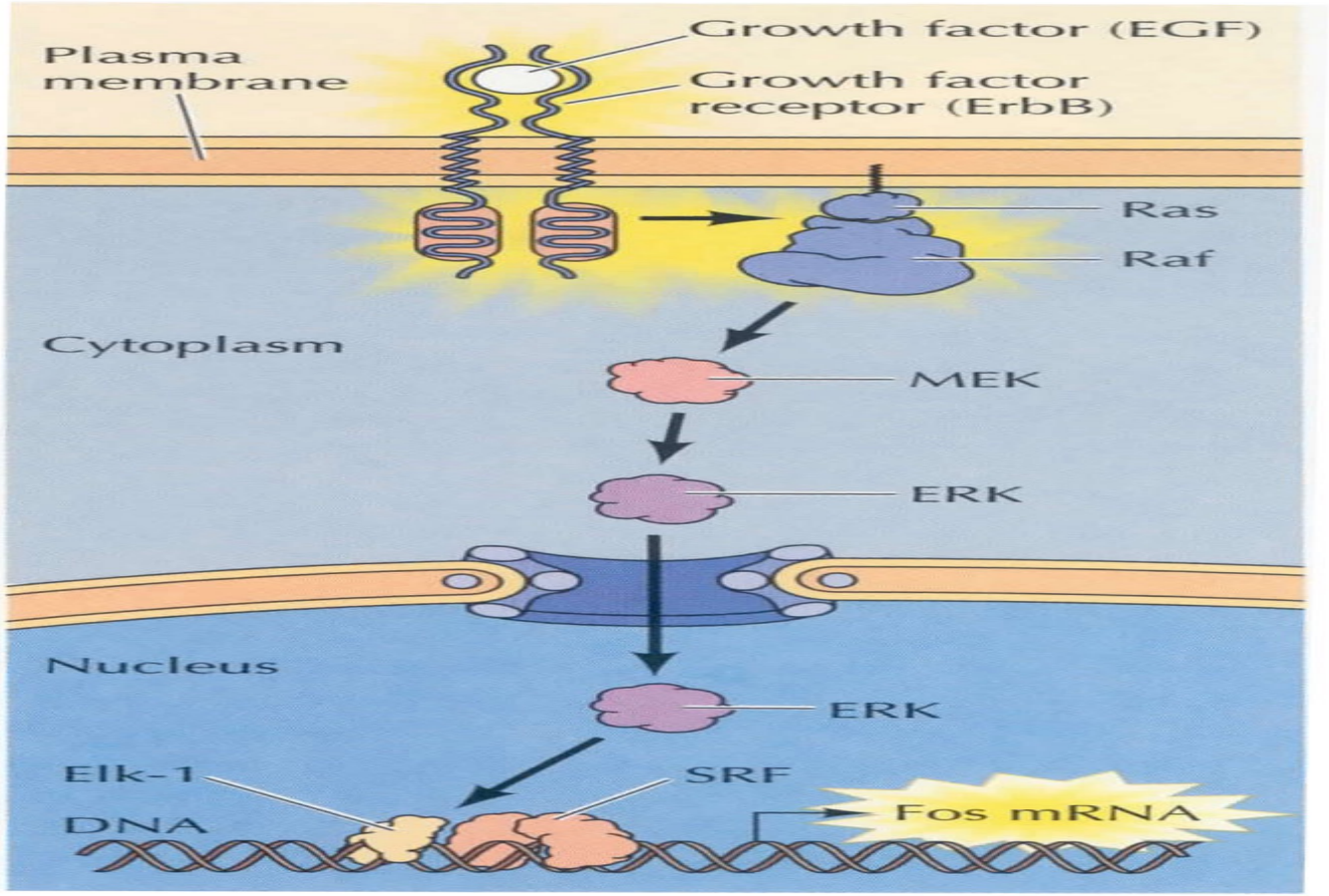
MEK

- RAF phosphorylates mitogen activated protein kinase kinase (MEK) increasing its activity.
- MEK1 and MEK2 are inhibited by trametinib
- Dabrafenib and trametinib are used in B-RAF mutated patients.

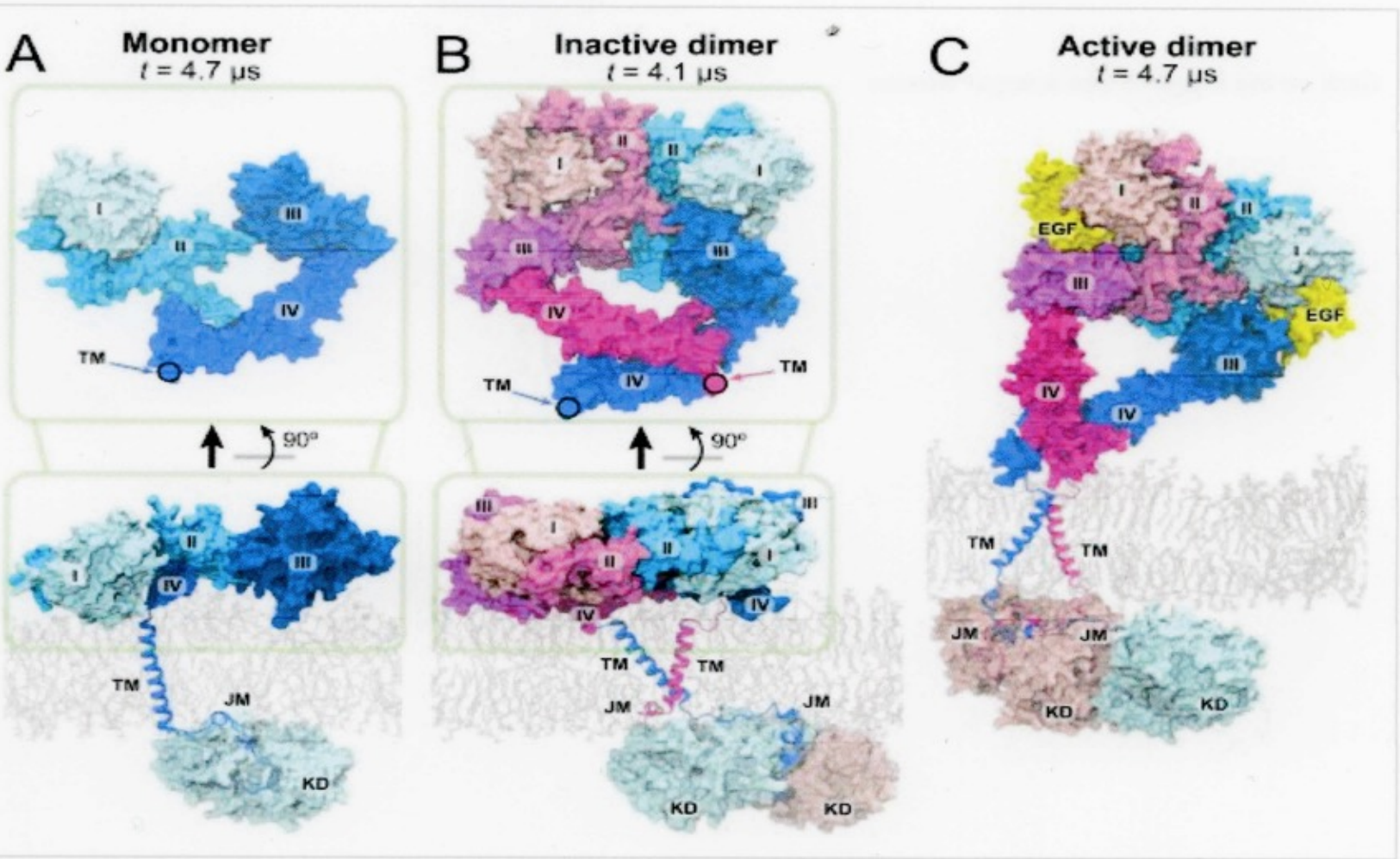
ERK

- 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.

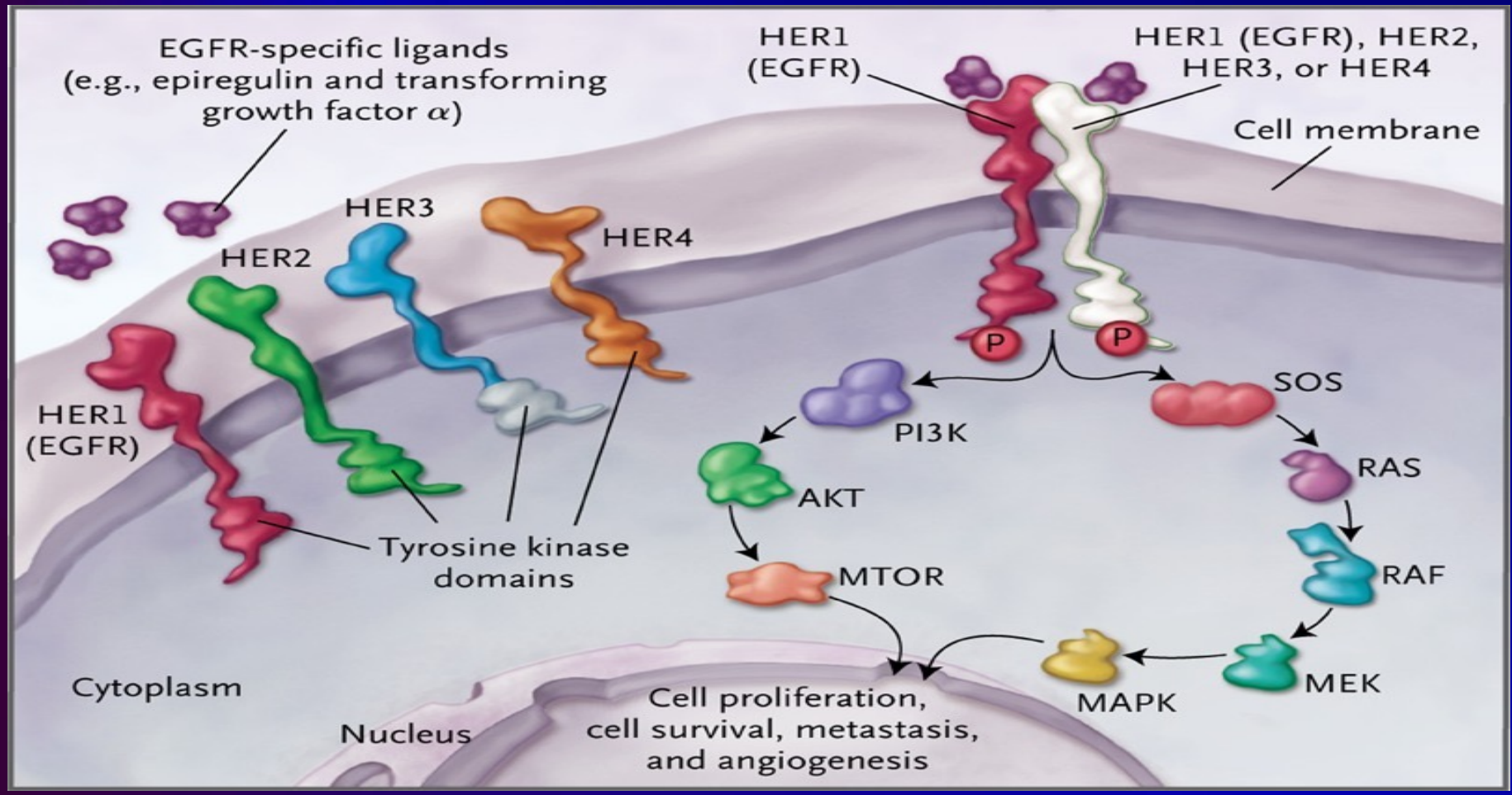
The EGFR stimulates cancer cell growth. *Molecular Biology of the cell; Alberts et al., 2001.*



EGFR homodimerization



ErbB RTKs and signal transduction



The EGFR forms heterodimers with HER2

- HER2 lacks a ligand binding site but forms heterodimers with the EGFR, HER3 and/or HER4.
- Trastuzumab is a mAb that is used to treat breast cancer patients who overexpress HER2

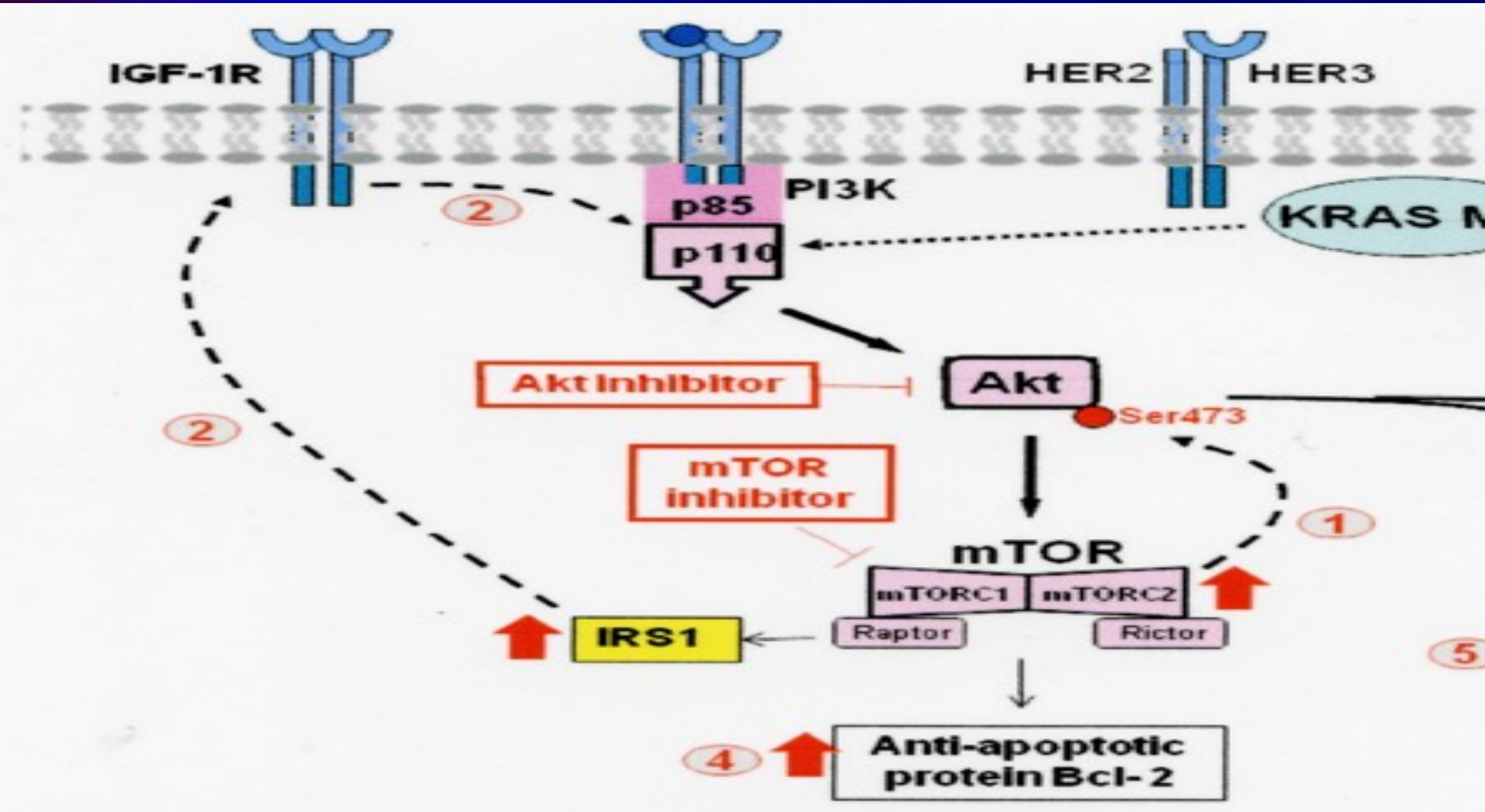
The EGFR forms heterodimers with HER3

- HER3 binds Neuregulin-1 and -2 with high affinity.
- HER3 has weak tyrosine kinase activity and must form heterodimers with the EGFR, HER2 or HER4 to become biologically active.
- Increased expression of HER3 results in resistance to many therapeutic agents.

The EGFR forms heterodimers with HER4

- HER4 binds Neuregulins 1,2,3 and 4 with high affinity. It can form homodimers.
- HER4 undergoes alternative splicing and can stimulate or inhibit cancer growth.
- Neuregulin-1 HER4 interactions are altered in schizophrenia.

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.

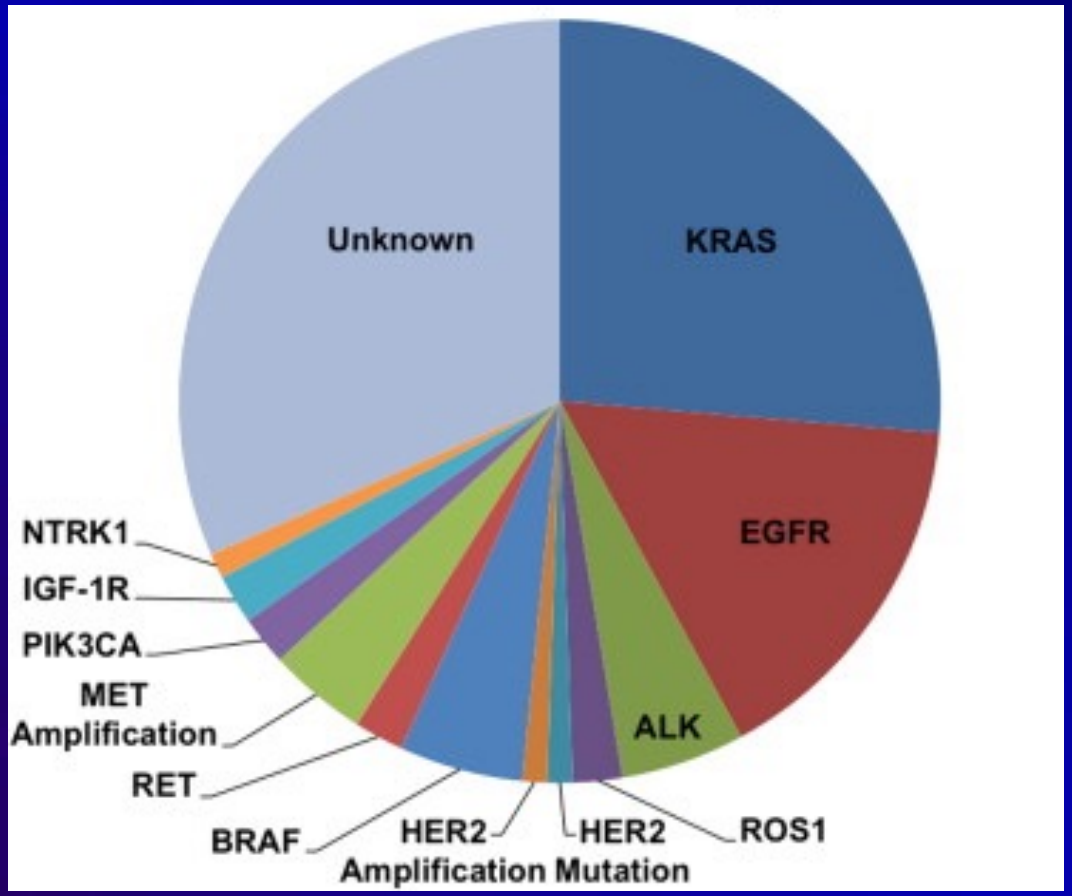
PTEN

- 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

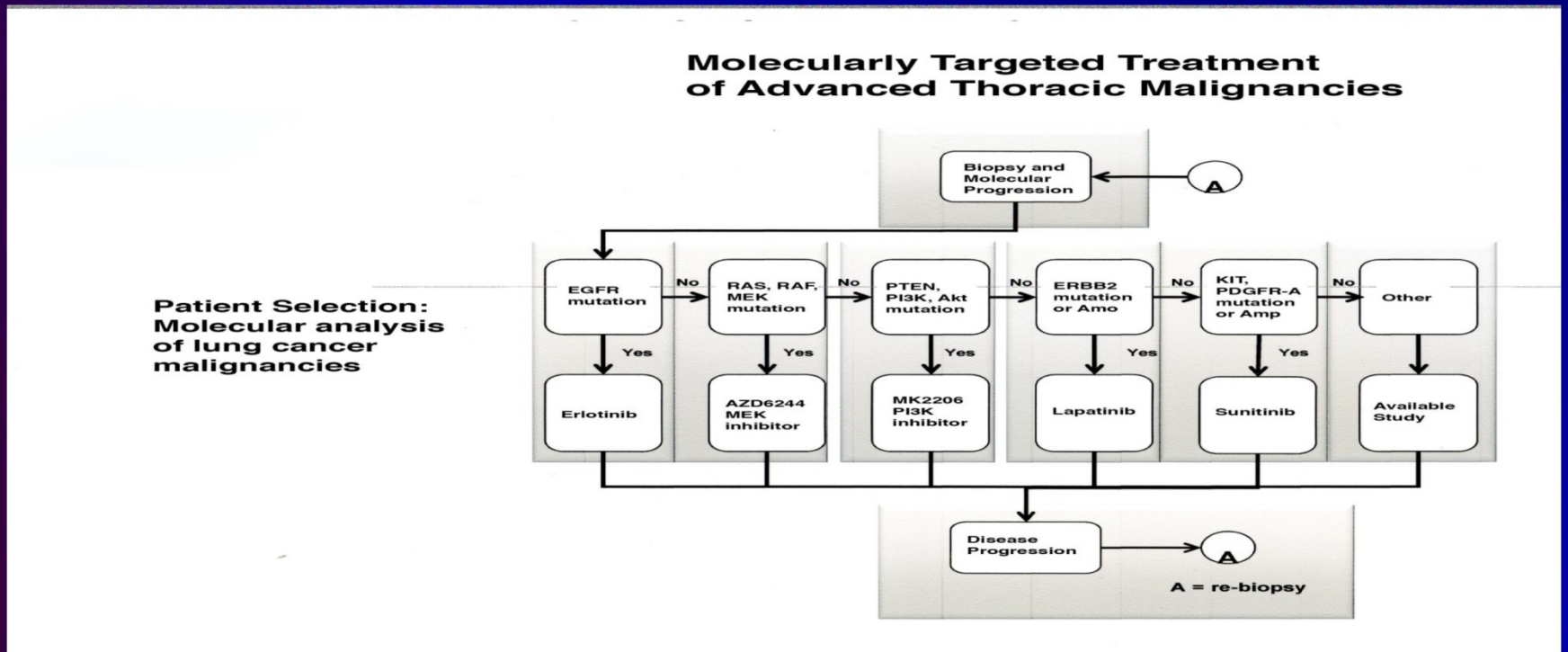
- AKT or protein kinase B prevents apoptosis of cells.
- AKT is a serine/threonine kinase which is phosphorylated at Ser473 increasing phosphorylation of mTOR.
- AKT promotes cellular survival by phosphorylating BAD and caspase-9 preventing apoptosis of cancer cells.
- AKT is mutated in breast cancer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

Personalizing Therapy for NSCLC Genetic Abnormalities in Lung Adenocarcinoma



Molecular medicine

Molecularly Targeted Treatment of Advanced Thoracic Malignancies

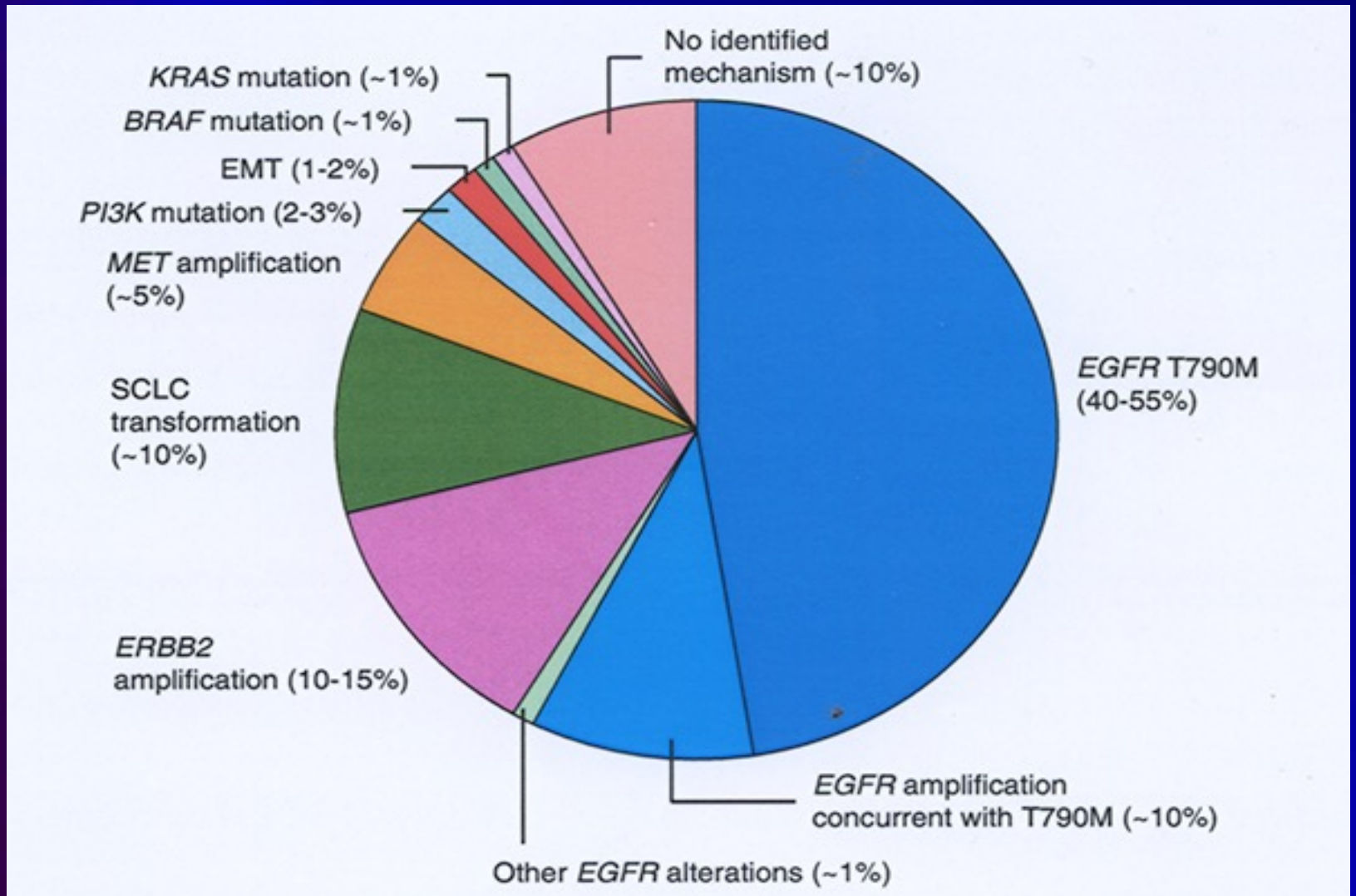


Erlotinib/gefitinib resistance

- NSCLC patients with L858R EGFR mutations develop resistance to erlotinib/gefitinib after 1 year due to a secondary mutations.
- Approximately 50% of the patients develop T790M mutations which can be treated with Osimertinib.

Secondary mutations in NSCLC

Westover et al., Ann Oncol. 2018



RTK TKIs

- 1st generation TKI (reversible)

Gefitinib, Erlotinib

- 2nd generation TKI (irreversible)

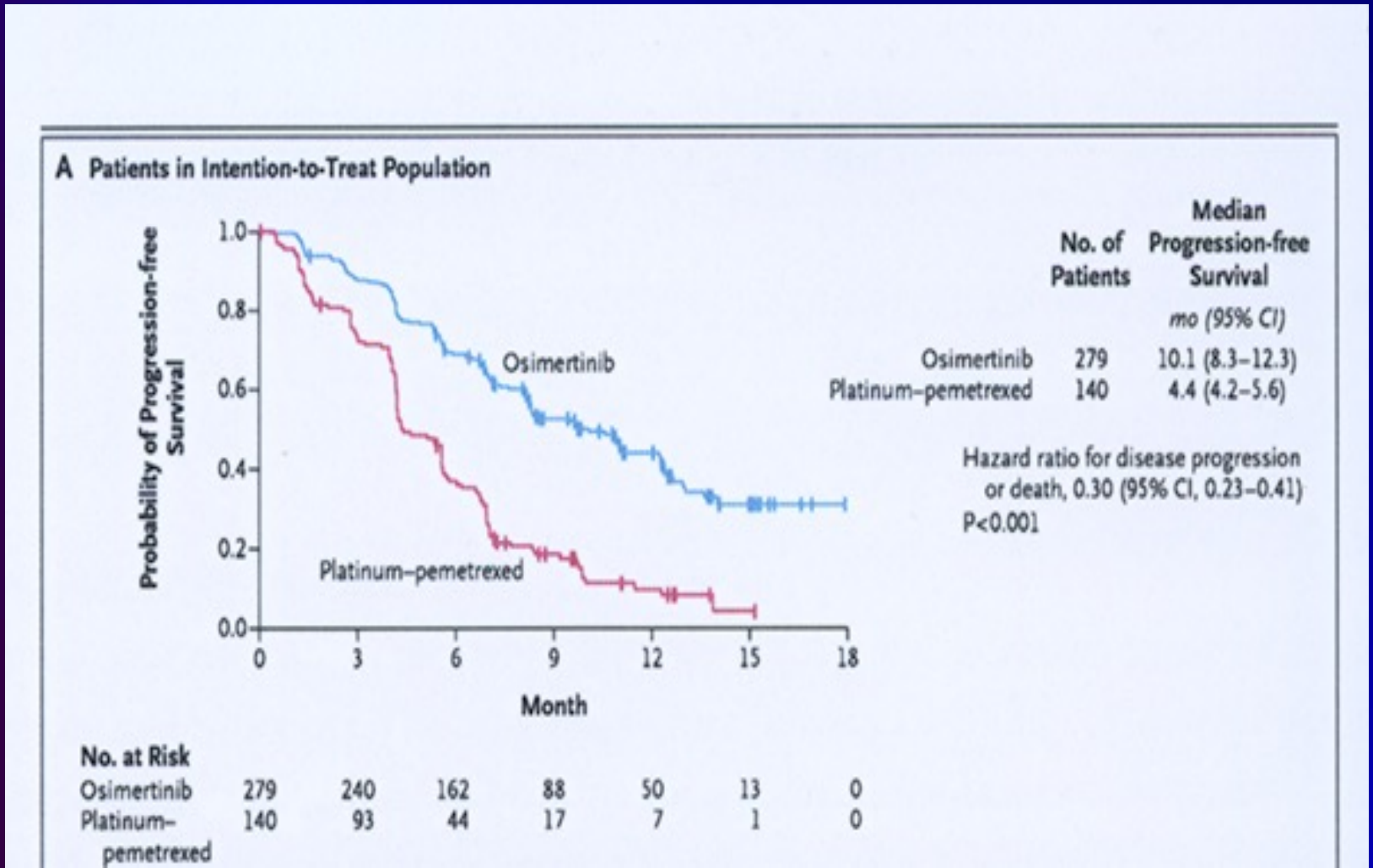
Afatinib

- 3rd generation TKI (irreversible)

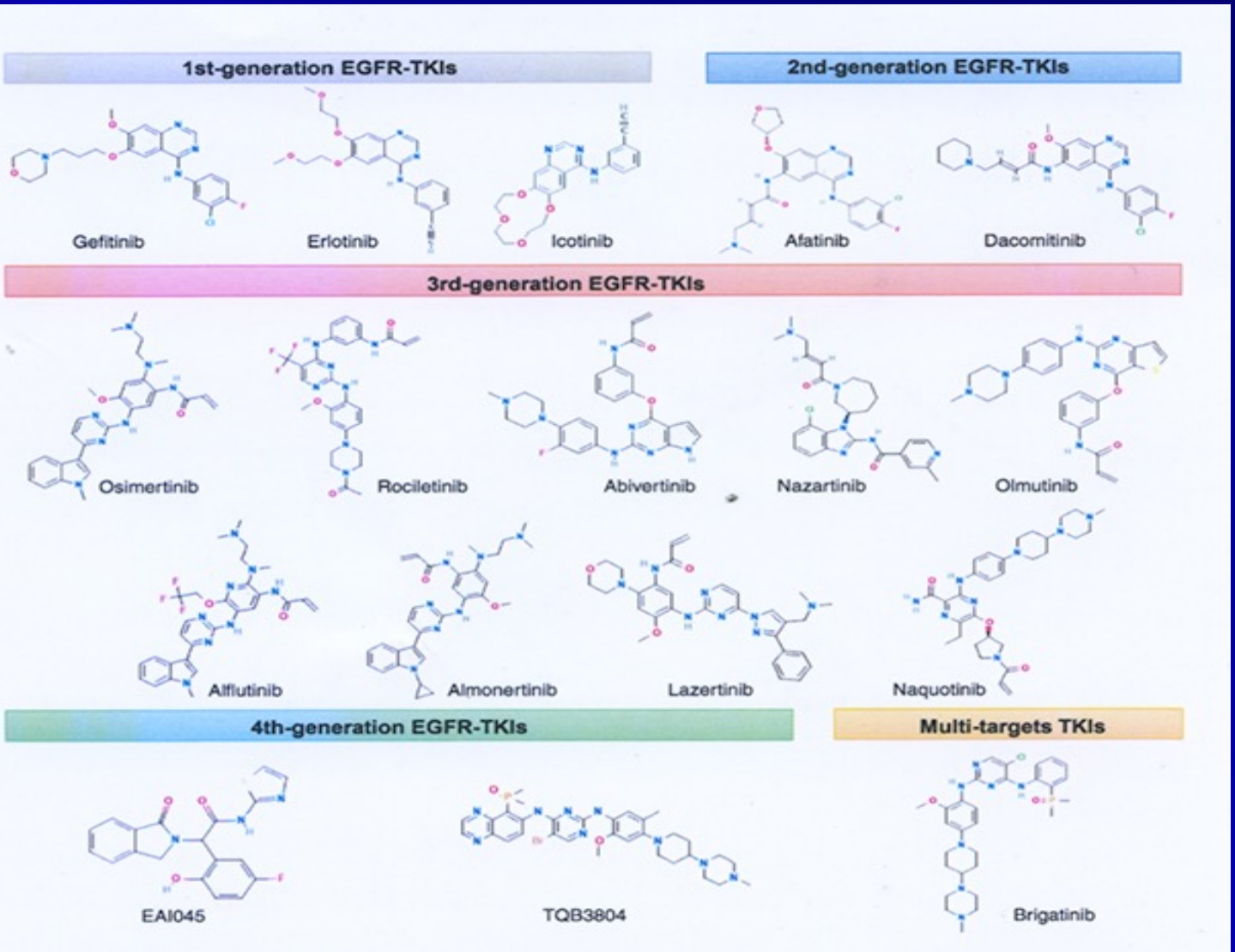
Osimertinib

Osimertinib improves overall survival in EGFR T790M patients

Mok et al., N Engl. J Med 2017

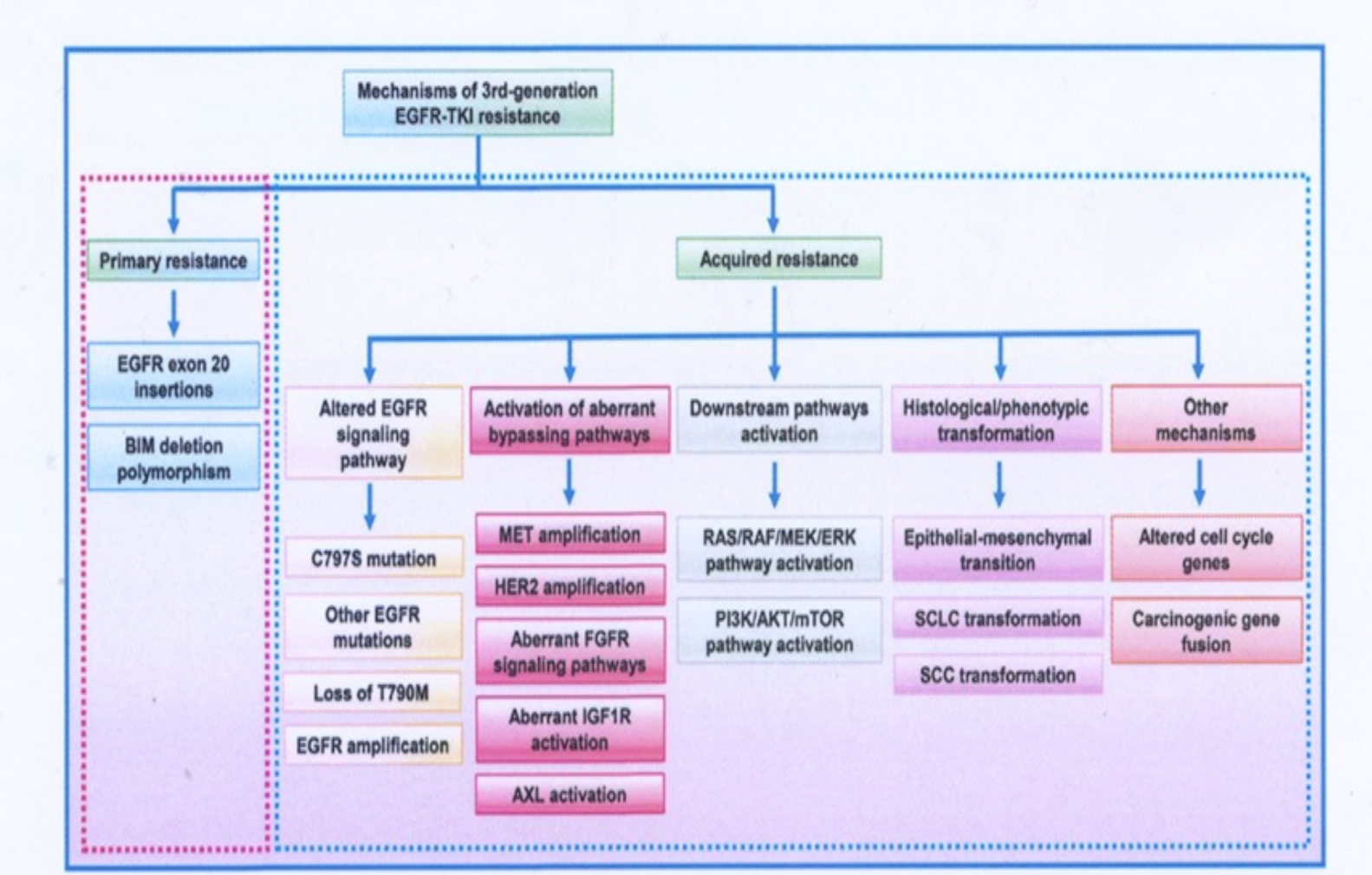


EGFR TKI structures



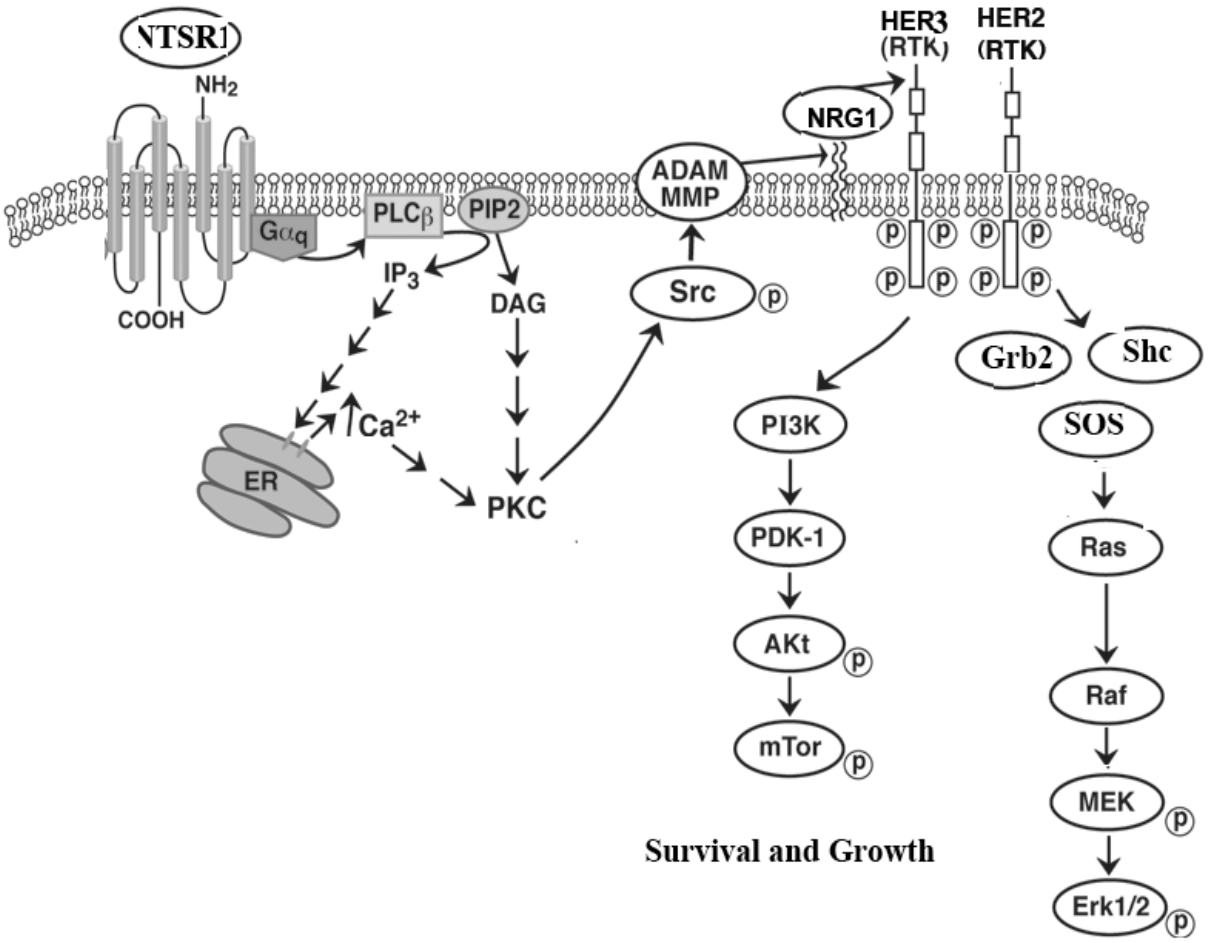
TKI resistance trial

He J. et al.,Int J Oncol 2021; 53:90

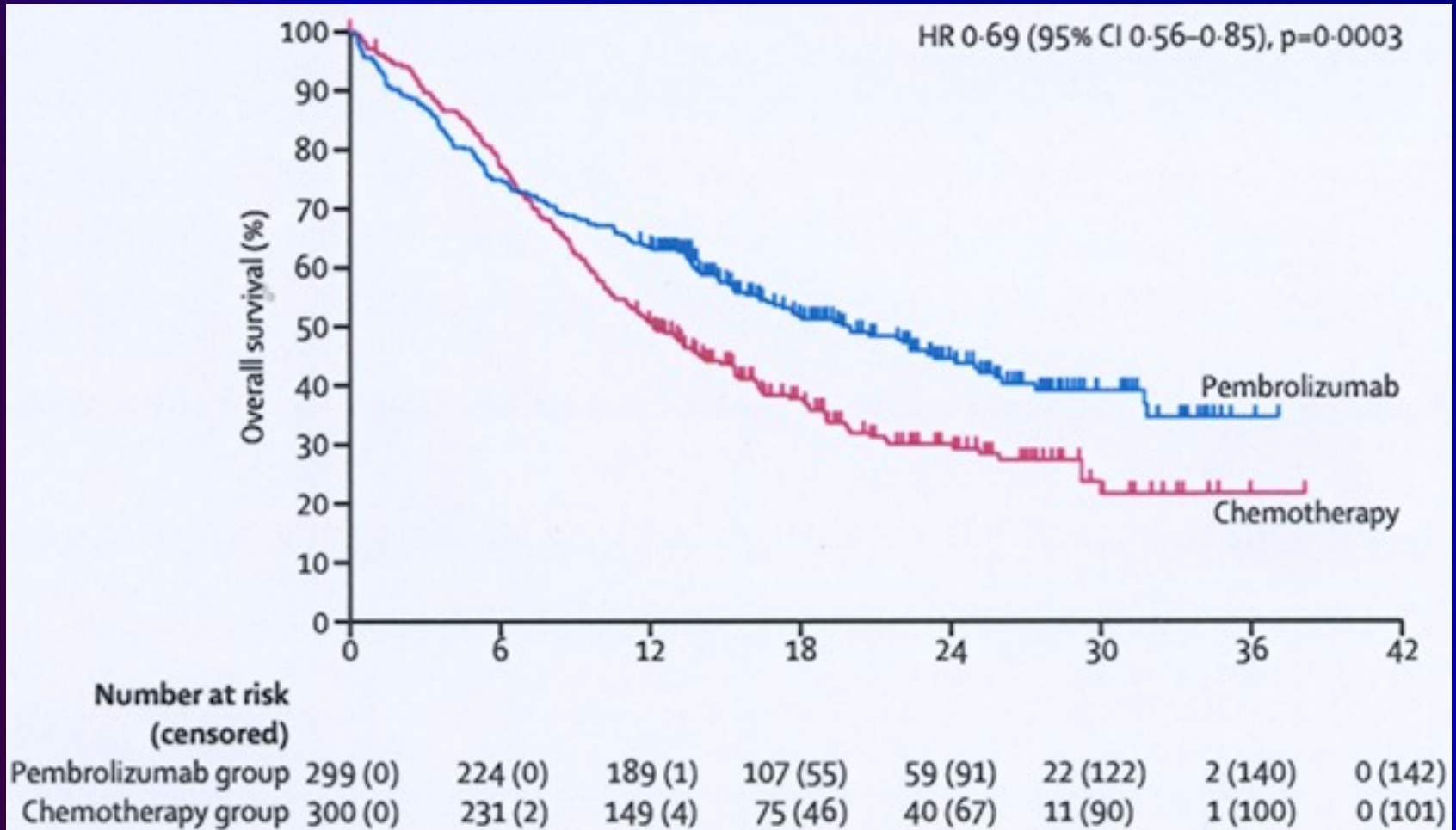


RTK transactivation.

Activation of GPCRs leads to tyrosine phosphorylation of RTK



Immune checkpoint inhibitors such as pembrolizumab increase overall survival in patients with PD-L1



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

- Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist, RS. Lung cancer. Lancet, 2021. PMID 34273294.
- Westover D, Zugazagoitia, J, Cho BC,
- Lovely CM, Paz-Ares L. Mechanisms of acquired resistance to first-and second-generation EGFR tyrosine kinase inhibitors. Ann Oncology 2018; 29: 110-119.