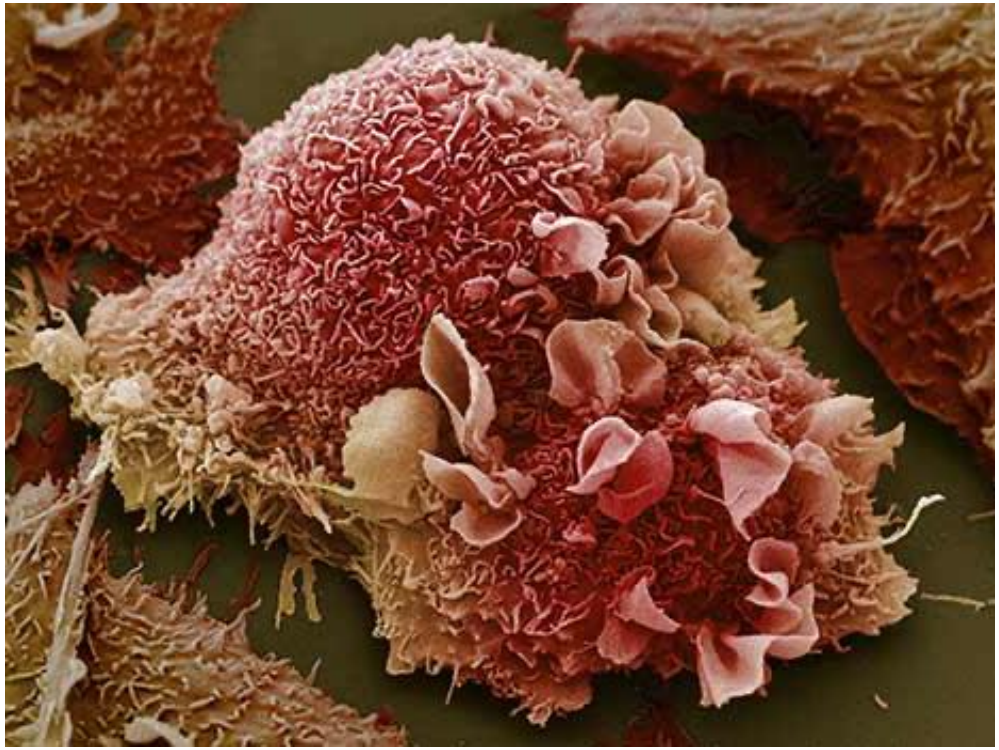
Basics of oncogenesis



Cancer

- The 2nd cause of death after cardiovascular disease
- In highly developed countries – the first

The most common cancers in Poland:

Women:

breast

lung

colon

endometrial cancer

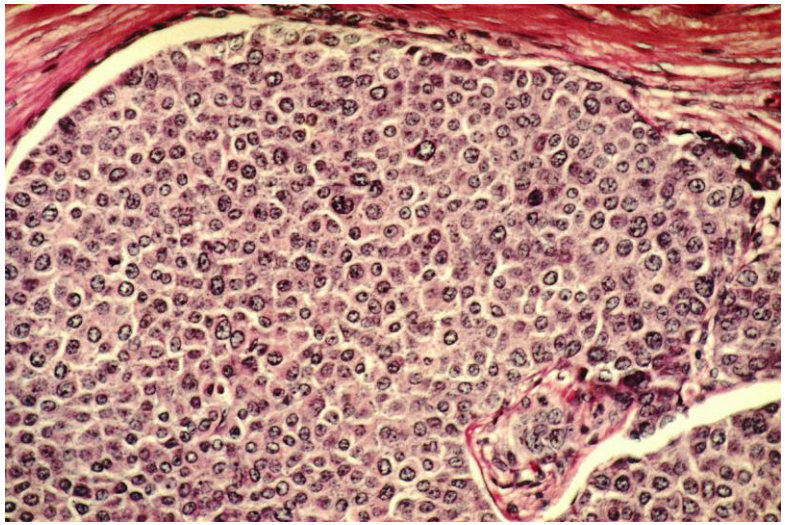
Men:

lung

prostate gland

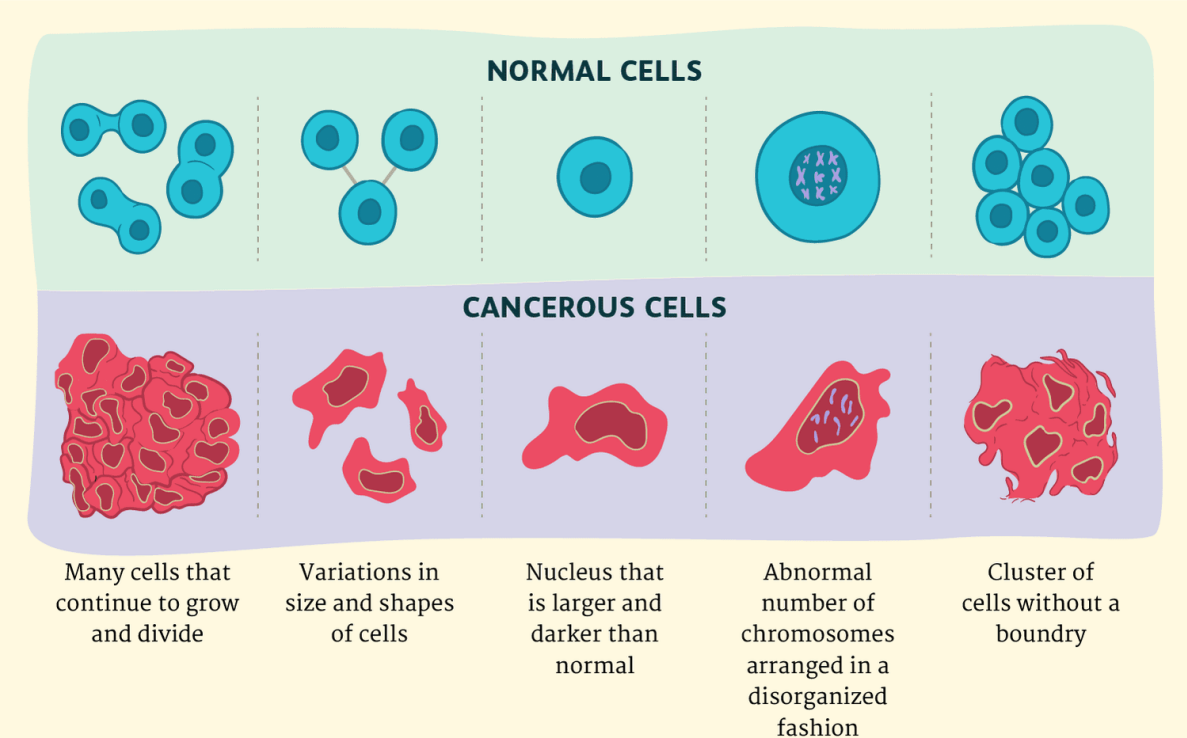
colon

bladder



Cancers

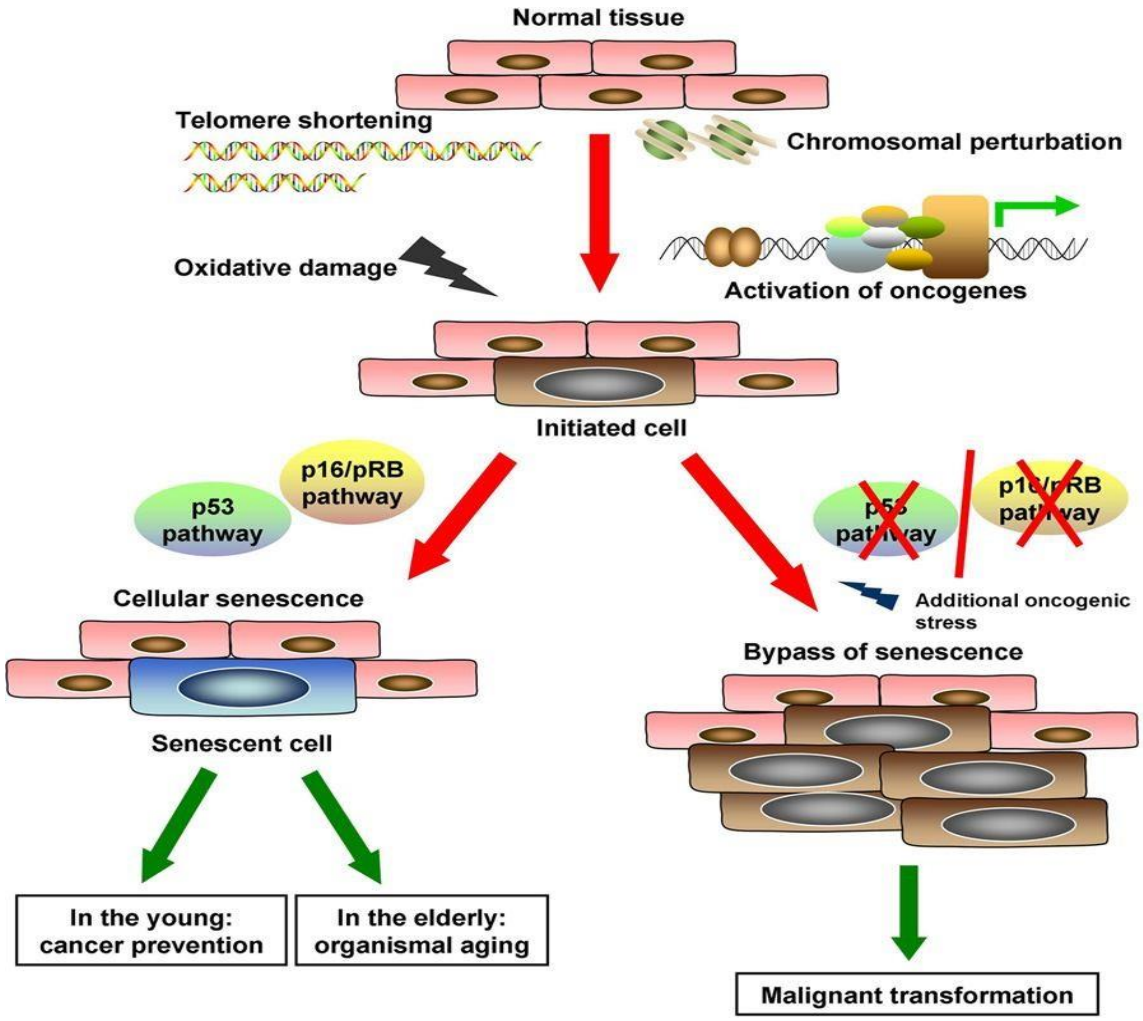
- Permanent, unregulated cell proliferation and greater resistance to cell death agents
- Failure to respond to proliferation inhibitory signals
- Lack of immune response – immune tolerance
- Clonal growth of cancerous tumors



Neoplastic transformation

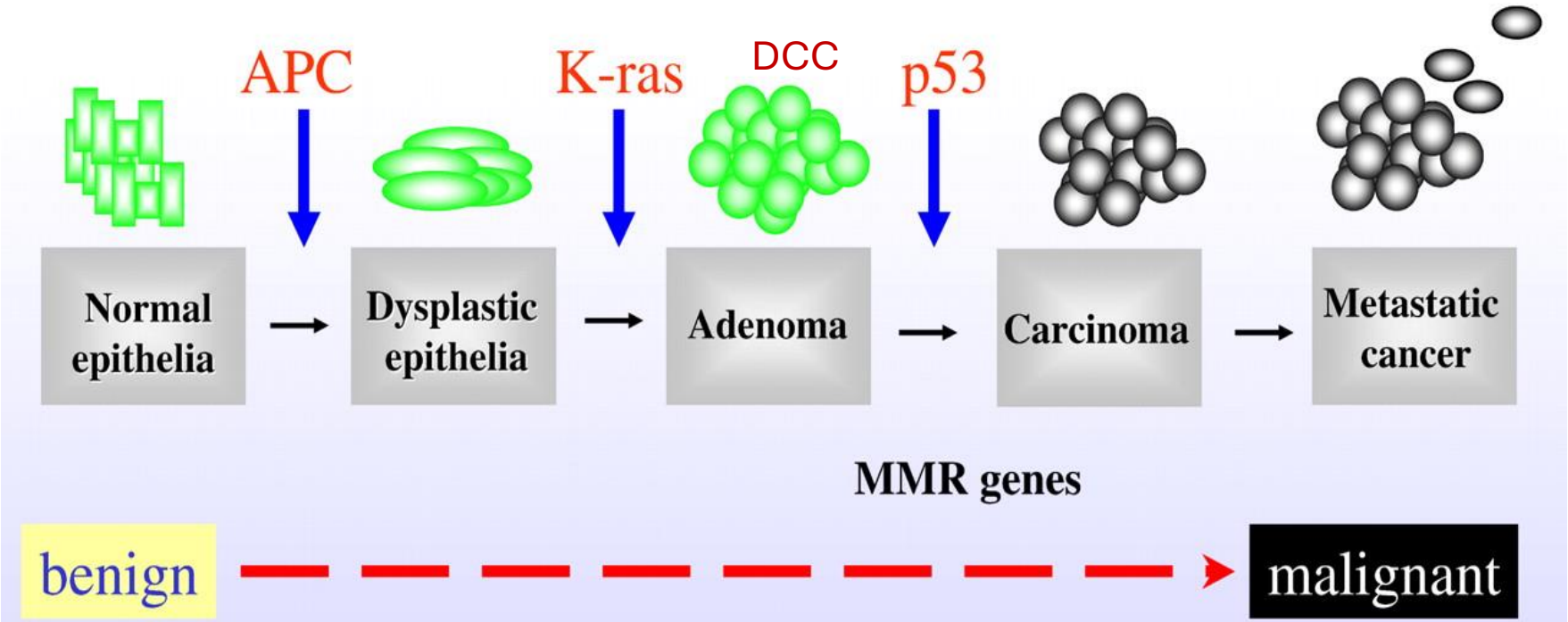
- Disturbed balance between the action of pro-cancer proteins (oncogenes) and proteins with opposite activity (tumor suppression genes and genes stabilizing the genome)
- The causes: genetic (point mutations, deletions, translocations, amplifications), epigenetic (cytosine methylation), infective, and environmental.
- Multi-stage proces. The accumulation of numerous damages, takes several decades.

Cancer is the result of bypassing the pathway of ageing or apoptosis

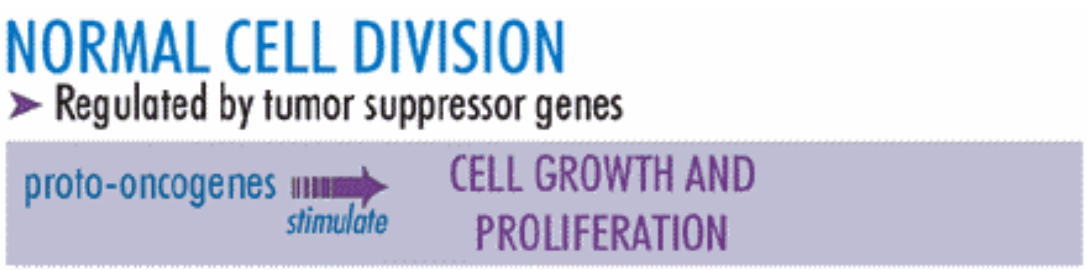


Cancer - accumulation of mutations

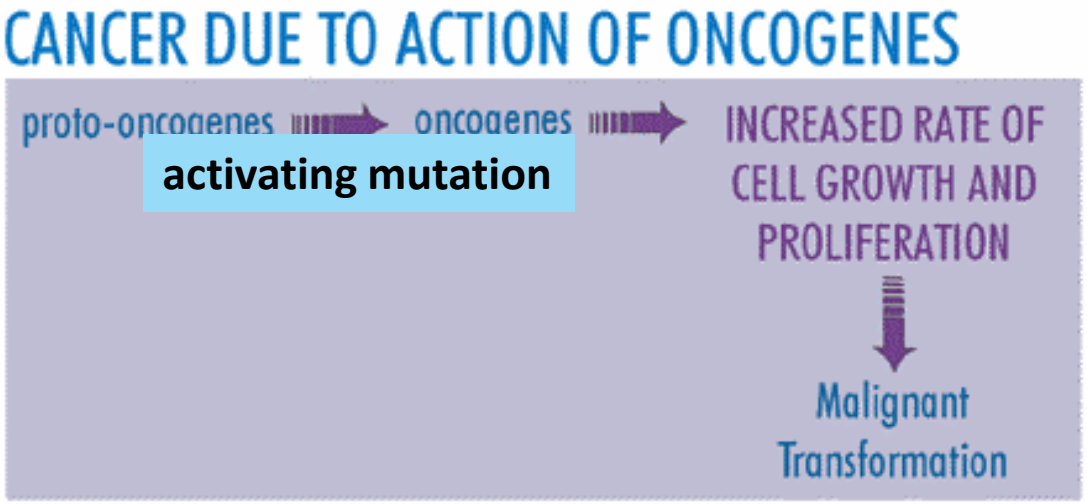
KRAS (small GTPase) - acts as an on/off switch in cell signaling. It controls cell proliferation, mutated, negative signaling is disrupted - cells can constantly multiply.



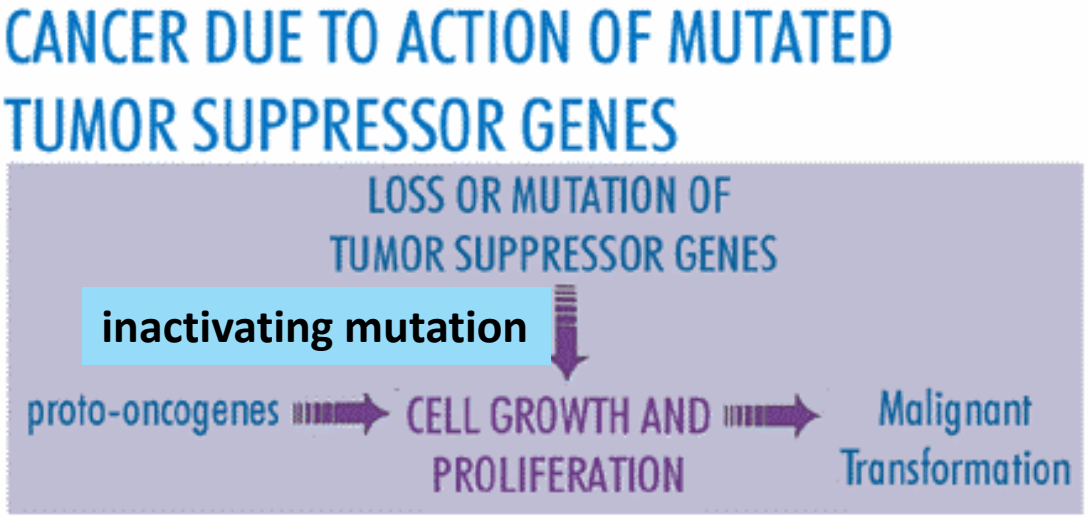
Fearon and Vogelstein model of colorectal cancer development



Mutations in:



- Protooncogene → oncogene

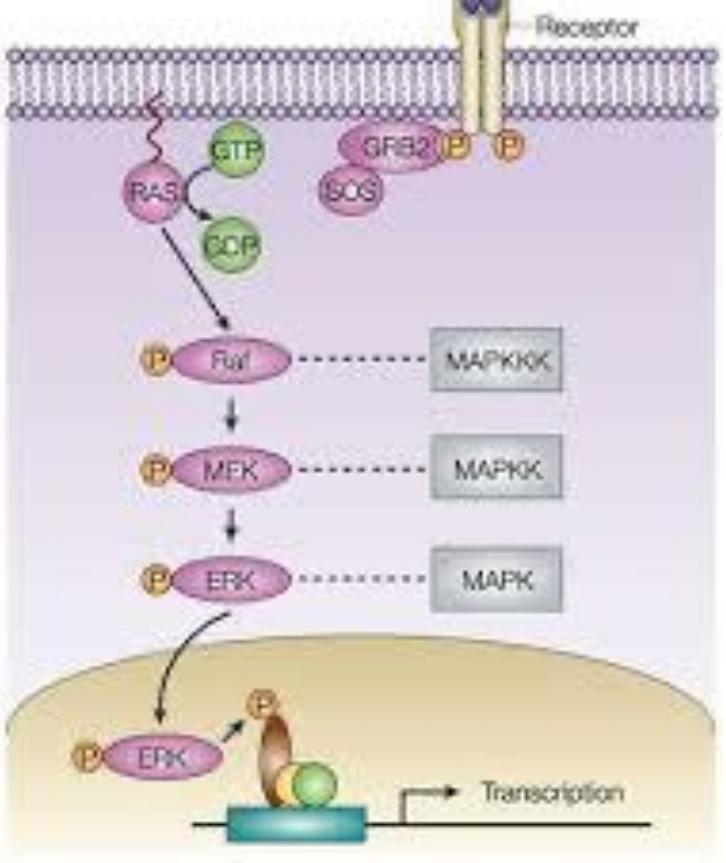


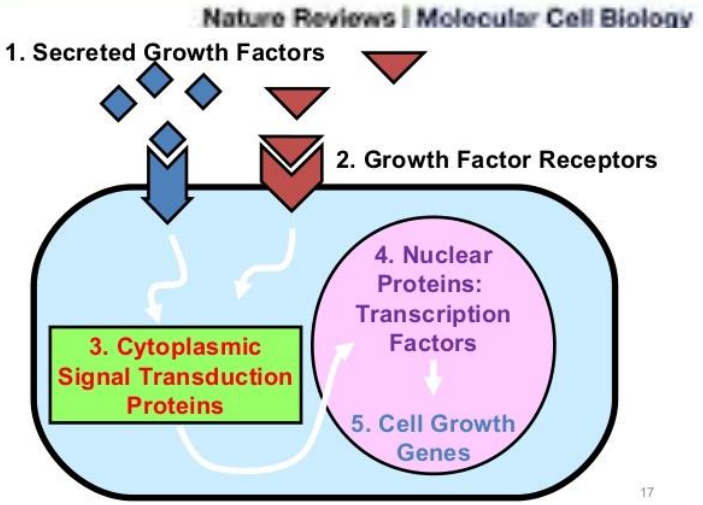
- Tumor suppressor genes (anti-oncogenes)
- DNA repair genes

Proto-oncogene - cell growth and differentiation

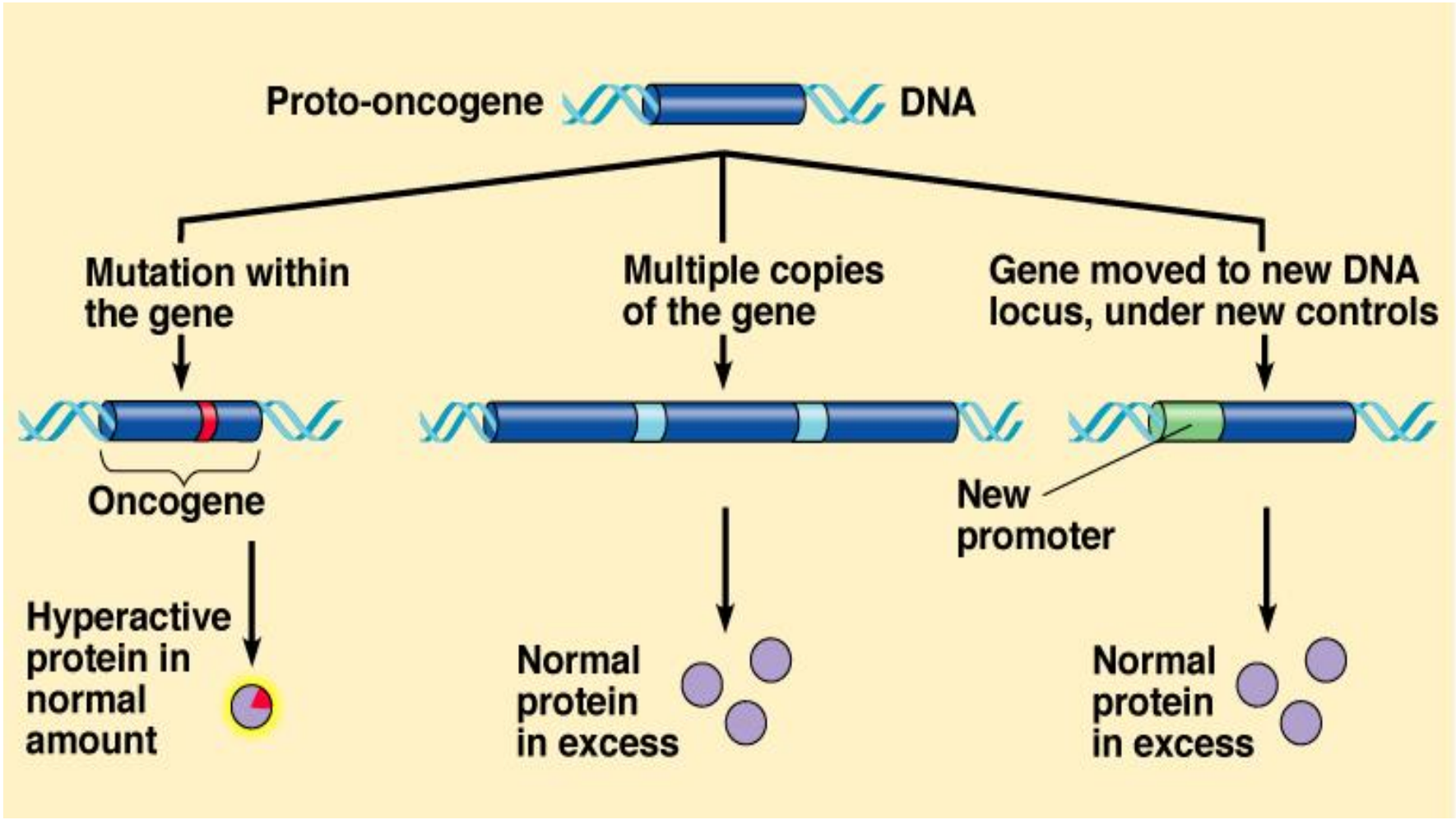
Normal gene that can become an oncogene due to mutation or increased expression:

- Tyrosine kinase receptors
- tyrosine kinases,
- signal transduction proteins (RAS, WNT),
- transcription factors (c-myc),
- Serine-threonine kinases (MAPKs, AKT),
- cyclins
- Cdk
- growth factors



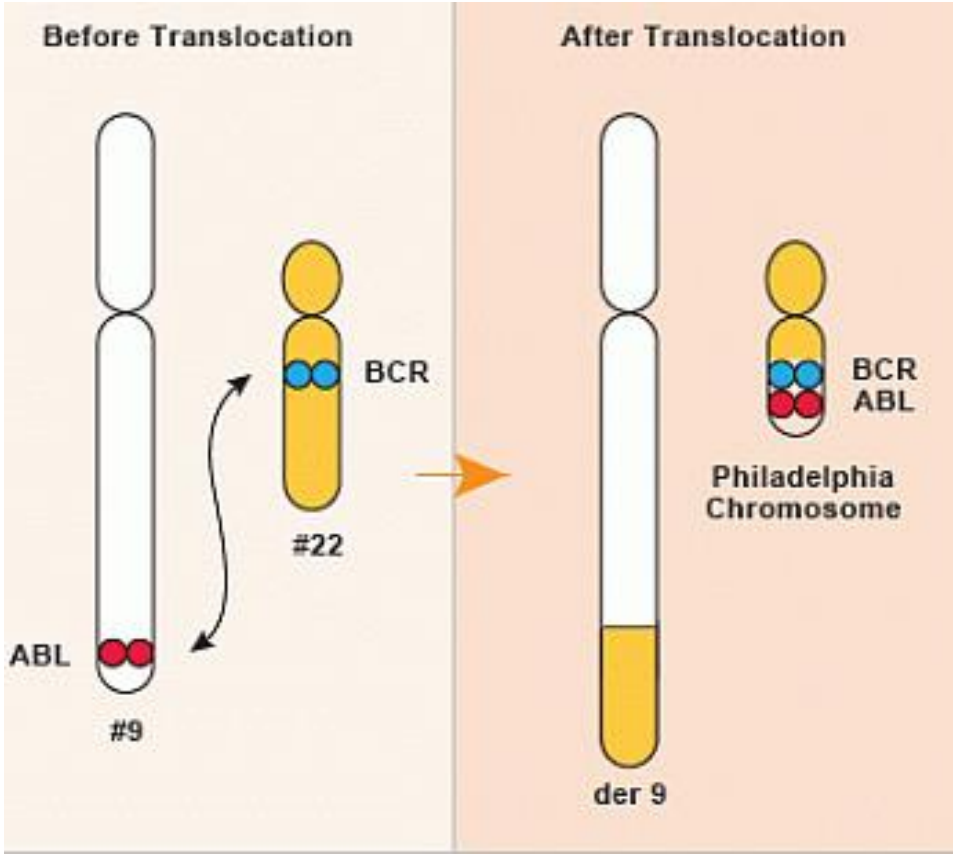


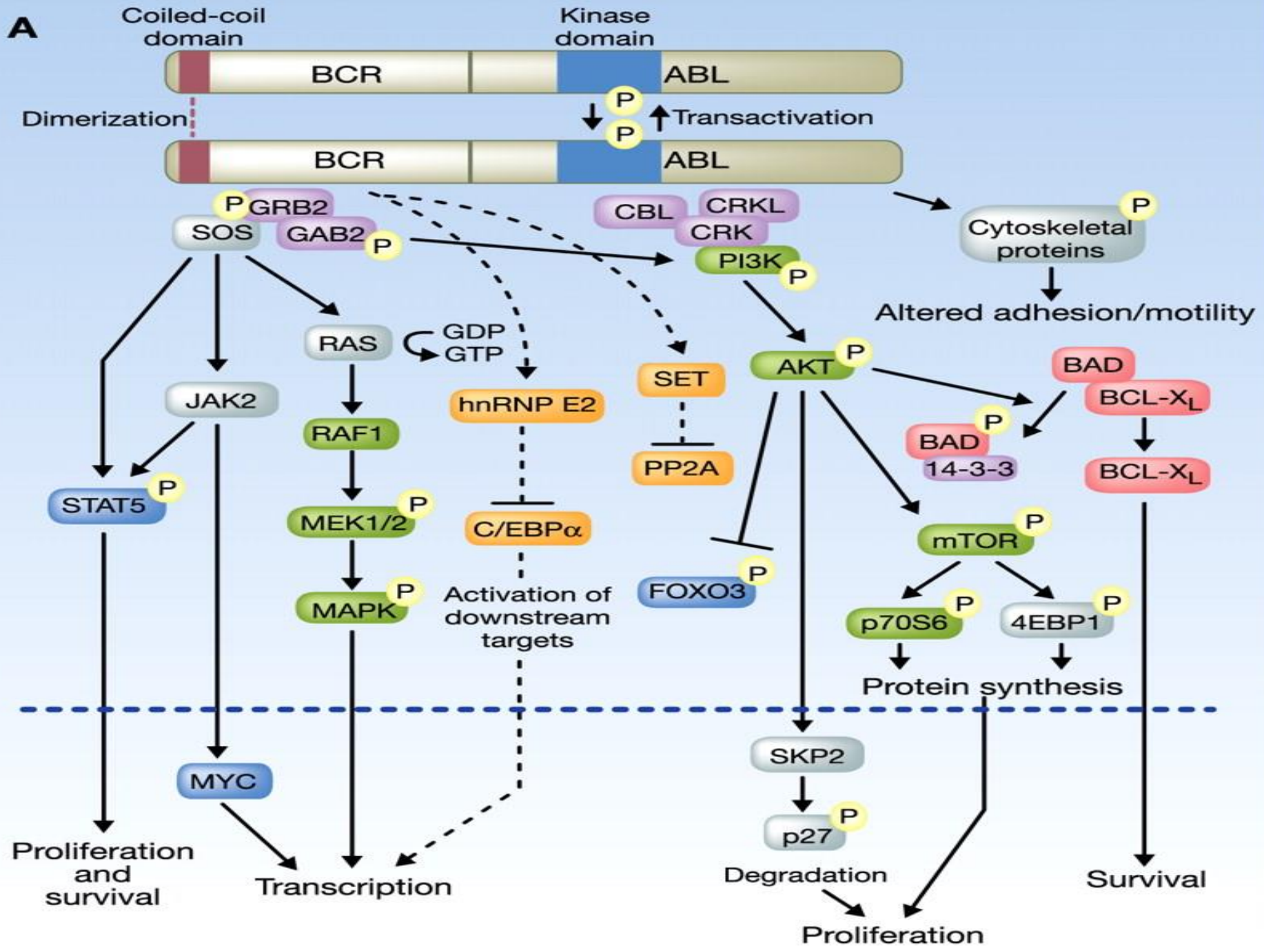
Proto-oncogene can become **oncogene**:



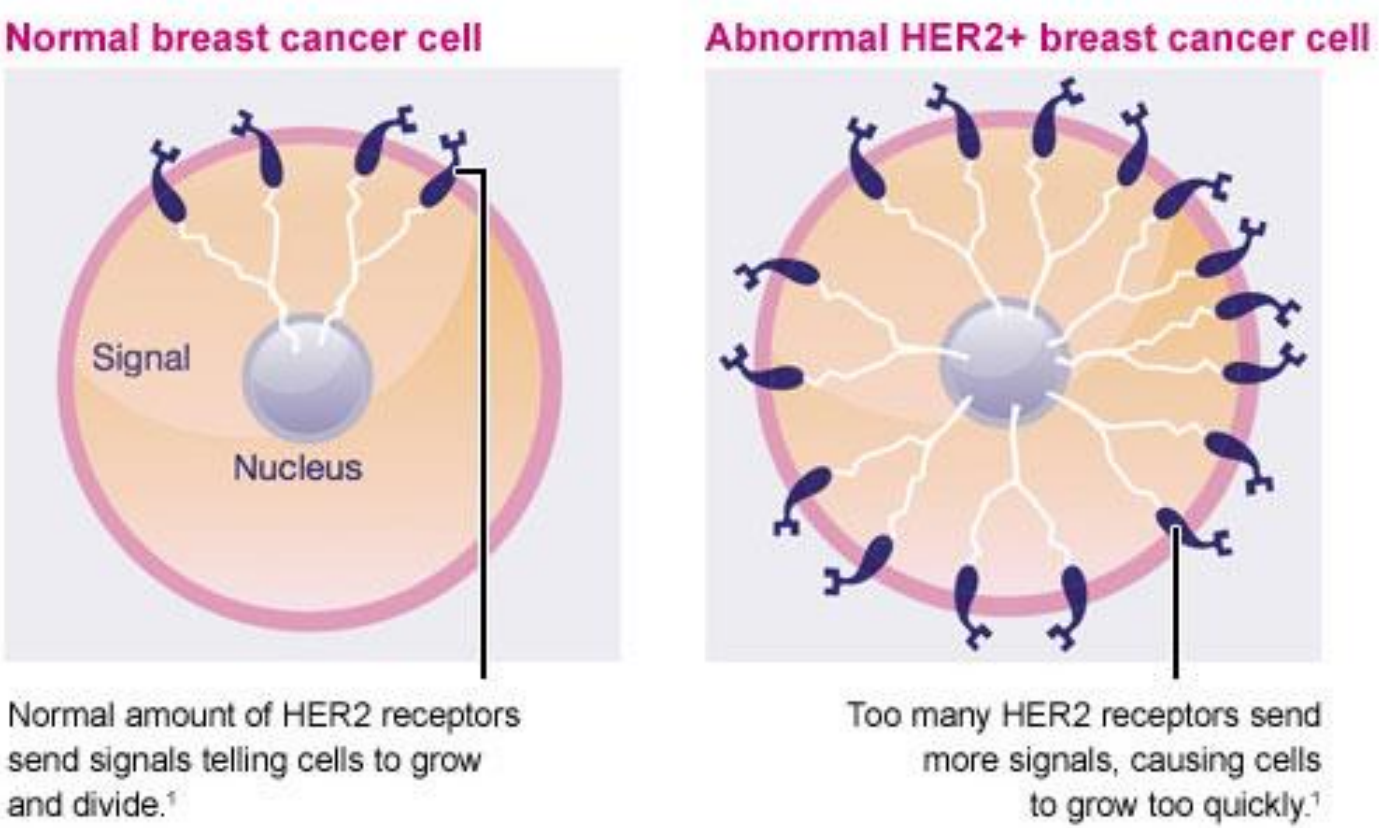
Chromosom Philadelphia- numeric chromosomal aberration – chronic myelogenous leukemia

- Translocation of the ABL gene from chromosome 9 to the gene on chromosome 22.
- BCR-ABL fusion gene - fusion protein - oncogene - deregulation of cell division
- Inhibited by Imatinib/Glivec, the first tyrosine kinase inhibitor

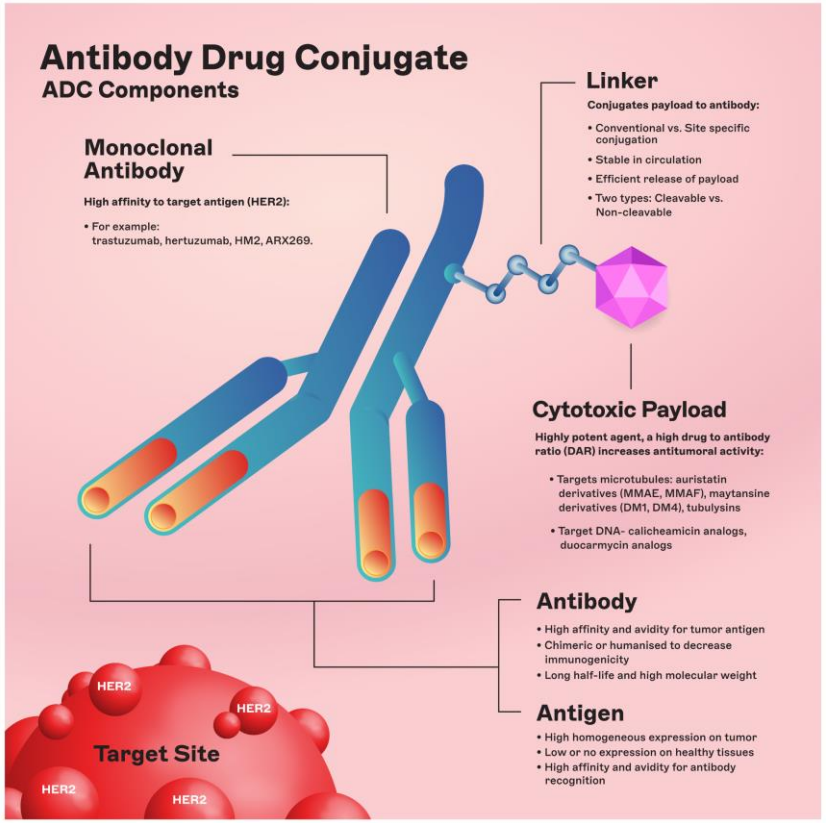



A
Coiled-coil domain
Kinase domain
BCR
ABL
Dimerization
P
P
Transactivation
BCR
ABL
P
GRB2
SOS
GAB2
P
CBL
CRKL
CRK
PI3K
P
Cytoskeletal proteins
P
Altered adhesion/motility
RAS
GDP
GTP
JAK2
hnRNP E2
SET
AKT
P
BAD
BCL-XL
P
BAD
14-3-3
BCL-XL
STAT5
P
RAF1
MEK1/2
P
C/EBPα
PP2A
mTOR
P
MAPK
P
Activation of downstream targets
FOXO3
P
p70S6
P
4EBP1
P
Protein synthesis
SKP2
MYC
p27
P
Proliferation and survival
Transcription
Degradation
Survival
Proliferation

Human epidermal growth factor receptor 2 – proto-oncogene. About 30% of breast cancers have amplified or overexpressed the HER2/neu gene. Overexpression of this receptor in breast cancer is associated with an increased risk of disease recurrence and a worse prognosis



Growth receptors – HER2



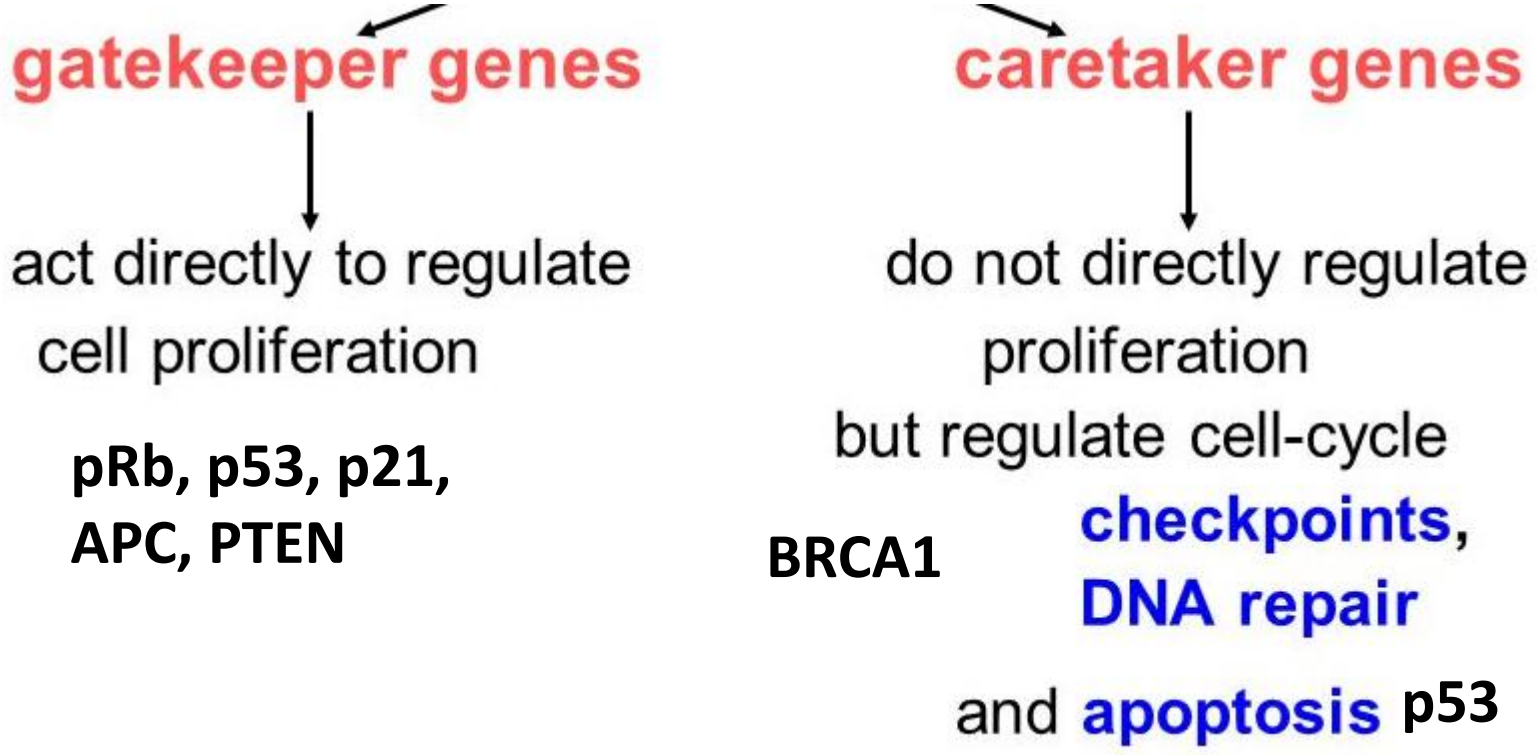
1. HER2 is a transmembrane receptor with tyrosine kinase activity
2. Activates PI3K/Akt and MAPK
3. Blocking HER2 causes G1 phase blocking, decreased angiogenesis, and induction of ADCC
4. Immunotherapy – anti-HER2 monoclonal antibodies, blocking CD20 in B-cell lymphomas – ADCC

Adverse reactions: cardiac arrhythmias and cardiomyopathy, toxic epidermal necrolysis, flu-like symptoms

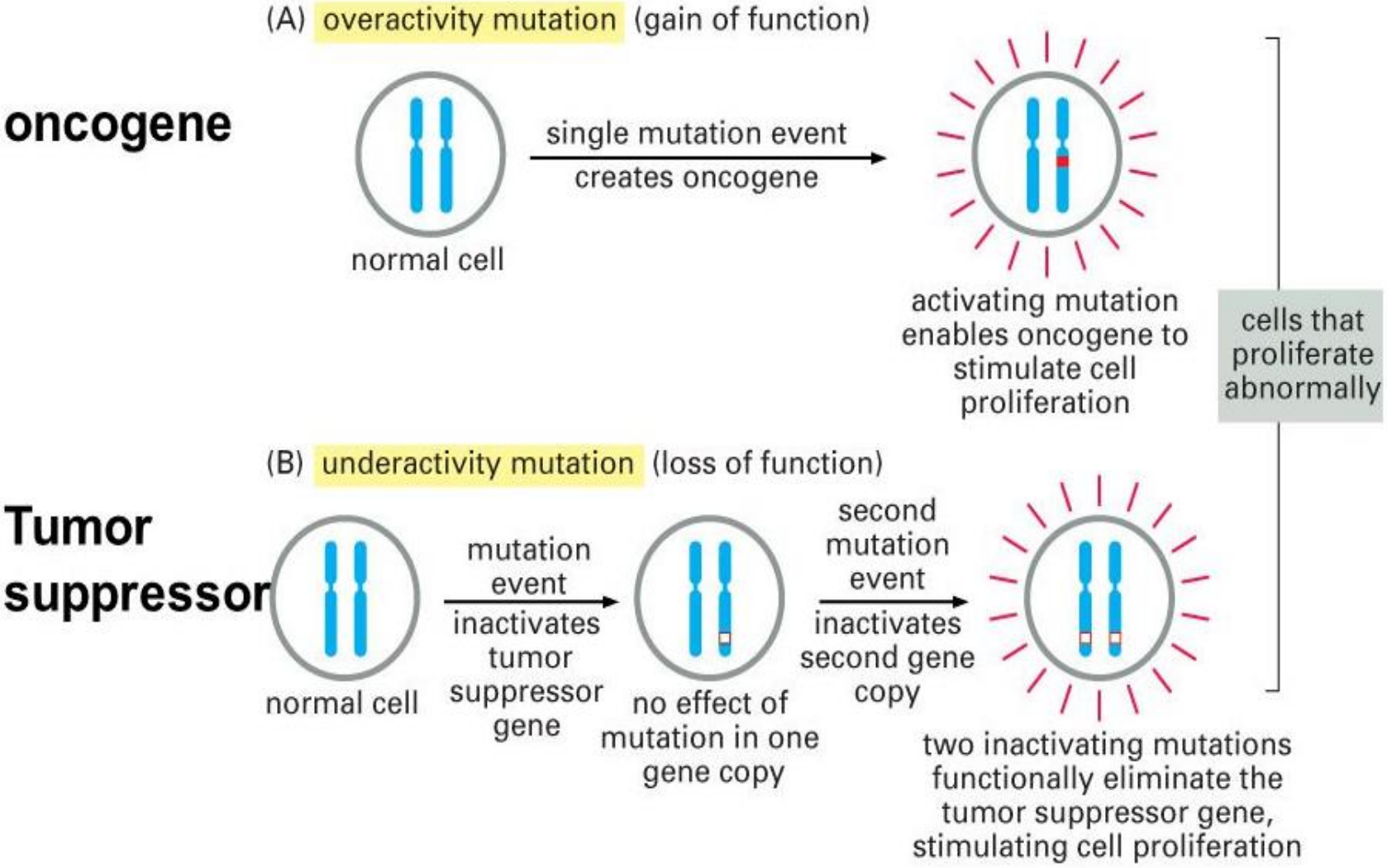
Tumor suppressor gene – anti-oncogene

- Repressive effect on cell cycle regulation - inhibition of cell cycle and cell signaling (pRb, p53, p21, APC, PTEN)

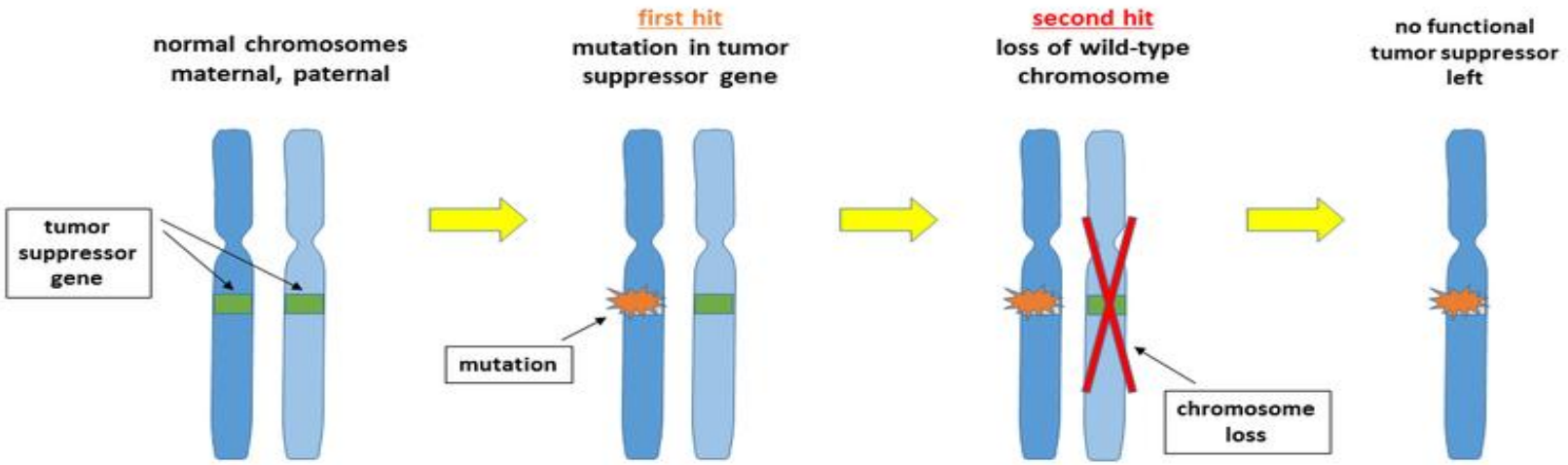
- DNA repair proteins (BRCA1) initiation of apoptosis (p53)



How do oncogenes and tumor suppressors work?

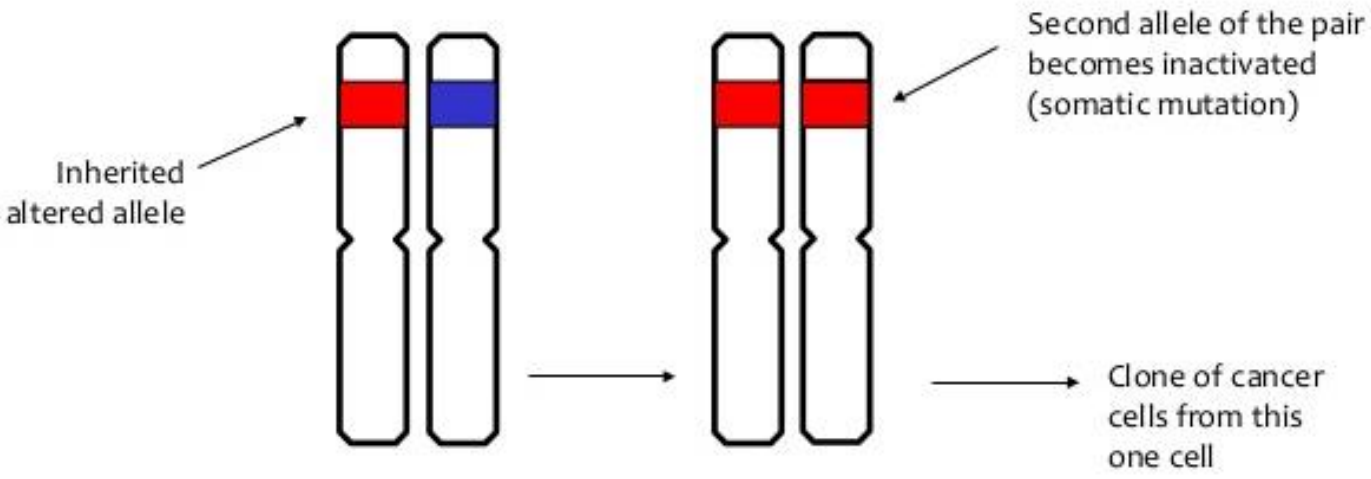


Non-hereditary cancer



Hereditary cancer

The changed allele is inherited – the second allele is inactivated



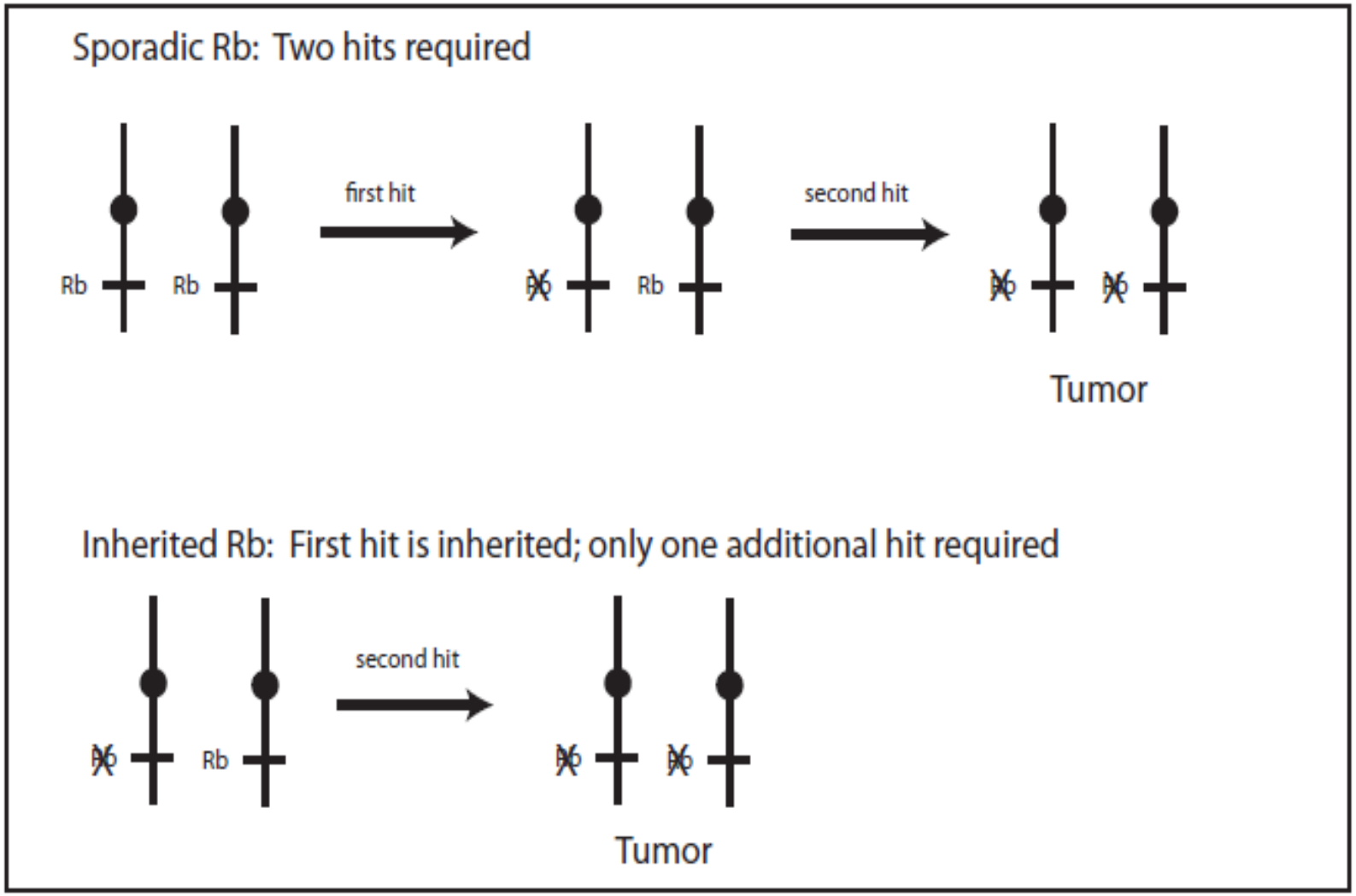
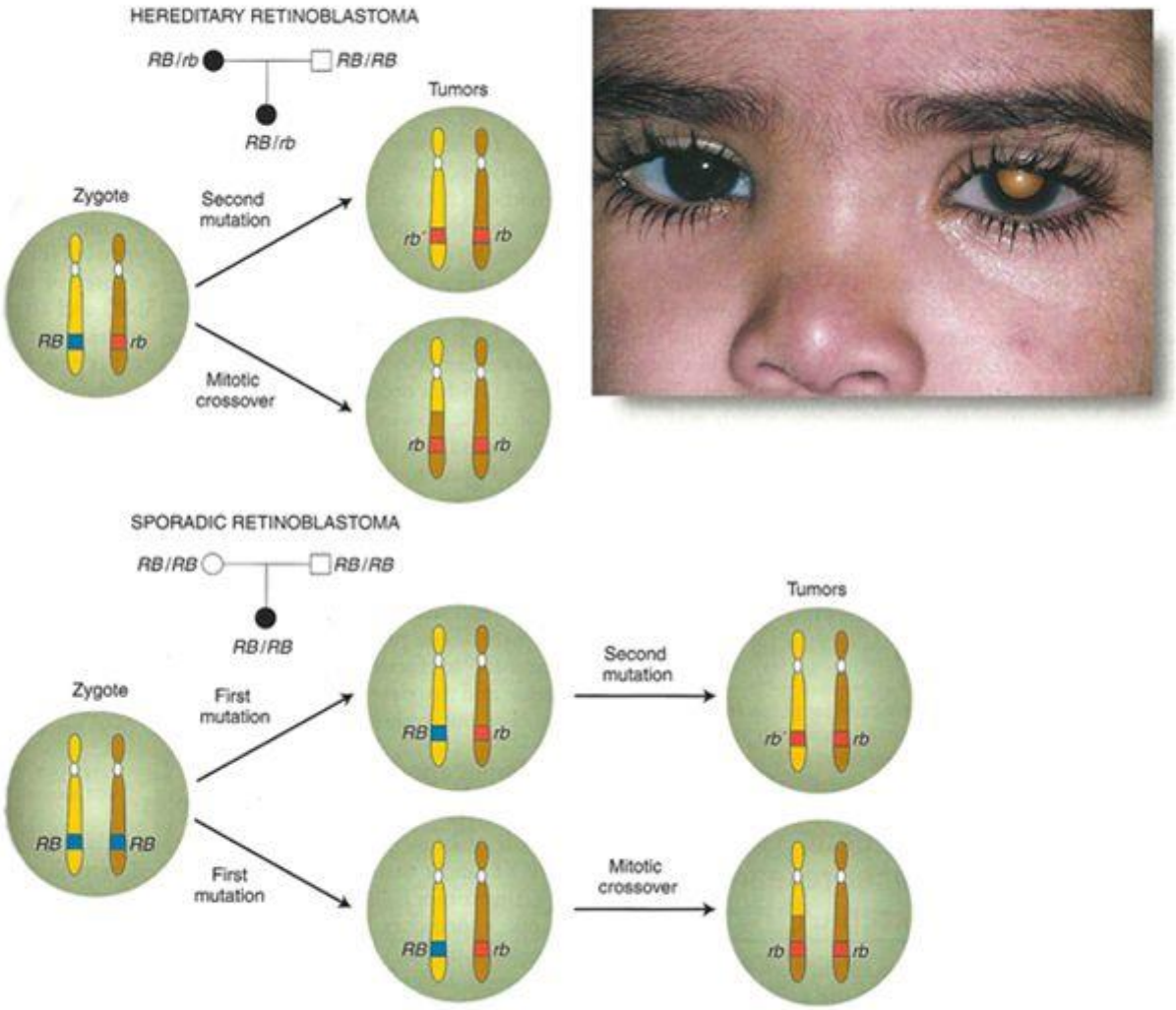
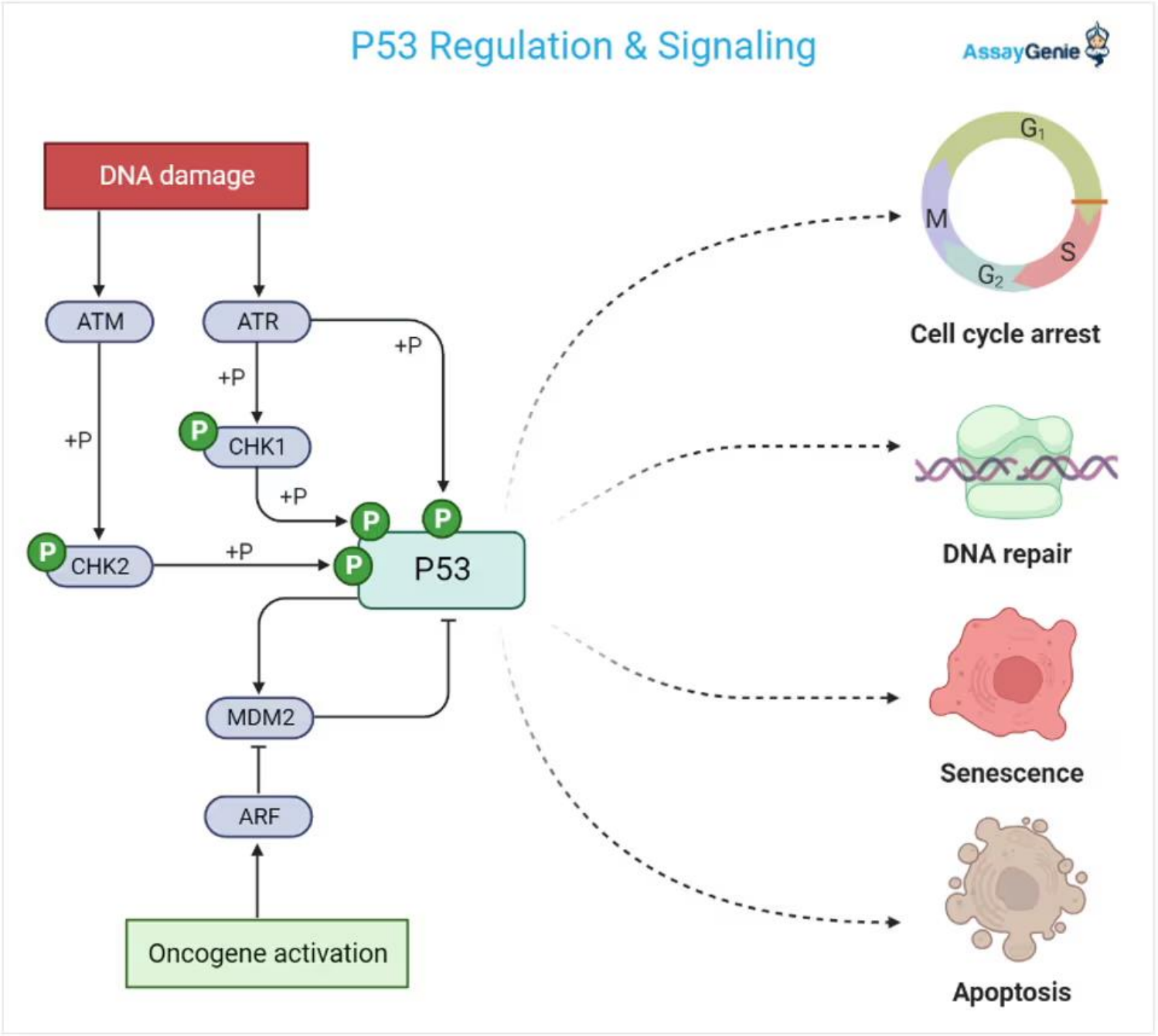


Figure 1. Knudson's two-hit hypothesis for retinoblastoma. *In sporadic Rb, both copies of RB1 (RB1) must be inactivated. This requires two mutational events "hits" which each inactivate one copy of RB1. In inherited Rb, the first hit is inherited.*

Familial vs. sporadic retinoblastoma

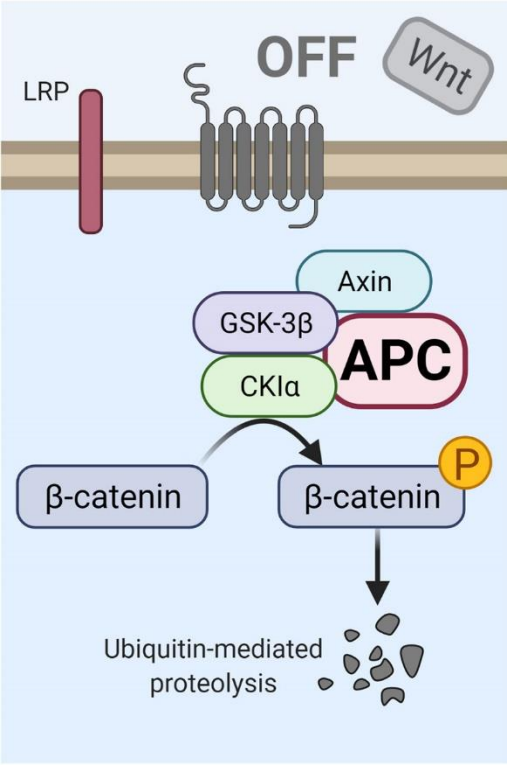


~90% of children with germline mutation will develop retinoblastoma. Remaining 10%, though do not develop the tumor transfer the mutation to their children.

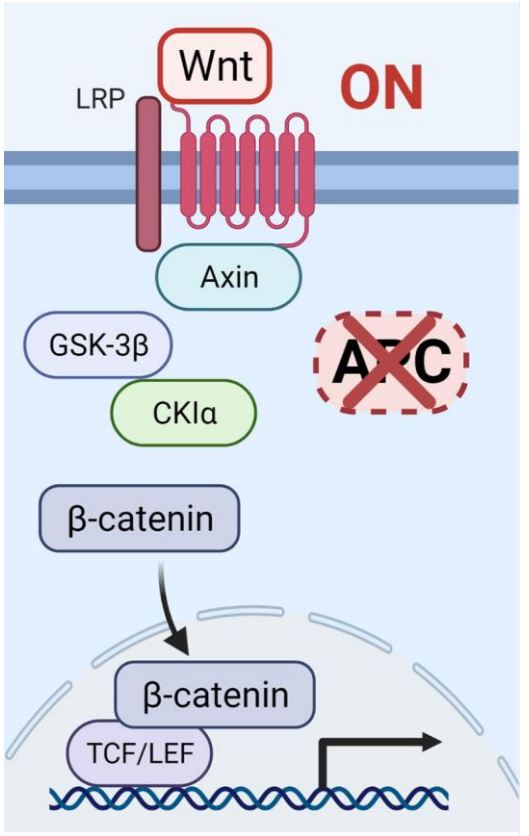

P53 Regulation & Signaling
AssayGenie
DNA damage
ATM
ATR
+P
+P
+P
+P
CHK1
+P
P
CHK2
+P
P
P
P53
MDM2
ARF
Oncogene activation
G1
M
S
G2
Cell cycle arrest
DNA repair
Senescence
Apoptosis

DNA mismatch repair (MMR) is a system for repairing erroneous insertion, deletion, and mis-incorporation of bases during DNA replication.

Double-strand breaks in DNA repair. BRCA1 and BRCA2 are unrelated proteins, but both are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA, or destroy cells if DNA cannot be repaired.



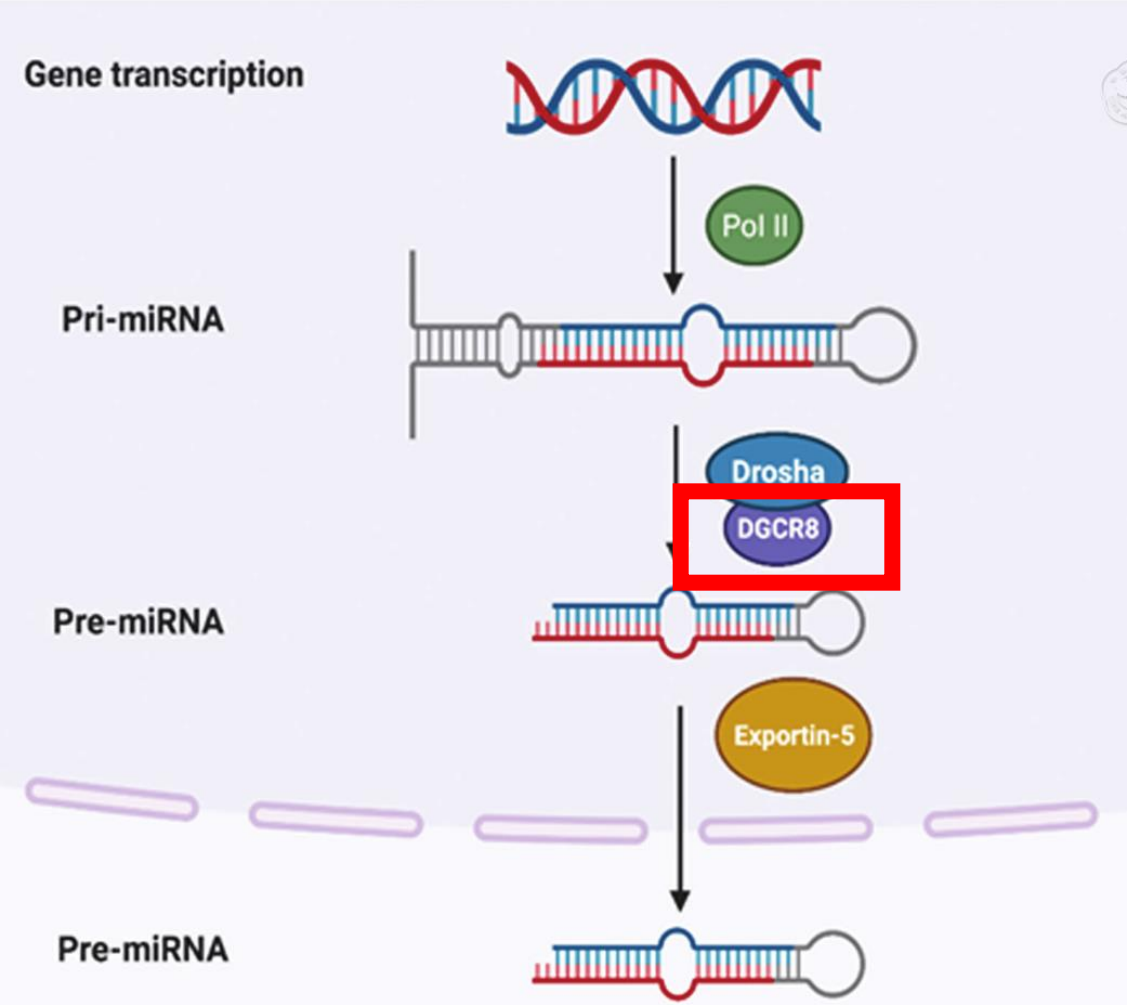
Adenomatous polyposis coli (**APC**) - a protein is a negative regulator that controls beta-catenin concentrations and interacts with E-cadherin, which are involved in cell adhesion. Mutations in the APC gene result in colorectal cancer and desmoid tumors.



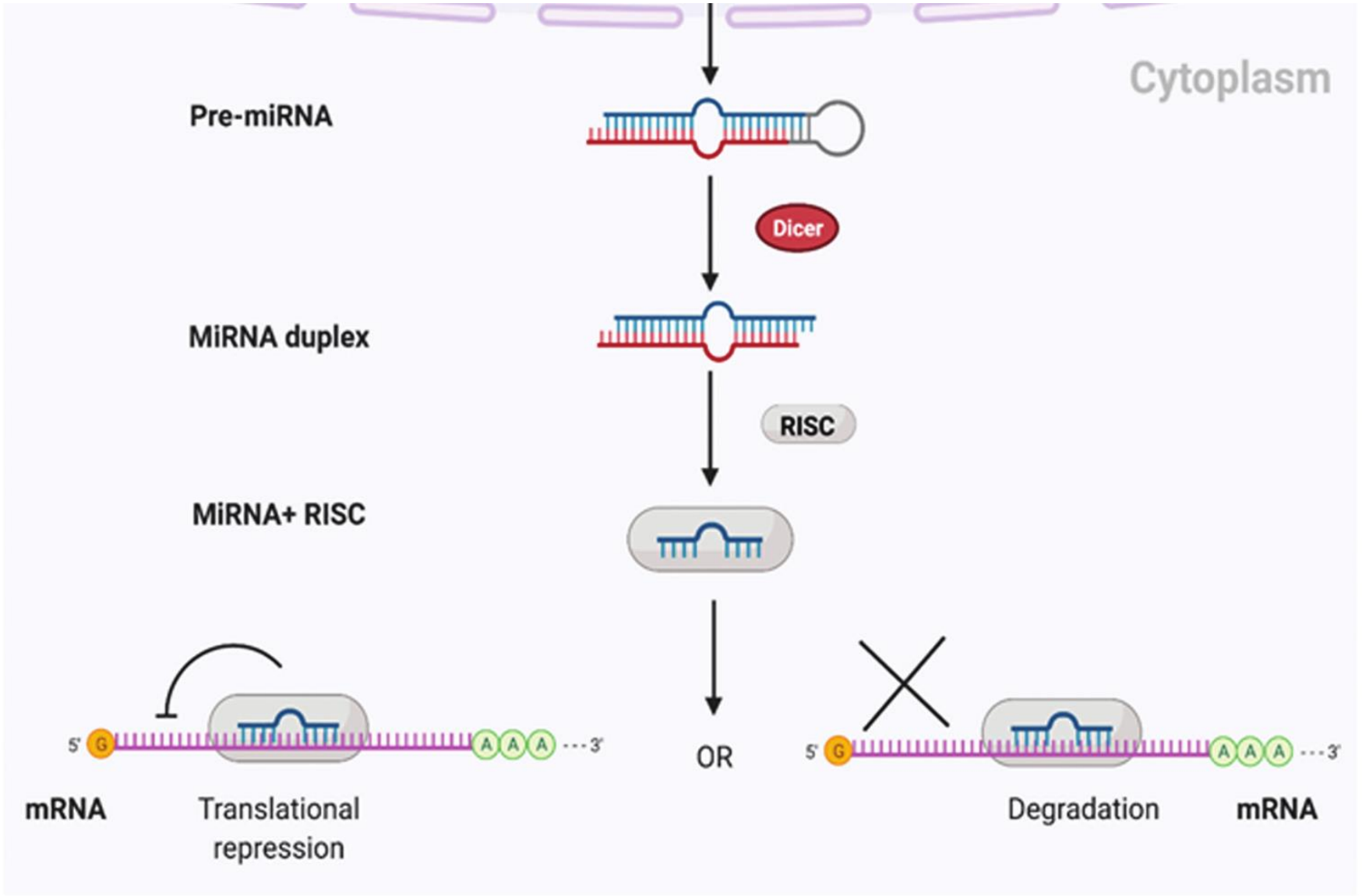
miRNA genes and their relationship to RNAi

miRNA genes encode primary single-stranded RNAs with a specific structure forming a double-stranded domain. Such pri-miRNA is cut by a complex whose main component is Drosha RNAse.

The pre-miRNA formed in this way travels to the cytoplasm and participates in RNAi there.



How does miRNA work?



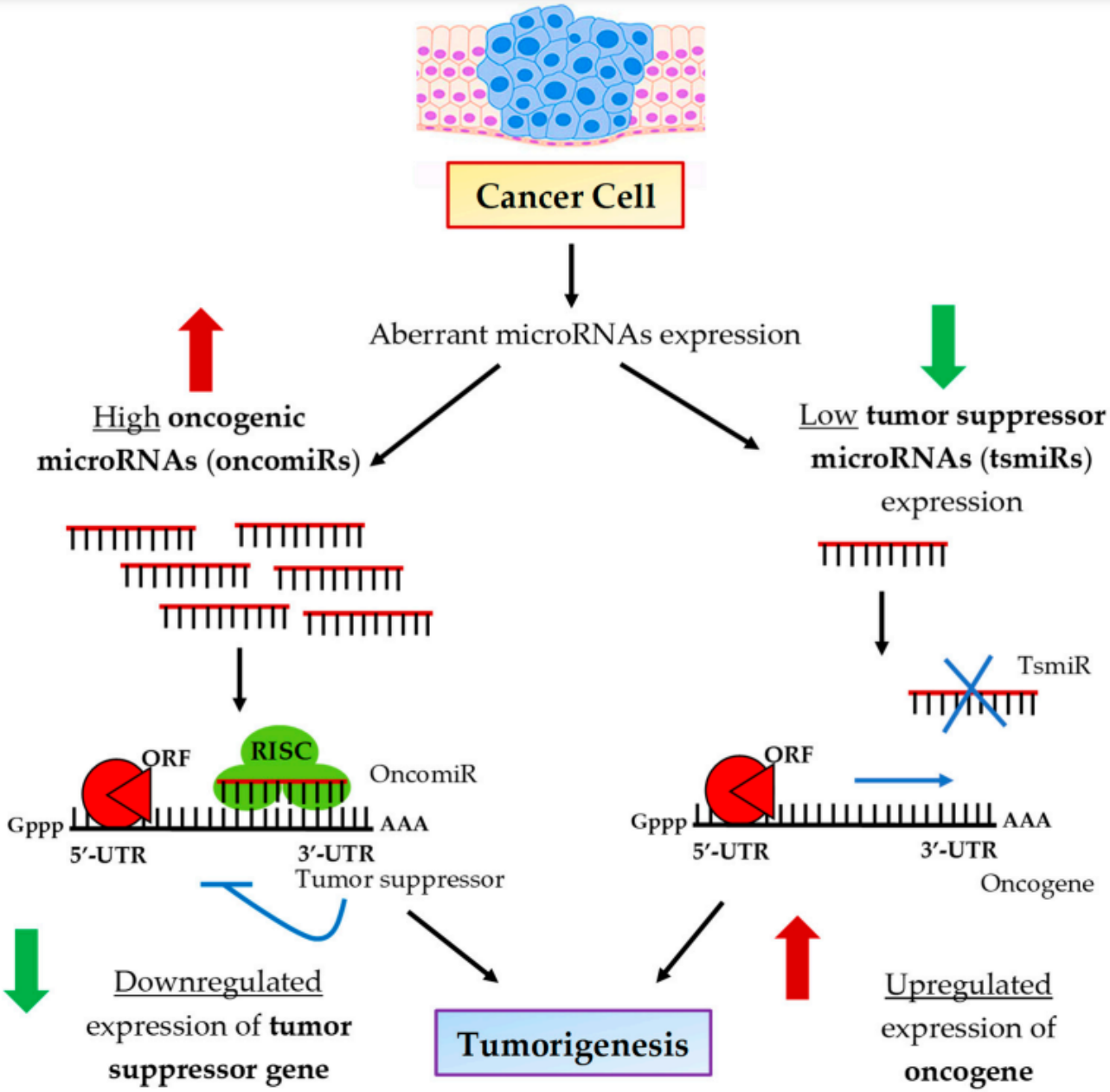
The role of miRNAs in cancer

OncomiRs are miRNA oncogenes that play a causal role in the formation and/or maintenance of tumor phenotypes.

OncomiR are powerful molecular tools that modulate many cancer-related genes.

Diagnostic marker

Therapy with miRNA


Cancer Cell
Aberrant microRNAs expression
High oncogenic microRNAs (oncomiRs)
Low tumor suppressor microRNAs (tsmiRs) expression
TsmiR
ORF
RISC
OncomiR
Gppp
AAA
5′-UTR
3′-UTR
Tumor suppressor
ORF
Gppp
AAA
5′-UTR
3′-UTR
Oncogene
Downregulated expression of tumor suppressor gene
Tumorigenesis
Upregulated expression of oncogene

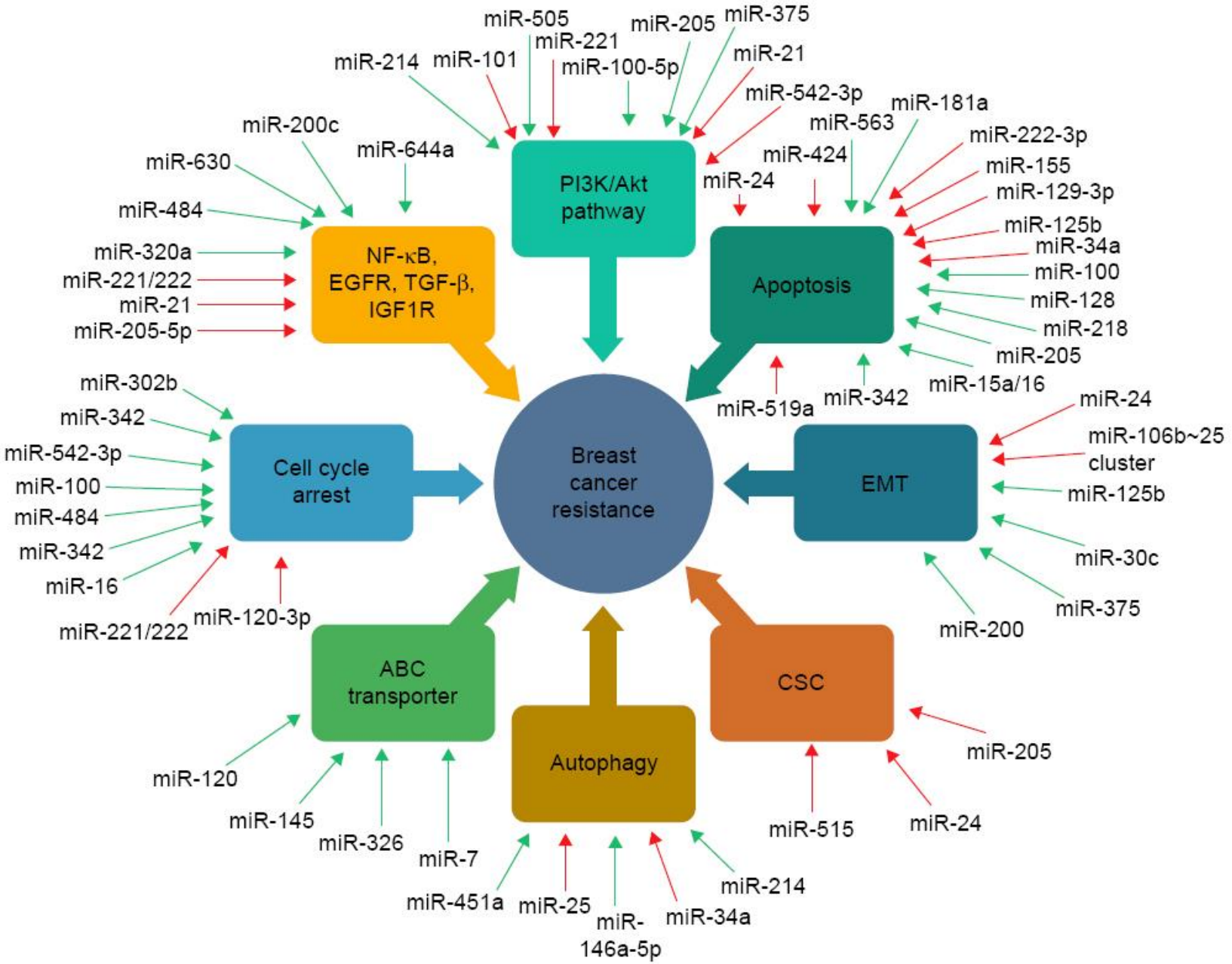


Figure 1 miRNA-mediated mechanisms involved in the regulation of BCa drug resistance. Red arrows indicate oncogenic miRNAs and green arrows represent cancer suppressor miRNAs.

Abbreviations: BCa, breast cancer; EGFR, epithelial growth factor receptor; CSC, cancer stem cell; TGF-β, transforming growth factor-β; EMT, epithelial–mesenchymal transition.

Cancer predisposition - hereditary disorders

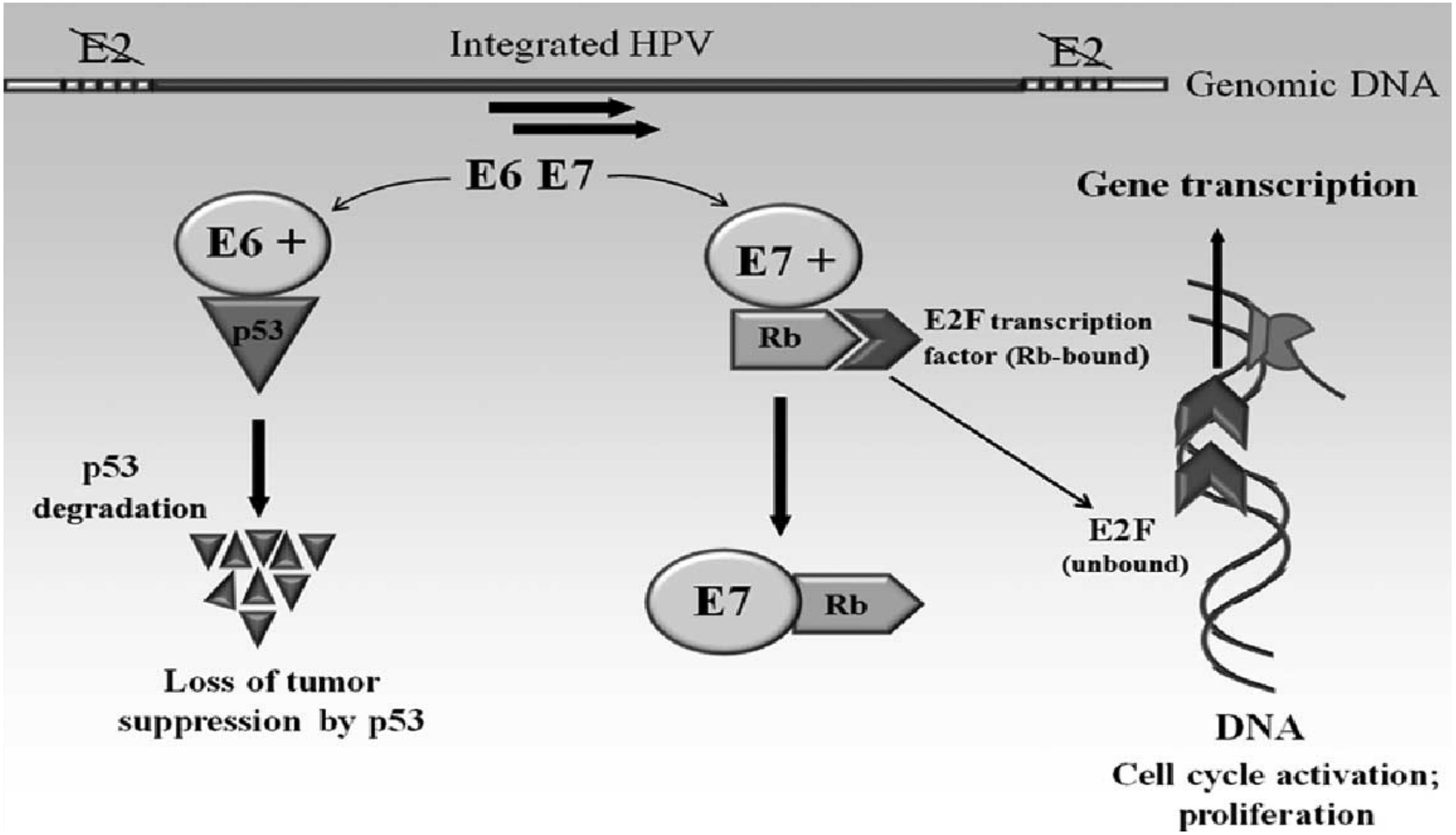
- **Li–Fraumeni syndrome** - **p53** mutation
- **Familial adenomatous polyposis (FAP)** - **APC** mutation, While polyps start out benign, malignant transformation into **colon cancer** occurs when they are left untreated
- **Lynch syndrome - Hereditary nonpolyposis colorectal cancer (HNPCC)** – mutations of genes involved in the DNA mismatch repair pathway **MLH1, MSH2**
- **Familial breast cancer** – **BRCA1, BRCA2** mutation (ds DNA breaks repair)
- **Retinoblastoma** - **pRB** mutation

15% of cancers are due to infections -, **hepatitis B, hepatitis C**, **human papillomavirus**, **Epstein–Barr virus** and **human immunodeficiency virus (HIV), Helicobacter pylori**

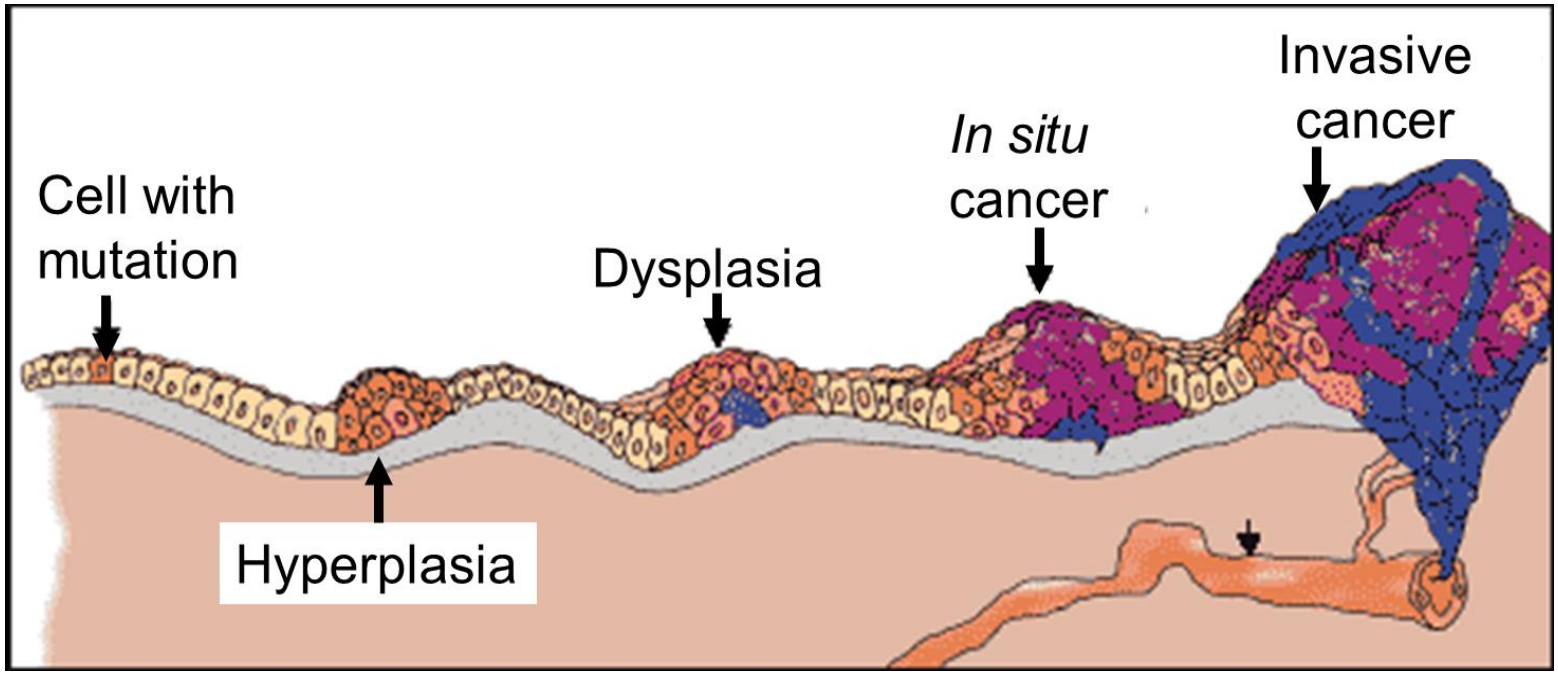
Virus	*Type of Cancer*
Epstein-Barr virus	Burkitt's lymphoma
Human papillomavirus	Cervical cancer
Hepatitis B virus	Liver cancer
Human T-cell lymphotrophic virus	Adult T-cell leukemia
Kaposi's sarcoma-associated herpesvirus	Kaposi's sarcoma
Helicobacter pylori	gastric carcinoma

HPV - human papilloma virus - DNA virus

- benign tumors - papillomas or warts (e. g. HPV1, HPV6, HPV11)
- HPV 16 and 18 - cervical, penile and oral cancers
- E genes - expressed immediately after infection of a cell, and a late (L) genes encoding capsid proteins
- **E6** - degradation of p53, **E7** – inactivation of pRb

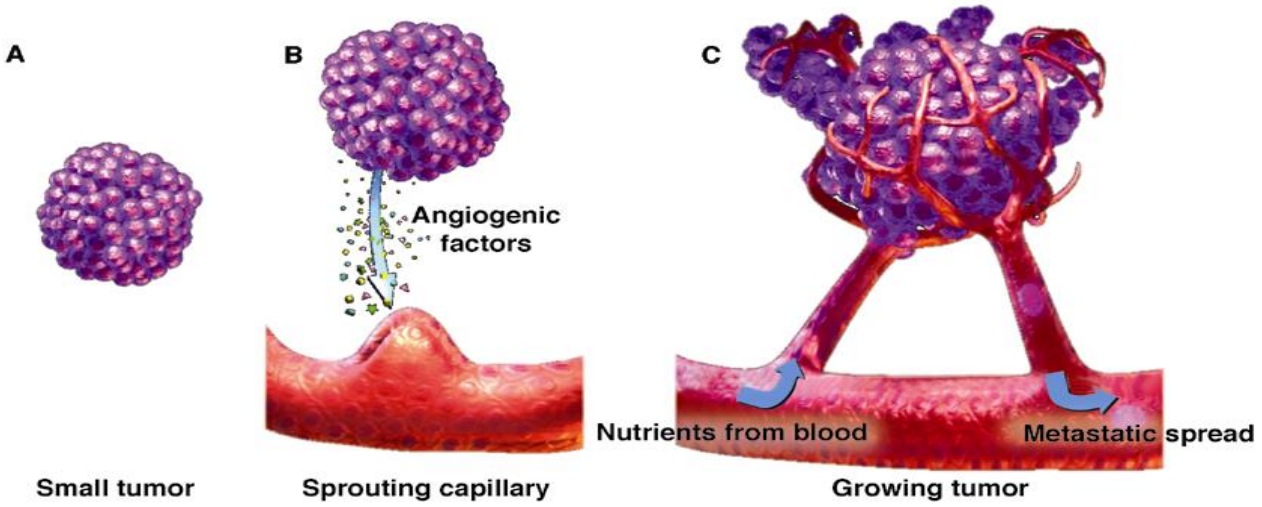


Cancer progression



- **hyperplasia** (an increase in the number of normal cells in an organ or tissue
- **dysplasia** (more immature cells),
- **metaplasia -** transformation of one differentiated cell type to another
- **cancer in situ**,
- **invasive cancer (malignant)** - formation of new tumors (**metastasis**)

Hypoxia and necrosis in the center of the tumor stimulates vessel formation



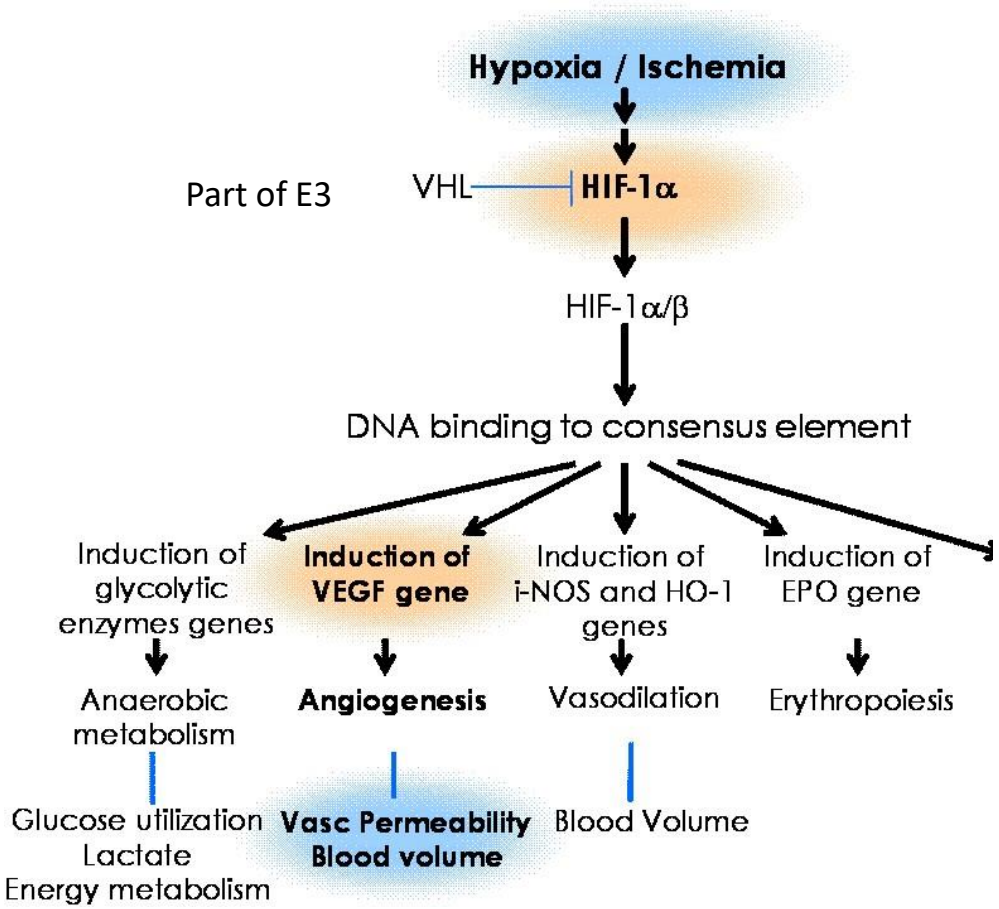
Angiogenesis – new blood vessels are formed from pre-existing vessels – development, wound healing, transition of tumors from a benign state to a malignant one

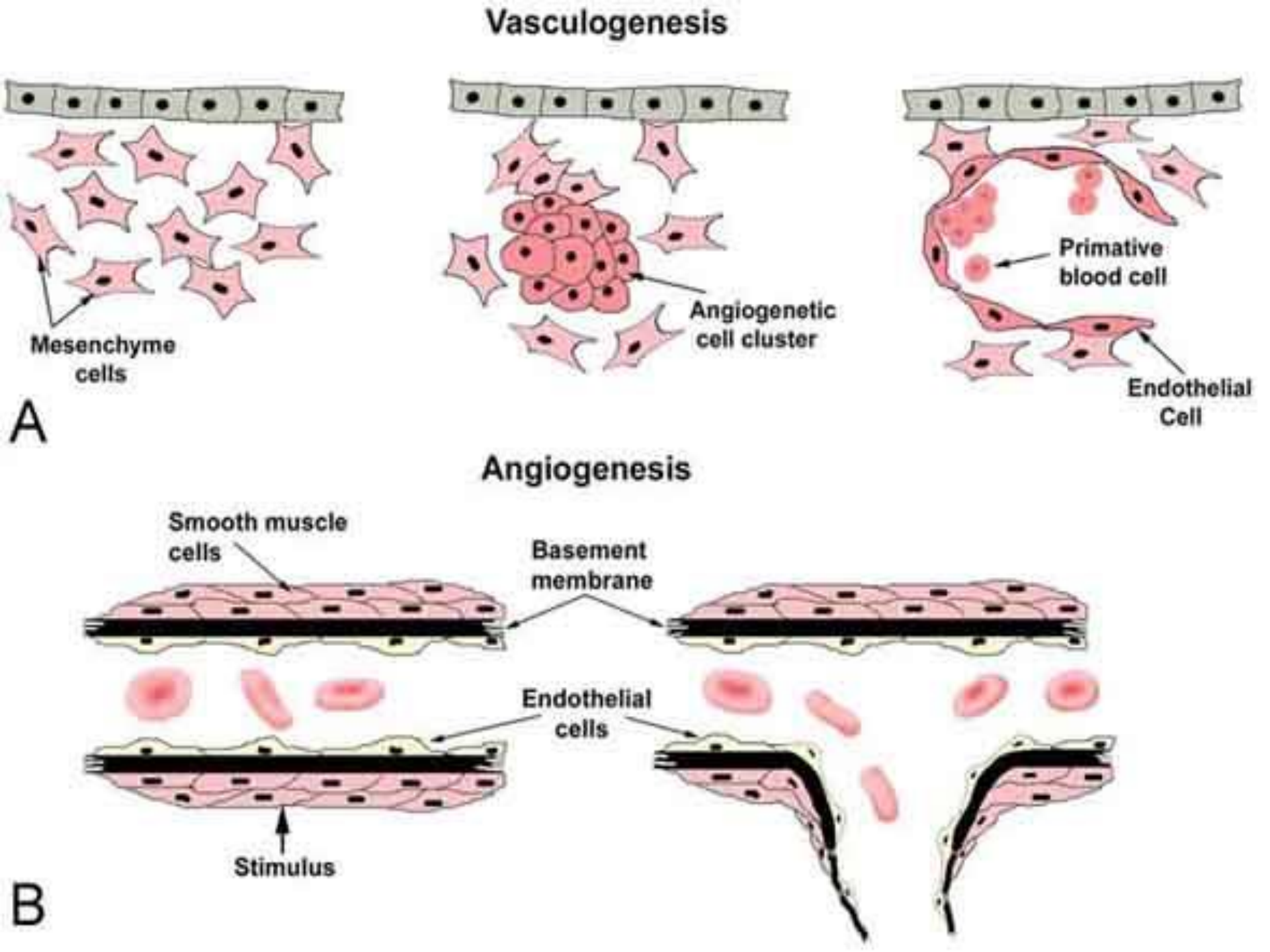
Vasculogenesis – de novo formation of blood vessels from mesoderm cell precursors - embryonic development of circulatory system. In adults – from stem cells - after cardiac ischemia, endometriosis, tumor growth

Hypoxia-inducible factors

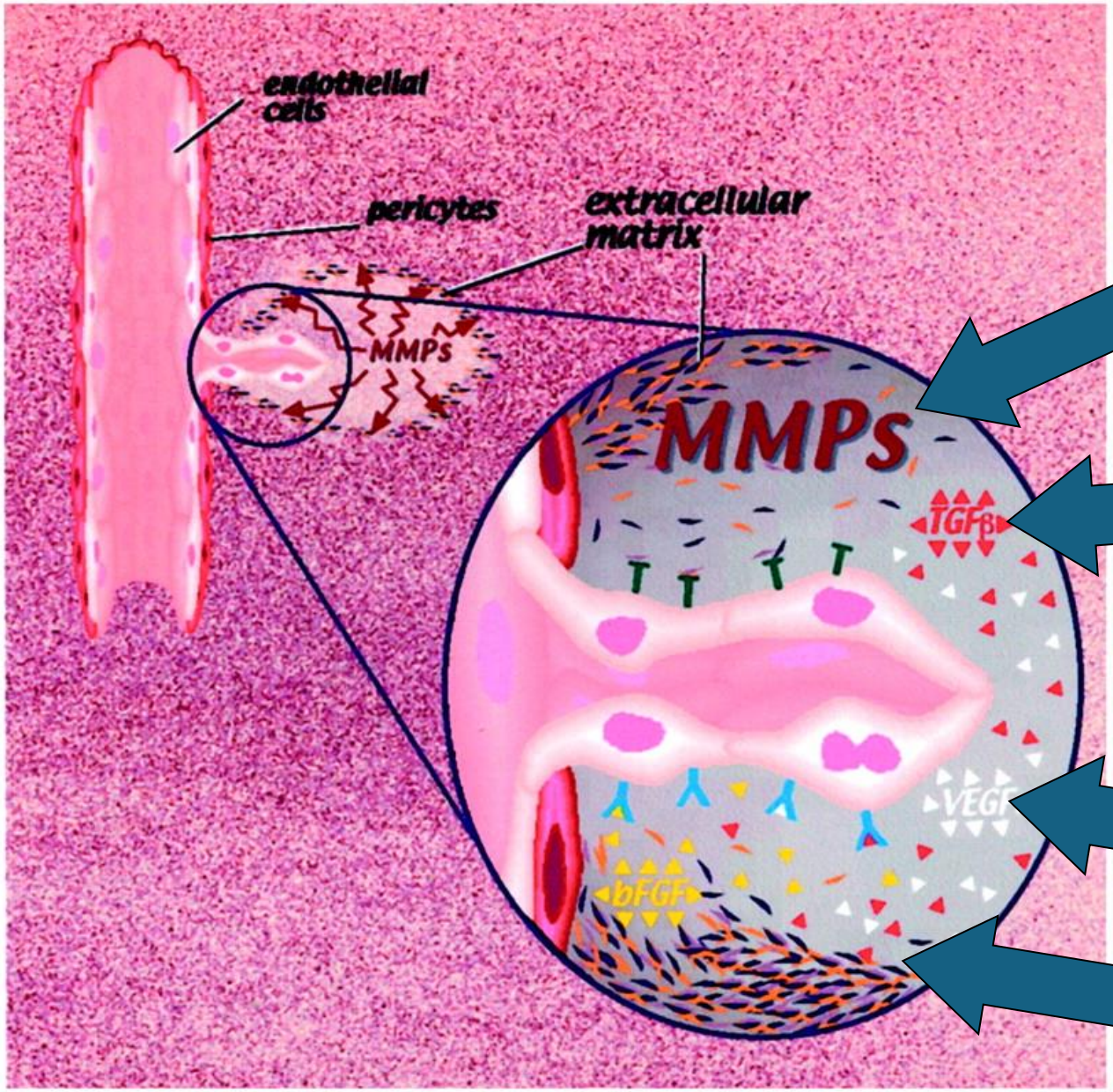
transcription factors that respond to decreases in available oxygen in the cellular environment

Hypoxia keeps cells from differentiating (stem cells) and promotes the formation of blood vessels in embryos and tumors.




Vasculogenesis
Mesenchyme cells
Angiogenetic cell cluster
Primative blood cell
Endothelial Cell
A
Angiogenesis
Smooth muscle cells
Basement membrane
Endothelial cells
Stimulus
B

Angiogenesis from existing blood vessels



MMPs

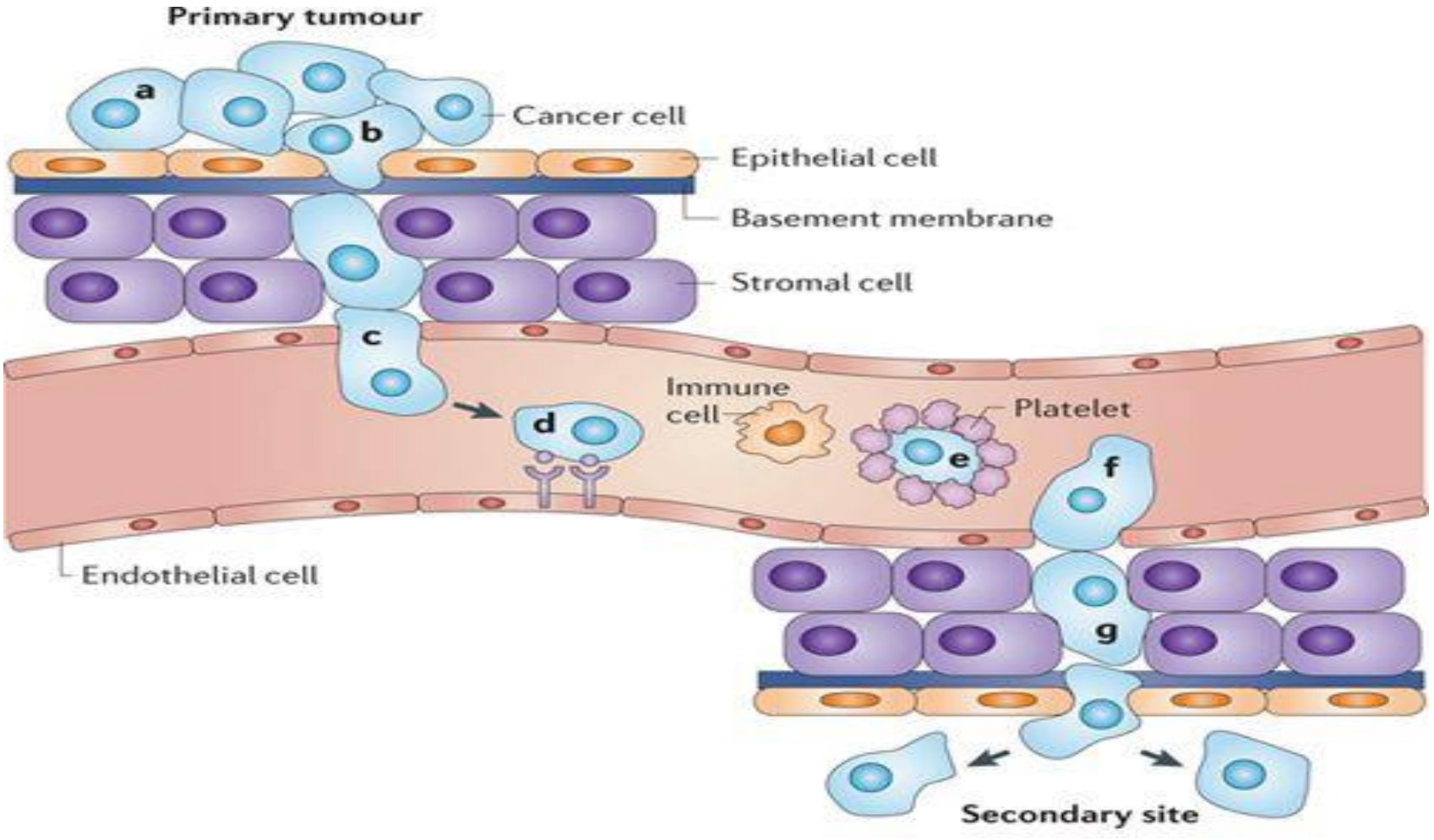
TGFb

VEGF

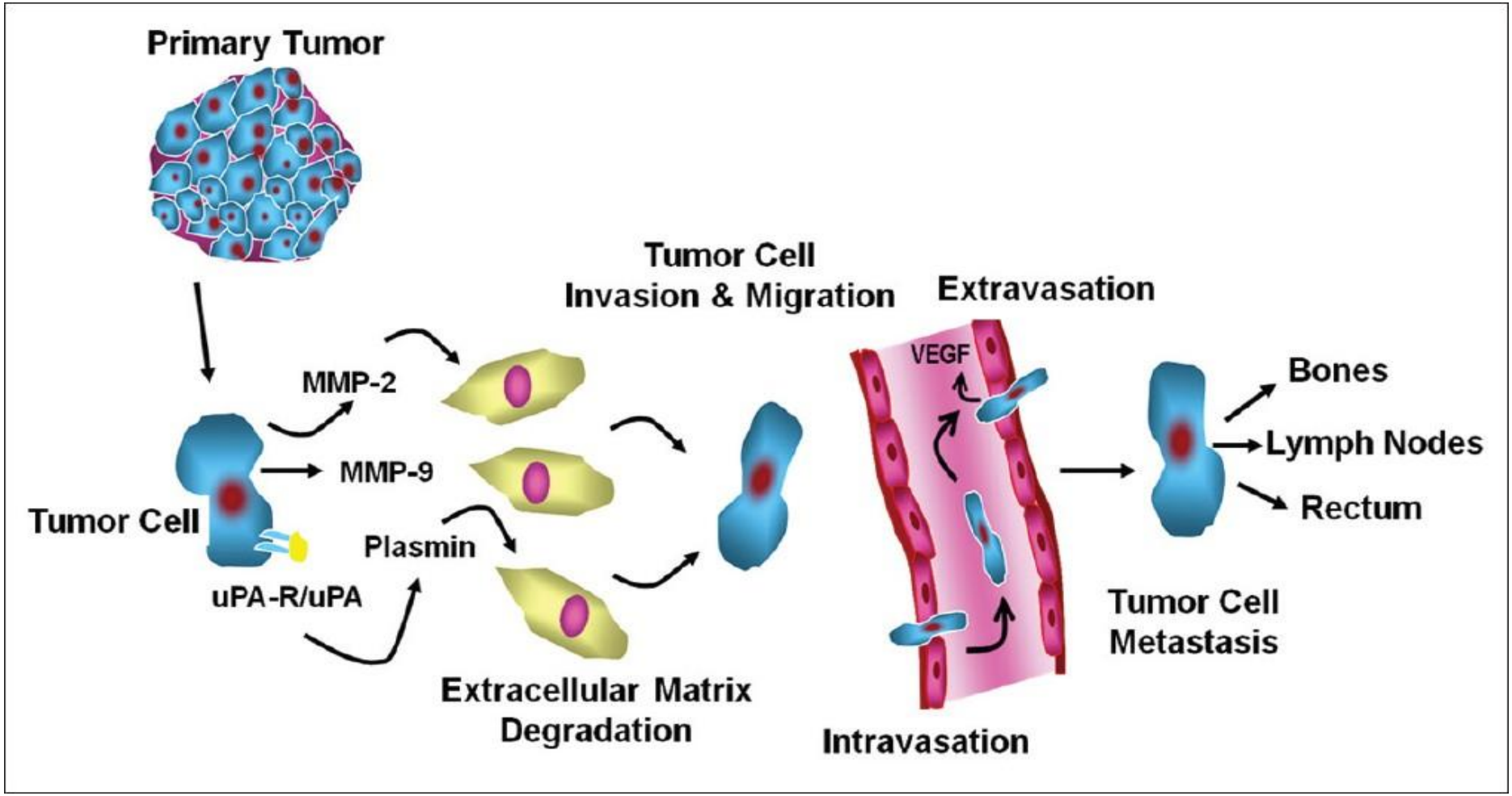
bFGF

Metastasis - the spread of cancer cells from the primary cancer to another part of the body.

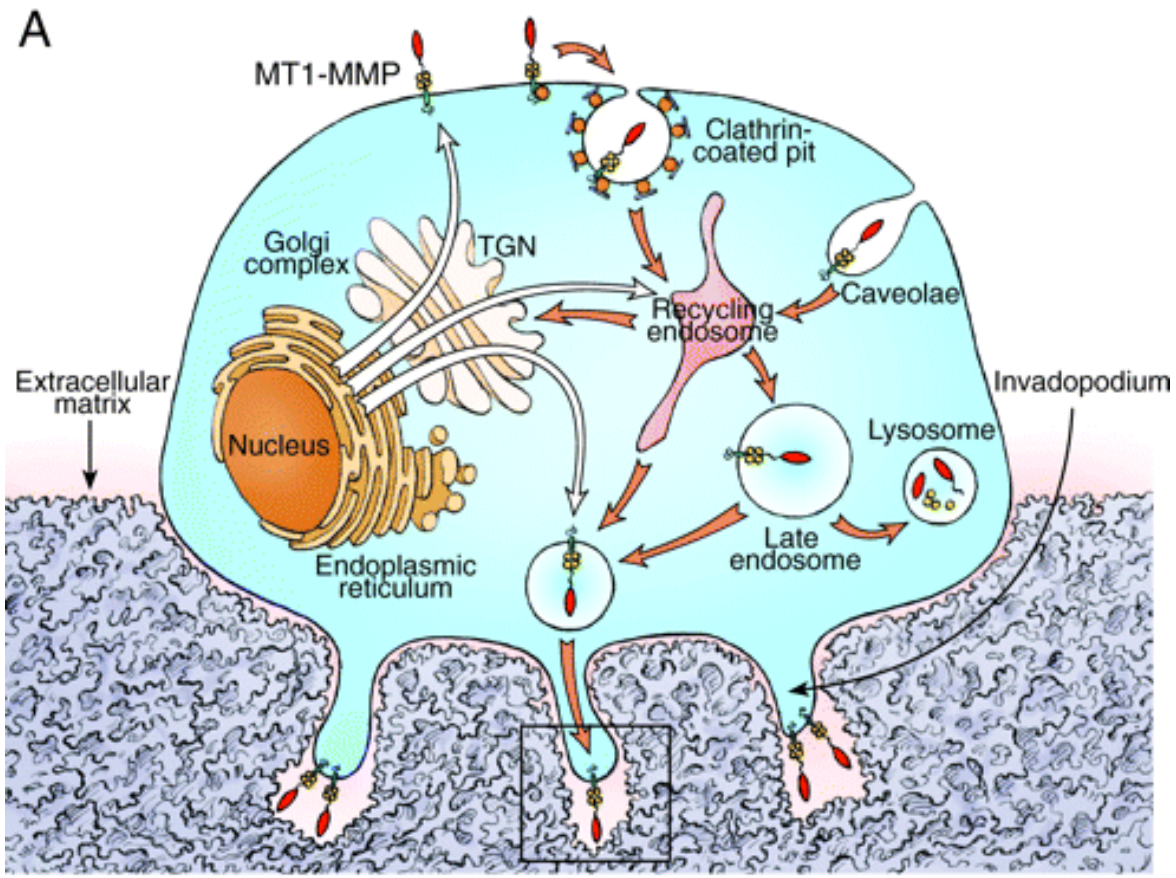
- cancer cells break away from the primary tumor, travel through the blood or lymph system, and form a new tumor in other organs or tissues of the body.



Tumor cells produce extracellular **matrix metalloproteinases (MMP)**

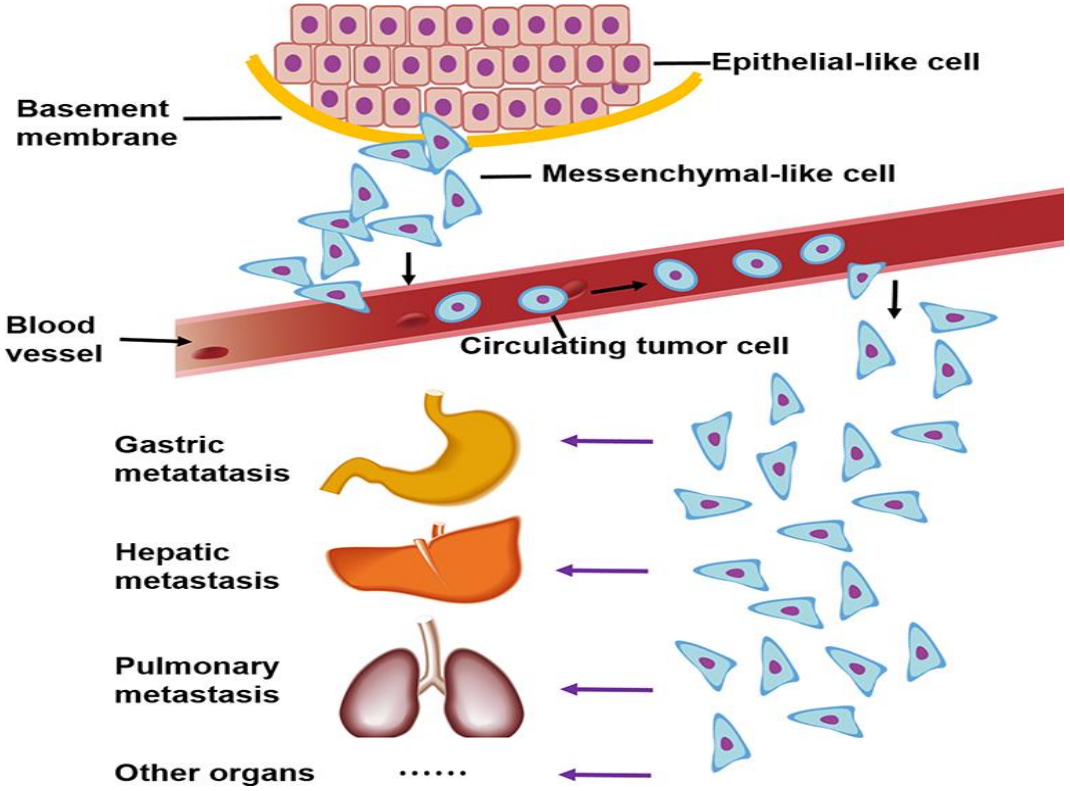


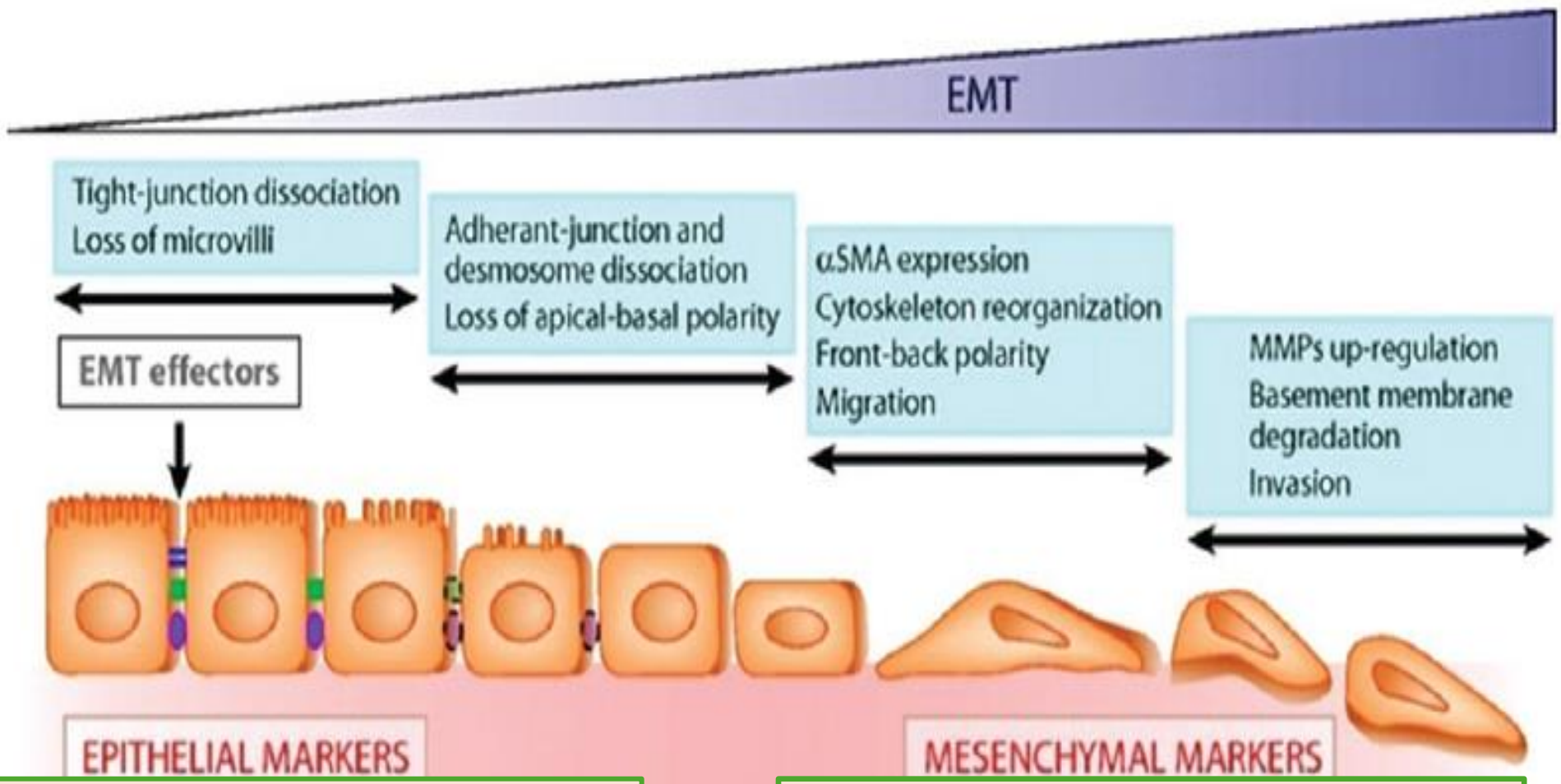
Metastasis starts with the migration of cancer cells from a primary tumor to distant tissues.



Epithelial-mesenchymal transition (EMT)

- characteristic for many metastatic cells
- highly differentiated epithelial cells are converted into undifferentiated mesenchymal cells with migration and invasive properties

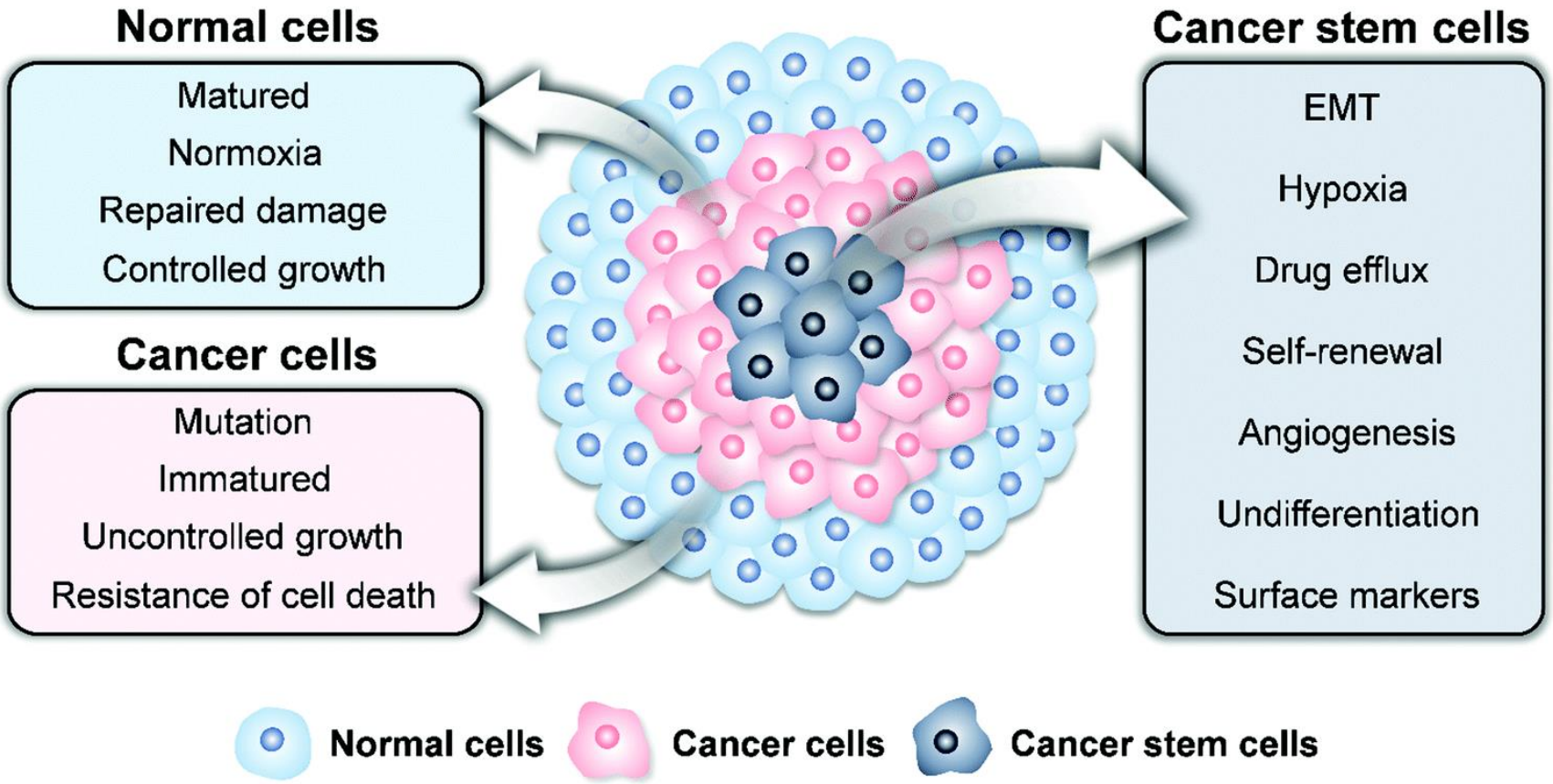


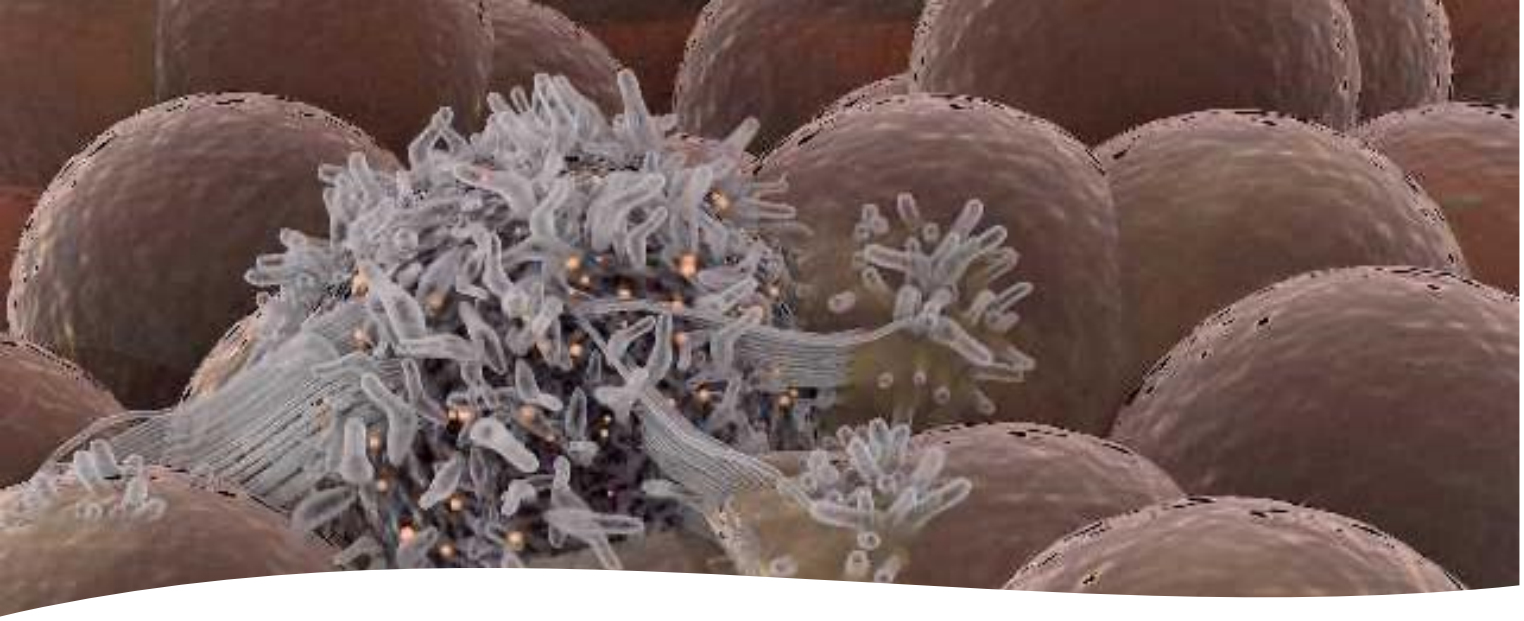


Epithelial cells – keratins, polarized - strong cell-cell connections (E-cadherins), strong cell - ECM connections (integrins) - unable to movement

Mesenchymal cells – vimentin, N-cadherins, migration, factors of EMT - EGF, FGF, HGF, TGFb, Snail/Slug/Twist/ZEB1,2 transcription factors - increase MMPs production

Stem cell theory of cancer



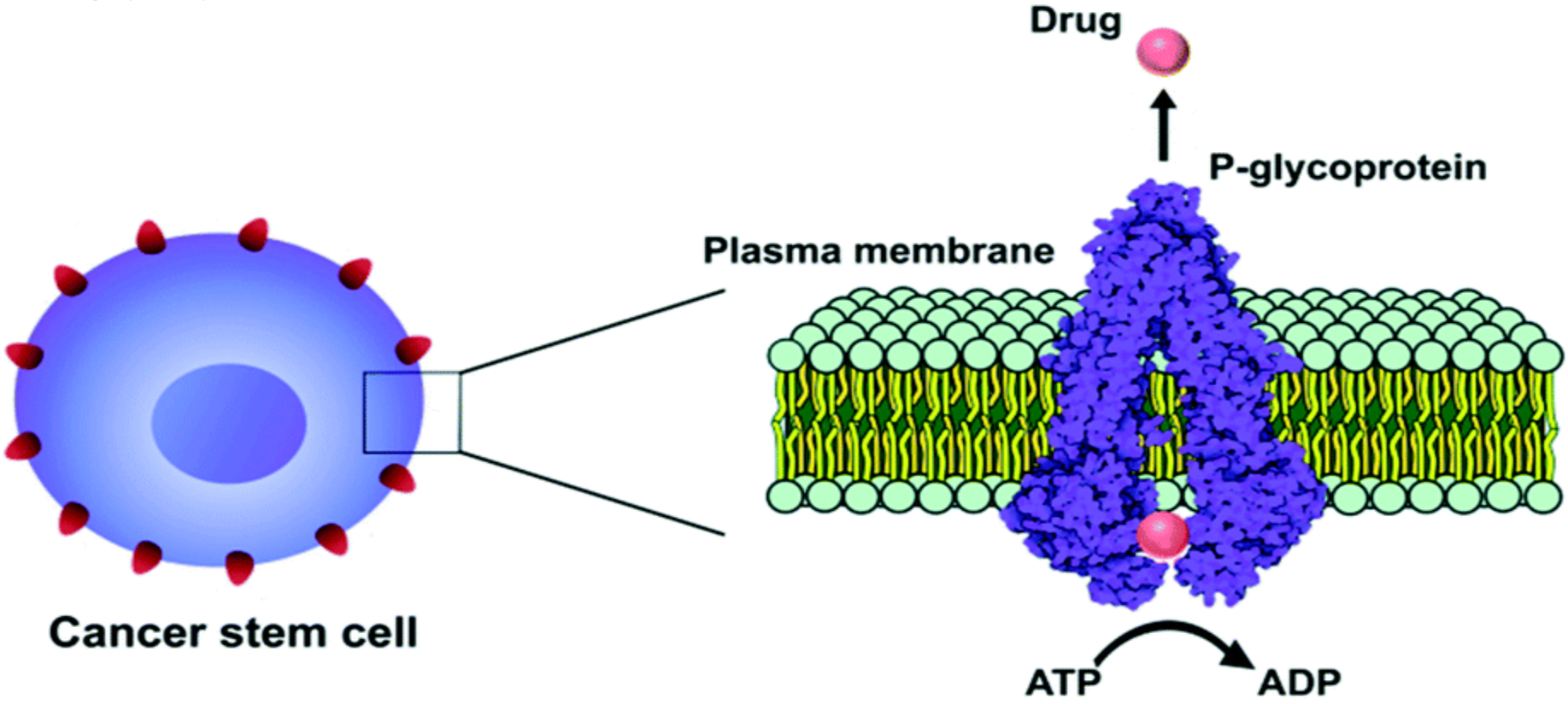


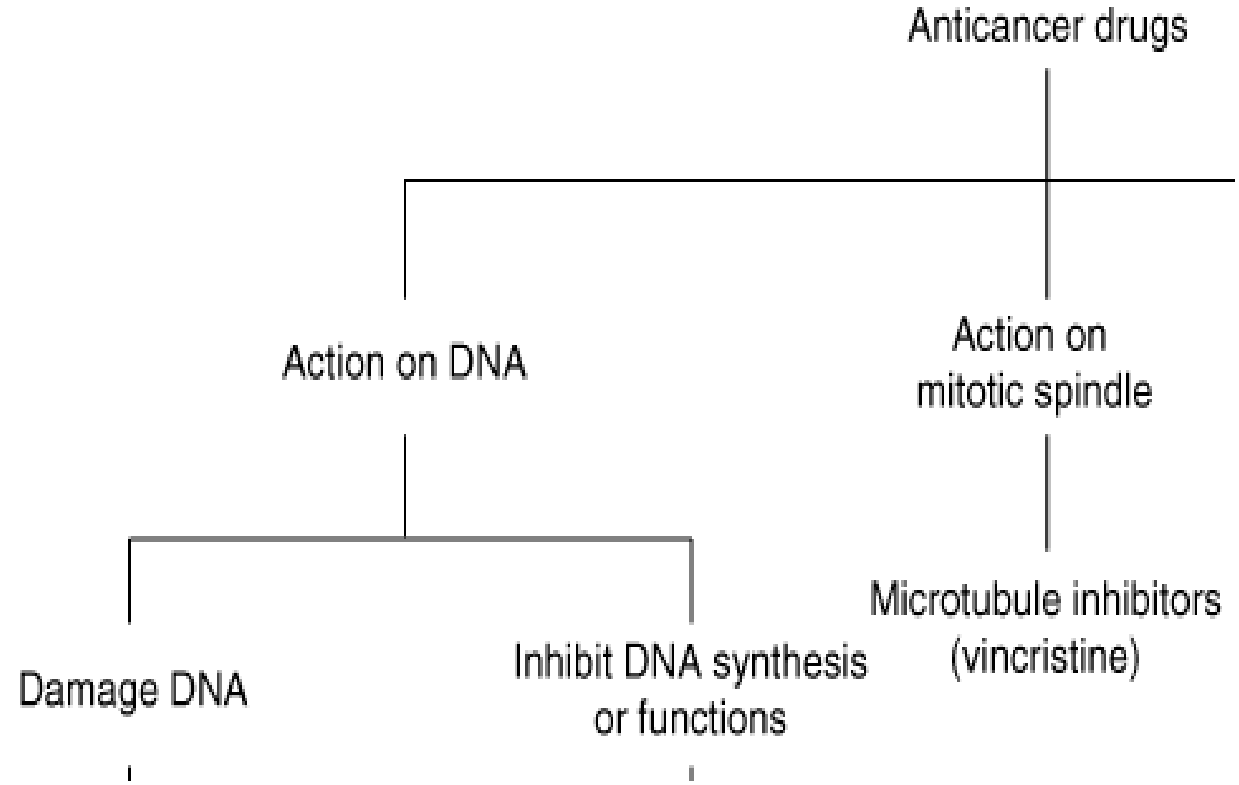
Cancer stem cells – hypothesis

- Stem cells divide very rarely, they are in the **G0 phase** of the cycle and are **difficult to kill**
- They usually **live in niches** hidden from the influence of the environment (at the bottom of crypts, deep in the hair follicle, in the recesses of the bone marrow)
- They exhibit **high expression of gp-100 – multi-drug resistance (MDR)**
- They are usually **resistant to the induction of apoptosis**

Multiple drug resistance

- the ability of cancer cells to survive and grow despite different anti-cancer therapies
- ATP-binding cassette transporters (ABC transporters) - carry molecules out of the cell against concentration gradient, using energy from ATP hydrolysis – efflux of antitumor drugs
- P-glycoprotein 1





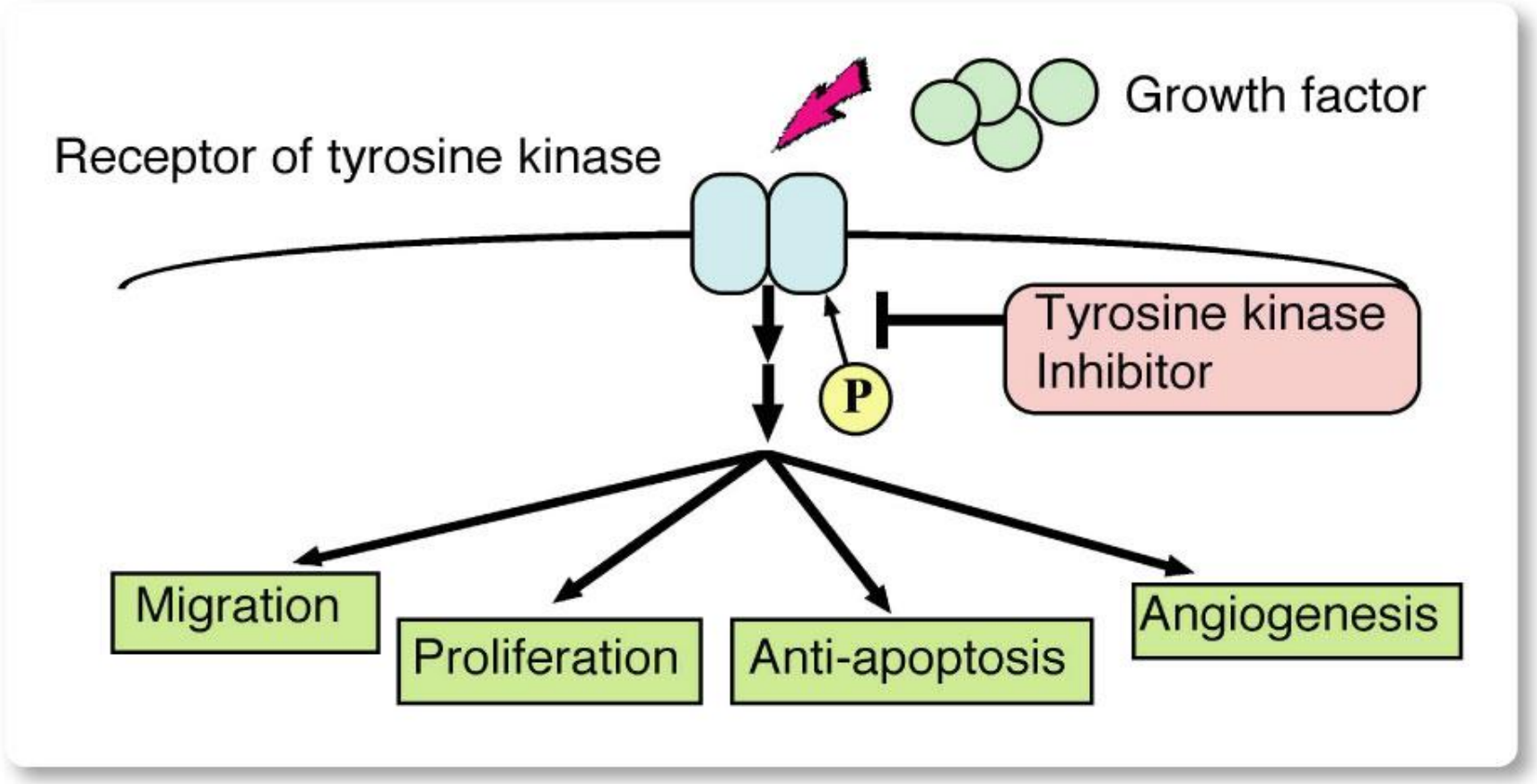
Anti-microtubule agents – inhibit microtubule function - vincristine and vinblastine, taxol

Alkylating agents - two DNA bases that are cross-linked

Antimetabolites – similar to nucleotides, in replication incorporated into DNA - Fluorouracil (5-FU)

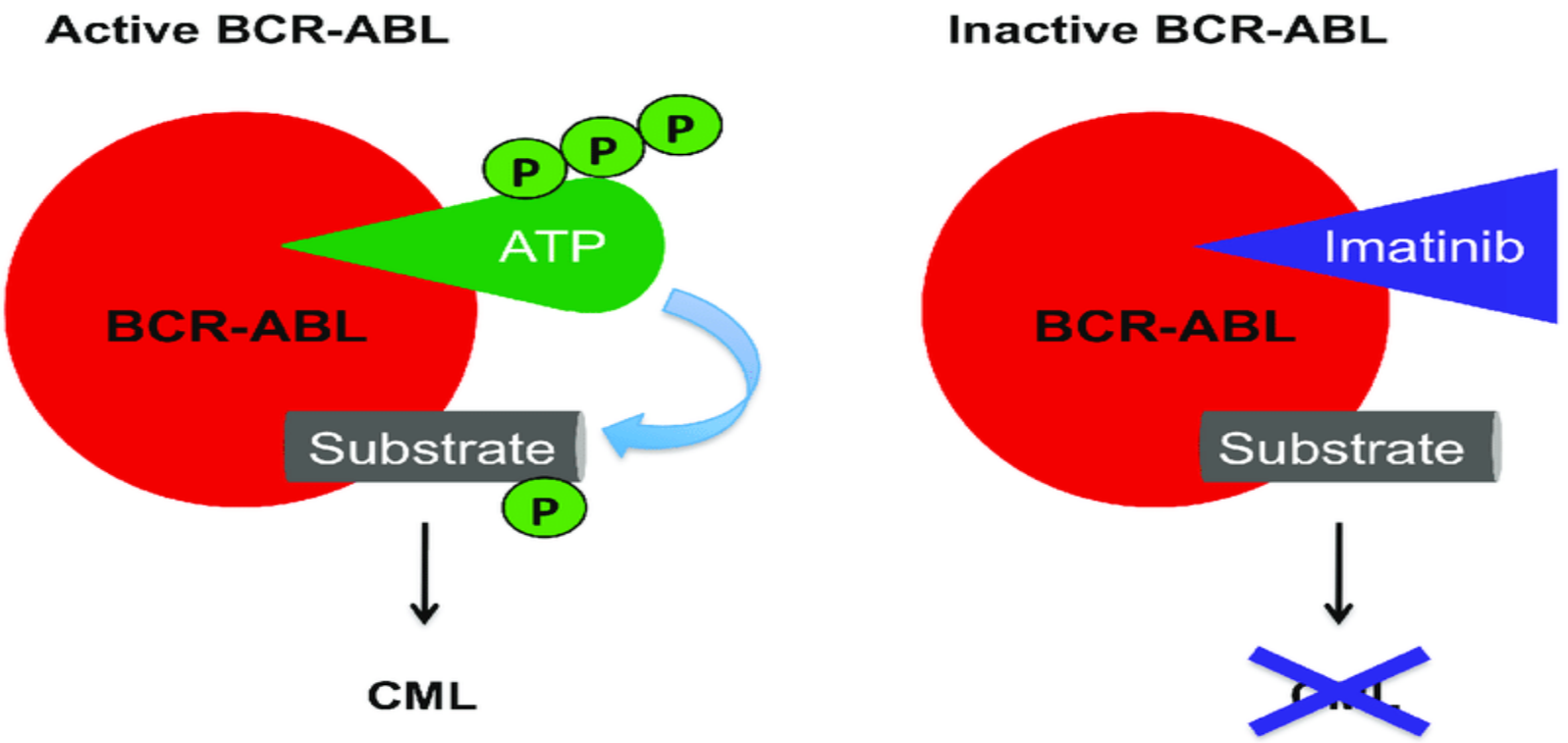
Common side effects: fatigue, nausea and vomiting, mouth sores, hair loss, low levels of several types of blood cells

New generation of anti-cancer drugs - **Tyrosine kinase inhibitors**

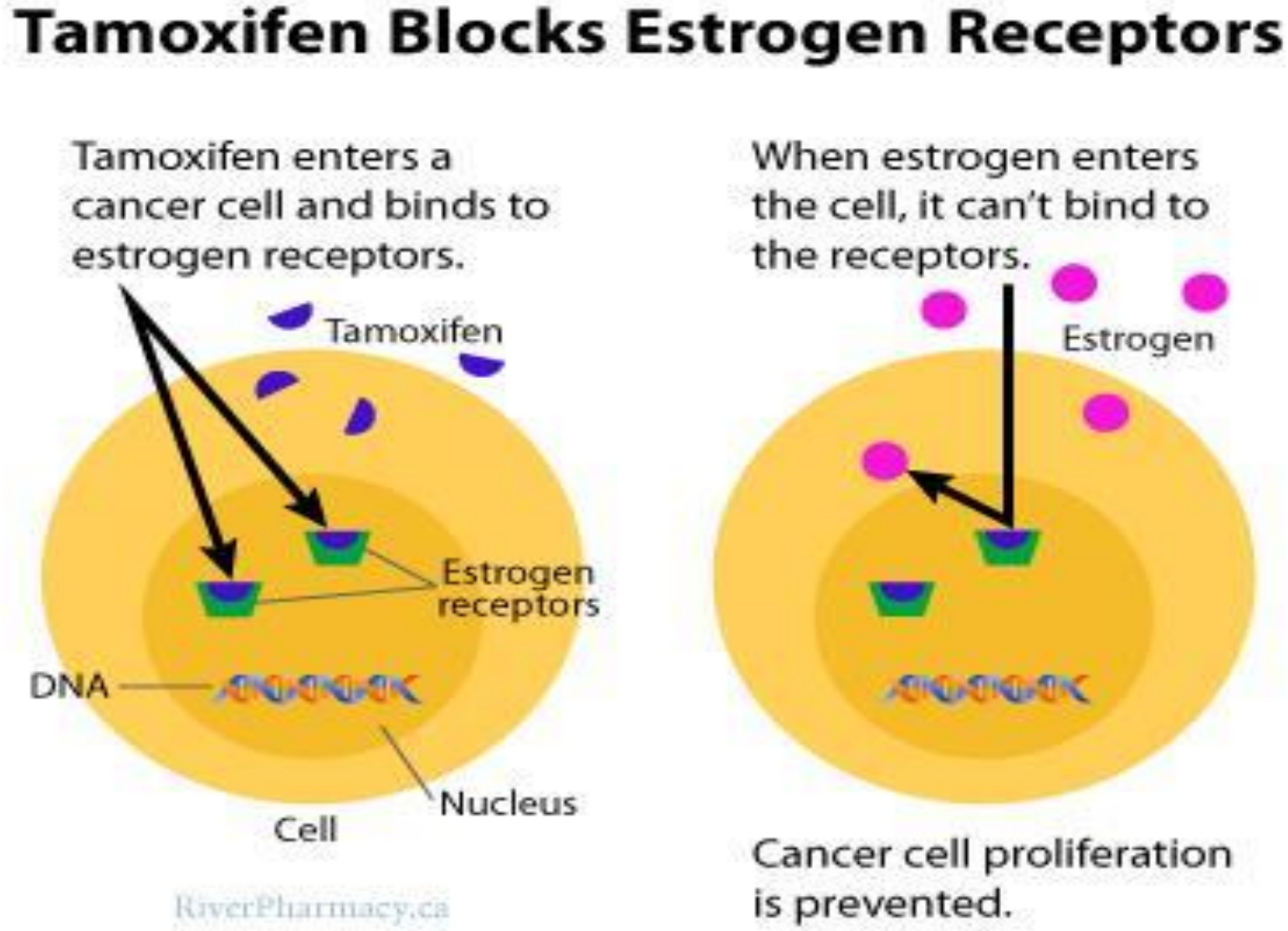


Gleevec (Imatinib) - stops the Bcr-Abl tyrosine-kinase

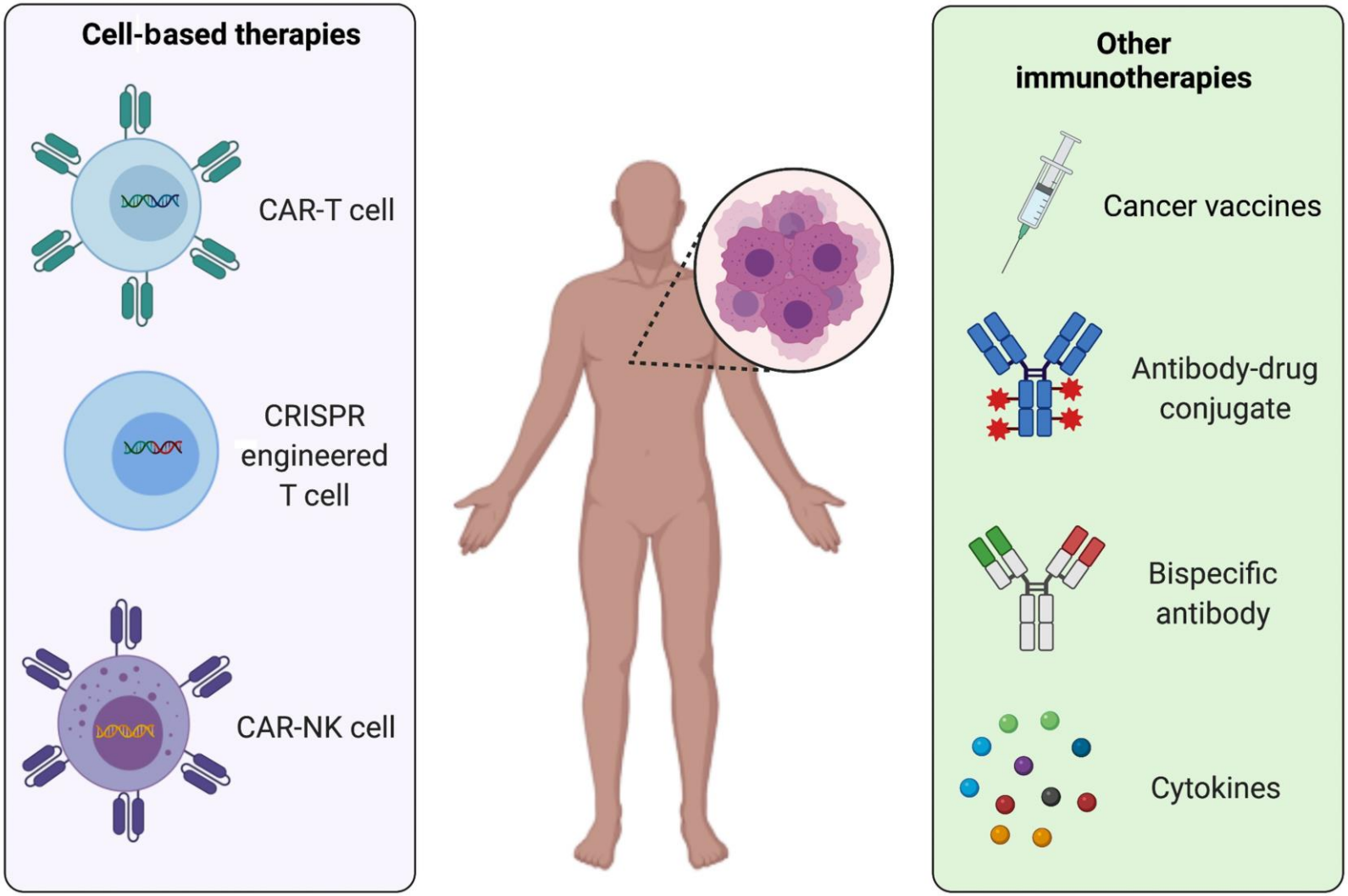
- leukemia with Philadelphia chromosome
- ATP binding is essential for BCR-ABL to phosphorylate substrates and activate pathways that promote cell survival and proliferation leading to CML. Imatinib can bind to the inactive BCR-ABL at its ATP binding site, and inhibit the phosphorylation of substrates



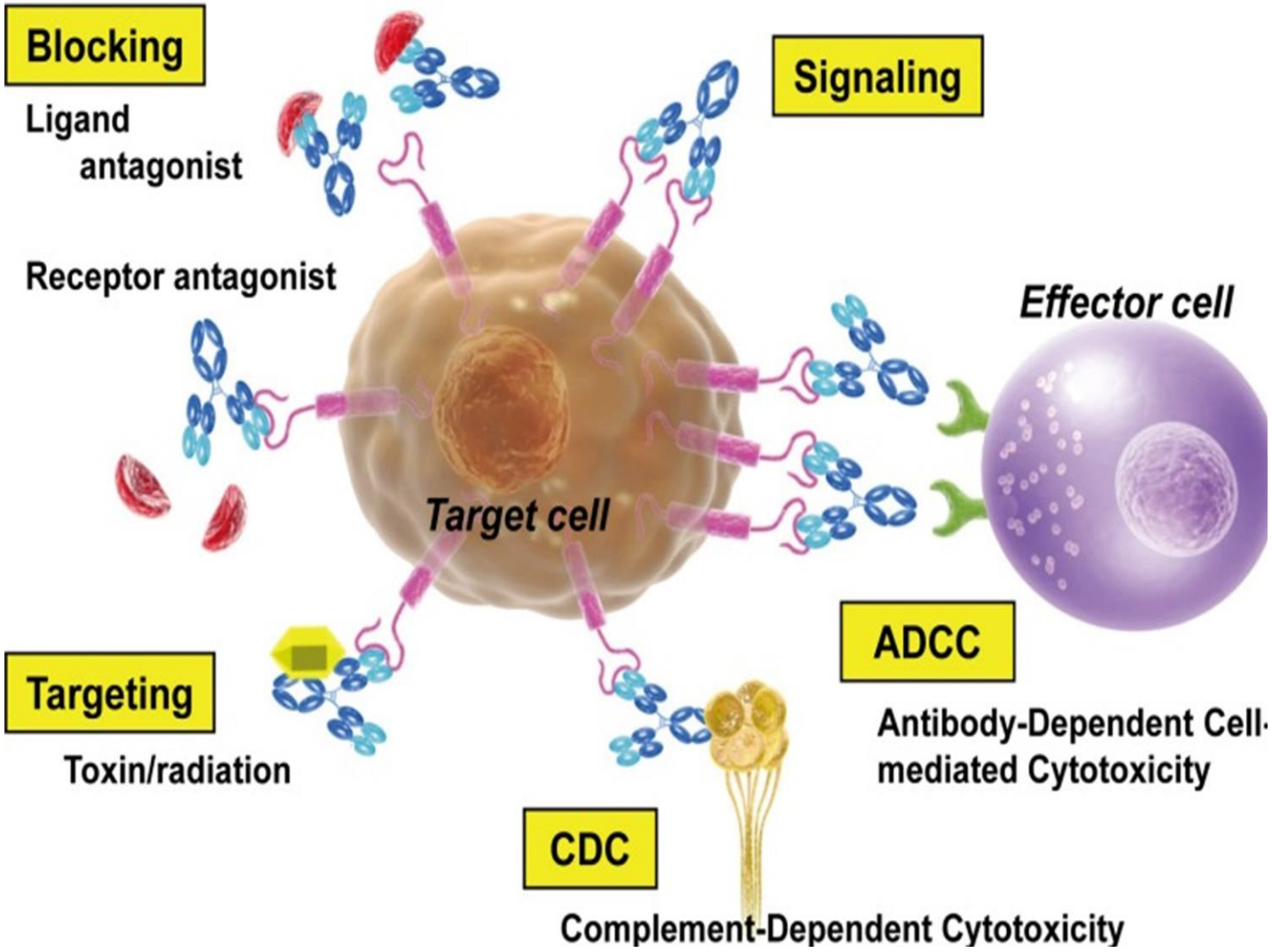
Estrogen receptor inhibitor in breast cancer – tamoxifen
Letrozole – Letrozole - aromatase inhibitor, postmenopausal women



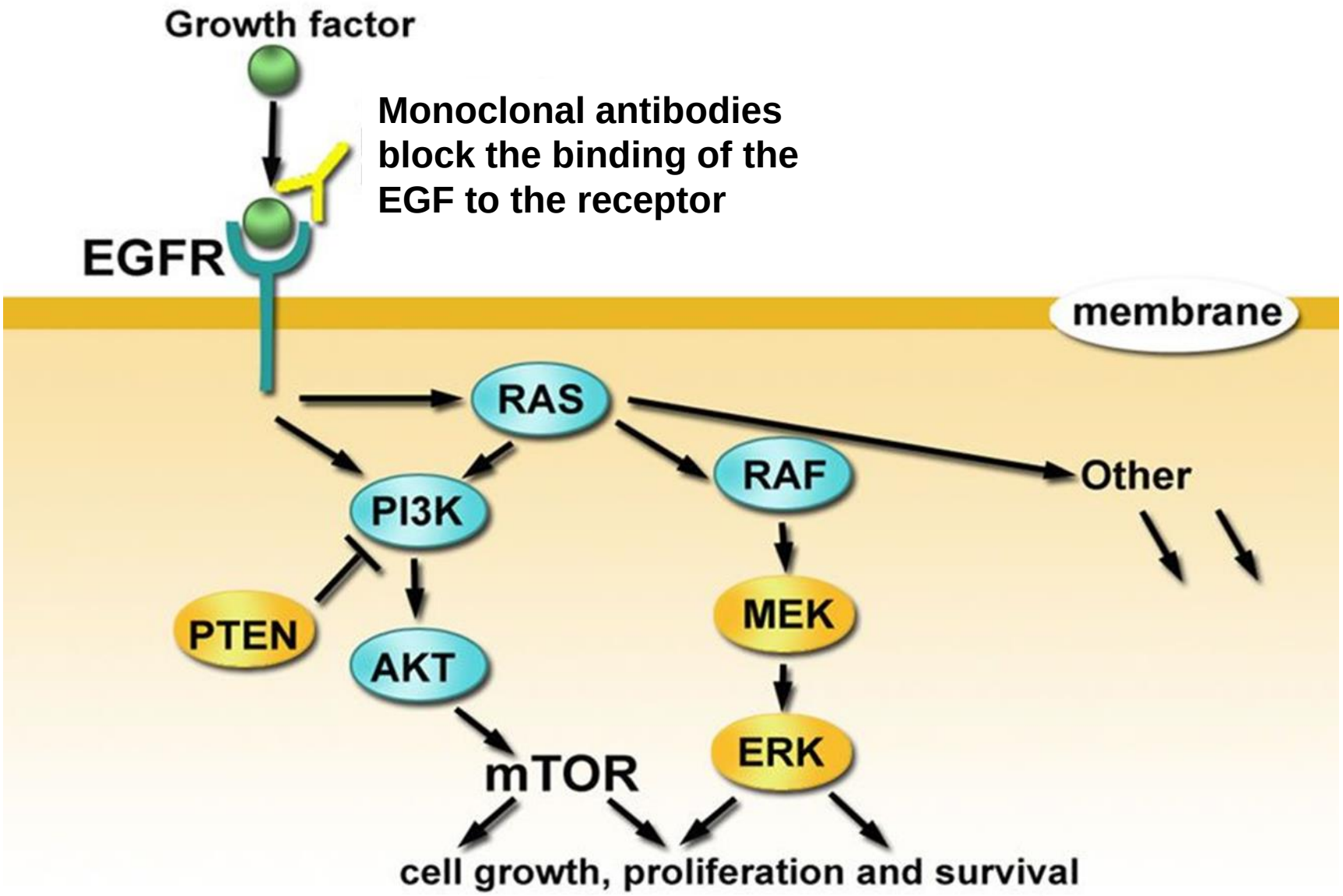
Approaches for cancer immunotherapy



Monoclonal antibodies



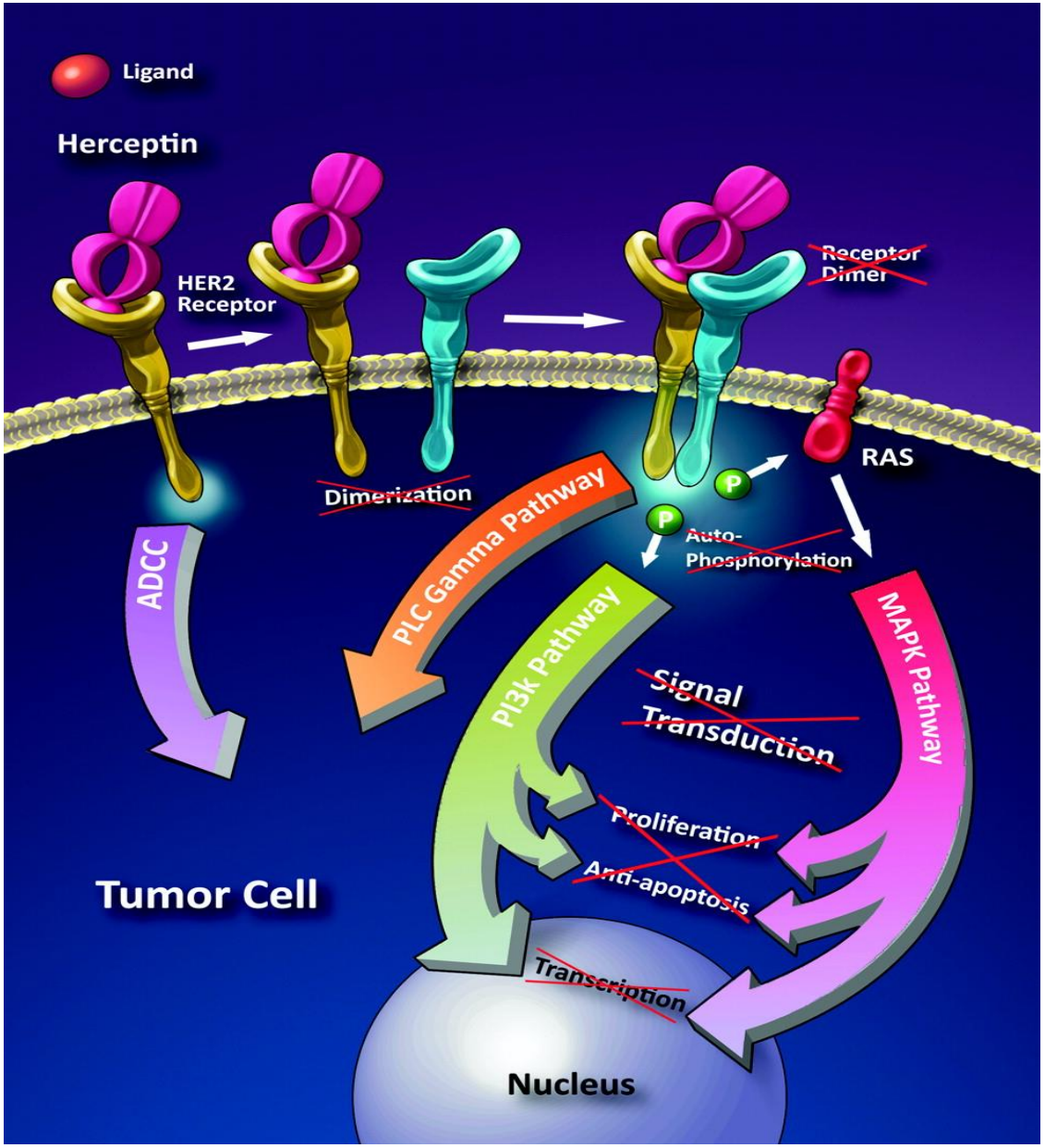
Epidermal growth factor receptor (EGFR) inhibitor, colorectal cancer, lung cancer



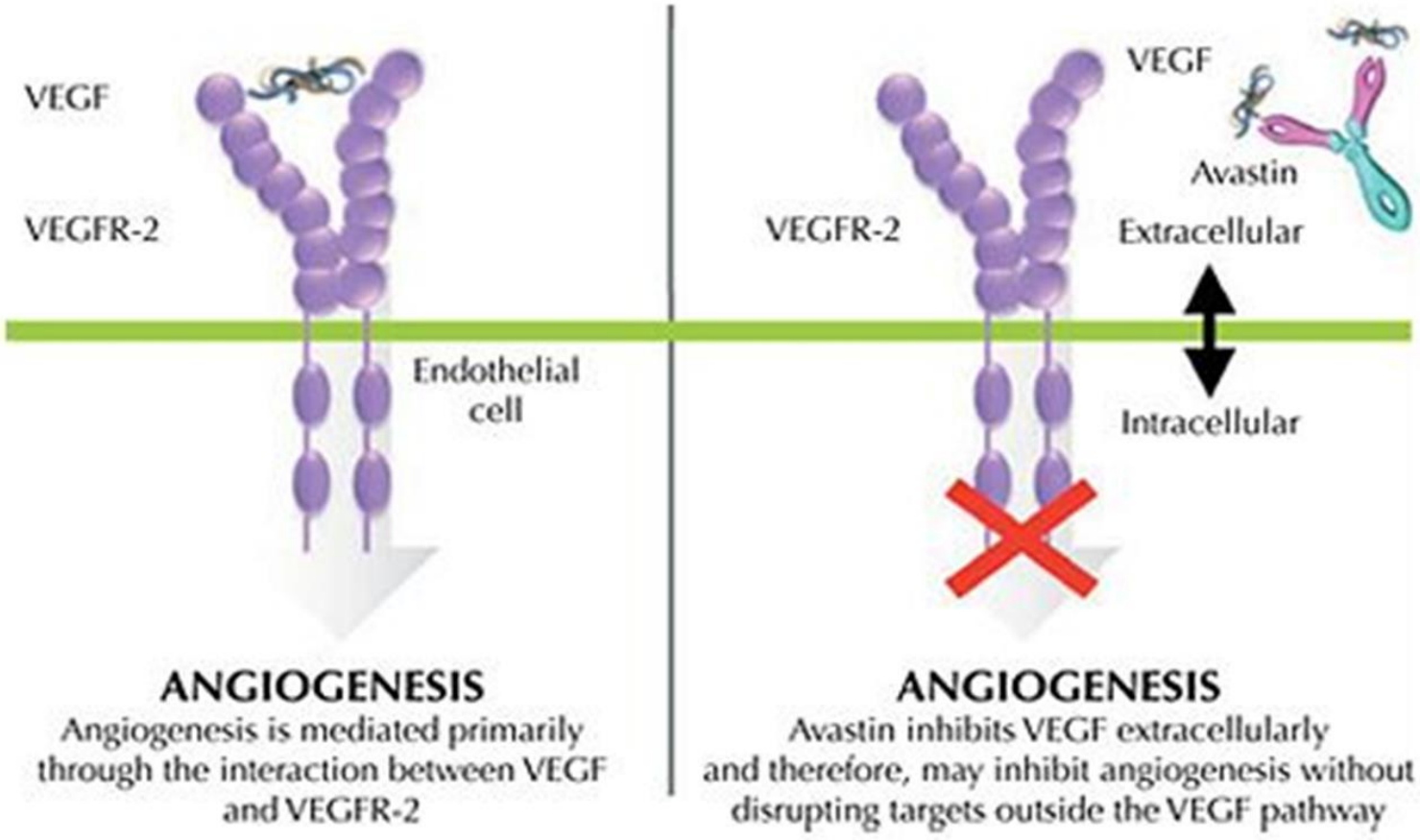
Breast cancer

Human epidermal receptor 2 (HER2) positive breast cancer (amplified gene for HER-2) - the most aggressive subtype of breast cancer

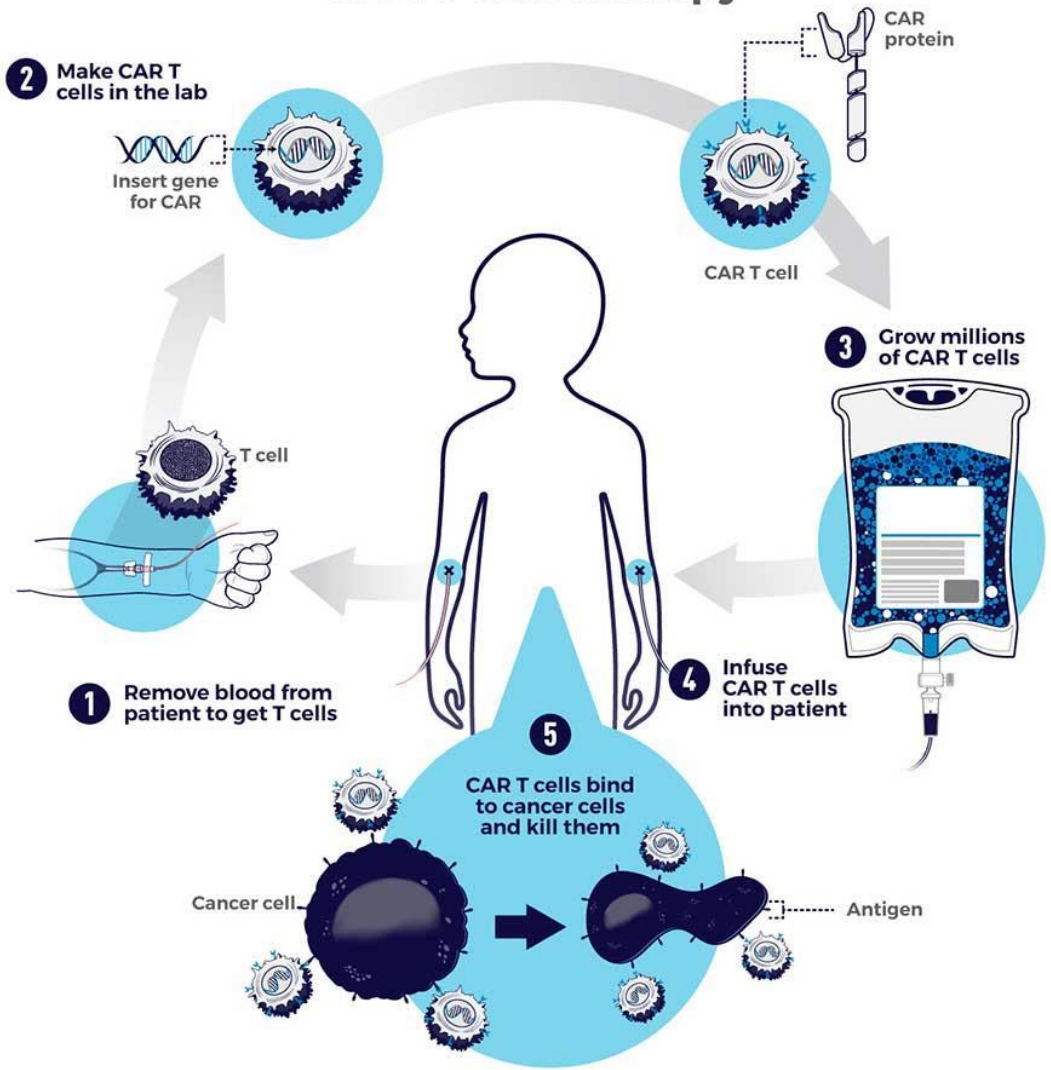
Herceptin (Trastuzumab)



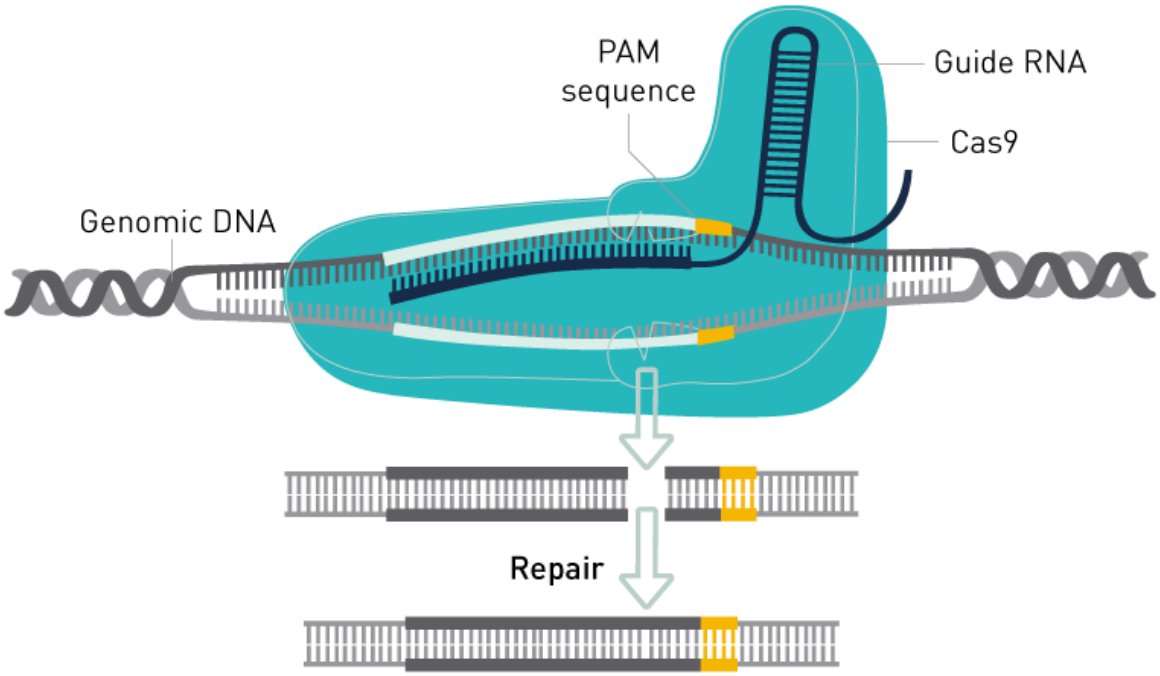
Anti-angiogenic targets for monoclonal antibody - cancer therapy
- anti-VEGF antibody - suppress tumor angiogenesis - colon cancer, lung cancer, glioblastoma



CAR T-Cell Therapy



CAR T-cell therapy is a type of treatment in which a patient's T cells are genetically engineered in the laboratory so they will bind to specific proteins (antigens) on cancer cells and kill them. **(1)** A patient's T cells are removed from their blood. Then, **(2)** the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T cells in the laboratory. The gene encodes the engineered CAR protein that is expressed on the surface of the patient's T cells, creating a CAR T cell. **(3)** Millions of CAR T cells are grown in the laboratory. **(4)** They are then given to the patient by intravenous infusion. **(5)** The CAR T cells bind to antigens on the cancer cells and kill them.



Component	Function
crRNA	Contains the guide RNA that locates the correct segment of host DNA along with a region that binds to tracrRNA (generally in a hairpin loop form), forming an active complex.
tracrRNA	Binds to crRNA and forms an active complex.
sgRNA	Single-guide RNAs are a combined RNA consisting of a tracrRNA and at least one crRNA.
Cas9 (most commonly)	An enzyme whose active form is able to modify DNA. Many variants exist with different functions (i.e. single-strand nicking, double-strand breaking, DNA binding) due to each enzyme's DNA site recognition function.
Repair template	DNA molecule used as a template in the host cell's DNA repair process, allowing insertion of a specific DNA sequence into the host segment broken by Cas9.