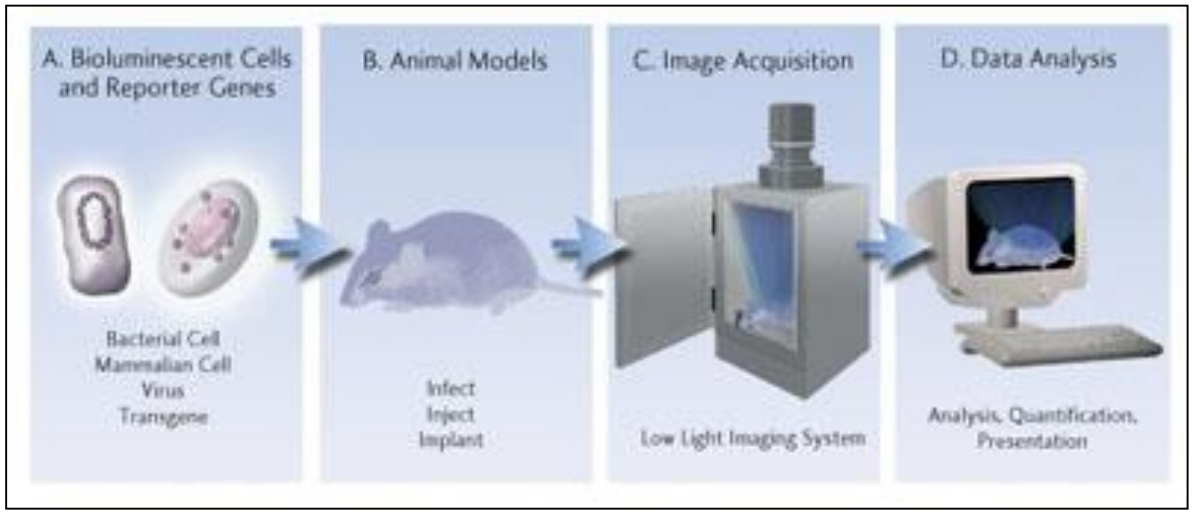
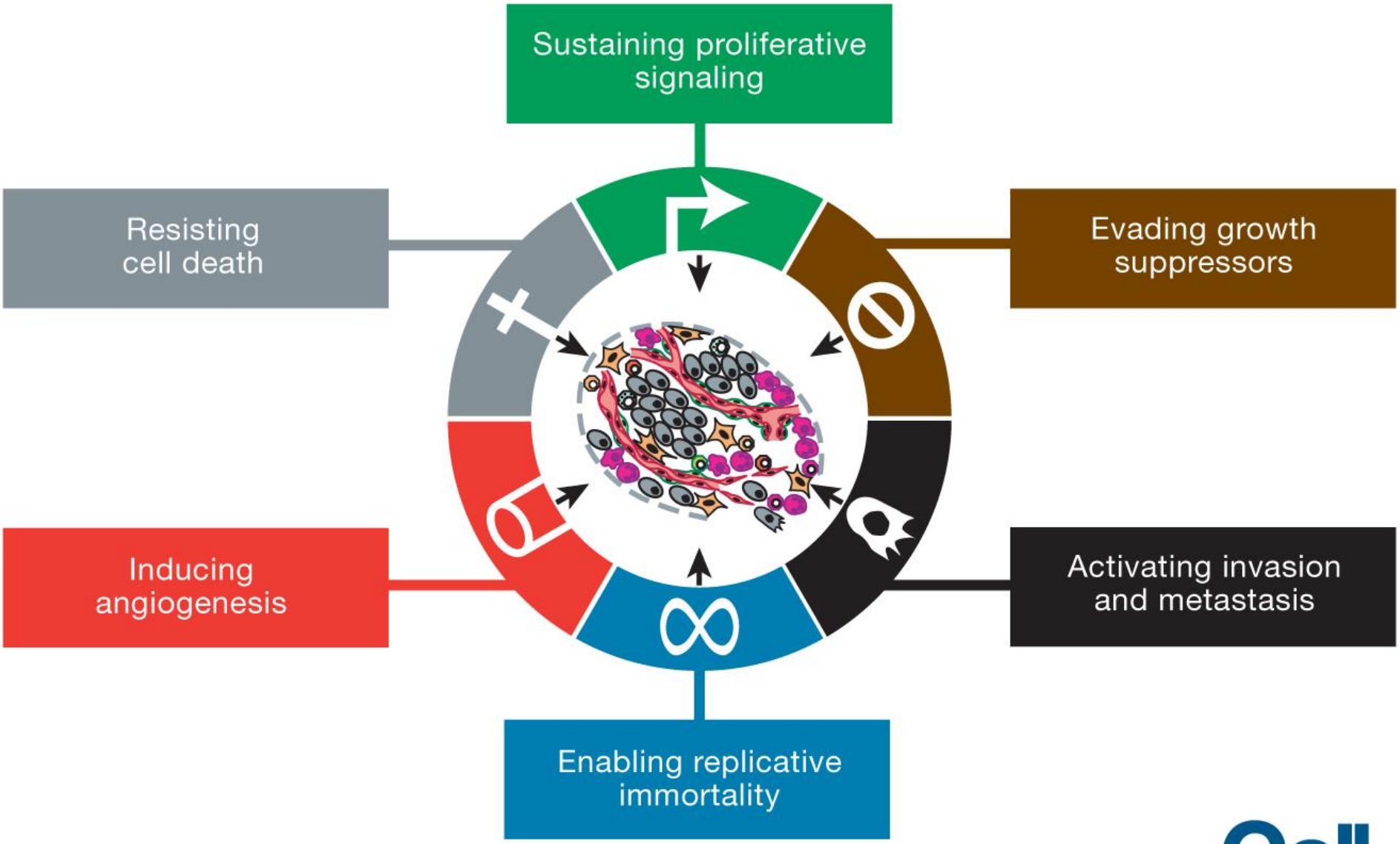


OCT5715 - Modelos Experimentais em Oncologia: Aula 1

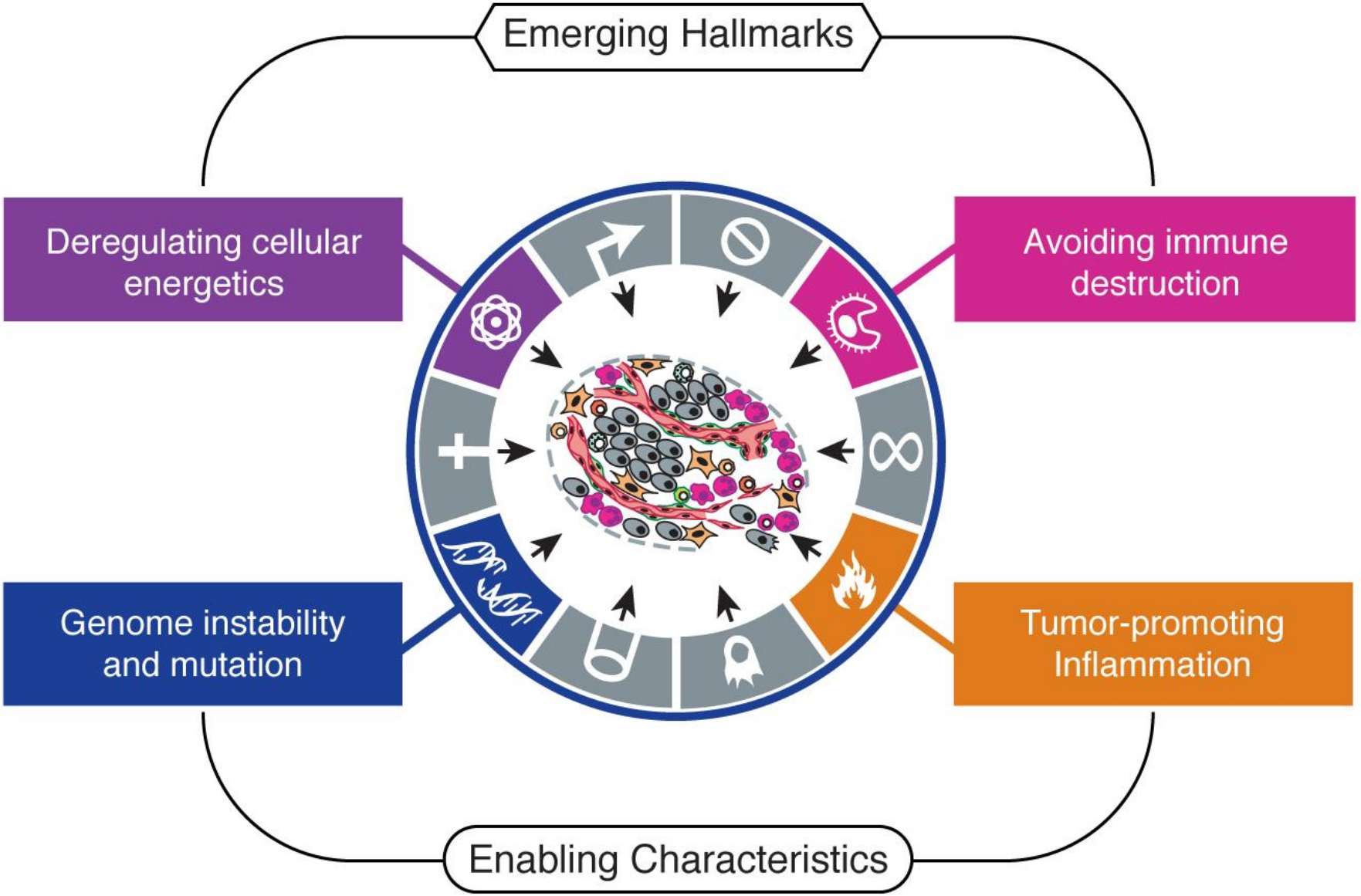
Prof. Dr. Harley Francisco de Oliveira

FMRP-USP



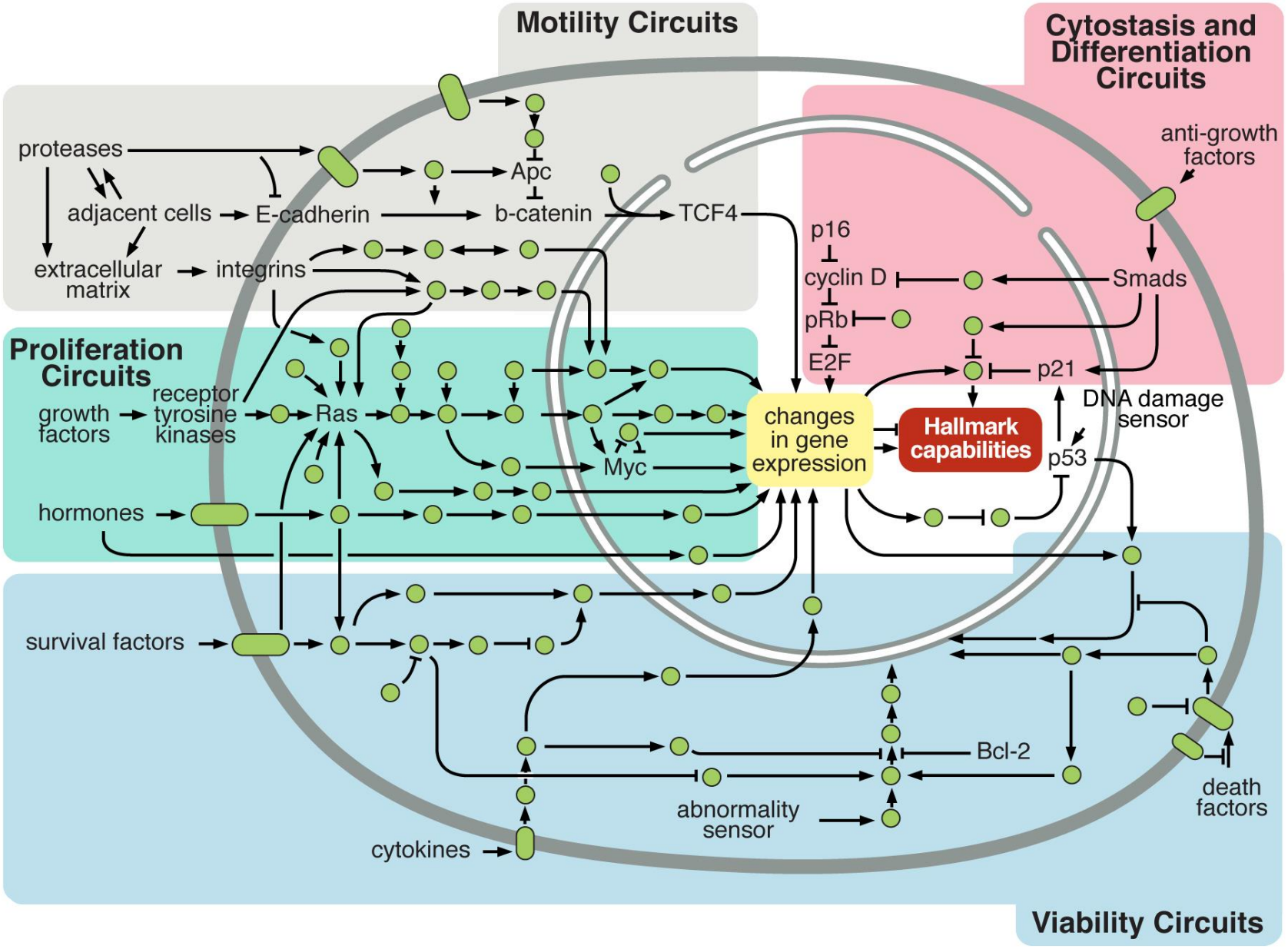
Hanahan and Weinberg, 2011

Cell PRESS



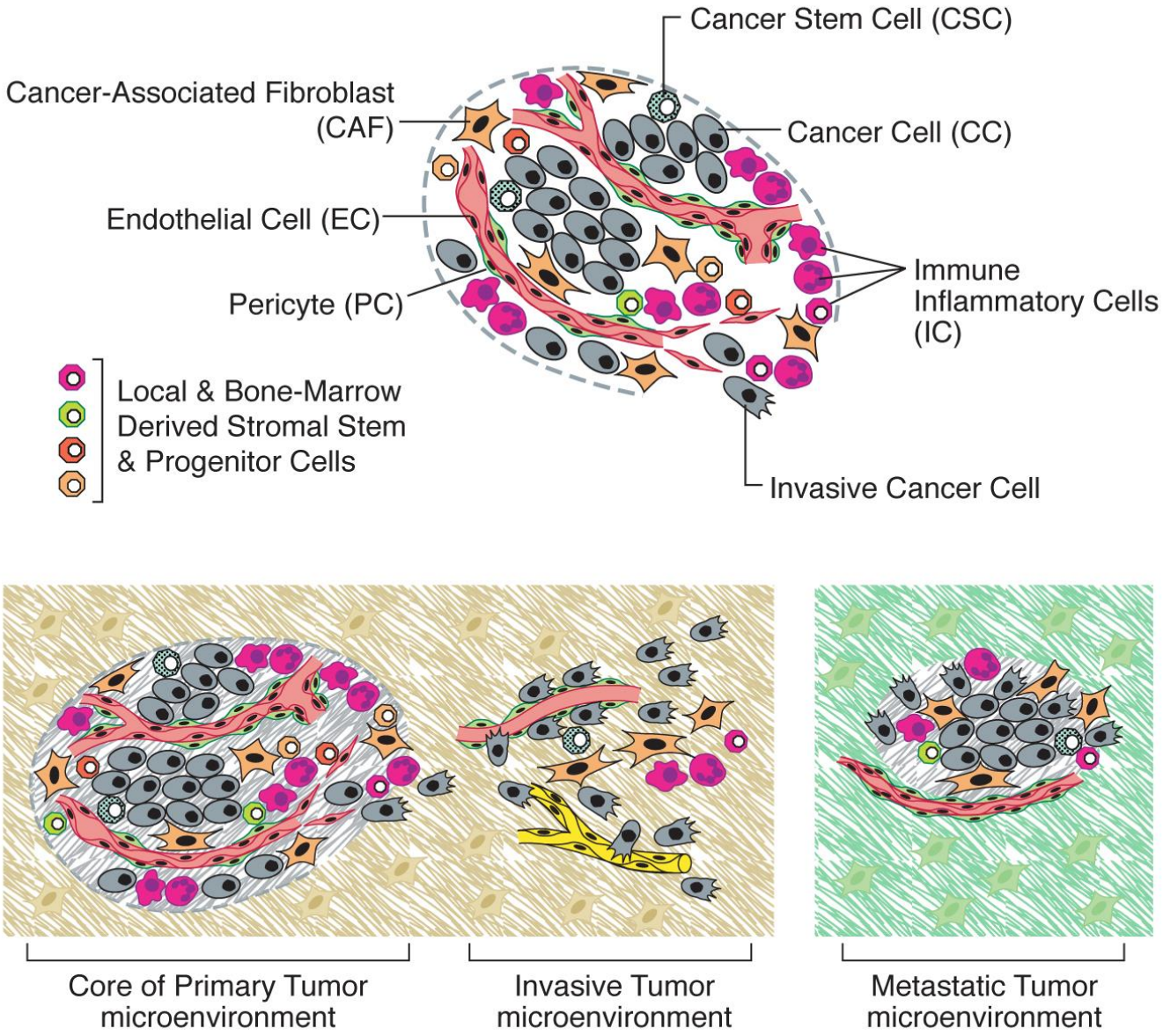
Hanahan and Weinberg, 2011

Cell PRESS



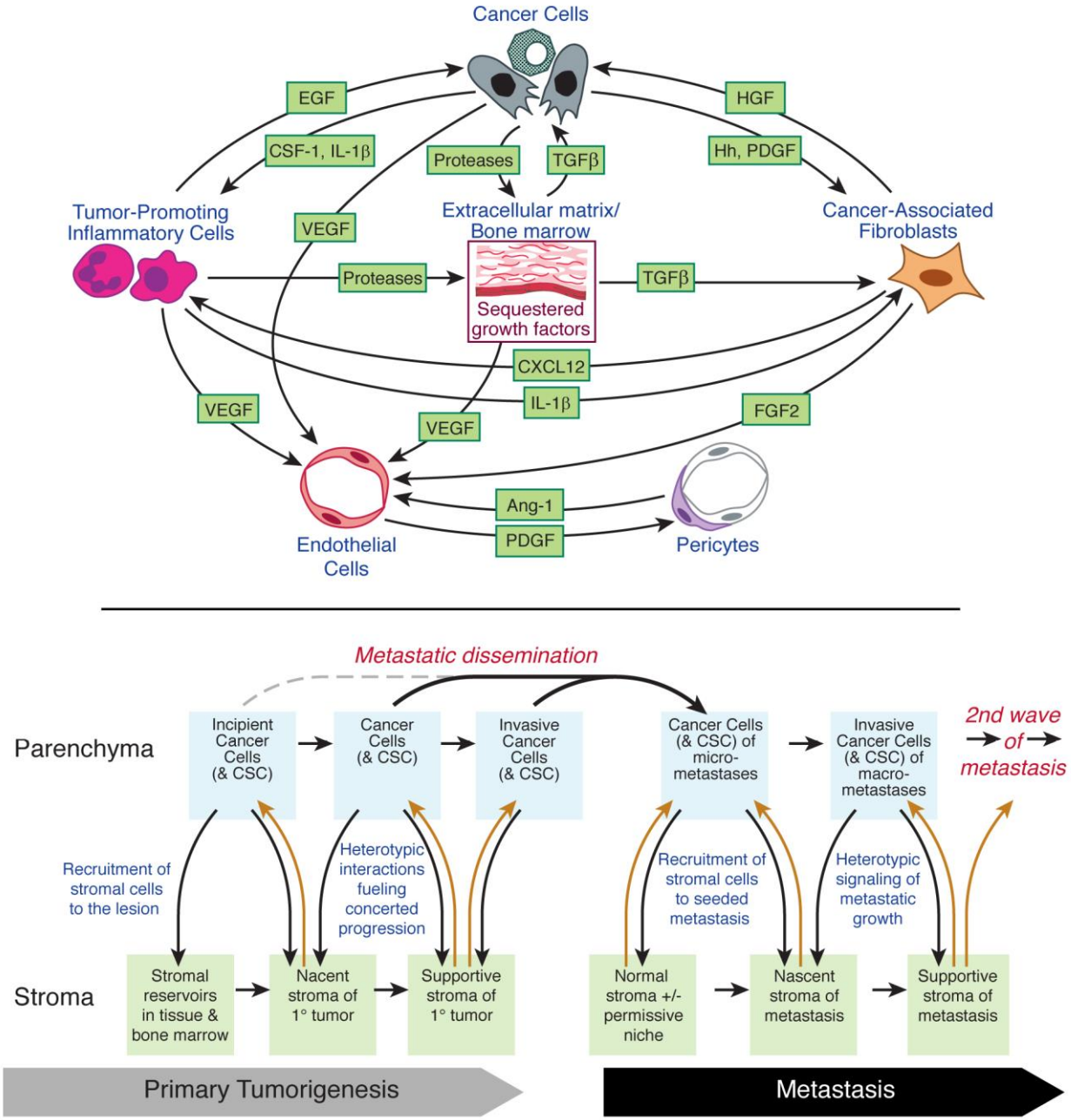
Hanahan and Weinberg, 2011

Cell PRESS



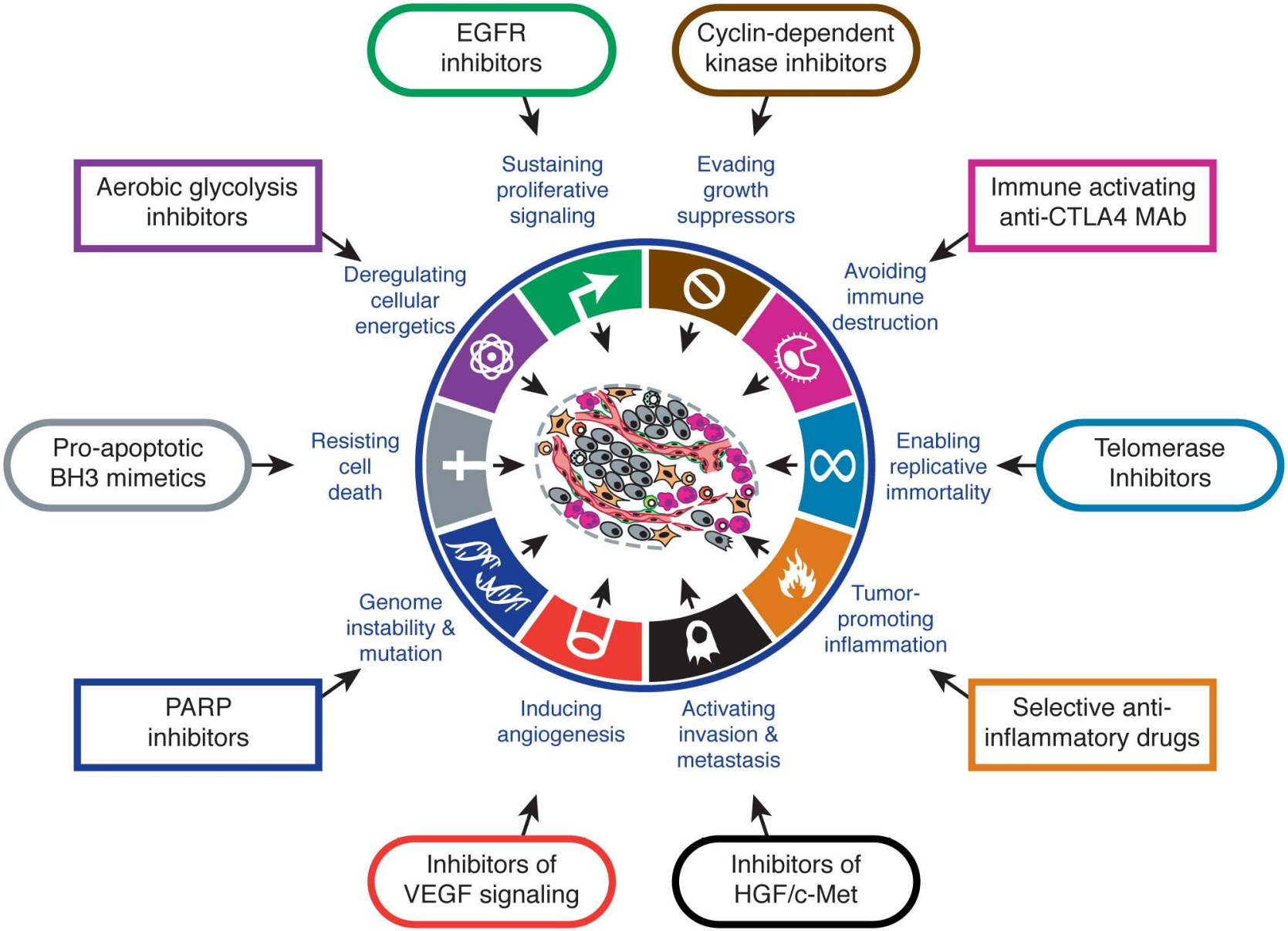
Hanahan and Weinberg, 2011

Cell PRESS



Hanahan and Weinberg, 2011

Cell PRESS



Hanahan and Weinberg, 2011

Sustaining proliferative signaling

- Growth signaling in normal cells is a highly regulated process where in proliferative signals are activated whenever necessary and deactivated when no longer necessary; this tight regulation ensures cell homeostasis. However, in cancer cells this regulation is compromised.

Sustaining proliferative signaling

- Increasing growth factor production;
- Stimulating normal cells in the microenvironment to provide cancer cells with growth factors;
- Increasing the number of receptors on the cell surface;
- Structurally altering receptors to facilitate cancer cell signaling;
- Activating proteins in the downstream signaling pathway.

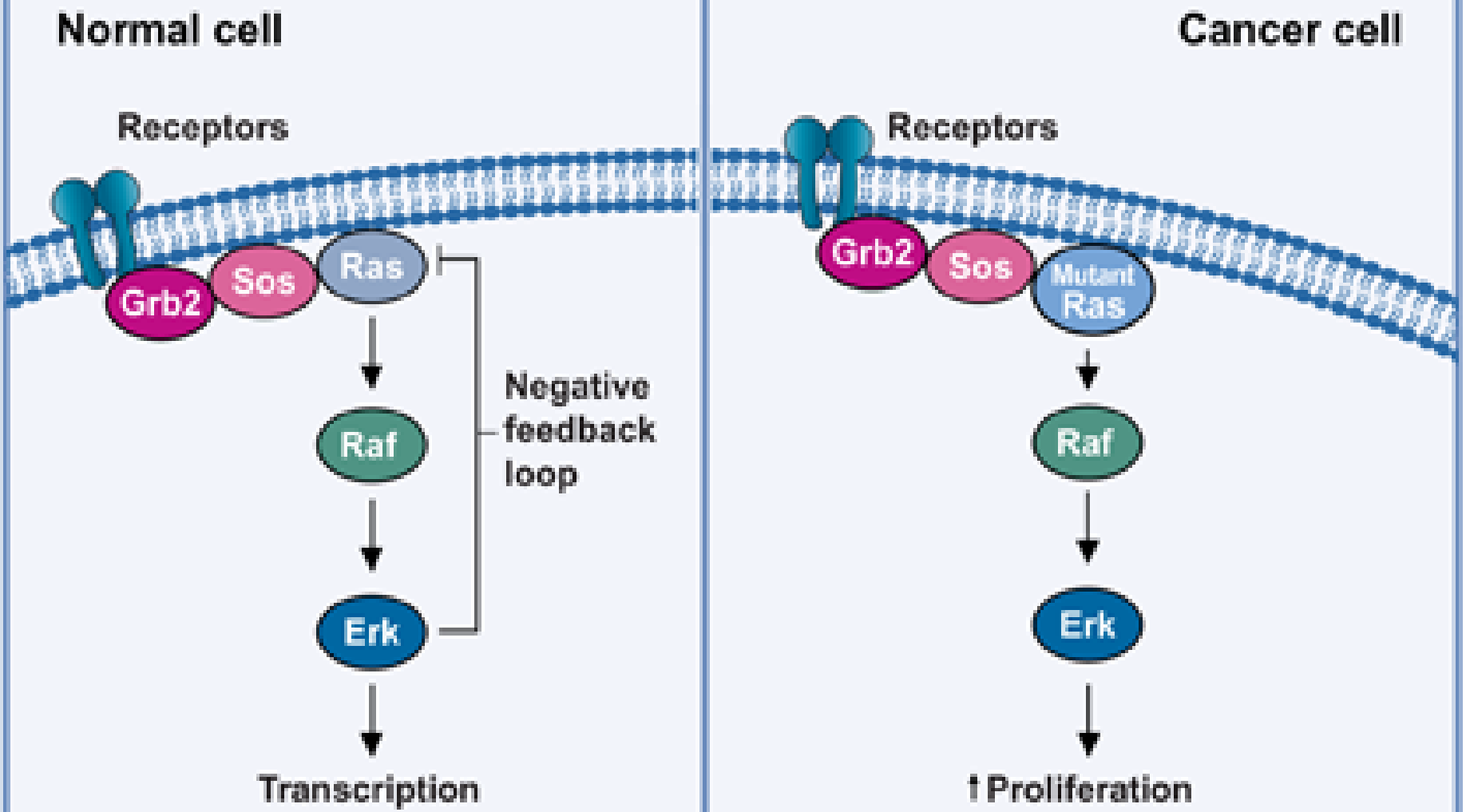
Sustaining proliferative signaling

- Cancer cells to disrupt negative feedback loops - dampen a signaling pathway whenever a mitogenic signal is hyperactivated. ex: RAS oncoprotein and PTEN.
- Oncogenic activity of RAS is not the result of overactive RAS signaling but rather the disruption of normal negative feedback mechanisms operated by the oncogenic GTPase.
- Loss-of-function mutations in Phosphatase and TENsin homolog (PTEN), which amplify phosphatidylinositol 3-kinase (PI3K) signaling.

Sustaining proliferative signaling

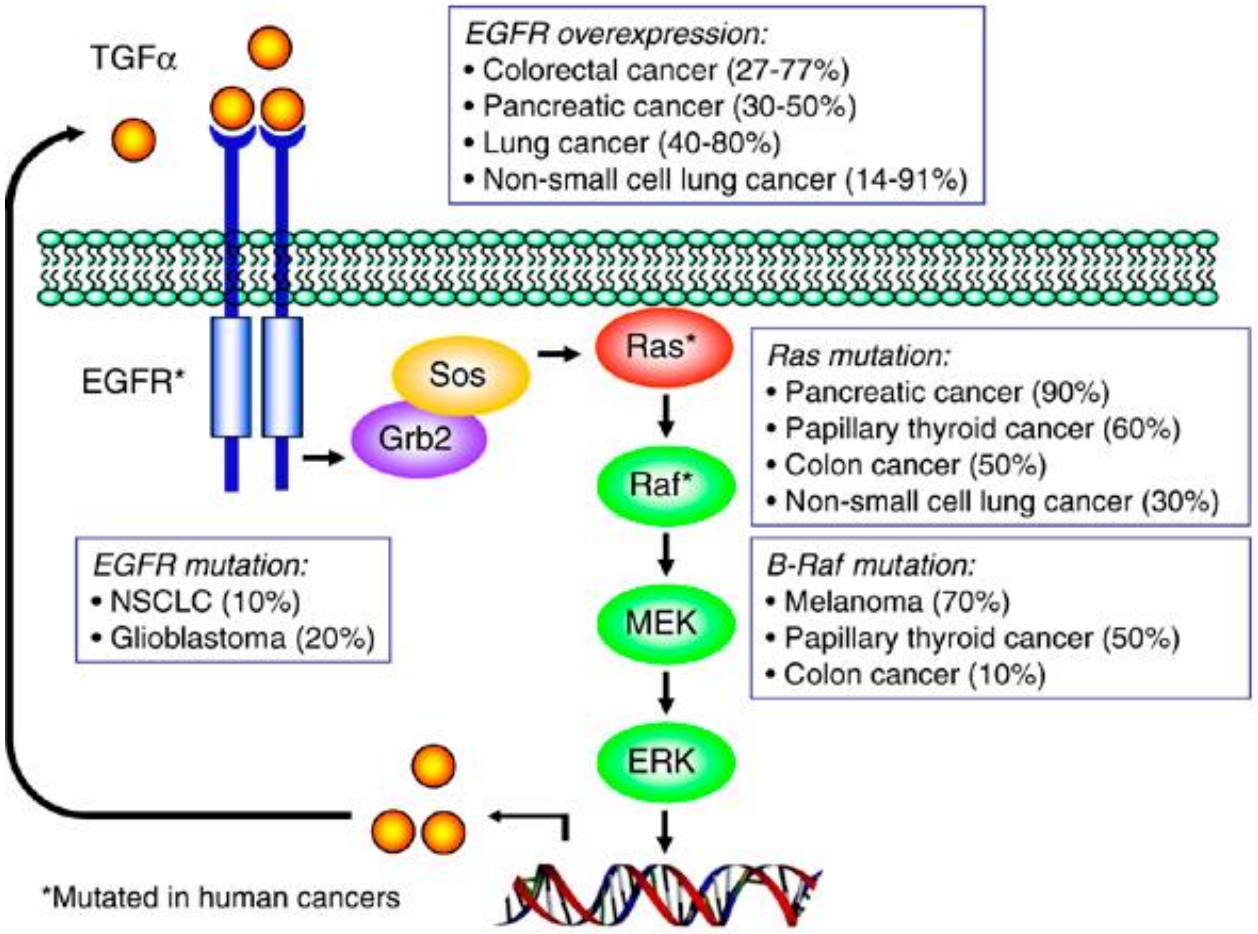
- Key Signaling pathways:
  - Ras Pathway;
  - MAPK Signaling;
  - PTEN Pathway;
  - PI3K Pathway;
  - mTOR Pathway;
  - AKT Signaling;
  - Cyclins and Cell Cycle Regulation;

Figure 9. Sustaining proliferative signaling[2]



2 - Bardeesy N, Sharpless NE. RAS unplugged: negative feedback and oncogene-induced senescence. Cancer Cell. 2006;10.

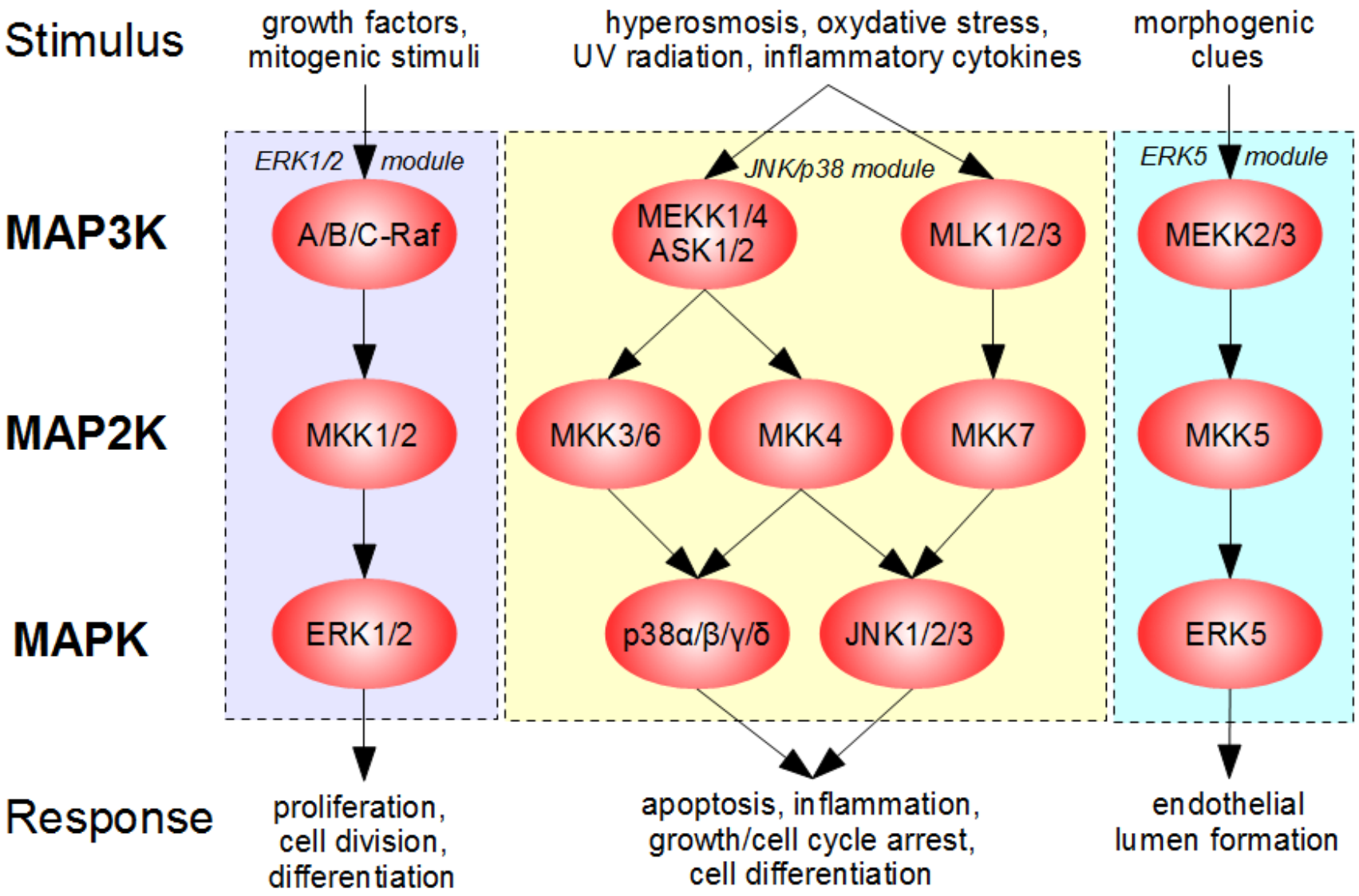
Oncogene activation of the ERK MAPK cascade



Mitogen-activated protein kinases - MAPk

- CMGC (CDK/MAPK/GSK3/CLK) kinase group.
- Cyclin-dependent kinases (CDKs).
- Involved in directing cellular responses to a diverse array of stimuli, such as mitogens, osmotic stress, heat shockand proinflammatory cytokines.
- Regulate proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis - among many others.

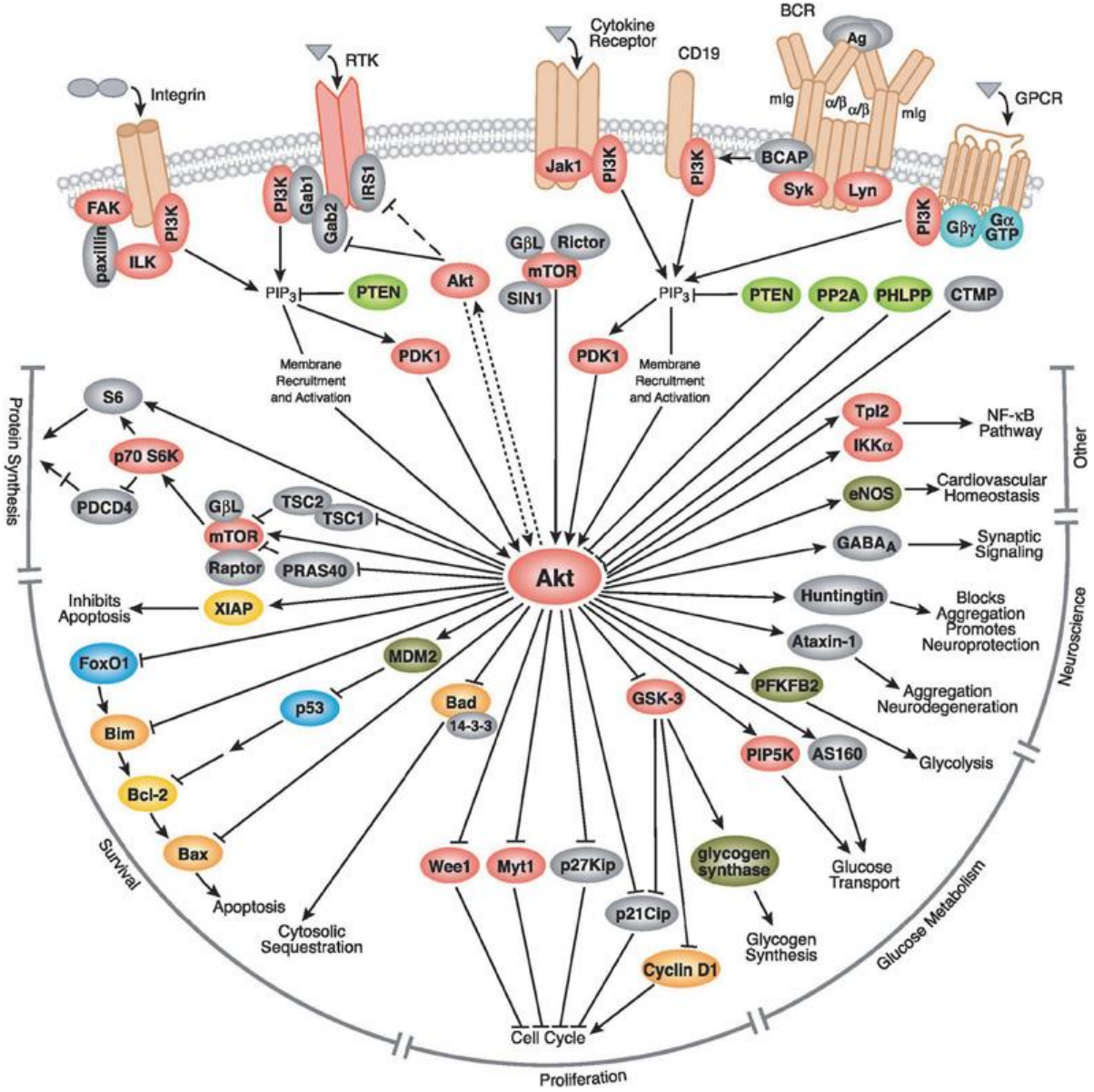
Simplified overview of mammalian MAPK cascades



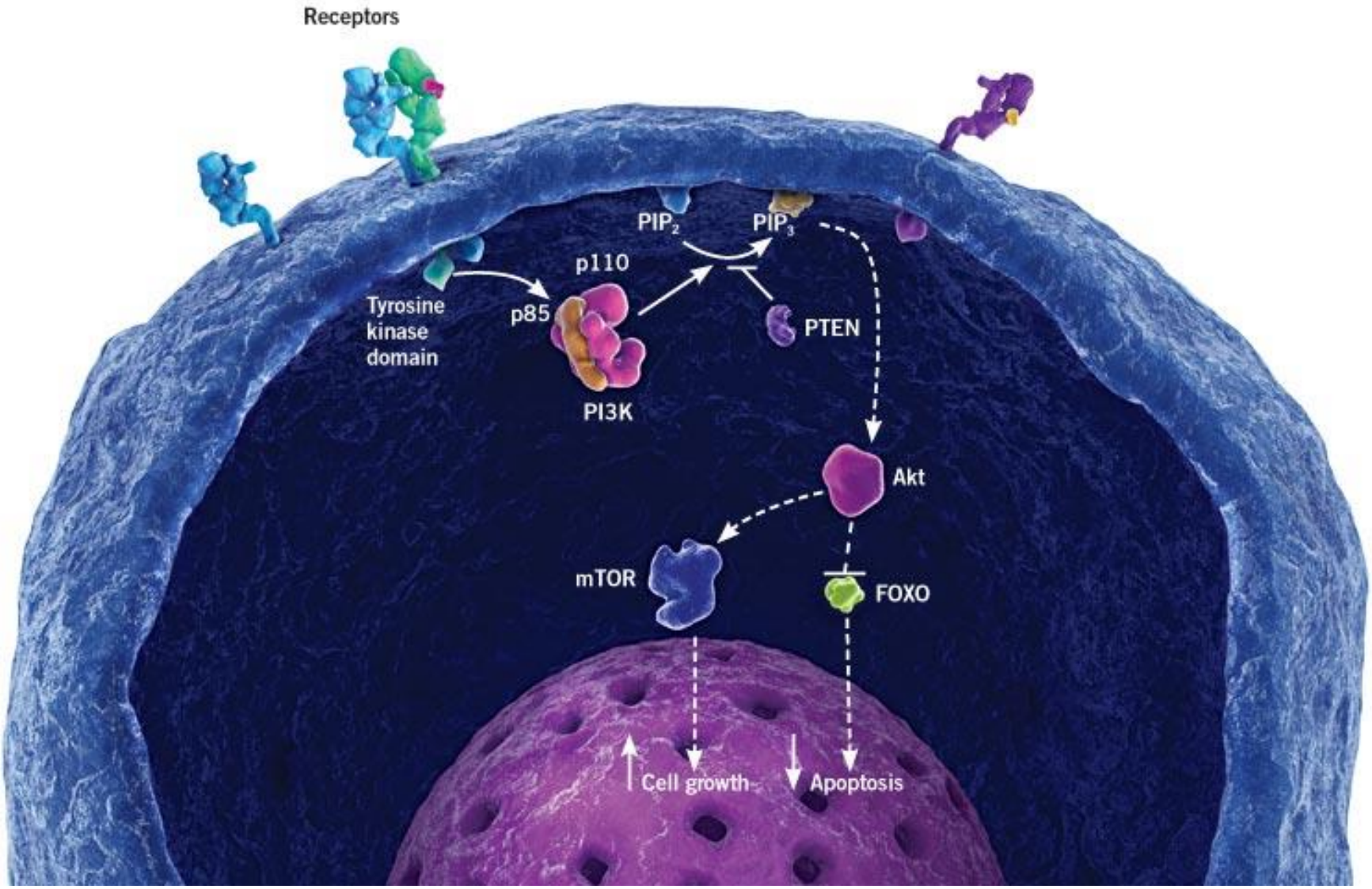
Targeting PI3K/Akt/mTOR signaling: The shutdown of cell survival signaling

- **Activation of the PI3K/Akt/mTOR signaling pathway regulates[1]:**
- Cell growth
- Cell proliferation
- Cell survival

PI3K/Akt/mTOR signaling



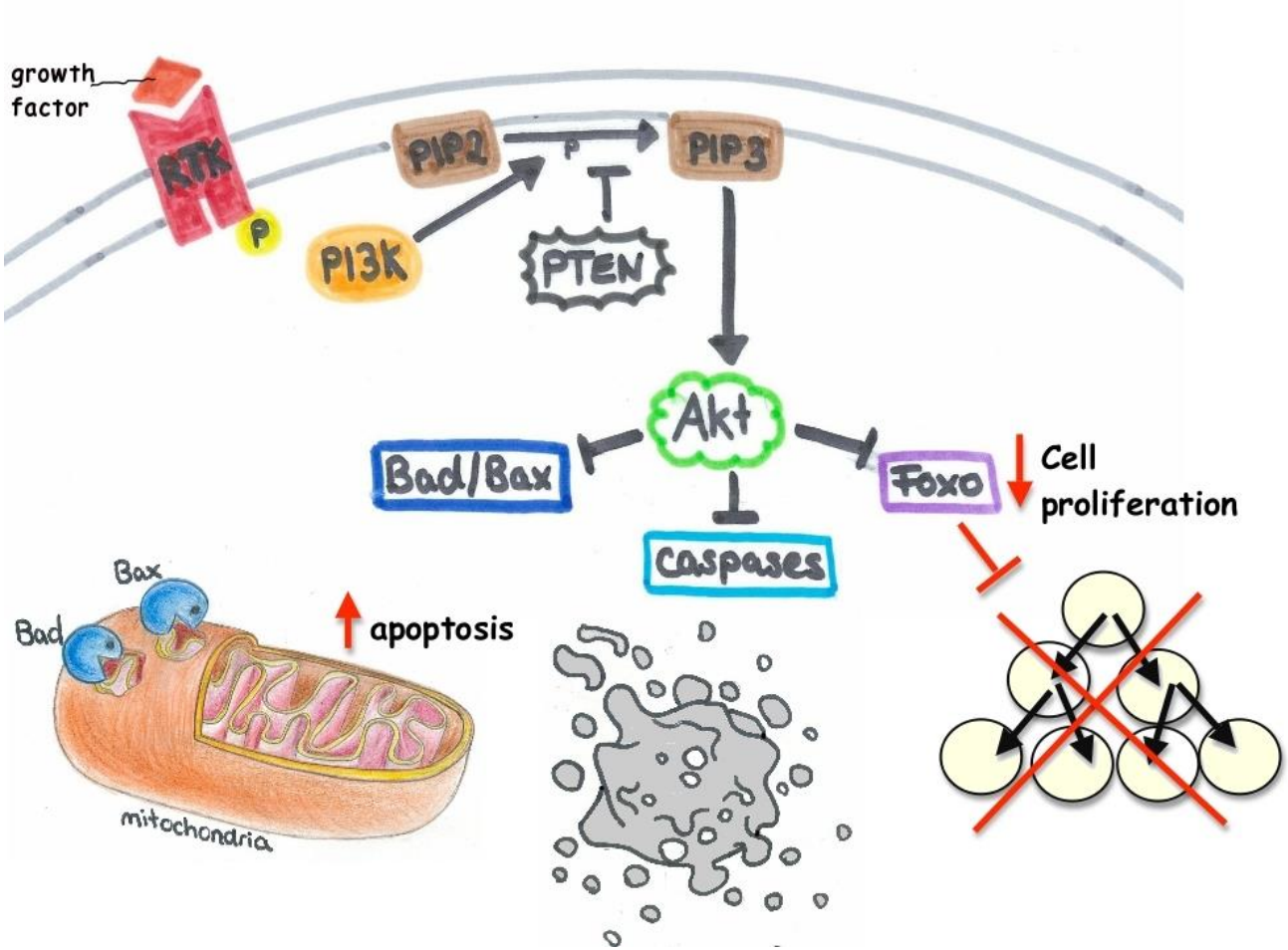
PI3K/Akt/mTOR signaling



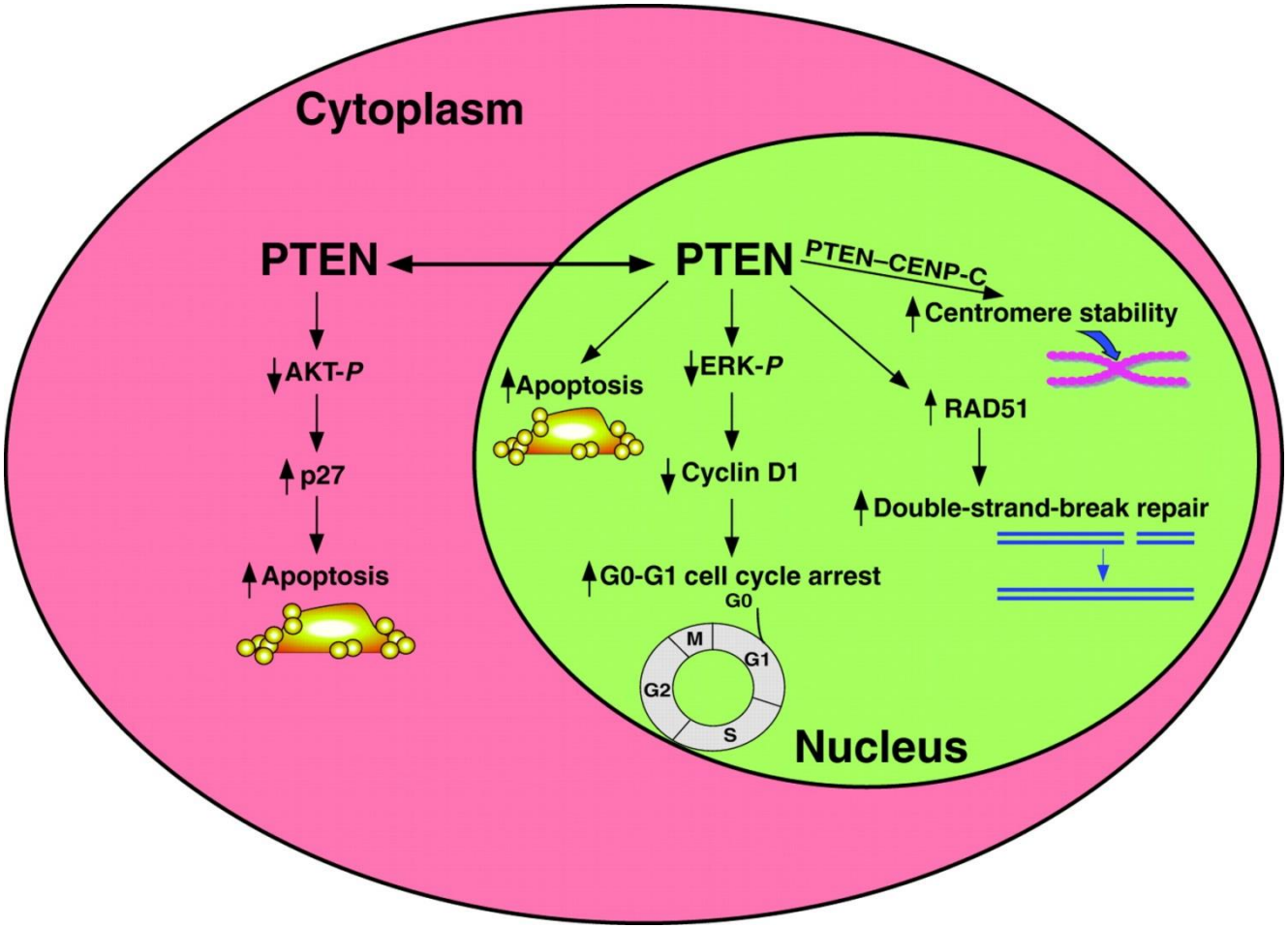
Phosphatase and tensin homolog (PTEN)

- Mutations of this gene are a step in the development of many cancers.
- Tumor suppressor gene through the action of its phosphatase protein product.
- This phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly.
- It is one of the targets of an oncomiR, MIRN21.
- This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency.
- PIP3 – PIP2 in cells and functions as a tumor suppressor by negatively regulating Akt/PKB signaling pathway.

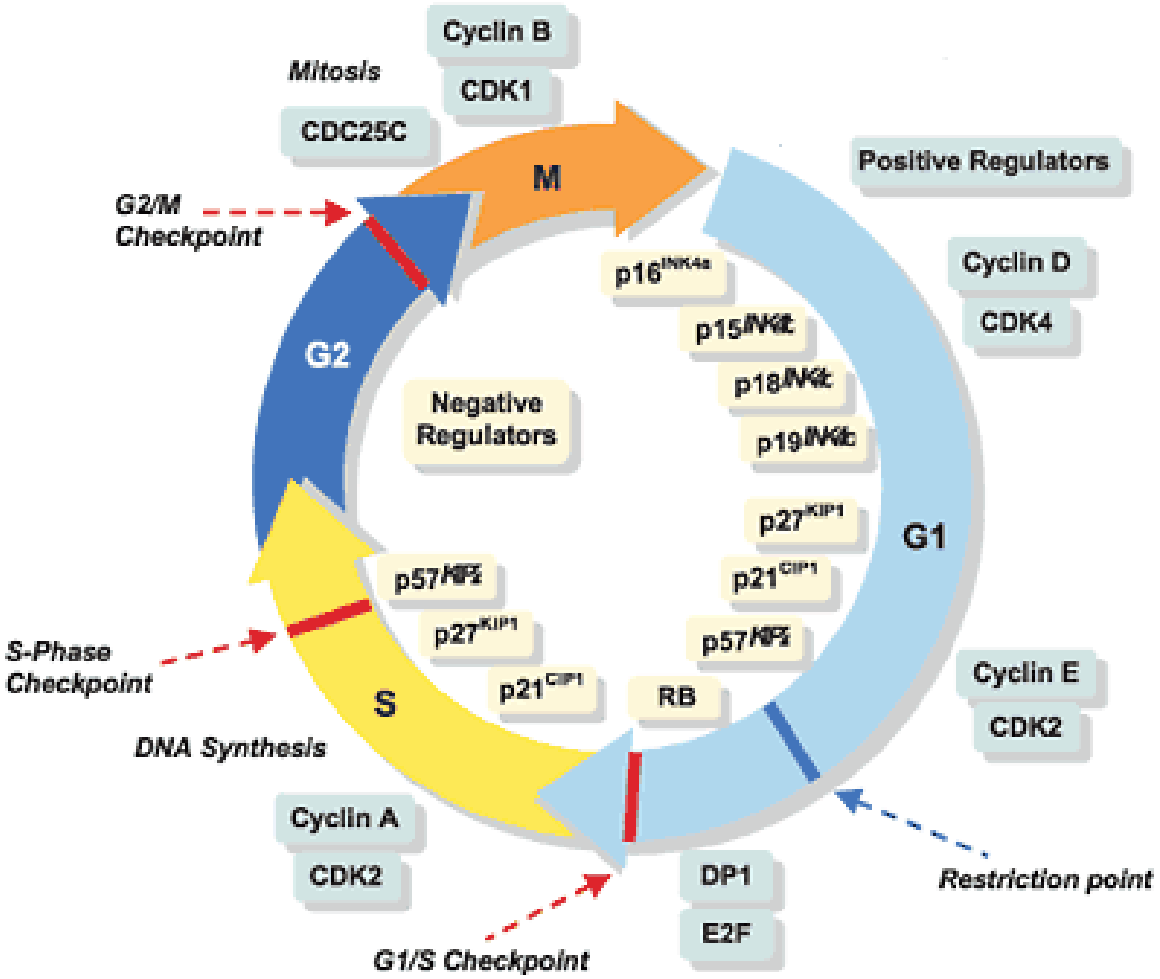
PTEN


growth factor
RTK
P
PIP2
PIP3
PI3K
PTEN
Akt
Bad/Bax
Foxo
Cell proliferation
caspases
Bax
Bad
apoptosis
mitochondria

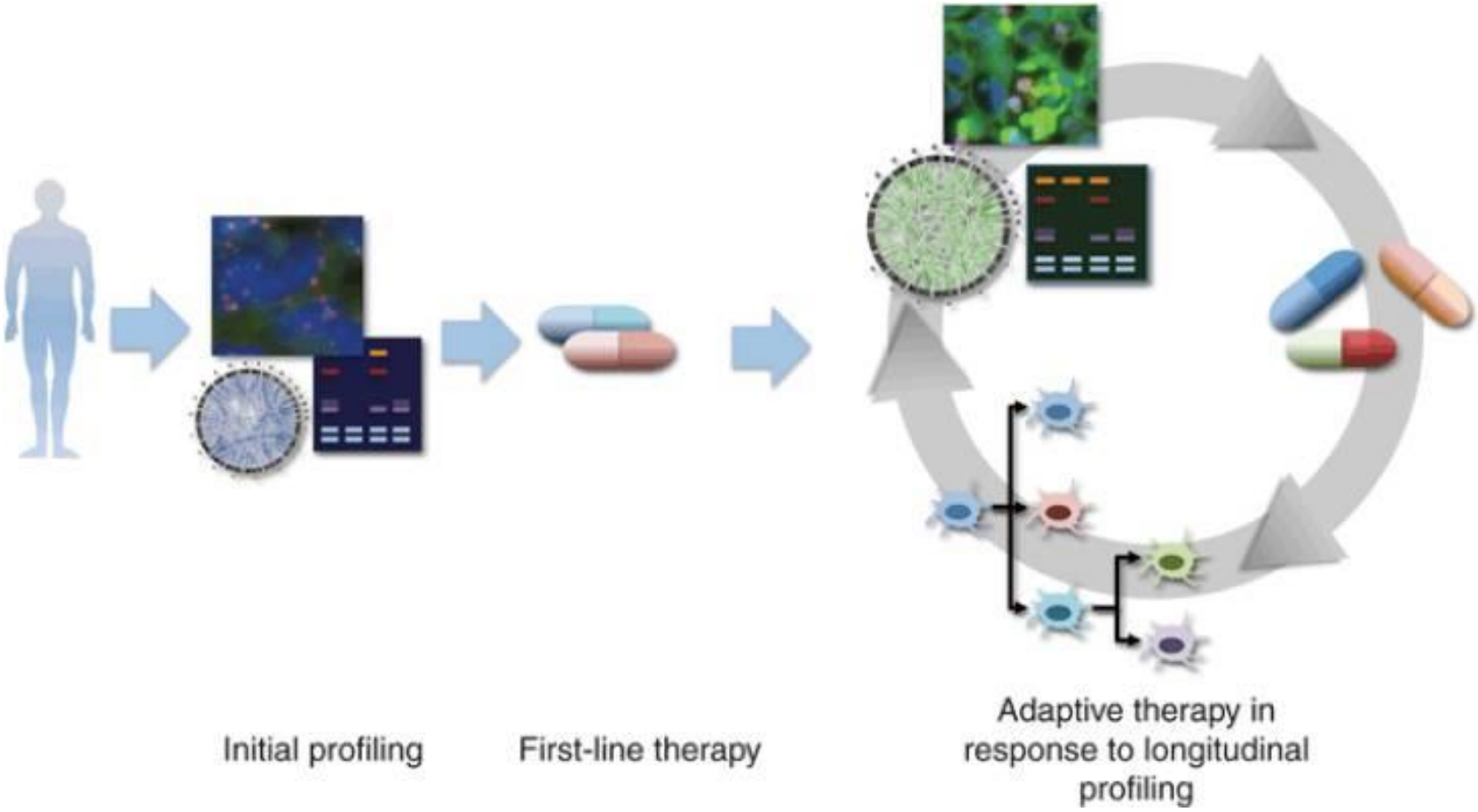
PTEN


Cytoplasm
PTEN
PTEN
PTEN–CENP-C
Centromere stability
AKT-P
Apoptosis
ERK-P
RAD51
p27
Cyclin D1
Double-strand-break repair
Apoptosis
G0-G1 cell cycle arrest
G0
M
G1
G2
S
Nucleus

Cell Cycle Regulation: cyclins and cell cycle checkpoints



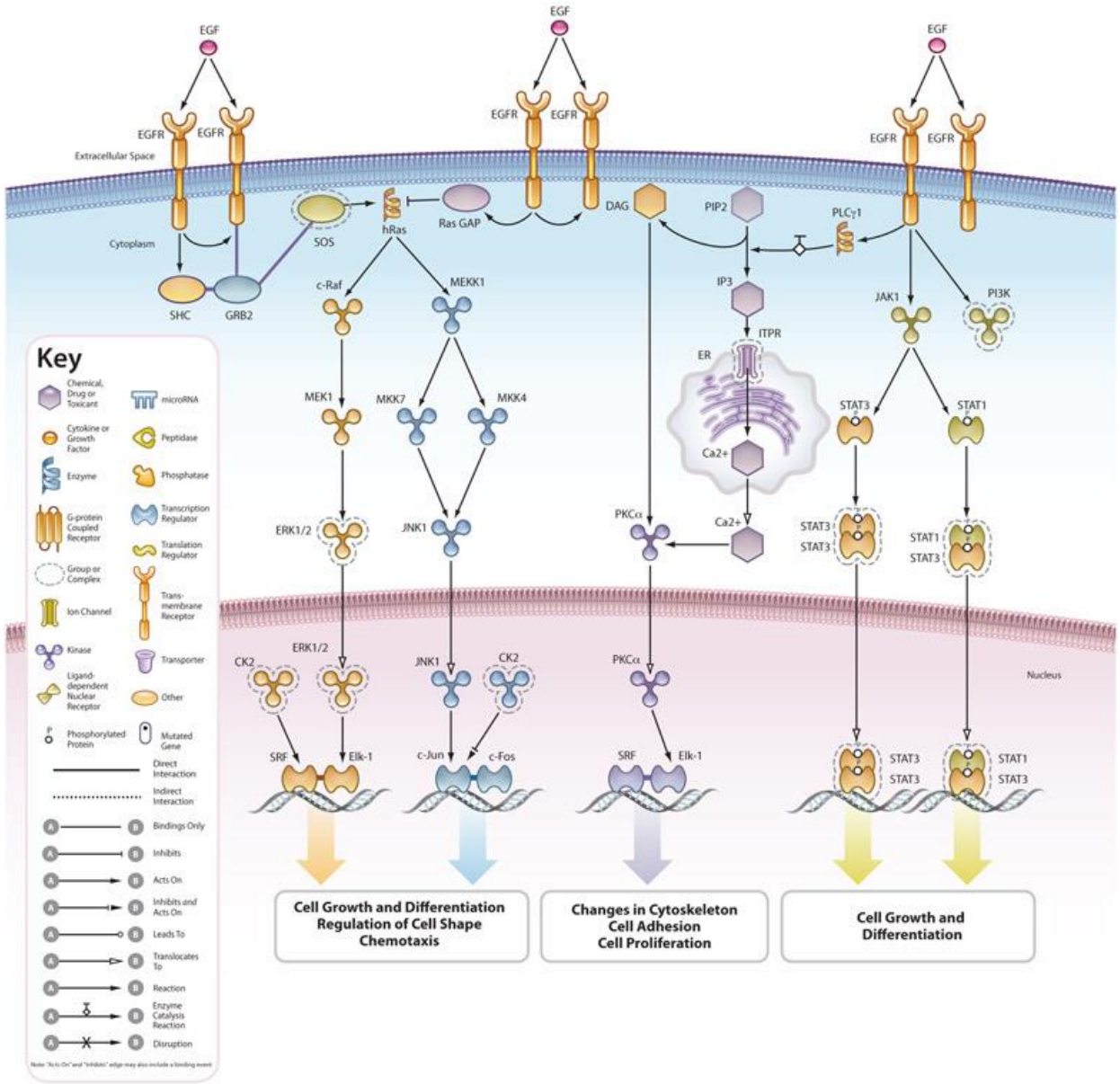
Translational medicine: Personalized cancer medicine



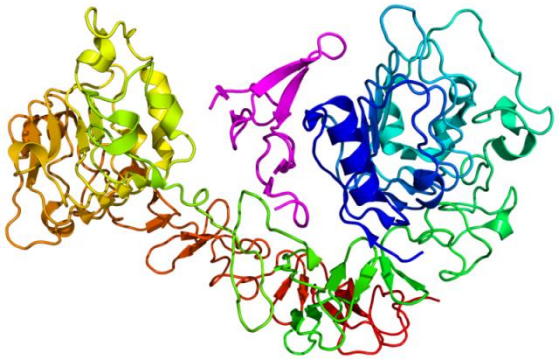
Model: Epidermal growth factor (EGF) family

- Regulate cell proliferation, migration and differentiation via tyrosine kinase receptors on target cells.
- The EGF receptor has a cytoplasmic tyrosine kinase domain, a transmembrane domain and an extracellular domain that binds to EGF.
- Ligand binding to the EGF receptor results in its dimerization, autophosphorylation and activation.
- Once activated, the EGF receptor transmits intracellular signals via the phosphorylation of several proteins.

Model: Epidermal growth factor (EGF) family



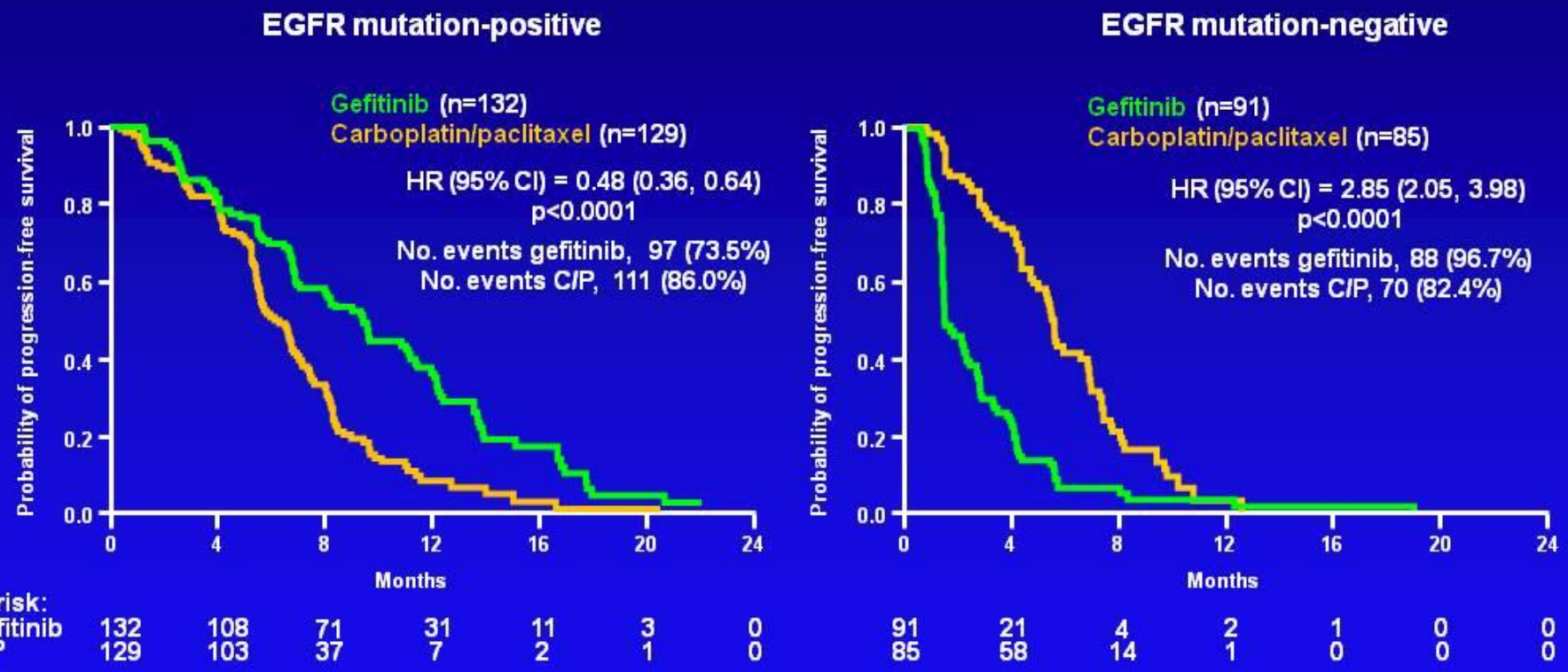
EGFR/ HER1 in cancer:



Below is a timeline of the major events that have bought about personalised treatment for non-small cell lung cancer

- 1962 - Stanley Cohen discovers EGF in mice
- 1977 - Identification of EGFR by Cohen
- 1980 - Demonstration that EGFR has intrinsic kinase activity
- 1981 - John Mendelsohn conducts research focusing on EGFR - proposes EGFR as anticancer target
- 1984 - Human EGFR cloned and sequenced
- Late 1980s - Mounting evidence for EGFR importance
- 1990 - First clinical trial of anti-EGFR agent (monoclonal antibody) confirms mode of action
- 1994 - New class of EGFR-tyrosine kinase inhibitors discovered
- 2001 - Anti-tumour activity shown in Phase II results
- 2002 - Gefitinib approved for non-small cell lung cancer in Japan
- 2004 – Erlotinib approved by FDA for non-small cell lung cancer
- 2004 - Discovery of EGFR mutations - exons 18–21 - linked to sensitivity to gefitinib
- 2005 - Results from the BR21 study
- 2004–2006 - Research into EGFR biomarkers widens
- 2006 - Panitumumab approved for colorectal cancer
- 2007- Results from key clinical trials
- 2007 - DxS develop EGFR29 Mutation Test Kit
- 2008 - Results from the IPASS study
- 2011 - Results from the EURTAC study

IPASS: Progression-free survival in EGFR-mutation + vs - patients



Treatment by subgroup interaction test, p<0.0001

Incidence of EGFR mutation: 261/437 = 59.7%

Mok et al 2008

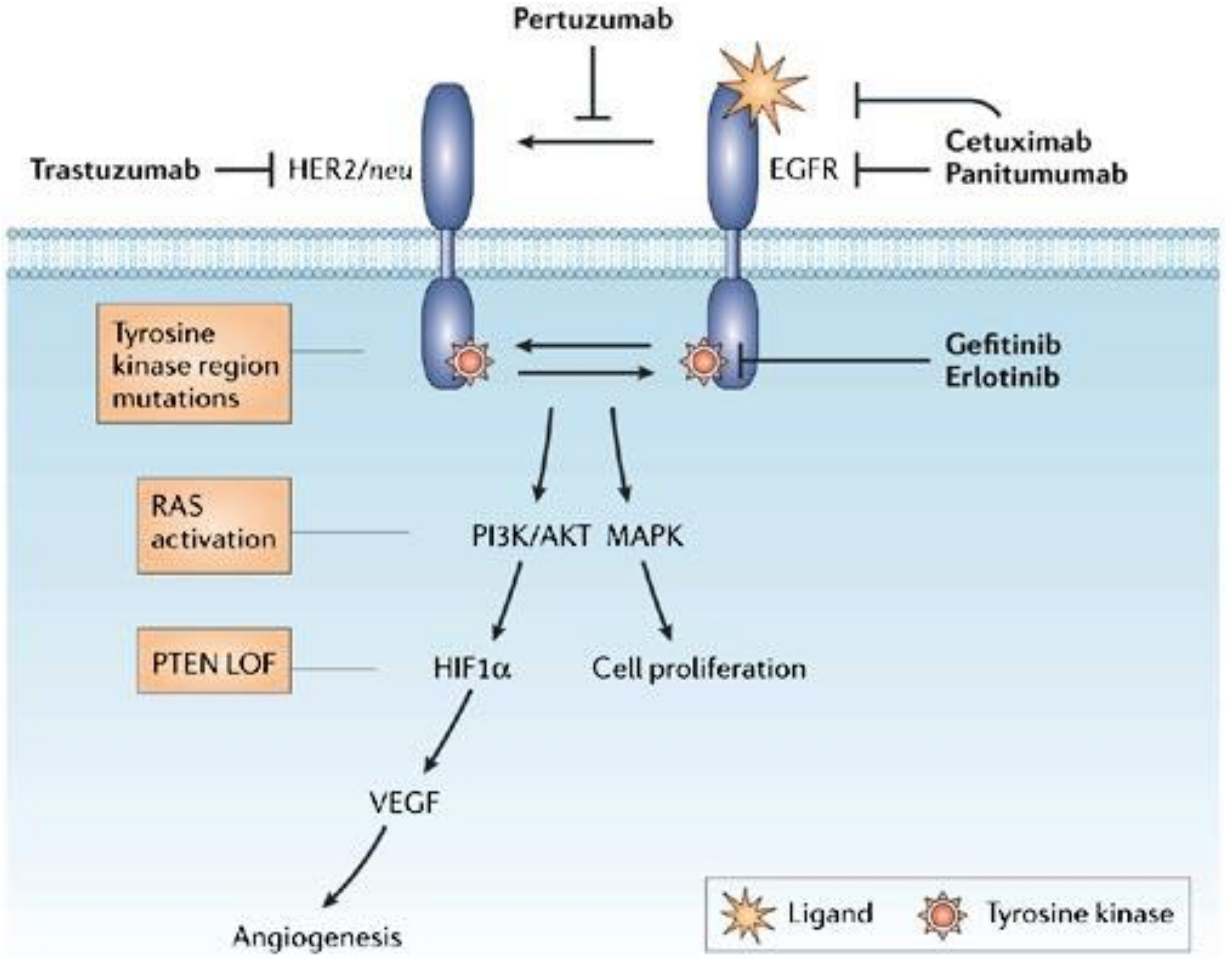
EGFR Mutations by Race and Other Clinical Characteristics

Subgroup	Total	East Asian	Non-East Asian
Smokers	11%	17%	4%
Never-smokers	54%	60%	35%
Adenocarcinoma	42%	49%	16%
Non-AdenoCa	3%	4%	1%
Male	16%	22%	1%
Female	46%	58%	20%

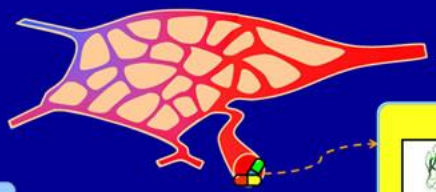
From trials: Paez, 2004; Pao 2004; Han 2005, Mitsudomi 2005, Eberhard 2005 Shigamatsu 2005, Huang 2005

Janne, ASCO Educational Session, 2007

EGFR Inhibitors



CETUXIMAB PATHWAY
In *Homo sapiens*



Cetuximab
DB00002

Epidermal growth factor
P01133

ACTIVATION

EGFR
P00533

GRB2
P62993

SOS1
Q07889

ACTIVATION

PI3K catalytic subunit α
P42336

PI3K regulatory subunit α
P27986

ACTIVATION

Raf-1
P04049

2 Zn⁺⁺

ACTIVATION

RAC-alpha serine/threonine protein kinase
P31749

ACTIVATION

Cancer Cell

MAP kinase kinase 1
Q02750

RAC-alpha serine/threonine protein kinase
P31749

ACTIVATION

ACTIVATION

ACTIVATION

Mitogen-activating protein kinase 1
P28482

Mg⁺⁺

ACTIVATION

G1/S-specific cyclin-D1
P24385

Nucleus

Serine/threonine protein Kinase mTOR
P42345

JUN
P05412

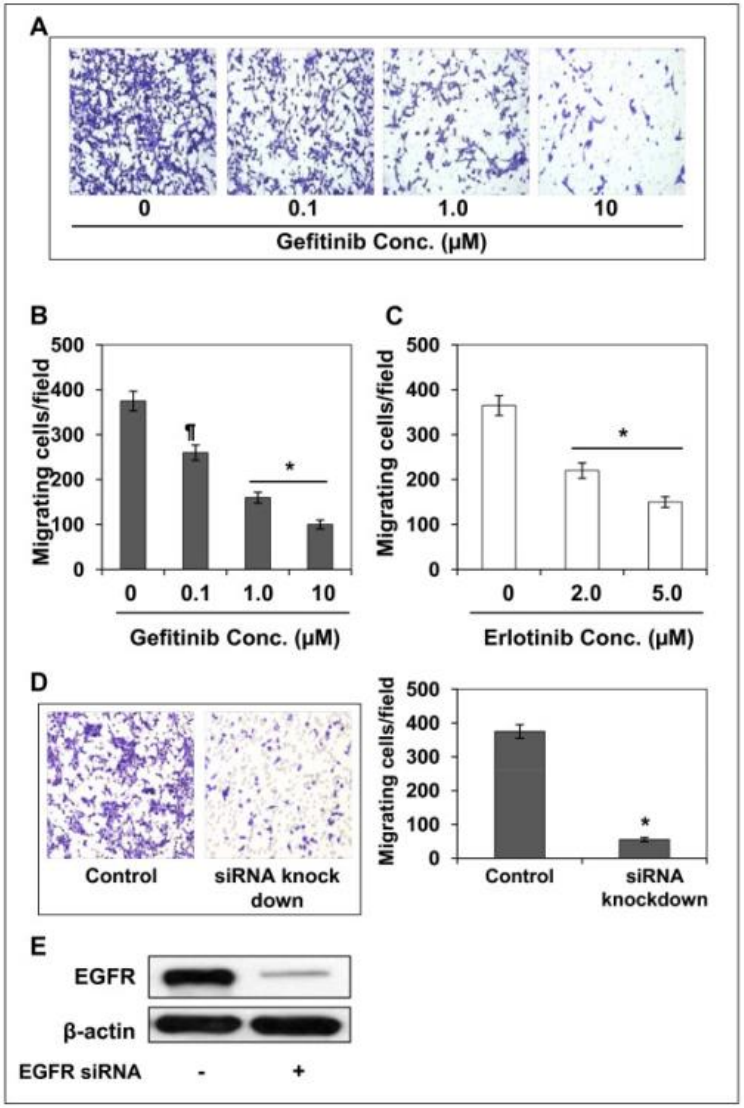
FOS
P01100

MYC
P01106

ACTIVATION

Tumour Growth

Inhibitor the invasive potential of head and neck cutaneous squamous cell carcinoma cells by targeting EGFR expression and epithelial-to-mesenchymal transition



EGFR-Expressing Tumors

Examples of EGFR-Expressing Tumors	Examples of Non–EGFR-Expressing Tumors
• Non–small-cell lung cancer	• Small-cell lung cancer
• Colorectal cancer	• Sarcomas
• Breast cancer	• Lymphoma/leukemia
• Pancreatic cancer	• Myeloma
• Prostate cancer	
• Ovarian cancer	
• Head and neck cancer	
• Esophageal cancer	
• Glioblastoma multiforme	

Ciardiello F, Tortora G. *Eur J Cancer.* 2003;39:1348-1354.
Salomon DS et al. *Crit Rev Oncol Hematol.* 1995;19:183-232.

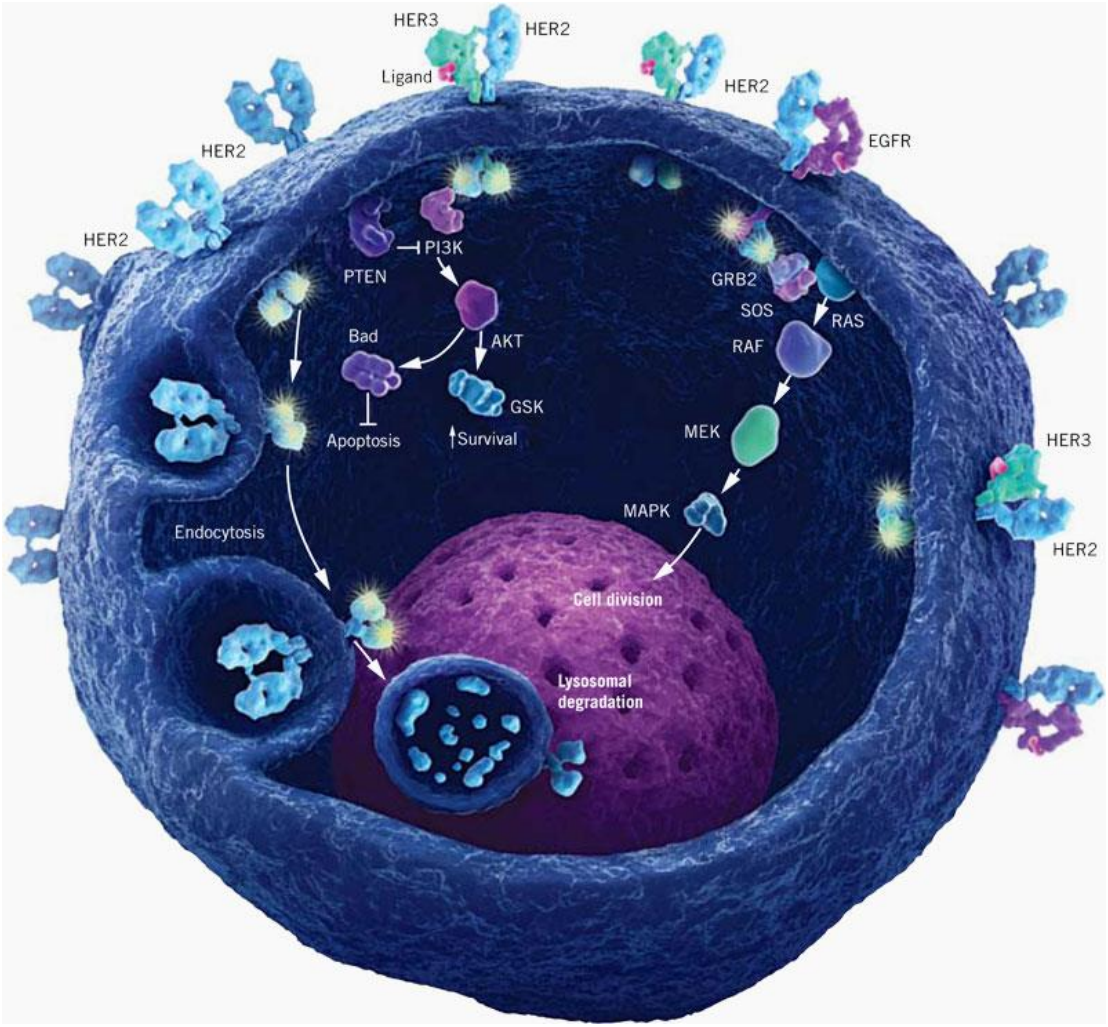
Incidence of EGFR Expression in Selected Solid Tumors

Tumor Type	EGFR Expression (%)
NSCLC	40-80
SCCHN	95
Colorectal	25-77
Glioblastoma	40-60
Breast	14-91
Esophageal	35-88
Pancreatic	30-50
Prostate	41-100
Ovarian	35-70

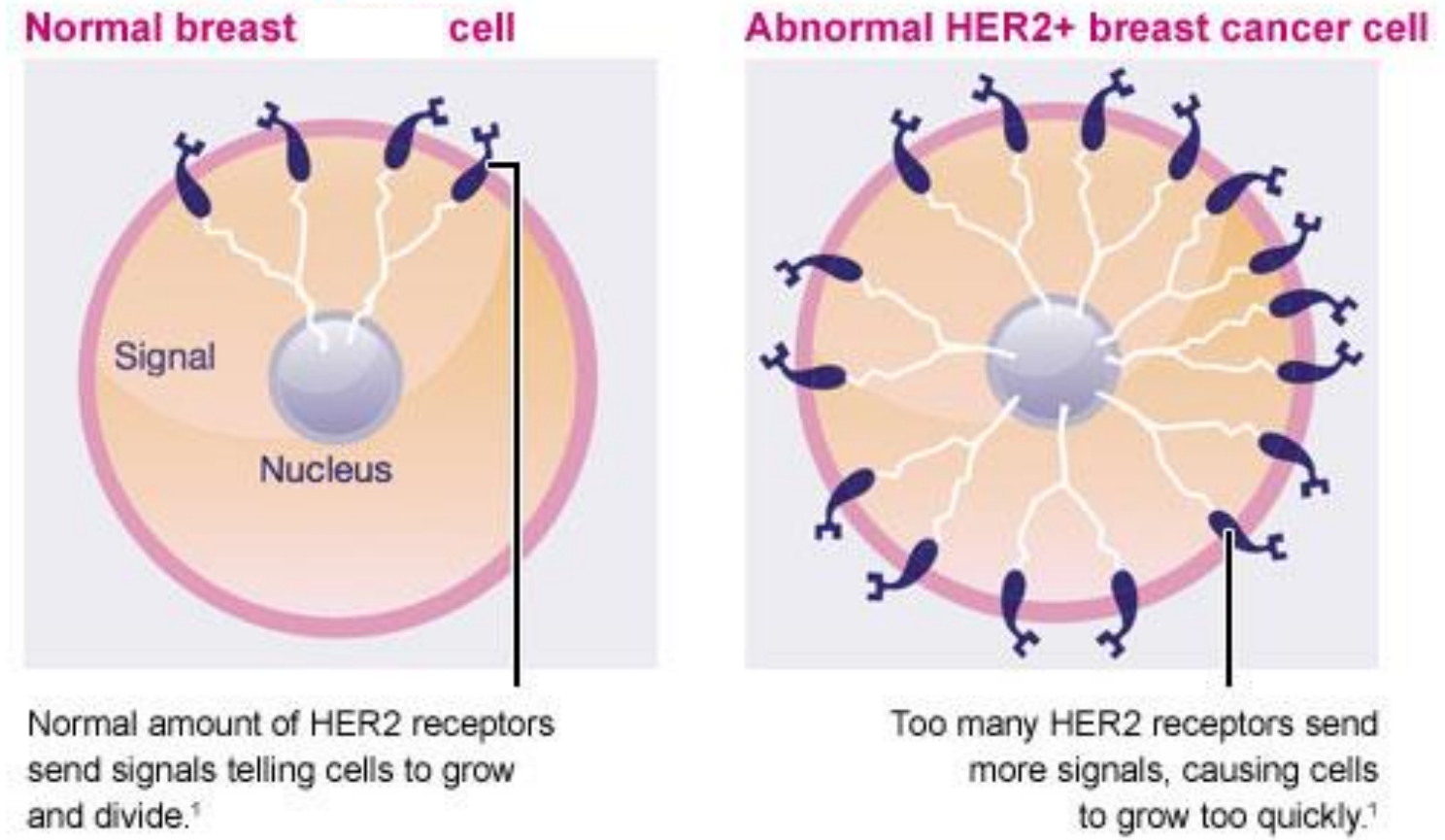
SCCHN = Squamous cell carcinoma head and neck.

Reproduced with permission from Arteaga C. Semin Oncol. 2003;30(suppl 7):3-14.

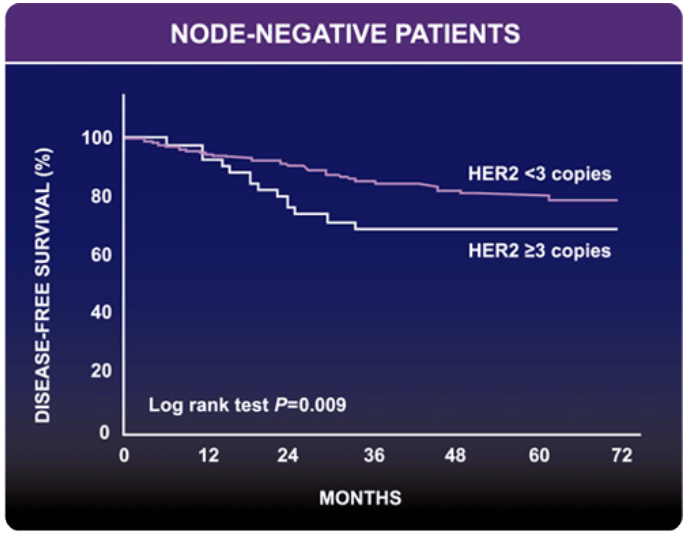
HER2 / c – erb2

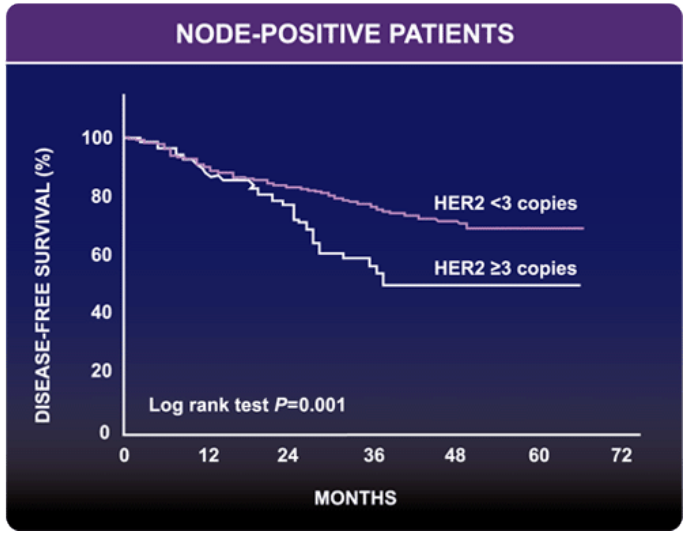

HER3
HER2
Ligand
HER2
EGFR
HER2
HER2
PI3K
PTEN
GRB2
SOS
RAS
Bad
AKT
RAF
GSK
Apoptosis
Survival
MEK
HER3
MAPK
Endocytosis
HER2
Cell division
Lysosomal degradation

HER2 / c – erb2: Breast cancer



HER2 / c – erb2: Breast cancer





HER2 / c – erb2

- **How is HER2 measured?**
- Fluorescence in situ hybridization (FISH), which detects gene amplification by measuring the number of copies of the *HER2* gene in the nuclei of tumor cells
- Immunohistochemistry (IHC), which measures the number of HER2 receptors on the cell surface and therefore detects receptor overexpression
- Other methods of HER2 testing have been used increasingly in clinical studies and may eventually be incorporated into routine practice. These include:
- Chromogenic in situ hybridization (CISH), which measures gene amplification using a light microscope rather than a fluorescent microscope, which is required for FISH
- Reverse-transcriptase polymerase chain reaction (RT-PCR), which detects *HER2* gene amplification

Highlights from the EGF Signaling Pathway

Gene Symbol	Name	Cellular Functions	Disease Associations	Subcellular Locations	Upstream Regulators	Binding Partners	Downstream Interactors	Antibodies	Small Molecules
STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	proliferation apoptosis expression in transformation differentiation	tumorigenesis obesity enterocolitis Crohn's Disease hyperphagia	nucleus cytoplasm focal adhesions nuclear foci plasma membrane	IL6 IL10 IL2 IL21 Interferon Alpha	FOS EGFR PRKCD DIRAS3 IL2RB	TERT IL10 HIF1A CDKN1A SOCS3	SAB2500883, Anti-STAT3 antibody produced in goat	
EGFR	epidermal growth factor receptor	proliferation apoptosis migration transformation survival	cancer tumorigenesis neoplasia psoriasis endometriosis	cell surface plasma membrane nucleus cytoplasm caveolae	EGF TNF CBL lysophosphatidic acid HBEGF	EGF AXL GRB2 CBL SRC	Mapk MAPK1 Akt Erk1/2 MAPK3	E2158, Monoclonal Anti-EGF Receptor antibody produced in mouse	Inhibitor: PZ0129, CP-380736
c-Raf	v-raf-1 murine leukemia viral oncogene homolog 1	transformation proliferation apoptosis cell cycle progression cell death	transformation proliferation apoptosis cell cycle progression cell death	cytoplasm nucleus perinuclear region plasma membrane filamentous network	EGF TP53 JAK1 OSM HRAS	HRAS YWHAZ MAP2K1 YWHAB RB1	MAPK1 RB1 HMGA2 MAP2K1 Mapk	R2404, Monoclonal Anti-Raf-1/c-Raf antibody produced in mouse	Inhibitor: G8418, GW5074
JNK1	mitogen-activated protein kinase 8	apoptosis cell death proliferation survival differentiation	tumorigenesis hypertrophy insulin resistance cancer heart failure	nucleus cytoplasm focal adhesions plasma membrane mitochondria	TNF EGF IL1B EGFR IGF1	JUN MAPK8IP1 MAP2K4 MAP2K7 THRB	CDKN1B JUN APP AP-1 MAP3K11	SAB4200178, Monoclonal Anti-JNK antibody produced in mouse	Inhibitor: S6687, SP600125
c-Jun	jun proto-oncogene	apoptosis proliferation transformation expression in cell death	tumorigenesis cancer neoplasia Alzheimer's Disease dedifferentiation	nucleus cytoplasm perinuclear region Golgi apparatus apical processes	TNF IL1B beta-estradiol TGFB1 lipopolysaccharide	FOS PTGS2 MAPK8 TAF1 ATF2	HIF1A SPP1 IL6 IL8 ESR2	SAB4300306, Anti-JUN (Ab-91) antibody produced in rabbit	
PKCa	protein kinase C, alpha	apoptosis proliferation activation in migration phosphorylation in	neurodegenerative disease diabetes rheumatoid arthritis malignant neoplasm cardiomyopathy	cytoplasm nucleus plasma membrane principal piece cytoskeleton	phosphatidylserine EGF beta-estradiol 15(S)-HETE D-glucose	ITGB1 AKAP12 EGFR CAV1 SELL	Erk1/2 APP PDE3A MAPK1 IGF2	WH0005578M1, Monoclonal Anti-PRKCA antibody produced in mouse	Inhibitor: K1838, K-252a
STAT1	signal transducer and activator of transcription 1, 91kDa	apoptosis expression in proliferation response differentiation	infection tumorigenesis pneumonia cancer fibrosis	nucleus cytoplasm mitochondria neuromuscular junctions	IFNG Interferon Alpha IFNA2 IL6 IFNB1	EIF2AK2 IFNGR1 FOS STAT2 PIN1	IRF1 CDKN1A IRF7 CASP1 CD40	SAB4300328, Anti-STAT1 (Ab-701) antibody produced in rabbit	
EGF	epidermal growth factor	proliferation migration apoptosis growth activation in	Alzheimer's Disease diabetes mellitus polycystic kidney disease schizophrenia cancer	apical membrane basolateral membrane cell surface Golgi apparatus clathrin-coated vesicles	ERBB2 ERBB3 ADAM10 CHUK PI4KA	EGFR ERBB3 ERBB2 PIK3R2 TAT	EGFR MAPK1 MAPK3 FLT1 Erk1/2	E2520, Monoclonal Anti-Epidermal Growth Factor antibody produced in mouse	Inhibitor: S2871, Suramin sodium salt
GRB2	growth factor receptor-bound protein 2	growth proliferation differentiation signaling transformation	Crohn's disease leiomyomatosis cardiac fibrosis hypertrophy uterine cancer	centrosome cytosol perinuclear region plasma membrane axons	F2 EGF Bcr IGF1 SHC1	SHC1 CBL SOS1 EGFR GAB1	MAPK3 EGFR ERBB2 RAF1 CBL	SAB2500491, Anti-GRB2 antibody produced in goat	
MEK1	mitogen-activated protein kinase kinase1	apoptosis proliferation transformation differentiation migration	tumorigenesis neoplasia hypertrophy cardiofaciocutaneous syndrome hyperalgesia	cytoplasm midbody nucleus centrosome mitotic spindle	EGF LEF RAF1v RAC1 TNF	MAPK1 RAF1 MAPK3 PEBP4 KSR1	MLANA MAPK1 DCT SILV TYRP1	SAB4502408, Anti-MEK1 antibody produced in rabbit	Inhibitor: P215, PD 98,059
hRas	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	transformation proliferation growth apoptosis senescence	tumorigenesis cancer neoplasia papillomatosis neurodegeneration	nucleus plasma membrane Golgi apparatus cytoplasm lamellipodia	Cd3 CD28 AXIN1 FTase IL6	RAF1 RALGDS RIN1 Blnk Raf	reactive oxygen species CDKN1A MAPK1 Erk1/2 Mapk	SAB4501441, Anti-HRASH, N-Terminal antibody produced in rabbit	Antagonist: E7781, Erastin
c-Fos	FBJ murine osteosarcoma viral oncogene homolog	transformation apoptosis proliferation expression in growth	cancer rheumatoid arthritis endometriosis neoplasia seizures	nucleus cytoplasm perinuclear region Golgi apparatus cell periphery	beta-estradiol TNF IL1B EGF ESR2	JUN STAT3 PTGS2 SRF IL8	IL6 CSF2 ESR2 IL8 CFLAR	SAB2104186, Anti-FOS antibody produced in rabbit	