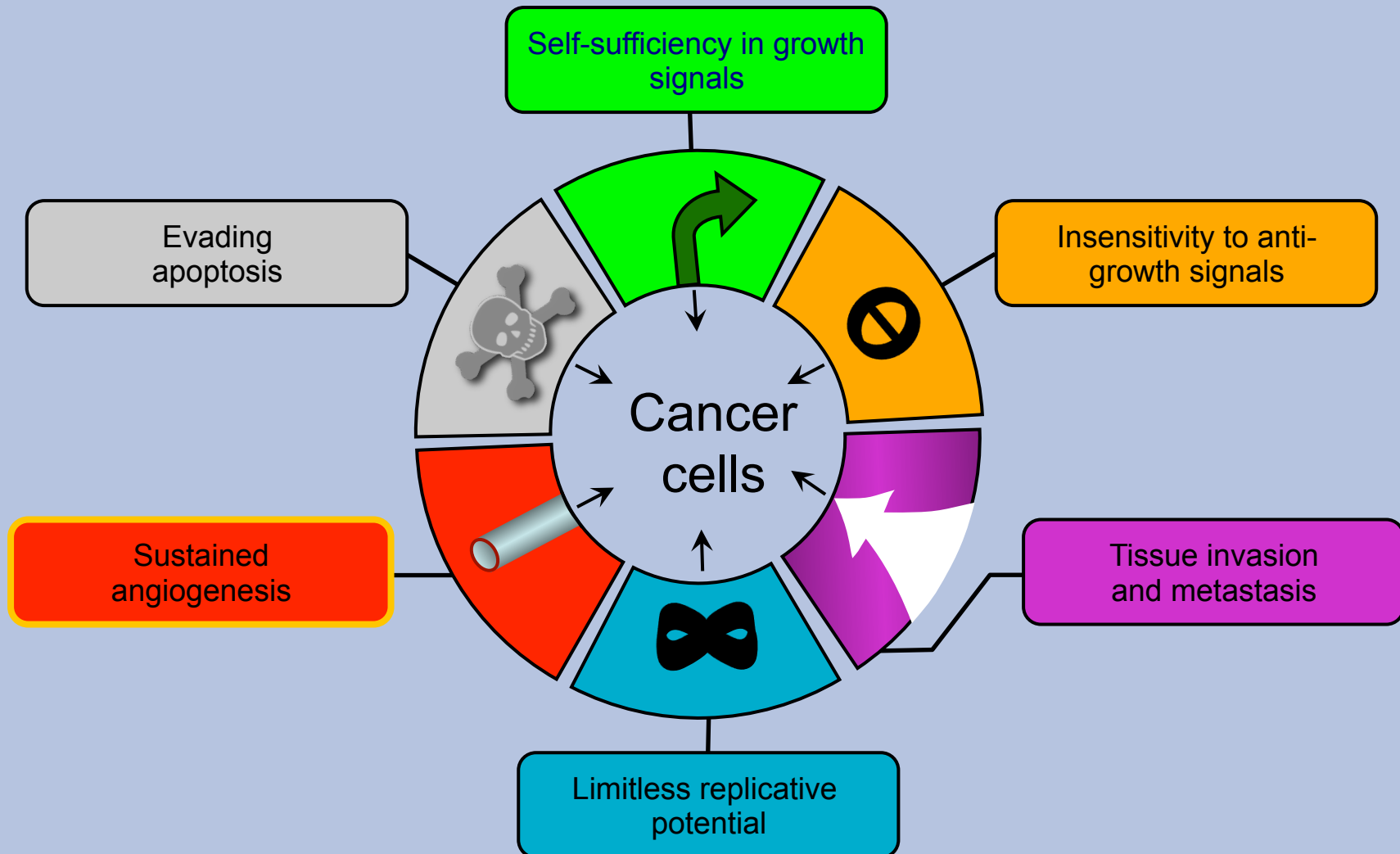
A photograph of an iceberg floating in the ocean. The visible tip of the iceberg is a small, rectangular block of white ice. Below the waterline, a much larger and more complex structure of ice is visible, illustrating the concept of the tip of the iceberg. The water is a deep blue, and the sky is a clear, lighter blue.

# Biología molecular del cáncer – Introducción básica

Mauricio Lema Medina MD



# Hallmarks of cancer



Adapted from Hanahan, et al. Cell 2000



Emerging Hallmarks

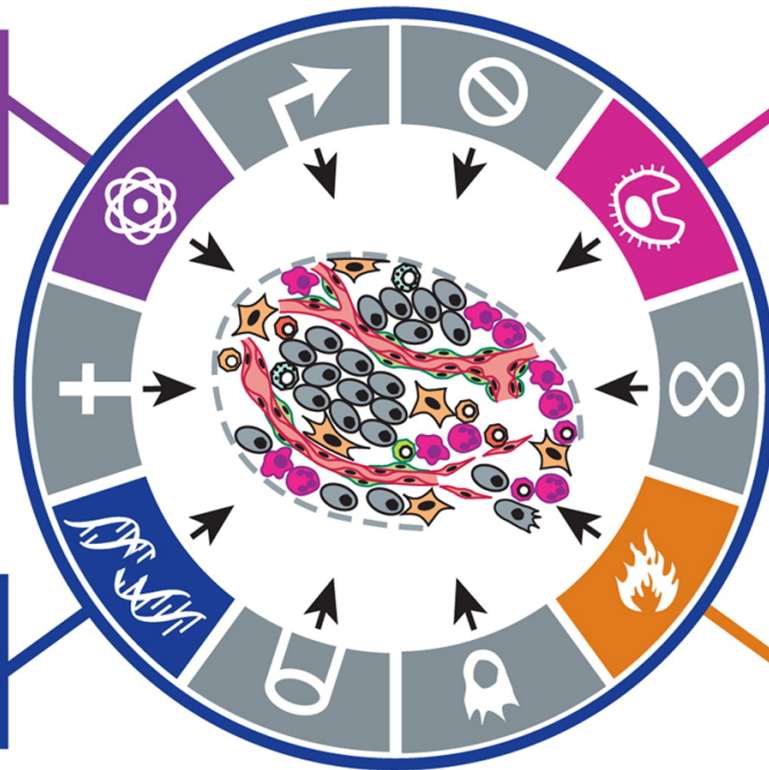
Deregulating cellular energetics

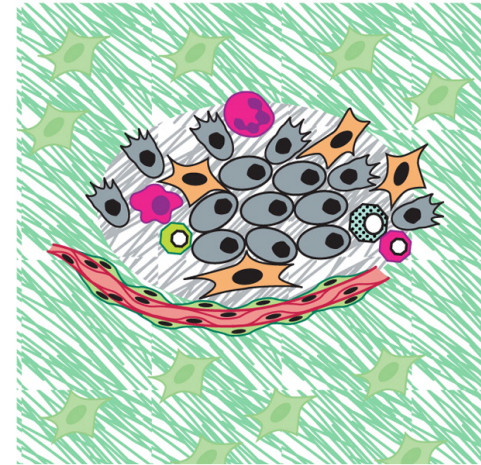
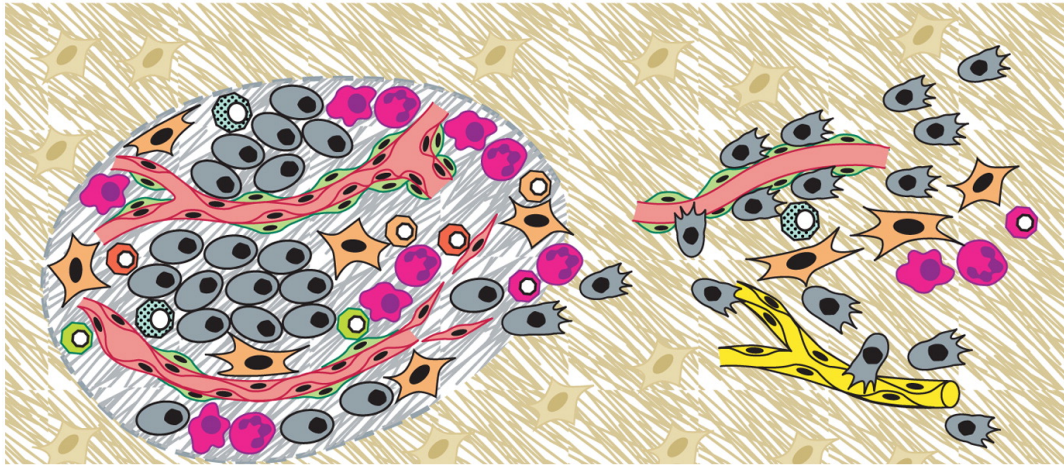
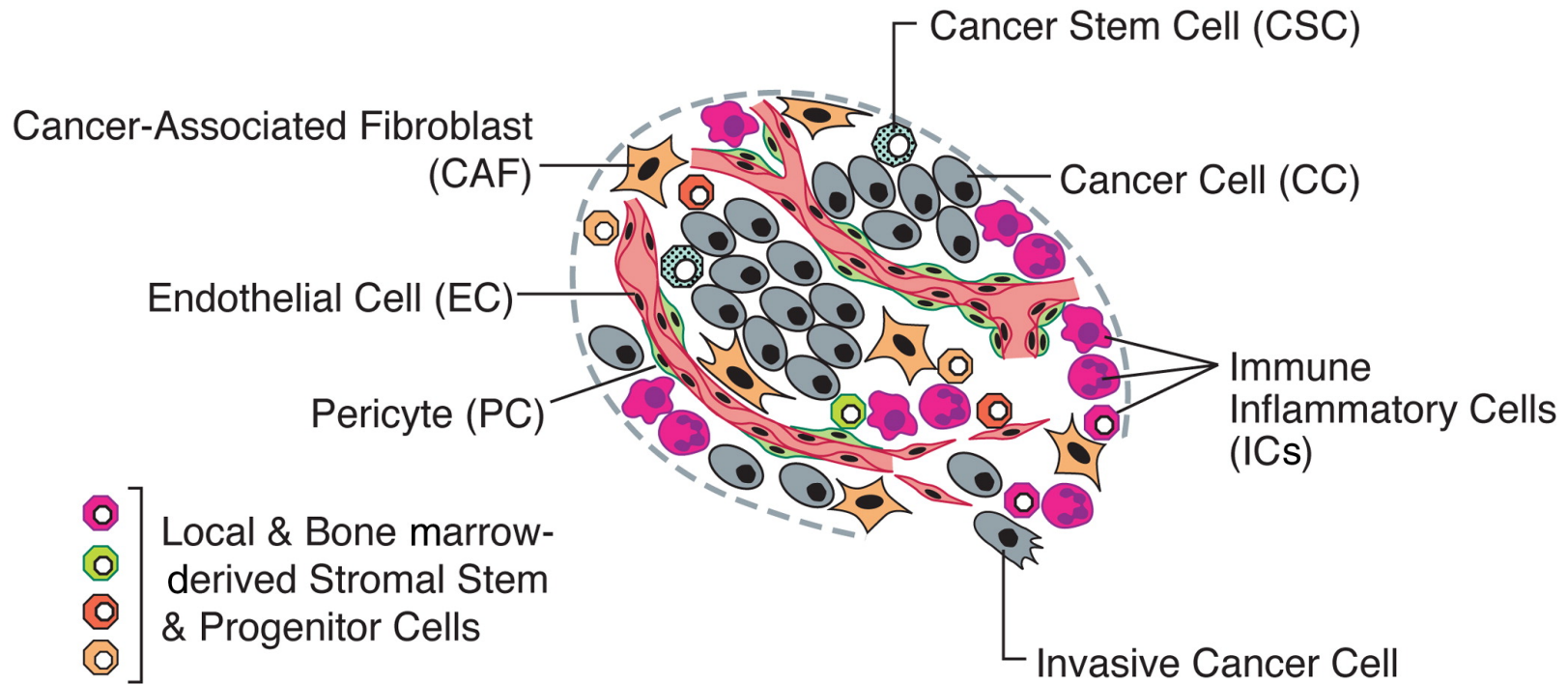
Avoiding immune destruction

Genome instability and mutation

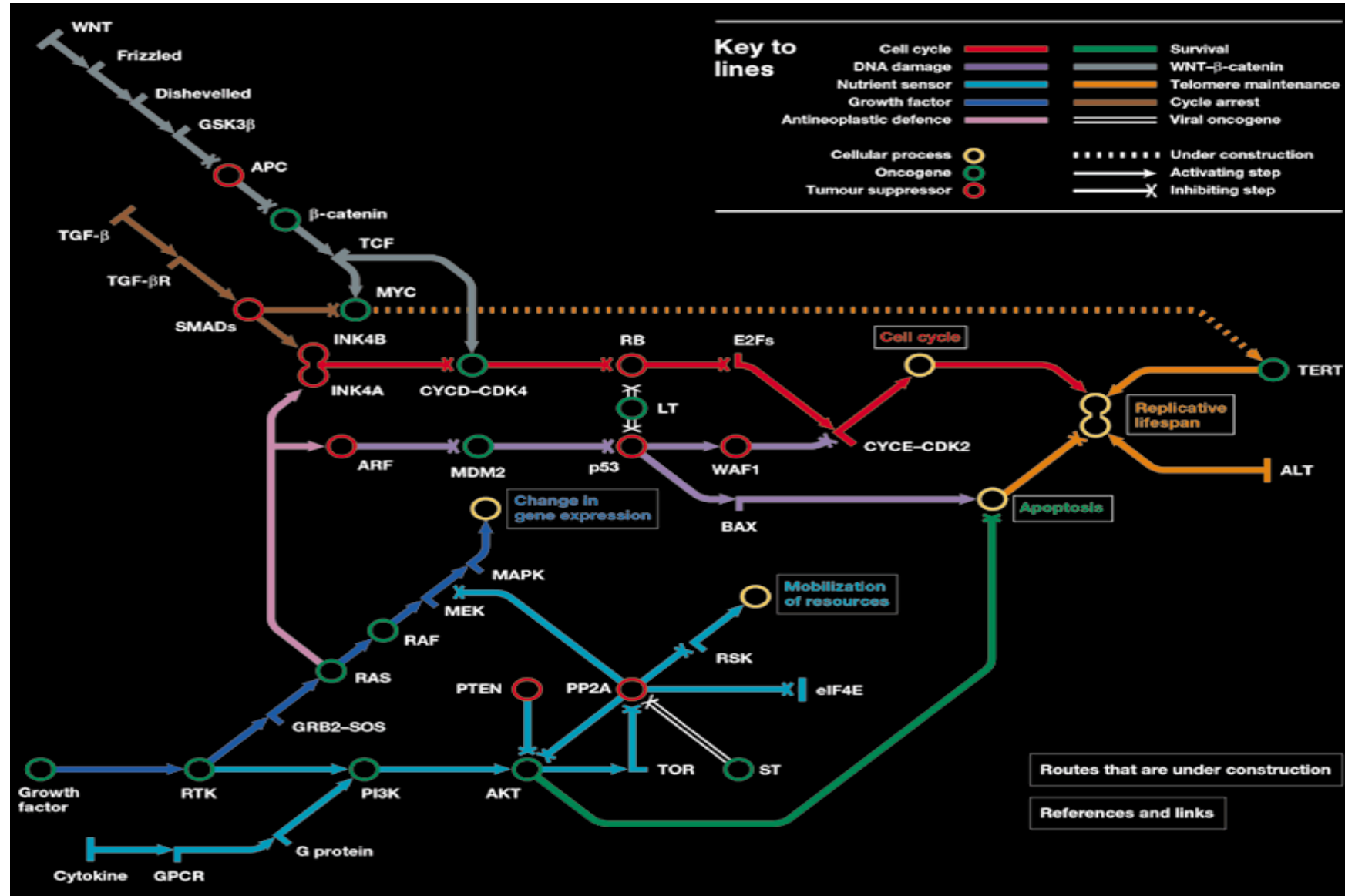
Tumor-promoting Inflammation

Enabling Characteristics





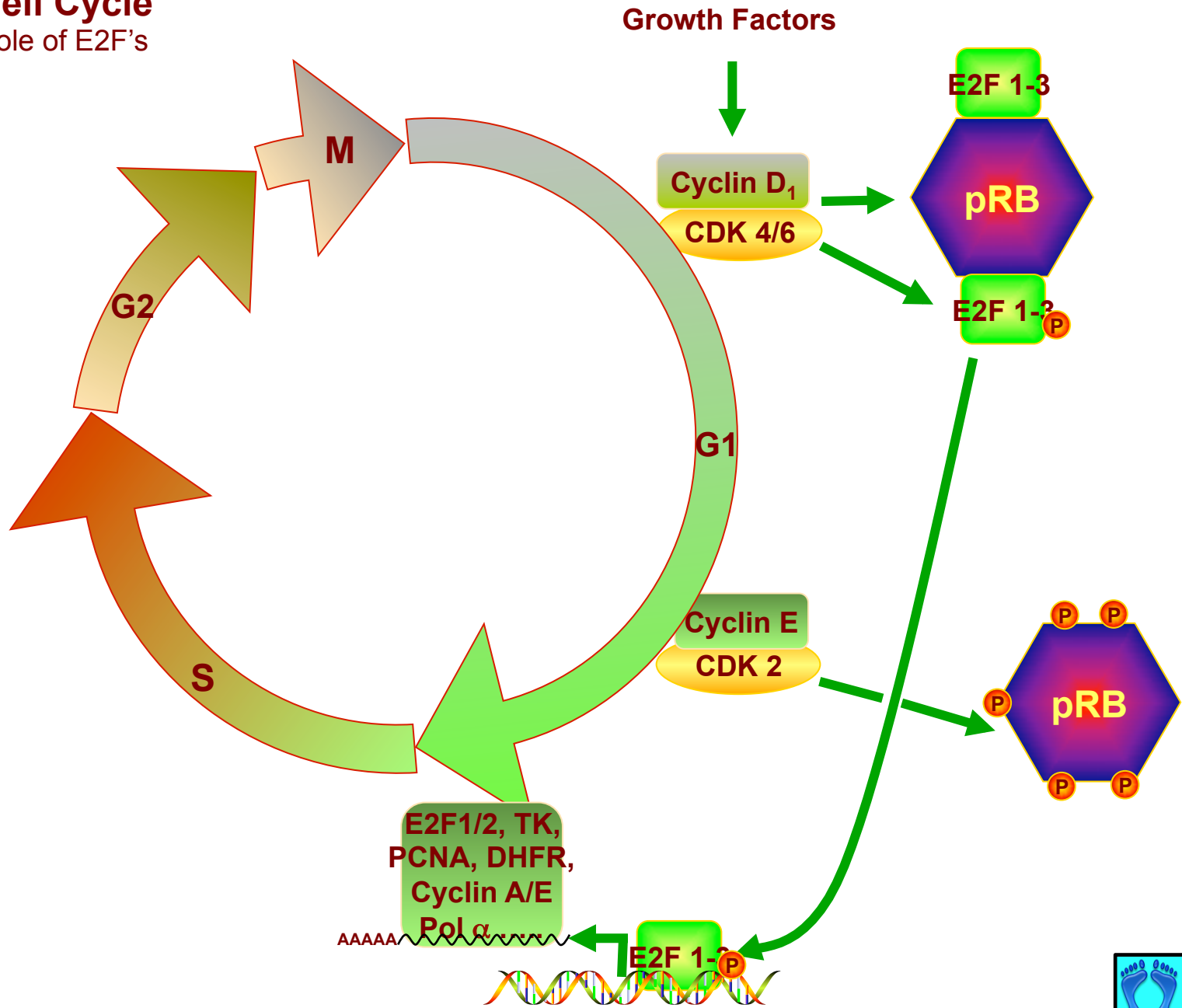
# The complex “sub pathway world”



**G1 CHECKPOINT**

# Cell Cycle

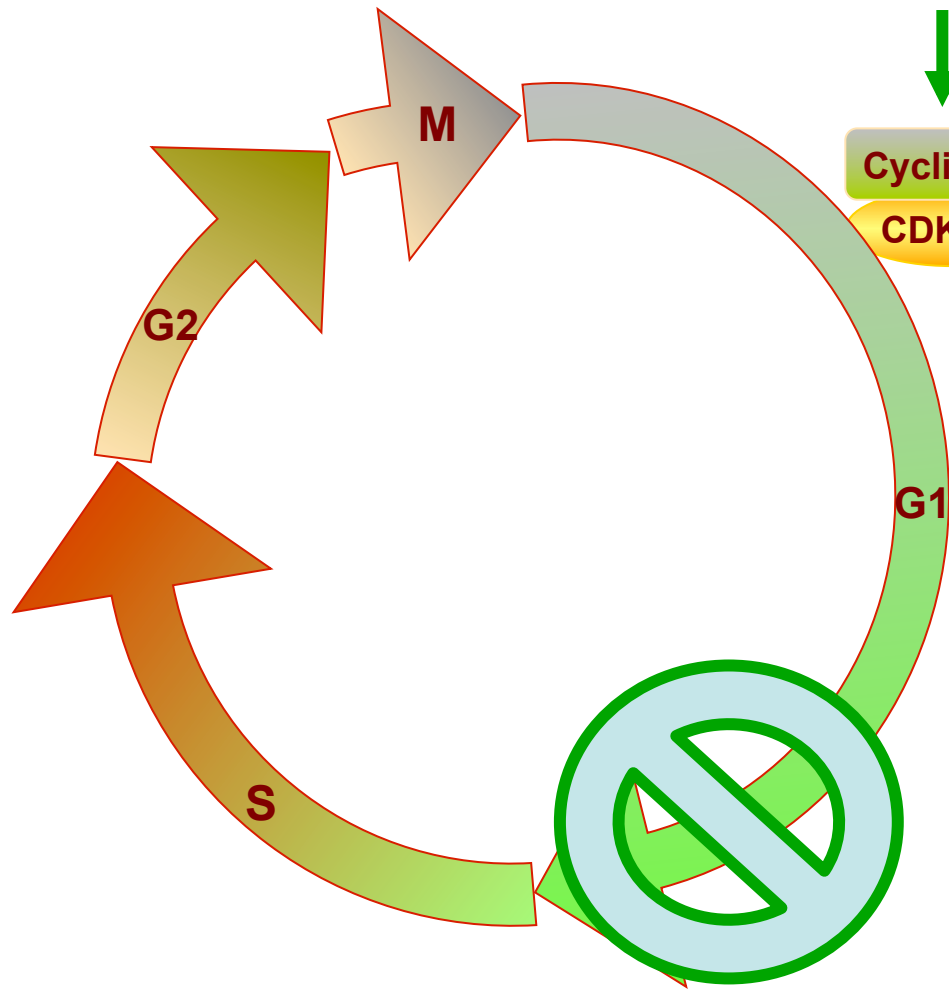
Role of E2F's



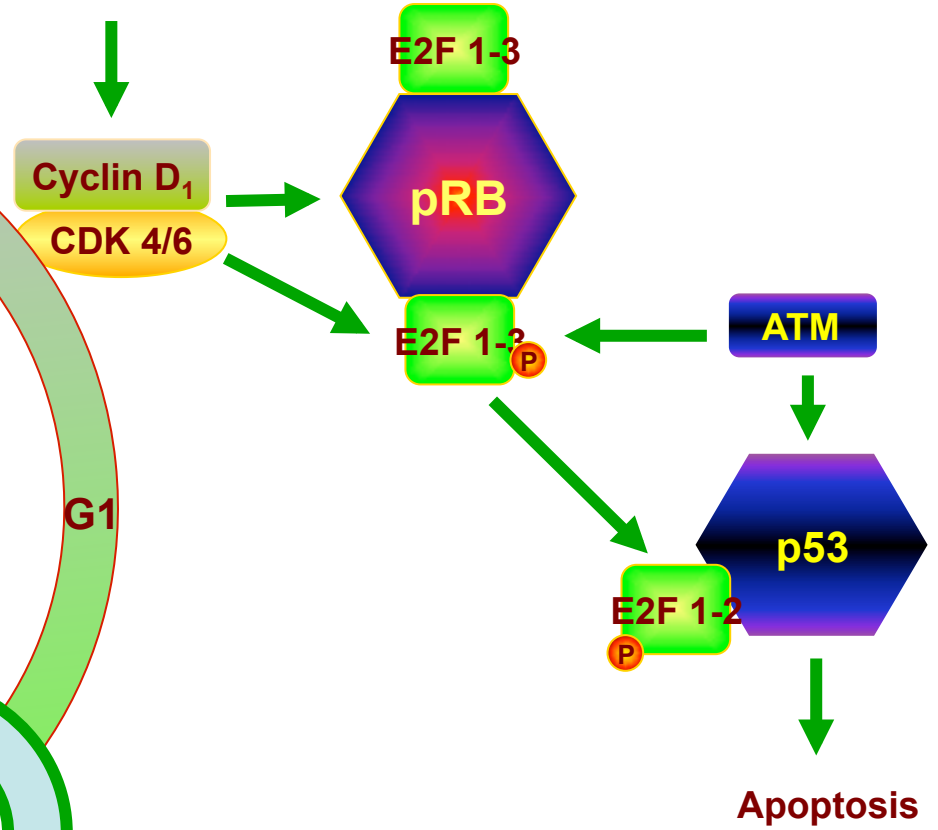


# Cell Cycle

Role of E2F's

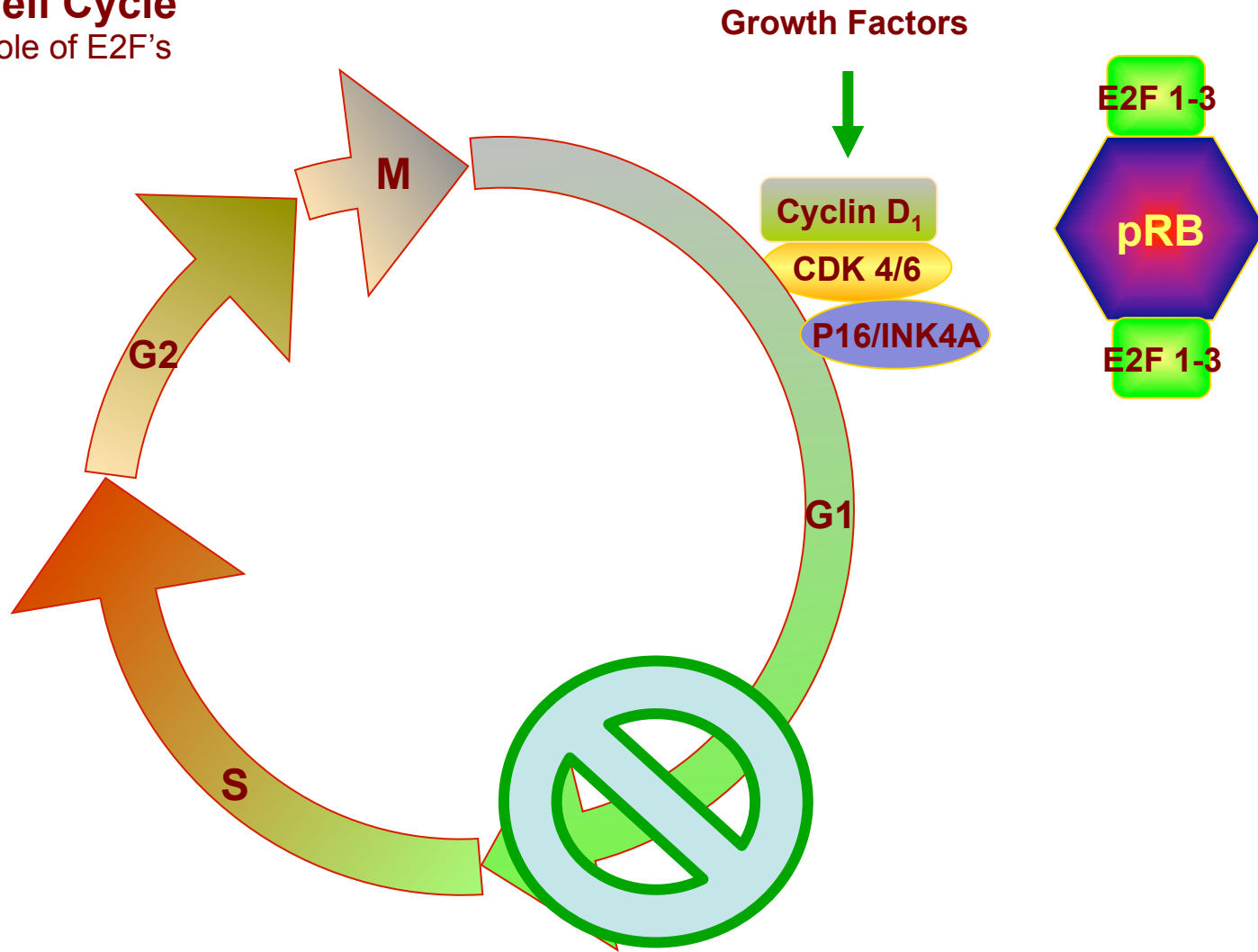


Growth Factors

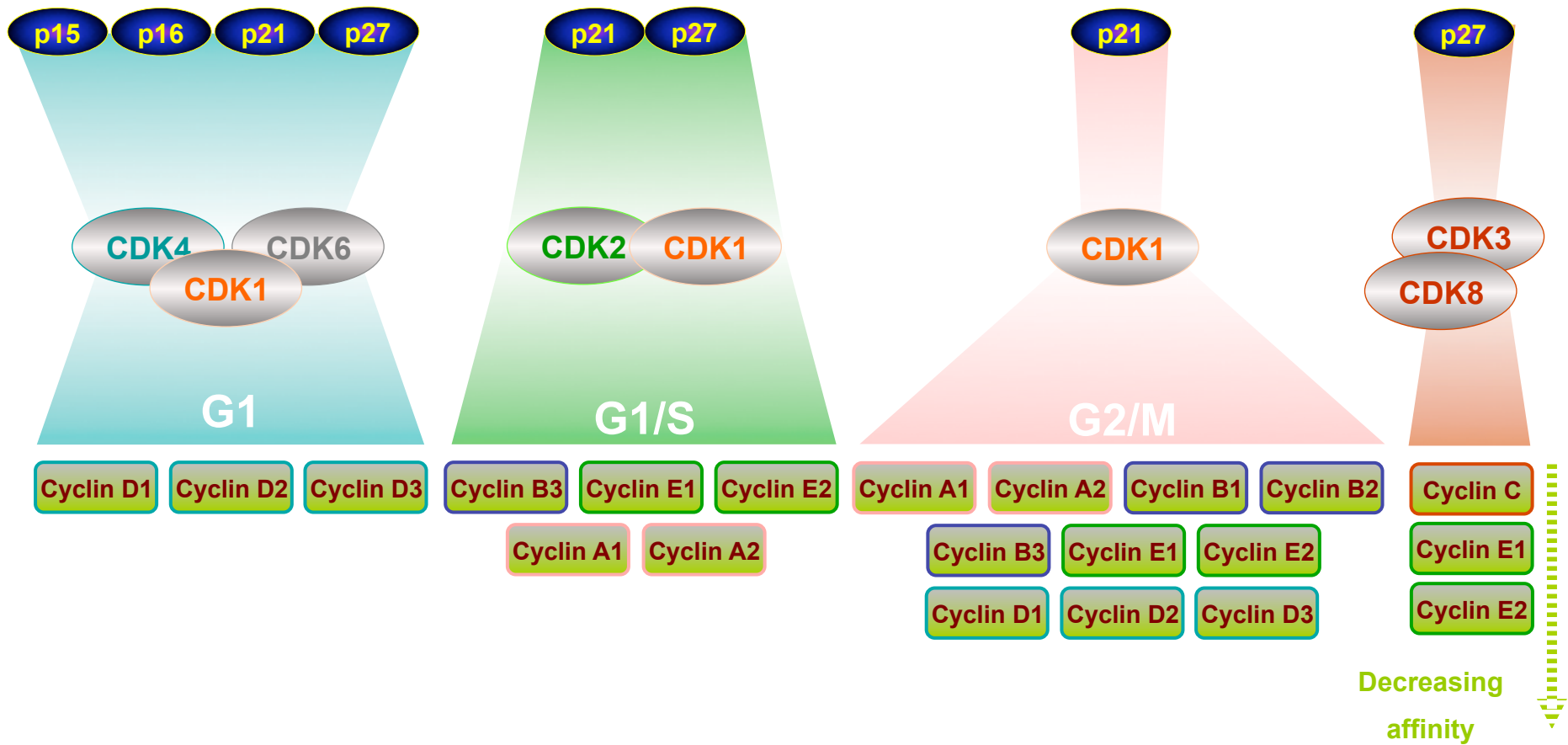


# Cell Cycle

Role of E2F's



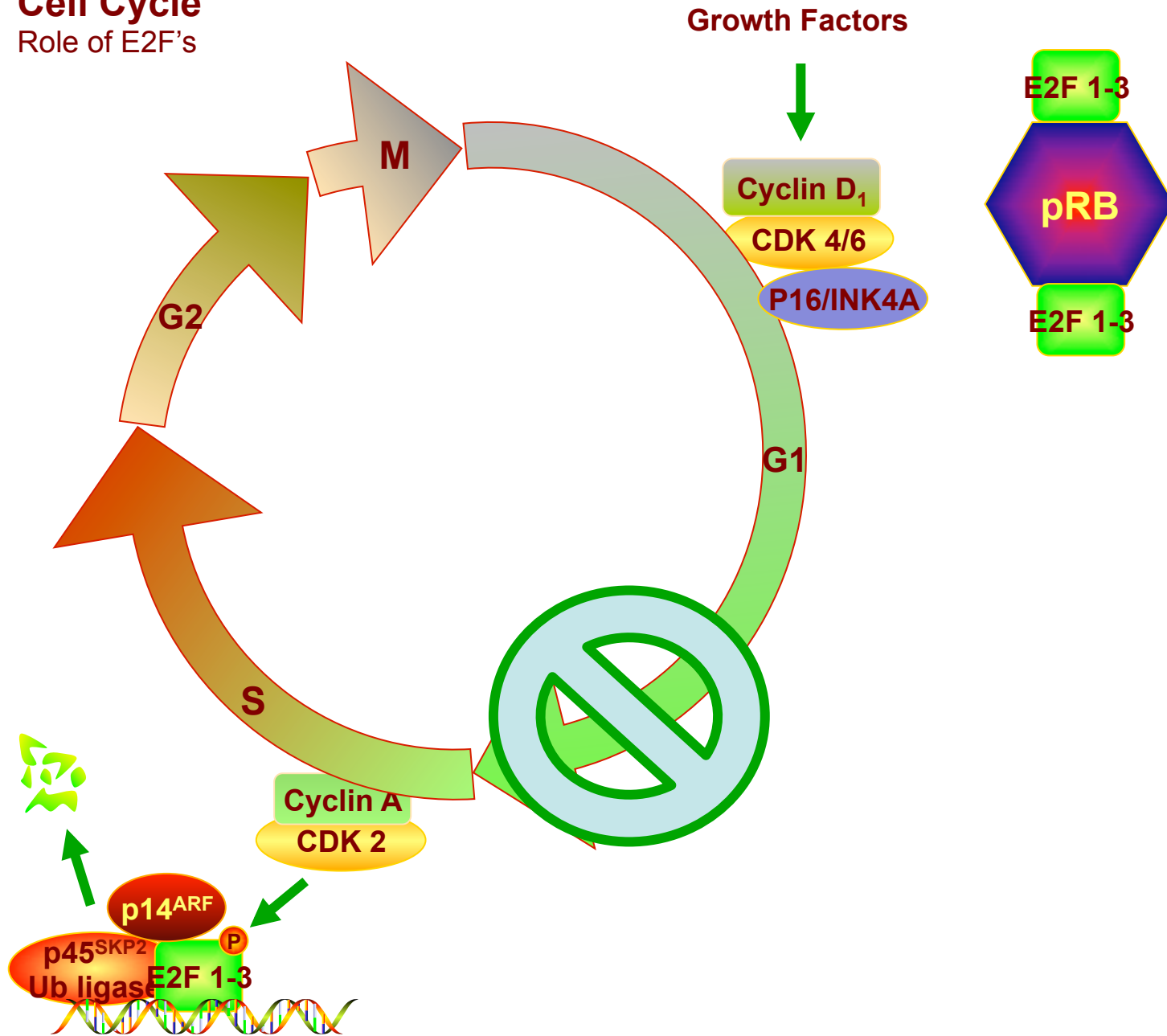
# Cyclin-CdK-CDKI binding specificities





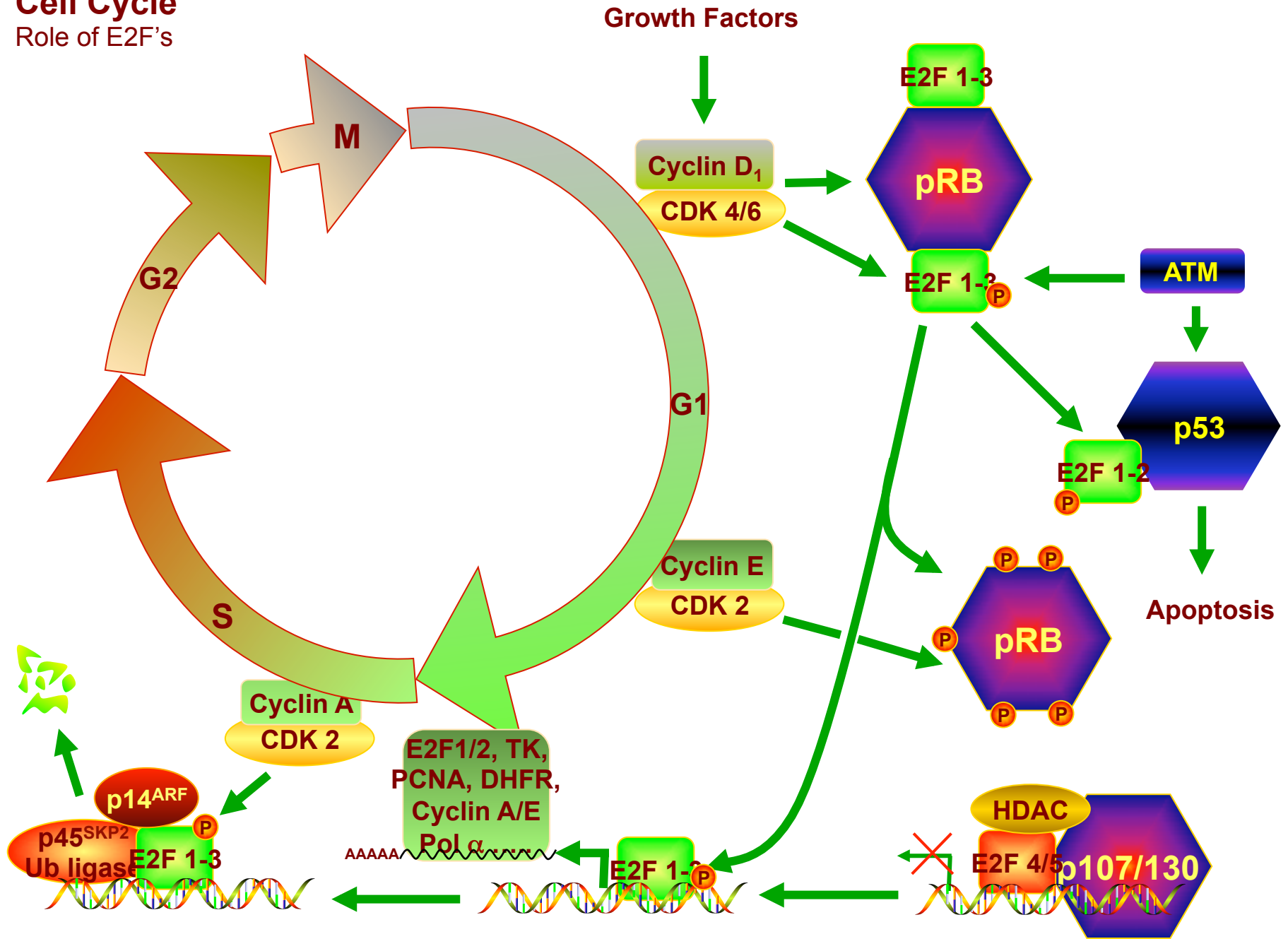
# Cell Cycle

Role of E2F's



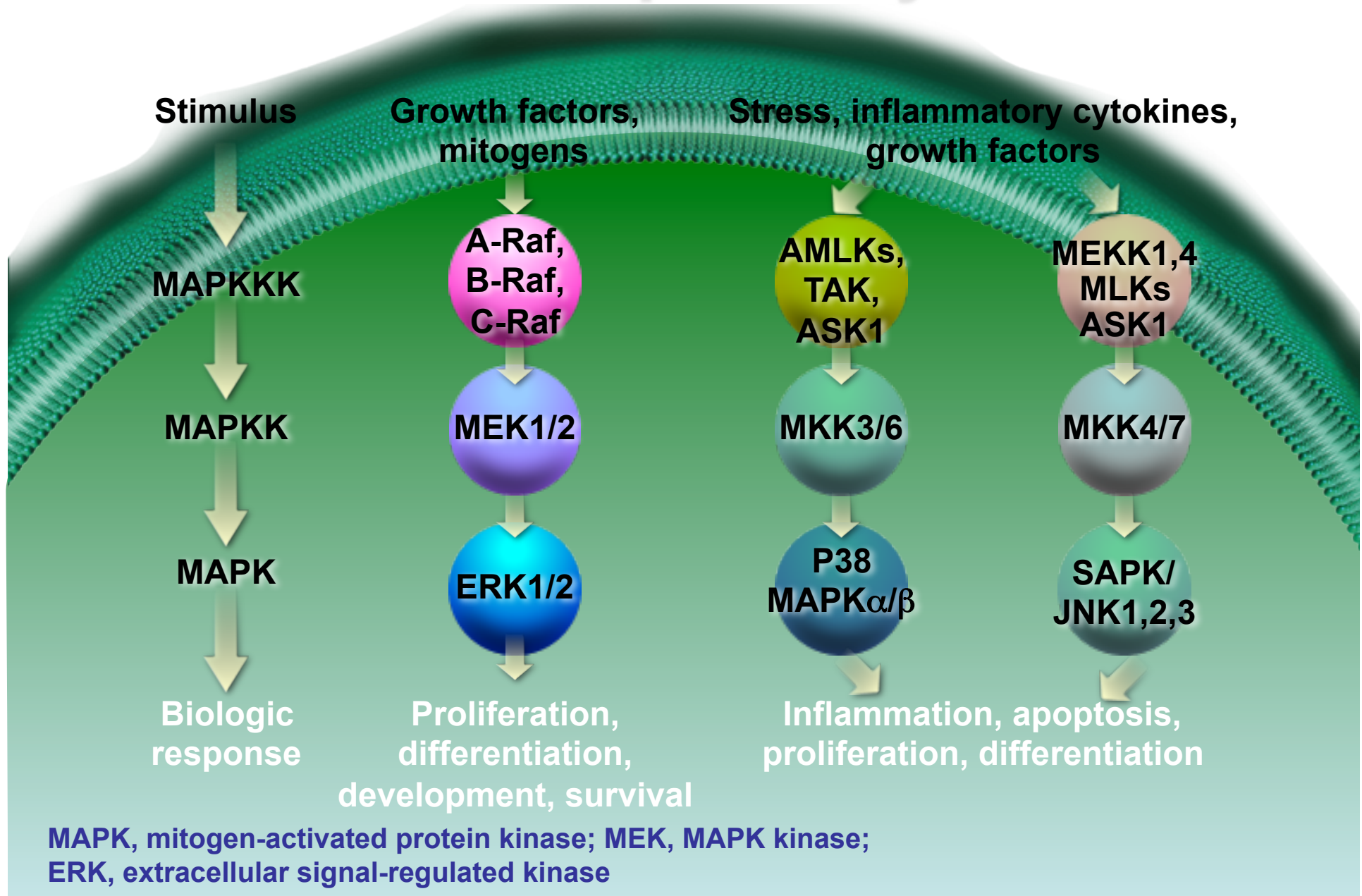
# Cell Cycle

Role of E2F's

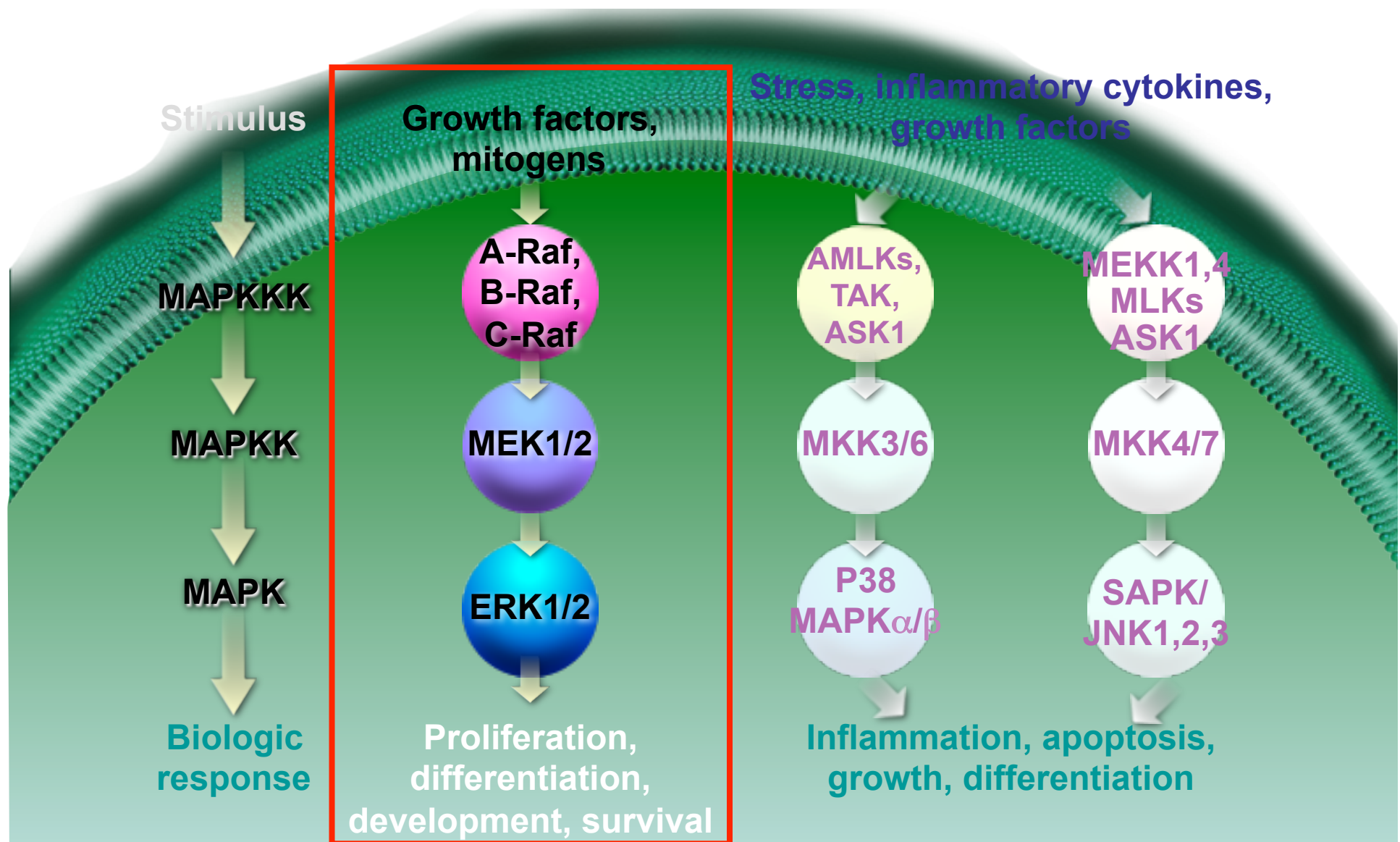


**PROLIFERATIVE STP**

# MAPK pathways

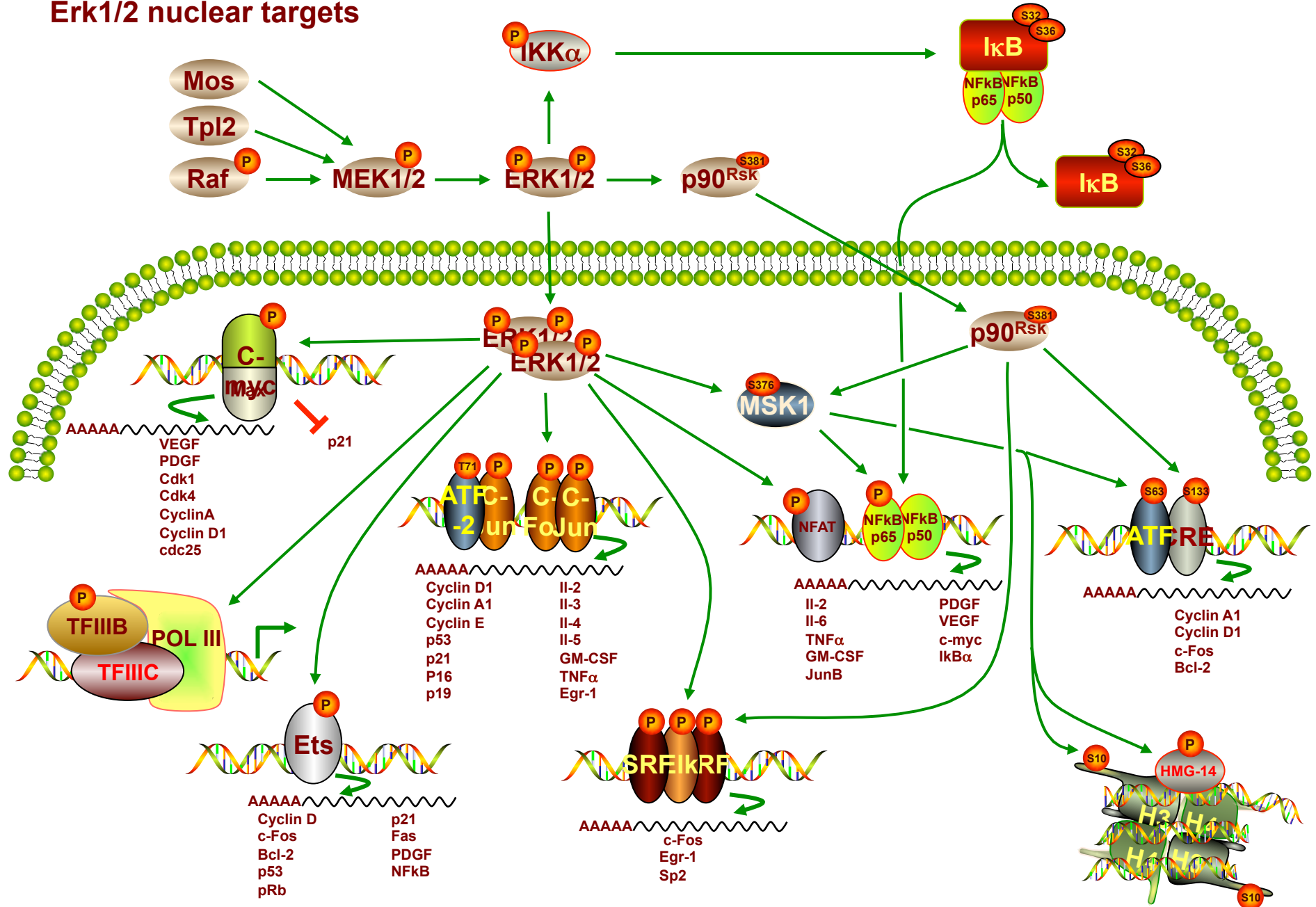


# MAPK pathways



MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; ERK, extracellular signal-regulated kinase

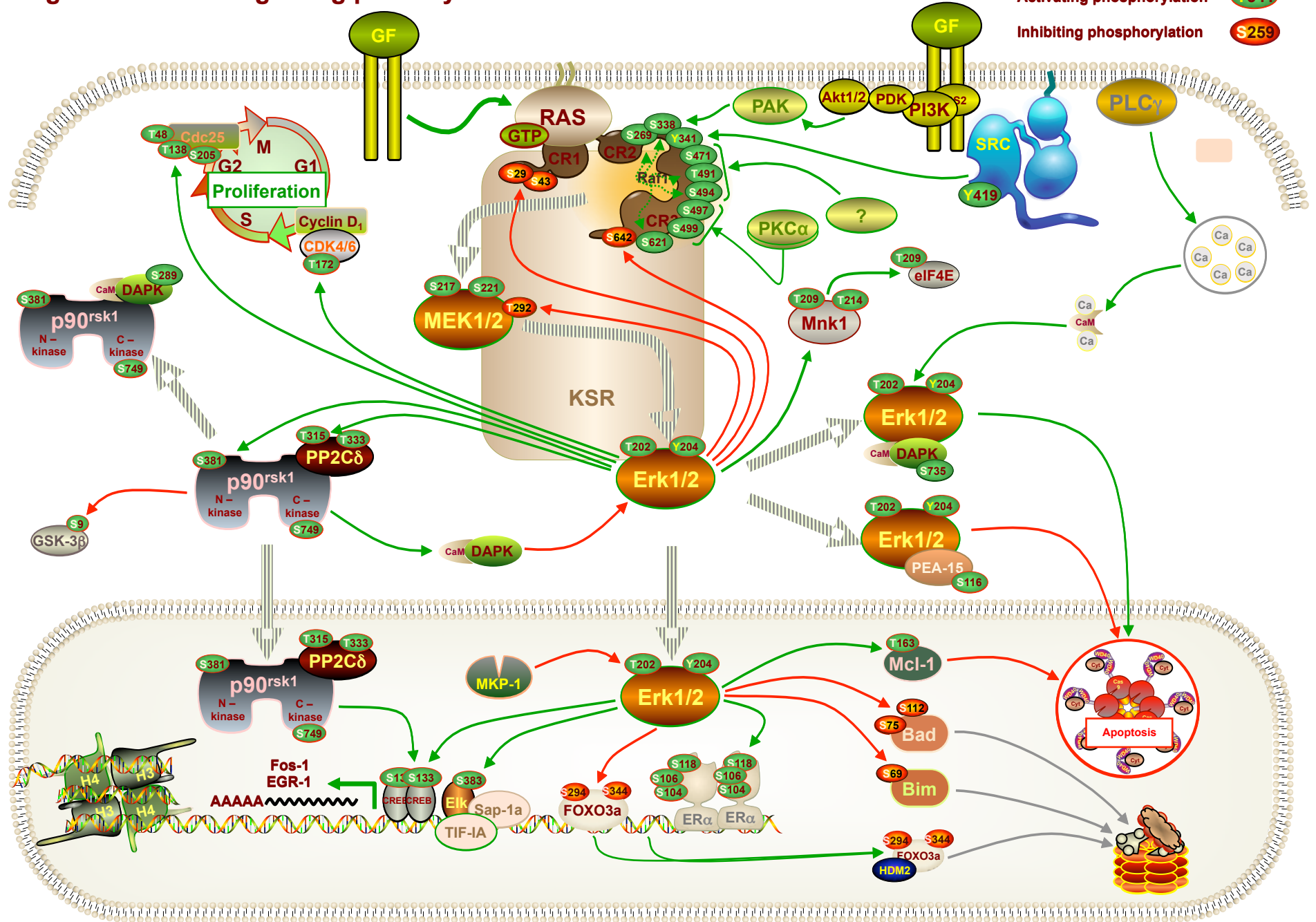
# Erk1/2 nuclear targets



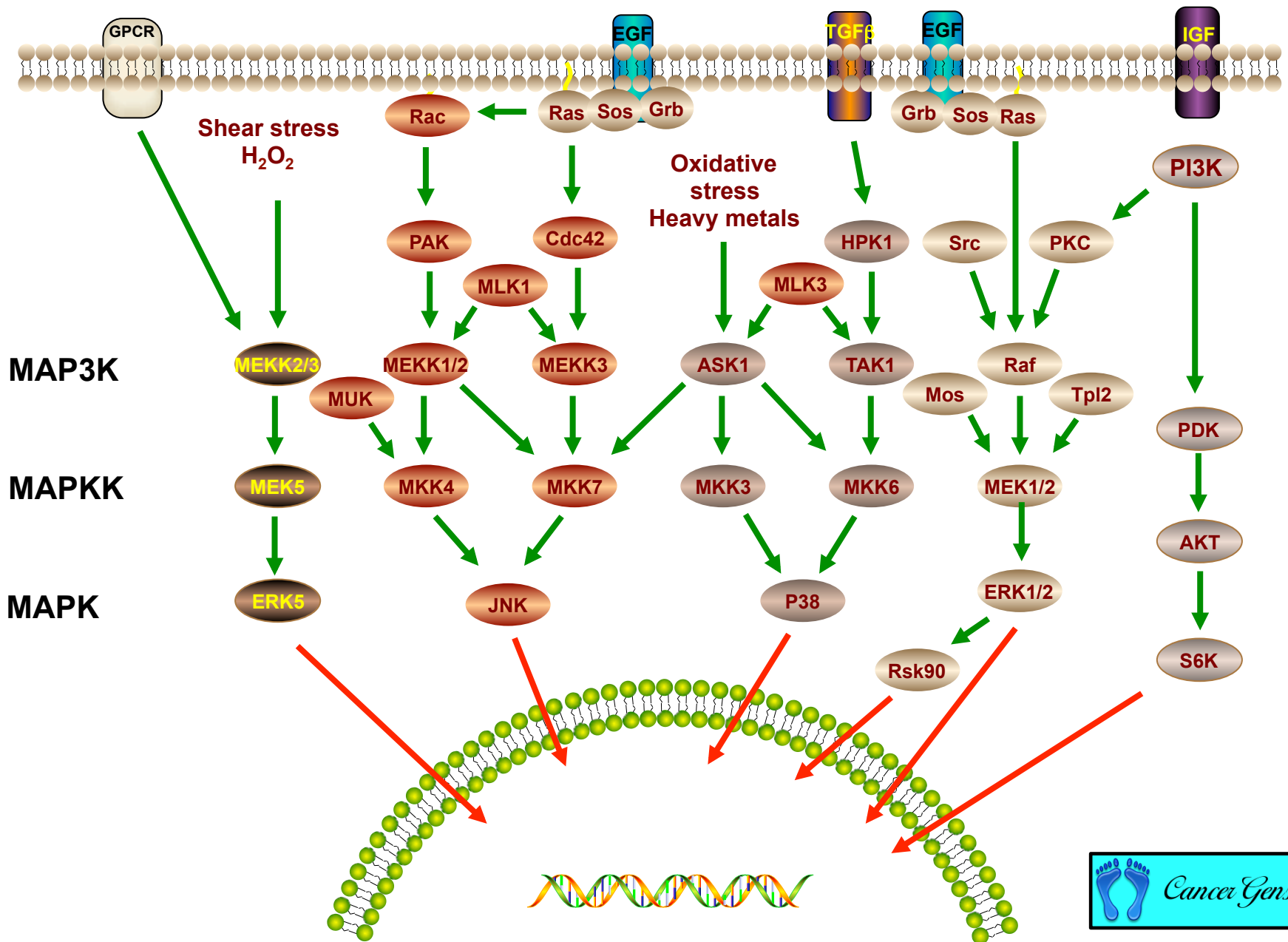


# Regulation of Erk signaling pathway

Activating phosphorylation **Y341**  
 Inhibiting phosphorylation **S259**

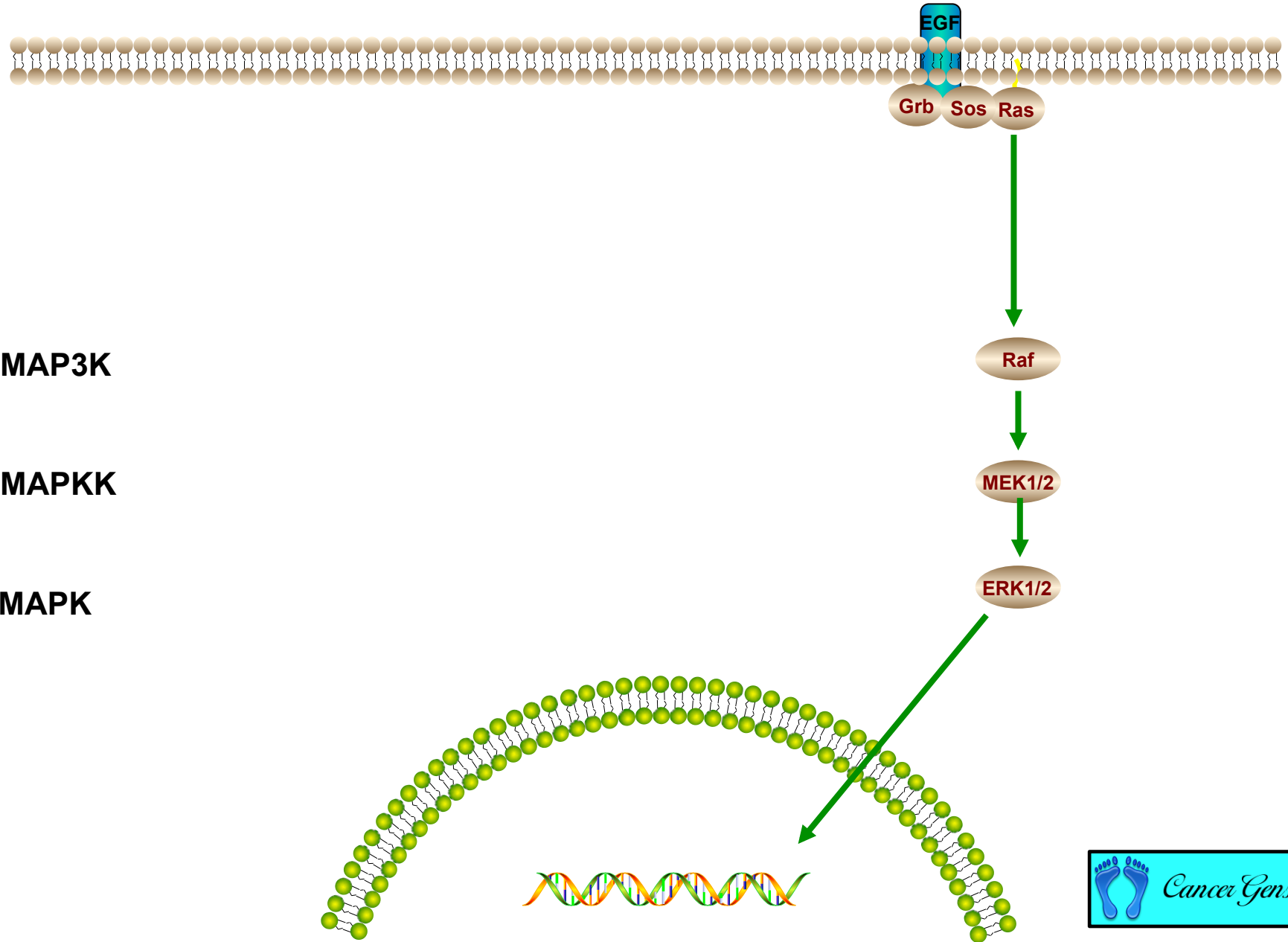


# MAPK signaling cascades

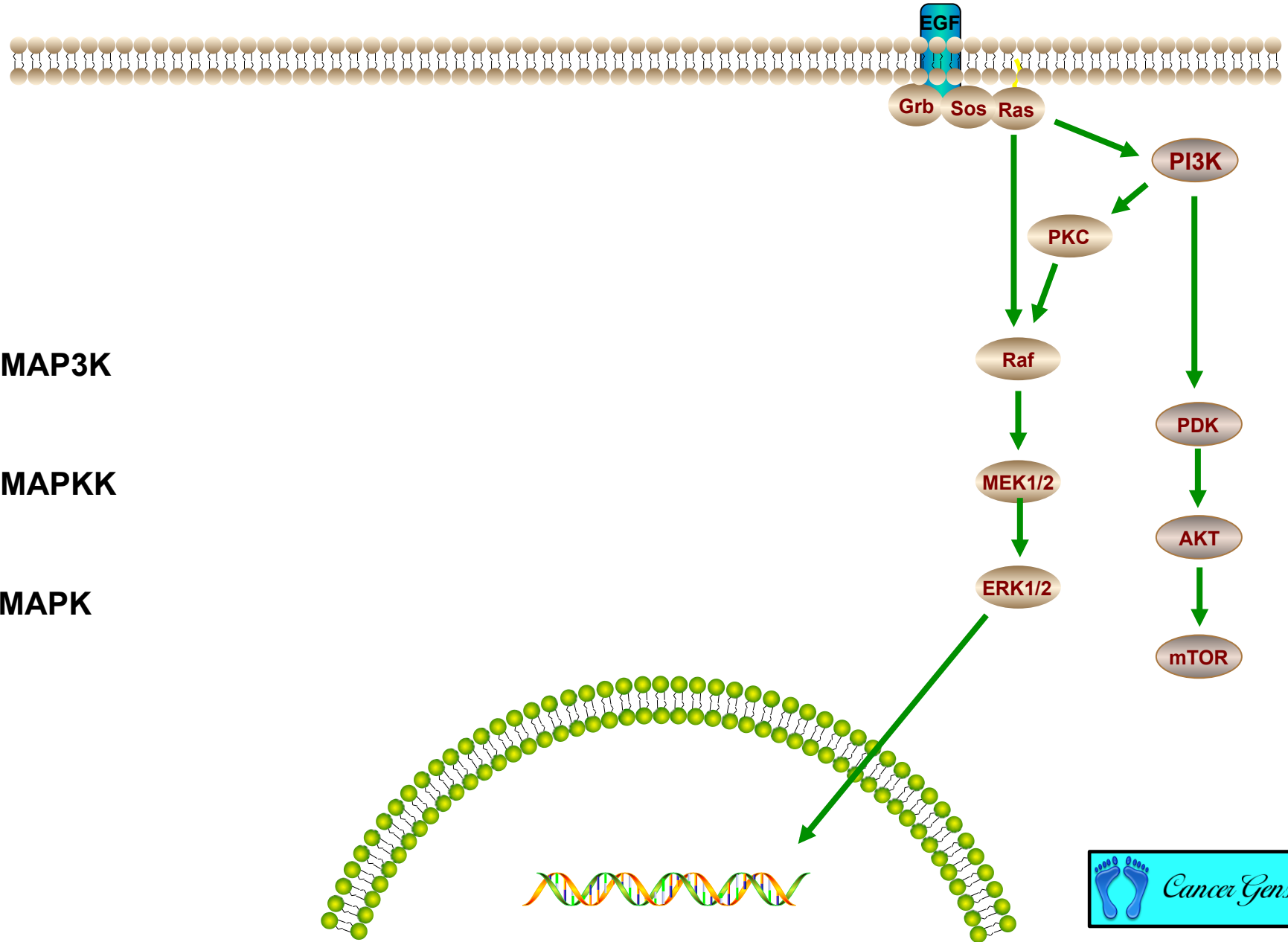




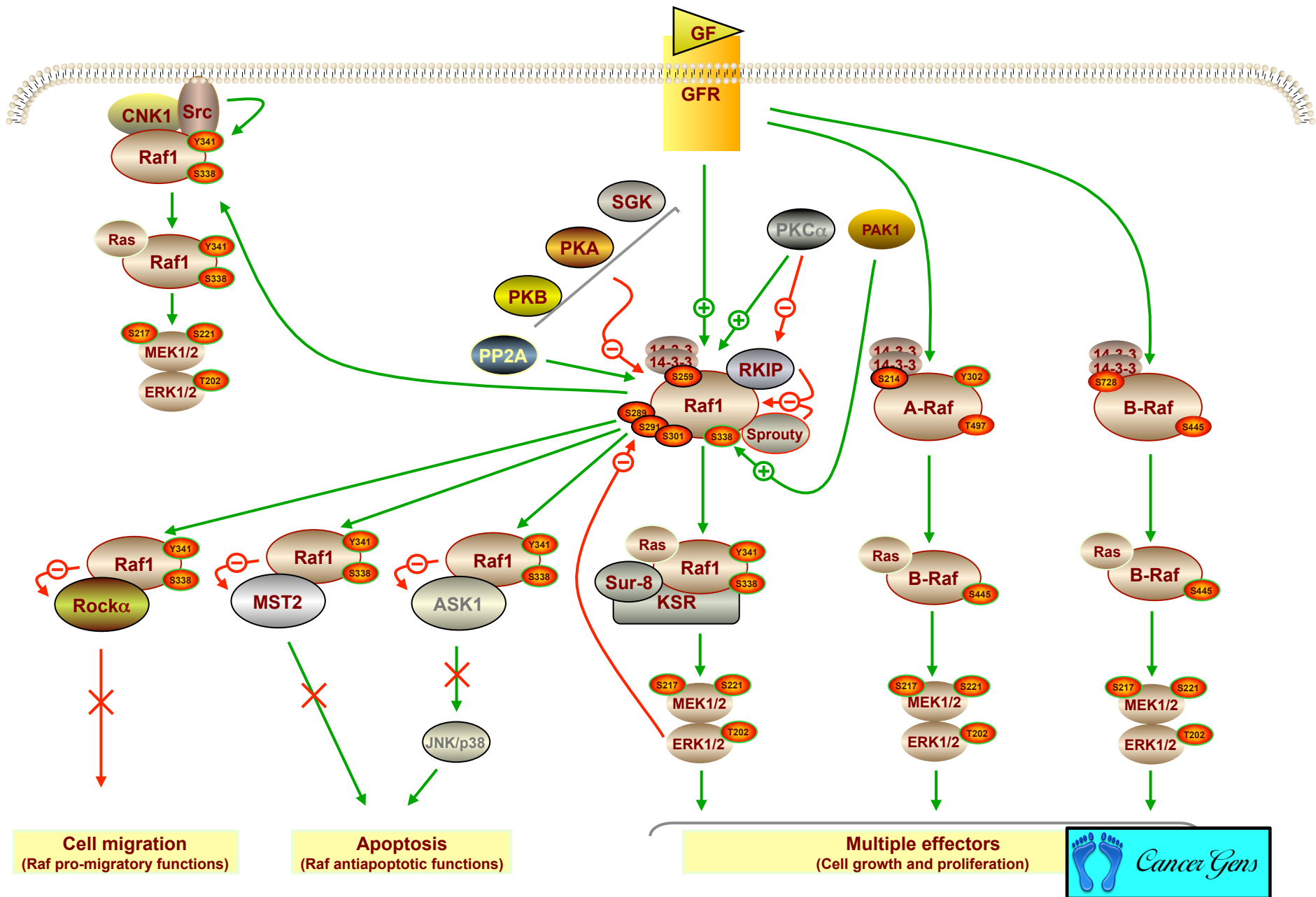
# MAPK signaling cascades



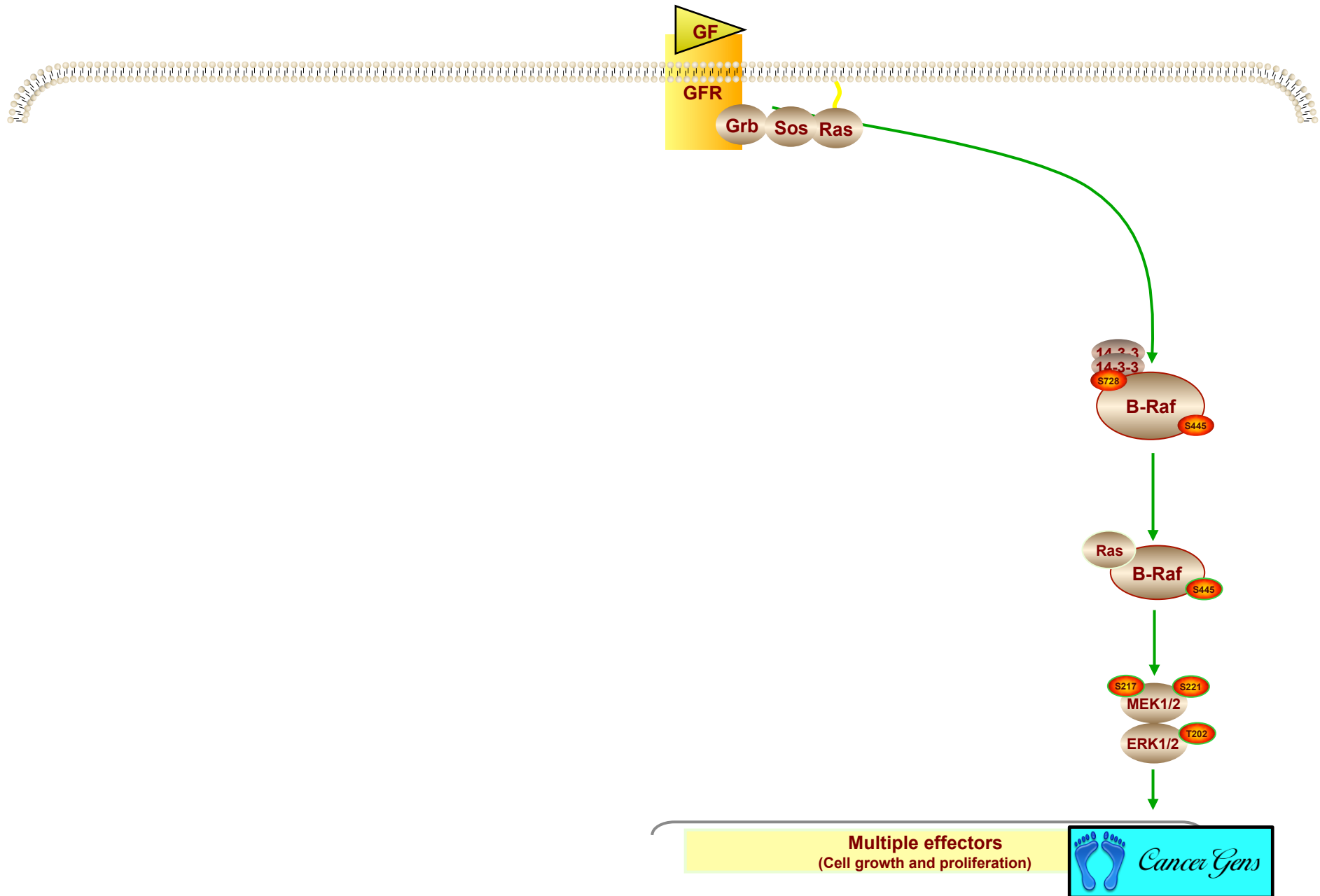
# MAPK signaling cascades



# Raf protein kinases – Targets and regulation

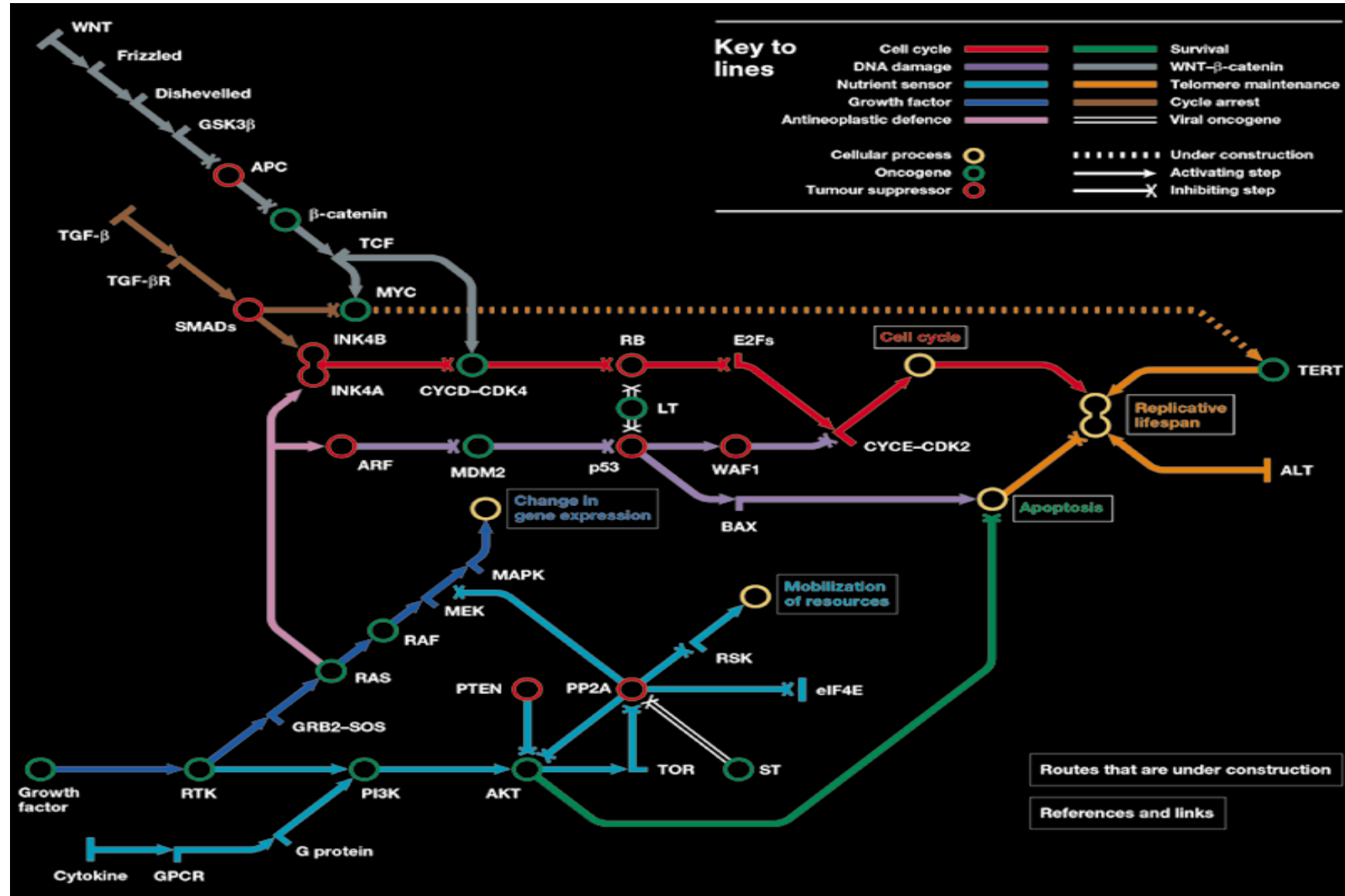


# Raf protein kinases – Targets and regulation



# **The EGFR family**

# The complex “sub pathway world”



Injections to newborn mice of crude submaxillary gland preparation  
cause premature eyelid opening / precocious tooth eruption  
(Stanley Cohen -1960)<sup>1</sup>



<sup>1</sup>. Cohen, S. (1960) *Proc. Natl. Acad. Sci. USA* 46, 302-311

**Injections to newborn mice of crude submaxillary gland preparation cause premature eyelid opening / precocious tooth eruption**  
(Cohen, S -1960)<sup>1</sup>

**EGF directly stimulated the proliferation of epidermal cells in organ cultures of chick embryo skin**  
(Cohen, S -1965)<sup>2</sup>

**Fibroblasts in culture responded to EGF with enhanced DNA synthesis**  
(Armelin, H -1973)<sup>3</sup>

**EGFR is membrane-bound**  
(Carpenter, G and Cohen, S -1976)<sup>4</sup>

**EGFR is a Tyrosine-Kinase**  
(Ushiro, H and Cohen, S -1980)<sup>5</sup>

**Aminoacid sequence / Homology to the *Erb-B* ...**  
(Yarden Y, et al -1984)<sup>6</sup>

1. Cohen, S. (1960) *Proc. Natl. Acad. Sci. USA* 46, 302-311

2. Cohen, S. (1965) *Dev. Biol.* 12, 394-407.

3. Armelin, H. (1973) *Proc. Natl. Acad. Sci. USA* 70, 2702-2706.

4. Carpenter, G. and Cohen, S. (1976) *J. Cell Biol.* 71, 159-171.

5. Ushiro, H. and Cohen, S. (1980) *J. Biol. Chem.* 255, 8363-8365.

6. Yarden, Y., et al. (1984) *Nature* 309, 418-425



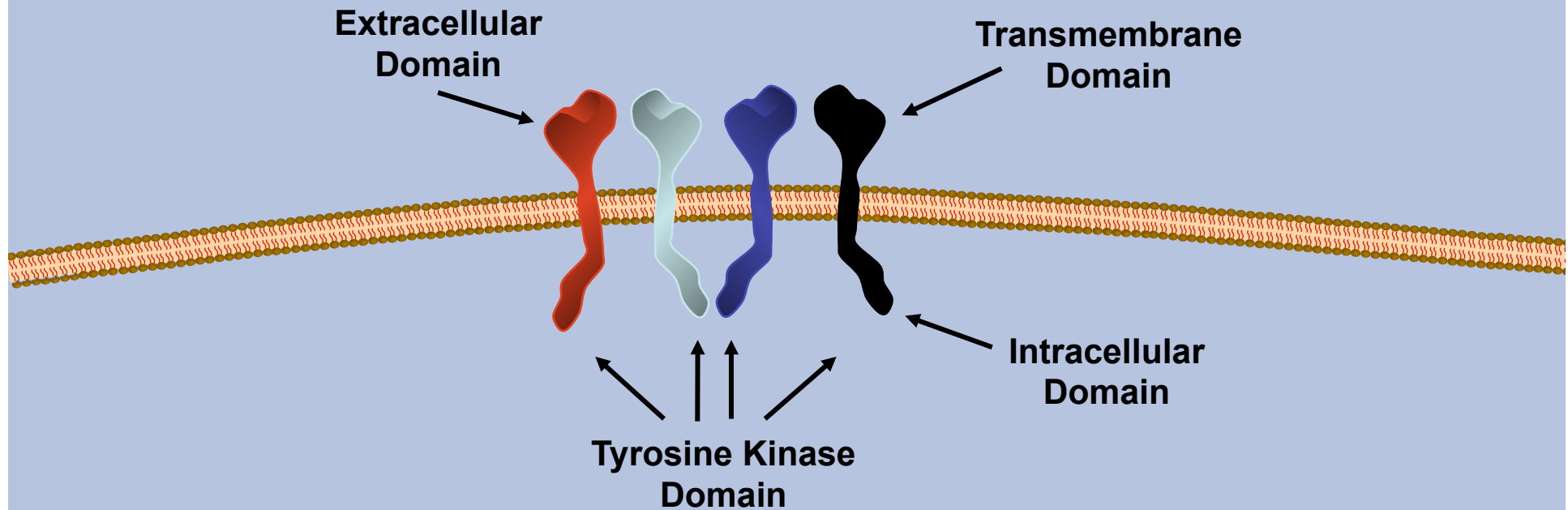


## Nobel Prize (1986): Stanley Cohen

1. Cohen, S. (1960) *Proc. Natl. Acad. Sci. USA* 46, 302-311
2. Cohen, S. (1965) *Dev. Biol.* 12, 394-407.
3. Armelin, H. (1973) *Proc. Natl. Acad. Sci. USA* 70, 2702-2706.
4. Carpenter, G. and Cohen, S. (1976) *J. Cell Biol.* 71, 159-171.
5. Ushiro, H. and Cohen, S. (1980) *J. Biol. Chem.* 255, 8363-8365.

# EGF Pathway

- **EGFR: transmembrane protein**



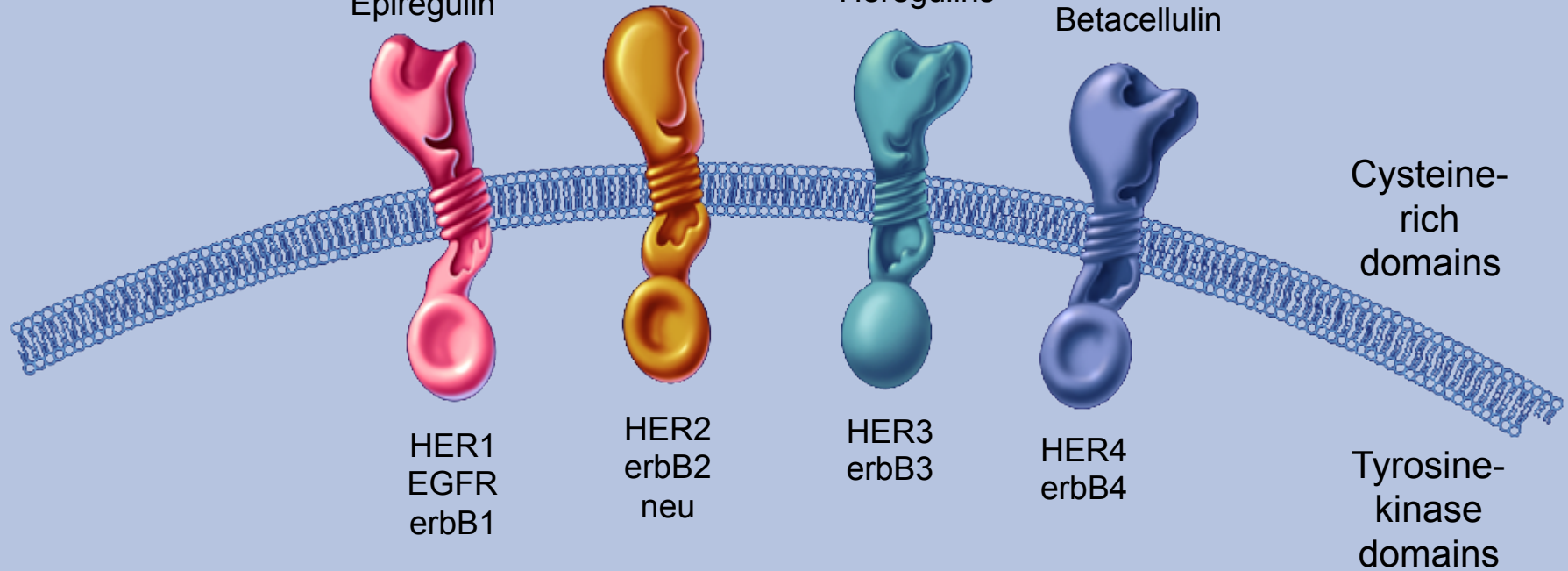
Adapted from:  
Ciardiello F, et al. N Engl J Med. 2008;358:1160-1174.

# HER/erbB family

Ligands:

EGF  
TGF- $\alpha$   
Amphiregulin  
Betacellulin  
HB-EGF  
Epiregulin

NRG2  
NRG3  
Heregulins  
Betacellulin



HER1  
EGFR  
erbB1

HER2  
erbB2  
neu

HER3  
erbB3

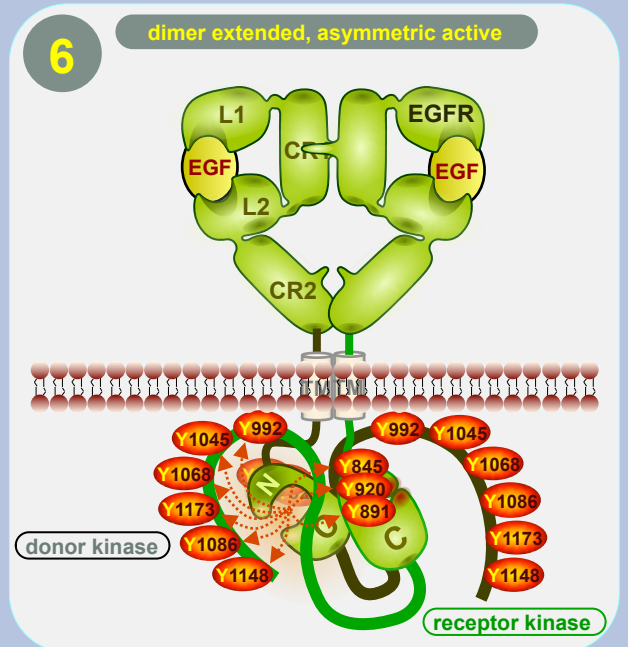
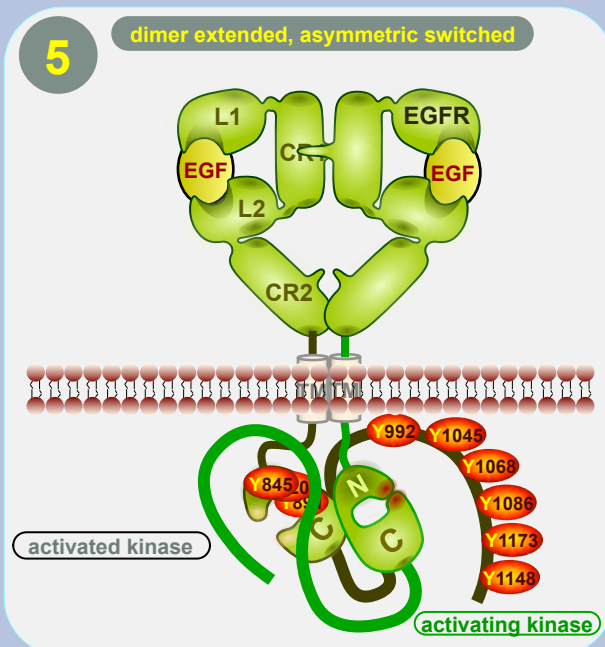
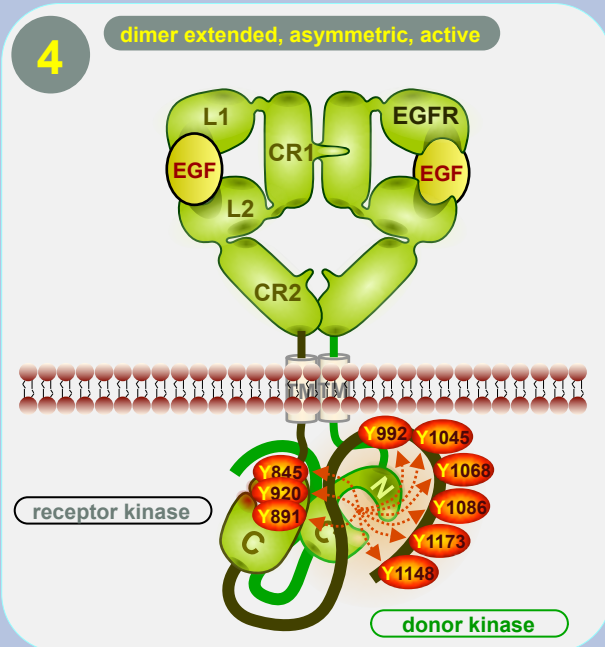
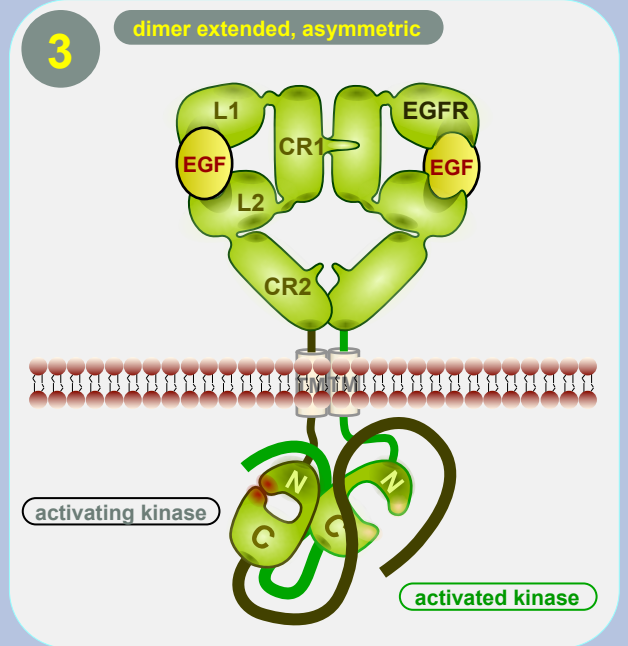
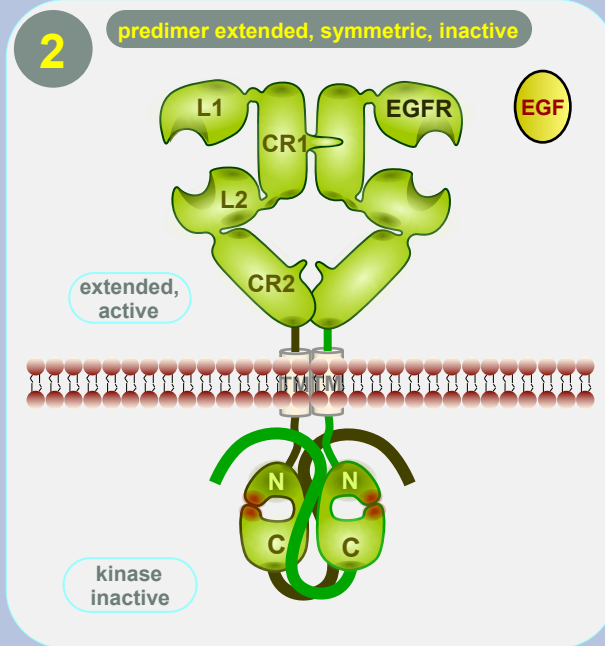
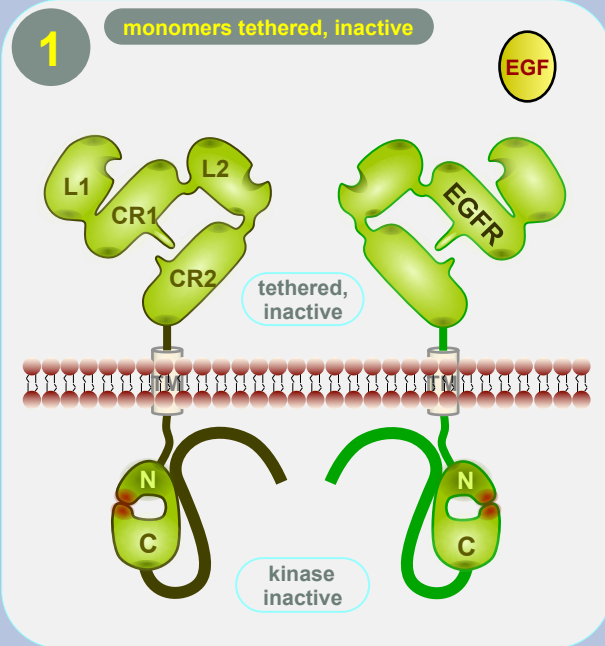
HER4  
erbB4

Salomon DS, et al. Crit Rev Oncol Hematol 1995;19:183–232  
Woodburn JR. Pharmacol Ther 1999;82:241–50

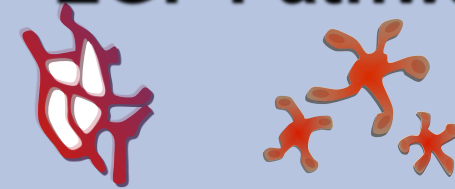
**HER1/EGFR**

# Stepwise EGFR ligand binding and tyrosine phosphorylation

Phosphotyrosine **Y1146**

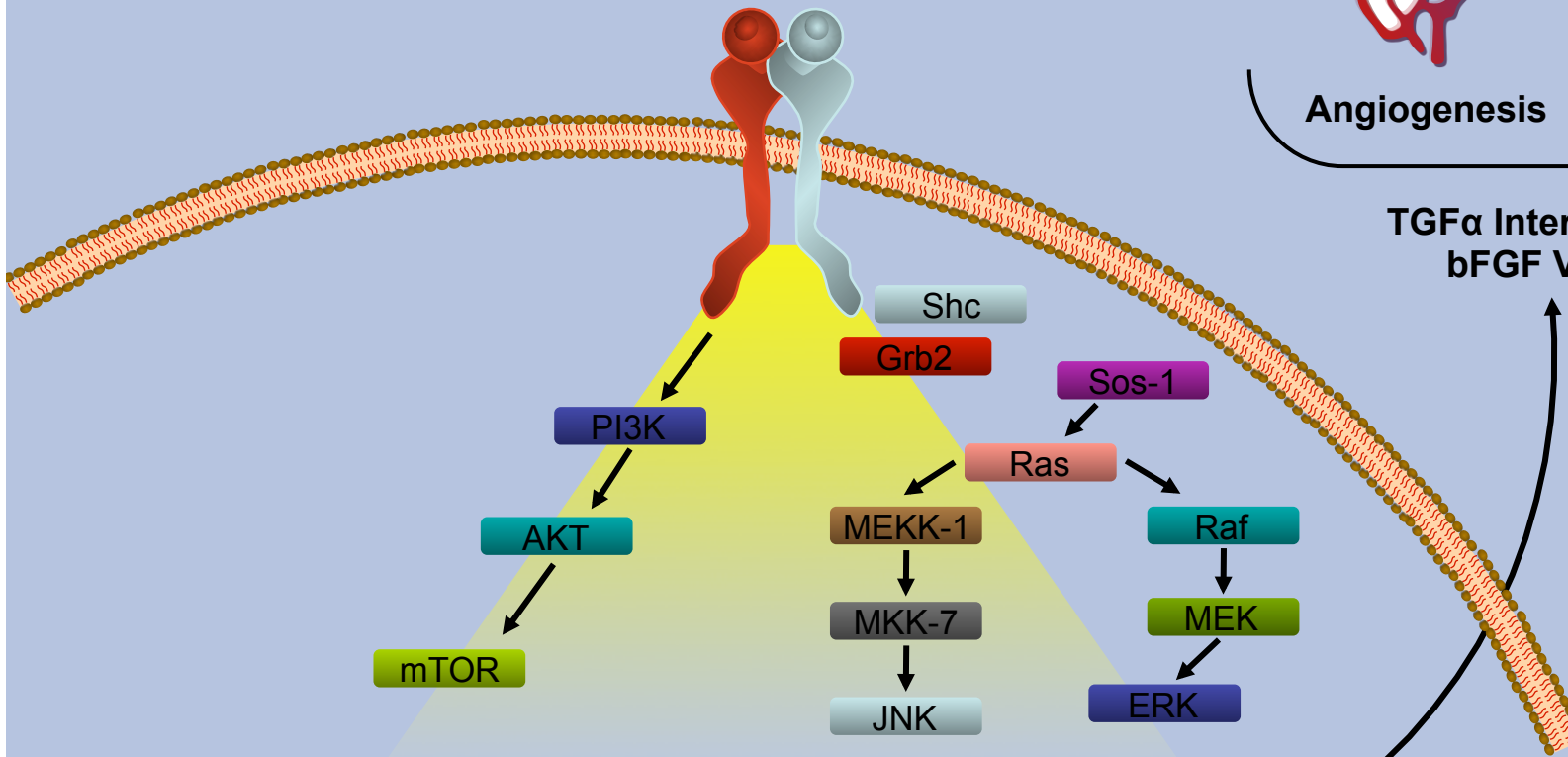


# EGF Pathway



Angiogenesis Metastasis

TGF $\alpha$  Interleukin-8  
bFGF VEGF



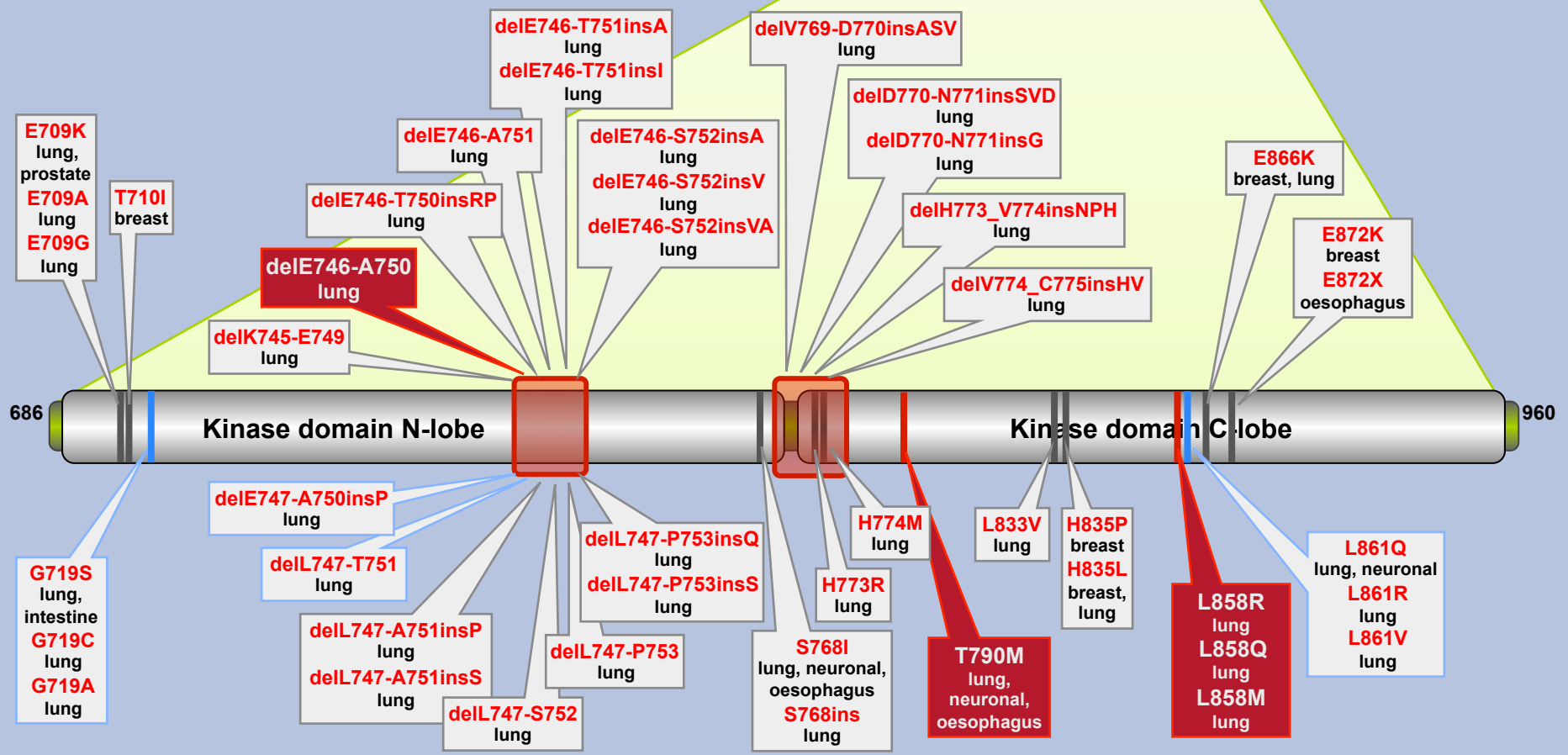
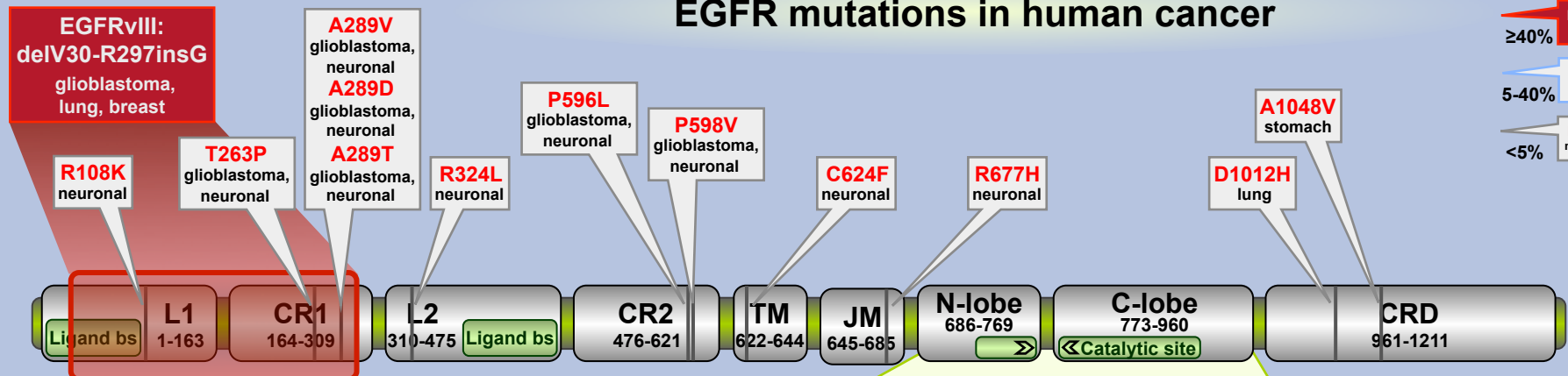
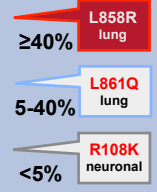
Apoptosis Resistance

Proliferation

Transcription

# EGFR mutations in human cancer

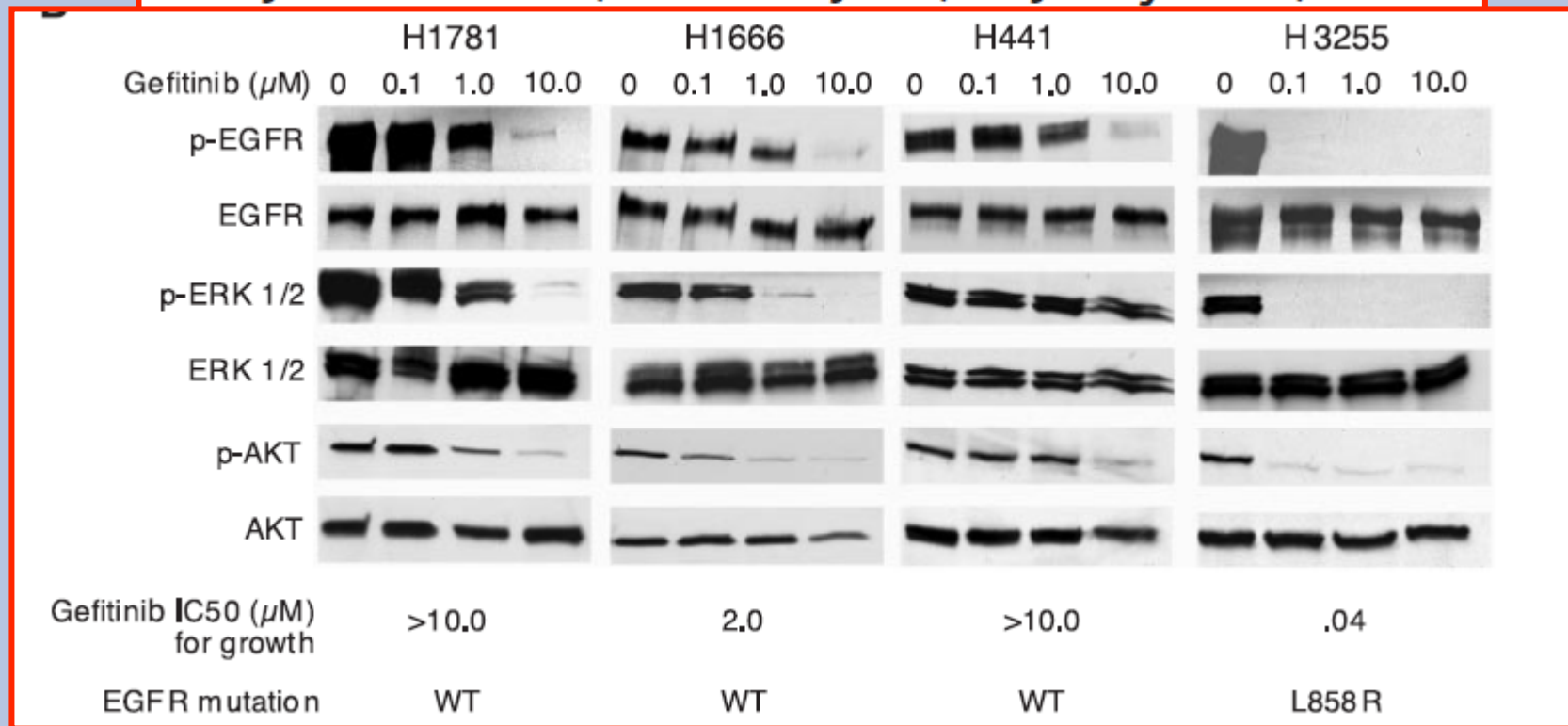
Frequency of mutation:





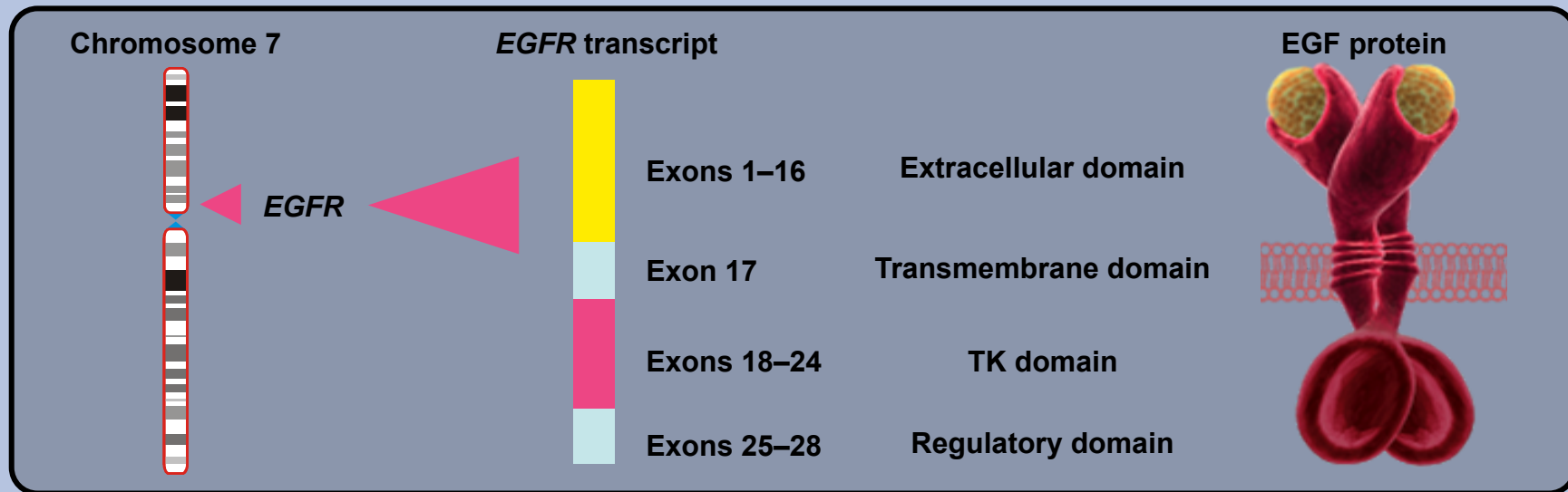
# EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy

J. Guillermo Paez,<sup>1,2\*</sup> Pasi A. Jänne,<sup>1,2\*</sup> Jeffrey C. Lee,<sup>1,3\*</sup>

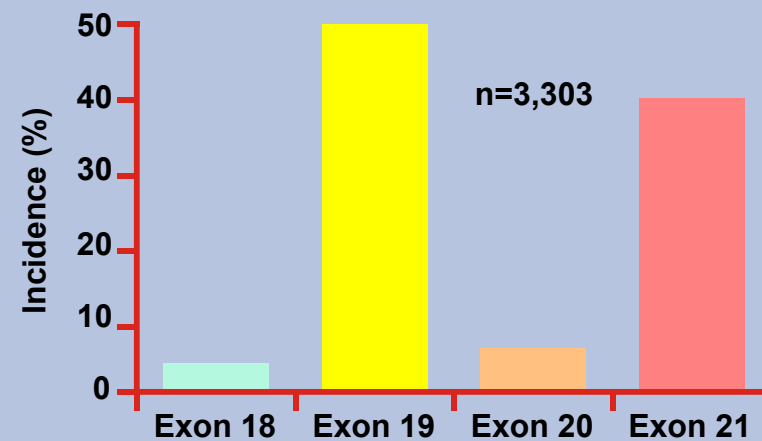




# EGFR mutation +ve NSCLC: different epidemiology

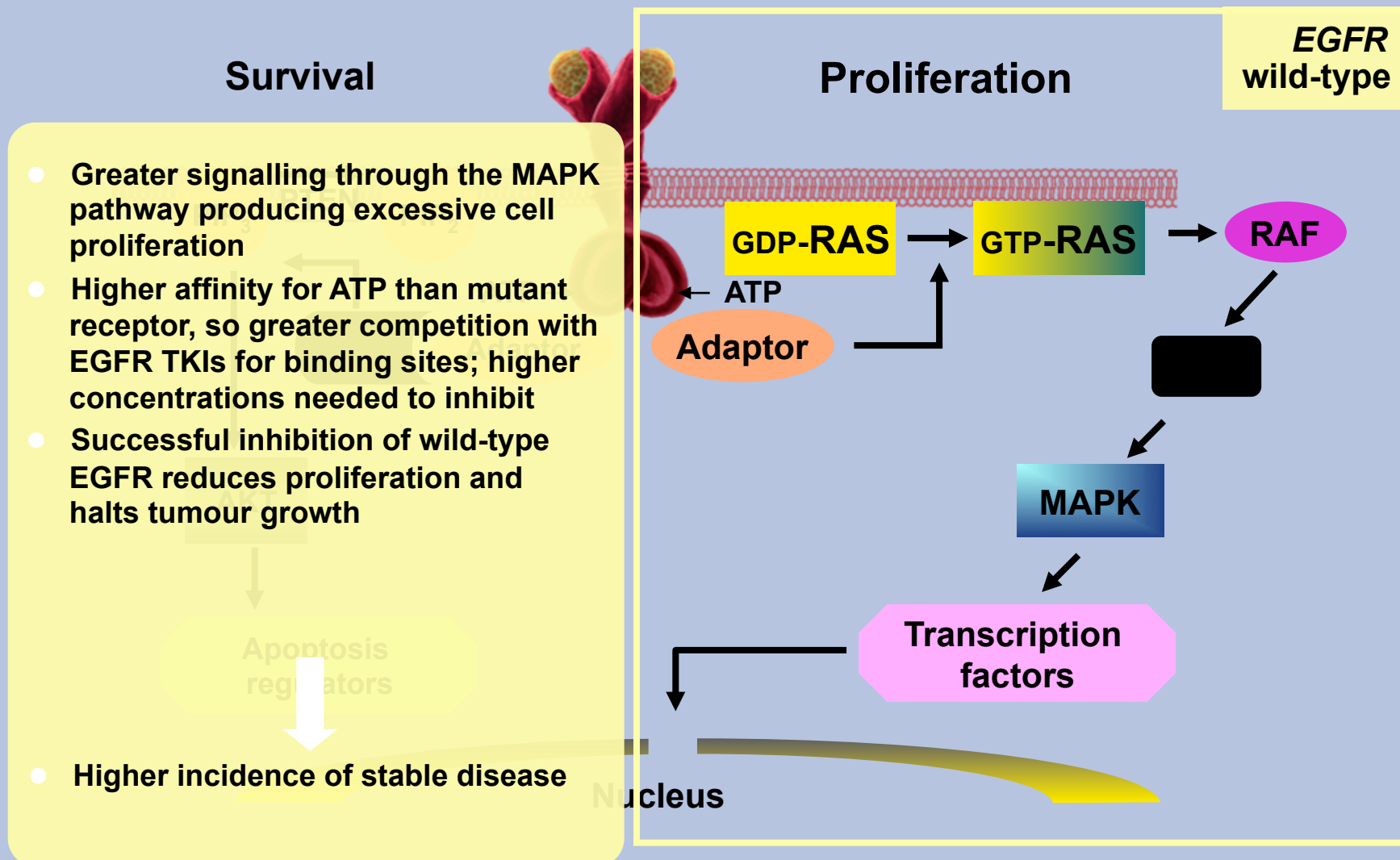


- Majority of mutations are exon 19 deletions or L858R point mutations in exon 21

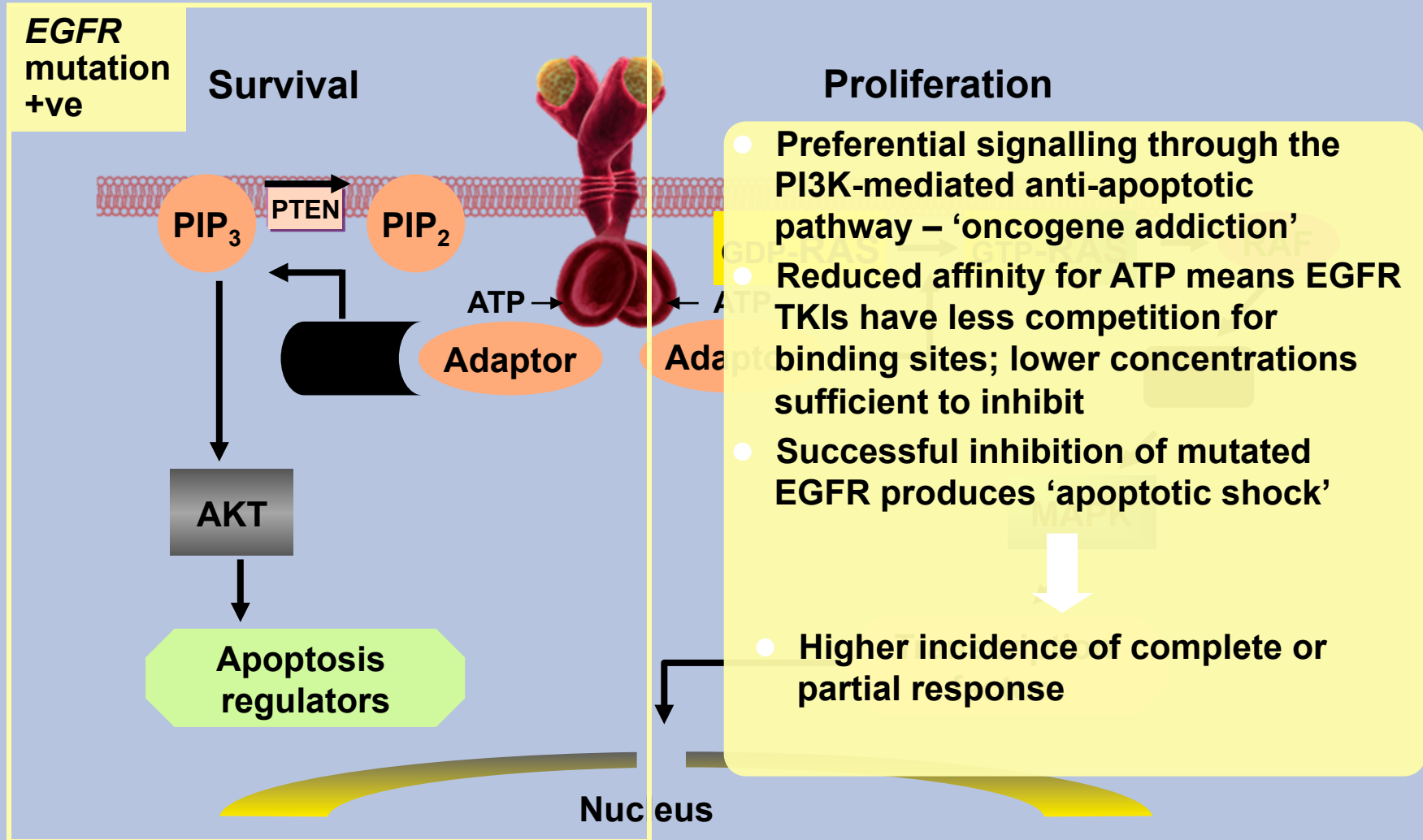


Shigematsu, et al. JNCI 2005; Murray, et al. JTO 2008

# EGFR in NSCLC: two distinct pathways

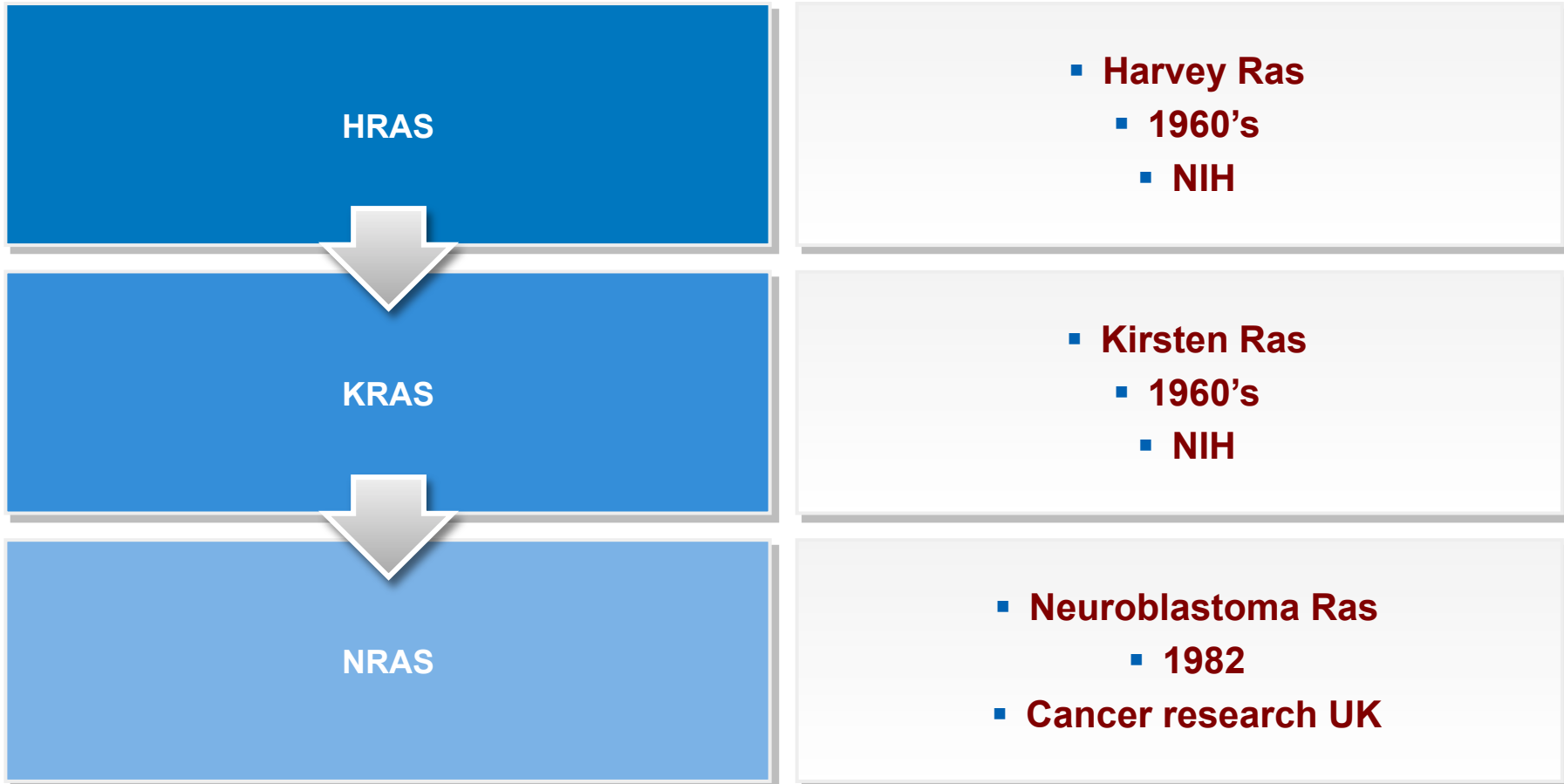


# EGFR in NSCLC: two distinct pathways

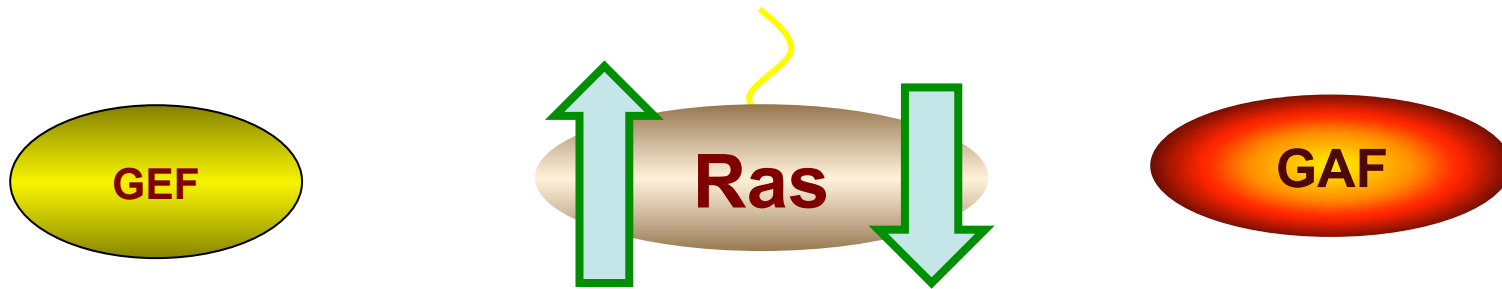


# Ras

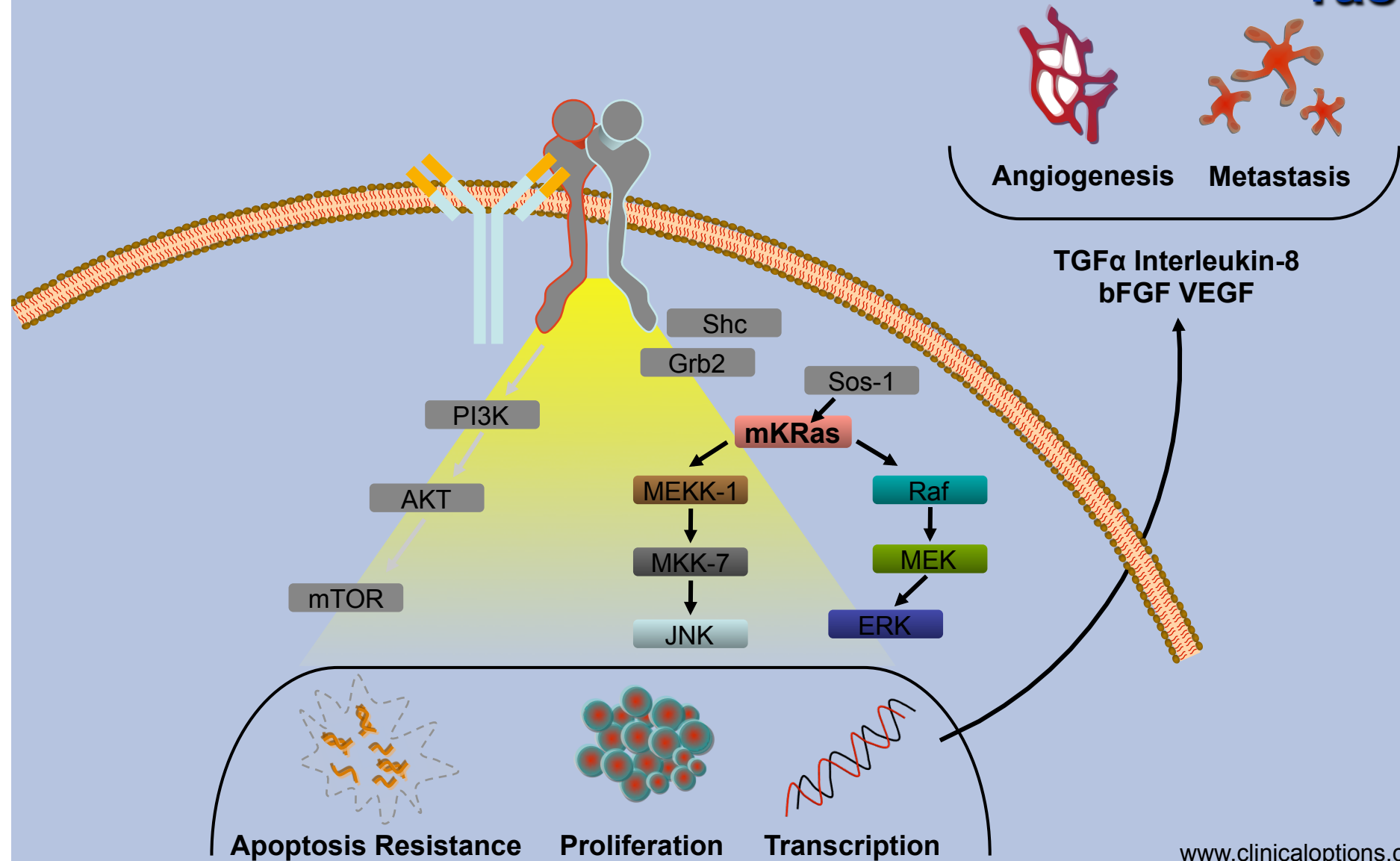
## Rat sarcoma (181 – 189 aas, p21)



# Ras



# EGF Pathway – Unscathed in mutant K-ras



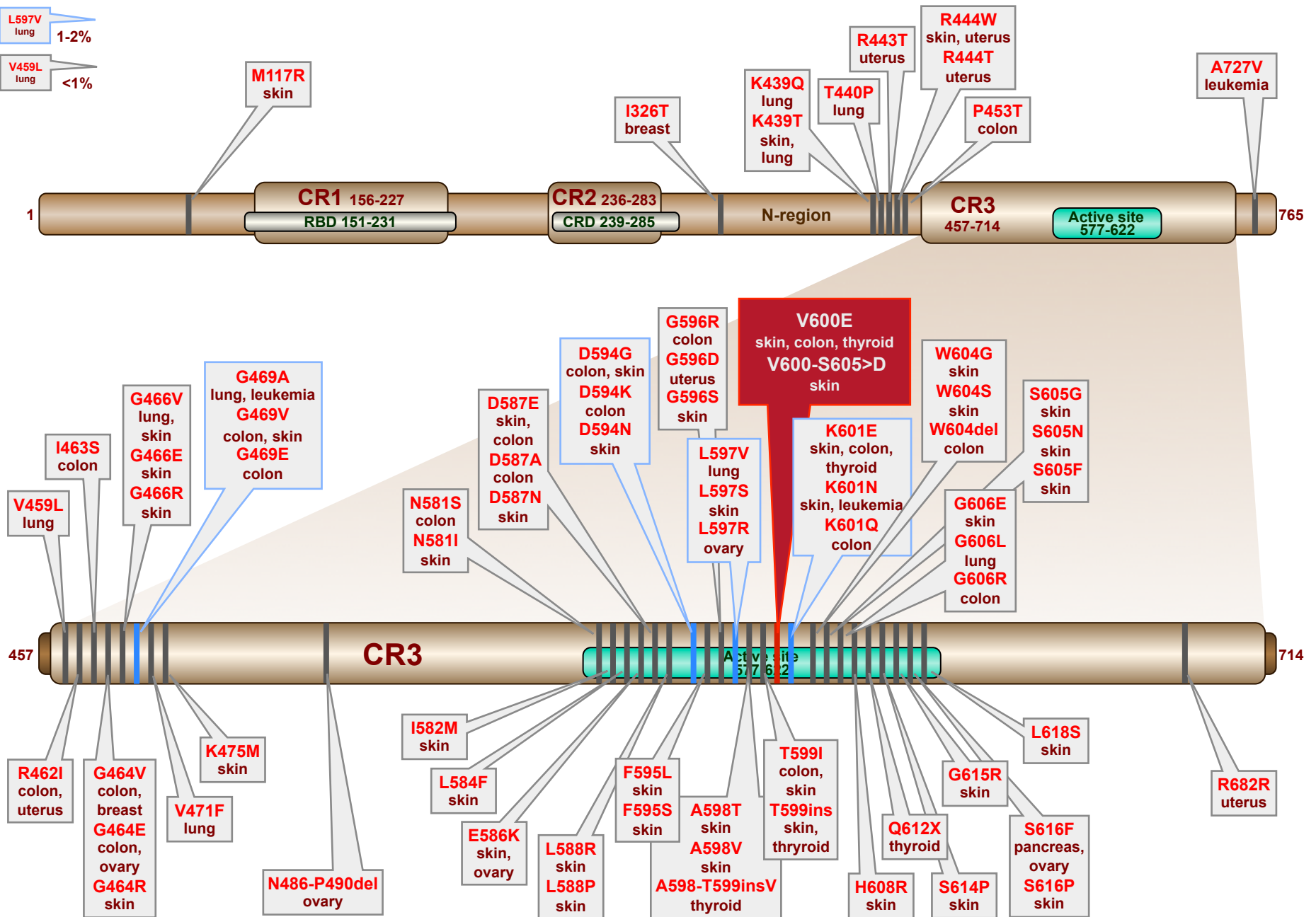
Frequency of mutation:

V600E  
skin  
≥80%

L597V  
lung  
1-2%

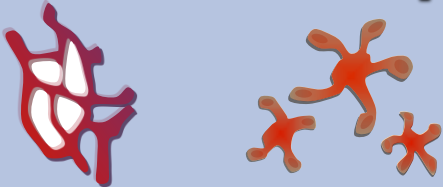
V459L  
lung  
<1%

# B-RAF mutations in human cancer



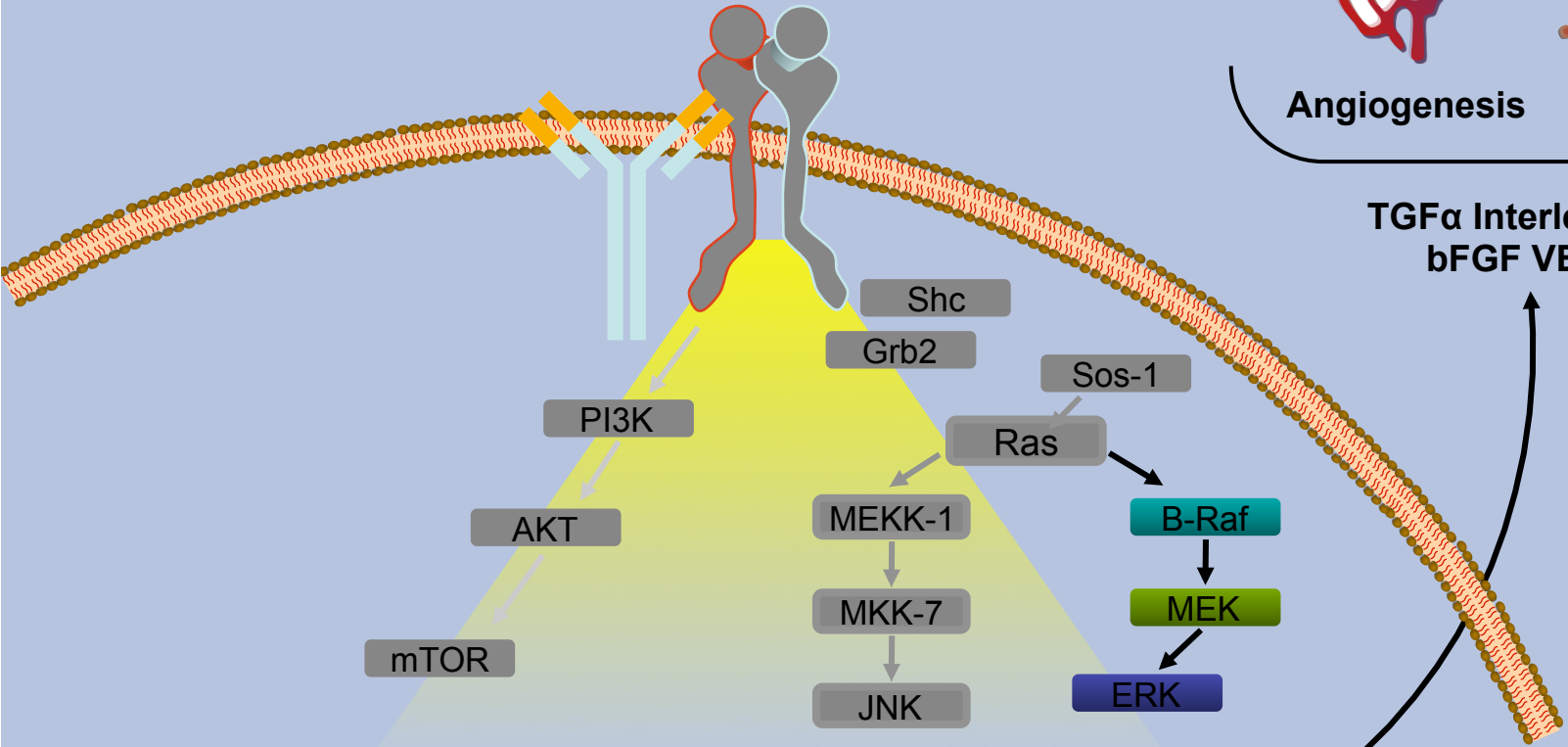


# EGF Pathway – Unscathed in mutant B-raf



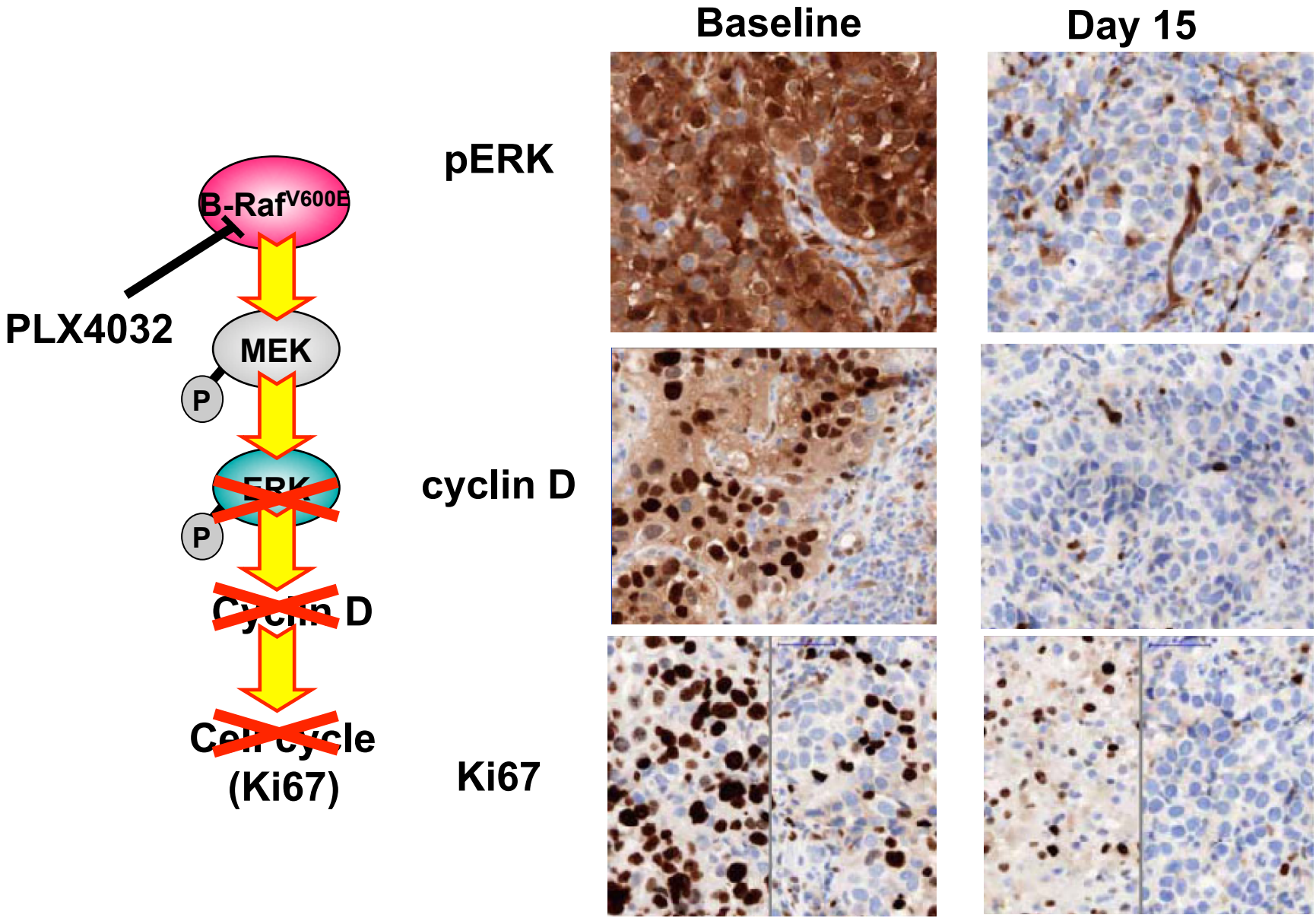
Angiogenesis    Metastasis

TGF $\alpha$  Interleukin-8  
bFGF VEGF

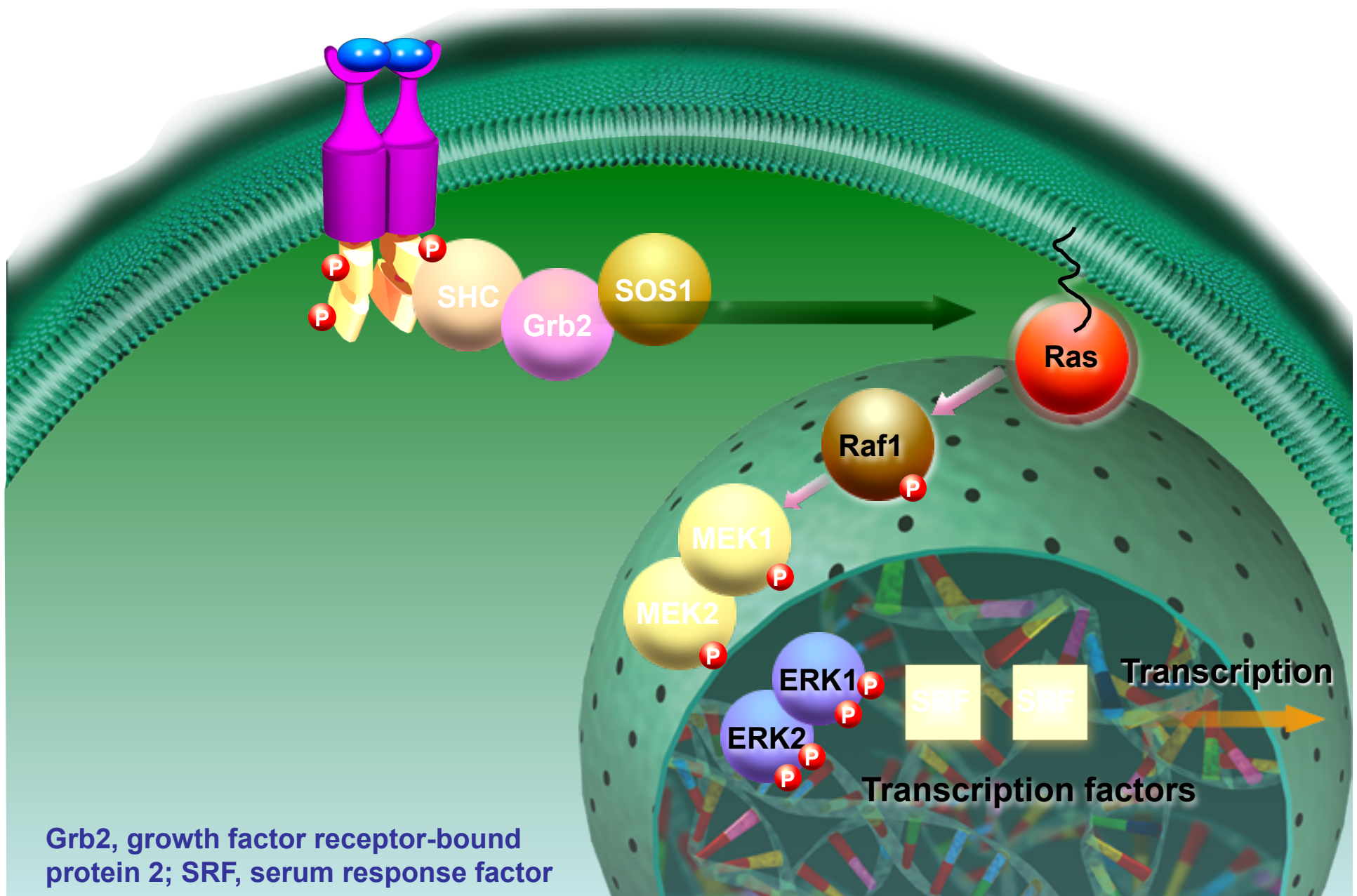


Apoptosis Resistance    Proliferation    Transcription

# Inhibition of MAPK signaling in BRAF<sup>V600E</sup> melanoma of patients treated with PLX4032

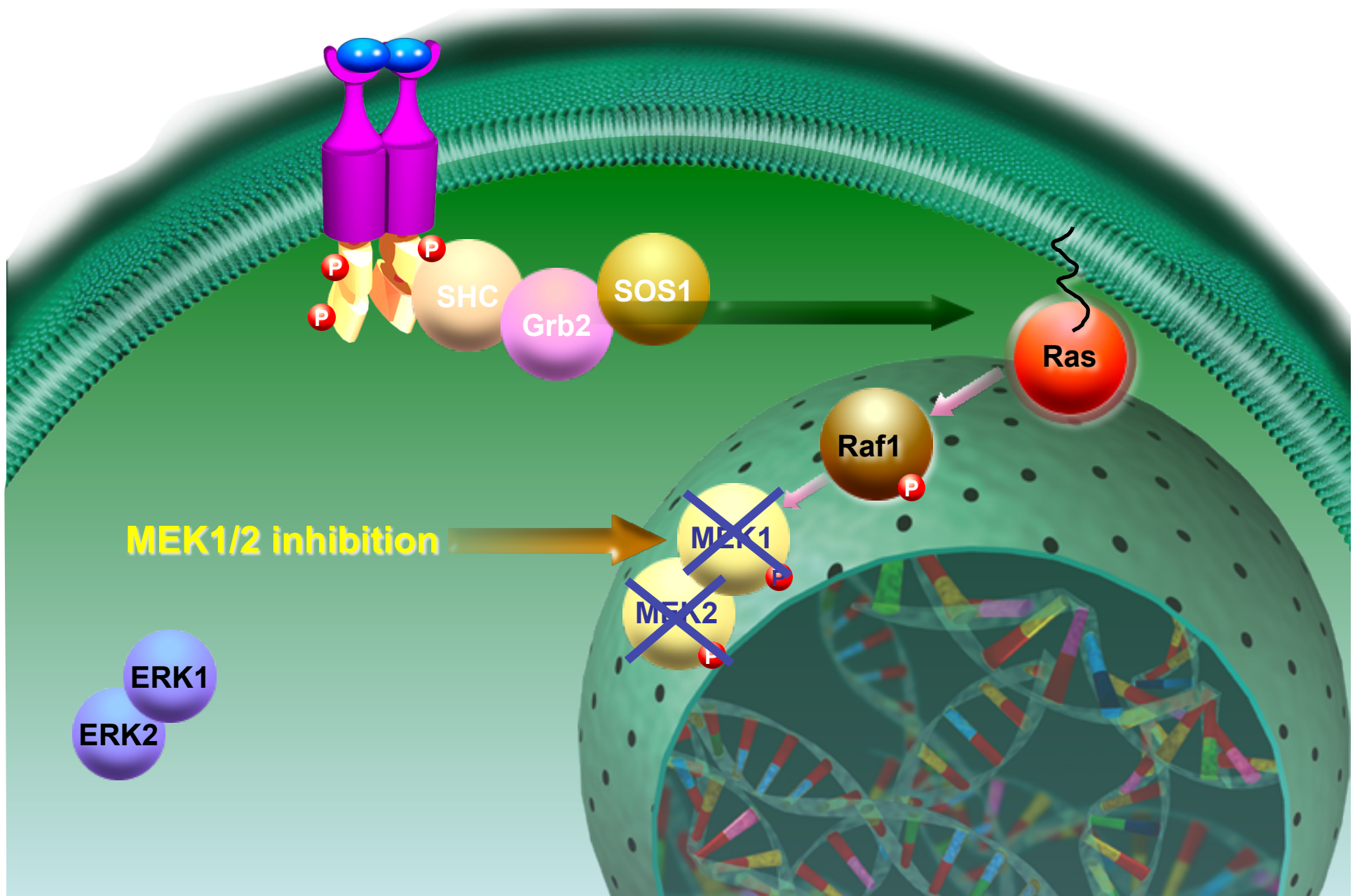


# The role of MEK1/2

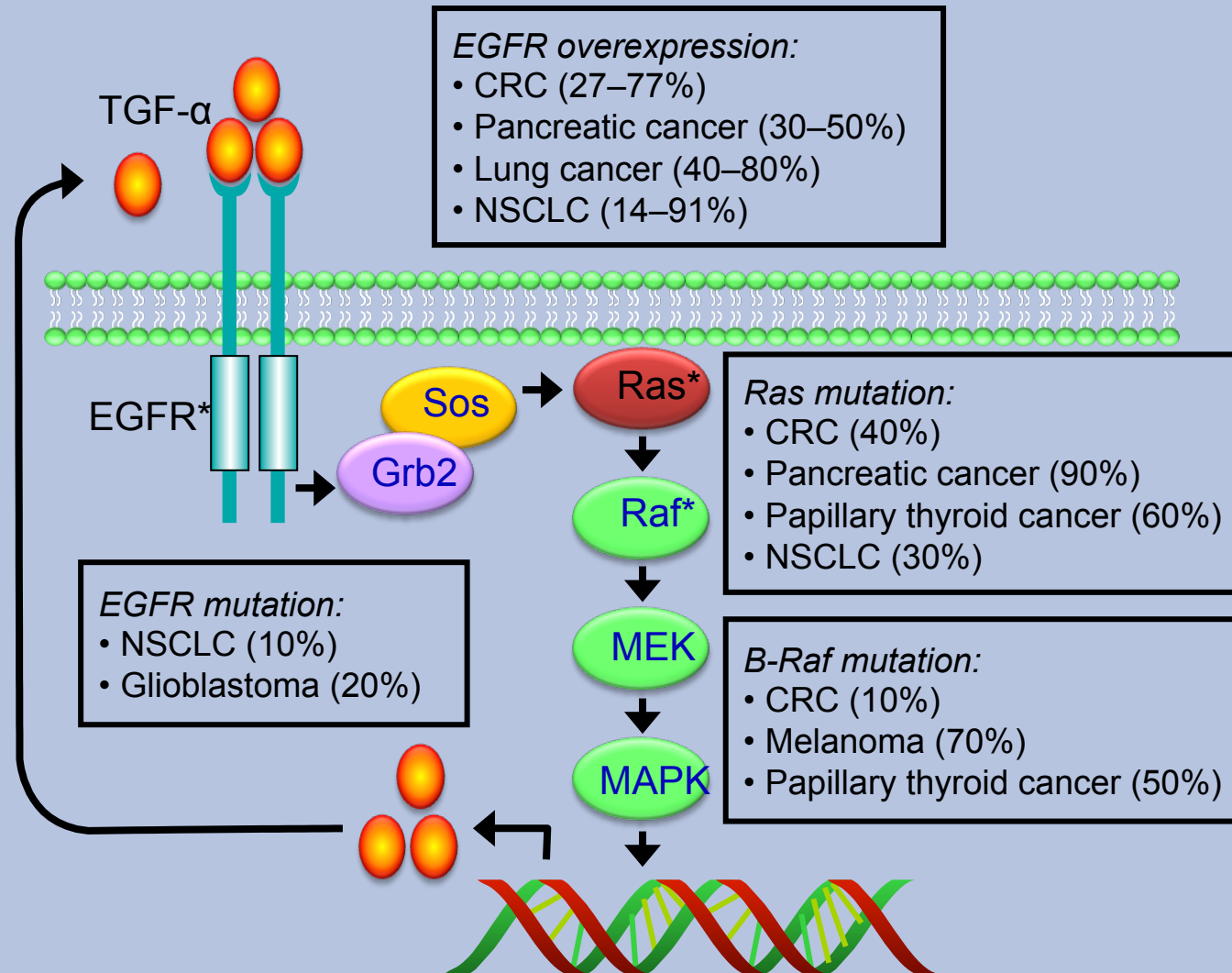




# MEK1/2 inhibition



# Targeting the EGFR pathway



\*Mutated in human cancers

Adapted from Roberts Der. Oncogene 2007

**HER2**

# Studies of the HER-2/*neu* Proto-oncogene in Human Breast and Ovarian Cancer

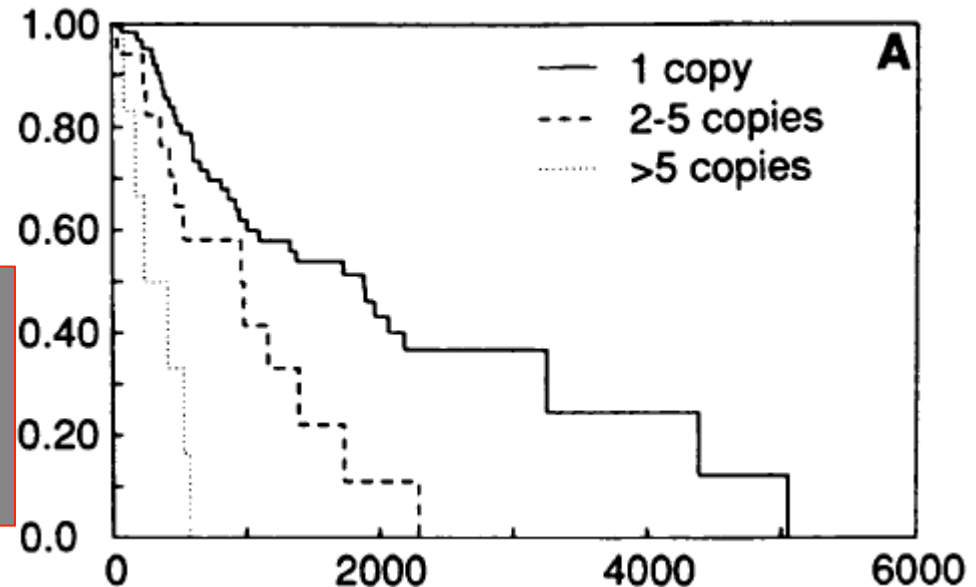
DENNIS J. SLAMON,\* WILLIAM GODOLPHIN, LOVELL A. JONES,  
JOHN A. HOLT, STEVEN G. WONG, DUANE E. KEITH, WENDY J. LEVIN,  
SUSAN G. STUART, JUDY UDOVE, AXEL ULLRICH, MICHAEL F. PRESS

## Breast Cancer

	Disease free survival		Overall survival	
	Uni- variate (P)	Multi- variate (P)*	Uni- variate (P)	Multi- variate (P)*
Nodes	<0.0001	<0.0001 [0.0818 ± 0.0214]	<0.0001	<0.0001 [0.0912 ± 0.0346]
HER-2/ <i>neu</i>	0.01	0.006 [0.1142 ± 0.0413]	0.041	0.045 [0.0864 ± 0.0288]
ER	0.235	0.60		
PGR	0.045	0.07		
Size	0.003	0.15		
Age	0.92	0.96		

\*Regression coefficients ± SE are shown in square brackets

Ovarian Cancer –  
Survival  
according to  
HER2 expression

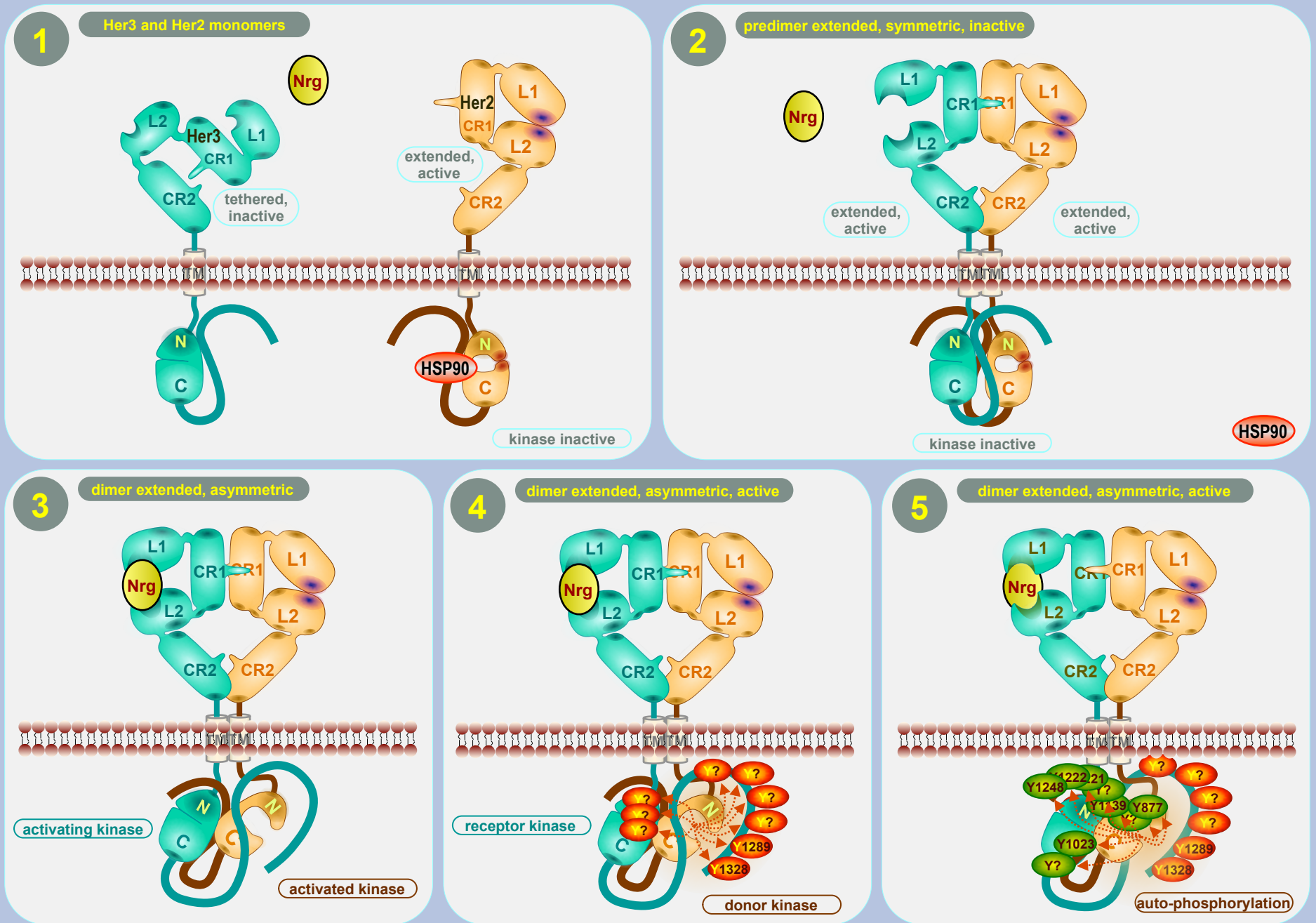




# Stepwise Her2/Her3 activation and tyrosine phosphorylation

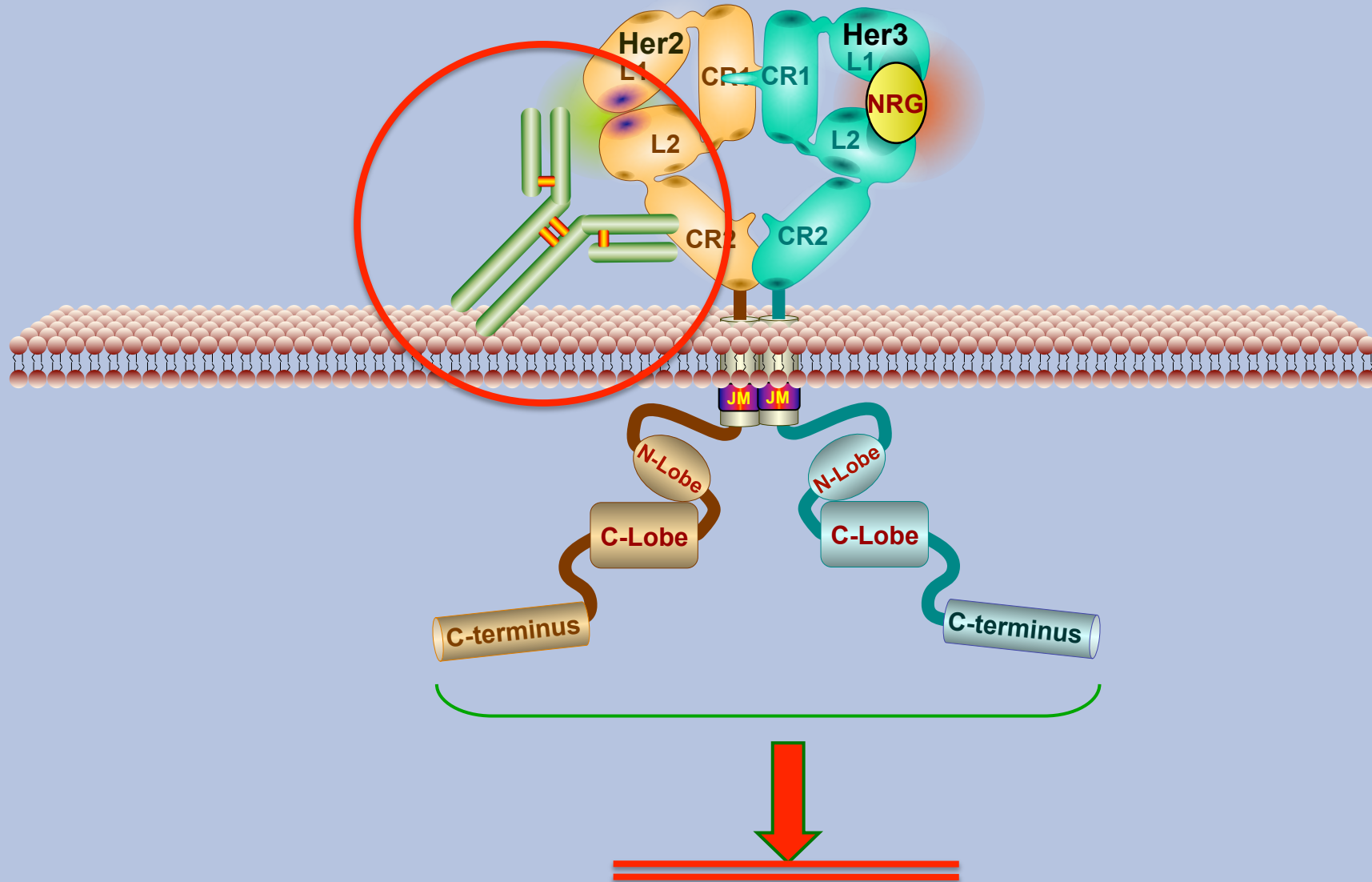
Crossphosphorylation **Y1146**

Autophosphorylation **Y1248**

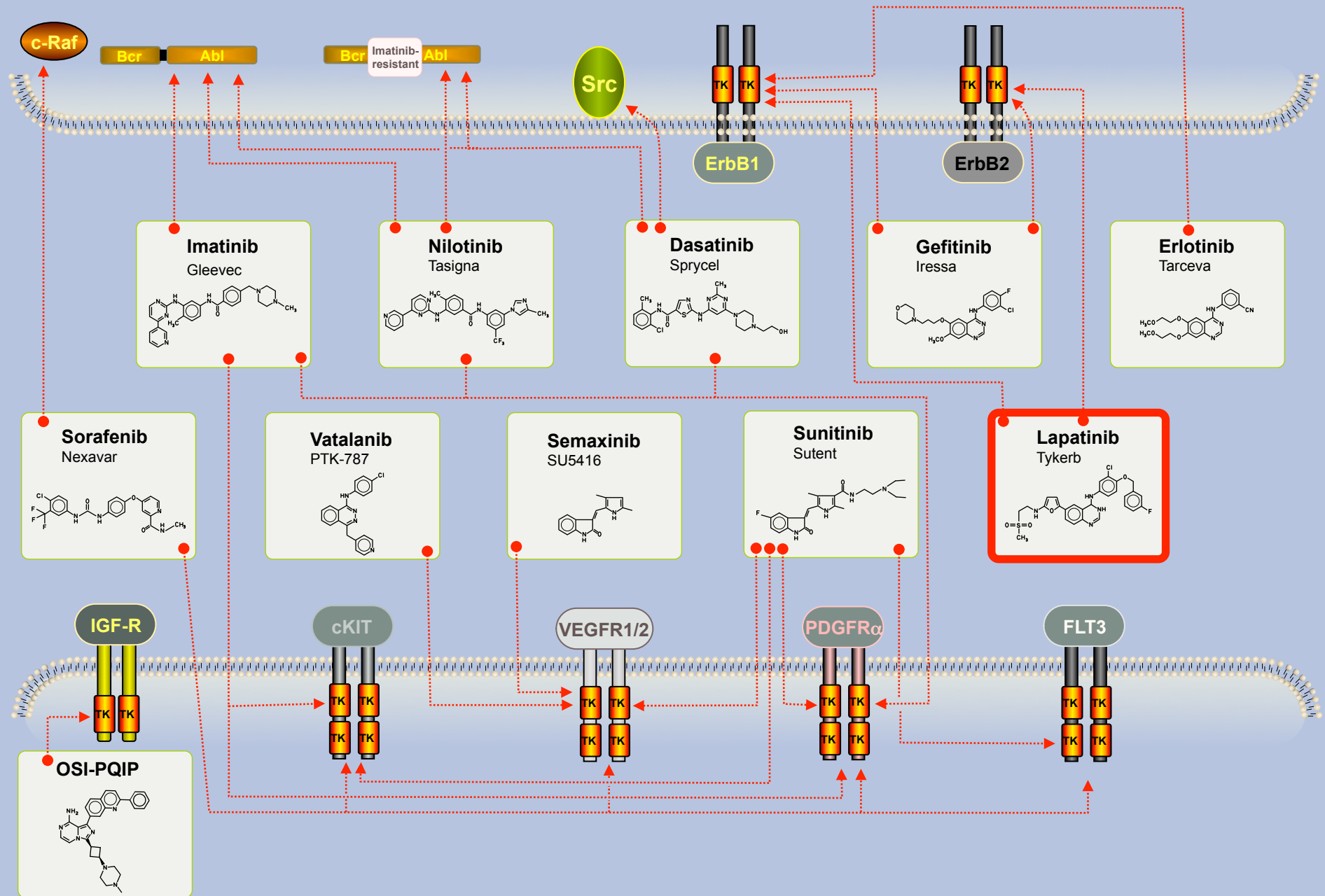


# Trastuzumab MOA

Her2/Her3  
Active heterodimer



# Specificity of BCR-ABL and RTK inhibitors

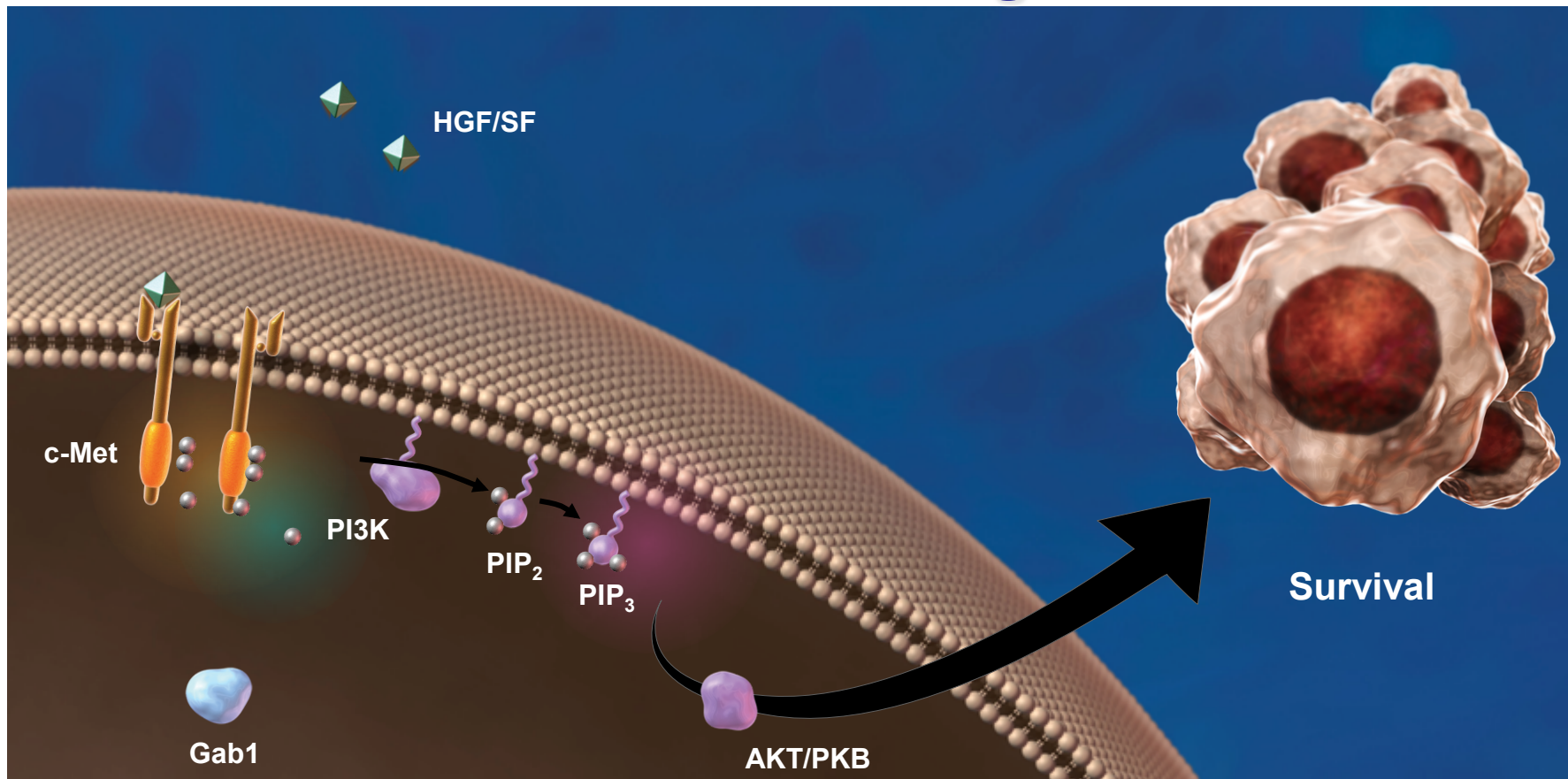


## HER2

- **Breast Cancer: Overexpression in 20%**
- **Gastric Cancer: Overexpression in 10-15%**

# **Other signal transduction pathways**

## c-MET as a target



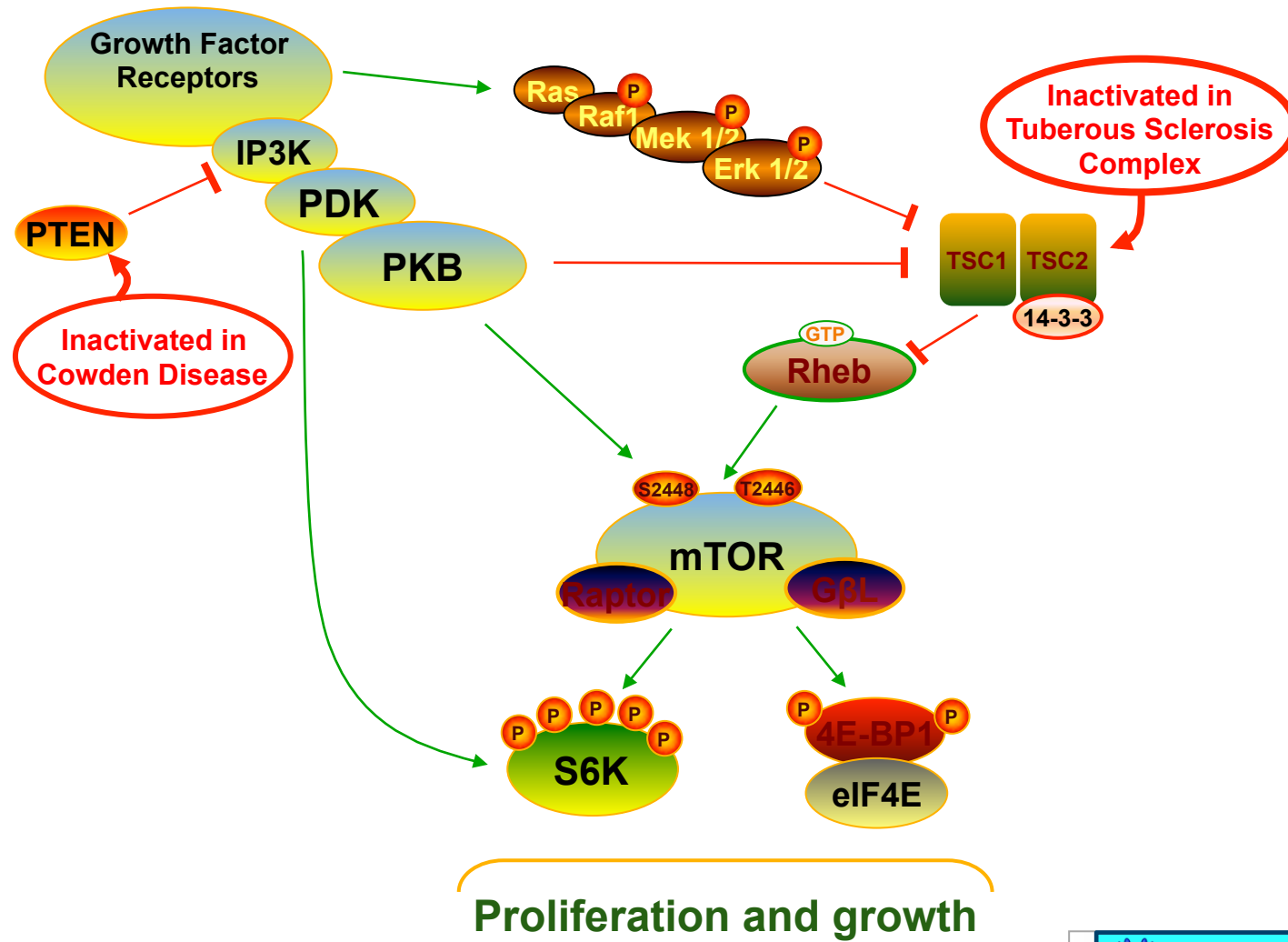
C-MET signaling mediates a number of normal cellular functions: proliferation, survival, migration and invasion

Disregulation of HGF/SF: c-MET has been associated with a number of human malignancies

C-MET inhibition represents a novel therapeutic approach

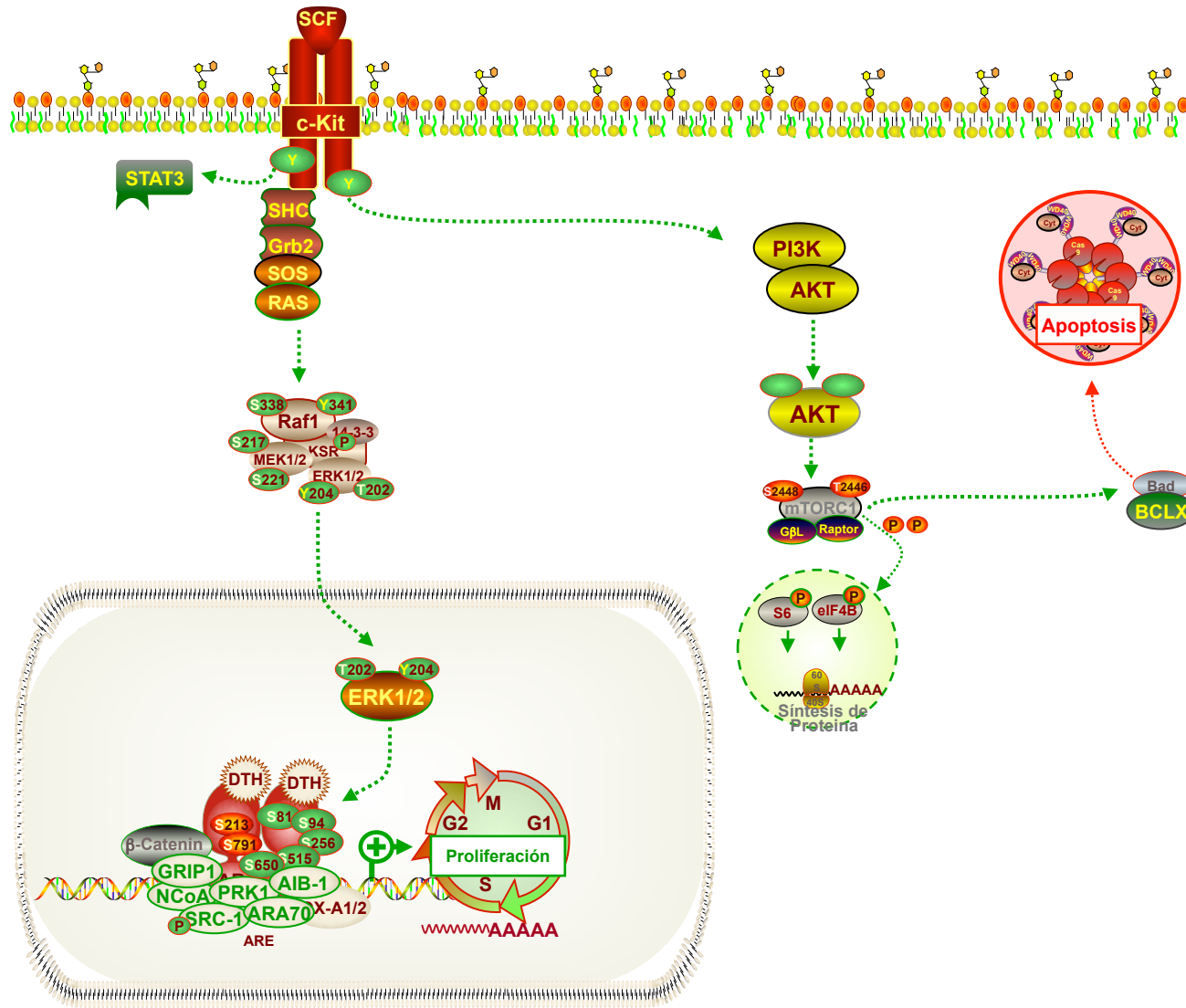
MET and EGFR inhibition are synergistic in NSCLC cell lines

# Vía del mTOR



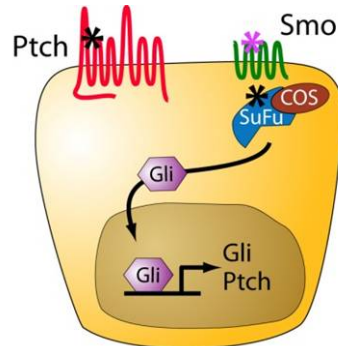


## Señalización por la vía del c-Kit



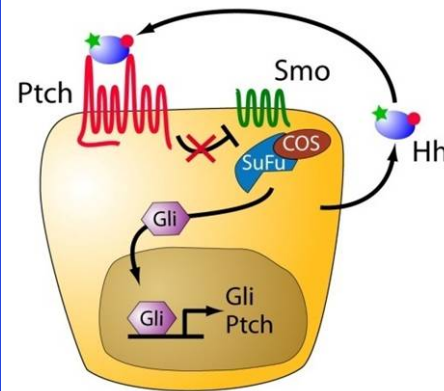
# Mechanisms of Hedgehog (Hh) Pathway Activation

## Type I Mutations



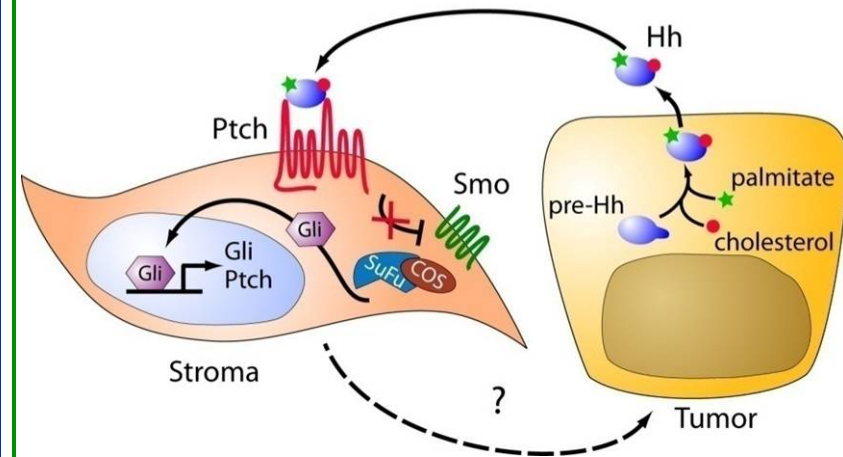
BCC  
Medulloblastoma

## Type II Autocrine signaling (tumor or stem cell)



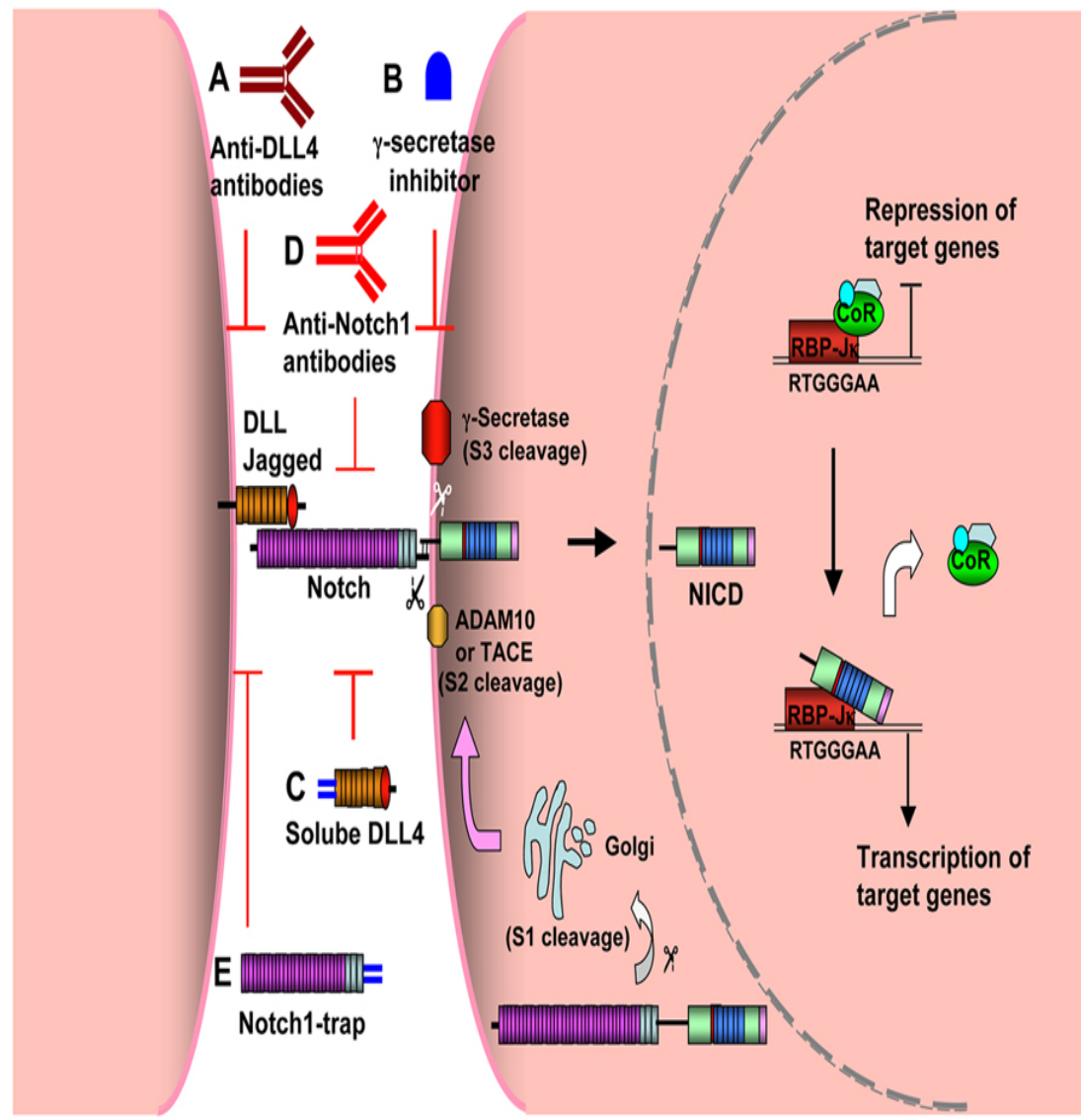
CML  
SCLC  
Pancreatic cancer  
Breast cancer  
Glioma

## Type III Paracrine signaling (stromal involvement)



Pancreatic cancer  
Colon cancer  
Lymphoma  
Prostate cancer

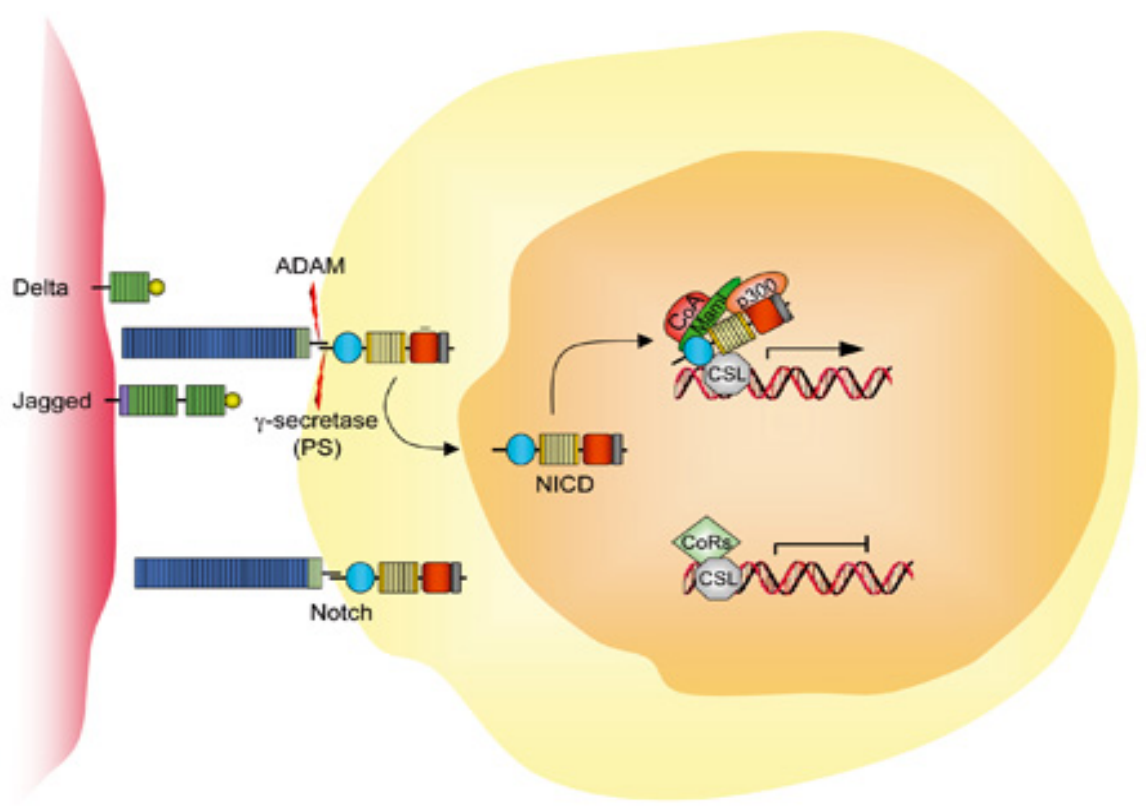
# Targeting Notch pathway: RO4929097



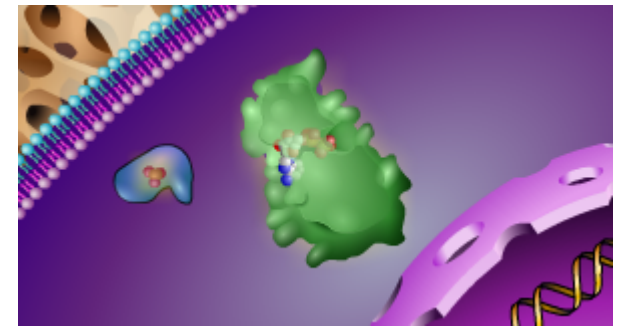
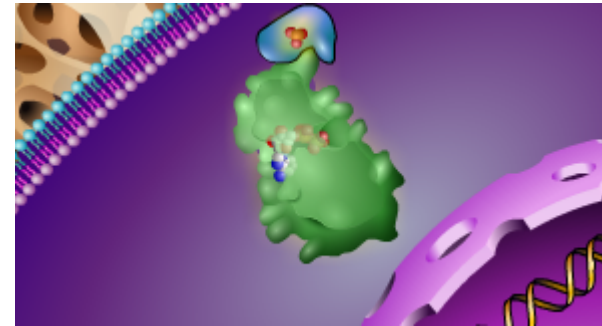
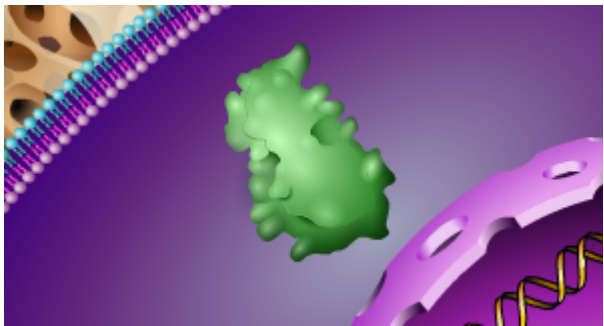
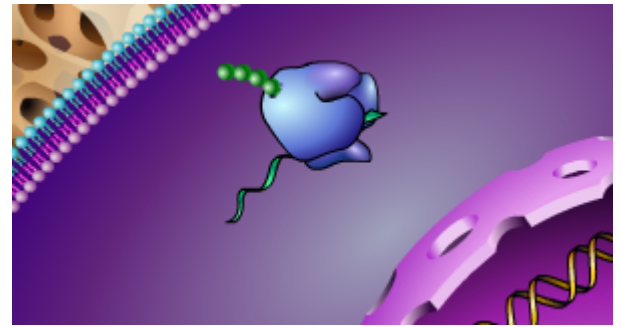
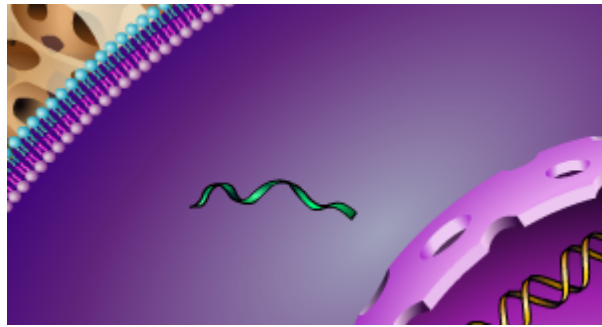
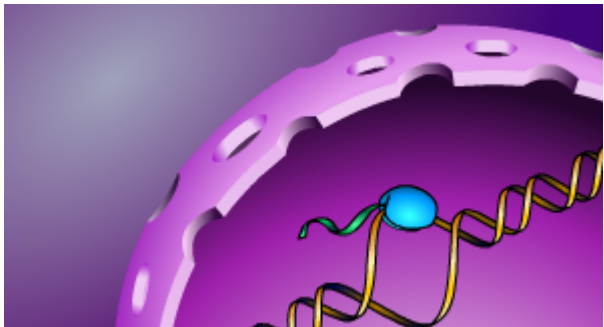
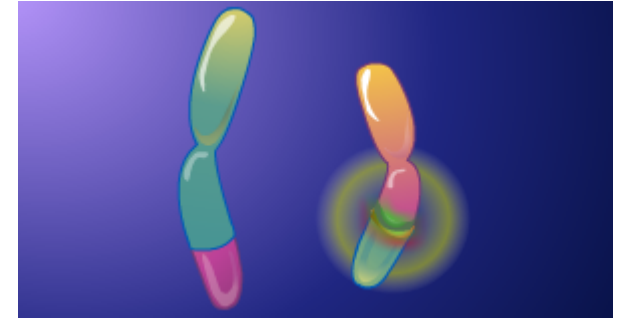
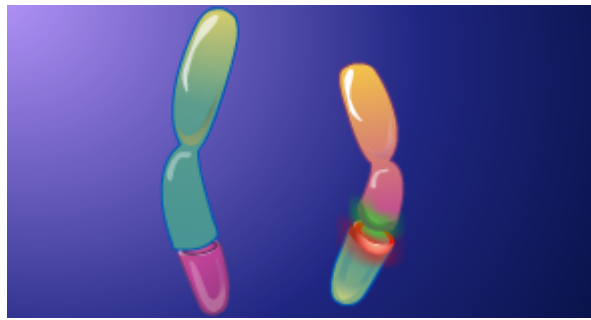
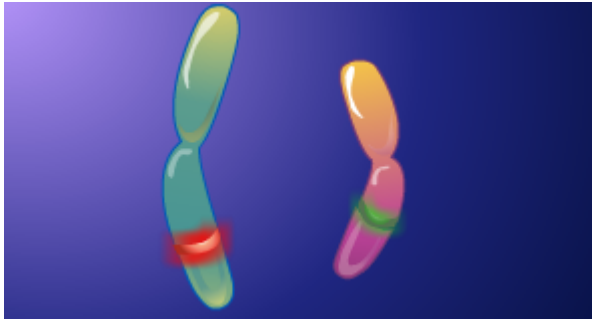
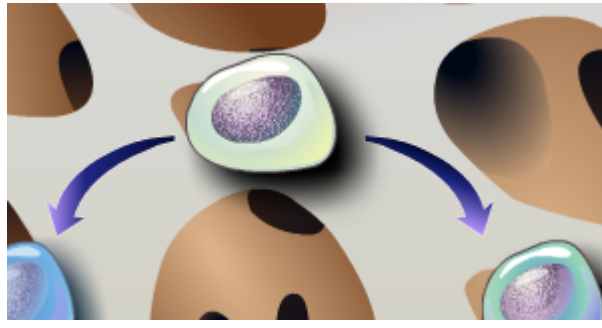
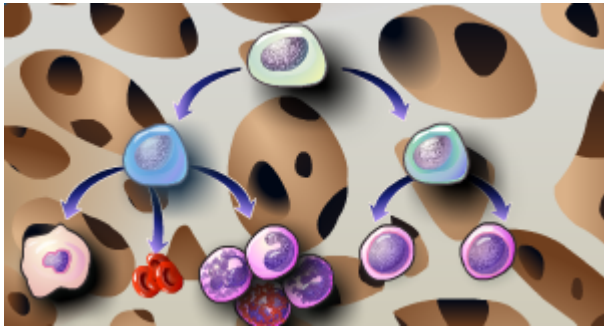
- The notch signaling pathway is involved in cell fate decisions during normal development and has also oncogenic function in several solid tumors

- $\gamma$ Secretase is a large intramembrane protease complex which is a key mediator in the notch signaling pathway

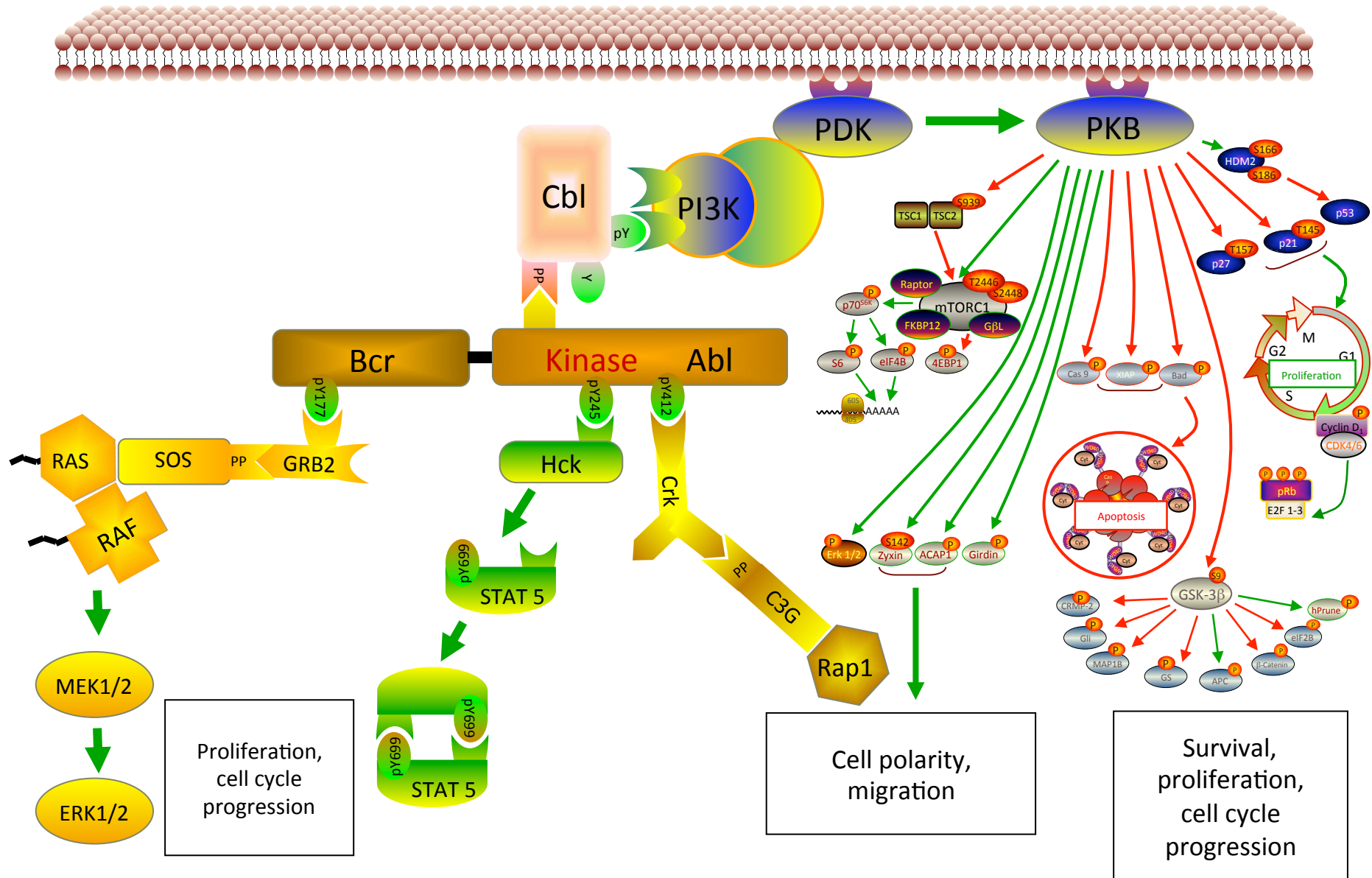
- By preventing Notch activation,  $\gamma$ secretase inhibitor may inhibit tumor growth



**BCR-ABL**

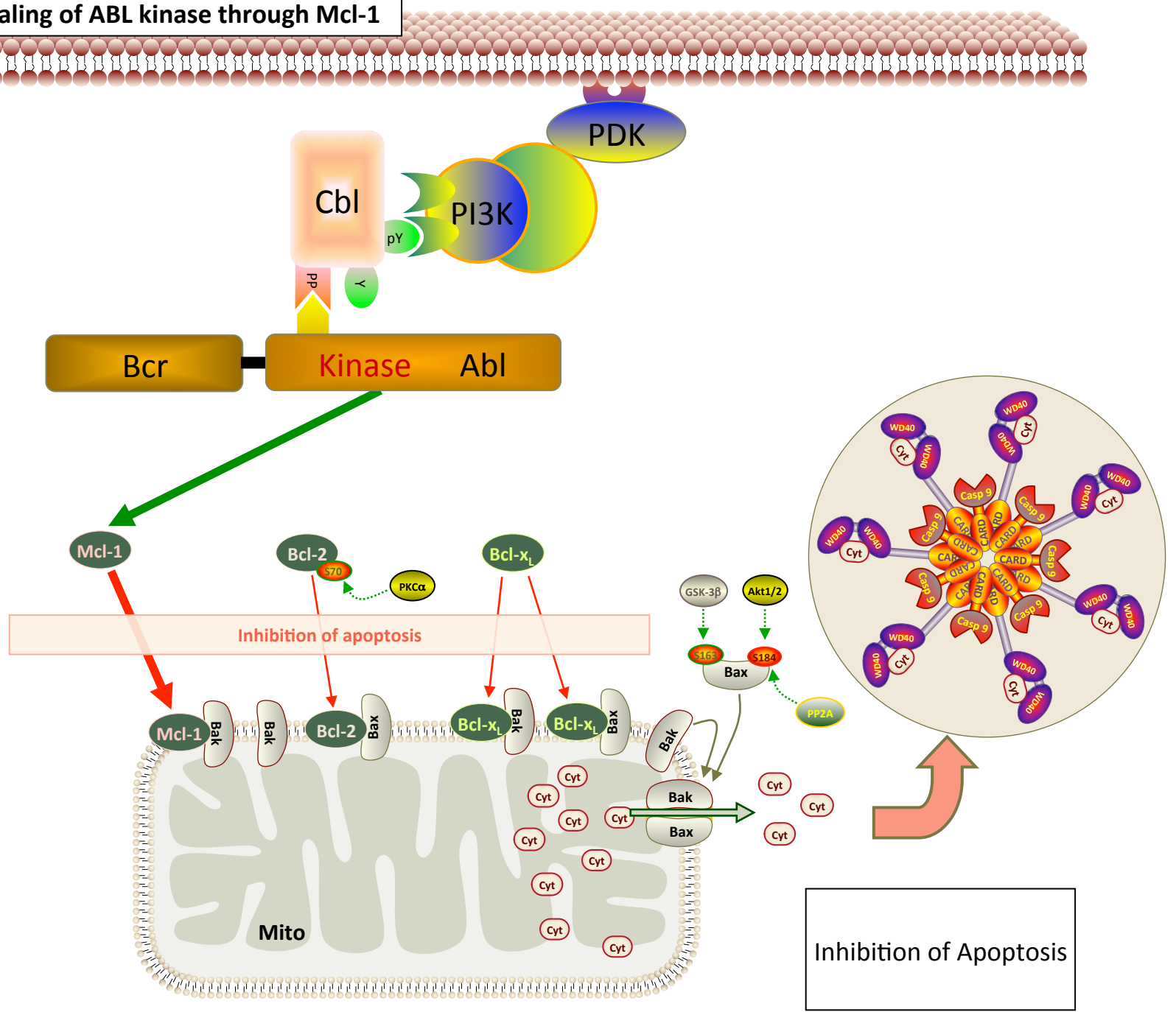


Bcr-Abl STP I

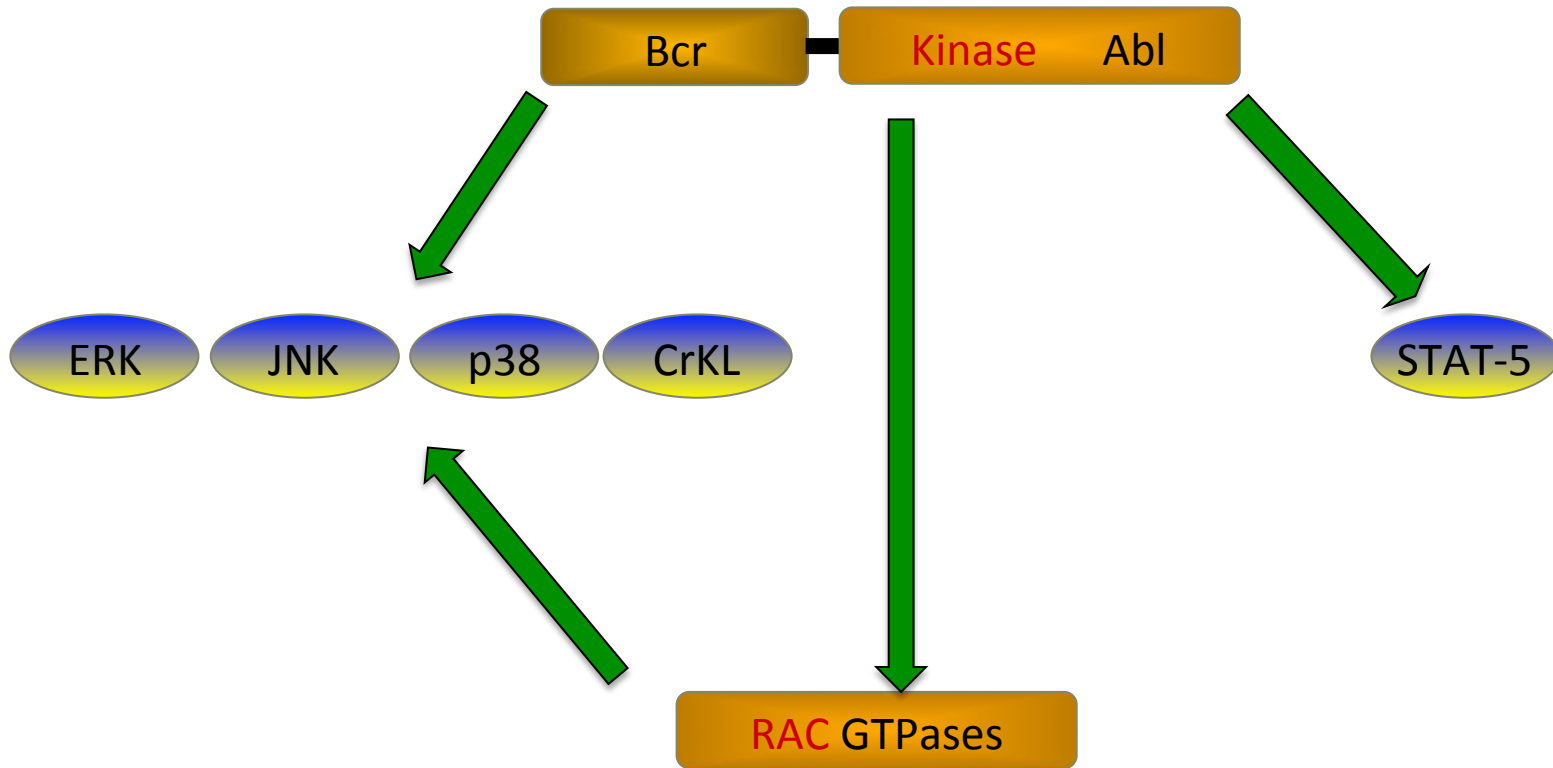
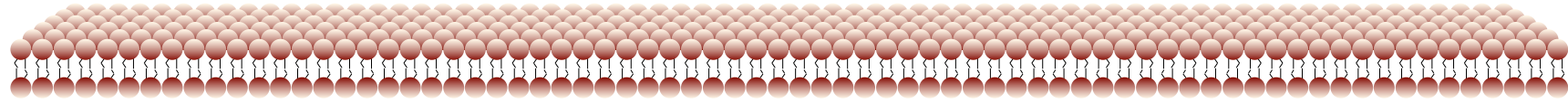


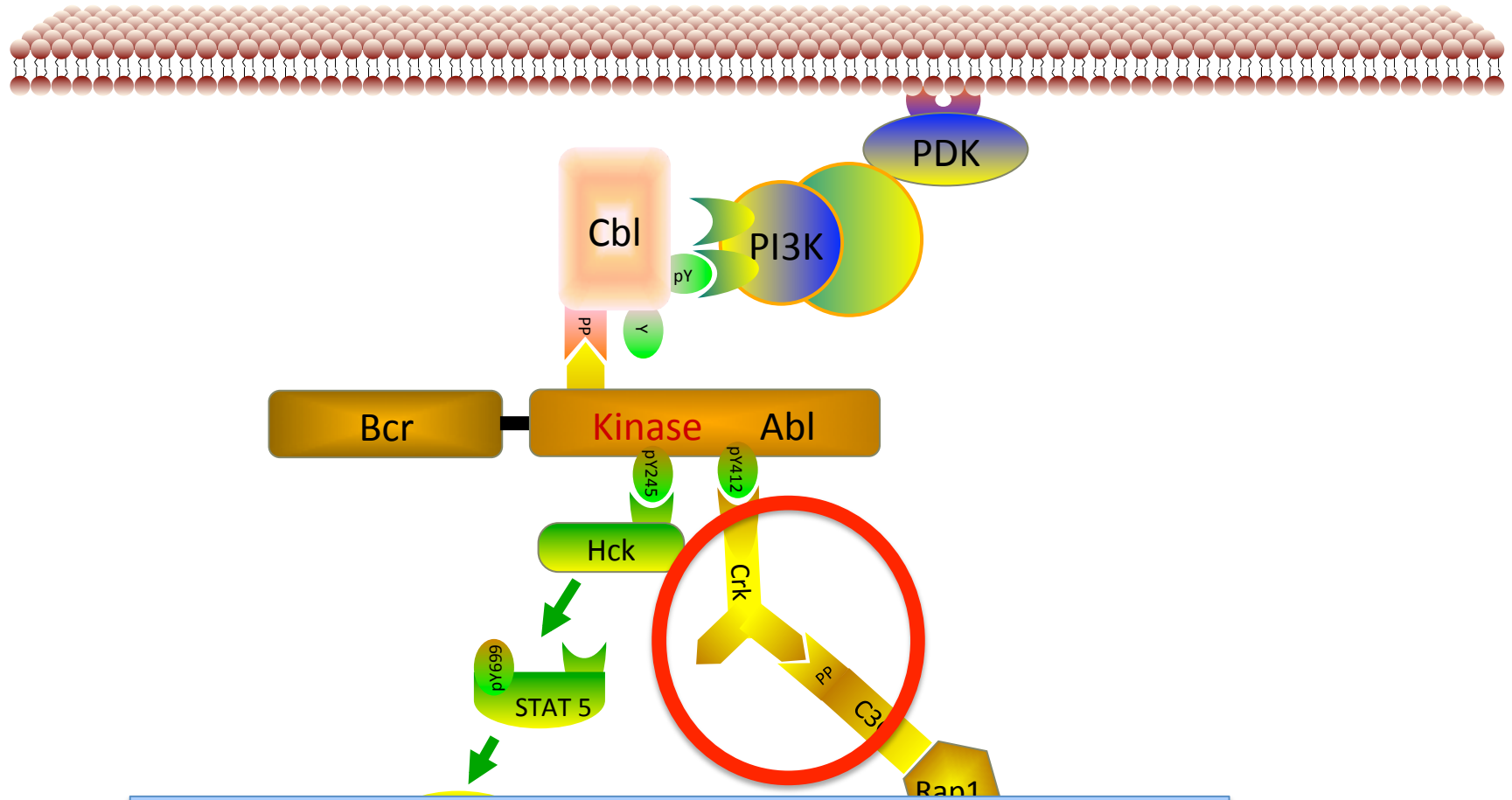


# Antiapoptotic signaling of ABL kinase through Mcl-1

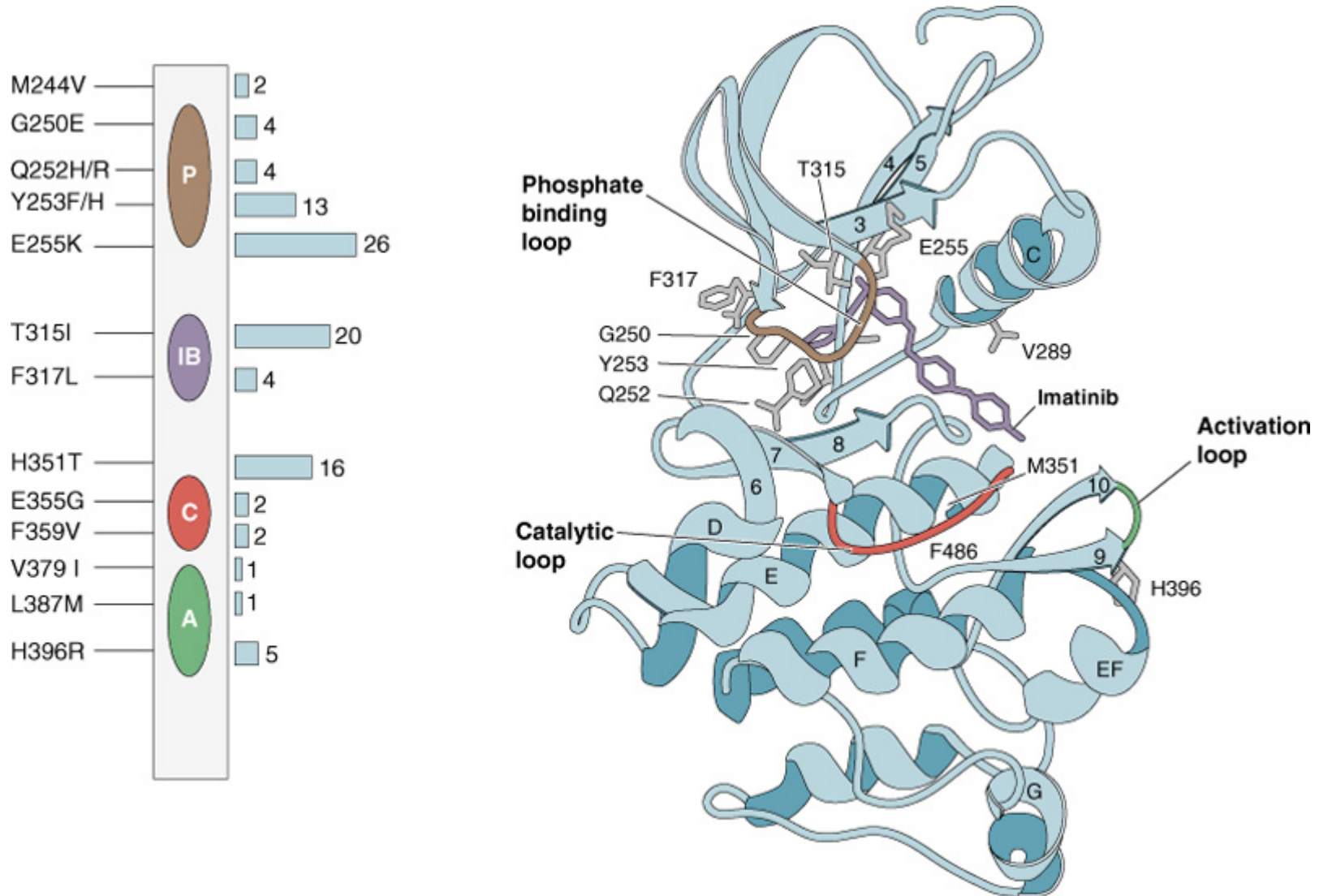


Bcr-Abl STP III



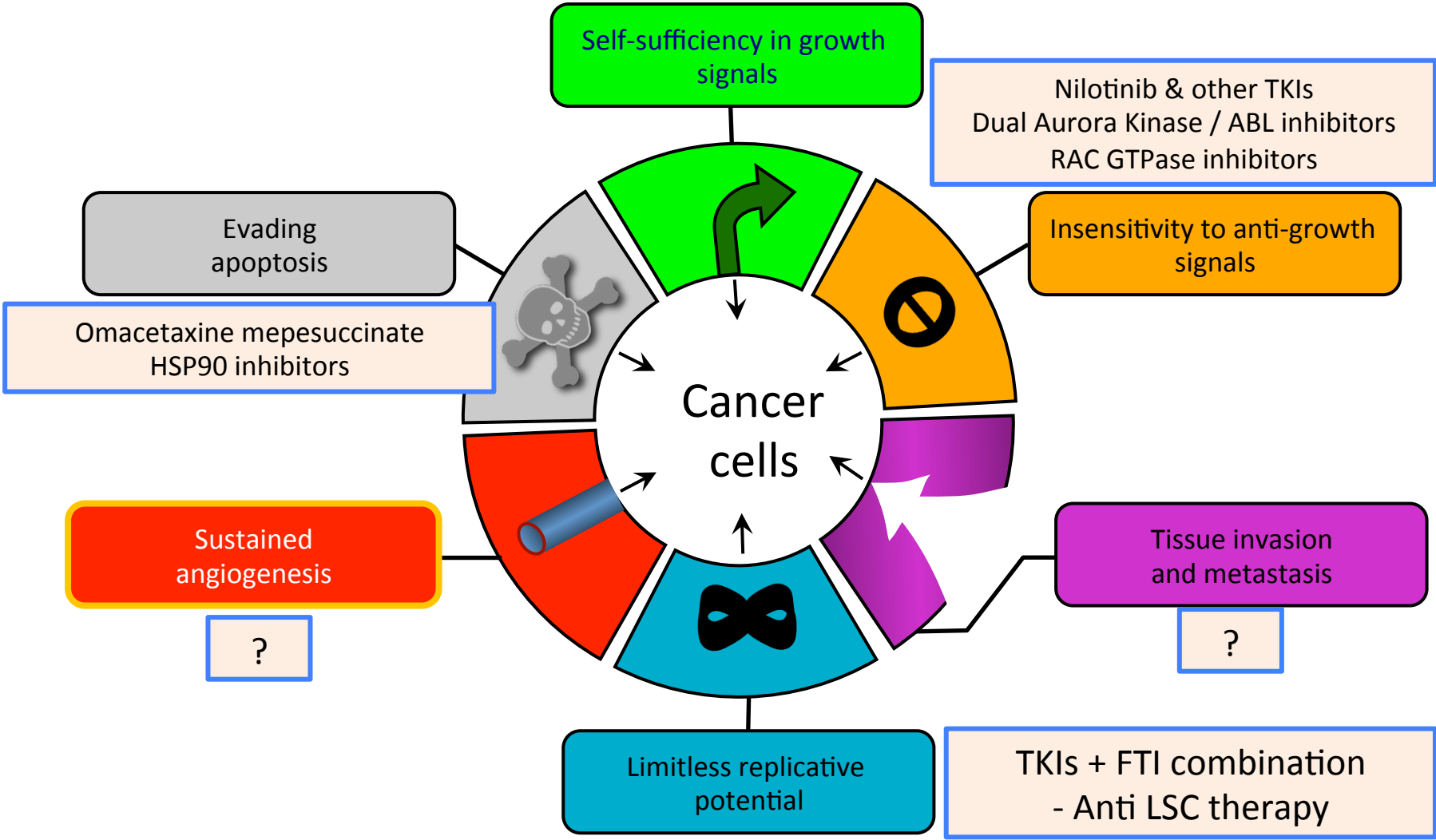


**CrkL Phosphorylation**  
 Is the hallmark of ABL kinase biologic activity



(From Tauchi T, Ohyashiki K: *Leuk Res* 28[Suppl 1]:S39-S45, 2004, with permission; based on data compiled from Shah NP, Nicoll JM, Nagar B, et al: Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib [STI571] in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2:117, 2002; and Druker BJ: *Semin Hematol* 40:50, 2003.)

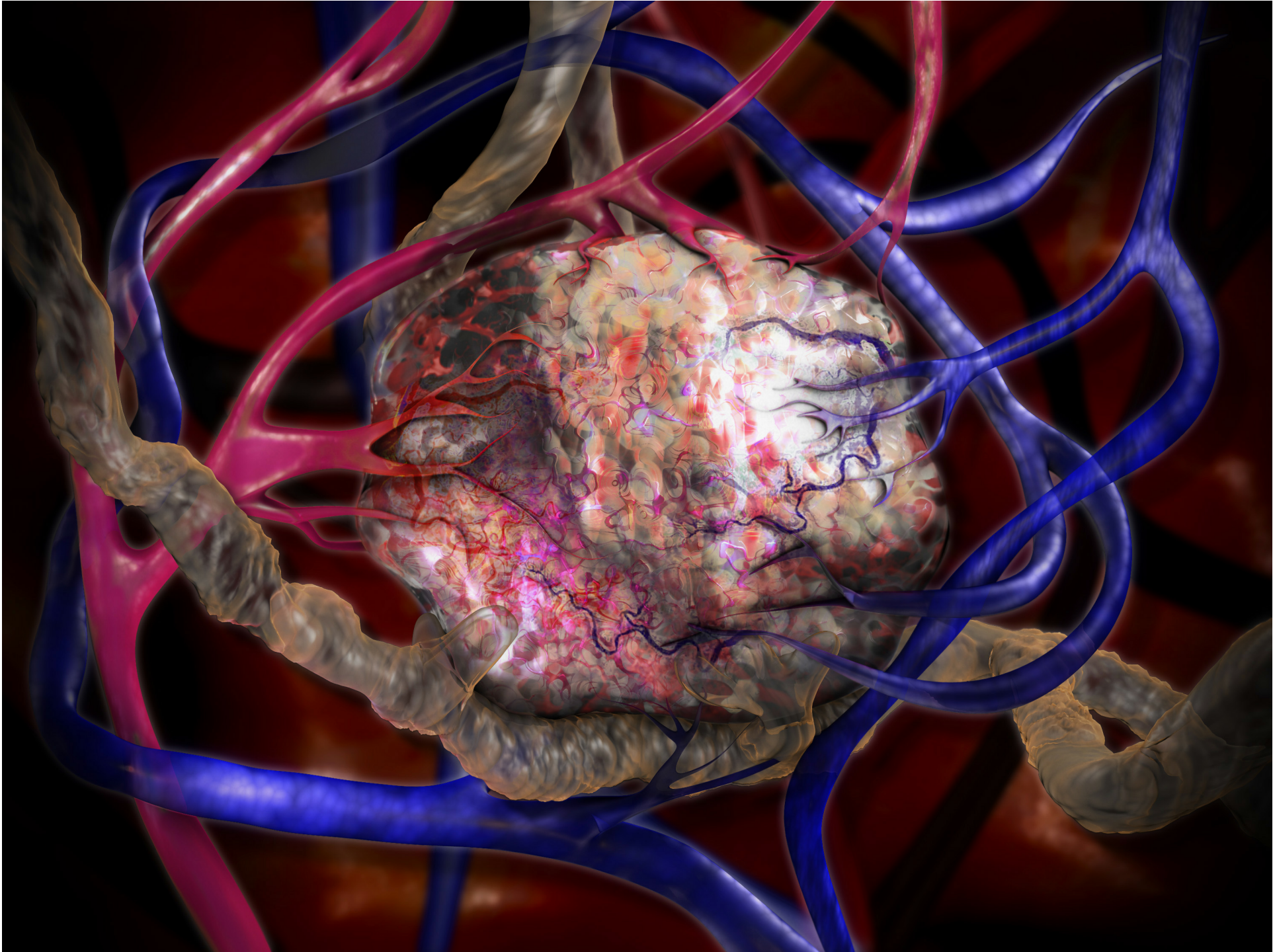
# Several strategies are being actively tackled in CML... May WE prevail!



Adapted from Hanahan, et al. Cell 2000

# **VEGF (Angiogenesis)**

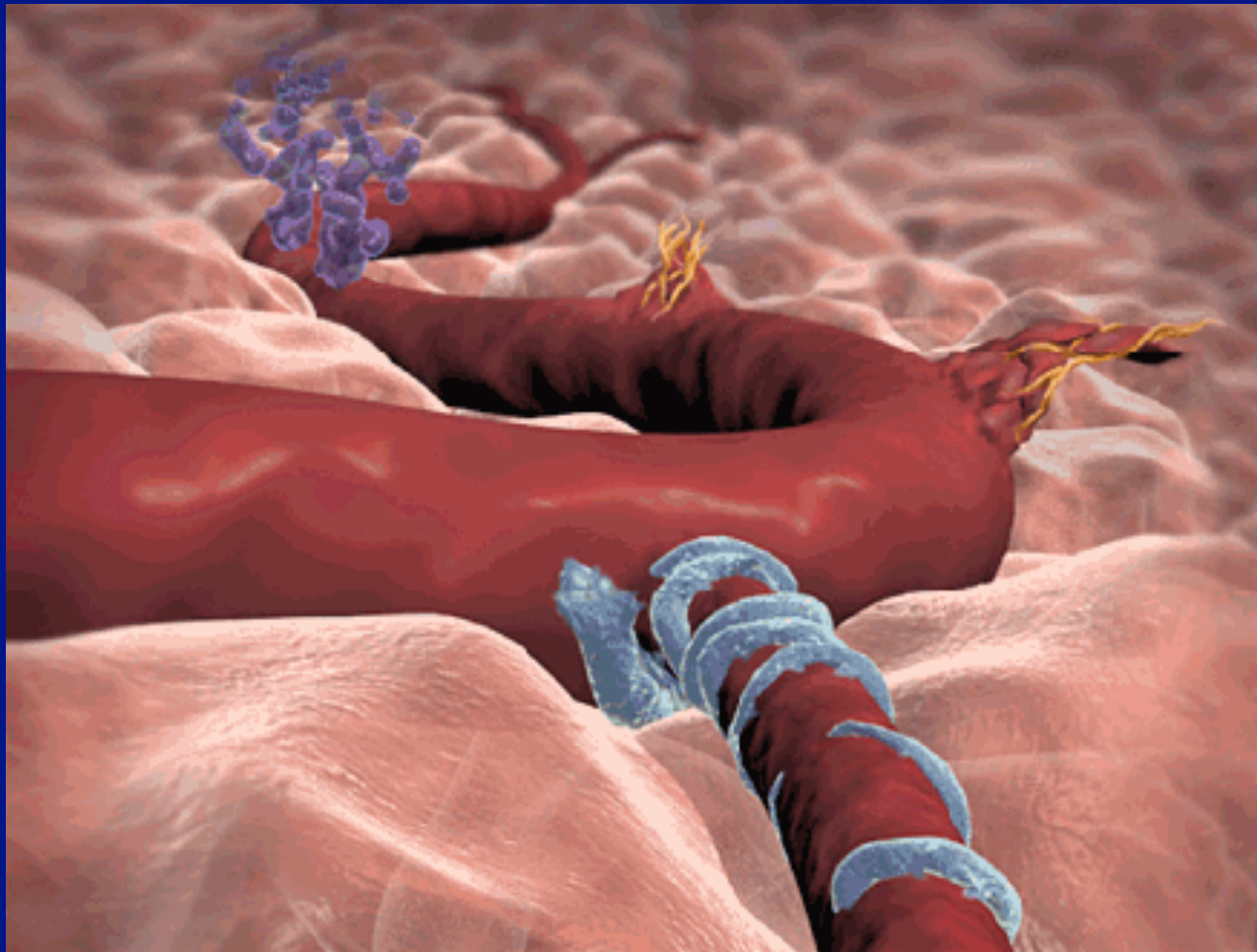






# Angiogenesis is the process of new blood vessel formation from existing vasculature

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# Physiologic versus pathologic angiogenesis

Generation of new blood vessels from existing vasculature

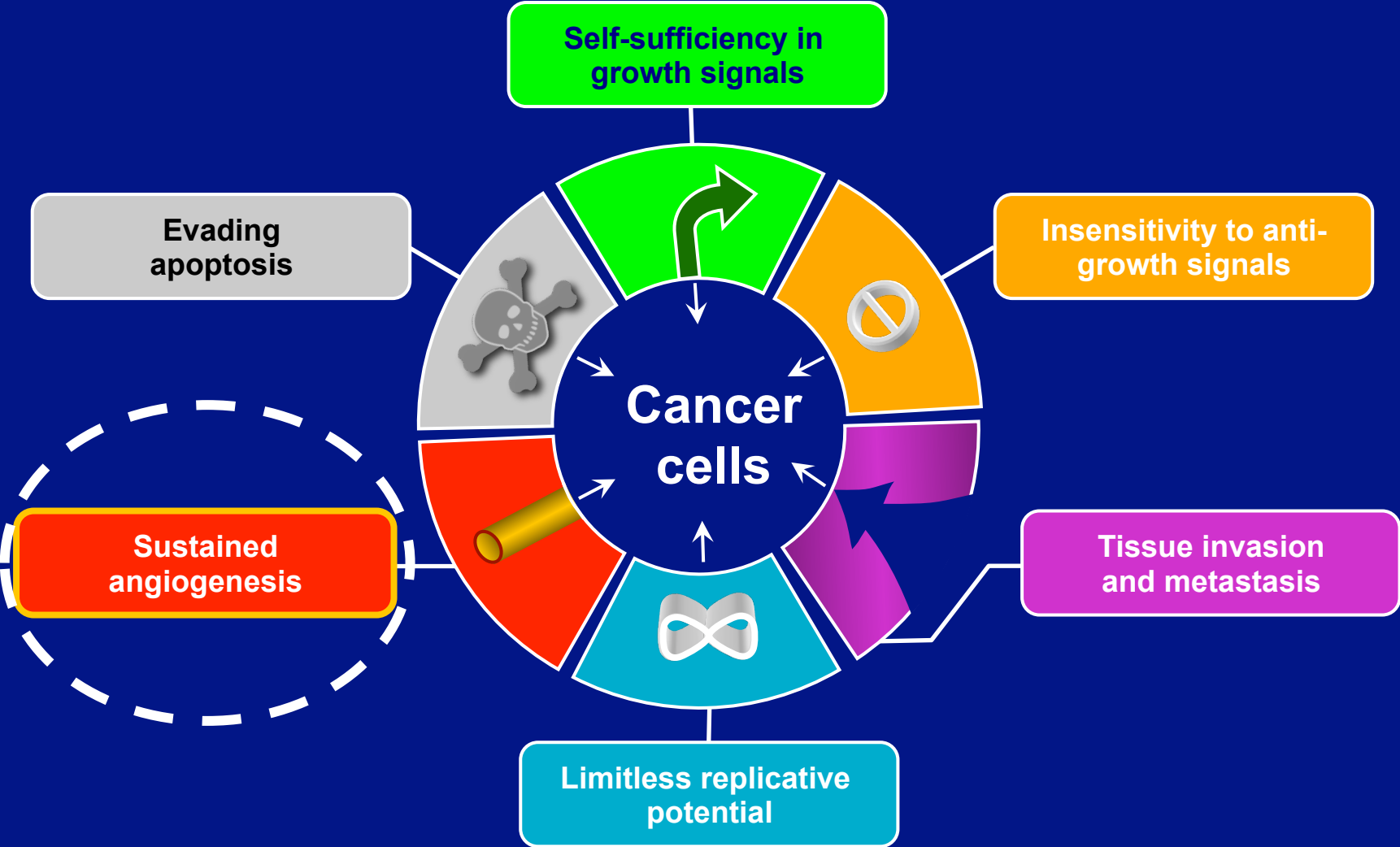
- **Physiologic**

- longitudinal bone growth
- wound healing
- secondary sexual development
  - corpus luteum formation
- intermittent, localized, tightly regulated
- vessels well-formed, grid-like

- **Pathologic**

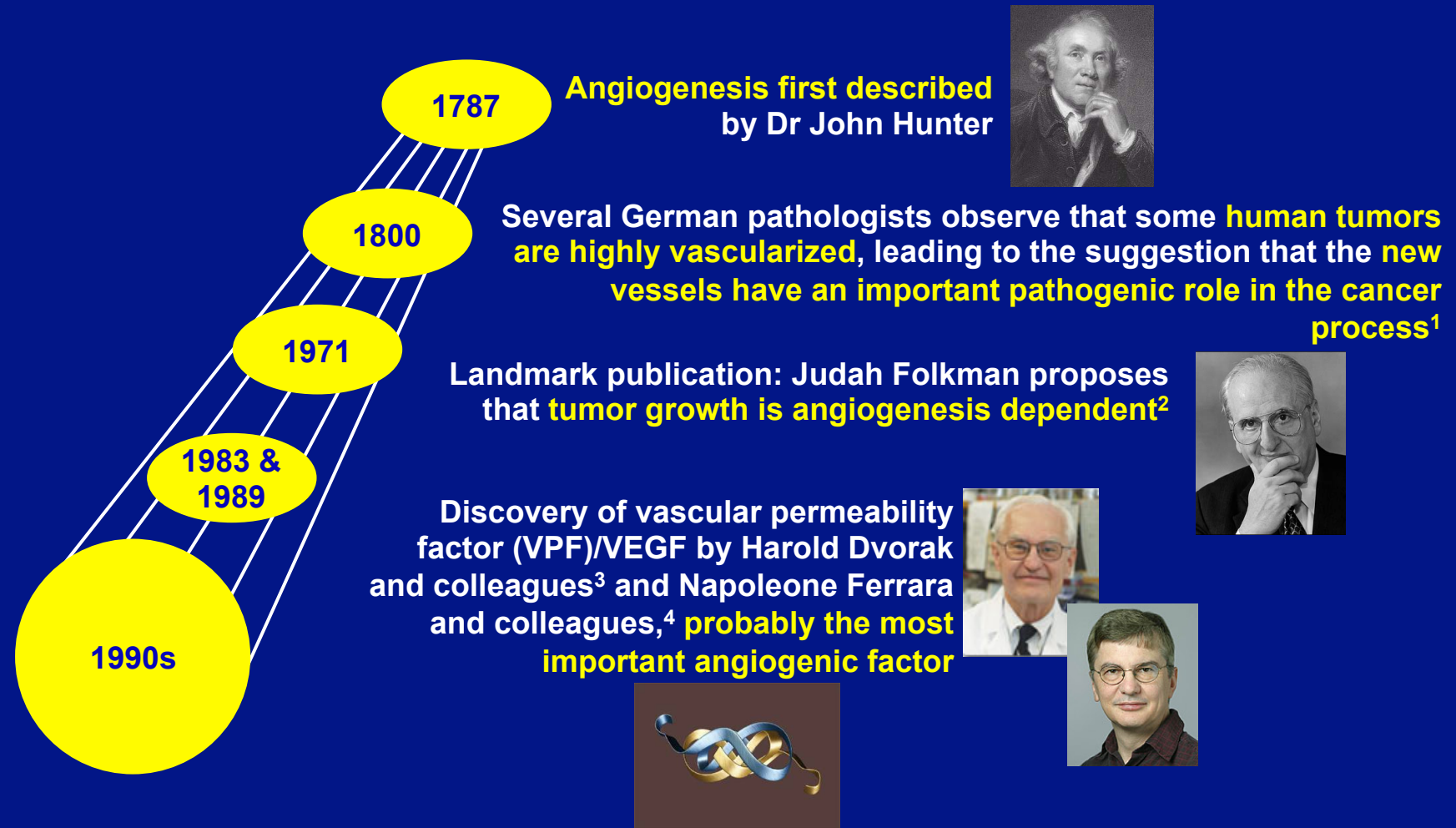
- tumor growth
- rheumatoid arthritis
- psoriasis
- retinopathies
- age-related macular degeneration
- sustained, localized
- vessels tortuous, leaky
- growth factor/s dependent

# Sustained angiogenesis is a hallmark of cancer



Adapted from Hanahan, et al. Cell 2000

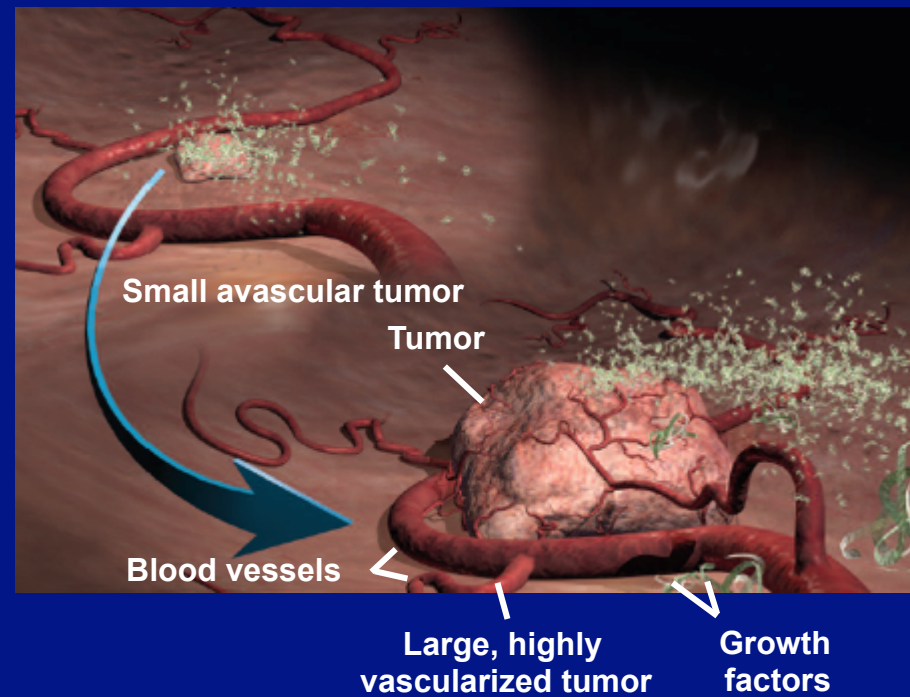
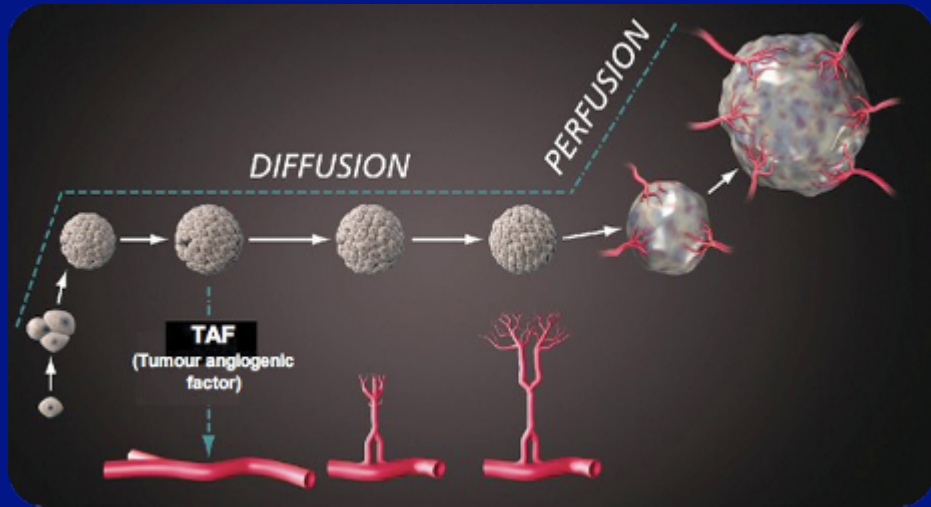
# Key landmarks in understanding angiogenesis and its role in tumor development



1. Ferrara. Nat Rev Cancer 2002; 2. Folkman. NEJM 1971; 3. Senger, et al. Science 1983  
4. Ferrara, Henzel. Biochem Biophys Res Commun 1989

# Angiogenesis is essential to tumor development

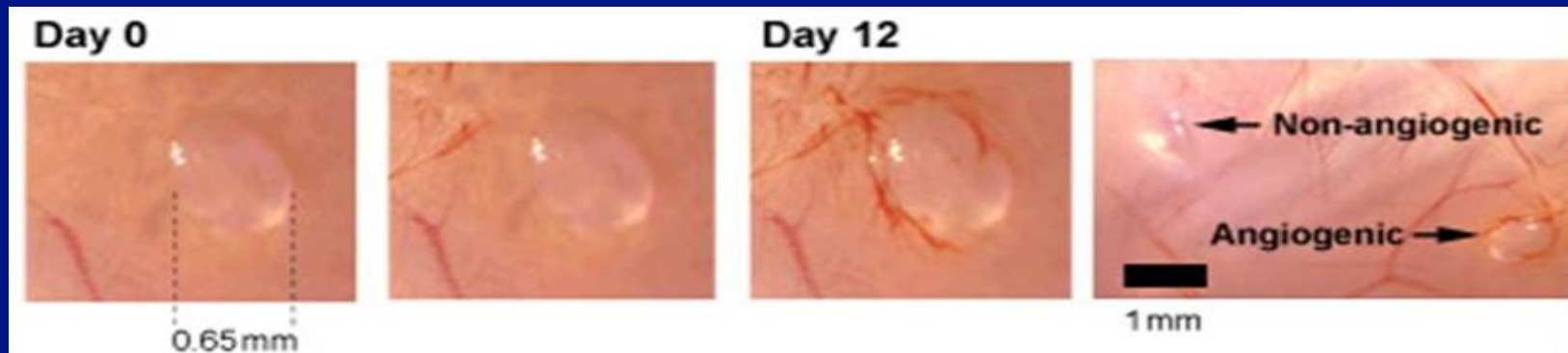
- An independent blood supply is required for a tumor to grow beyond 2mm in diameter<sup>1,2</sup>
- Larger tumors rely on their vasculature for survival and further growth<sup>1,2</sup>



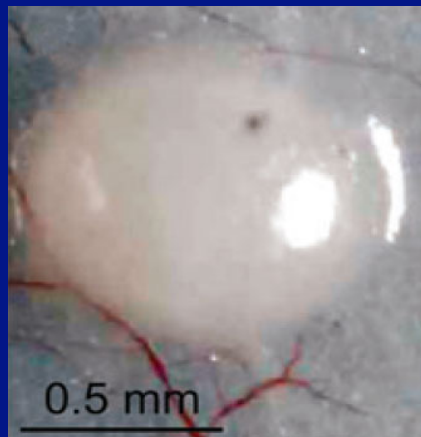


# Visualizing the angiogenic switch

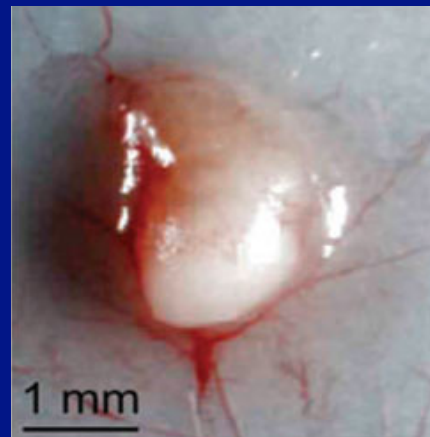
The images below depict **neovascularization** in a rat tumor model over 12 days following implantation of a tumor



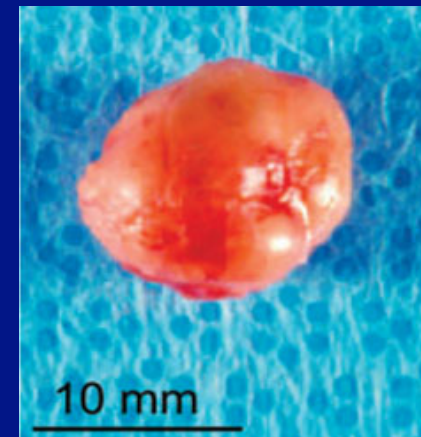
**Non-angiogenic tumor**



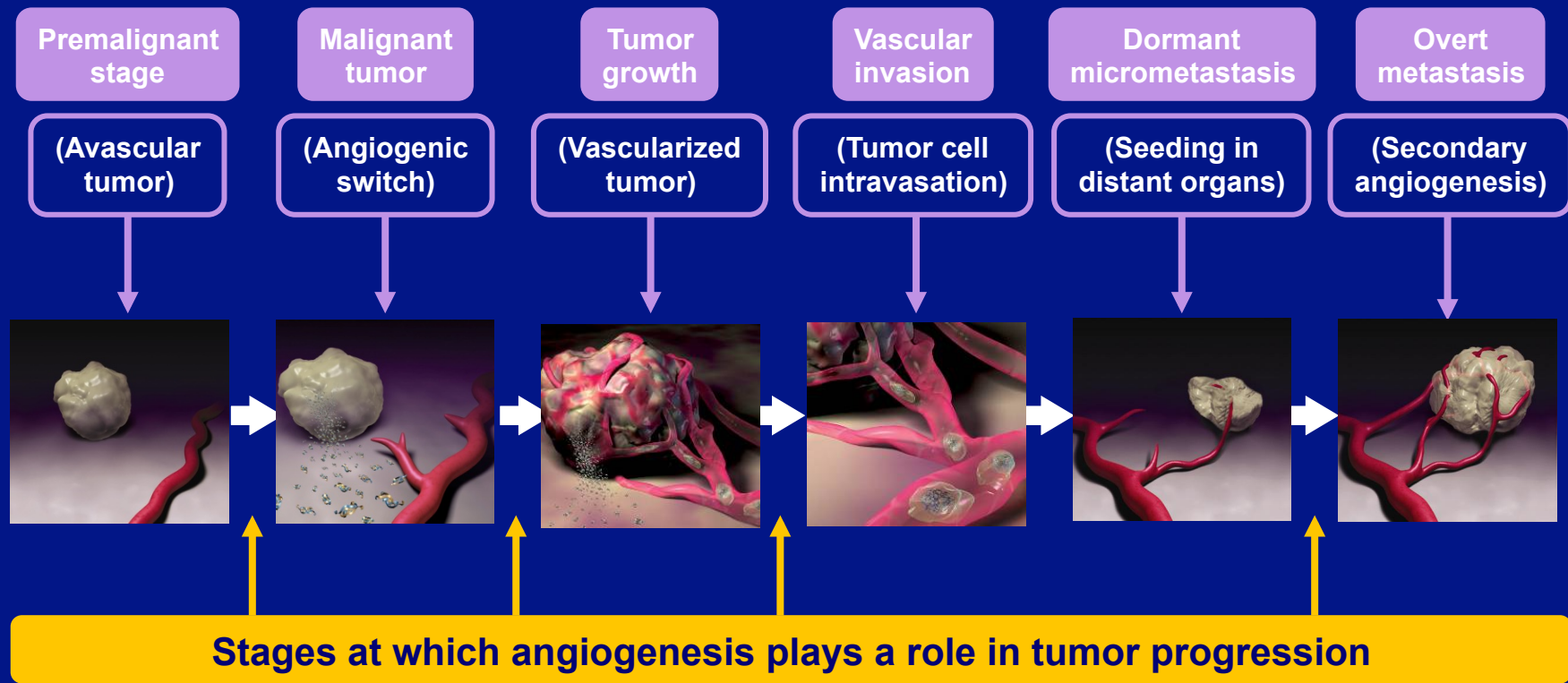
**Initial vascularization**



**Post-angiogenic switch**



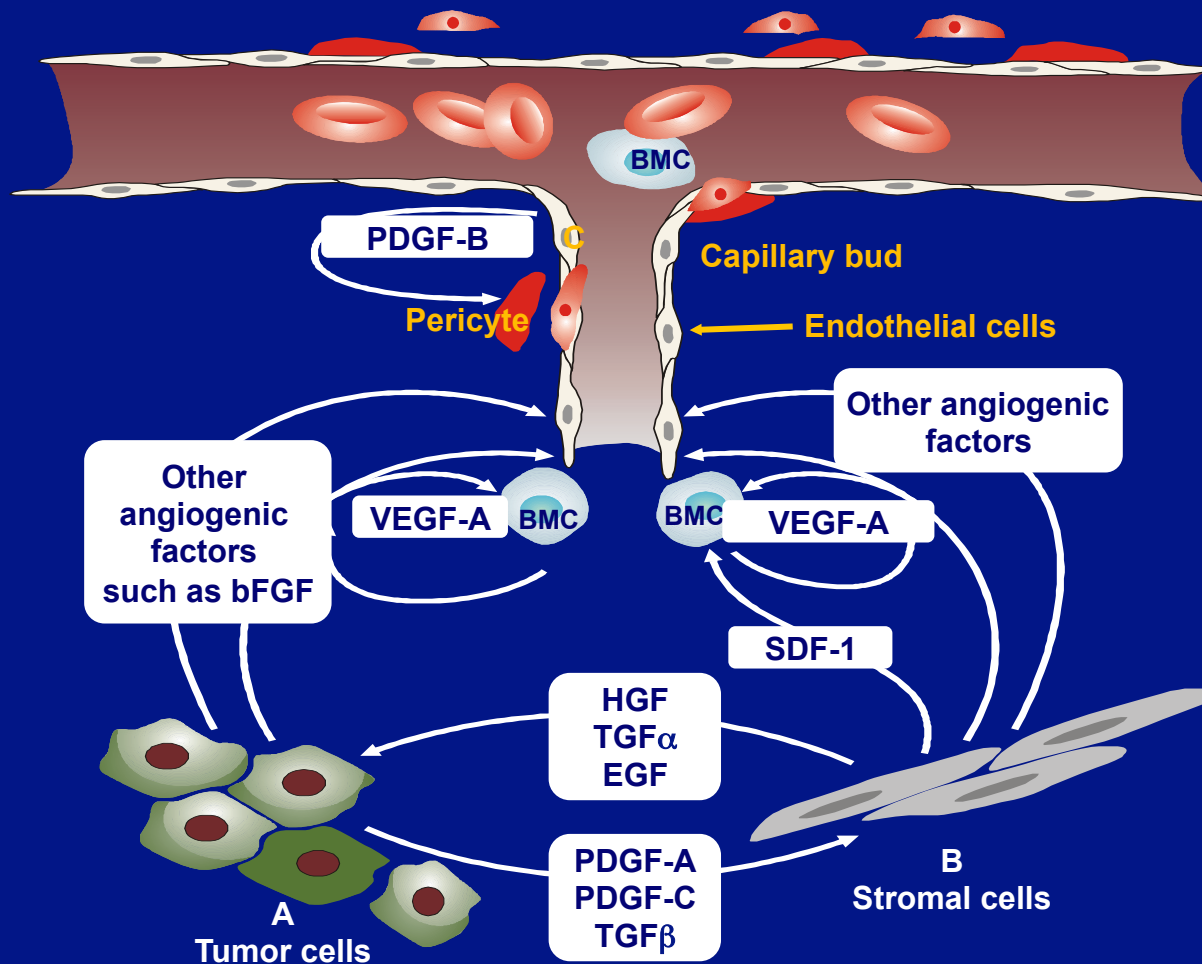
# Angiogenesis is involved throughout tumor formation, growth and metastasis



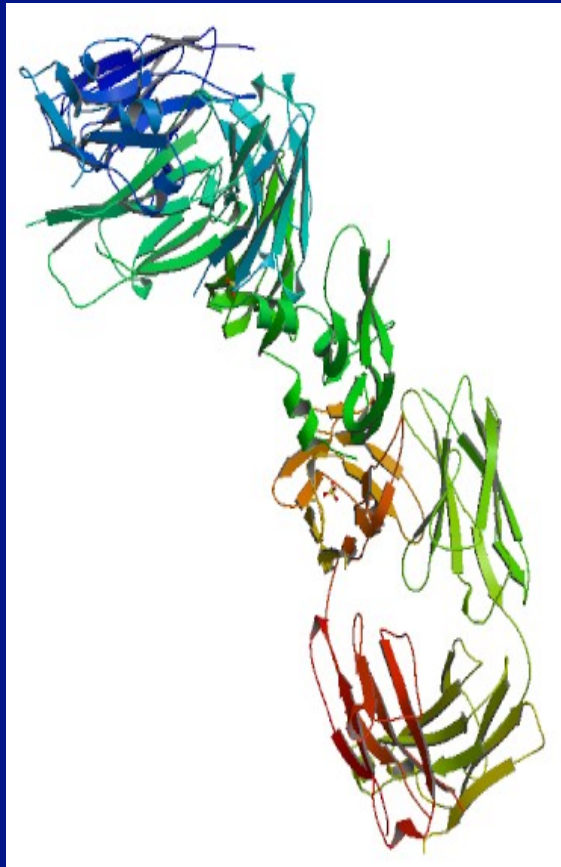
## Tumour growth depends on angiogenesis



# Tumor angiogenesis is complex: many cell types and many factors are involved



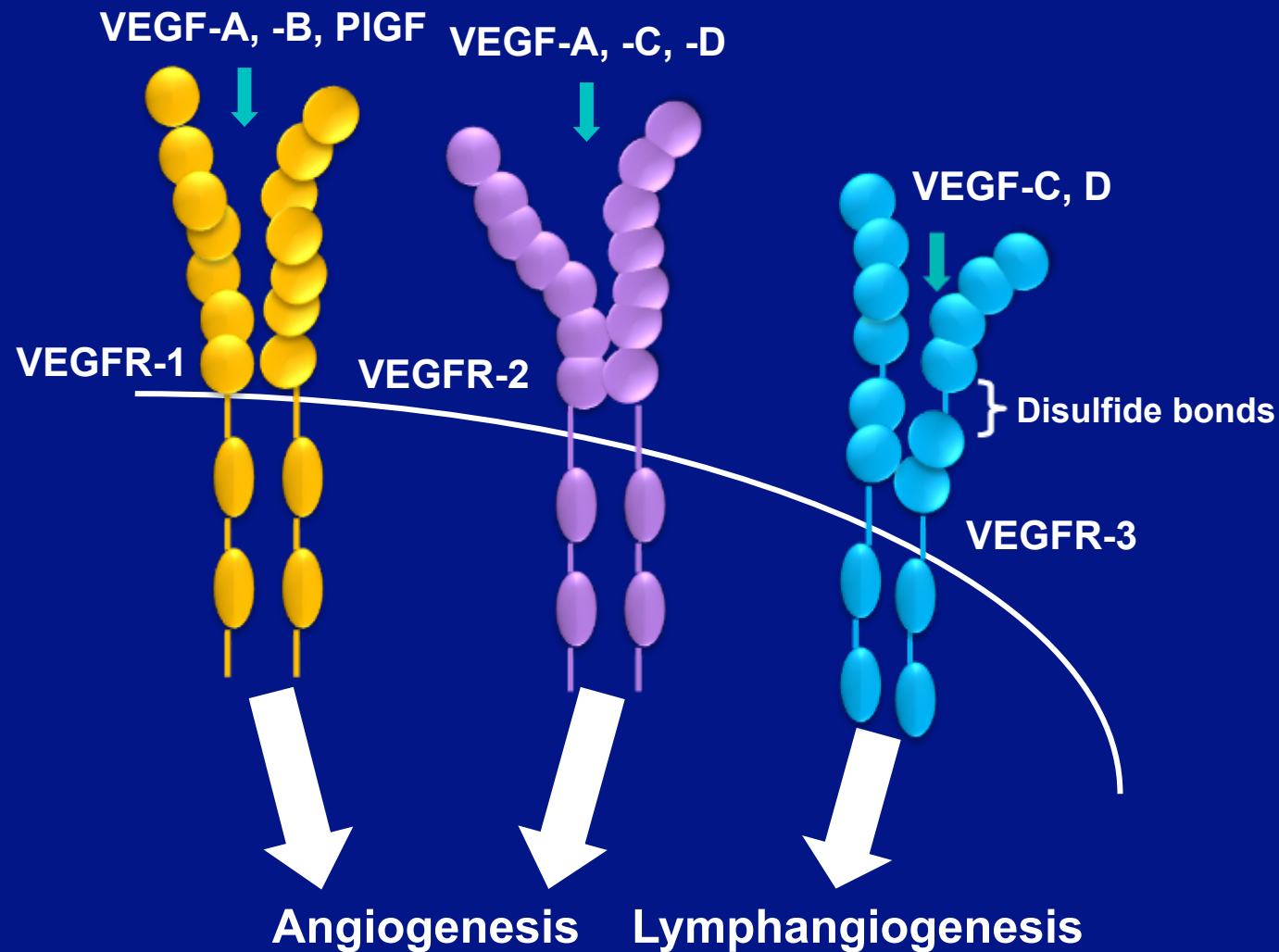
# VEGF is at the center of the angiogenic pathway



- Also known as vascular permeability factor (VPF)
- aka: VEGF-A; related molecules are VEGF-B, C, and D
- Central mediator of angiogenesis
- Mitogen for endothelial cells
- 45KDa heparin binding homodimeric glycoprotein
- Regulates angiogenesis
- Promotes survival of immature vasculature
- Binds to membrane receptor tyrosine kinases
- Four molecular species arising from the same gene
  - VEGF121, VEGF165\*, VEGF189, VEGF206

\*Predominant molecular species

# The VEGF family of isotypes and receptors



Adapted from Hicklin, Ellis. JCO 2005

# Functions of the VEGF family of receptors

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## VEGFR-1<sup>1,2</sup>

**Crucial to embryonic angiogenesis**

**Does not appear to be critical in pathogenic angiogenesis. Present on activated vascular endothelial cells, dendritic cells, HSCs, leukemic tumor cells**

## VEGFR-2<sup>1,3</sup>

**Most important VEGF receptor in tumor angiogenesis**

**Mediates the majority of VEGF angiogenic effects.**

**Present on vascular endothelial cells, circulating endothelial precursors, dendritic cells, leukemic cells**

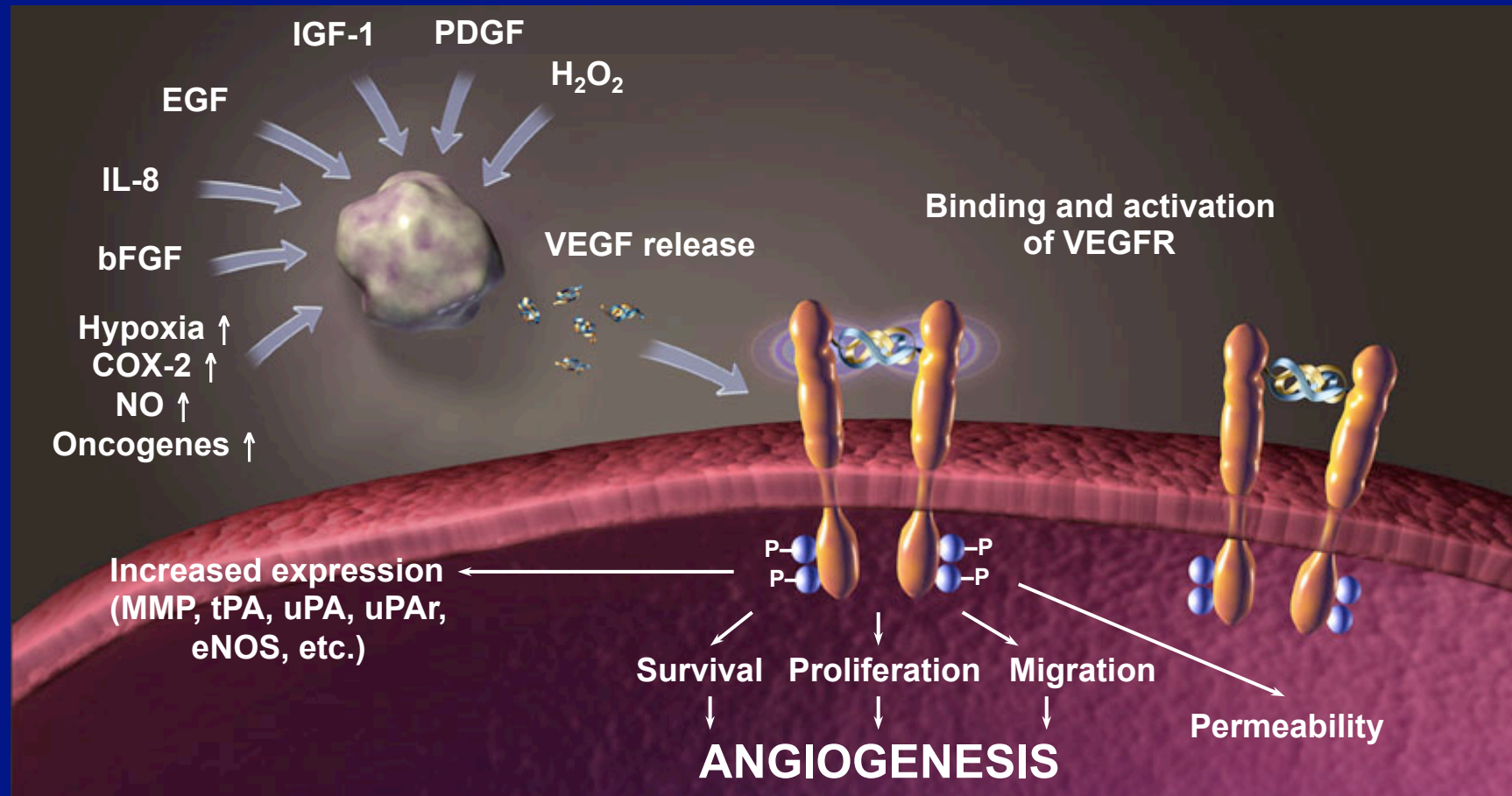
## VEGFR-3<sup>1,4</sup>

**Found only in lymphatic endothelial cells**

**Associated with lymph node metastasis**

---

# Tumor characteristics and environment promote VEGF expression



# Cancer-causing genes implicated in tumor angiogenesis

- **Activated oncogenes**

- Ras
- Src
- ErbB2/HER2
- EGFR
- HPV16
- Bcr-Abl
- n-myc; c-myc

- **Mutated/inactivated tumor suppressor genes**

- p53
- PTEN
- VHL
- p16

- **Effect**

- VEGF↑ bFGF↑ TSP-1↓
- VEGF↑
- VEGF↑ TSP-1↓
- VEGF↑ IL-8↑ bFGF↑
- VEGF↑
- VEGF↑
- VEGF↑ TSP-1↓
  
- VEGF↑ TSP-1↓
- VEGF↑
- VEGF↑
- VEGF↑

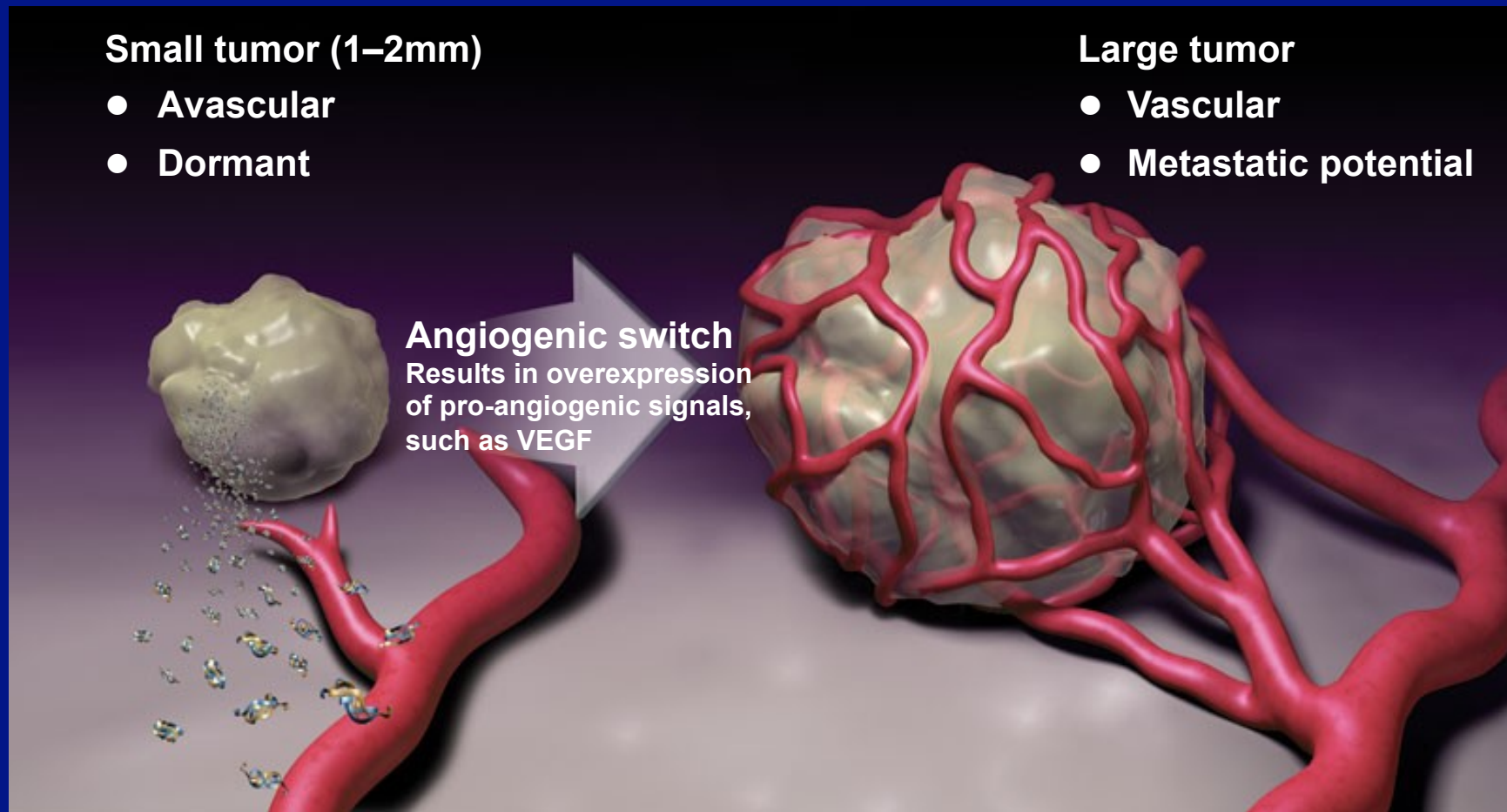
# Overexpression of pro-angiogenic signals, such as VEGF, enables tumors to progress

## Small tumor (1–2mm)

- Avascular
- Dormant

## Large tumor

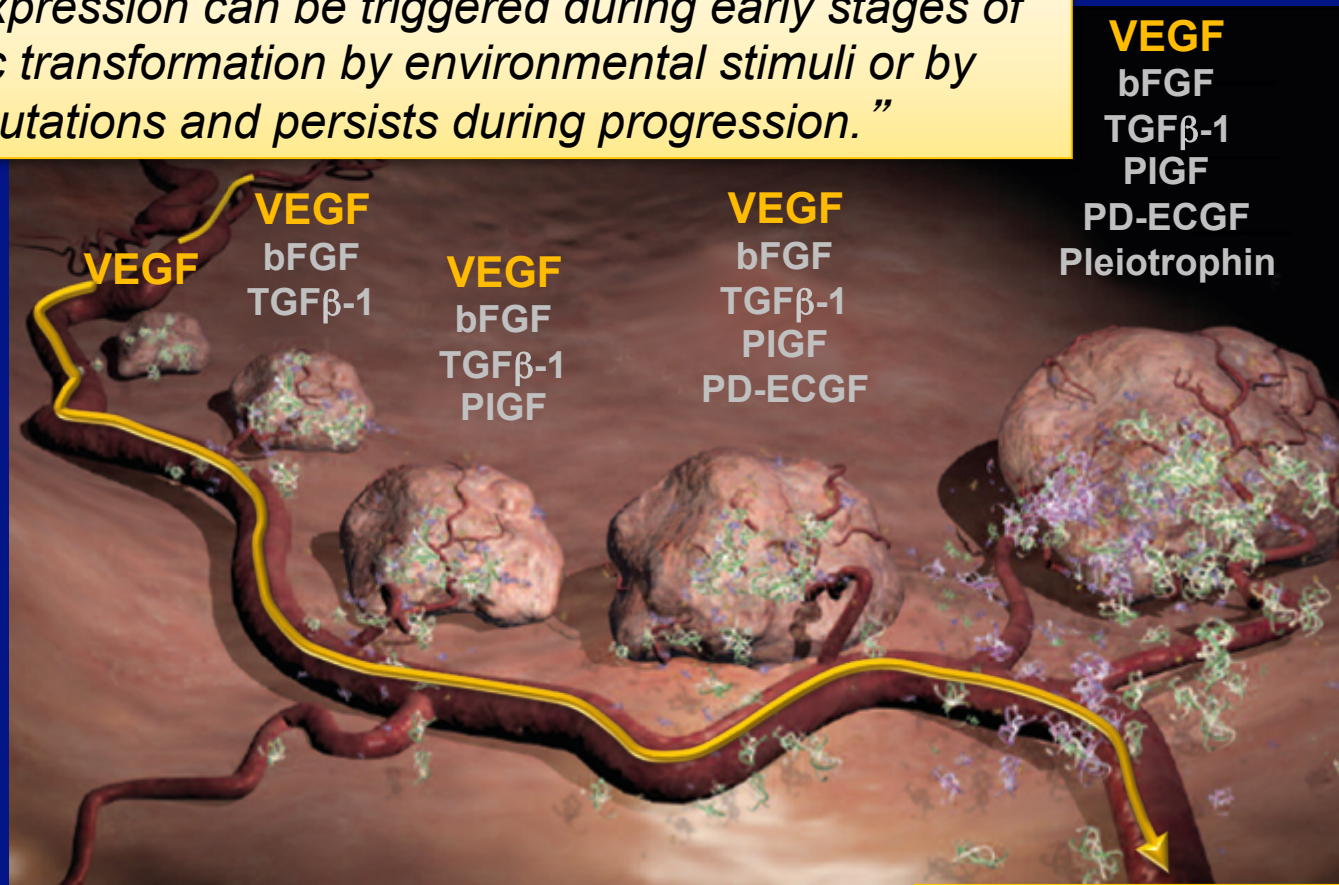
- Vascular
- Metastatic potential





# VEGF is the only angiogenic factor present throughout the tumour life cycle

*“VEGF expression can be triggered during early stages of neoplastic transformation by environmental stimuli or by genetic mutations and persists during progression.”*

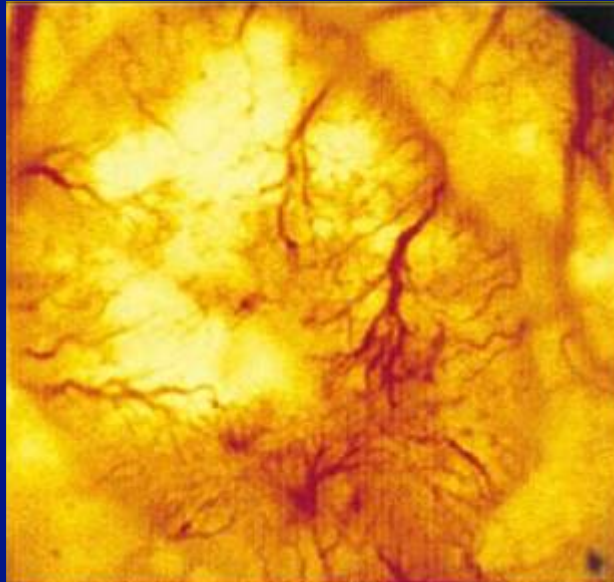


**Tumour life cycle**

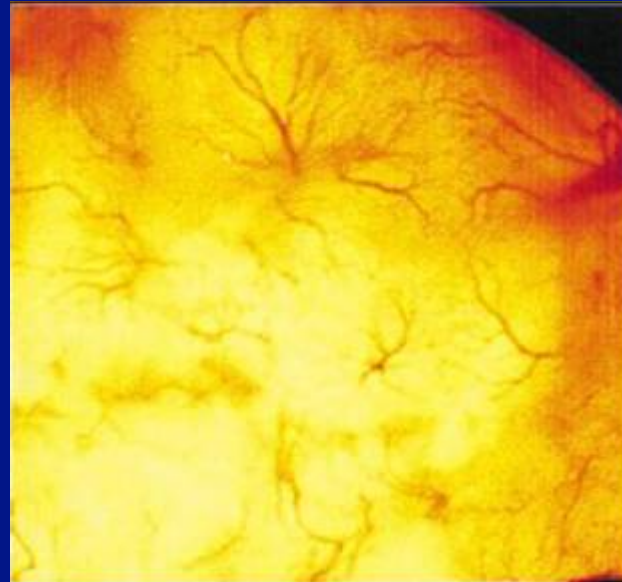
Folkman. Cancer. In: Principles and Practice of Oncology 2005  
Bergers, et al. Nat Rev Cancer 2003; Jain, et al. Nat Clin Pract Oncol 2006  
Inoue, et al. Cancer Cell 2002

## Regression of tumor vasculature: decreased vascular volume

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Control (saline)



Anti-VEGF mAb

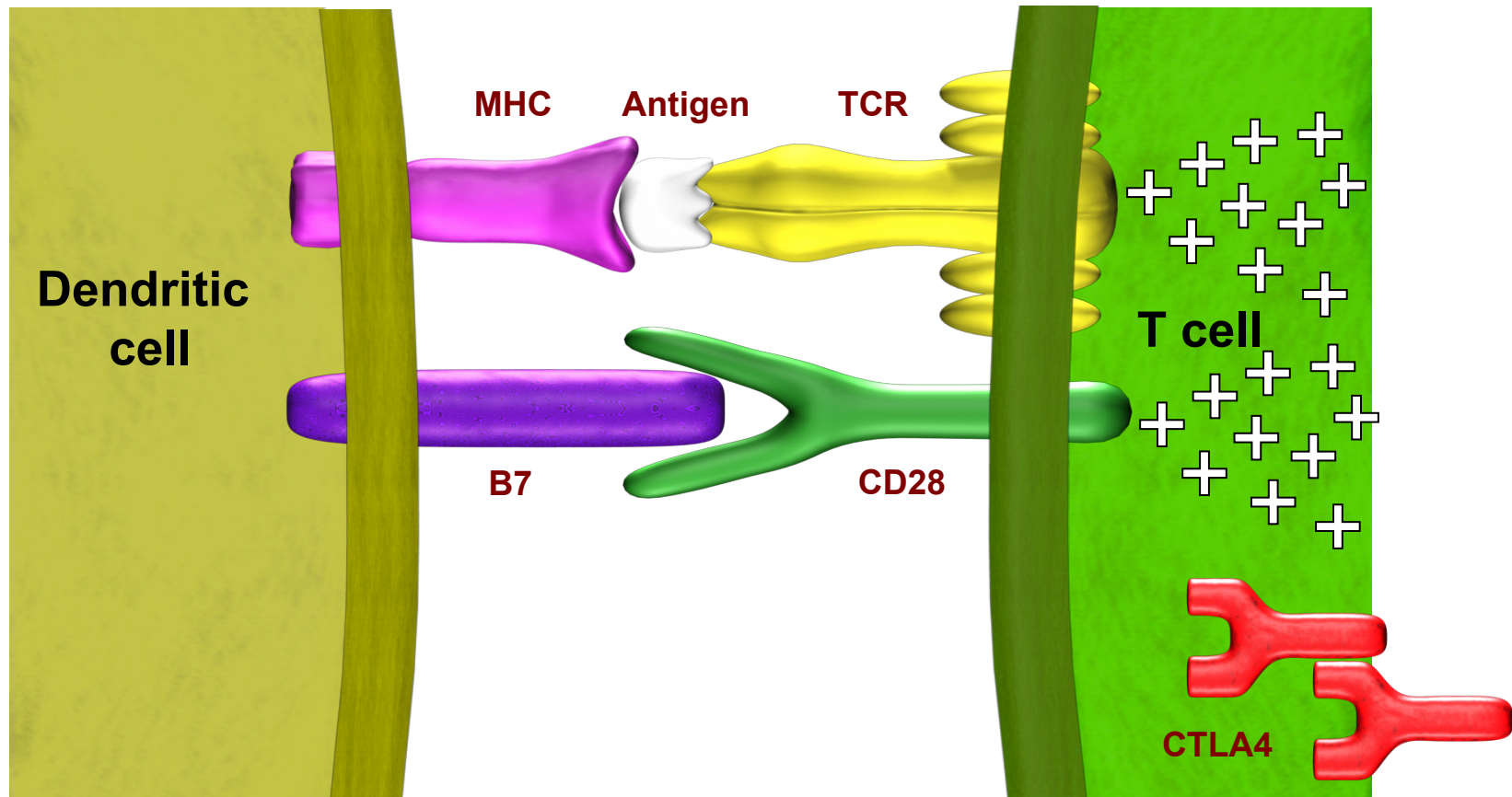
- **Decreased tumor vascular volume was observed in a colon adenocarcinoma xenograft model after administering an anti-VEGF mAb**
  - **by day 7 small vessels were not visible (right)**

# **Antiangiogenic Therapy in Cancer**

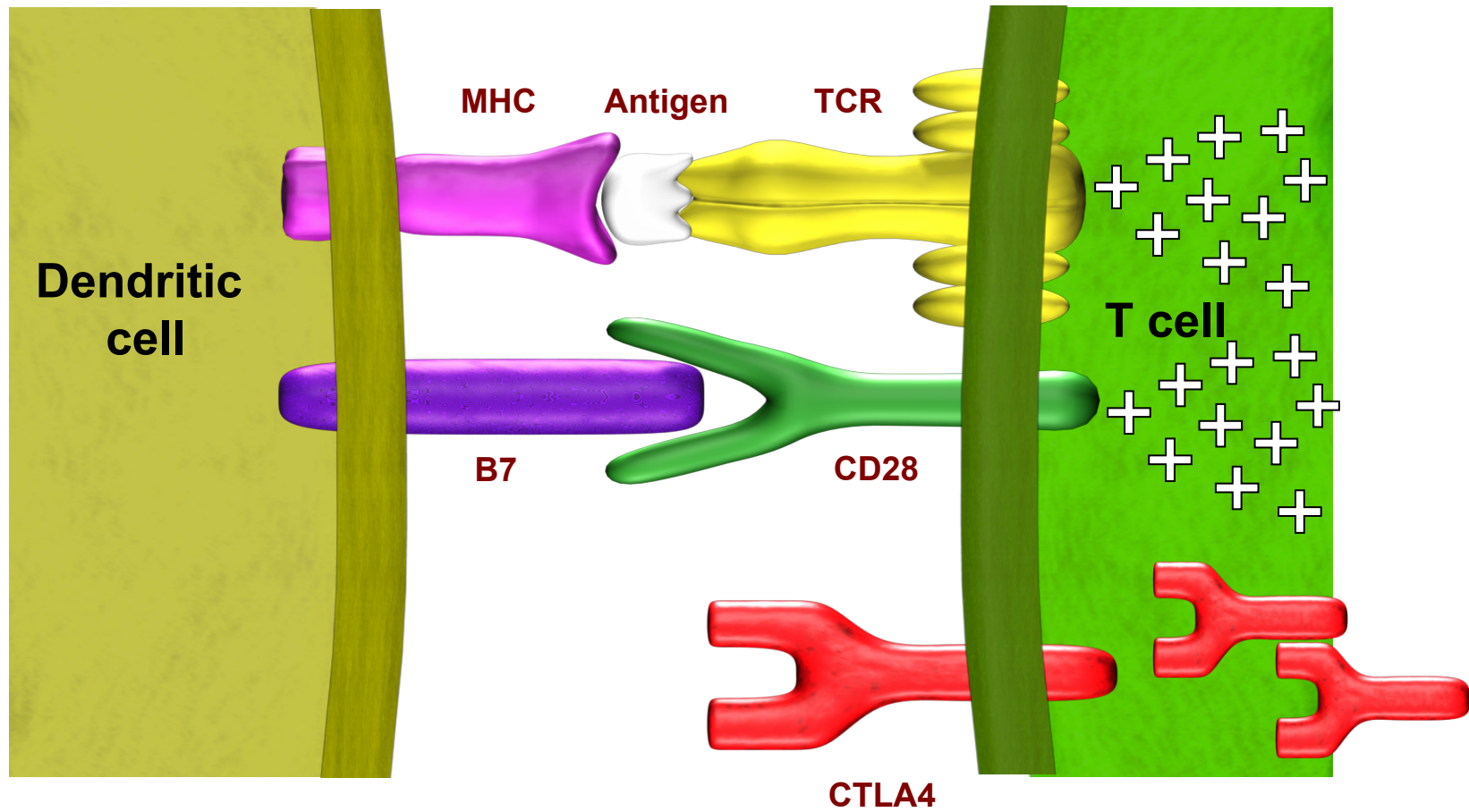
- **Metastatic Colo-rectal cancer**
- **Metastatic Non-Squamous NSCLC**
- **Glioblastoma**
- **Ovarian Cancer**
- **Advanced Cervix Cancer (GOG 240, 2013)**
- **Renal Cell Carcinoma**
- **Hepatocellular Carcinoma**
- **Breast Cancer (FDA approved withdrawn due to political reasons)**
- **Neuro-Ectodermal Tumors**
- **GIST**

# **EVADING APOPTOSIS**

# T Cell Activation by TCR and Co-stimulation Through CD28

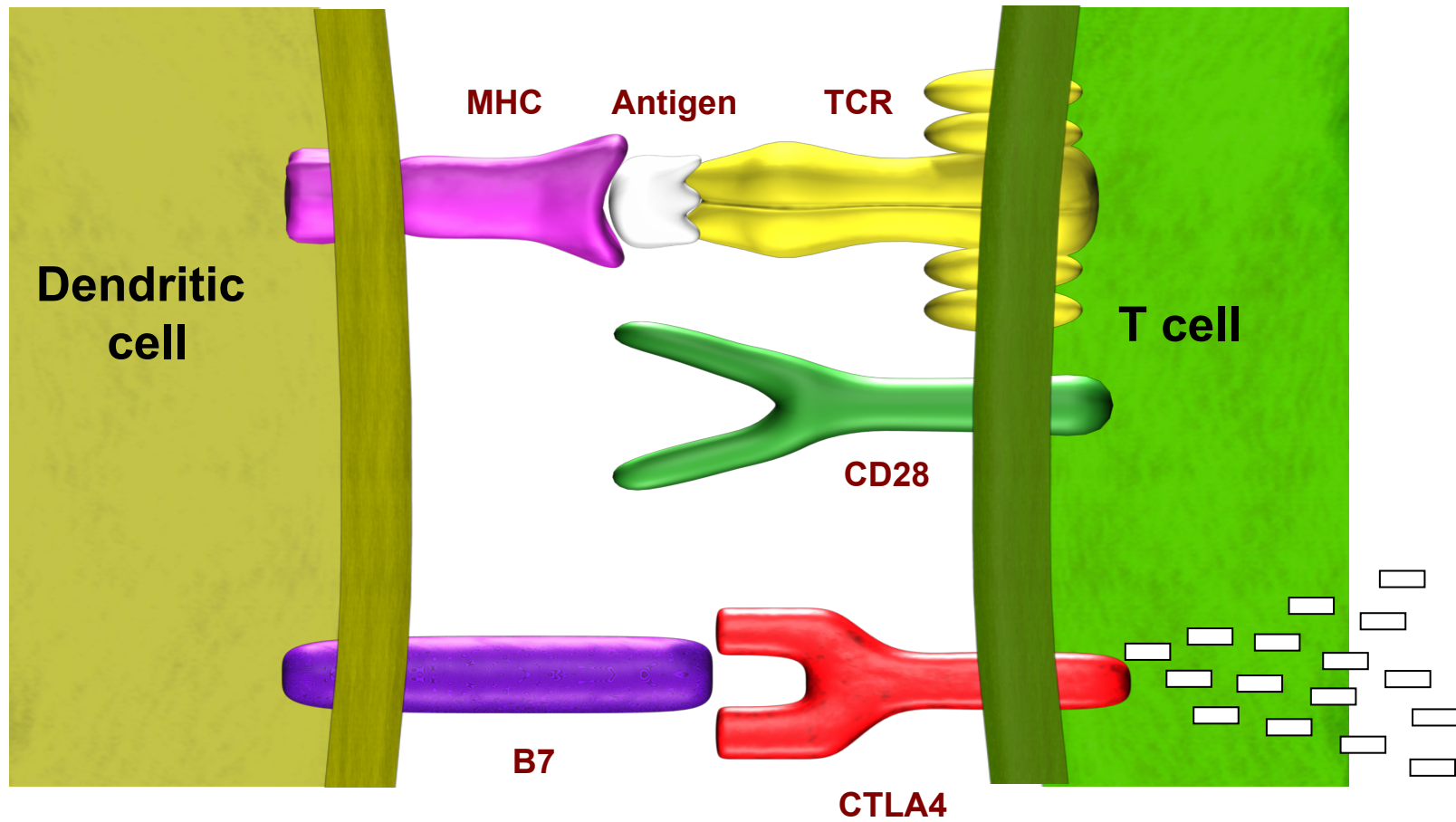


# CTLA4 Receptors Are Up-Regulated Following T-Cell Activation



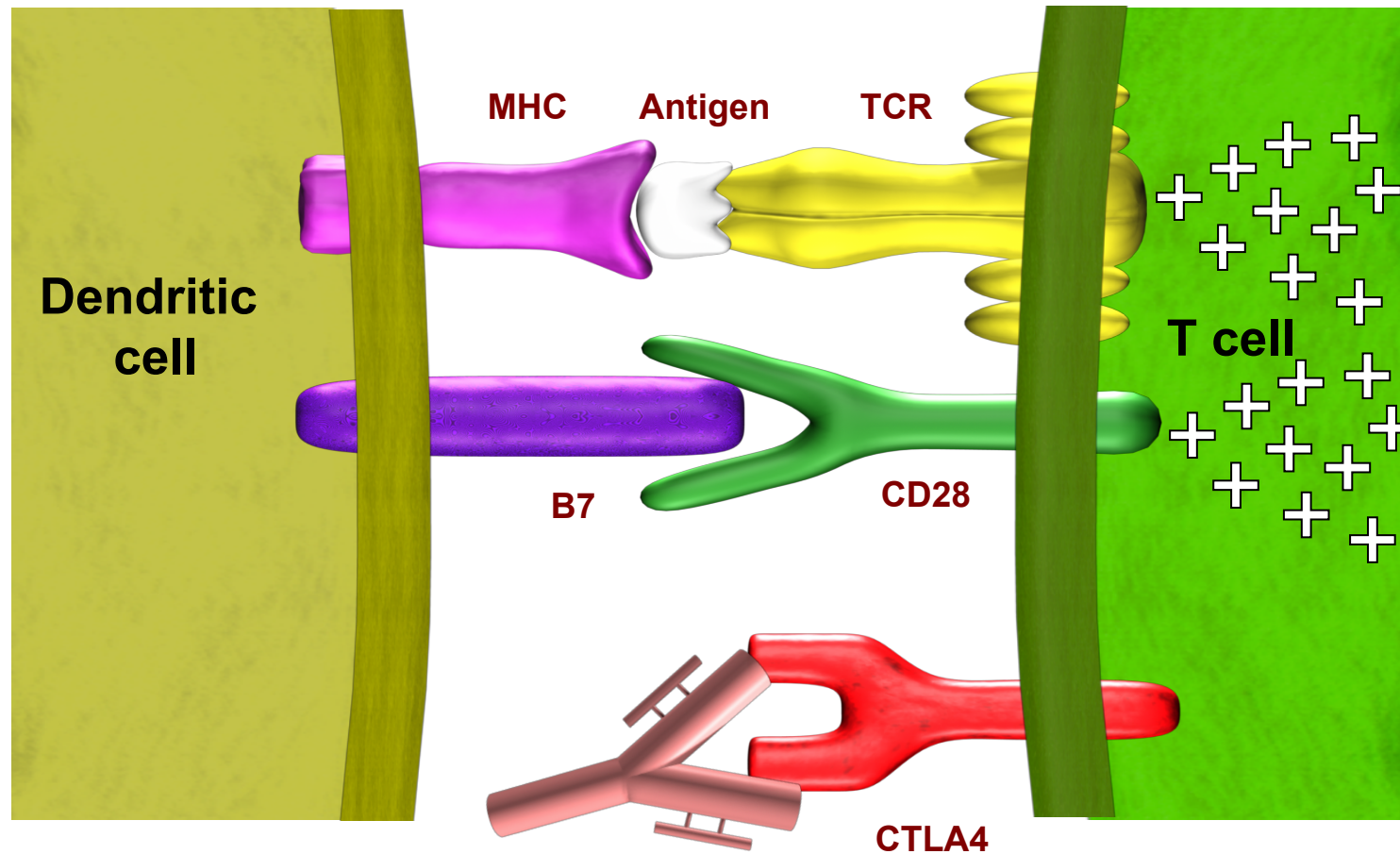


# CTLA4 Negatively Modulates T-Cell Activation



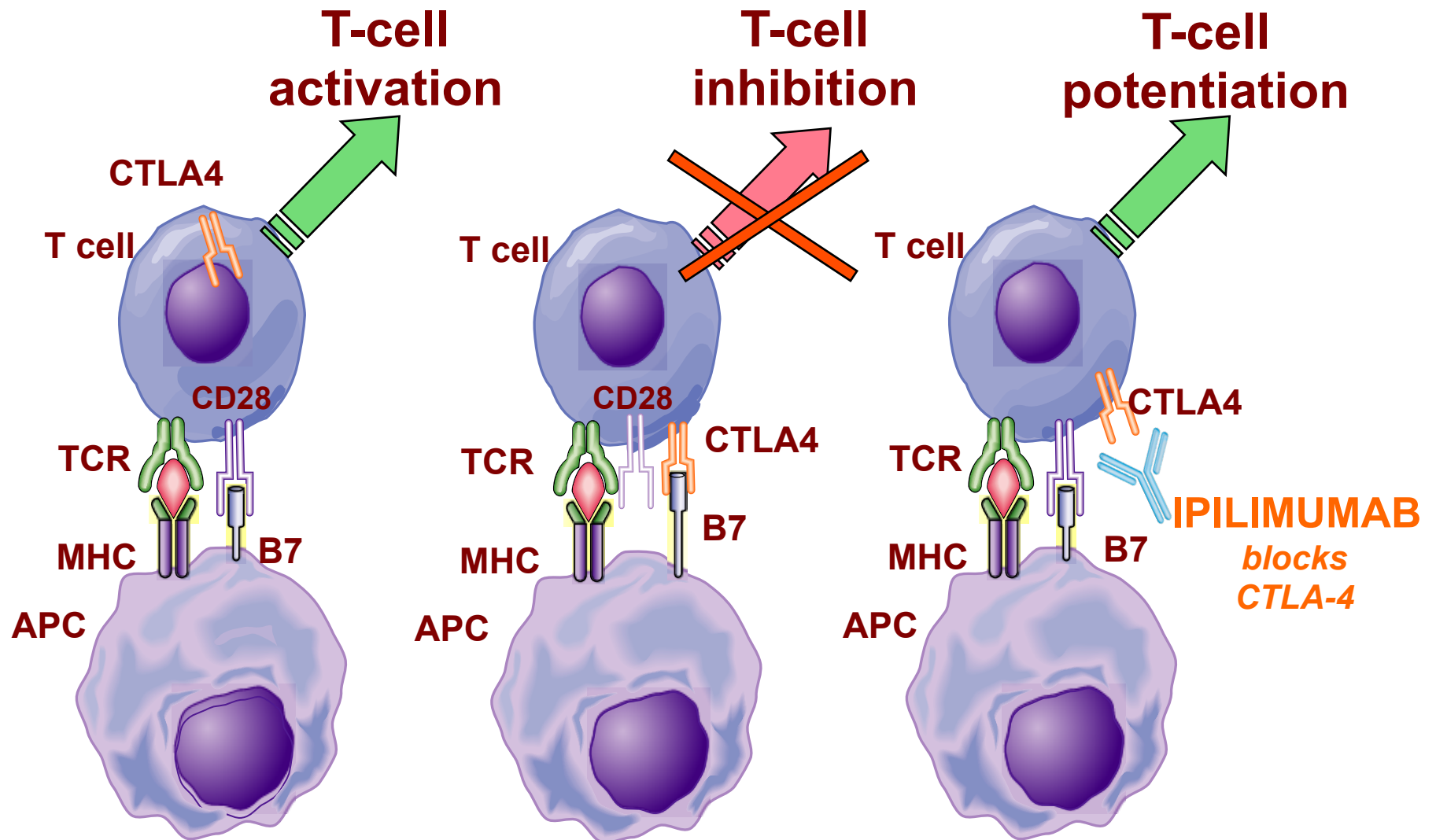


# Blocking Antibodies to CTLA4 Allow Positive Signaling from Costimulatory Molecules to T Cells



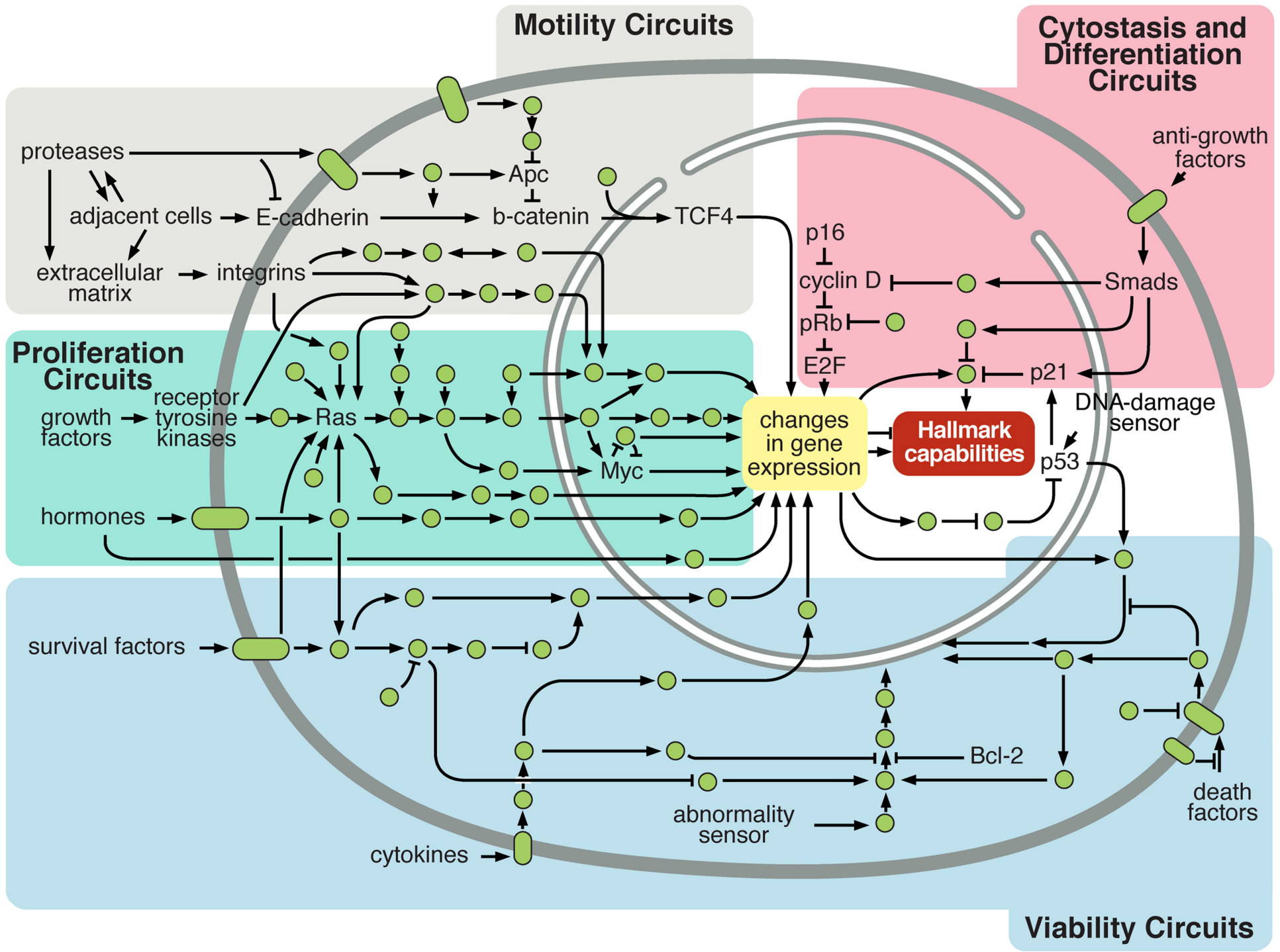
Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734-1736.

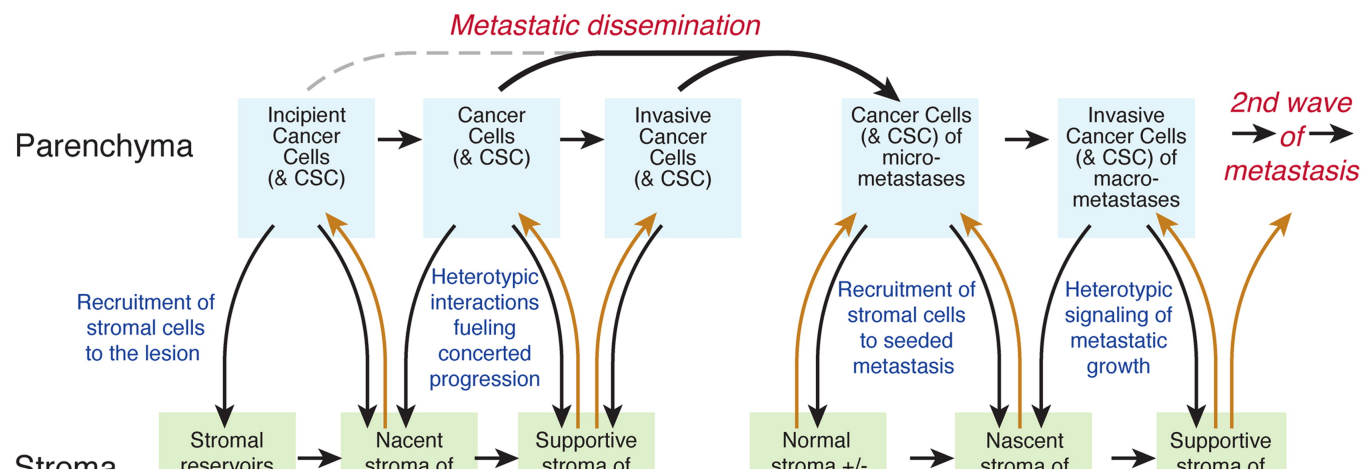
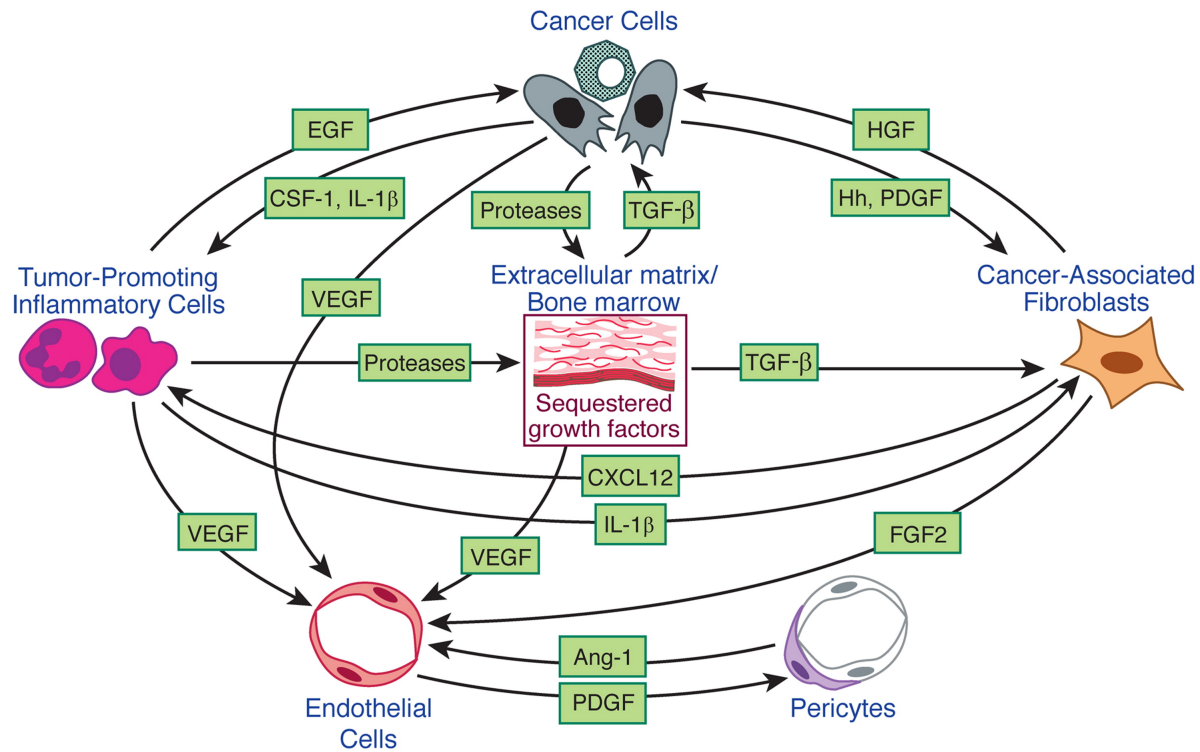
# Ipilimumab: Mechanism of Action



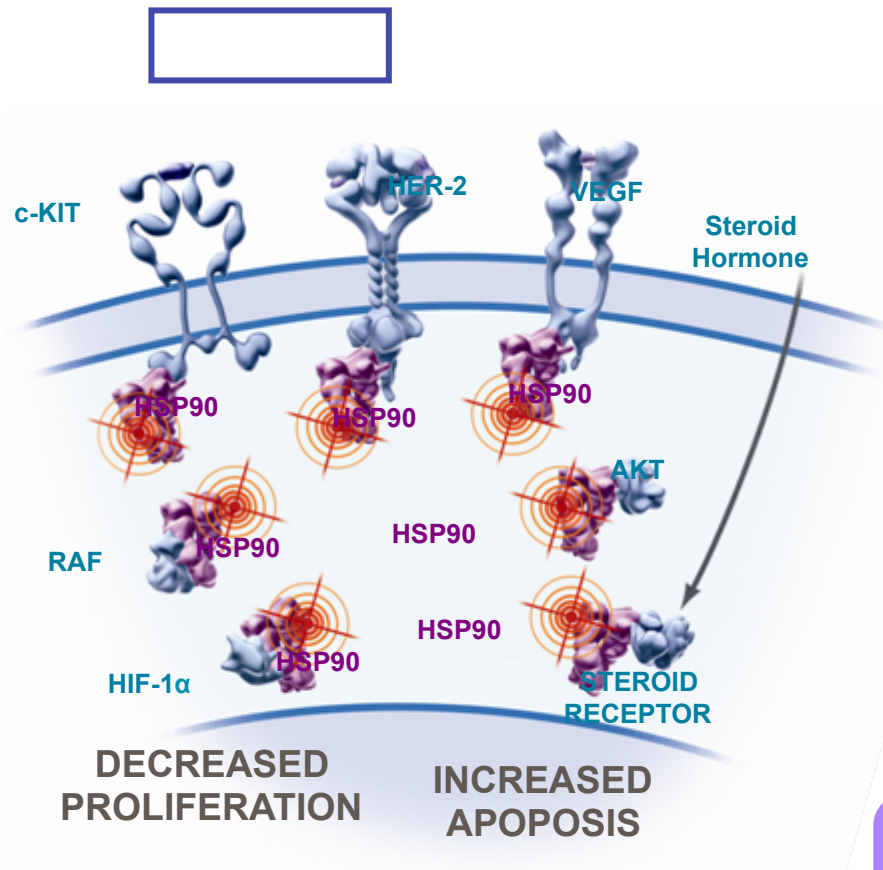
## **Anti CTLA-4 therapy**

- **Malignant Melanoma**
- **Possibly**
  - **Prostate Cancer**
  - **NSCLC**





# Novel Potent HSP90 Inhibitors

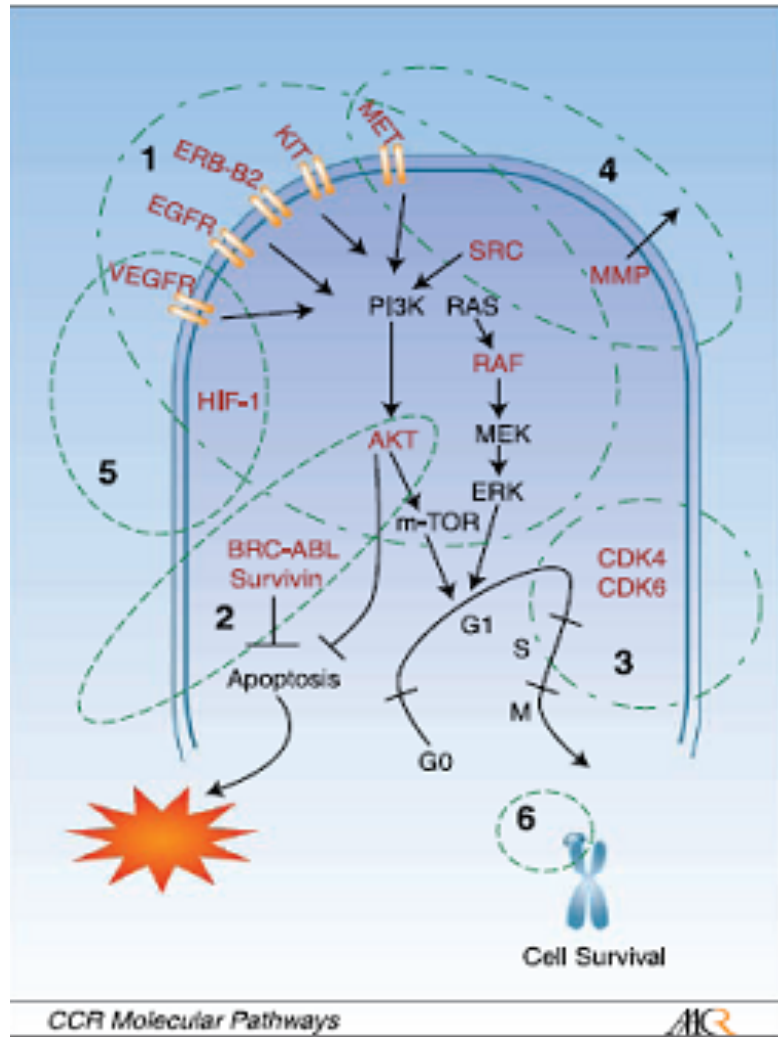


- HSP90 is a chaperone protein which is involved in the folding of a large number of client proteins
- Key oncogenic client proteins include:
  - Transcription factors (e.g. estrogen receptor, androgen receptor)
  - Signaling kinases and their mutated forms (e.g. ErbB2, Akt, Ras, Raf, IKK, Bcr-Abl, FAK, IGF-1R, NPM-Alk, etc.)
- Upregulated and in a more active state in cancer cells

**Inhibition of HSP90 leads to simultaneous inhibition of multiple pathways resulting in an anti-tumor effect**



# HSP90 is a chaperone protein to “client proteins” which are involved in all 6 hallmarks of cancer



Every protein in red is an HSP90 client

- Growth factor independence
- Inhibition of apoptosis
- Resistance to anti-growth signals
- Tissue invasion and metastasis
- Sustained angiogenesis
- Unlimited replicative potential



# Key Client Proteins of HSP90 and Associated Diseases

Cancer indication	Key HSP90 client proteins	Additional client proteins
Breast	HER-2, estrogen and progesterone receptor	EGFR, Akt, VEGFR, IGF1R, Raf-1
Stomach	HER-2, C-Met, Akt	EGFR, VEGFR, TS, PDGFR, HIF-1a
NSCLC	EGFR, C-Met	VEGFR, K-Ras, AKT
Pancreas	K-ras, IGF-1R, VEGFR, ERK	Akt, EGFR, HIF-1a, IL-6R
Myeloma	IGF1R, IL-6R, IKK	Raf, Akt
Glioblastoma	EGFR, VEGFR, PDGFR	HER2, PI3K, C-Met, NF1, Akt, K-ras etc

