Translational Medicine and New Therapies for the Treatment of GIST

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What are the problems with the current therapeutic options for recurrent/ metastatic GIST?

- Primary Gleevec Resistance
  - KIT WT
  - PDGFRA mutants
- Gleevec and Sutent not curative
  - Primarily “static” not “cidal”
- Requires lifelong therapy with imatinib for stable disease
- Secondary Gleevec resistance within 2 years in 50% of patients
- Sunitinib stabilizes subset of patients for median of 6-8 months
- No FDA approved 3rd line therapy.
  - Sorafenib is only FDA approved (not for GIST) 3rd line therapy.

New Strategies/Hope for Treating GIST

- Novel KIT inhibitors that target KIT and PDGFRA resistance mutants.
- Target KIT downstream signaling.
- Destroy KIT.
- Target KIT survival pathways / induce apoptosis.
- Augment the immune system to kill GIST cells.
- Targeting chromatin remodeling/epigenetics/transcription factors.
- Targeting GIST stem cells.

3rd line KIT inhibitors

- Nilotinib - second generation tyrosine kinase inhibitor that inhibits KIT, PDGFRα, and BCR-ABL which has higher intracellular concentrations compared with imatinib.
- Sorafenib - multikinase inhibitor that blocks KIT, VEGFR, PDGFR, RAF kinases (MAP kinase pathway) with demonstrated activity in *KIT* exon 17 mutants that are resistant to IM and sunitinib.
- Dasatinib - multikinase inhibitor that inhibits KIT, PDGFR, ABL, EPHA2, and SRC family kinases and is active against PDGFRA D842V and *KIT* exon 17 mutants in vitro.
- All have demonstrated activity in imatinib-/sunitinib-resistant GIST.
- Many other 2nd generation KIT inhibitors in various phases of development and study (e.g. – regorafenib, motesanib, vatalanib, amuvatinib, motesanib, tivozanib, dovitinib).

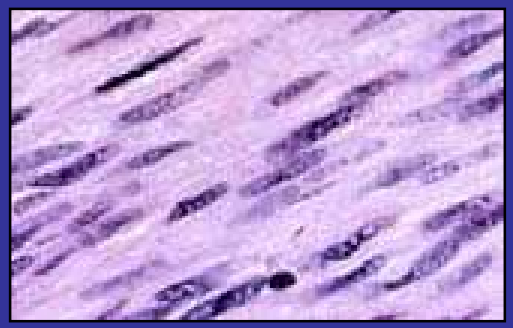
PDGFRA inhibitor

- CP-868,596
- Potent PDGFRA D842V inhibitor
- Clinical trial being conducted at Oregon Health Sciences University and Fox Chase Cancer Center.

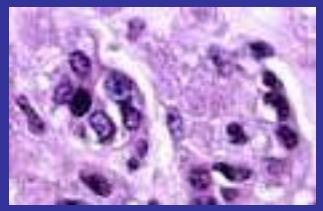
The limitation of KIT inhibitors

- Patients with generalized/multifocal resistance have different secondary *KIT* mutations in different progressing nodules.
- Theoretically need to cover most if not every conceivable activating mutation.
- GIST will find a way to escape from mono-therapy.
- The GIST field is moving slowly towards combination therapy.

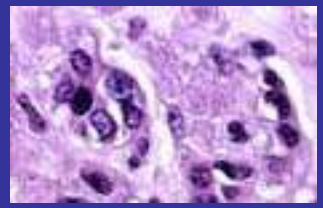
Variety of secondary *KIT* mutations in a single patient with a primary *KIT* exon 9 mutation and generalized resistance



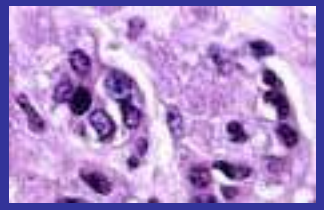
Primary *KIT* exon 9 mutant



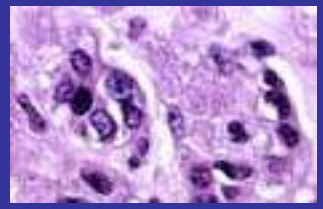
Exon 9 / N822K



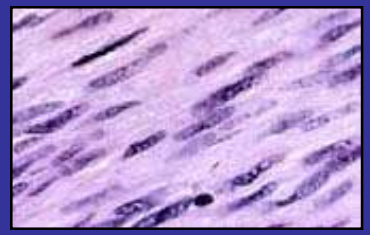
Exon 9 / D820E



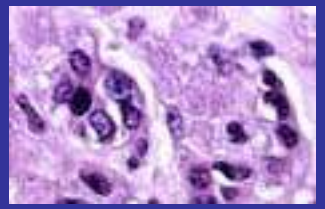
Exon 9 / N822Y



Exon 9 / D820G



Exon 9 / V654A



Exon 9 / N822H

Courtesy of Dr. Jonathan Fletcher

Switch Pocket Kinase Inhibitors

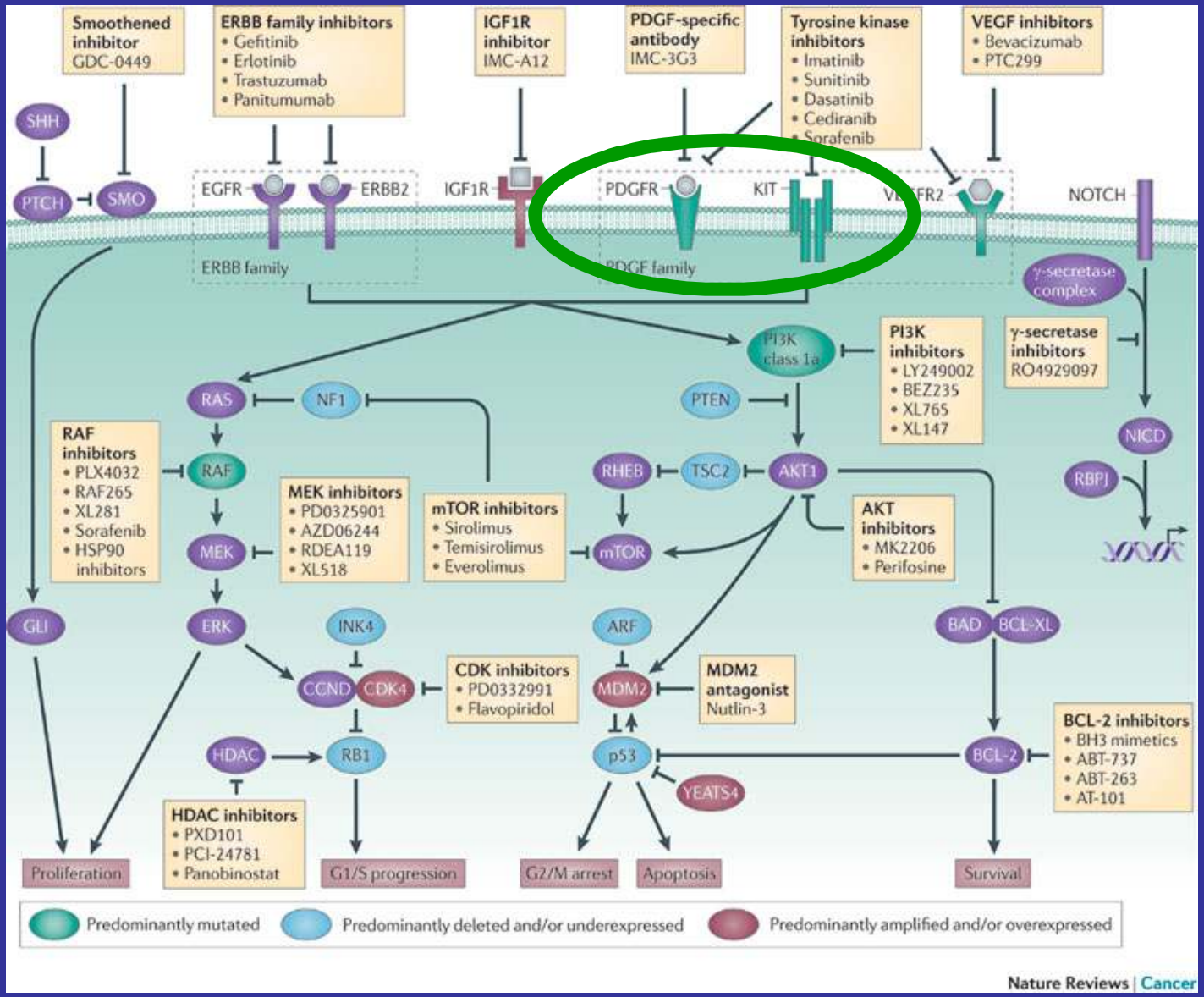
- Traditional KIT / PDGFRA kinase inhibitors compete with ATP.
- Switch pocket inhibitors work by an entirely different mechanism where they stabilize the inactive conformation of KIT / PDGFRA by binding the “pocket” that allows switching from inactive to active forms of the proteins. They trap the proteins in an inactive conformation.
- Active at nanomolar (very low) concentrations.
- Since mechanism of action is different / novel, these inhibitors have the potential to inhibit most Gleevec/Sutent KIT / PDGFRA mutants.

Heinrich MC et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 10007).

Targeting KIT downstream signaling

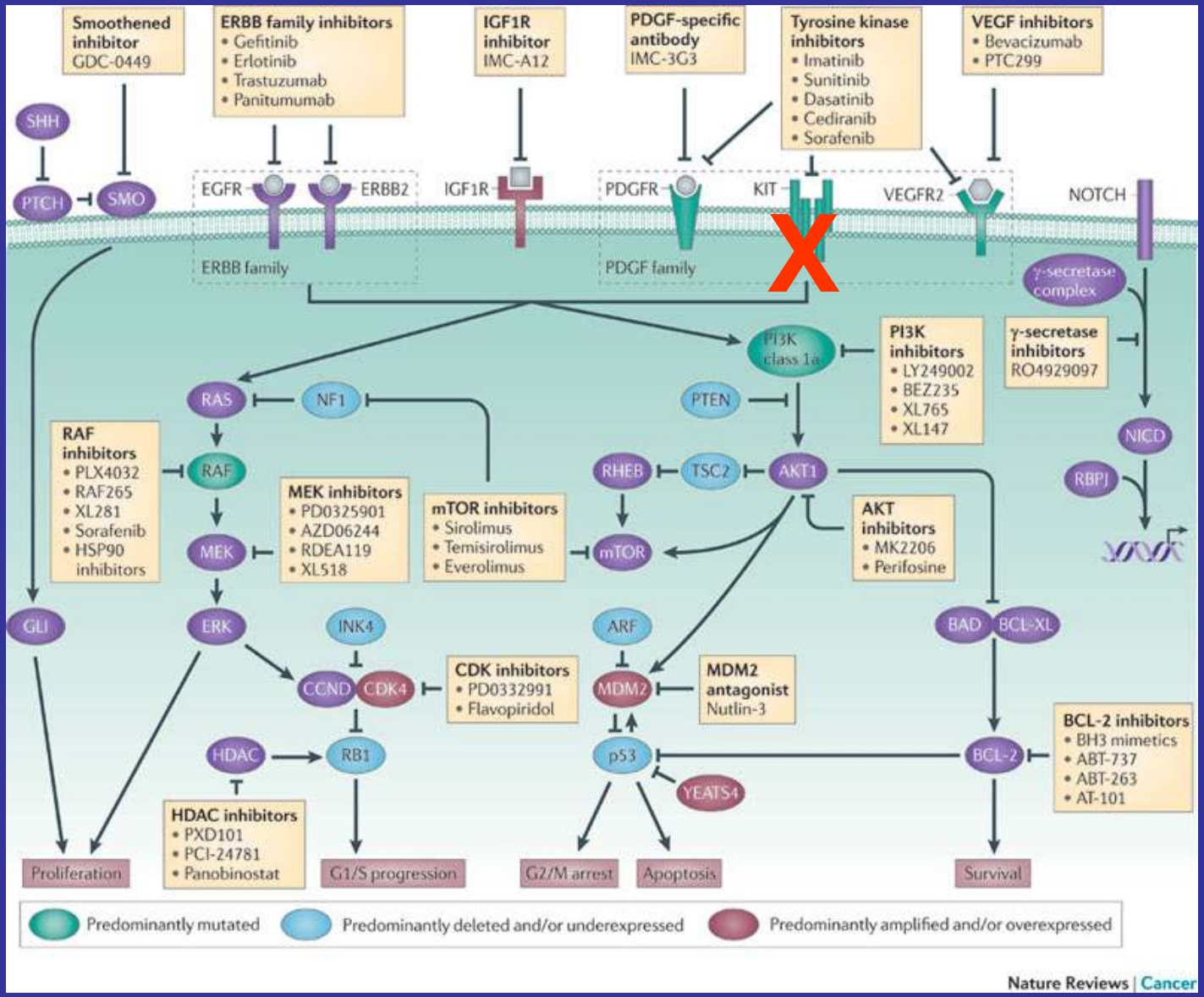
- Cells contain networks of proteins that cause cells to divide, grow, move (metastasize) and survive.
- Think of KIT / PDGFRA as the accelerator that controls the engine using a car analogy.
- Gleevec / KIT inhibitors are like the brakes for the car.
- Downstream signaling molecules are like the transmission of the car – once it is engaged, if the brakes fail, the car moves forward and is out of control.

Target KIT downstream signaling



Taylor BS et al. Nat Rev Cancer 2011;11:541-57

Target KIT downstream signaling



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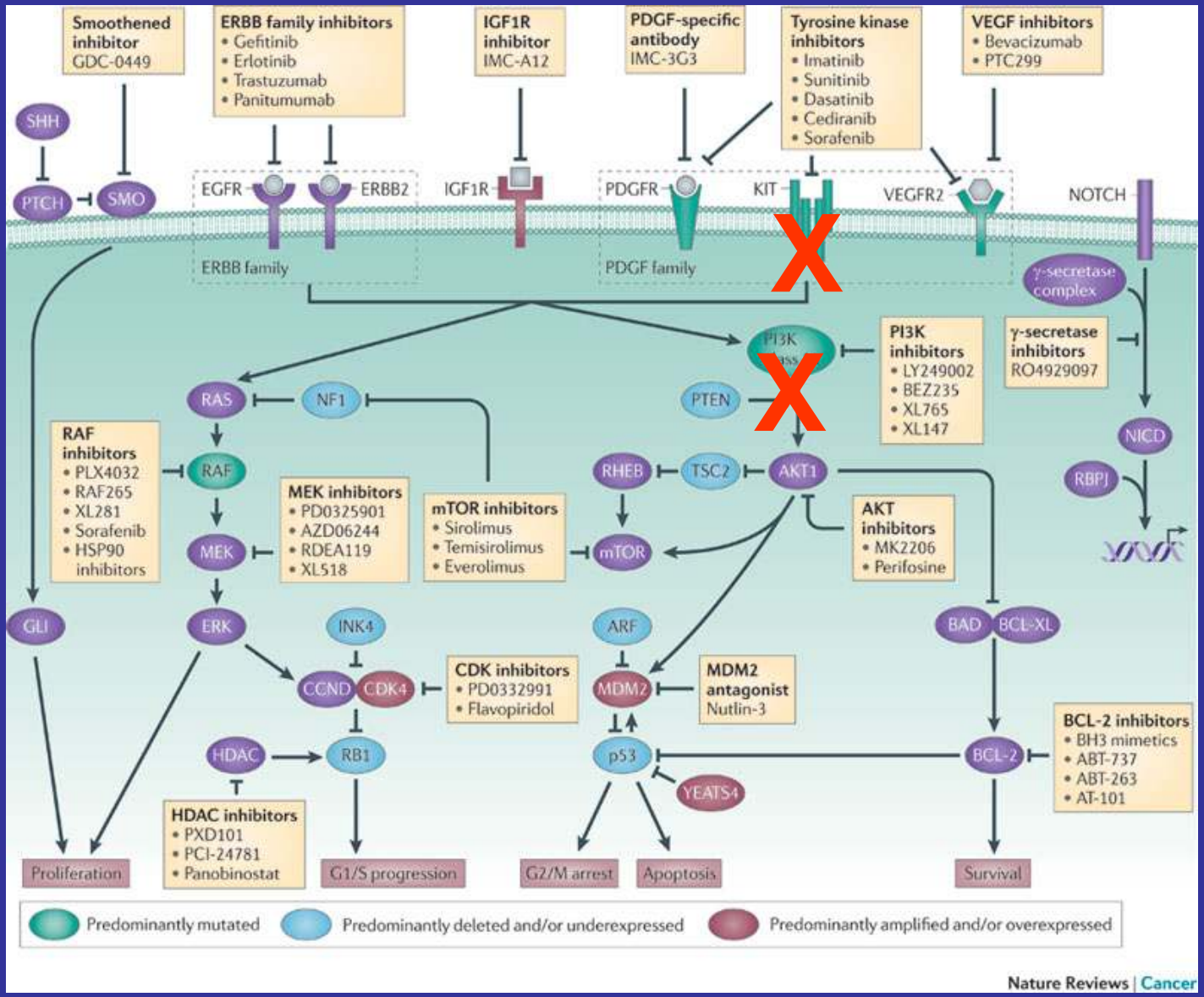
Target KIT downstream signaling



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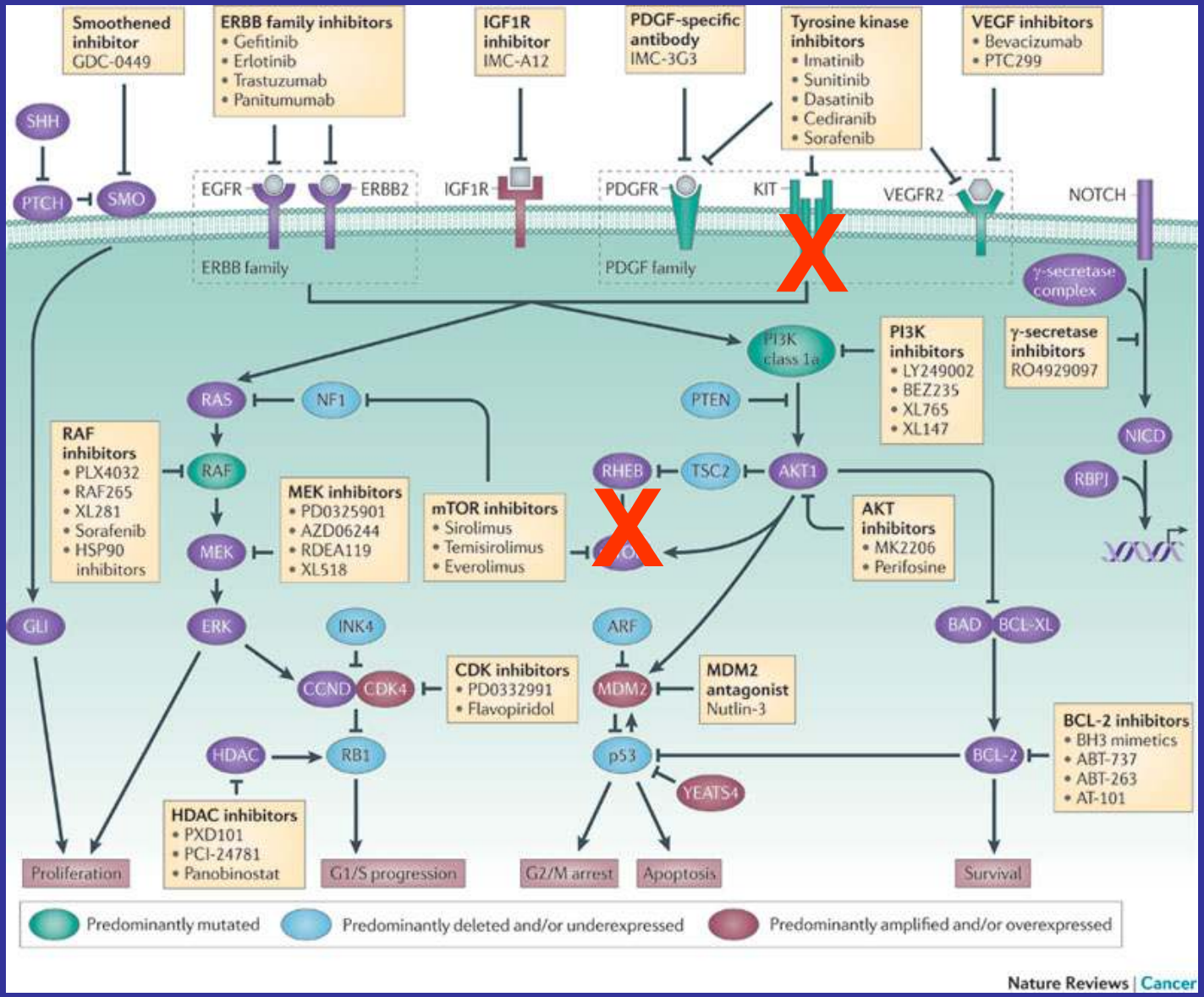
Target KIT downstream signaling



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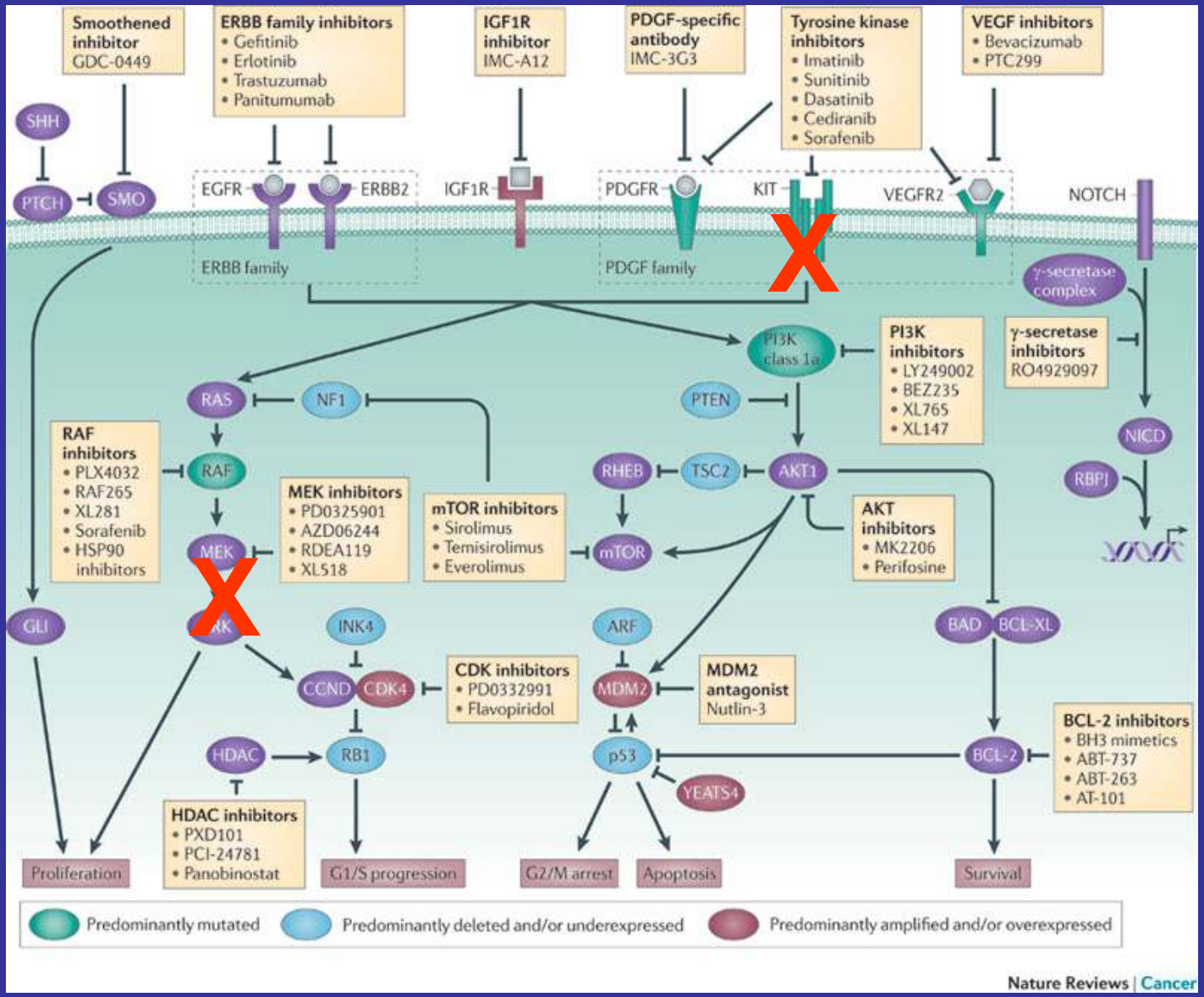


Taylor BS et al. Nat Rev Cancer 2011;11:541-57

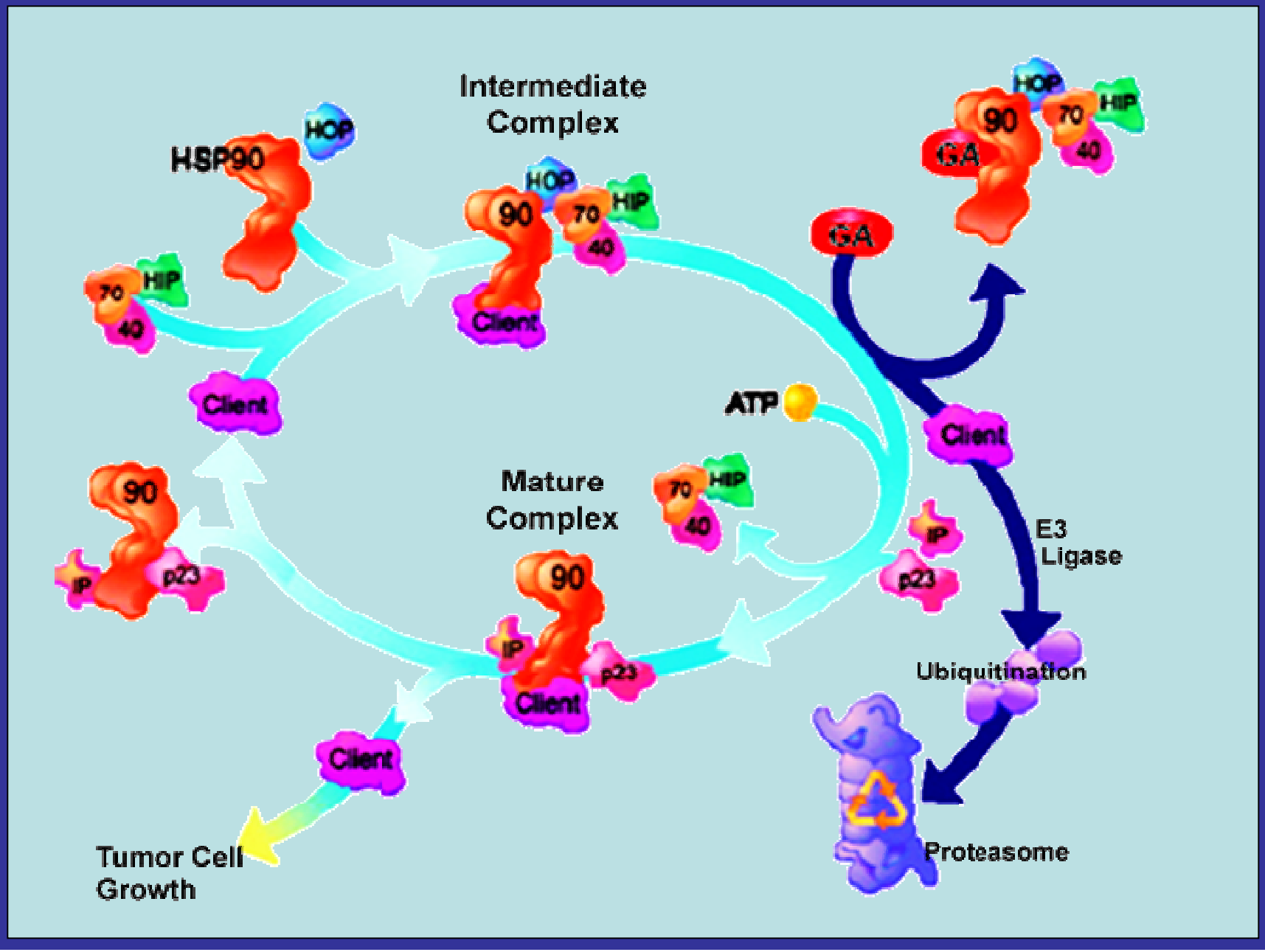
Target KIT downstream signaling

- PI3K inhibitors
- mTOR inhibitors
- Dual PI3K / mTOR inhibitors
- MEK inhibitors
- Can be used in combination with KIT / PDGFRA inhibitors
  - Minimize resistance by targeting two or more proteins in the KIT / PDGFRA pathway
  - It's like simultaneously putting on the brakes and disabling the transmission using a car analogy. If the brakes fail, the car still can't move since the transmission is also disabled.
- Downside of combination therapy is that it is sure to be more toxic than monotherapy.

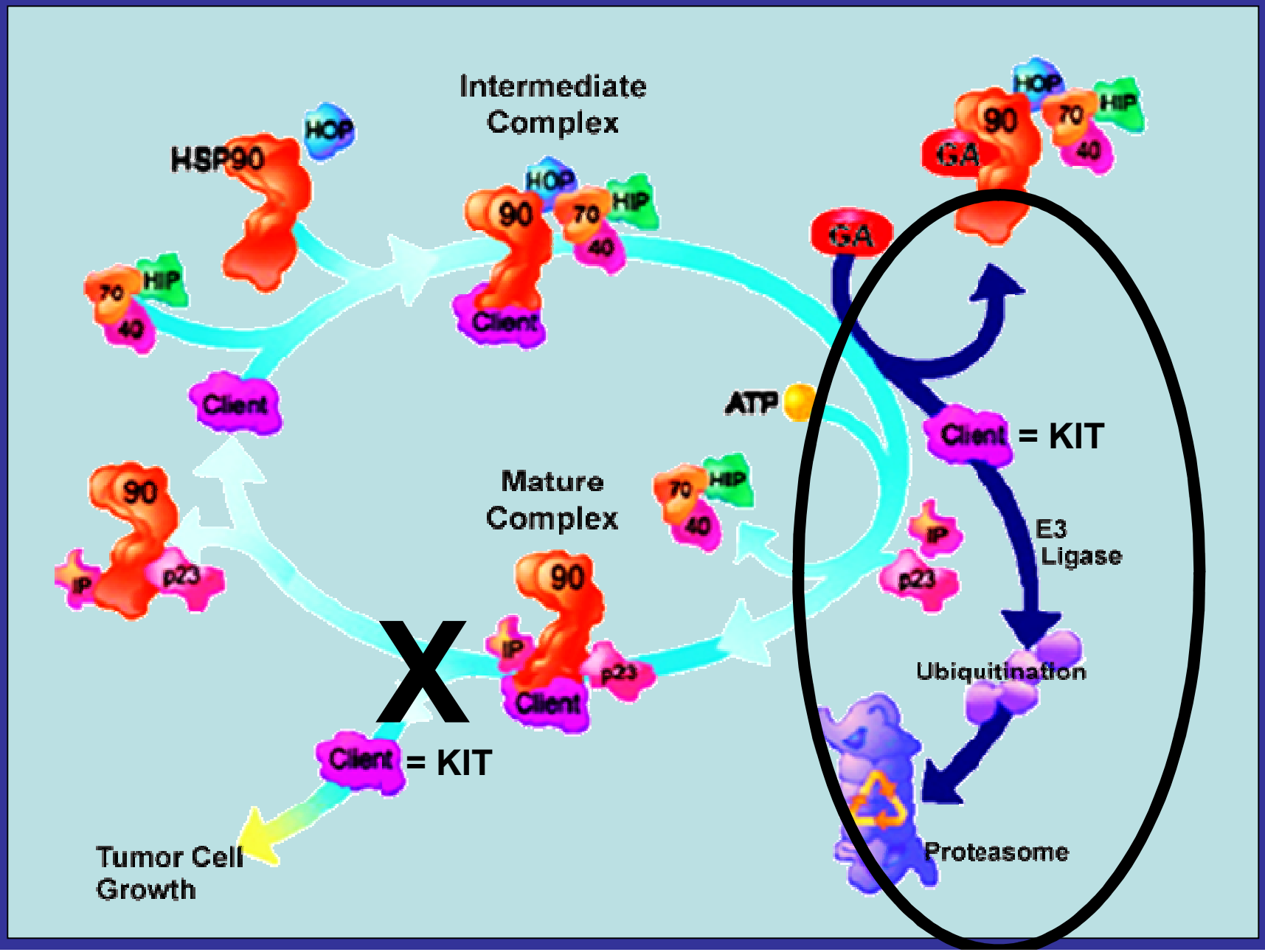
Destroying KIT by HSP90 inhibition

- There are cellular mechanisms for making (protein translation) and destroying (ubiquitin mediated degradation) KIT.
- HSP90 (a type of chaperone protein) stabilizes KIT and other “client” proteins by making sure that they are “folded” correctly.
- If proteins like KIT are not folded correctly then they are targeted for degradation.
- The idea is that inhibition of HSP90 should destabilize KIT, leading to improper folding and accelerating protein degradation.

HSP90 pathway



HSP90 pathway



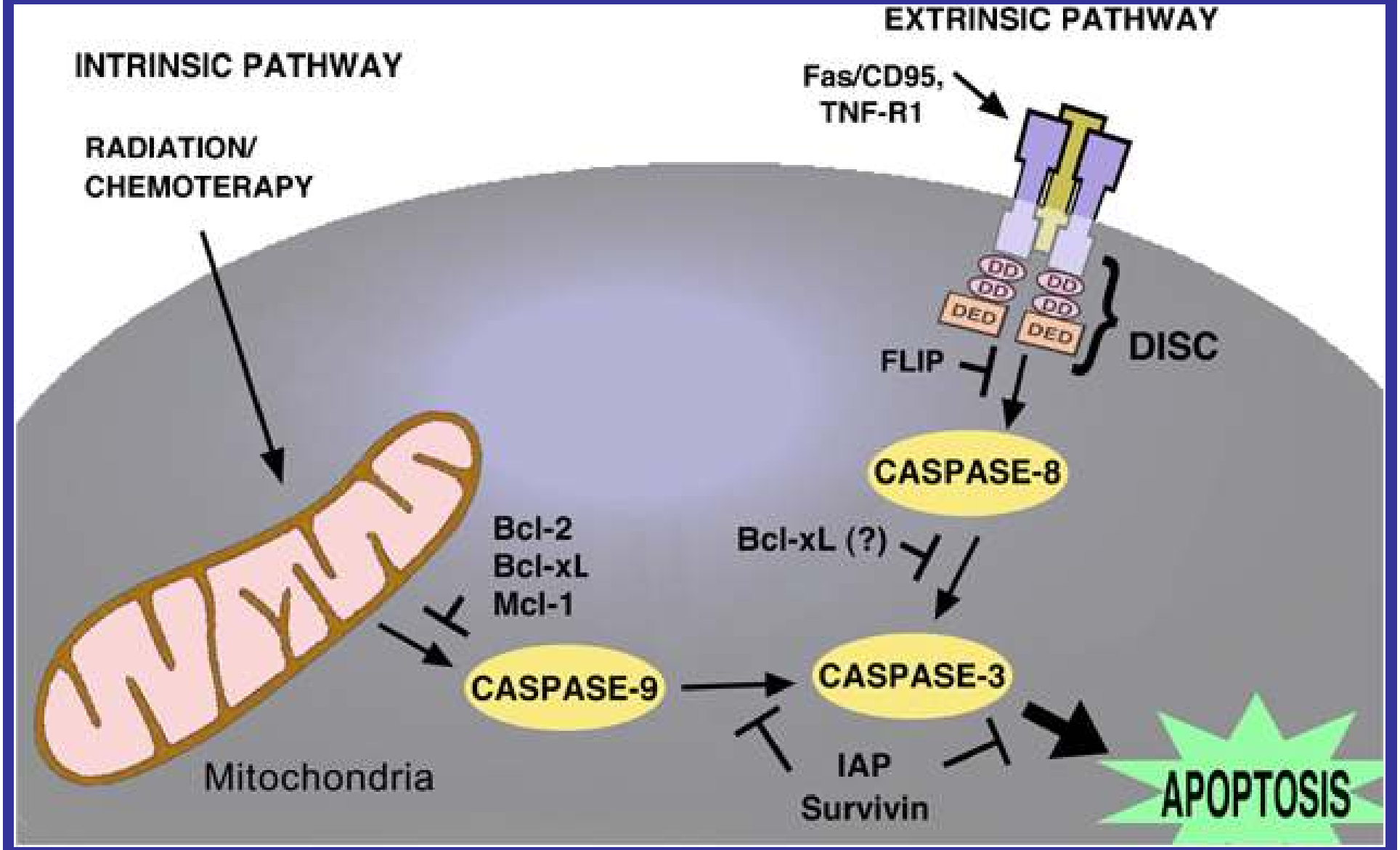
Downside of HSP90 inhibition

- Not specific for KIT so it potentially has high toxicity.
- There is a lot of effort going into making better HSP90 inhibitors that are selective for KIT.

What is apoptosis?

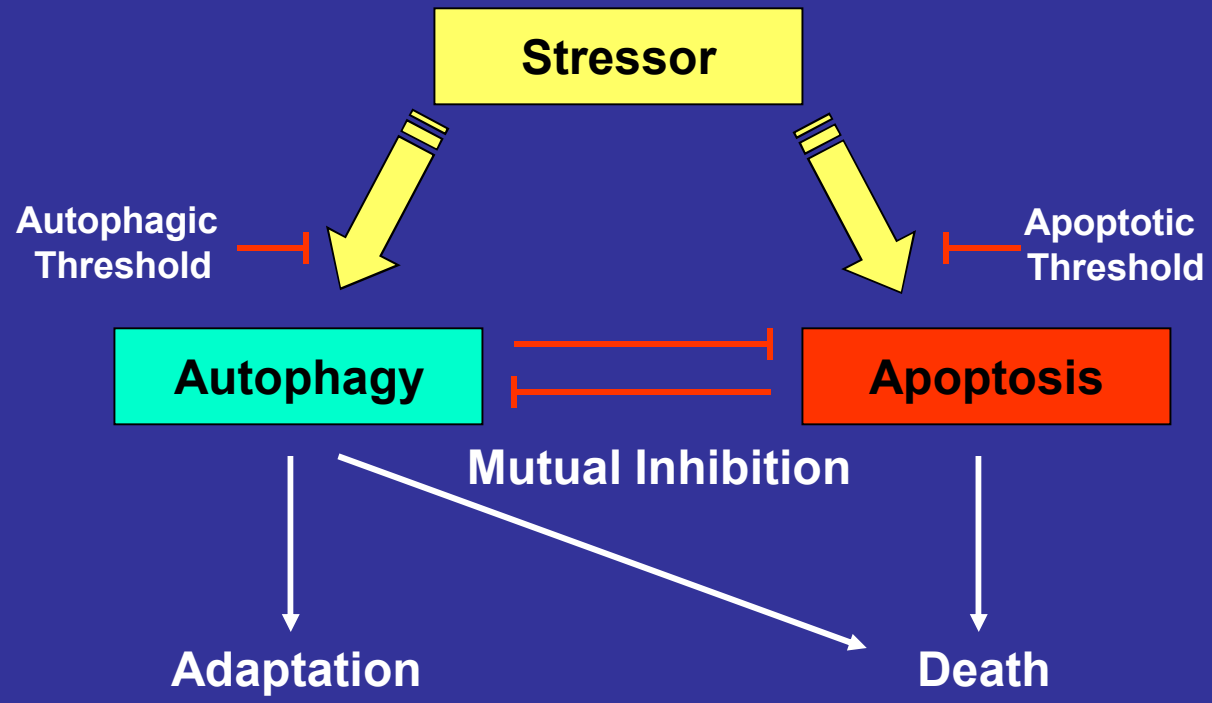
- Programmed cell death
  - For the good of the organism, cells are programmed to kill themselves when they are damaged beyond repair.
  - It's a "fail safe" mechanism.
- Apoptosis is an enzymatic process and therefore, can potentially be targeted with drugs that activate apoptosis or inhibit it.
- Since we are talking about cancer cells (GIST cells) we are talking about activating apoptosis to kill GIST cells.

Apoptosis


EXTRINSIC PATHWAY
INTRINSIC PATHWAY
Fas/CD95,
TNF-R1
RADIATION/
CHEMOTERAPY
DD
DD
DD
DD
DED
DED
DISC
FLIP
CASPASE-8
Bcl-2
Bcl-xL
Mcl-1
Bcl-xL (?)
CASPASE-9
CASPASE-3
Mitochondria
IAP
Survivin
APOPTOSIS

Target KIT survival pathways / induce apoptosis - Autophagy

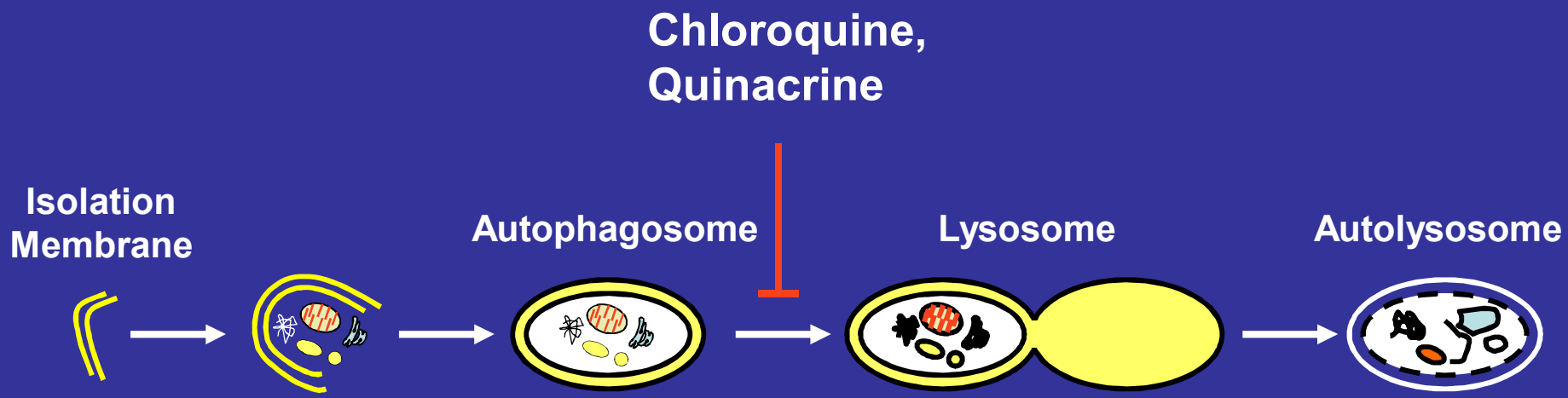
- Autophagy is a primitive cell survival pathway that is activated during cellular stress to help cells (even cancer cells) survive.
- KIT inhibition (e.g. imatinib) activates autophagy.
- Autophagy opposes apoptosis so inhibiting autophagy activates apoptosis.
- Co-treatment with KIT inhibitor and autophagy inhibitors (e.g. chloroquine) increases GIST cell death (apoptosis).


Stressor
Autophagic Threshold
Apoptotic Threshold
Autophagy
Apoptosis
Mutual Inhibition
Adaptation
Death

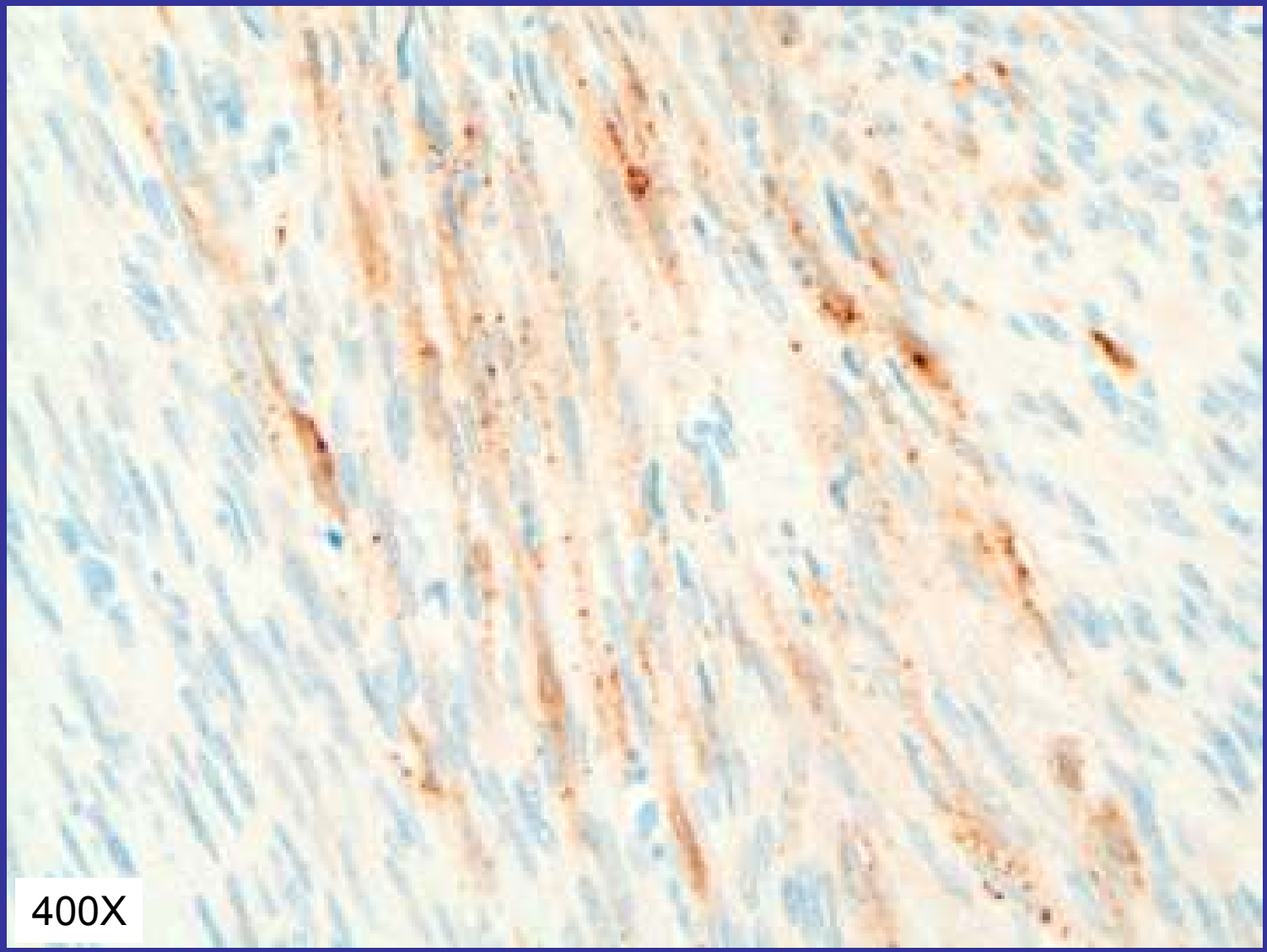
How does autophagy work

- Autophagy means self eating in Greek
- Organelles such as mitochondria are digested to generate energy during energy depleted states.
- Damaged organelles that could activate programmed cell death (apoptosis) are engulfed and digested to avoid apoptosis.

Autophagy

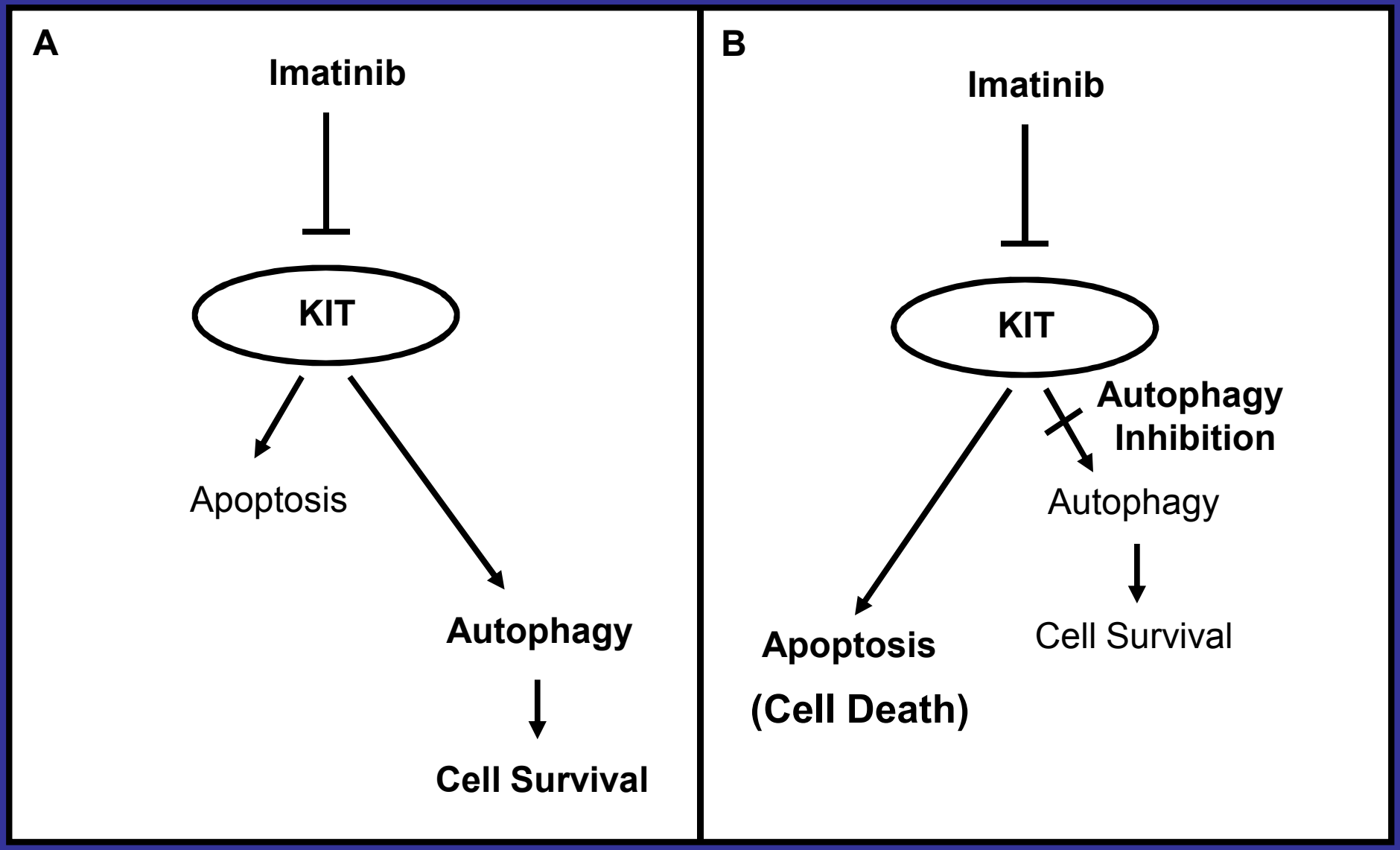

Chloroquine,
Quinacrine
Isolation
Membrane
Autophagosome
Lysosome
Autolysosome

Immunohistochemistry for LC3 in Human GISTsTreated With Imatinib



Gupt A et al. *Proc Natl Acad Sci USA* 2010:107:14333-8

Target KIT survival pathways / induce apoptosis



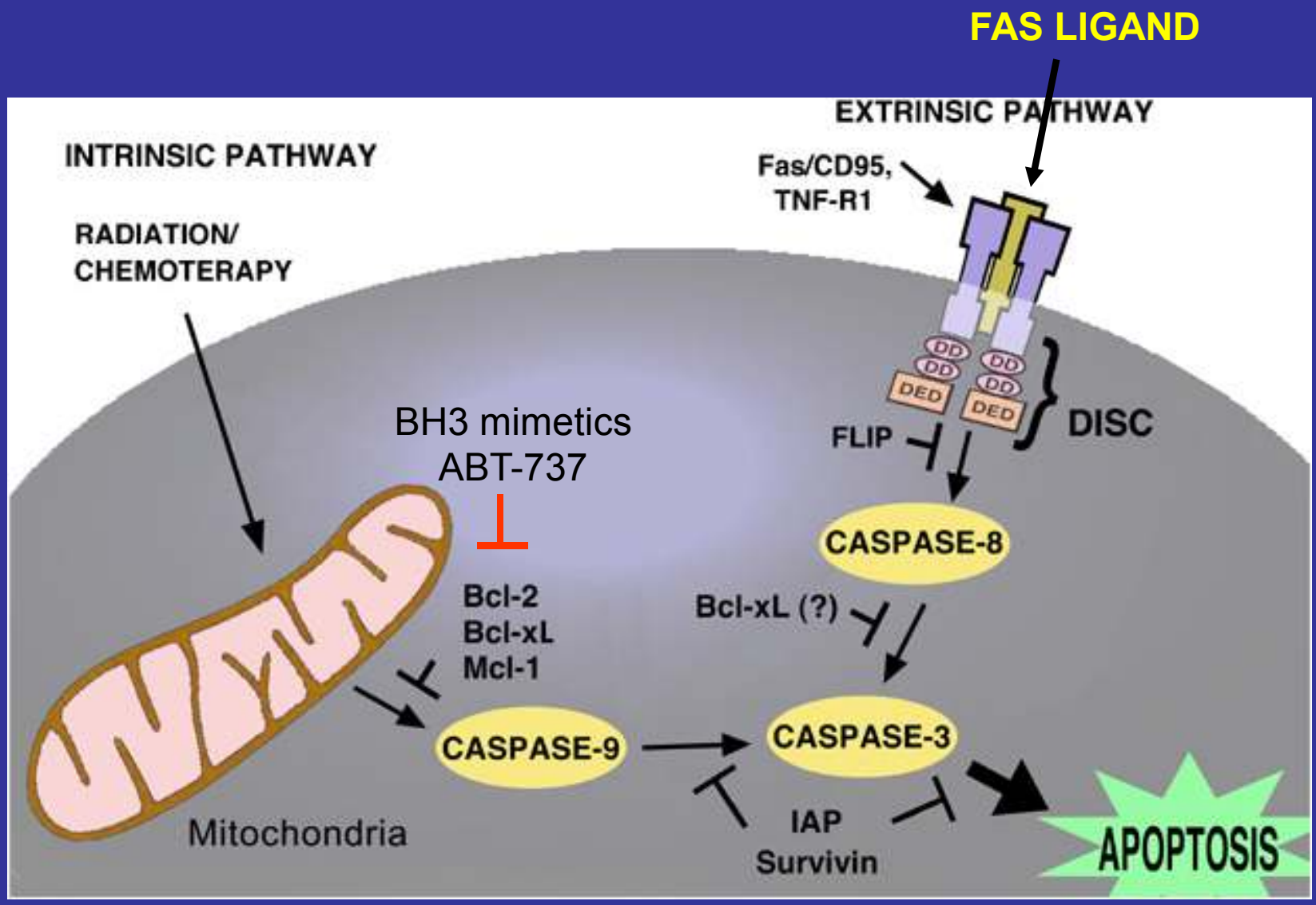
Rubin and Debnath *Autophagy* 2010;6:1190.

Induction of apoptosis

- BH3-mimetics, ABT-737 and other drugs activate apoptosis by inhibiting Bcl-2.
- Reynoso et al. showed that ABT-737, an inhibitor of Bcl-2, synergized with imatinib to increase cell death.
- Activators of apoptosis can be used in combination with KIT inhibitors.
- One downside is that inducers of apoptosis are not specific for cancer cells. Need to protect non-cancerous cells.

Reynoso D et al. *Mol Onc* 2011;5:93-104

Induction of apoptosis



Activation of the Immune System

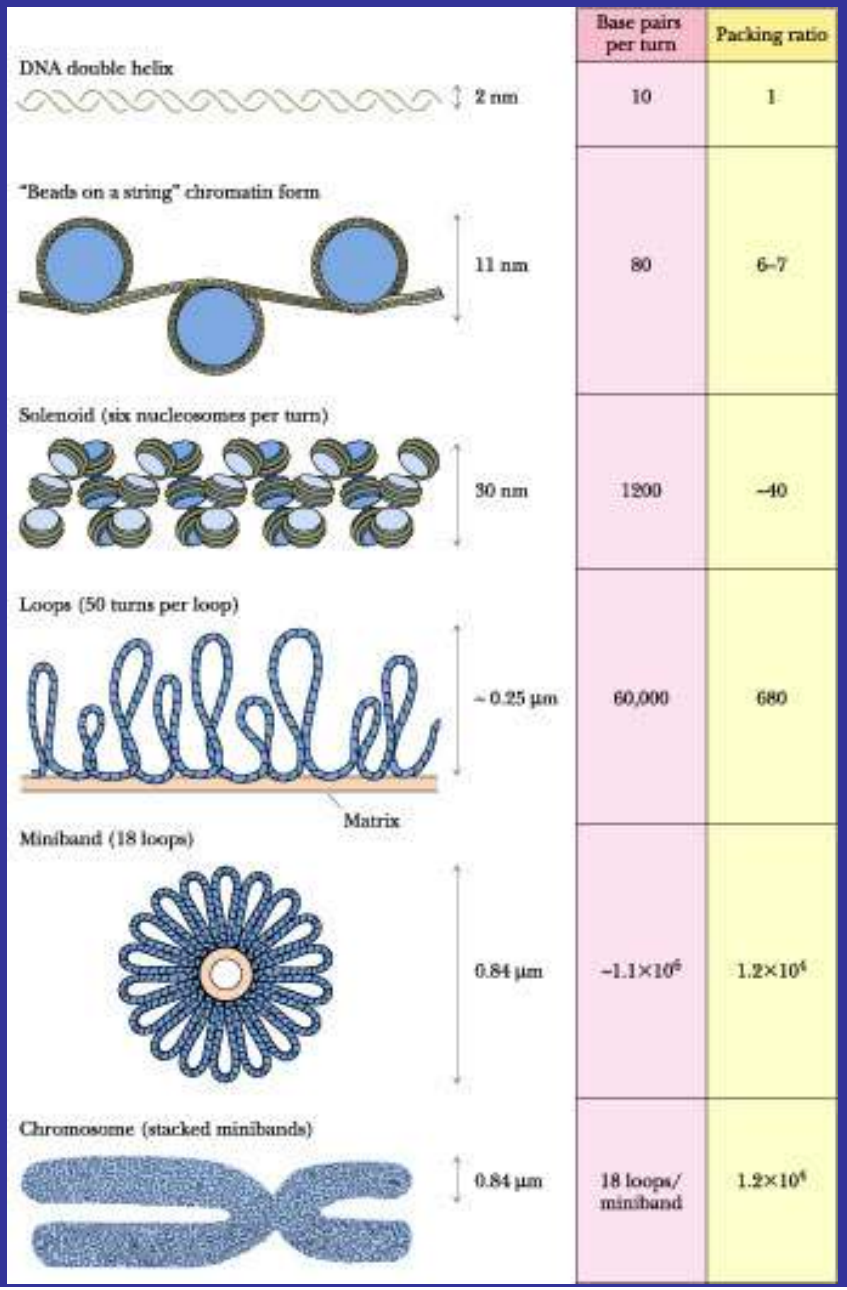
- Imatinib activates immune system, a previously unrecognized activity.
- Activates immune system by inhibition of GIST cell-specific indoleamine 2,3-dioxygenase (Ido).
- Ido produces chemicals that inhibit immune system.
- CTLA4 (cytotoxic T lymphocyte antigen 4) is an important T cell protein that facilitates immune tolerance of cancer cells.
- CTLA4 antagonists inhibit immune tolerance, thus activating the immune system against tumor (GIST) cells.
- Can be used in combination with KIT inhibitors.

Balachandran VP et al. *Nat Med* 2011;17:1094

Targeting Chromatin

- The DNA that is packaged into the nucleus is a meter/yard in length.
- DNA needs to be packaged so that it fits into a nucleus which is a microscopic structure that can only be seen with the aid of a microscope.
- Chromatin is DNA that is packaged by interacting with proteins.
- Local chromatin organization influences whether genes are expressed.
- Genes are transcribed into RNAs which are translated into proteins and proteins are what make cells “go”.
- The idea behind targeting chromatin is that global gene expression changes will be detrimental to tumor cells.

Chromatin Assembly



Base pairs per turn	Packing ratio
10	1
80	6–7
1200	~40
60,000	680
~1.1×10^6	1.2×10^4
18 loops/ miniband	1.2×10^4

The Central Dogma!

GENES (DNA)

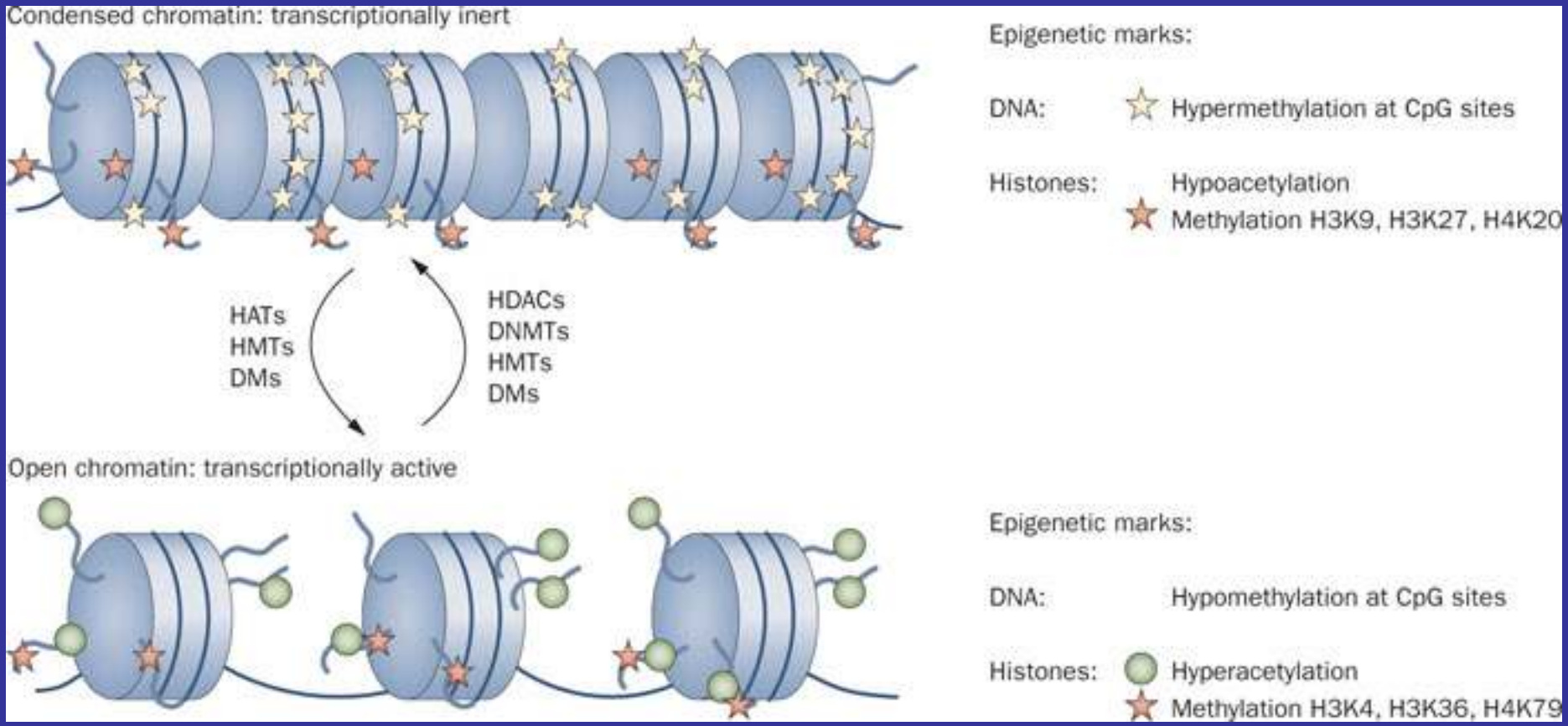
↓ TRANSCRIPTION

TRANSCRIPTS (RNA)

↓ TRANSLATION

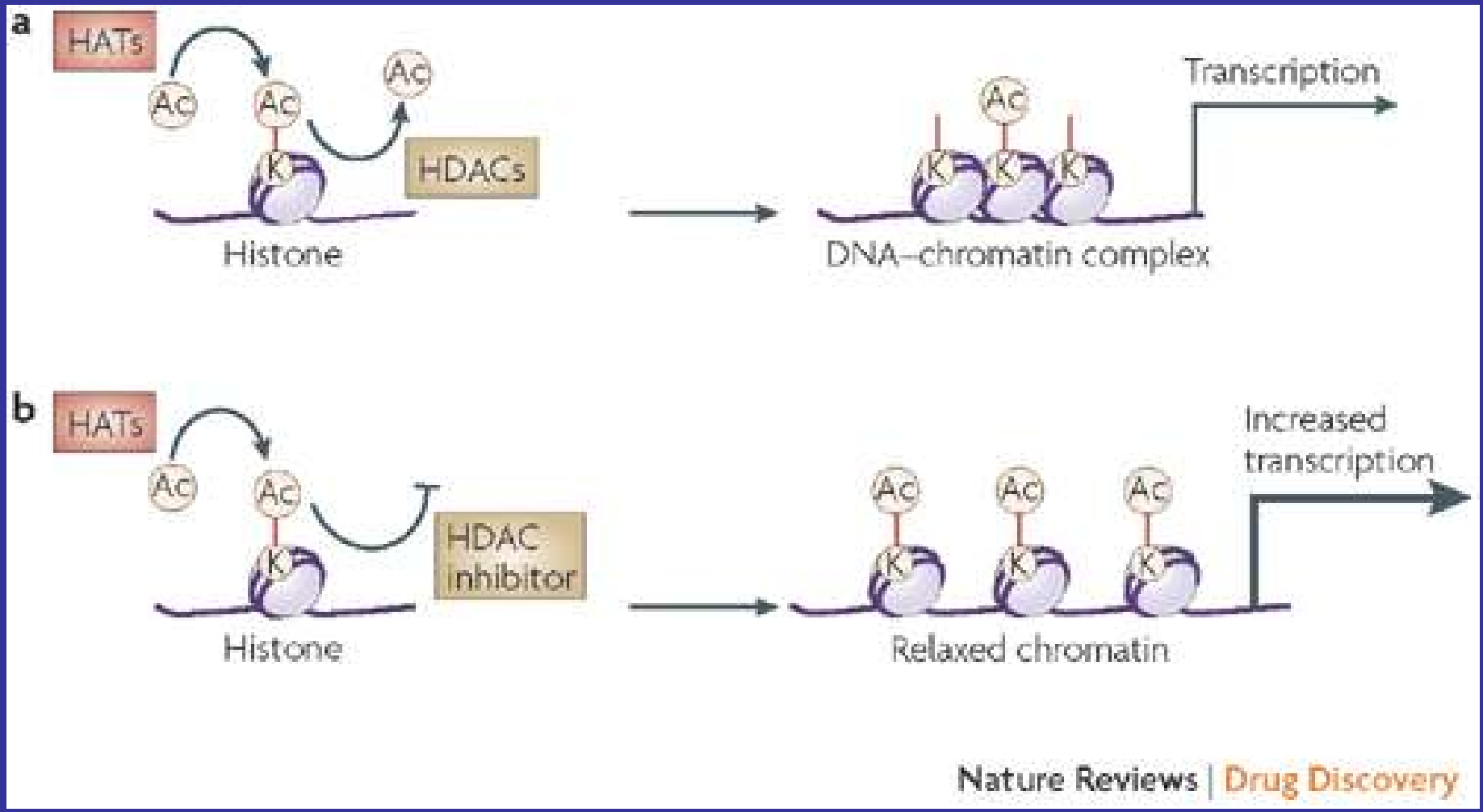
PROTEINS (AMINO ACIDS)

Chromatin Structure and Transcription



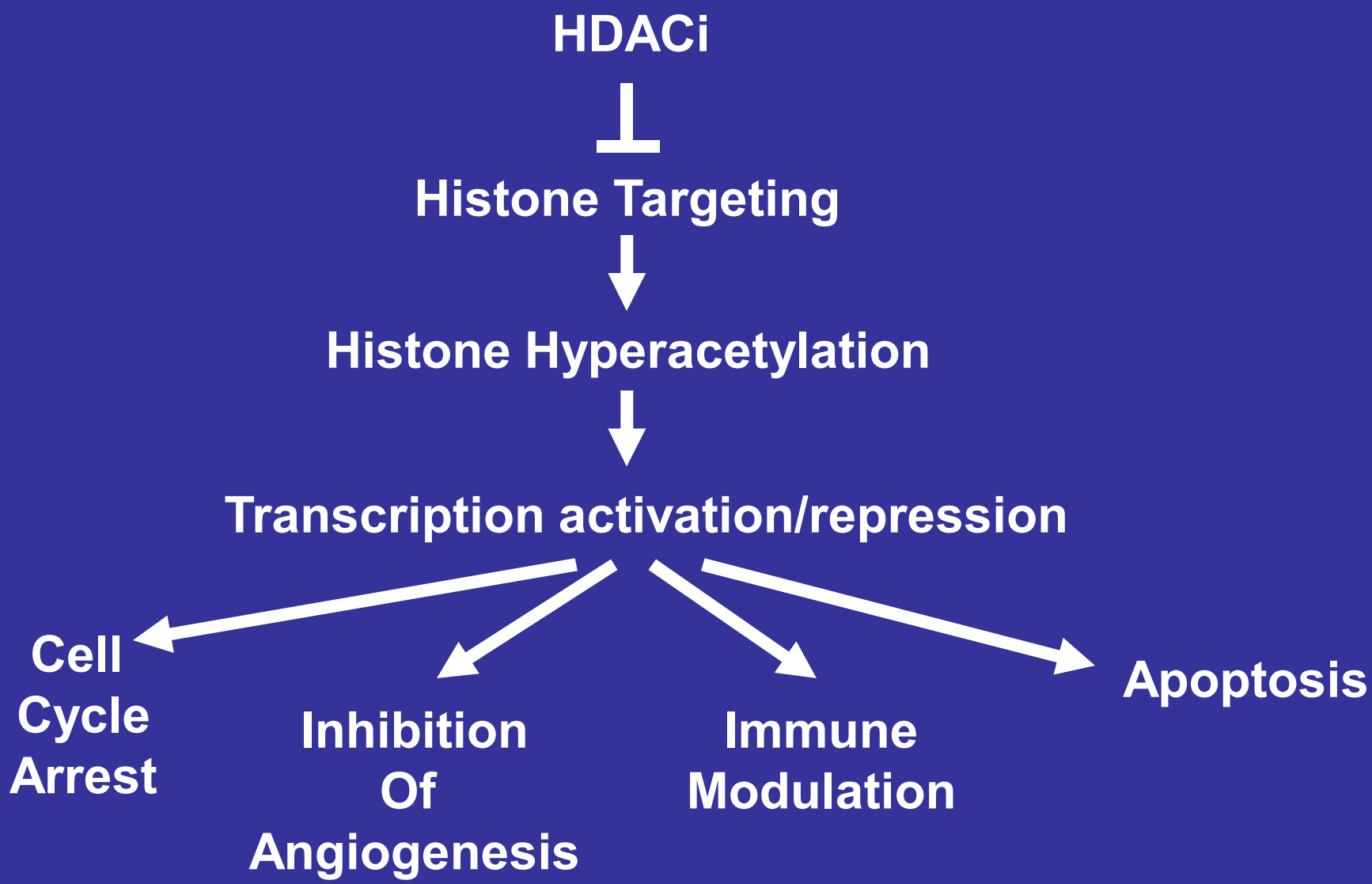
Perry, A. S. *et al.* (2010) *Nat. Rev. Urol.* doi:10.1038/nrurol.2010.185

Chromatin and Histone Acetylation



Kazantsev AG and Thompson LM. Nat Rev Drug Discov 2008;7:854-68.

Mechanism of action of HDAC inhibitors



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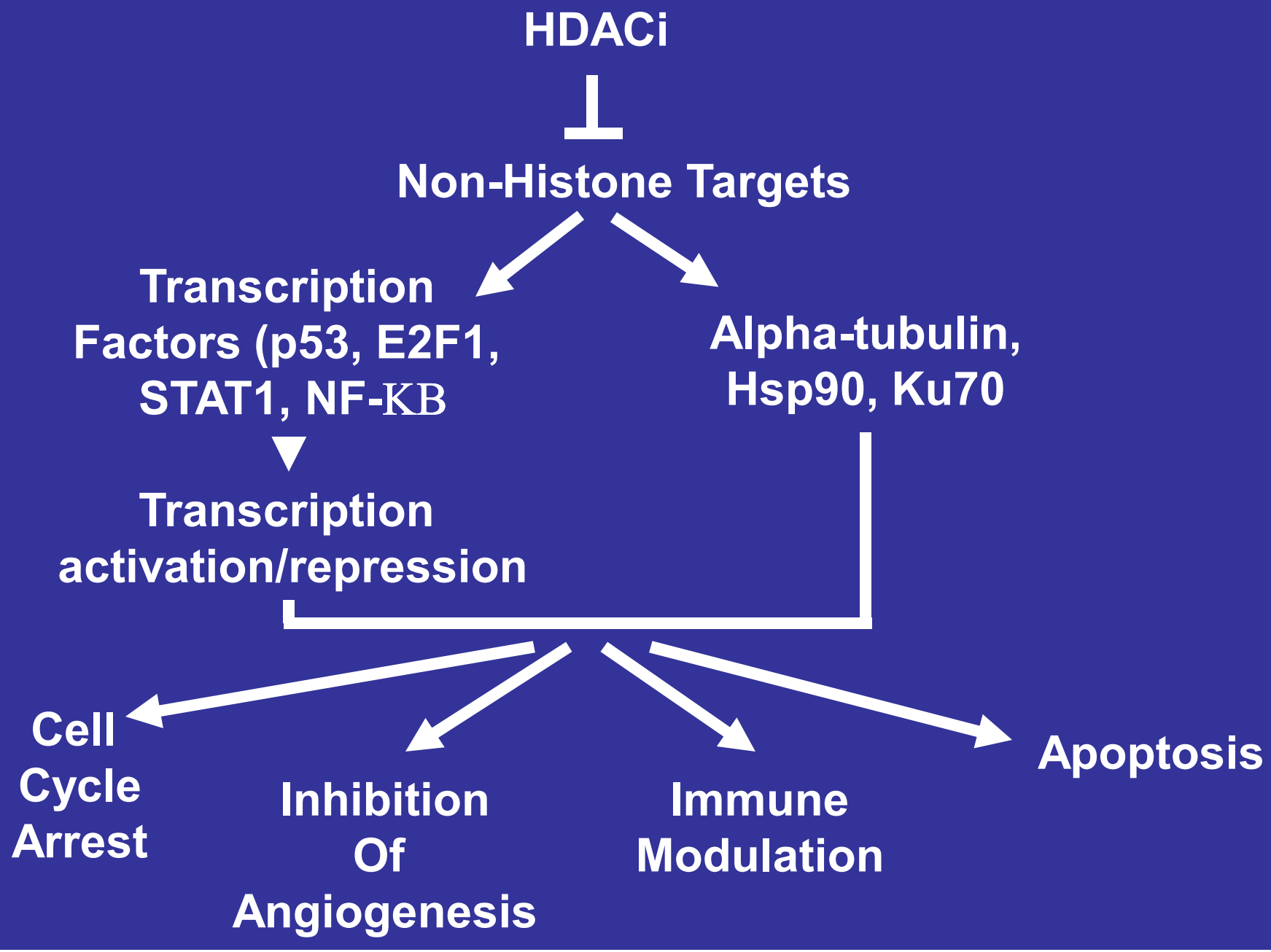


Evidence for activity of HDAC inhibitors

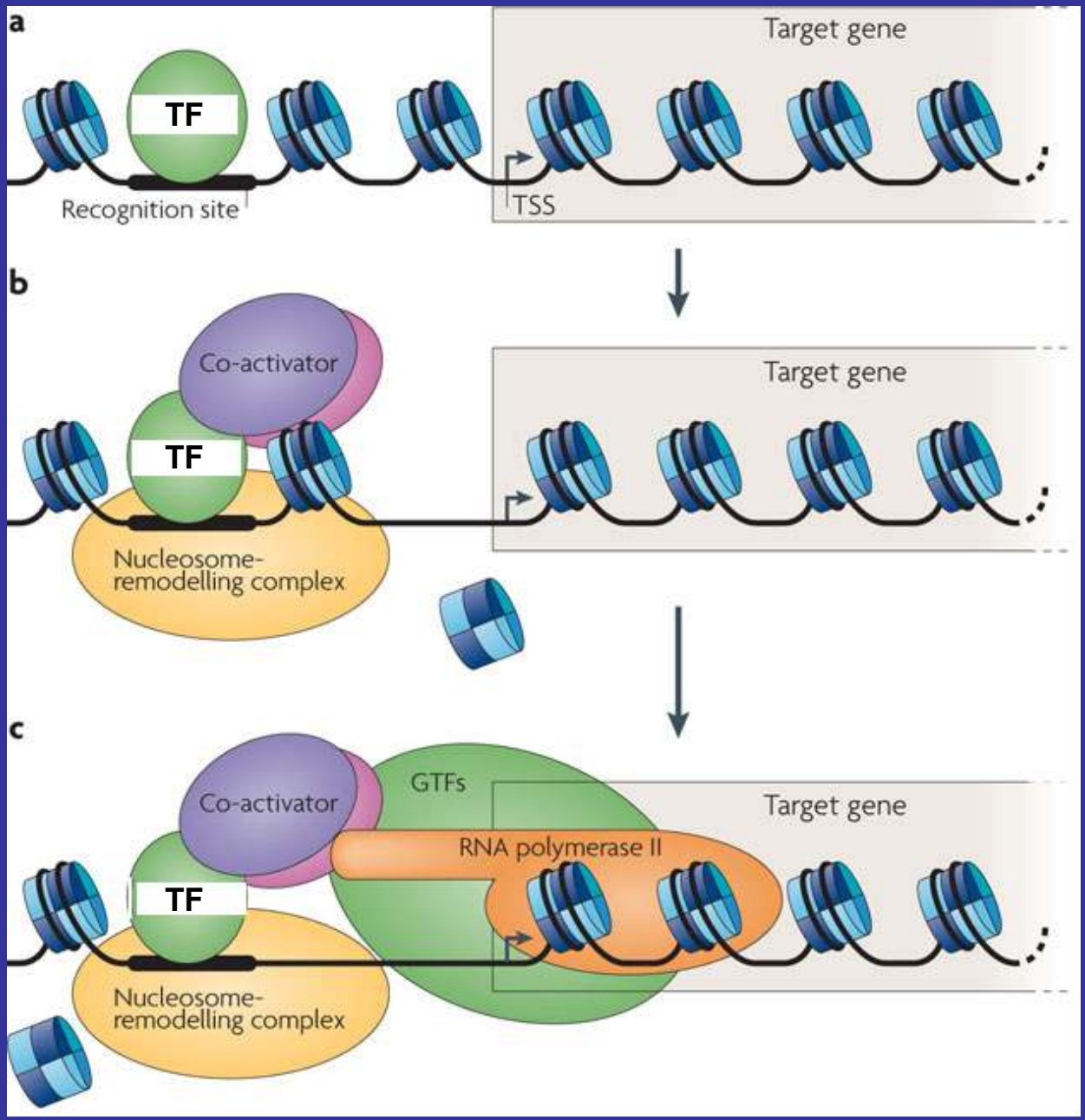
- Inhibition of proliferation seen KIT-positive but not KIT-negative GIST
- Suggests effect is through targeting KIT directly but never proven
- KIT activity, expression, and activation of downstream pathways strongly inhibited.
- Affects seen with several HDAC inhibitors: SAHA, LBH589, VPA, trichostatin A, NaButyrate.

Muhlenberg T et al. *Cancer Res* 2009;69:6941-6950.

What is a transcription factor?

- Transcription factors are proteins that bind DNA and modulate RNA transcription through interaction with “core” transcriptional machinery.
- Transcription factors control transcription.
- Remember that DNA is transcribed to RNA which is translated into proteins.

What is a transcription factor?



ETV1 is an important transcription factor for GIST

- ETV1 is a transcription factor.
- ETV1 is expressed in subtypes of interstitial cells of Cajal that give rise to GIST.
- ETV1 is universally expressed in GIST.
- Suppression of ETV1 inhibits GIST proliferation.
- ETV1 cooperates with KIT to unleash an oncogenic transcriptional program.

Chi P et al. *Nature* 2010;467:849-853

What are cancer stem cells?

- Cancer stem cells compose a variable subset of cells within a cancer that possess properties that allow a single cancer stem cell (self renewal) to establish a tumor composed of cancer stem cells and differentiated cancer cells.
- Differentiated cancer cells do not possess cancer stem cell properties. They cannot establish a tumor.
- Current theories suggest that cancer therapies are good at killing differentiated cancer cells without killing cancer stem cells.
- This is the reason that apparently successful therapies are not successful. They do not kill all the cancer stem cells so that the cancer stem cells re-establish tumors.

Do GIST stem cells exist?

- Isolated a population of $Kit^{low}CD44^{+}Cd34^{+}$ cells from mouse stomach that spontaneously transformed and had properties of cancer stem cells.
- These cells are resistant to Kit blockade by Gleevec.
- In a genetically engineered mouse model, these cells were resistant to Gleevec.
- We think that GIST stem cells do exist and that they are resistant to Gleevec.

Bardsley MR et al. *Gastroenterology* 2010;139:942-952

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