What's going on in the GIST Research World?

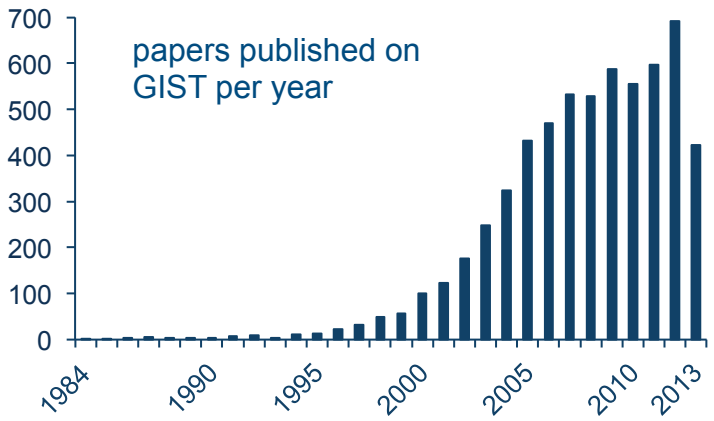
GIST Summit 2013

MD Anderson Cancer Center
September 14, 2013

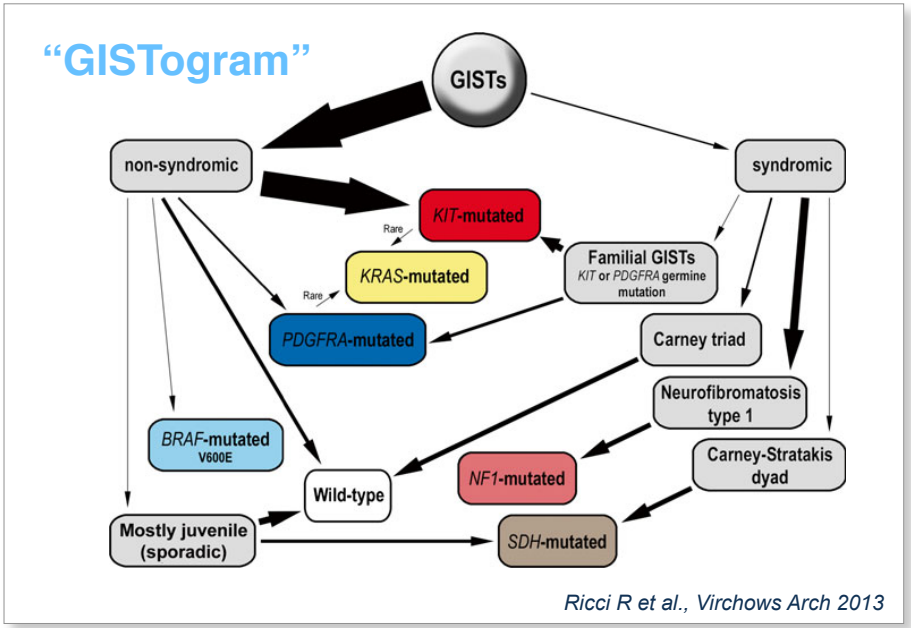
Anette Duensing, M.D.
Assistant Professor of Pathology
University of Pittsburgh Cancer Institute


On my way to
TEXAZZ!
TEXAZZ
UNIVERSITY OF TEXAS
cube

What's going on in the GIST research world?



more than 6000 since 1984!!

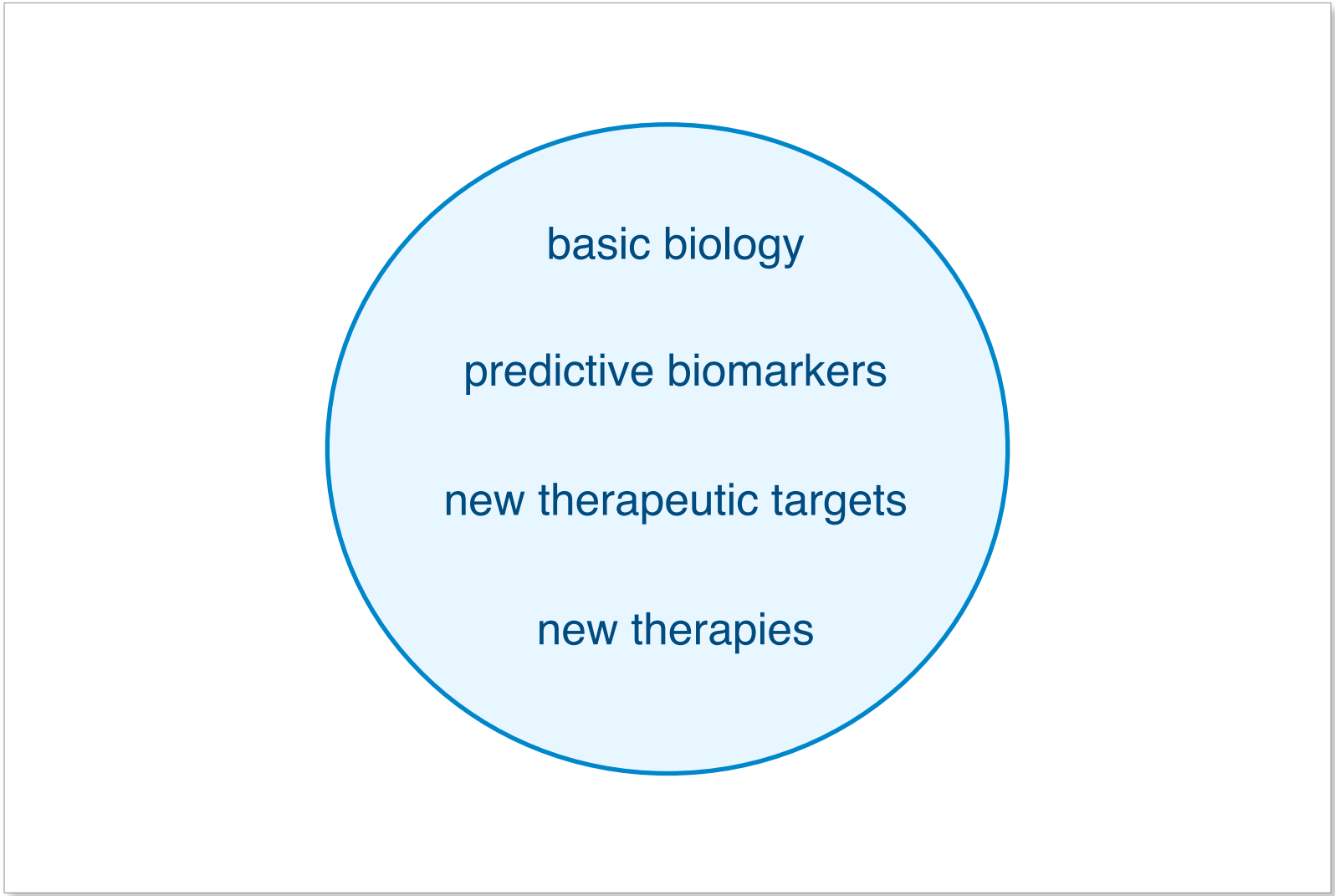


Ricci R et al., Virchows Arch 2013

- clinical trials
- predictive/diagnostic biomarkers
  - immunohistochemistry
  - CINSARC, aurora A
- wt/pediatric GIST
  - SDH expression/mutation
  - IGFR
- p53, cell cycle
- epigenetics, microRNA (ETV1)
- immunology
  - therapeutic (anti-KIT Ab, reg. T-cells, NK cells)
  - prognostic (immune infiltrate, neutrophil-lymphocyte ratio)

Pathway to Cure GIST

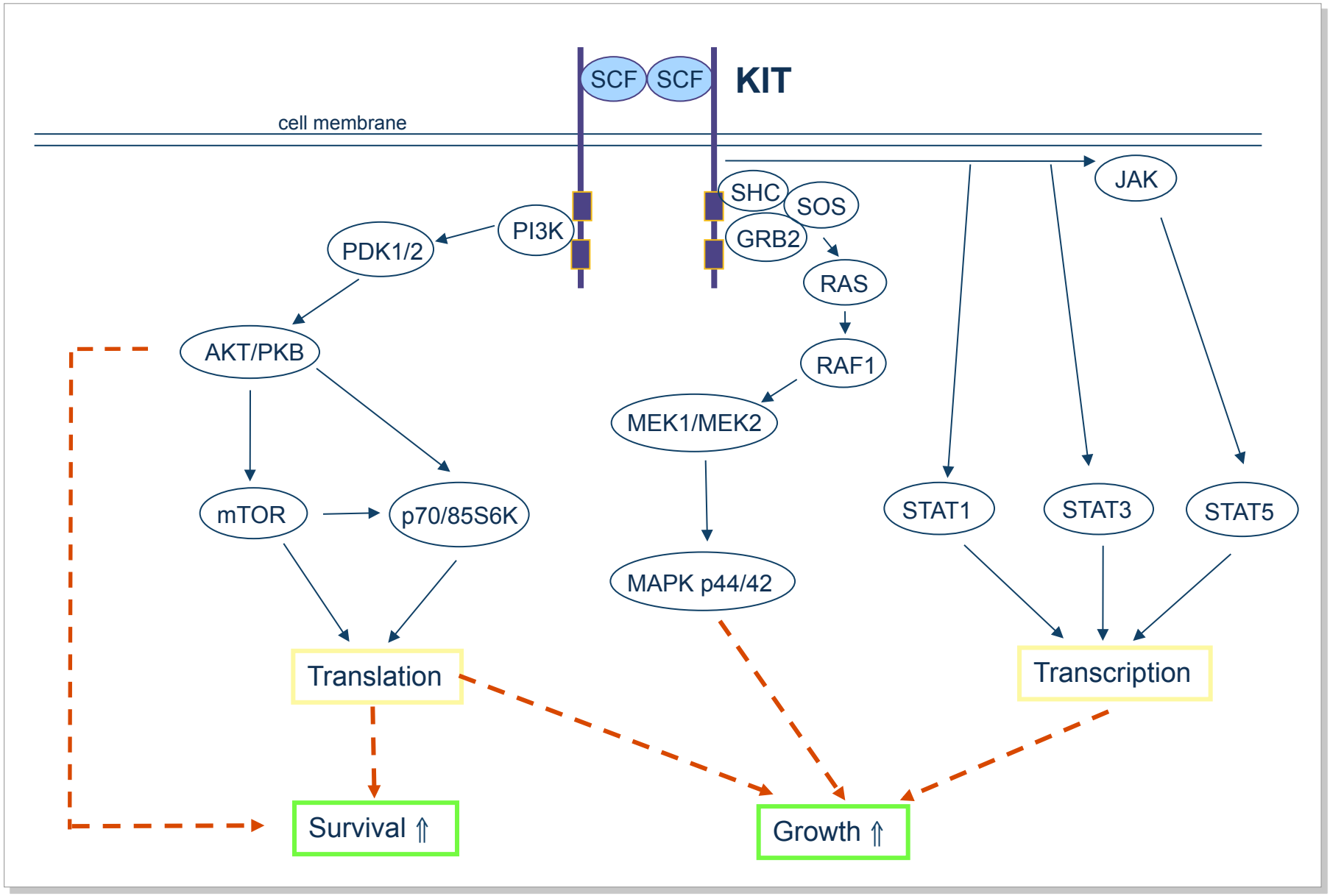
Pathway to Cure GIST
(and how to tackle the problem)



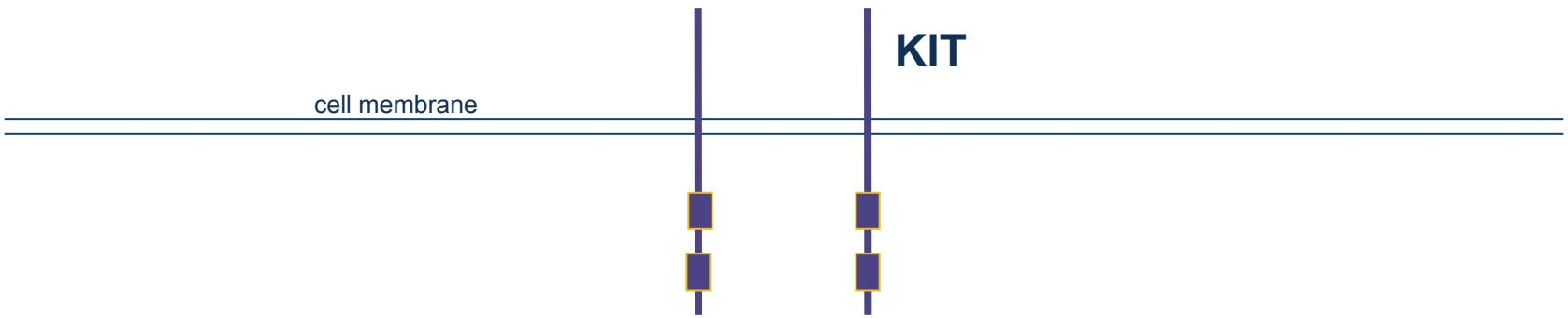
SLOW!

How did this KIT thing work again?

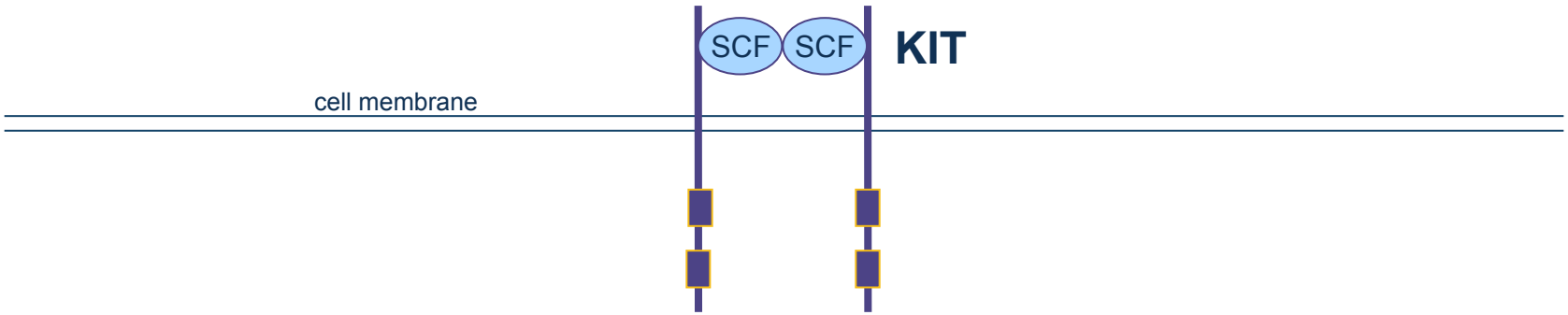
KIT signal transduction



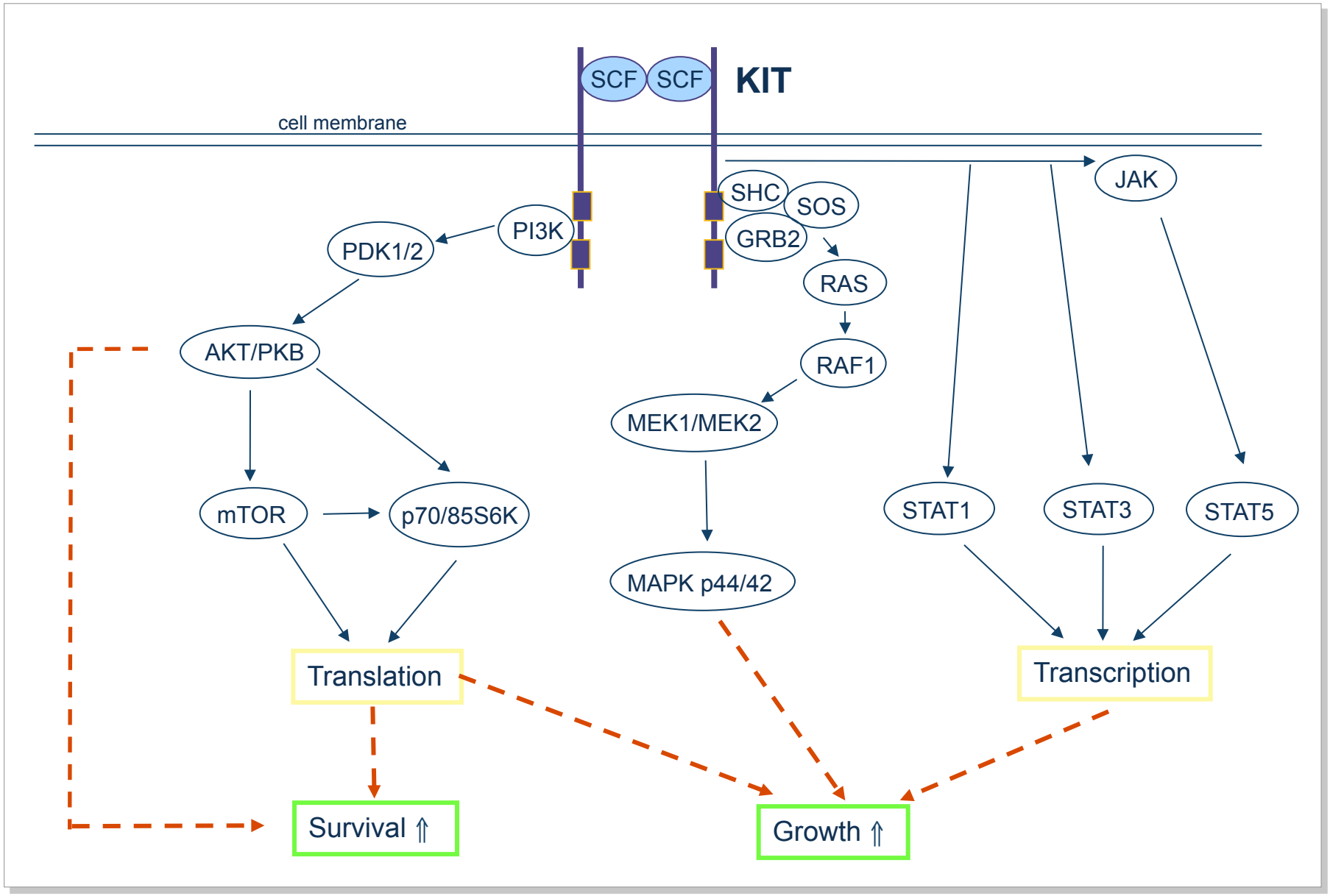
KIT signal transduction - inactive



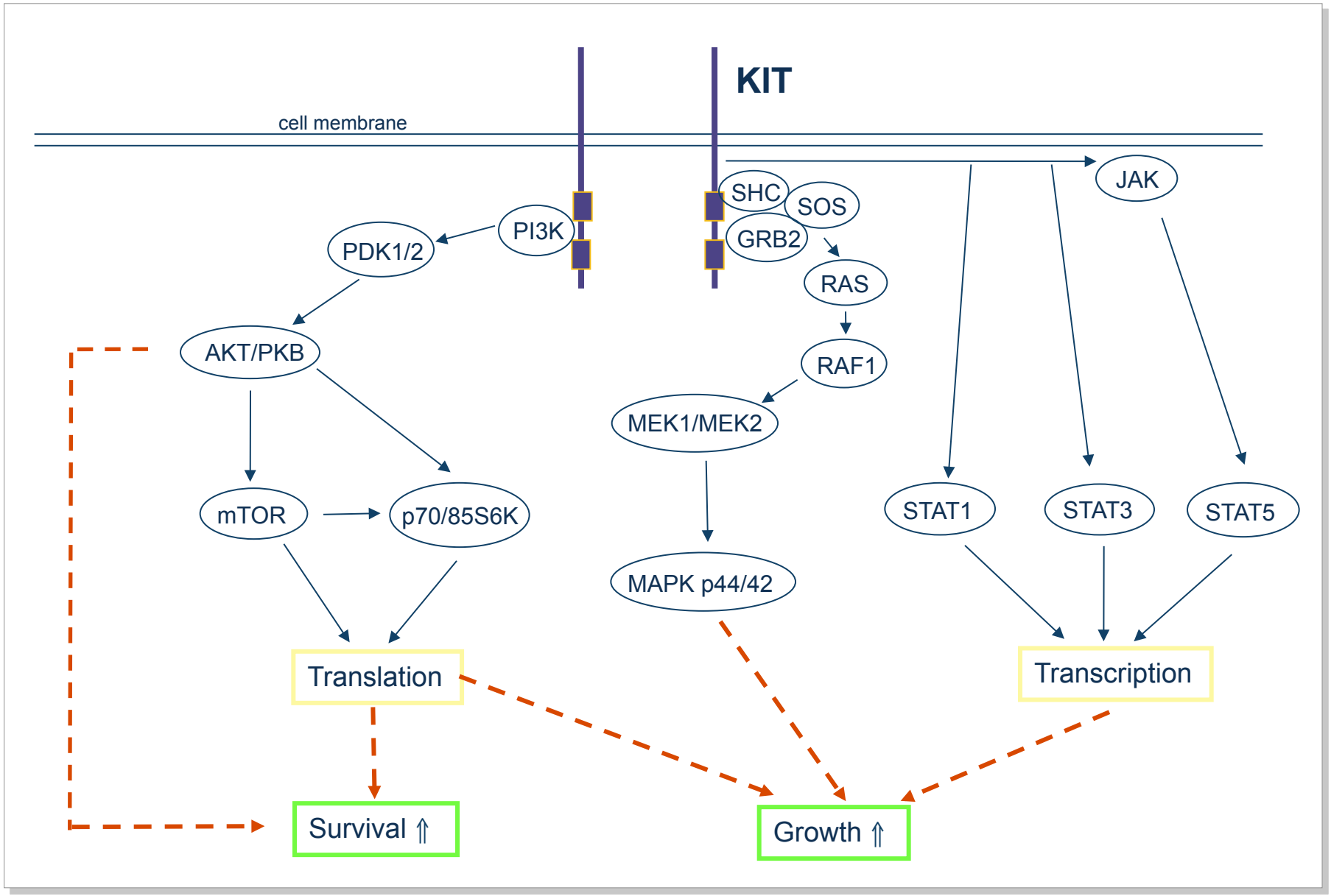
KIT signal transduction - activation



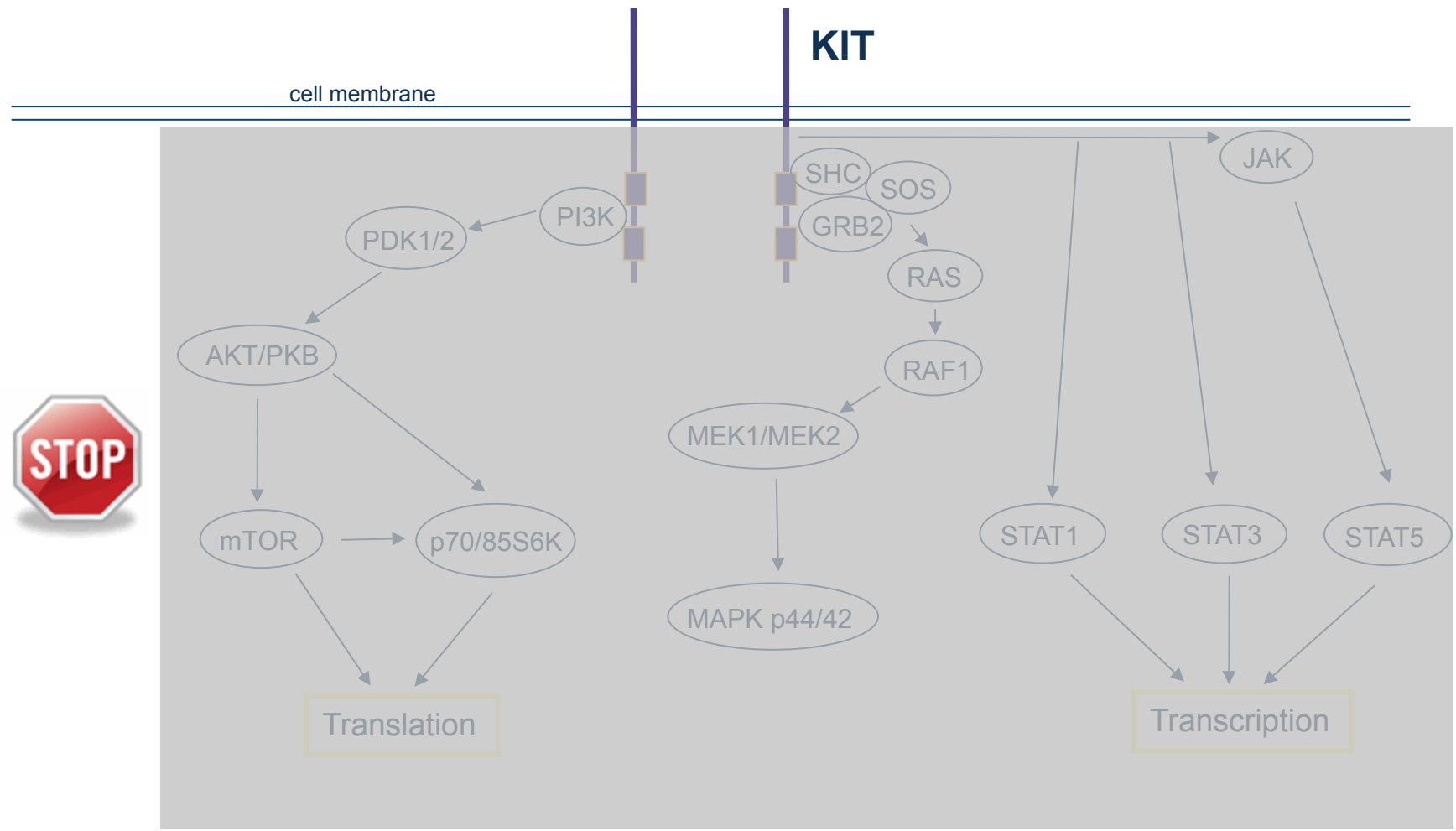
KIT signal transduction – active



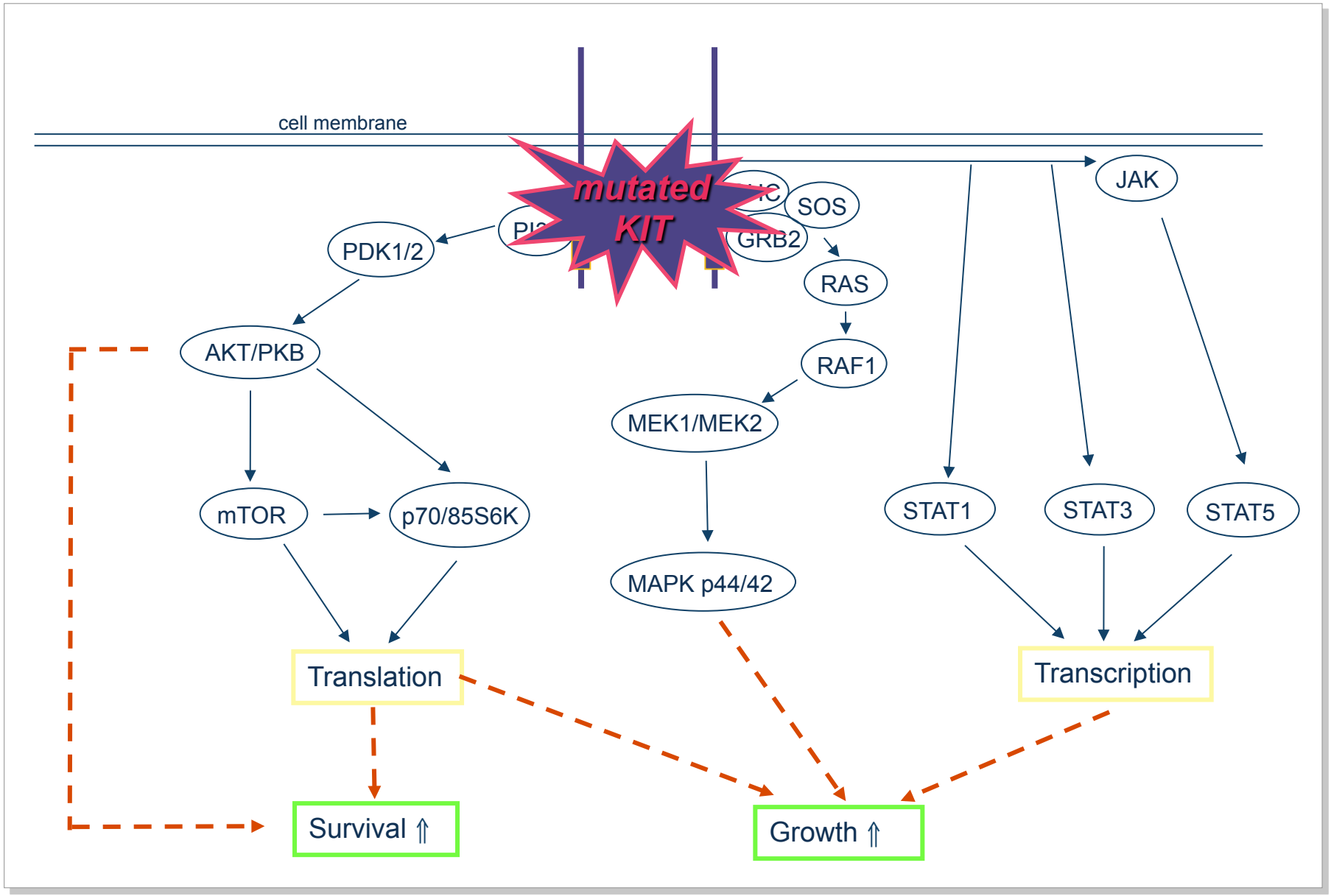
KIT signal transduction - inactivation



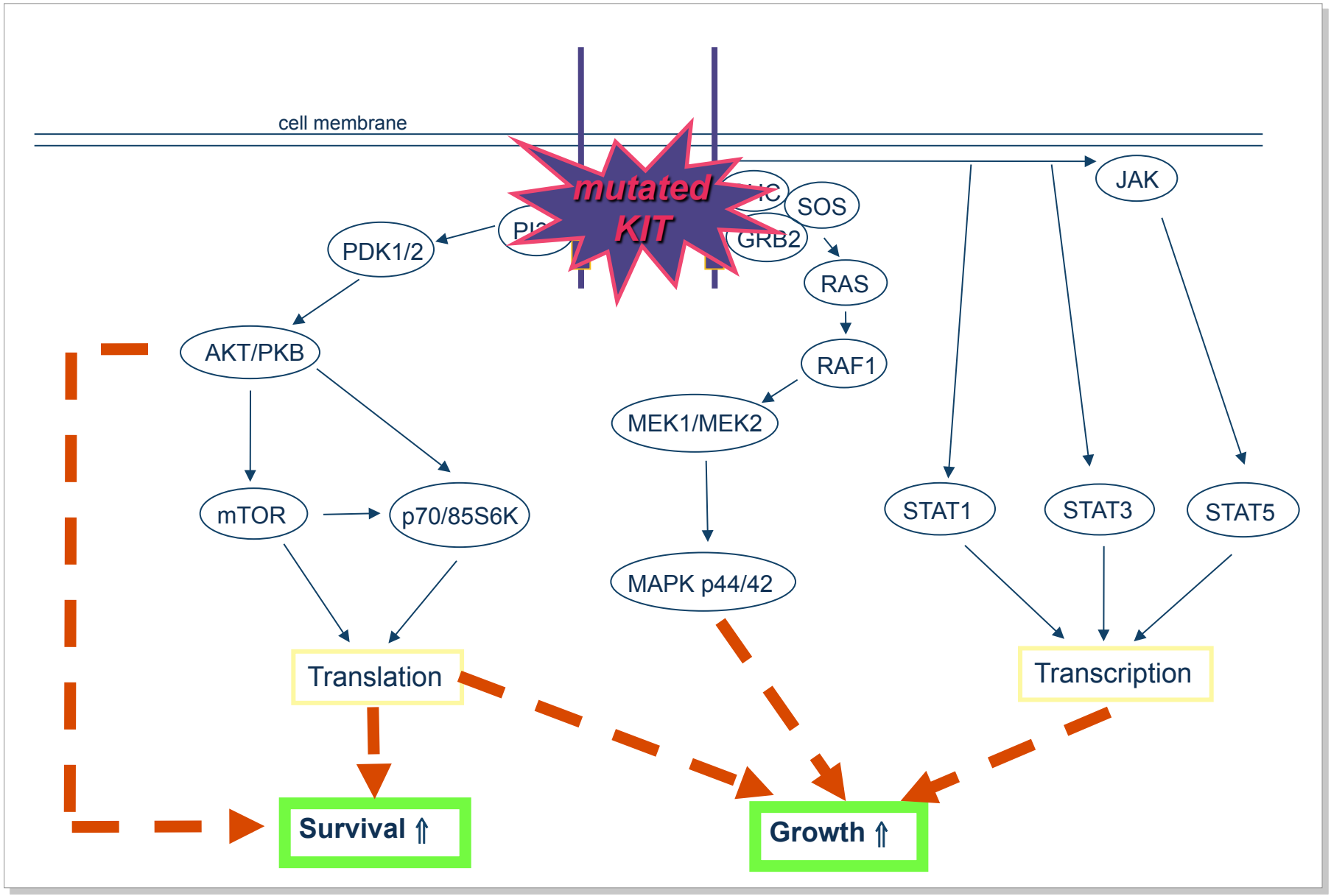
KIT signal transduction - inactivation



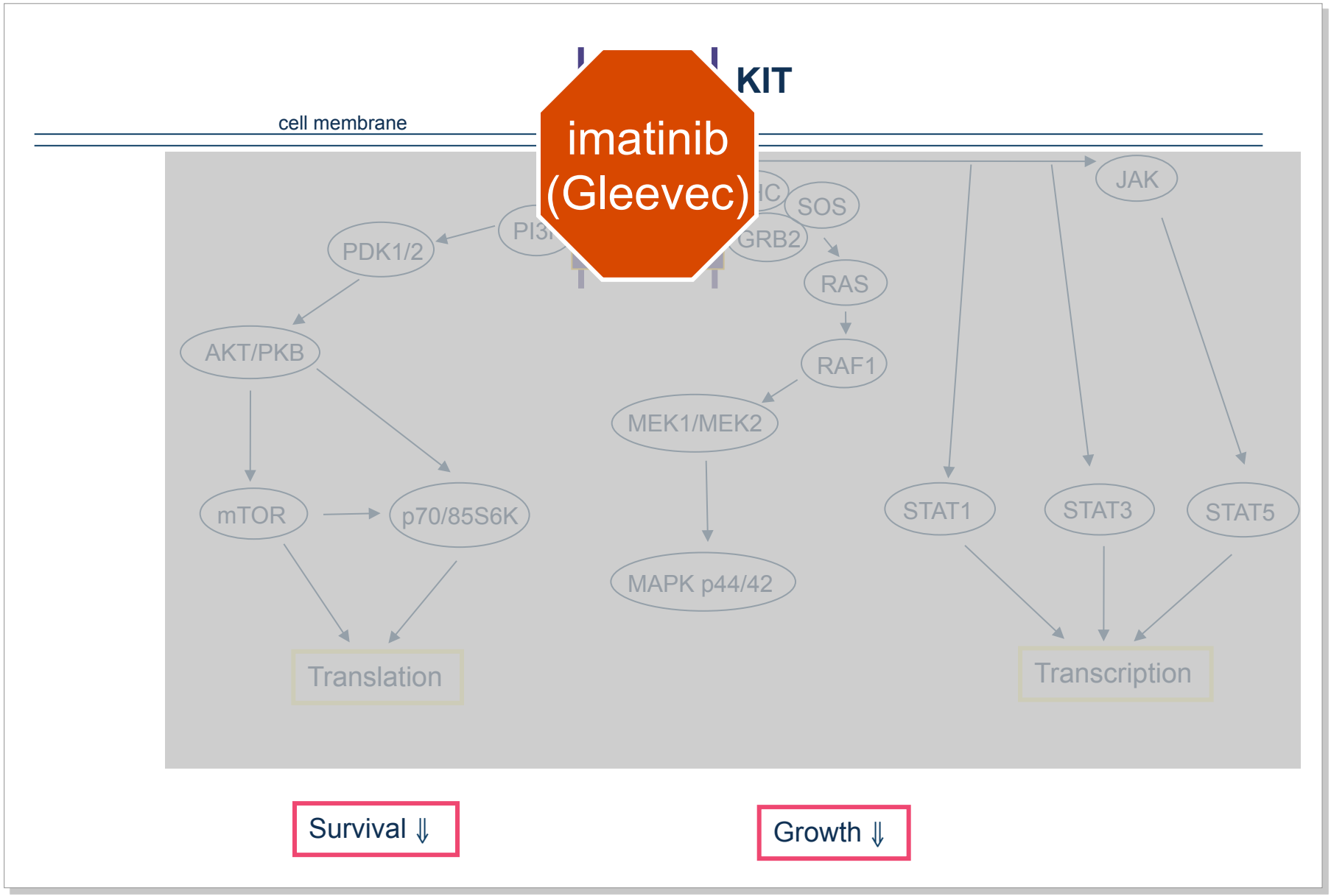
Mutant KIT signal transduction



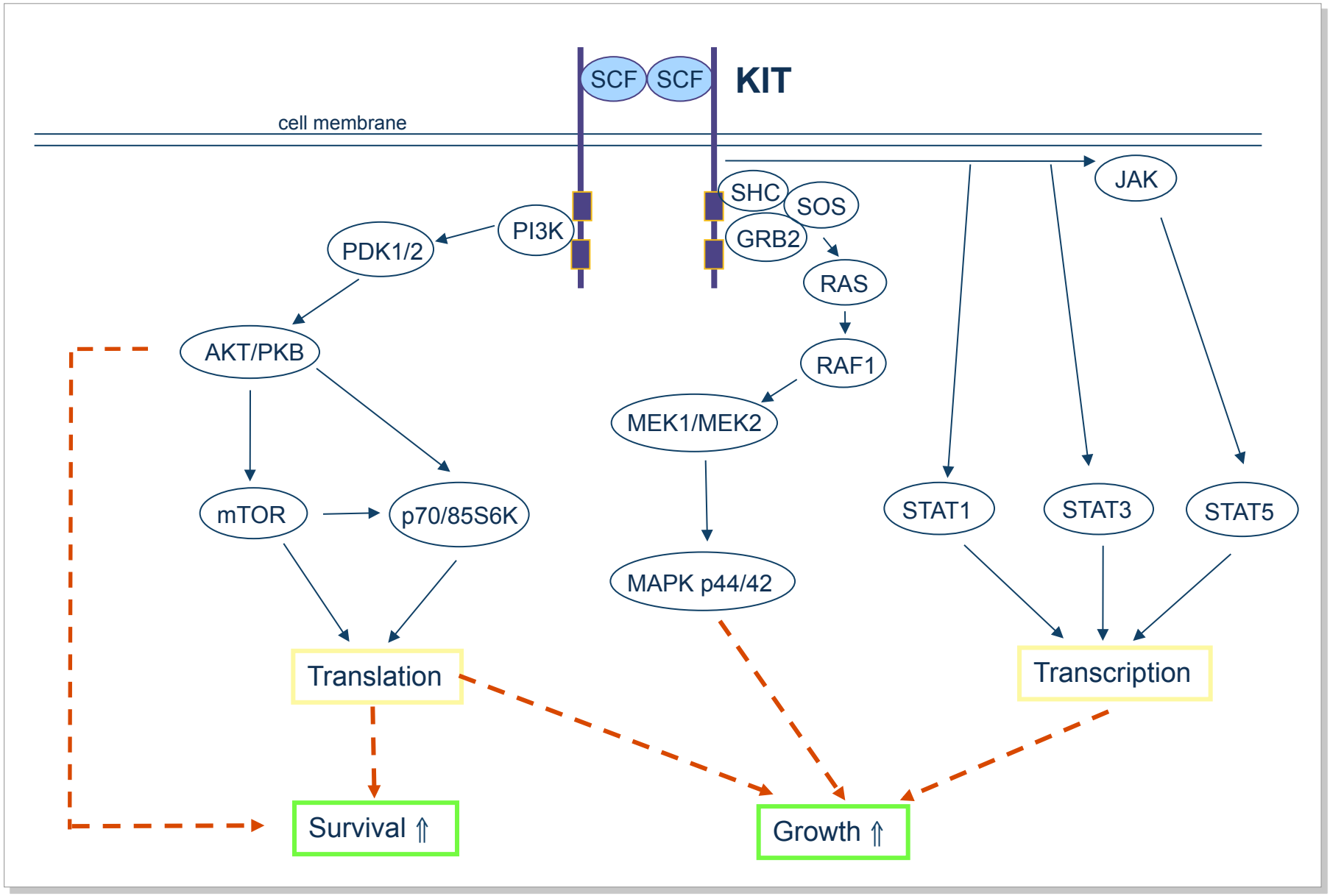
Mutant KIT signal transduction



Inhibition of KIT signal transduction



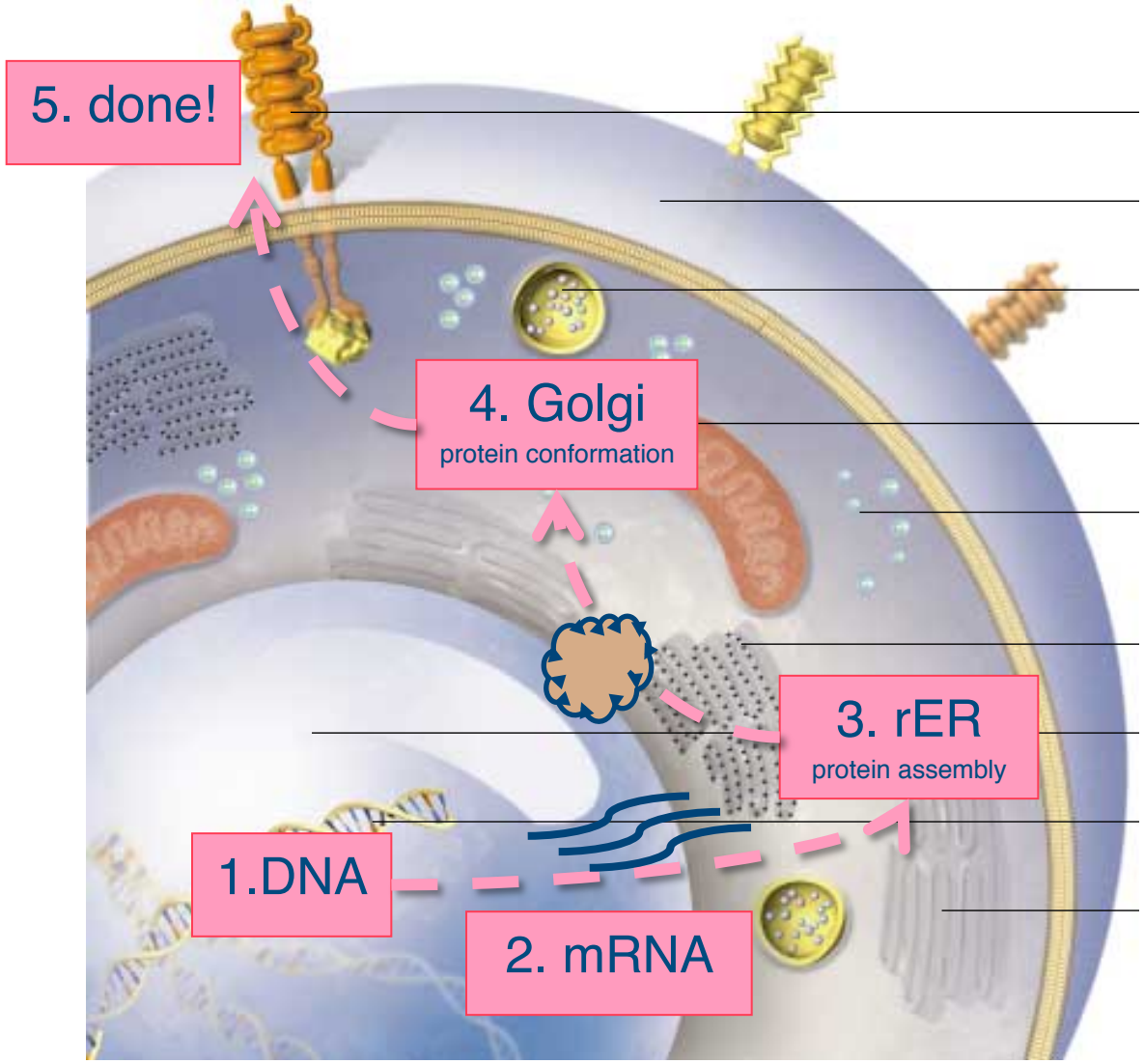
KIT signal transduction



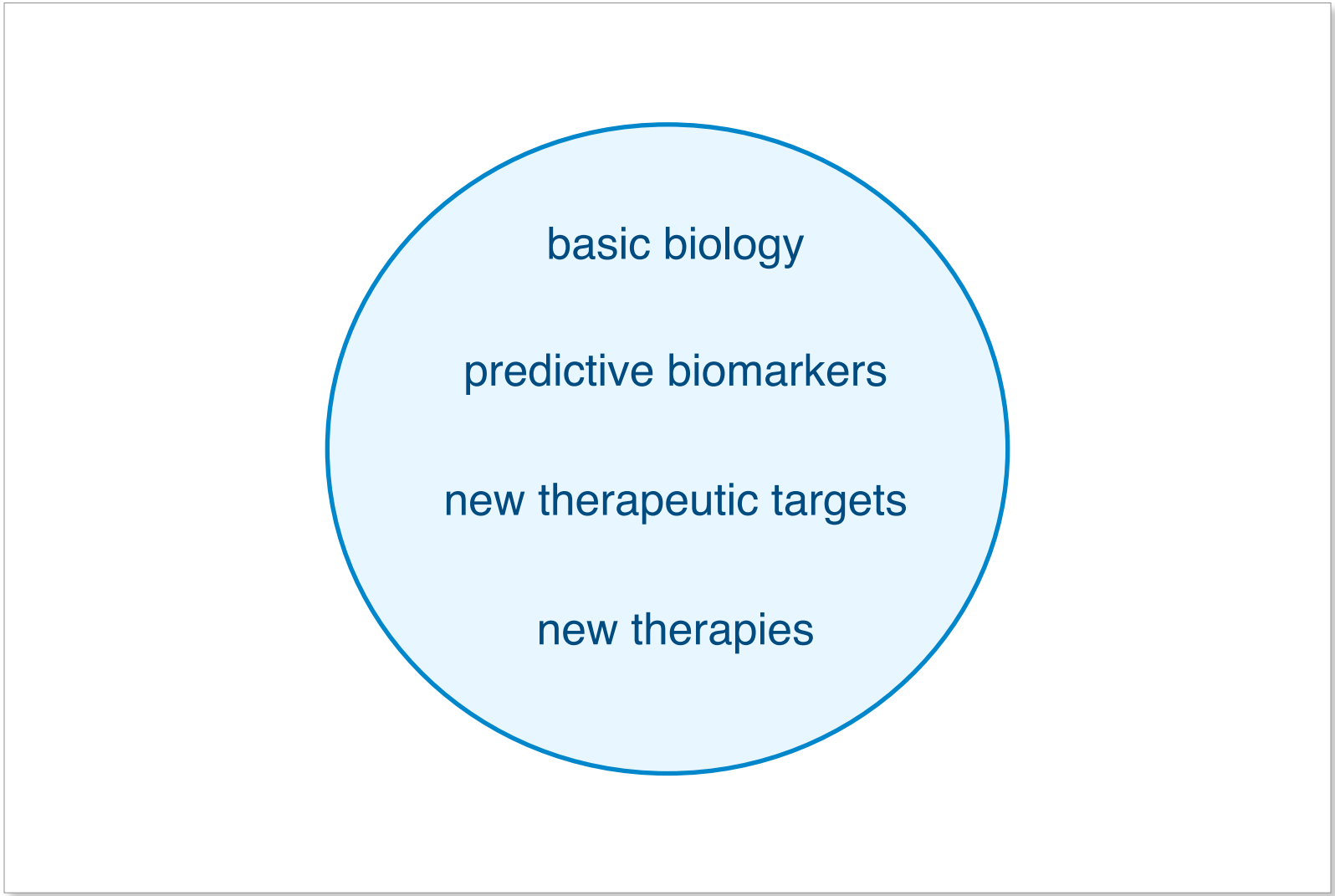
OK,

one more thing…

How does a cell function?
(from DNA to protein)



Pathway to Cure GIST
(and how to tackle the problem)

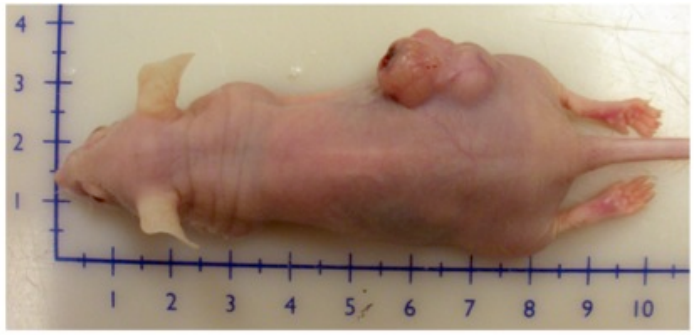


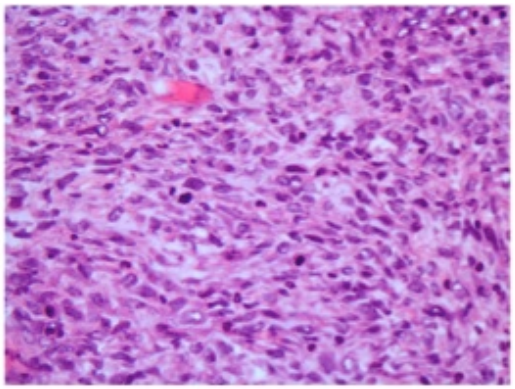
Basic Biology of GIST

GIST stem cells

Hypothesis:
$KIT^{low}PDGFRA^{low}CD34^{+}$ ICC stem cells cause GIST resistance to TKIs

- constitutively activating KIT mutations increase ICC stem cell numbers
- transformed ICC stem cells give rise to $KIT^{low/-}$ GIST-like tumors - similar to those found in some long-term imatinib-treated patients
- ICC stem cells are insensitive to imatinib

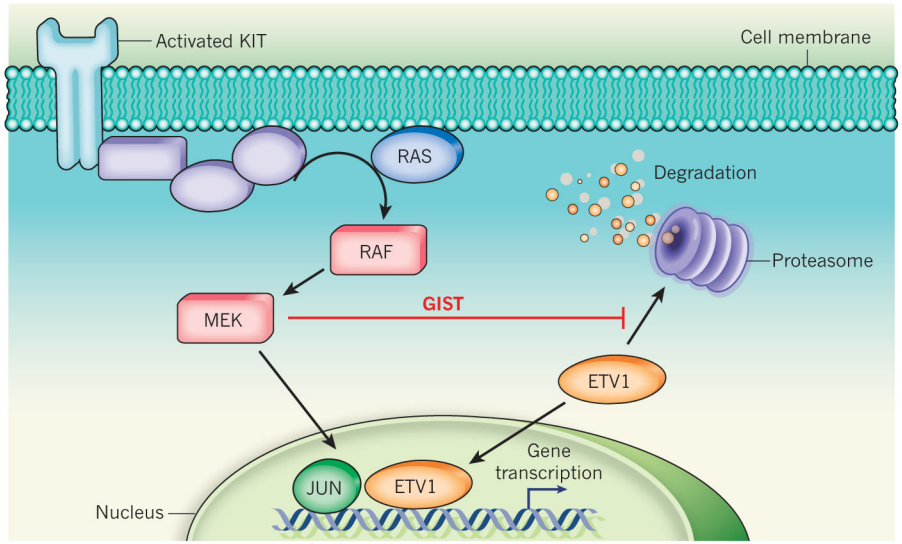




Bardsley MR et al., Gastroenterology 2010

ETV1

- “transcription factor”
- highly expressed in
  - GIST
  - ICC (“interstitial cells of Cajal”)
- needed for ICC/GIST development
- regulated by KIT
- potential therapeutic target



Chi P et al., Nature 2010
Heinrich MC and Corless CL., Nature 2010 [editorial]

So... You say to yourself... What the heck is ETV1?

ETV1 is chief engineering officer "Scotty" for the starship Enterprise.
KIT receptor is Captain KIRK barking orders for warp 9 tumor growth.

ETV1 (Scotty) implements the orders by making the necessary adjustments to the ship's engines or dilithium crystals. In other words ETV1 is the component in the cell's nucleus (the ship's engine room) that performs the actual task of revving up the engines...that is facilitating a change in the expression of certain genes that will promote growth and survival of the GIST cell.

ETV1 is a "transcription factor." and does it's job in the cell nucleus. It and other proteins bind directly on the DNA of a gene to activate gene expression. KIT is a cell surface receptor that acts like a satellite dish and television set to receive growth signals from the outside and then to broadcast them into the room (the insides of the cell).

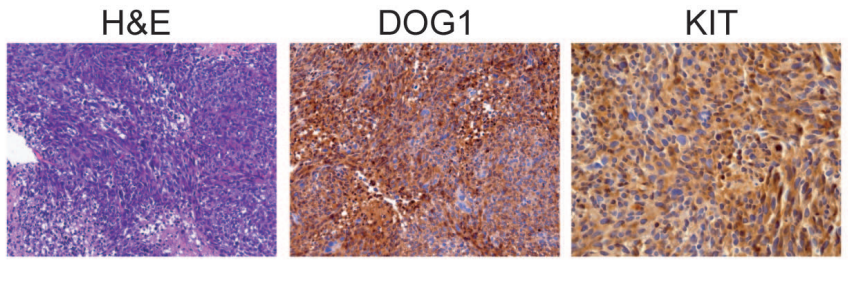
Tie up or disable Scotty (or ETV1) and then Captain Kirk's (KIT) orders can't be carried out. So far, our treatments (Gleevec, Sutent, Tasigna, Regorafenib, sta9090) have focused on shutting up KIRK. But we could go down to engine room instead and take out ETV1 (Scotty).

Symcox M, GIST Support International 2010

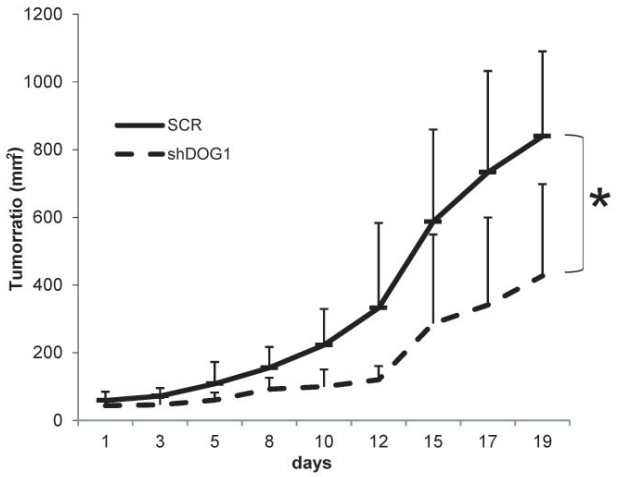
DOG1 in GIST

- diagnostic marker
- co-regulated with but not dependent on KIT
- specific target, because highly expressed
- tumor growth partially dependent on DOG1

- biochemical inhibitors with improved selectivity needed



GIST-T1 xenograft

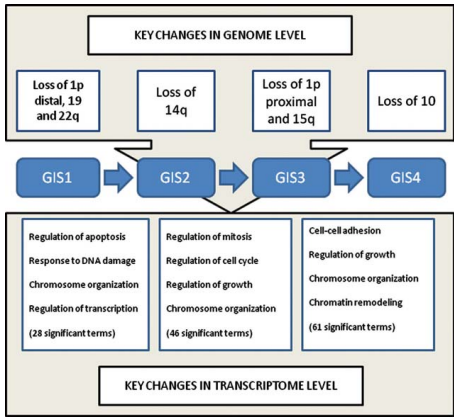


Simon S et al., Cancer Res 2013

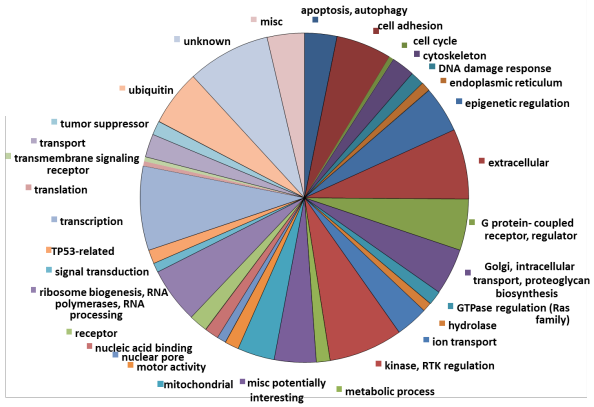
GIST whole genome (sequencing) studies

Goal: to identify additional mutated genes in GIST that can be targeted therapeutically or serve as biomarkers

- MDACC
  - whole genome copy number aberrations (CNA; array CGH)
  - whole genome gene expression
  - 42 GIST vs. 30 LMS
- Med. Uni. Vienna
  - whole genome CNA (SNP array)
  - whole exome sequencing focusing on target regions
  - 29 GIST (CNA), 13 exome
- OHSU
  - unbiased whole exome sequencing
  - GIST primary tumors and cell lines
  - 18 GIST patients, 4 cell lines



YlipääJ et al., Cancer 2011

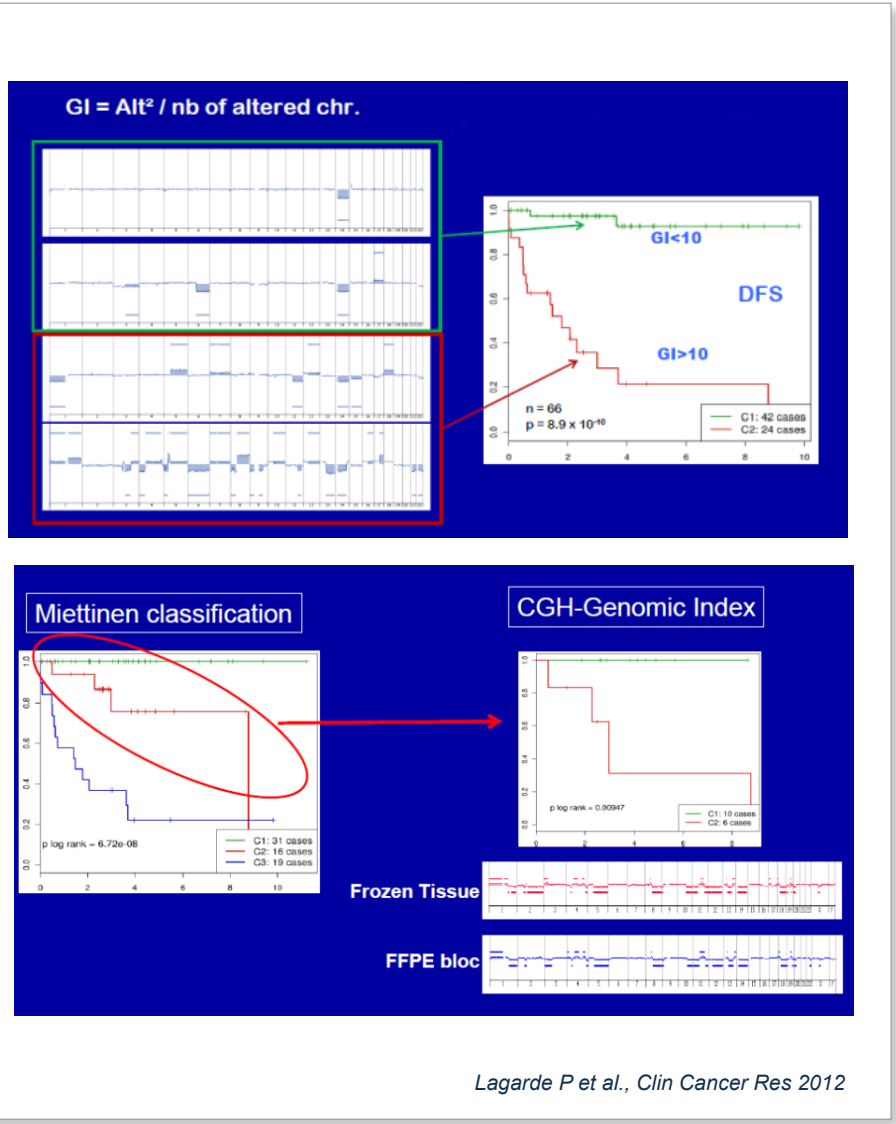


Corless CL, Heinrich MC, personal communication

Biomarkers

CINSARC GIST study

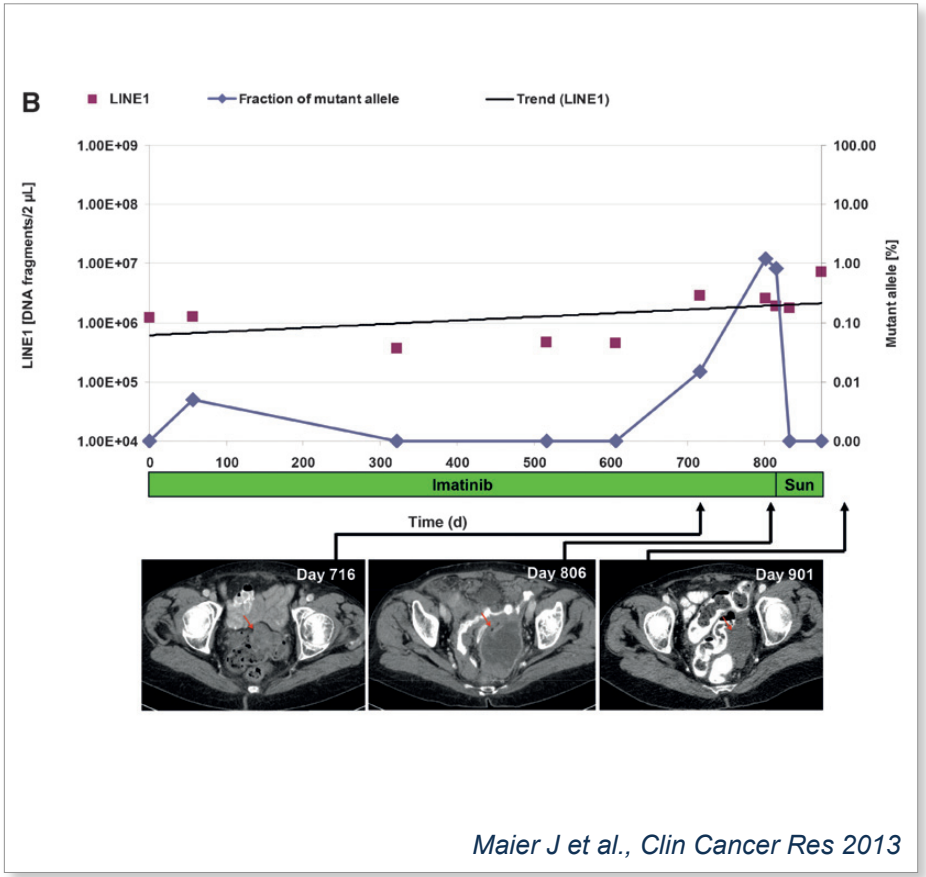
- Goal: prognostic markers for clinical outcome
- CINSARC(Complexity INdex in SARcomas) signatures and tumor Genomic Index (GI)
- more sensitive in prediction recurrence than current histopathologic risk scheme
- GI index is an independent prognostic factor
- identifies poor prognosis patients in intermediate-risk group



Lagarde P et al., Clin Cancer Res 2012

free circulating tumor DNA

- free DNA (→ not inside a cell) circulating in the blood
- increased in cancer patients
  - dying cells inside a tumor disintegrate and release DNA
  - live tumor cells can get into bloodstream, but disintegrate there
- detected with highly sensitive techniques
  - mutated KIT as low as 0.01% (1 in every 10,000!)



Maier J et al., Clin Cancer Res 2013

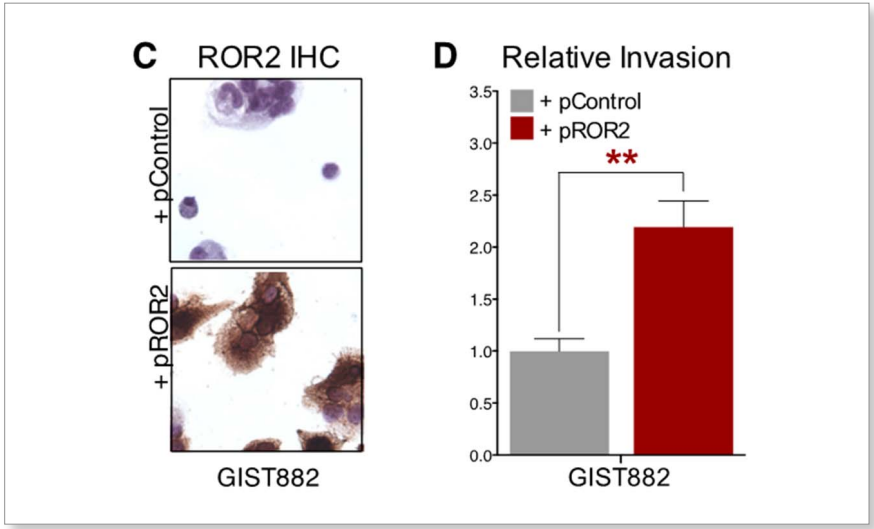
complete “liquid biopsy”

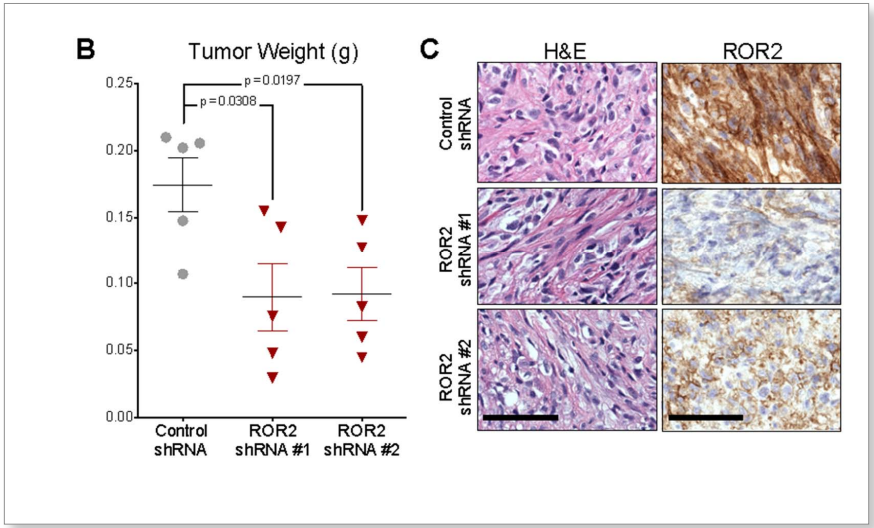
→ heterogeneity of tumors and metastases in GIST!

New Therapeutic Targets

ROR2 as therapeutic target in GIST

- receptor tyrosine kinase (ligand = Wnt5)
- enhances invasion in vitro (GIST and LMS)
- knockdown inhibits invasion and decreases tumor size in xenografts
- expression level correlates with outcome (high ROR2 = poor outcome)
- therapeutic target in GIST

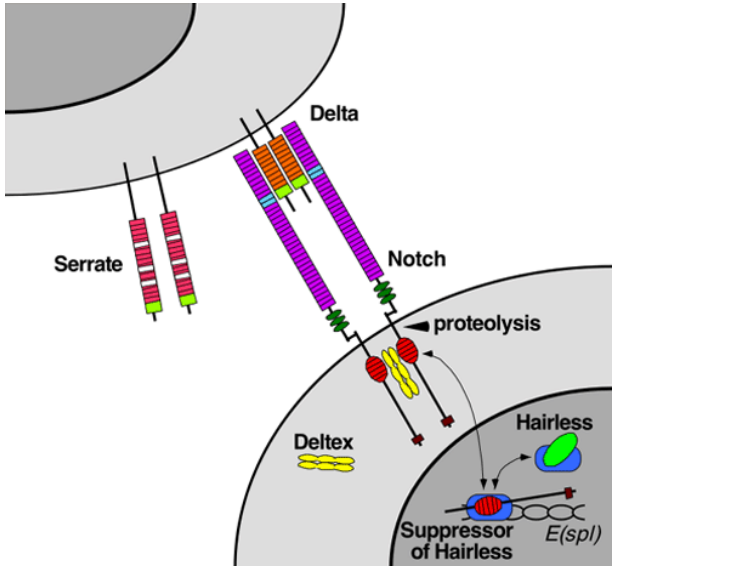




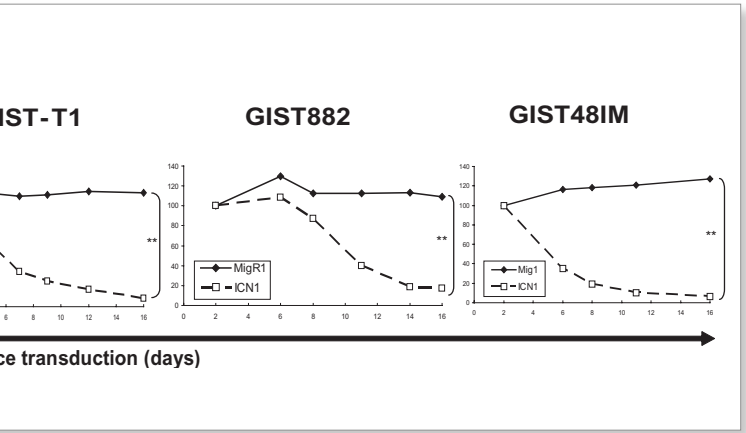
Edris B et al., J Pathol 2012

The Notch pathways as therapeutic target in GIST

- neural function and development
  → ICC?
- cell-to-cell signaling
  - receptor on one cell
  - ligand on second cell
- cell proliferation



Wikipedia



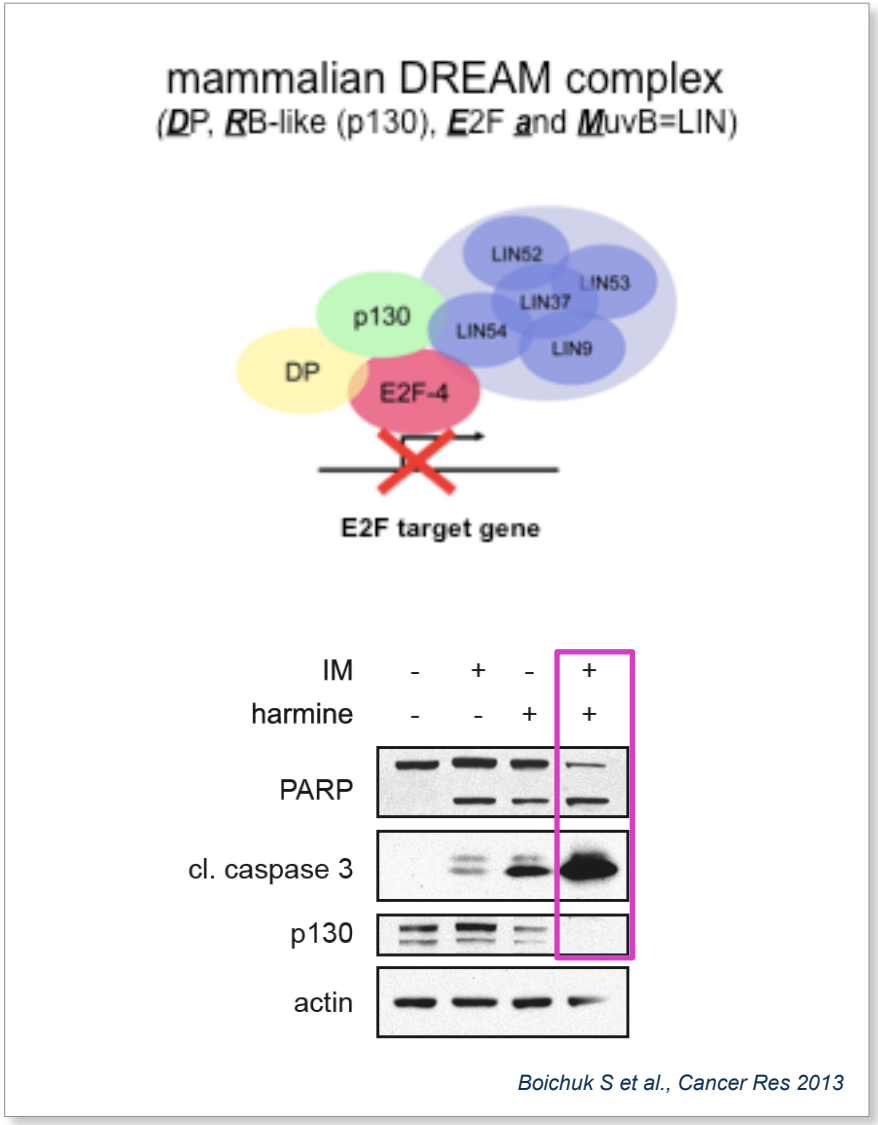
Dumont AG et al., Carcinogenesis 2012

The DREAM complex as therapeutic target in GIST

- imatinib leads to a reversible cell cycle exit in GIST cells (tumor cell “sleep”)
- cells are not dividing or growing – BUT are metabolically active and not dead
- potential reservoir for resistant clones

- Key molecular regulator: DREAM complex

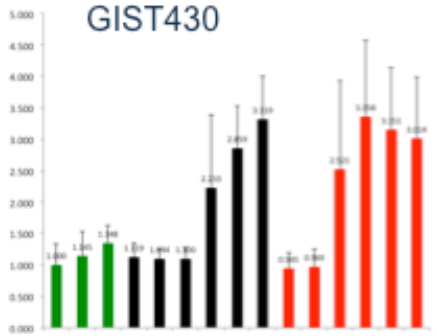
- can be targeted therapeutically to enhance apoptotic effect of imatinib



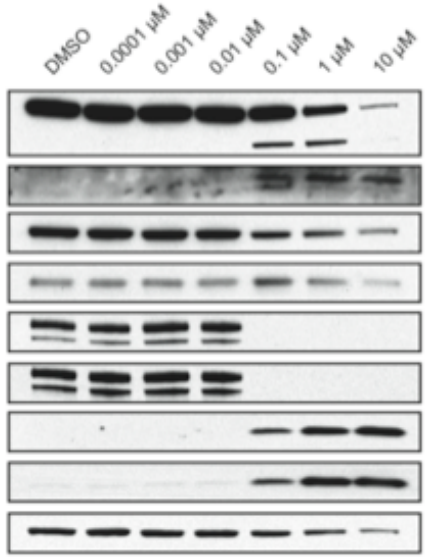
Targeting the ubiquitin-proteasome machinery in GIST

- the FDA-approved proteasome inhibitor bortezomib (Velcade®) induces apoptosis in GIST cells
  (Bauer S et al. Cancer Res. 2010)
- new therapeutic option for GIST patients, BUT suboptimal pharmacokinetics
- second-generation proteasome inhibitors
  - drug X:
    → *FDA-approved for multiple myeloma (MM)*
  - drug Y:
    → *Phase II for MM*

drug X



drug Y



Duensing Lab, work in progress

High-throughput gene knockdown studies
siRNA/shRNA library screens

siRNA libraries

GIST cells

focus on whole genome approaches

focus on "druggable" gene subgroups

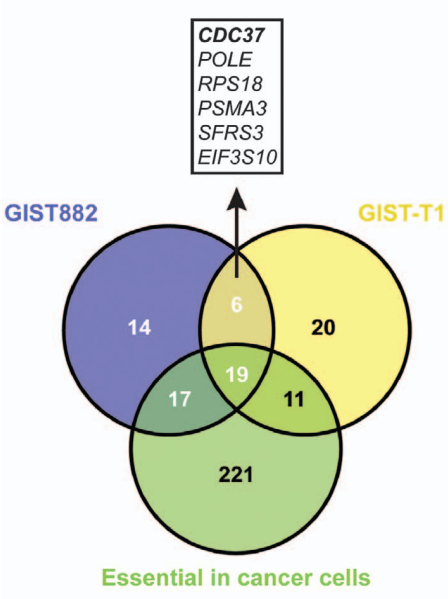
apoptosis
cell viability

Goal: identify important survival genes in GIST besides KIT/PDGFRA

High-throughput gene knockdown studies siRNA/shRNA library screens

genome-wide screen

- CDC37
  - crucial cofactor for KIT expression
  - interacts with oncogenic KIT
  - regulates expression and activation of KIT and downstream signaling intermediates
  - knockdown leads to KIT inhibition
- promising target for inactivating KIT



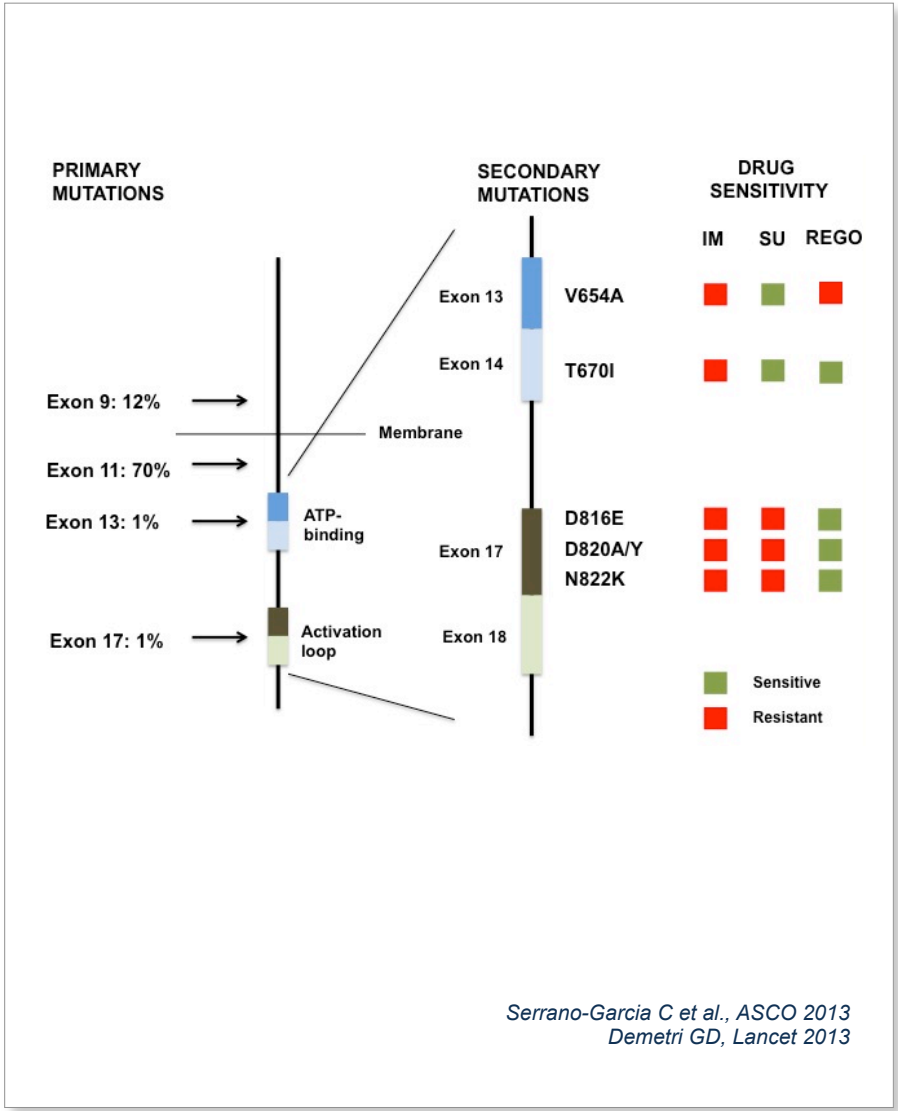
Marino-Enriquez A et al., Oncogene 2013

New Therapies

Regorafenib (Stivarga®)

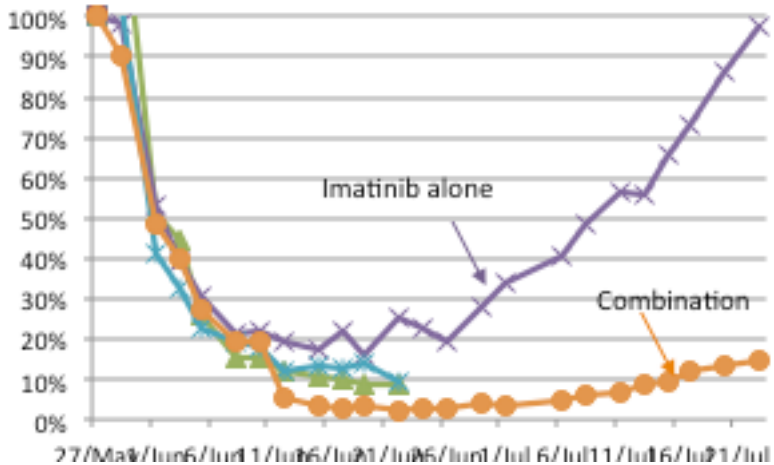
- oral multikinase inhibitor
  - KIT, PDGFR, FGFR, VEGFR2/3, TIE-2, B-RAF
- significant activity in patients with advanced GIST
- FDA-approval

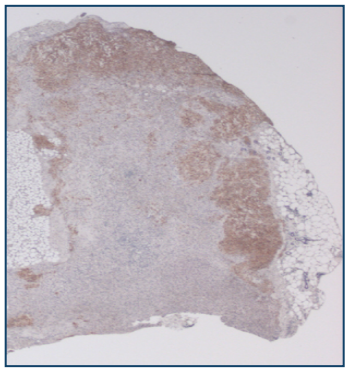
- potent inhibitor of *KIT* exon 11 mutations
- significant activity against *KIT* exon 17 (activation loop) secondary mutations
- less active against *KIT* exon 13 (V654A, ATP-binding pocket) mutations than SU



PI3K inhibitor GDC-0941

- significant reduction in tumor volume alone and in combination with imatinib
- higher histological reponse in combination treatment arm, especially apoptotic activity
- no tumor re-growth after treatment discontinuation in the combination treatment arm
- combination treatment (imatinib + GCD-0941) yields long-lasting effect (in mice!)





KIT/CD117

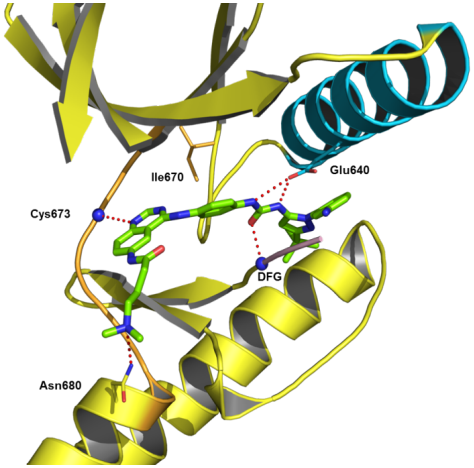
Grade 3 histologic response in a combination treatment

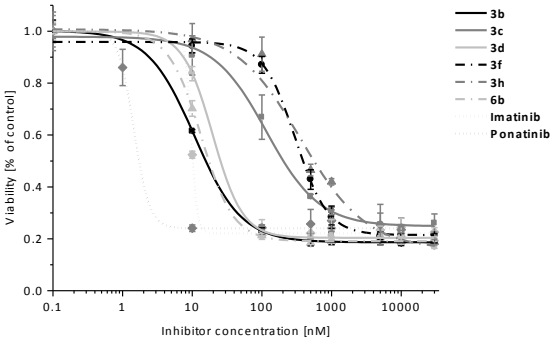
Floris G et al., Clin Cancer Res 2012

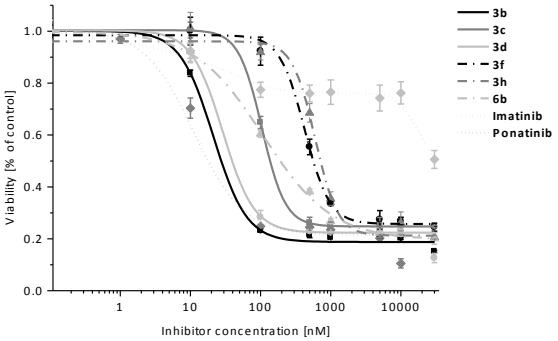
KIT-specific compounds against gatekeeper mutation (T670I)

Compound	R^1	R^2
3a	NO_2	NO_2
3b	H	NO_2
3c	NH_2	NH_2
3d	H	NH_2
3e	NH_2	NHFmoc
3f		NH_2
3g		NH_2
3h		NH_2
3i		NH_2
4a	H	NO_2
4b	H	NH_2
5a	NO_2	---
5b	NH_2	---
6a	NO_2	---
6b	NH_2	---

In silico-mutated KIT T670I

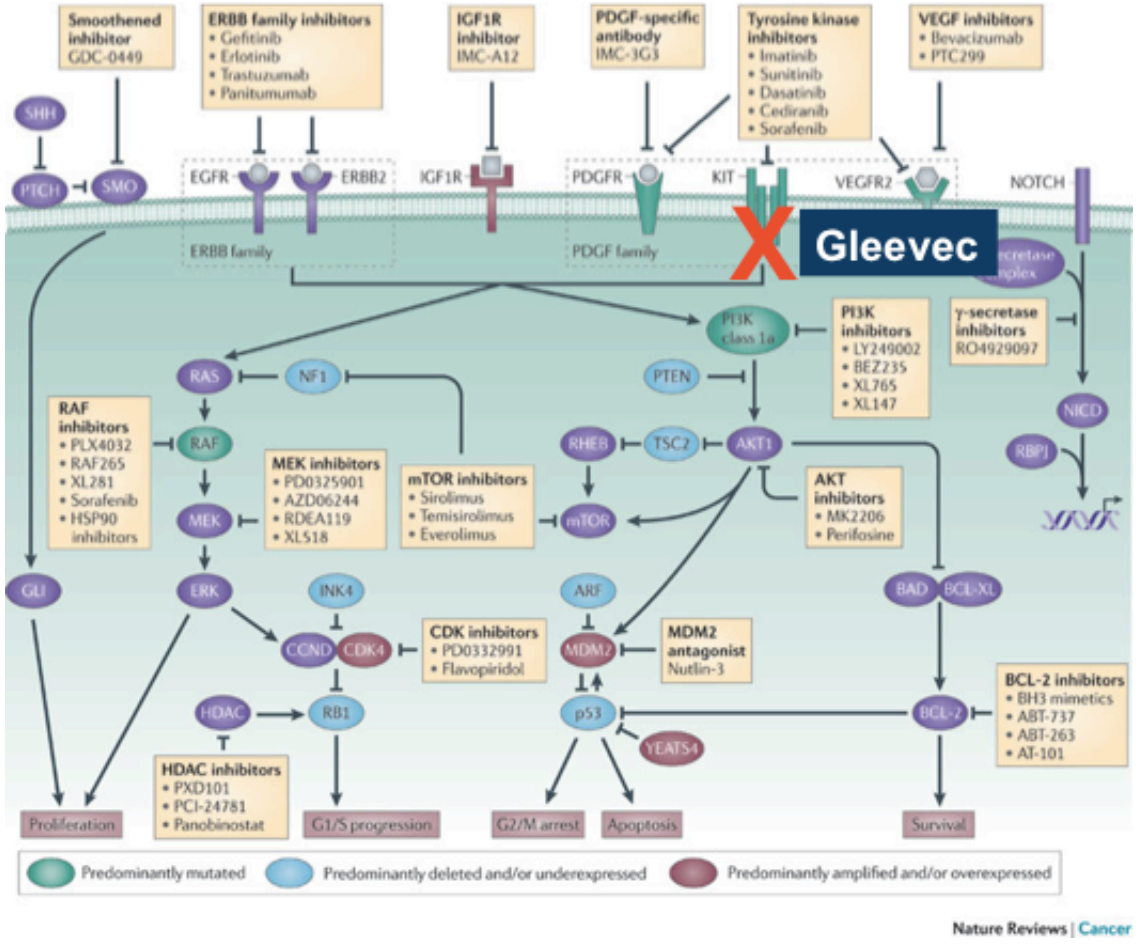






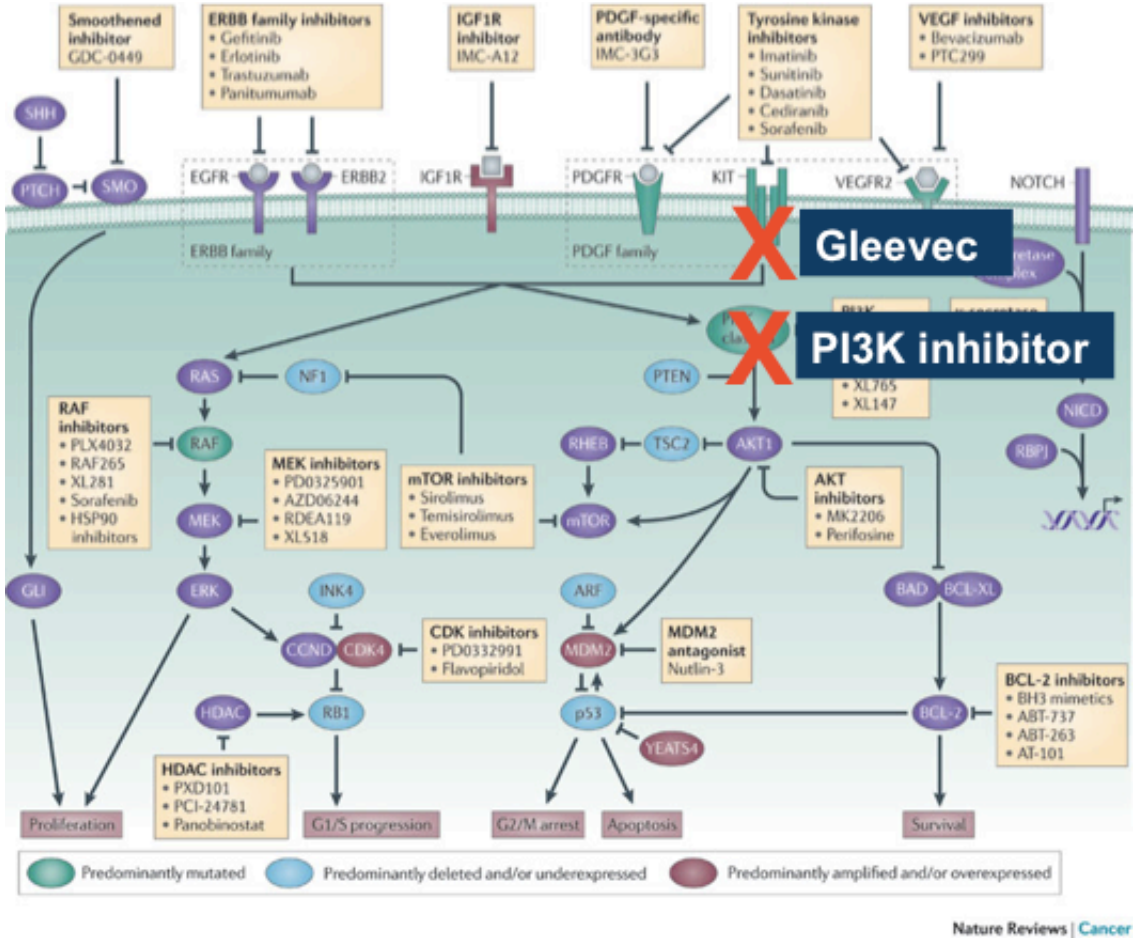
Richters A et al., J Med Chem 2013

Combined Therapies for GIST



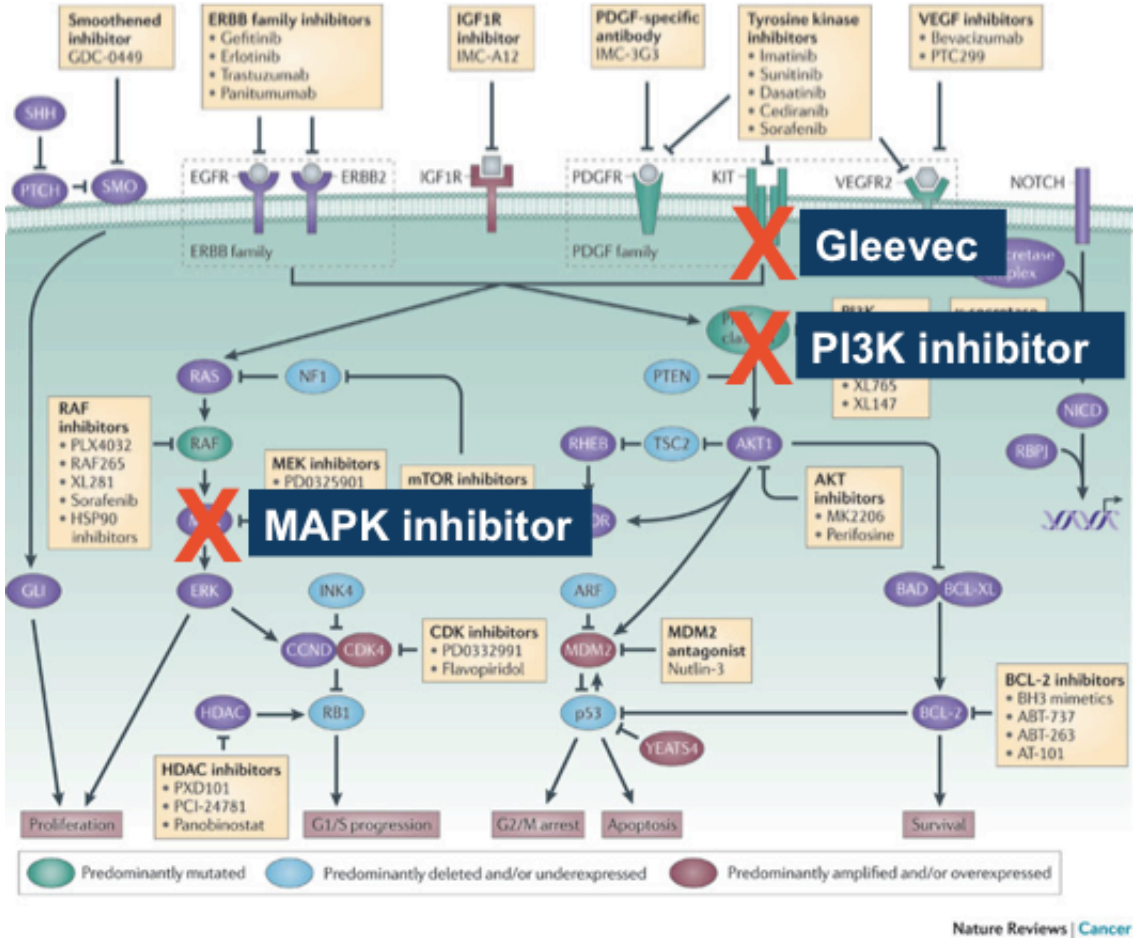
modified after Taylor BS et al. Nat Rev Cancer 2011

Combined Therapies for GIST



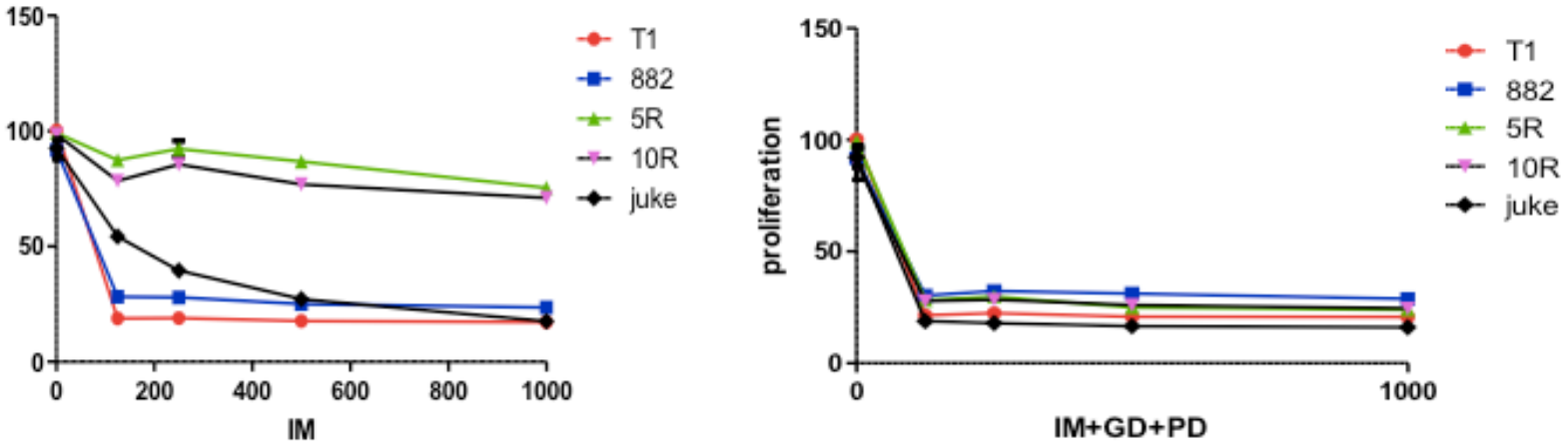
modified after Taylor BS et al. Nat Rev Cancer 2011

Combined Therapies for GIST



modified after Taylor BS et al. Nat Rev Cancer 2011

Combined Therapies for GIST

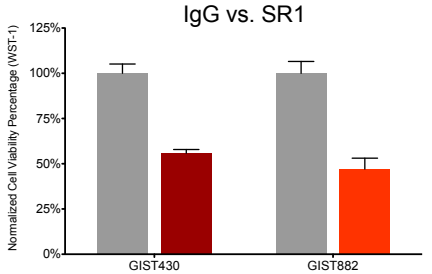


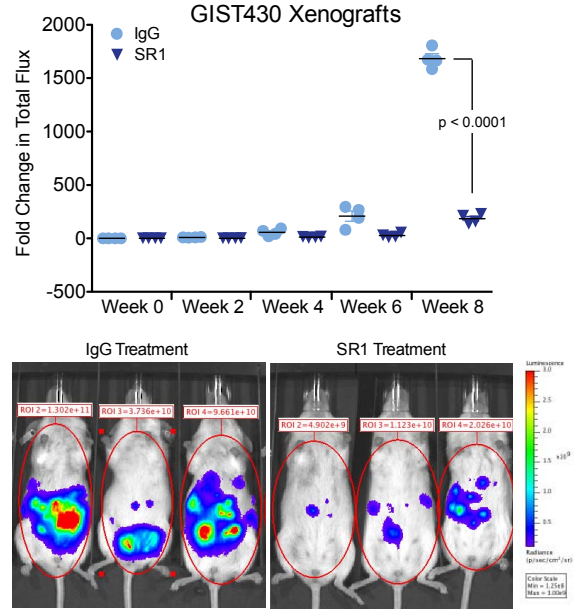
Rubin BL, personal communicaiton

The combination of imatinib, a PI3K inhibitor and a MAPK inhibitor can control all known GIST cell lines - while IM is unable to control GISTs with either resistant KIT mutations (5R) or those that have switched over to another oncogenic pathway (10R)

anti-KIT antibody as therapy in GIST

- TKI-resistant GIST still dependent on KIT expression and activation
- progressing disease can have several secondary resistance mutations → difficult to target with TKI
- anti-KIT mAb SR1
  - inhibits GIST cell viability
  - xenograft growth
- alternative to TKI therapy in GIST, especially in IM-resistant disease





Edris B et al., PNAS 2013

Drug compound screens

drug compound libraries

GIST cells

focus on kinase inhibitors

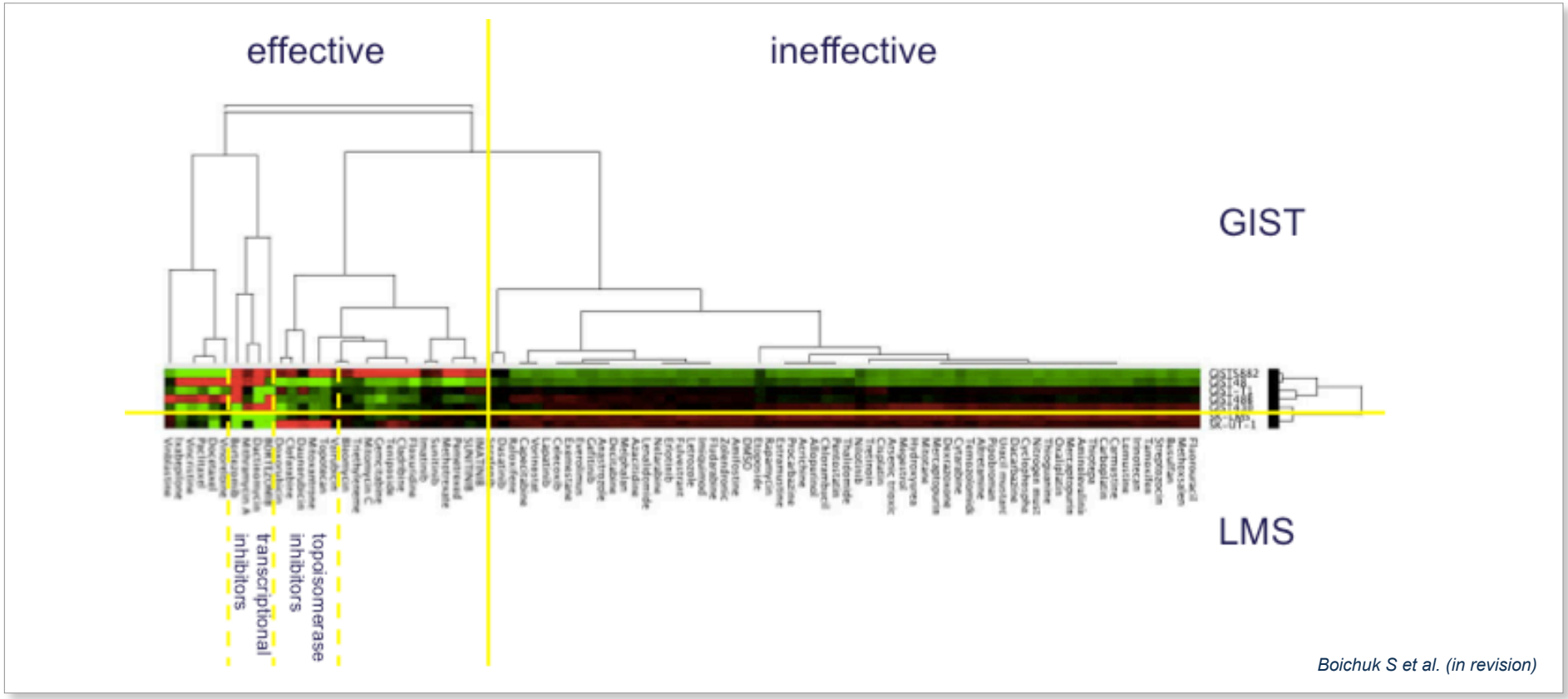
focus on FDA-approved drugs

treatment in 96-well plates

apoptosis
cell viability

Goal: identify active compounds for the treatment of GIST

Screen has identified two major FDA-approved drug classes effective in GIST

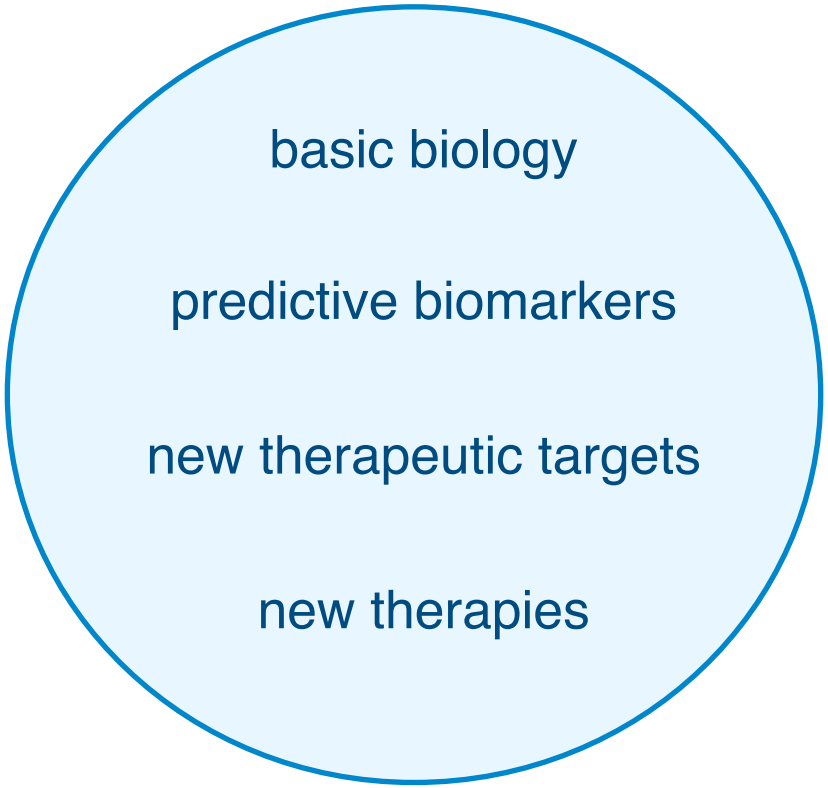


Boichuk S et al. (in revision)

- ~100 FDA-approved drugs tested → ***drug repurposing***
- ***transcriptional*** and ***topoisomerase* II** inhibitors are active in GIST cell lines and xenograft models
- clinical trials are being discussed

Summary:

What is going on in the GIST research world?



Summary:

What is going on in the GIST research world?

A lot is going on
in the GIST research world!!!

THANK YOU,

for your attention!

aduensin@pitt.edu