Targeting the Ras-MAPK Pathway for Cancer Therapy

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- Mechanisms of Ras-driven oncogenesis
- Mechanisms of Ras synthetic lethal interactions
- Target identification and therapeutic strategies in Ras mutant caners

I declare no conflict of interest

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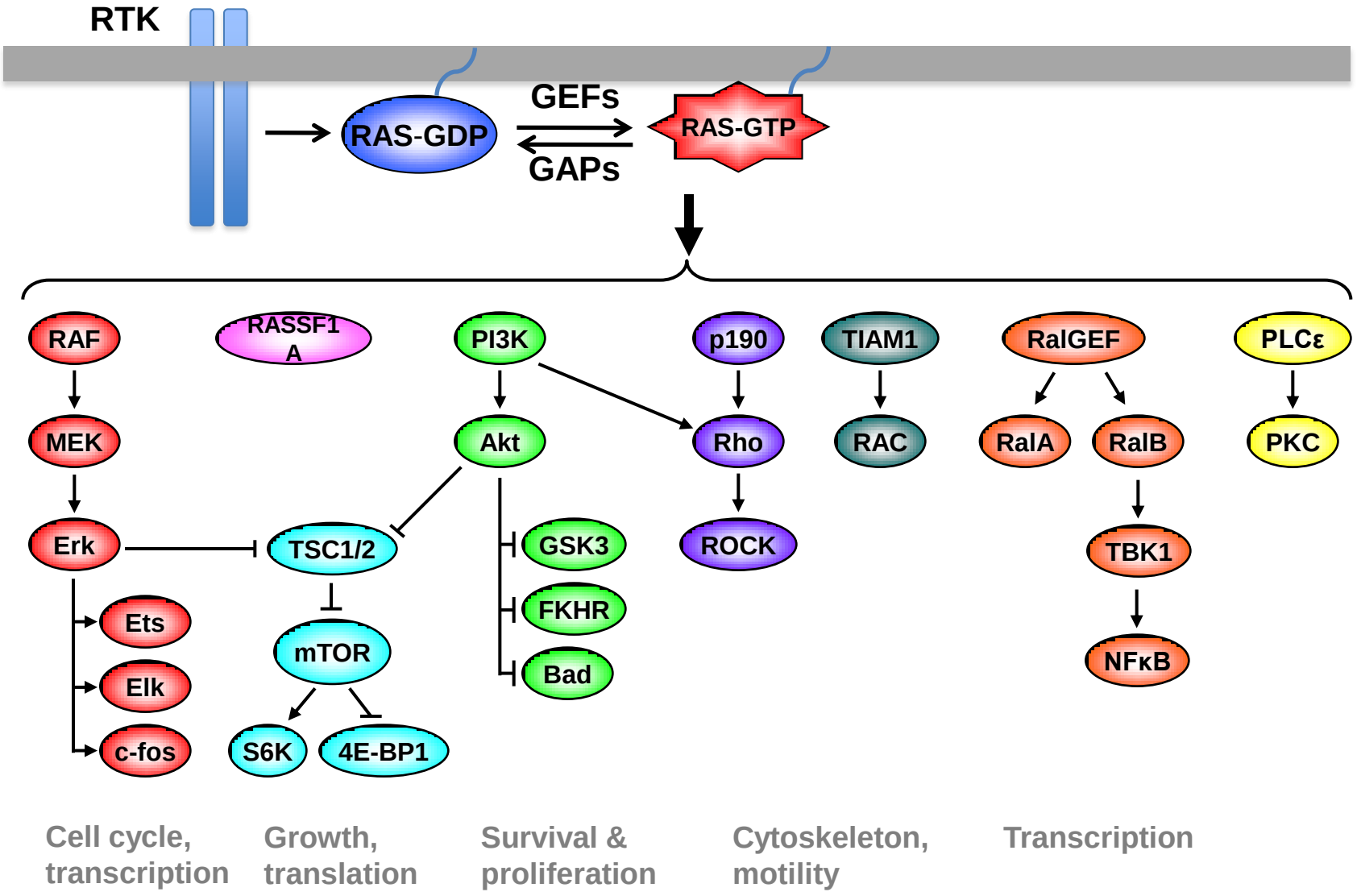
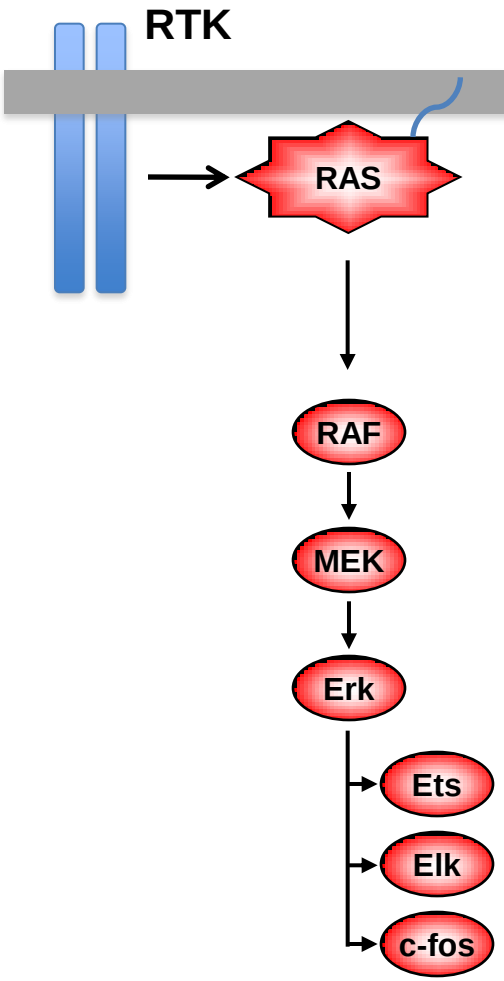
The Ras signaling network



Ras-MAPK pathway is essential for cell proliferation



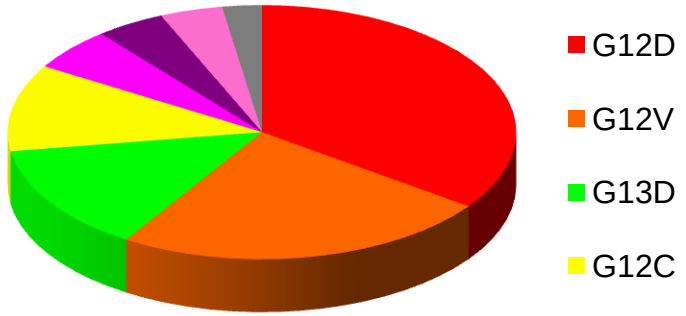
Drosten *et al.*, EMBO J., 2010
Genetic analysis of Ras signaling pathways in cell proliferation, migration and survival

Ras	RAF	MEK	ERK	PI3K	RalGDS	Proliferation
wt	wt	wt	wt	wt	wt	Yes
null	wt	wt	wt	wt	wt	No
null	Active	wt	wt	wt	wt	Yes
null	wt	Active	wt	wt	wt	Yes
null	wt	wt	Active	wt	wt	Yes
null	wt	wt	wt	Active	wt	No
null	wt	wt	wt	wt	Active	No
null	wt	wt	wt	Active	Active	No

Incidence of Ras mutation in human cancer

Tissue	K-ras	H-ras	N-ras	Total
Pancreas	**60%**	0%	2%	**62%**
Small intestine	**20%**	0%	**25%**	**45%**
Large intestine	**32%**	0%	3%	**35%**
Biliary tract	**32%**	0%	1%	**33%**
Skin	2%	5%	**19%**	**26%**
Salivary gland	4%	**16%**	0%	**20%**
Lung	**17%**	1%	1%	**19%**
Gastrointestinal tract	**19%**	0%	0%	**19%**
Ovary	**15%**	**0%**	4%	**19%**
Urinary tract	4%	**12%**	3%	**19%**
Cervix	8%	9%	1%	**18%**
Haem./lymph. tissue	5%	0%	**12%**	**17%**
Upper aerodigestive	4%	9%	3%	**16%**
Endometrium	**14%**	1%	0%	**15%**
Prostate	8%	6%	1%	**15%**
Thymus	**15%**	0%	0%	**15%**
Thyroid	3%	4%	7%	**14%**
Stomach	6%	4%	2%	**12%**
Liver	7%	0%	4%	**11%**
Testis	5%	0%	4%	**9%**

Lung, colon & pancreatic cancers



(Weng and Luo, analysis of Sanger COSMIC data)

(Modified from review by Karnoub & Weinberg, 2008.)

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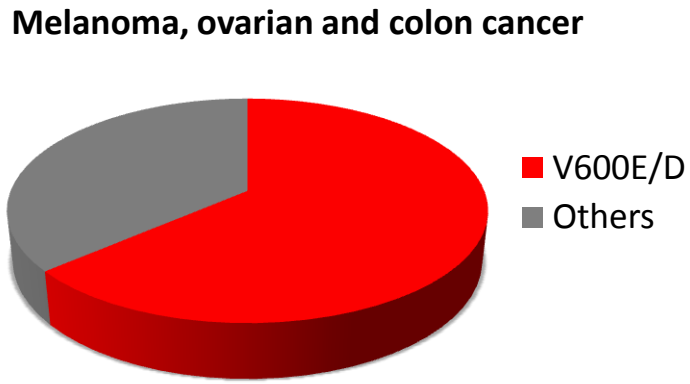
Incidence of BRAF mutation in human cancer

Tissue	BRAF
Melanoma	67%-80%
Thyroid	39%
Ovarian	14%
Colon	12%



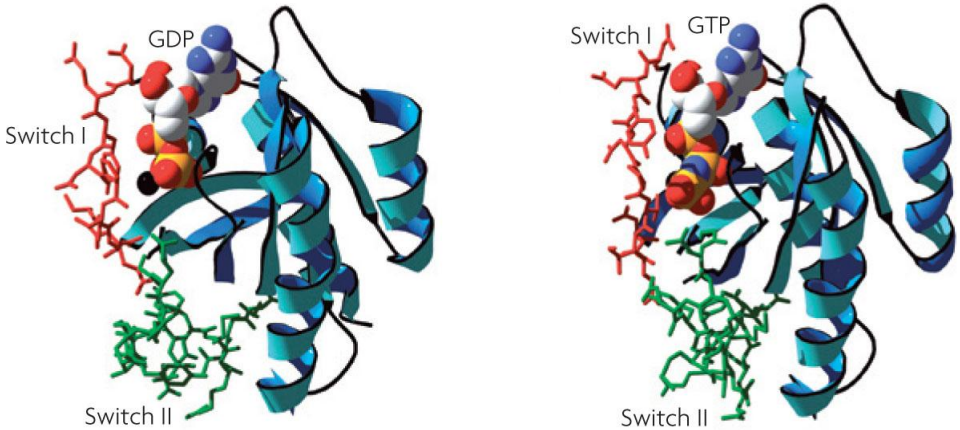
(Modified from Davies et al., 2002 and Vakiani and Solit, 2011)

The Ras GTPase switch

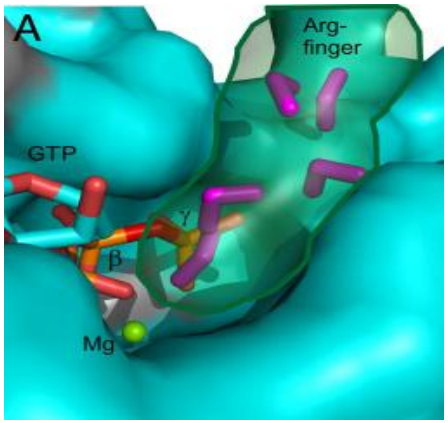
GEFs
(RasGRP, Shc, Grb2,
Sos, NR2B, RasGRF1)

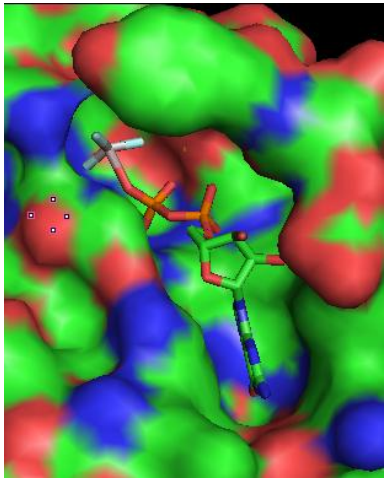
Ras-GDP
inactive

Ras-GTP
active

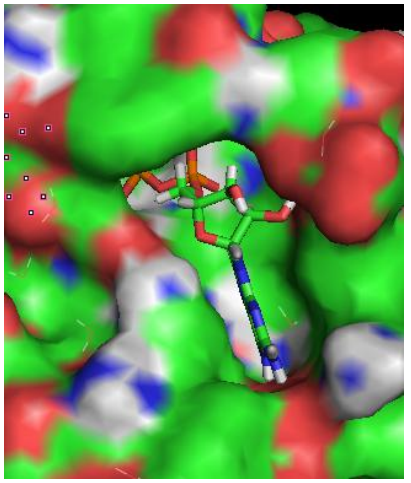


GAPs
(p120GAP, NF1, Capri, SynGAP, GAP1m,
GAP1IP4BP, RASAL1, DABZIP)





WT G12



Mut D12

(Karnoub & Weinberg, 2008, Kotting et al 2010)

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Strategies to inhibit mutant Ras function

- Target Ras itself
- Target Ras effectors
- Target Ras synthetic lethal interactions

Approaches to inhibit Ras: direct targeting of Ras

Small molecule activators of mutant Ras GTPase

Difficult because the GTP binding pocket is covered in the mutant.

Ras GTP binding pocket inhibitor

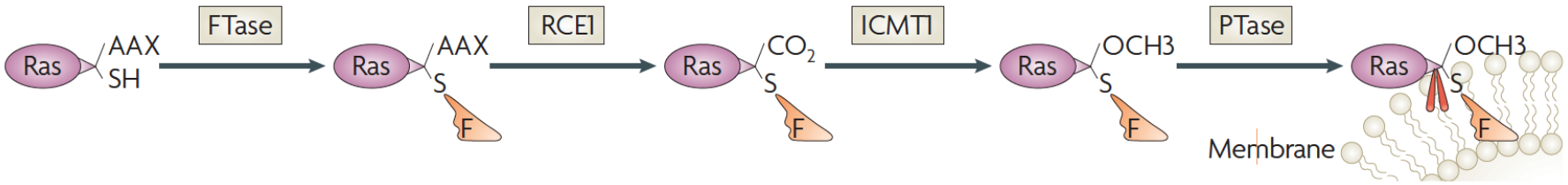
Difficult because Ras has high affinity for GTP (Km ≈ pM) and intracellular GTP concentration is high (mM).

Ras Effector domain binding inhibitor

Relatively large and flat interaction surface.

Proof-of-principle peptides have been tested.

Approaches to inhibit Ras: Ras prenylation



Kato *et al.*, PNAS, 1992

Isoprenoid addition to Ras protein is critical modification for its membrane association and transforming activity

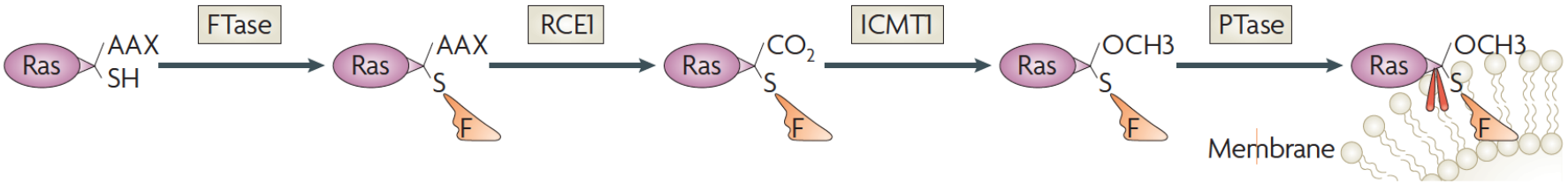
Table 1. Transforming activity, membrane association, and processing of Ki-Ras4B CAAX mutants

Substitution				Transforming activity*	Membrane association†	Processing‡
C	A_1	A_2	X			
C	V	I	M	1.00	++++	3
S	V	I	M	0.00	–	1
C				0.00	–	1

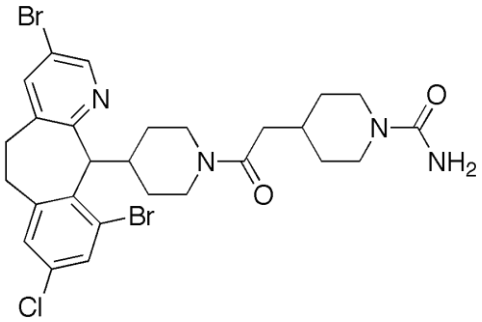
FTase inhibitors:

FTI-276
FTI-2148
L-739750
BZA-2B
R115777
SCH66336
RPR130401

Approaches to inhibit Ras: Ras modification

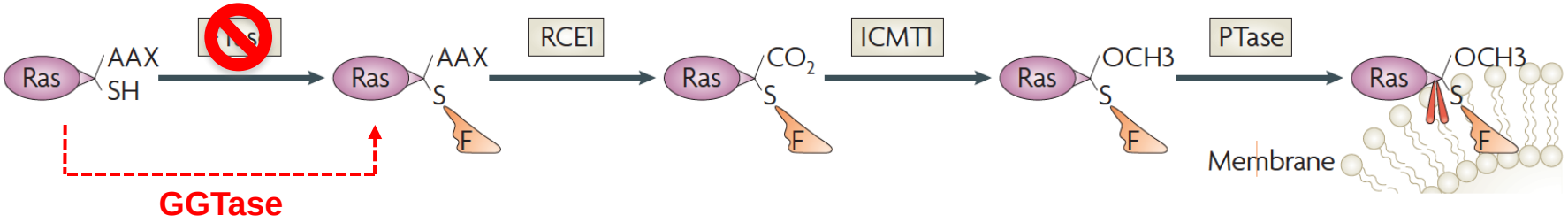


SCH66336/Lonafarnib



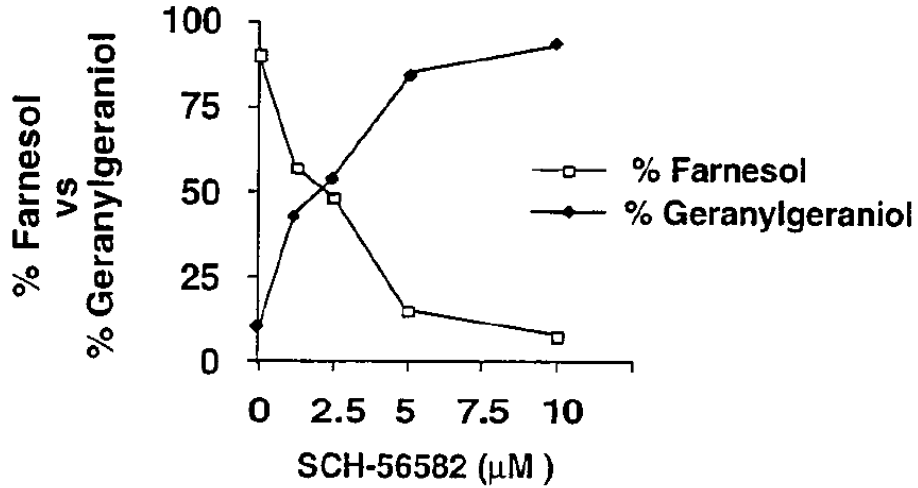
- Good selectivity and potency towards FTase in vitro (Liu et al., 1998).
- Anti-tumor activity not correlated with Ras mutation status.
- Affect many cellular proteins that require farnesylation.
- Phase II trial: single agent in metastatic colon cancer showed no objective response (Shema et al. 2002).
- Phase II trial: Combination with chemotherapy on-going.

Approaches to inhibit Ras: Ras modification



Whyte *et al.*, JBC, 1997

K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors

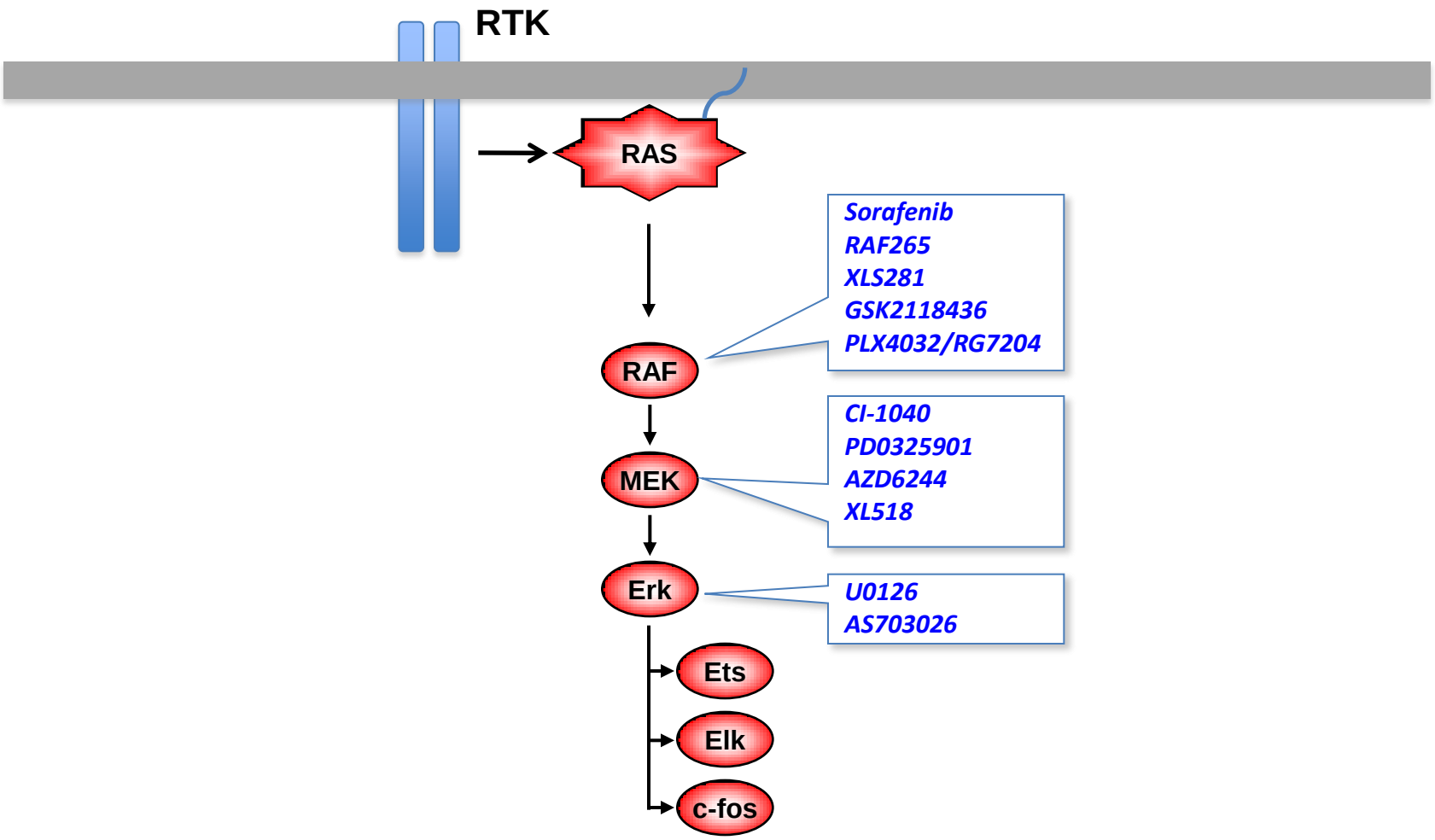


Dual-specific FTase and GTase inhibitors:

L-778123

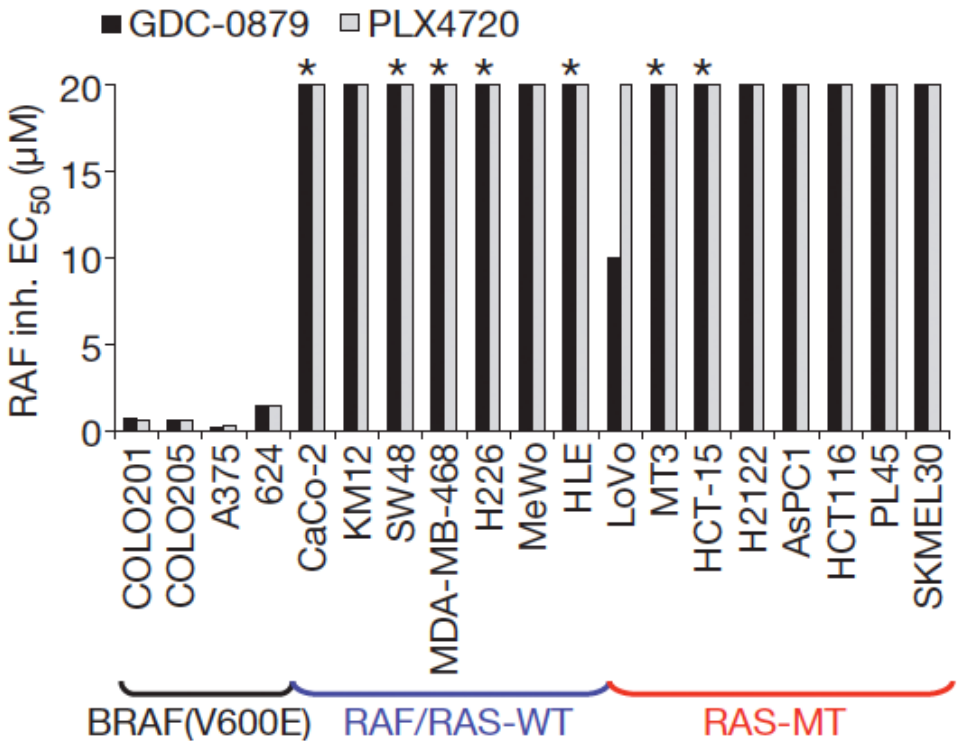
AZD3409

Ras-MAPK pathway is essential for cell proliferation



Targeting MAPK pathway: BRAF

BRAF inhibitors: ineffective in Ras-mutant cells due to trans-activation of CRAF
(Hatzivassiliou *et al.* Nature 2010, Poulikakos *et al.* Nature 2010, Heidorn *et al.* Cell 2010)

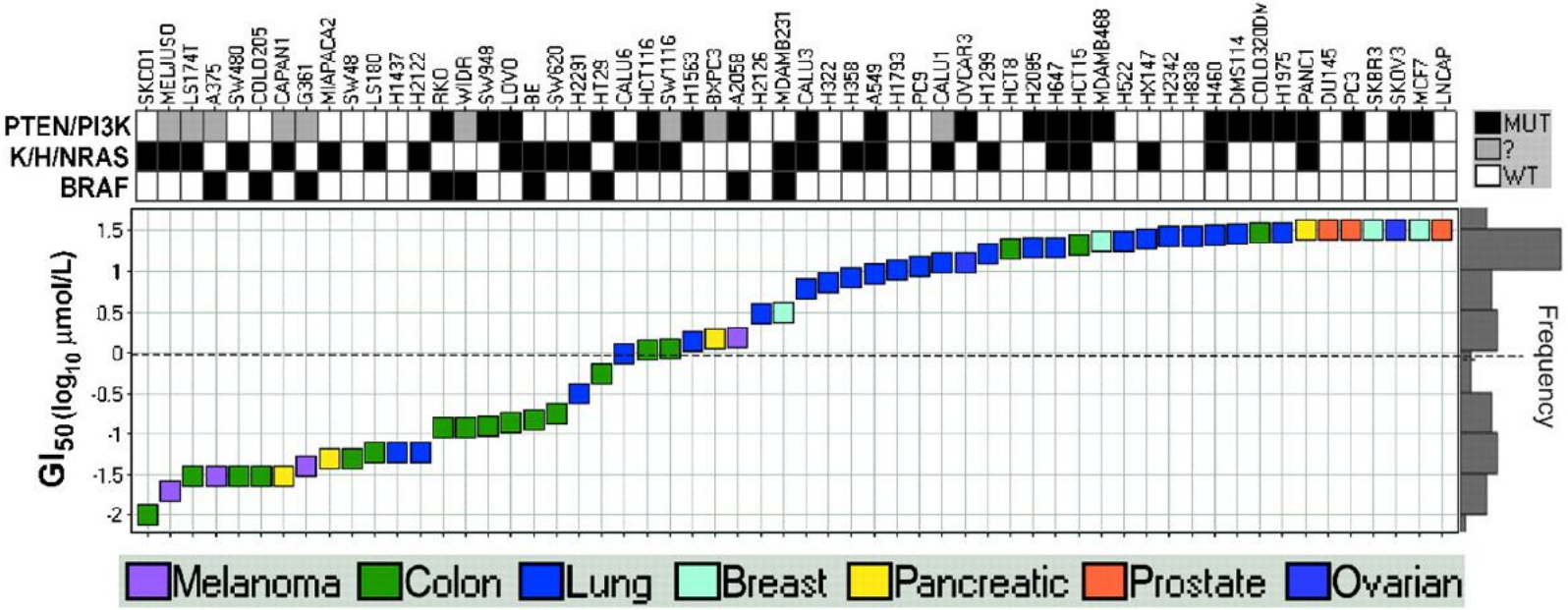


Targeting MAPK pathway: MEK

MEK inhibitors: sensitivity in some Ras mutant cells

Solit *et al.* Cancer Res 2008; Wee *et al.* Cancer Res 2009, Halilovic *et al.* Cancer Res 2010, Dry *et al.* Cancer Res 2010

AZD6244 (Dry et al. Cancer Res 2010)



Targeting MAPK pathway: MEK

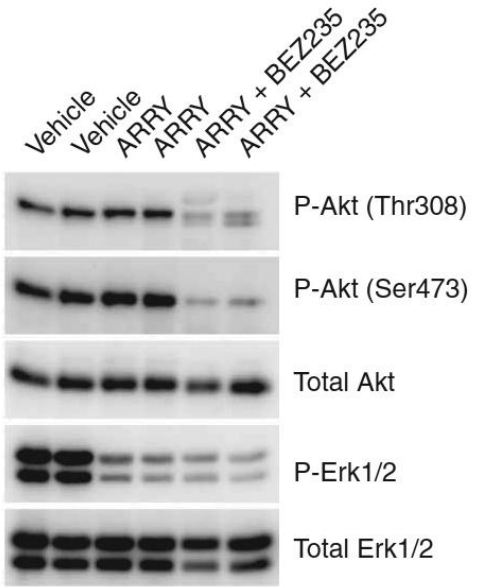
MEK inhibitor single agent trials are disappointing thus far

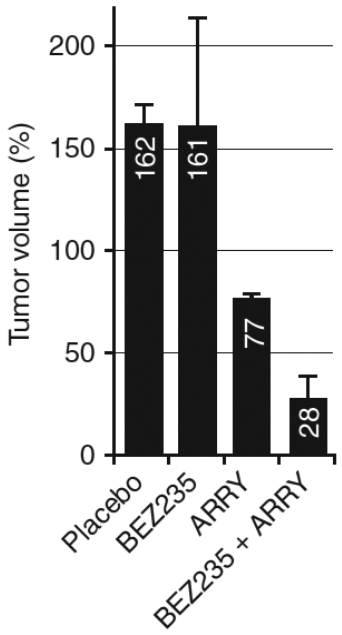
Ref	Agent	Phase	Indication	Improved Response
Rinehart *et al*. JCO 2004	CI-1040	Phase II	advanced NSCLC, breast, colon and pancreatic cancer (n =67), single arm open label	No
Haura *et al*. Clin Cancer Res 2010	PD-0325901	Phase II	advanced NSCLC (n=24), single arm open label	No
Hainsworth *et al.* J Thorac Oncol 2010	AZD6244	Phase II	advanced NSCLC (n = 84), vs. pemetrexed randomized open label	No
Bennouna *et al.* Invest New Drugs 2010	AZD6244	Phase II	vs. capecitabine in advanced colorectal cancer (n = 69), vs. capecitabine randomized open label	No

- No analysis of Ras mutation status
- Dose limiting toxicity of MEK inhibitors might prevent sufficient MEK inhibition in tumors

Combination therapy: MEK + PI3K

Engelman *et al.*, Nat Medicine 2008
Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PI3KCA H1047R murine lung cancers





Phase I trials of combination therapy

MEK Inhibitor	PI3K inhibitor
GSK1120212	BKM120
GSK1120212	GSK2126458
GDC-0973	GDC-0941

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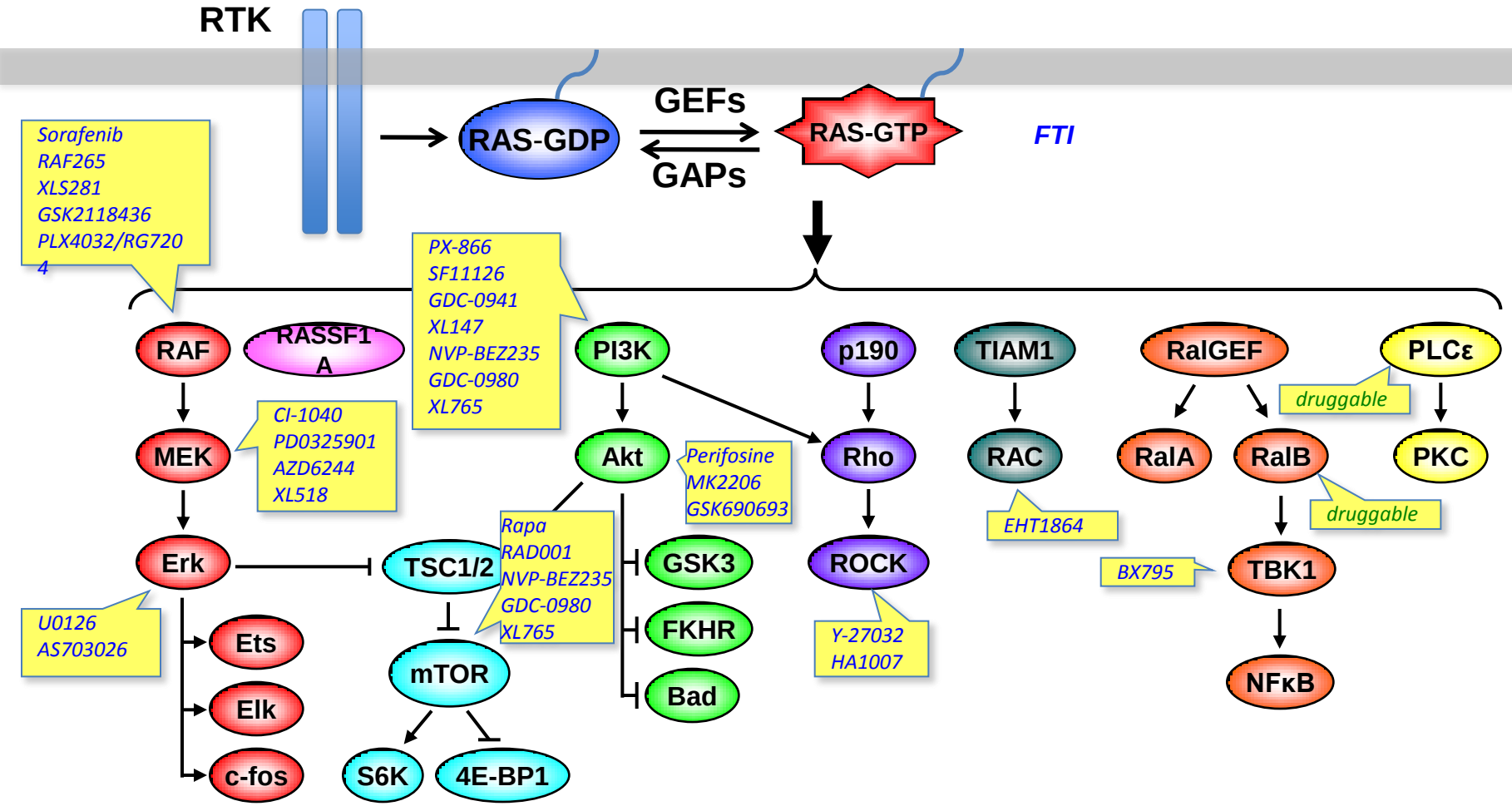
Challenges to drug combinations

- Define effective combination using *relevant* pre-clinical models
- Manage toxicity to gain therapeutic window



Synthetic lethality: the workaround?

Hartman *et al.*, Science 2001
Principles for the Buffering of Genetic Variation

Ras

Ras-selective drug target

Gene A	Gene B	Viability
wt	wt	alive
wt	null	alive
mutant	wt	alive
mutant	null	dead

RNAi screens for Ras synthetic lethal interactions

Reference	Synthetic Lethal Genes
Sarthy et al. 2007	Survivin, etc.
Scholl et al. 2009	STK33, etc.
Luo et al. 2009	PLK1, Ubc9 etc.
Barbie et al. 2009	TBK1 etc.
Puyol et al. 2010	CDK4

Challenges

- Context-dependent effect
- In vivo validation in relevant pre-clinical models
- In vivo therapeutic window

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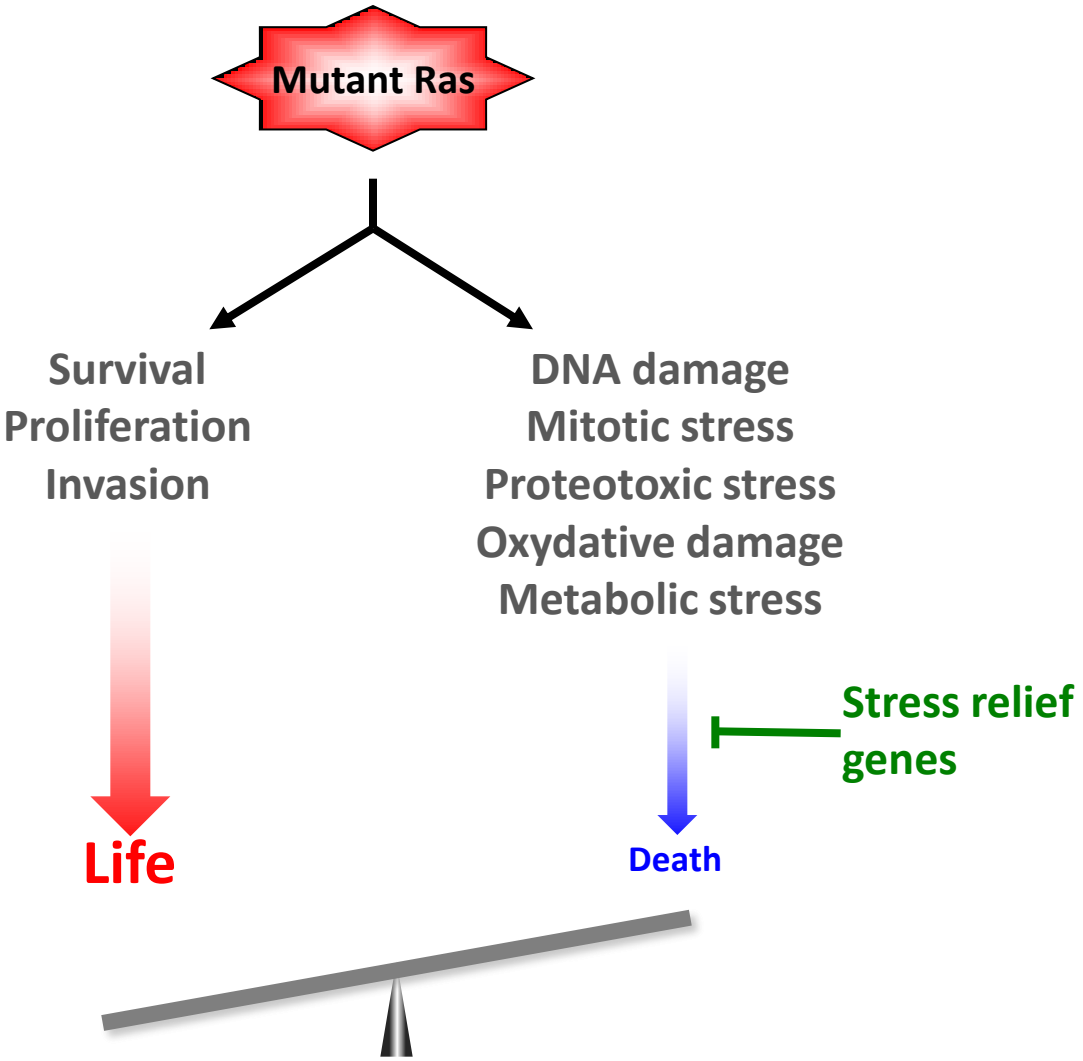
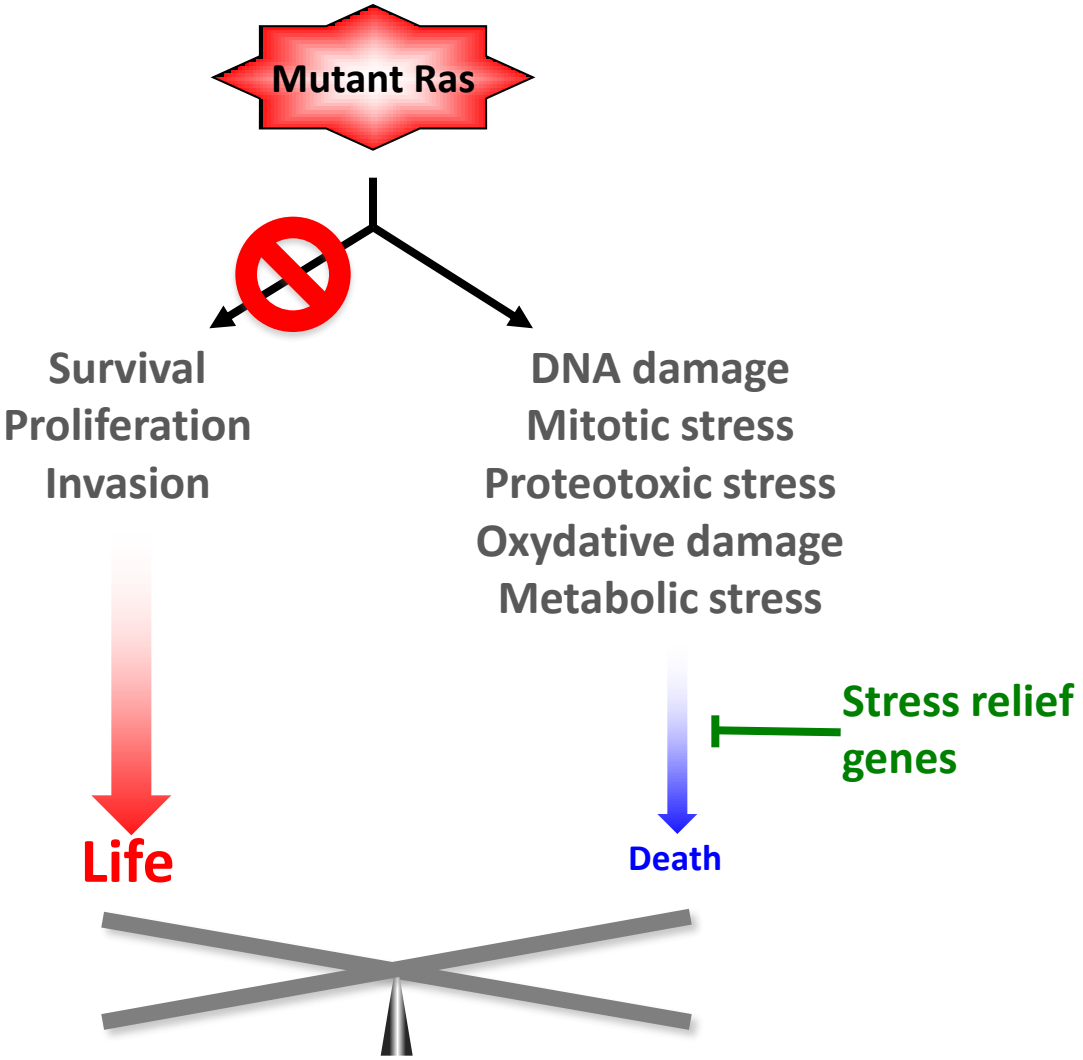
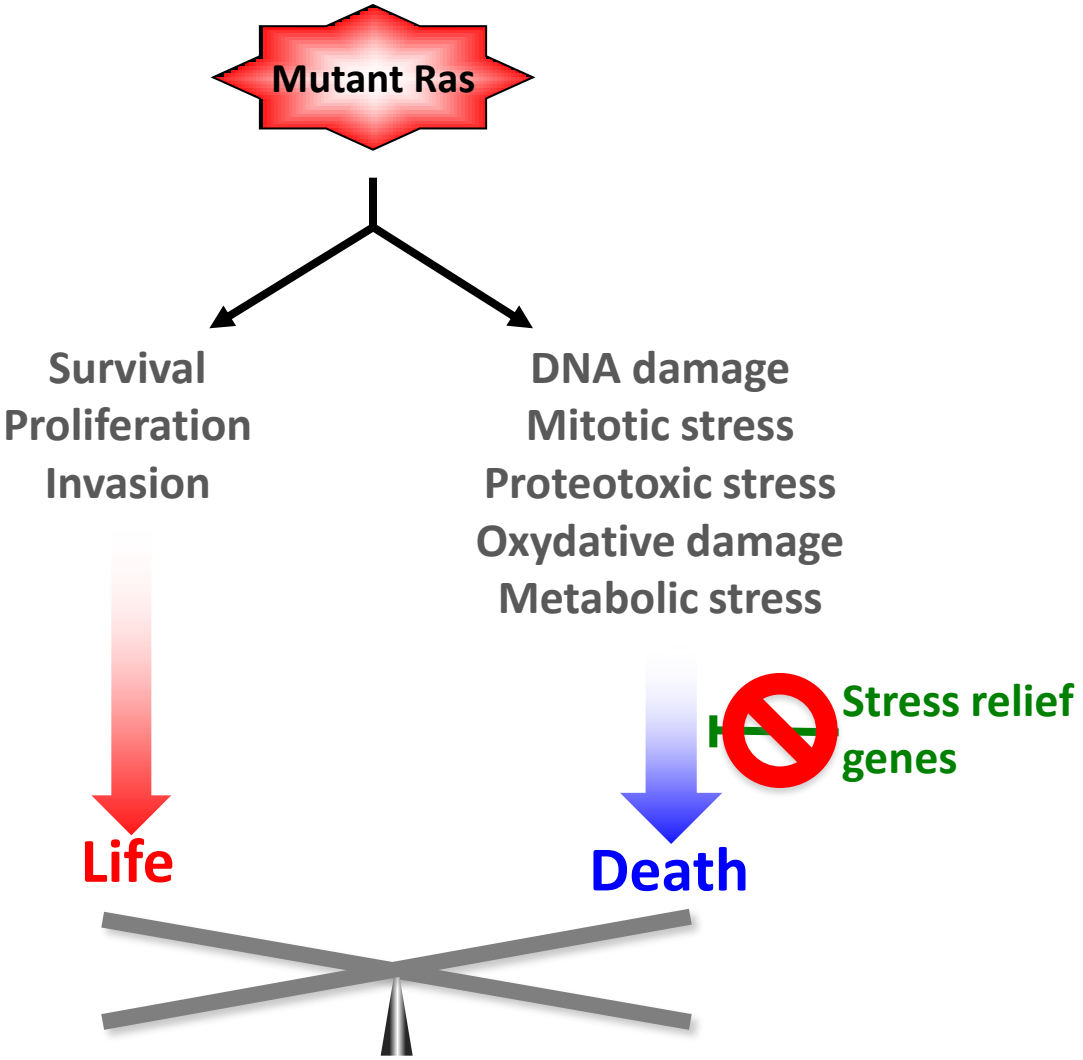
Targeting oncogene and non-oncongene addiction



Targeting oncogene and non-oncongene addiction



Targeting oncogene and non-oncongene addiction




Don't worry, I had the same thing, and they cured me!