RAS oncogene

The Ras Oncogene

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NIH NATIONAL CANCER INSTITUTE
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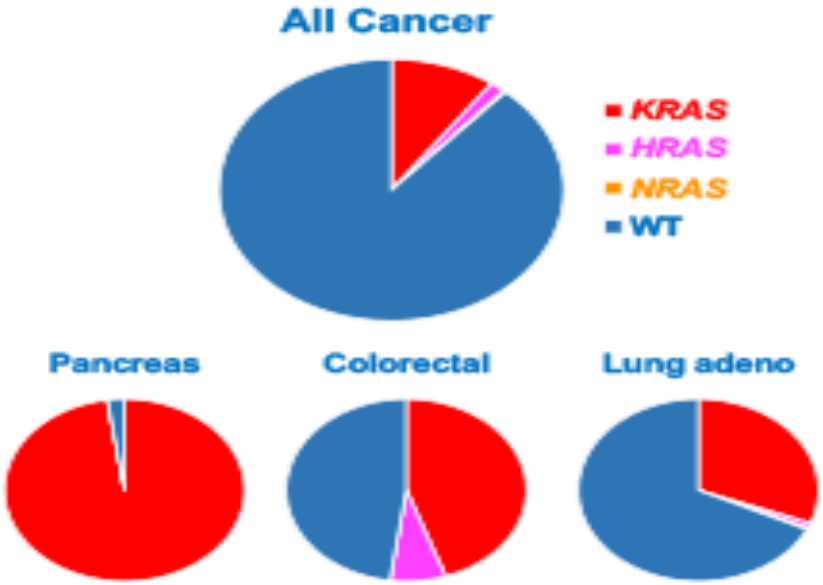
Research goals

Our research goals

- Understand mechanism of KRAS-driven oncogenesis
- Identify better therapeutic strategies for *KRAS* mutant tumors



Clinical Challenge

- ~ 200,000 new patients / year
- No effective targeted therapies

Outline

Lecture Outline

1. Discovery of the Ras oncogene
2. Ras signaling & oncogenesis
3. Targeting Ras and its signaling network
4. Synthetic lethal partners of the KRAS oncogene
5. Identify optimal target combinations for KRAS mutant cancer cells
6. Concluding thoughts

RAS oncogene

Discovery of the Ras Oncogene in Murine Tumor Viruses

1964	***Harvey (Nature, 1964)*** An Unidentified virus which causes the rapid production of tumors in mice
1967	***Kirsten & Mayer (J. NCI, 1967)*** Morphologic response to a murine erythroblastosis virus

Molecular characterization

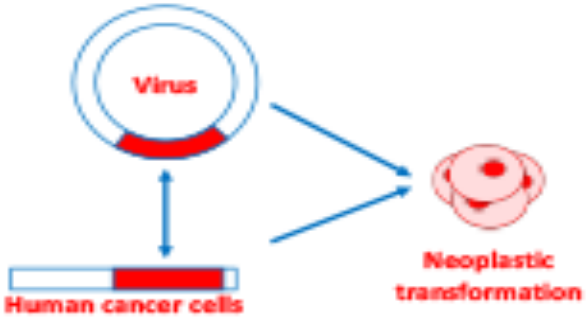
Molecular Characterization of the Viral Ras Protein

1979	***Shih ... Scolnick (Virology, 1979)*** Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus ***Scolnick... Shih (PNAS, 1979)*** Guanine nucleotide-binding activity as an assay for *src* protein of rat-derived murine sarcoma viruses
1980	***Willingham ... Scolnick (Cell, 1980)*** Localization of the src gene product of the Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry

Mammalian counterpart

Viral Ras Gene Has Mammalian Counterpart

1981	***Ellis ... Scolnick (Nature, 1981)*** The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes
1982	***Chang ... Lowy (Nature, 1982)*** Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus ***Santos ... Barbacid (Nature, 1982)*** T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes ***Parada ... Weinberg (Nature, 1982)*** Human EJ bladder carcinoma oncogene is homologous of Harvey sarcoma virus *ras* gene ***Goldfarb ... Wigler (Nature, 1982)*** Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. ***Der ... Cooper (PNAS, 1982)*** Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses



Point mutations

Human Ras Oncogene Has a Point Mutation

1982	***Tabin ... Weinberg (Nature, 1982)*** Mechanism of activation of a human oncogene ***Reddy ... Barbacid (Nature, 1982)*** A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene ***Taparowsky ... Wigler (Nature, 1982)*** Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. ***Capon ... Goeddel (Nature, 1982)*** Activation of Ki-ras2 gene in human colon and lung carcinomas by two different point mutations

Transforming oncogene

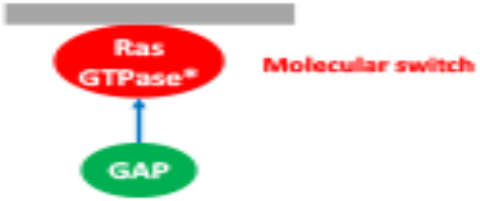
Human Ras Oncogene Encodes a Transforming Oncoprotein

1984 **1985**	***Stacey ... Kung (Nature, 1984)*** Transformtion of NIH 3T3 cells by microinjection of ***Ha-ras*** p21 protein ***Feramisco ... Sweet (Cell, 1984)*** Microinjection of the oncogene form of the human H-ras (T-24) protein results in rapid proliferation of quiescent cells ***Mulcahy ... Stacey (Nature, 1985)*** Requirement for ***ras*** proto-oncogene function during serum-stimulated growth of NIH 3T3 cells

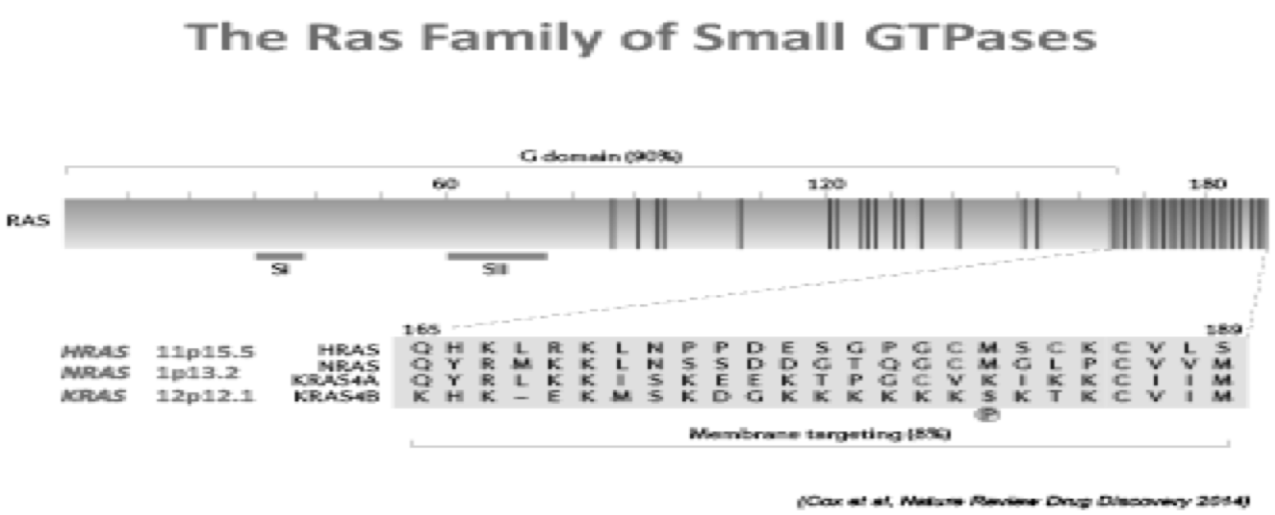
Biochemical properties

Biochemical Properties of the Ras Protein

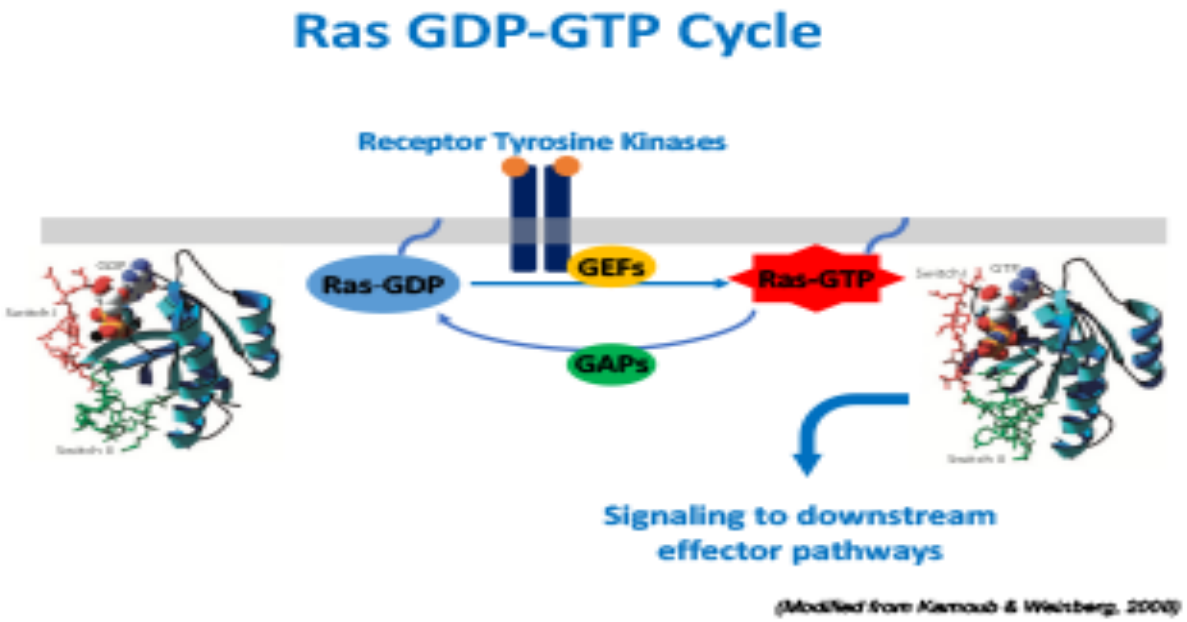
1984	***Willumsen & Lowy (Nature, 1984)*** The p21 ras C-terminus is required for transformation and membrane association
1984 **1985**	***Sweet ... Lowy (Nature, 1984)*** The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity ***Gibbs ... Scolnick (PNAS,1984)*** Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules ***Hanne ... Kung (PNAS, 1985)*** ***Ha-ras*** proteins exhibit GTPase activity: point mutations that activate ***Ha-ras*** gene products result in decreased GTPase activity
1987	***Trahey ... McCormick (Science, 1987)*** A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants



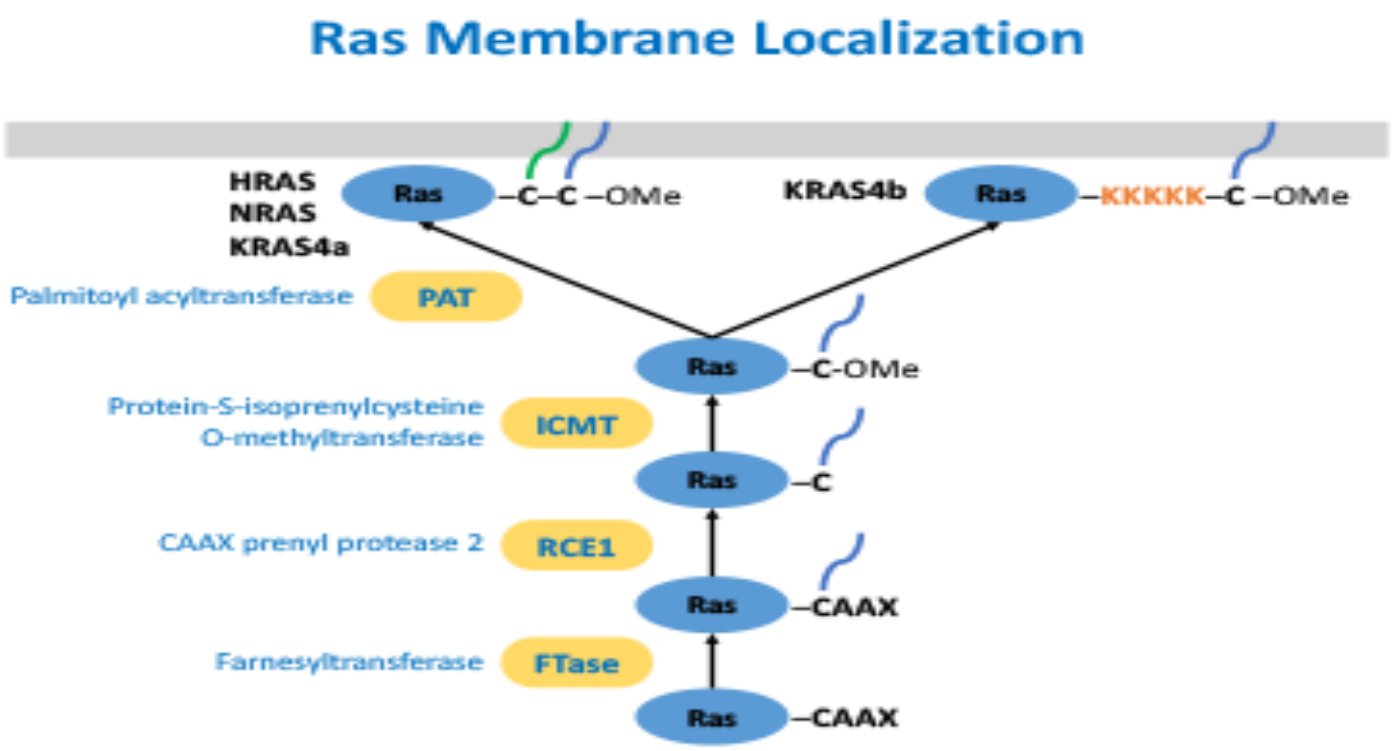
Ras family


The Ras Family of Small GTPases
G domain (90%)
60
120
180
RAS
SI
SII
165
189
HRAS 11p15.5
NRAS 1p13.2
KRAS 12p12.1
HRAS Q H K L R K L N P P D E S G P G C M S C K C V L S
NRAS Q Y R M K K L N S S D D G T Q G C M G L P C V V M
KRAS4A Q Y R L K K I S K E E K T P G C V K I K K C I I M
KRAS4B K H K – E K M S K D G K K K K K K S K T K C V I M
P
Membrane targeting (8%)
(Cox et al, Nature Review Drug Discovery 2014)

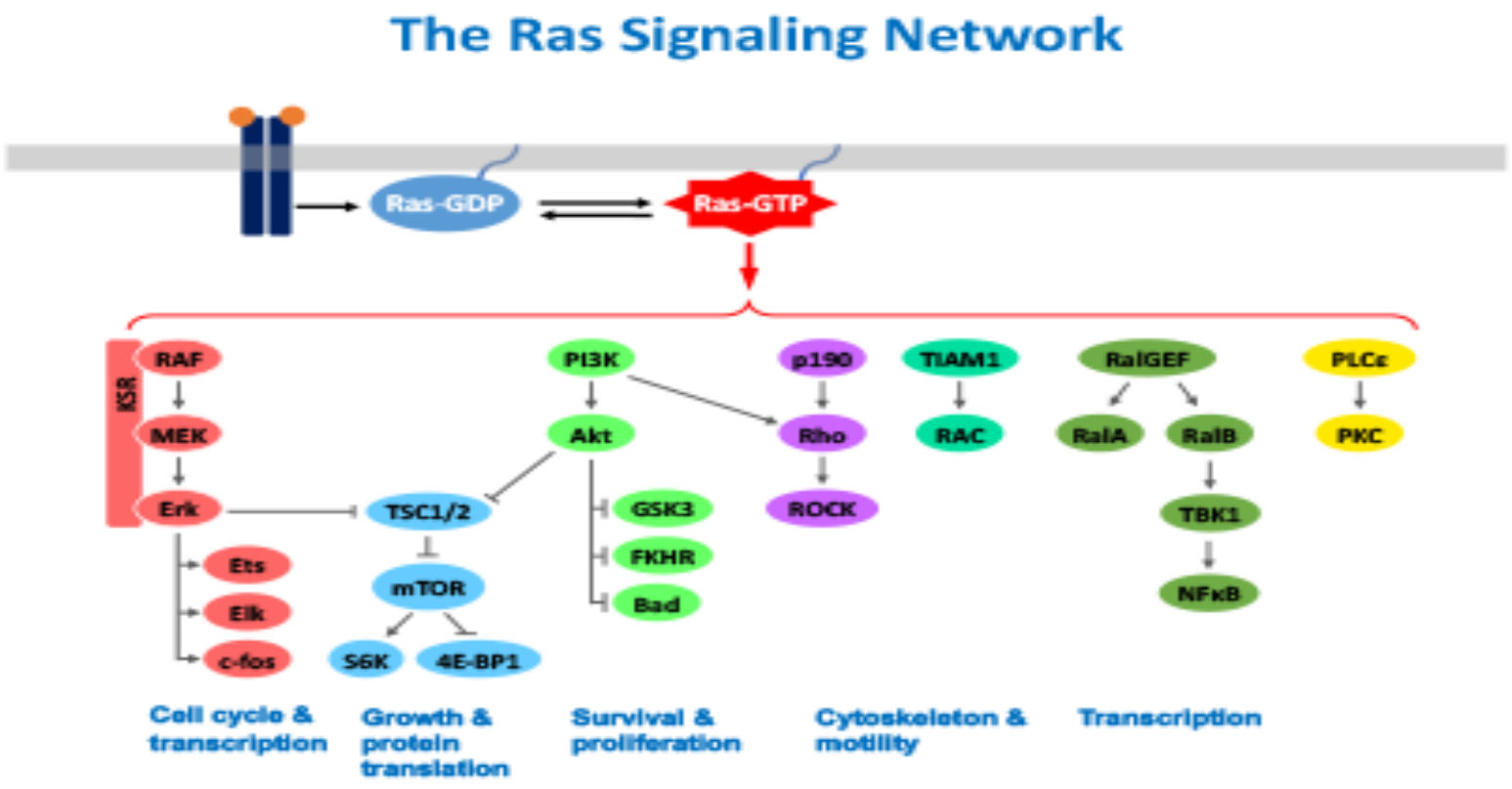
Ras GDP-GTP Cycle



Ras membrane localization



Ras signaling network



Ras mutations

Incidence of Ras mutations in human cancer

Ras mutations
occur in
10% of human cancer

PANCREAS — KRAS	95%
COLORECTAL — KRAS	45%
LUNG — KRAS	35%
AML — NRAS	15%
MELANOMA — NRAS	15%
BLADDER CANCER — HRAS	10%

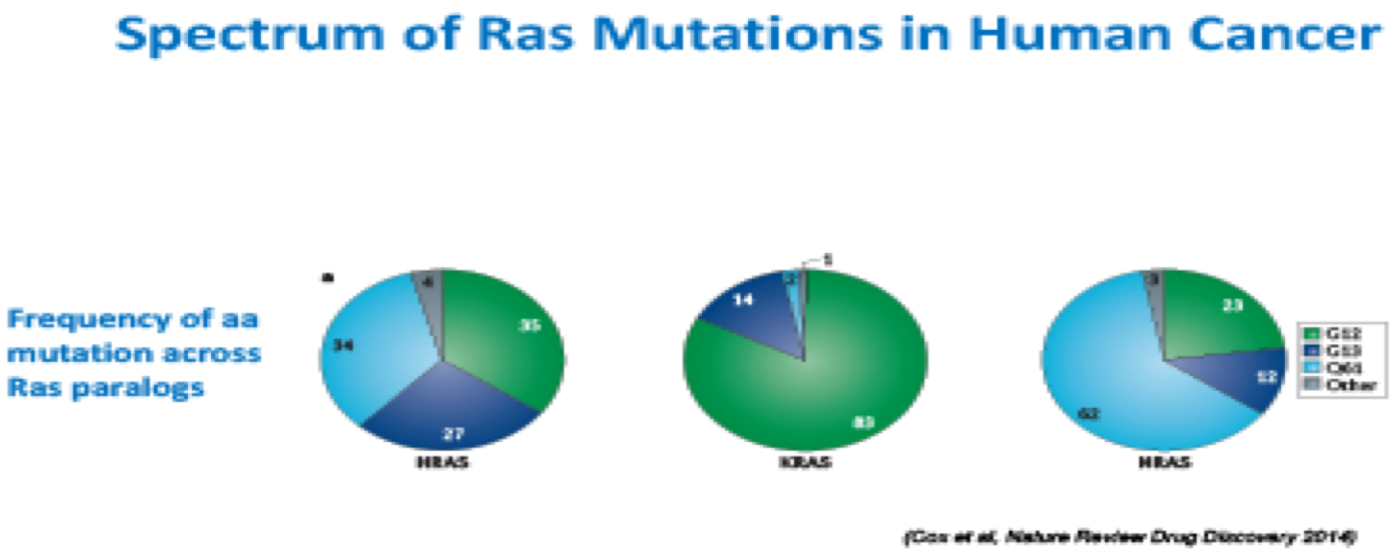
(NCI Ras Initiative)

Table 1 | Frequency of RAS mutations in human cancers*

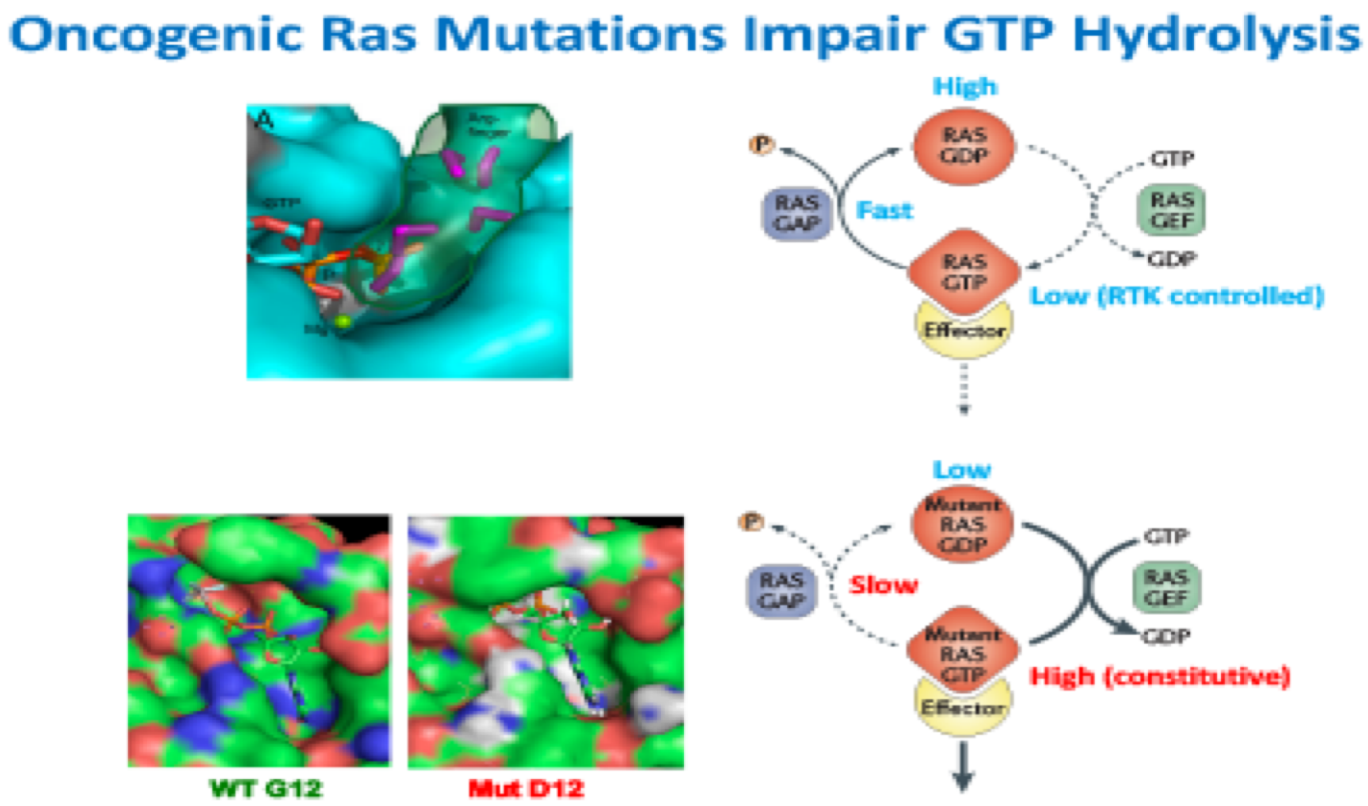
Cancer	% KRAS	% NRAS	% HRAS	% All RAS
Pancreatic ductal adenocarcinoma	93.7	0.0	0.0	93.7
Colorectal adenocarcinoma	44.7	7.5	0.0	52.2
Multiple myeloma	21.8	18.9	0.0	40.6
Lung adenocarcinoma	30.9	0.9	0.3	32.2
Skin cutaneous melanoma	0.8	27.0	1.0	28.1
Uterine corpus endometrioid carcinoma	21.4	3.0	0.4	24.8
Uterine carcinosarcoma	12.3	1.8	0.0	14.3
Thyroid carcinoma	1.0	8.5	3.9	13.5
Acute myeloid leukaemia	3.1	6.7	1.8	11.6
Bladder urothelial carcinoma	3.1	1.4	5.9	10.6
Gastric adenocarcinoma	11.4	0.9	0.0	12.0
Cervical adenocarcinoma	8.3	0.0	0.0	8.3
Head and neck squamous cell carcinoma	0.9	0.3	4.7	5.9
Diffuse large B cell lymphoma	5.2	0.0	0.0	5.2
Oesophageal adenocarcinoma	3.8	0.0	0.6	4.4
Chronic lymphocytic leukaemia	1.9	2.5	0.0	4.4
Lung squamous cell carcinoma	3.3	0.0	1.1	4.4
Small cell lung carcinoma	1.4	0.0	1.4	2.8
Renal papillary cell carcinoma	1.3	0.0	0.8	2.1
Adrenocortical carcinoma	0.0	0.0	1.7	1.7
Chromophobe renal cell carcinoma	0.0	1.5	0.0	1.5
Hepatocellular carcinoma	0.8	0.4	0.0	1.1
Breast invasive carcinoma	0.7	0.4	0.3	1.4
Cervical squamous cell carcinoma	1.3	0.0	0.0	1.3
Ovarian serous adenocarcinoma	0.6	0.8	0.0	1.3
Adrenocortical carcinoma	1.1	0.0	0.0	1.1

(Cox et al, Nature Review Drug Discovery 2014)

Mutation spectrum



GTP hydrolysis

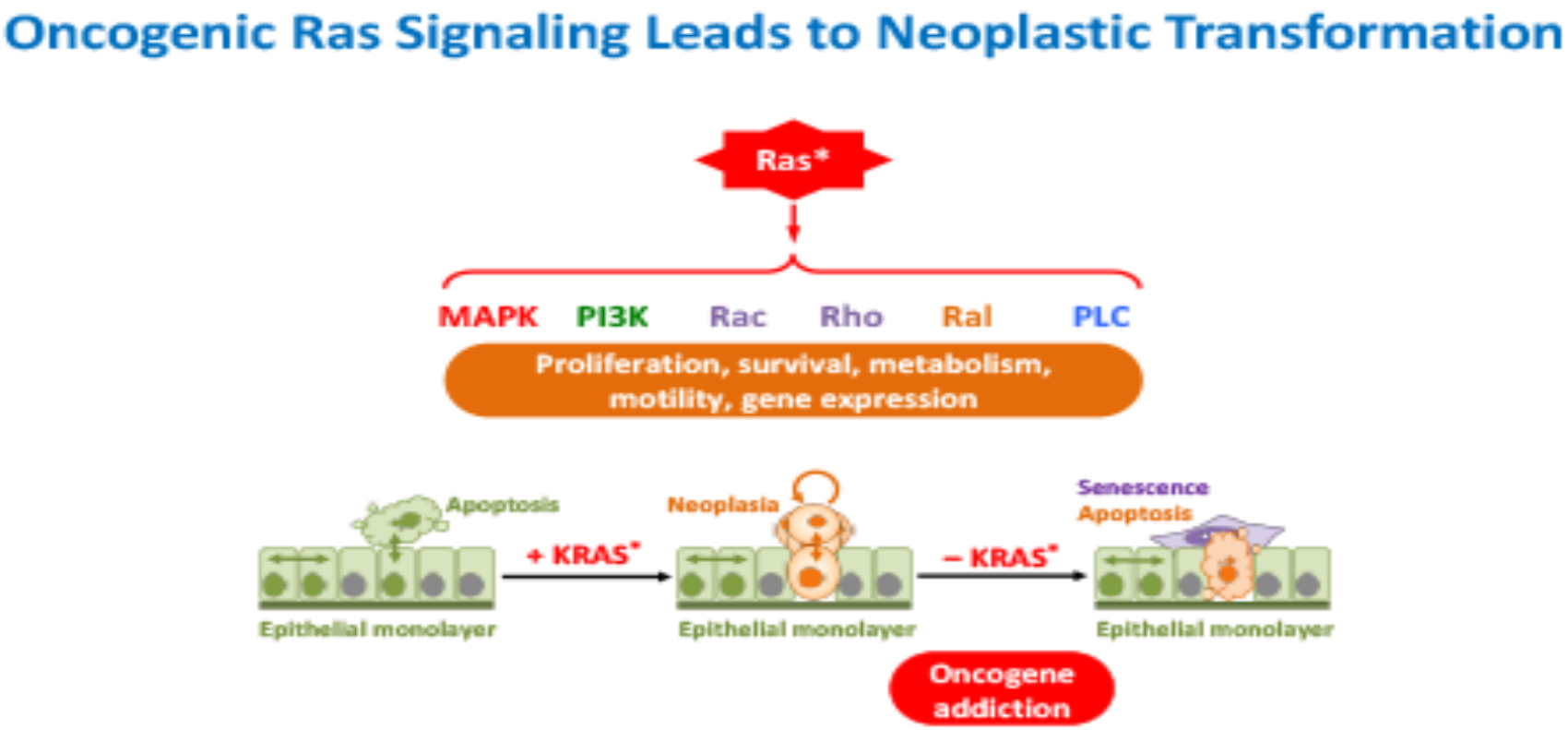


Oncogenic driver

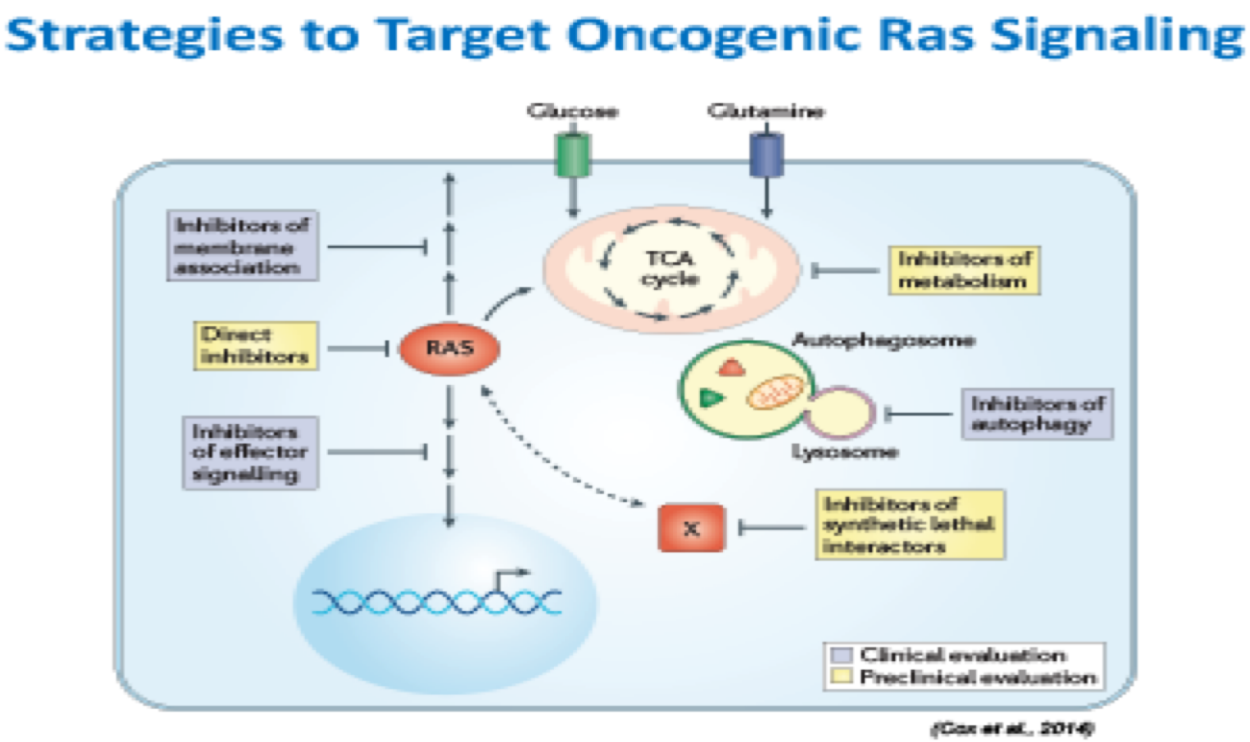
KRAS is a Major Oncogenic Driver in Adenocarcinomas

	Early Neoplasia	Adenoma	Adeno-carcinoma
Lung	KRAS	KRAS TP53	KRAS TP53 LKB1 ...
Pancreas	KRAS	KRAS CDKN2A	KRAS CDKN2A TP53 ...
Colon	APC	APC KRAS	APC KRAS TP53 ...

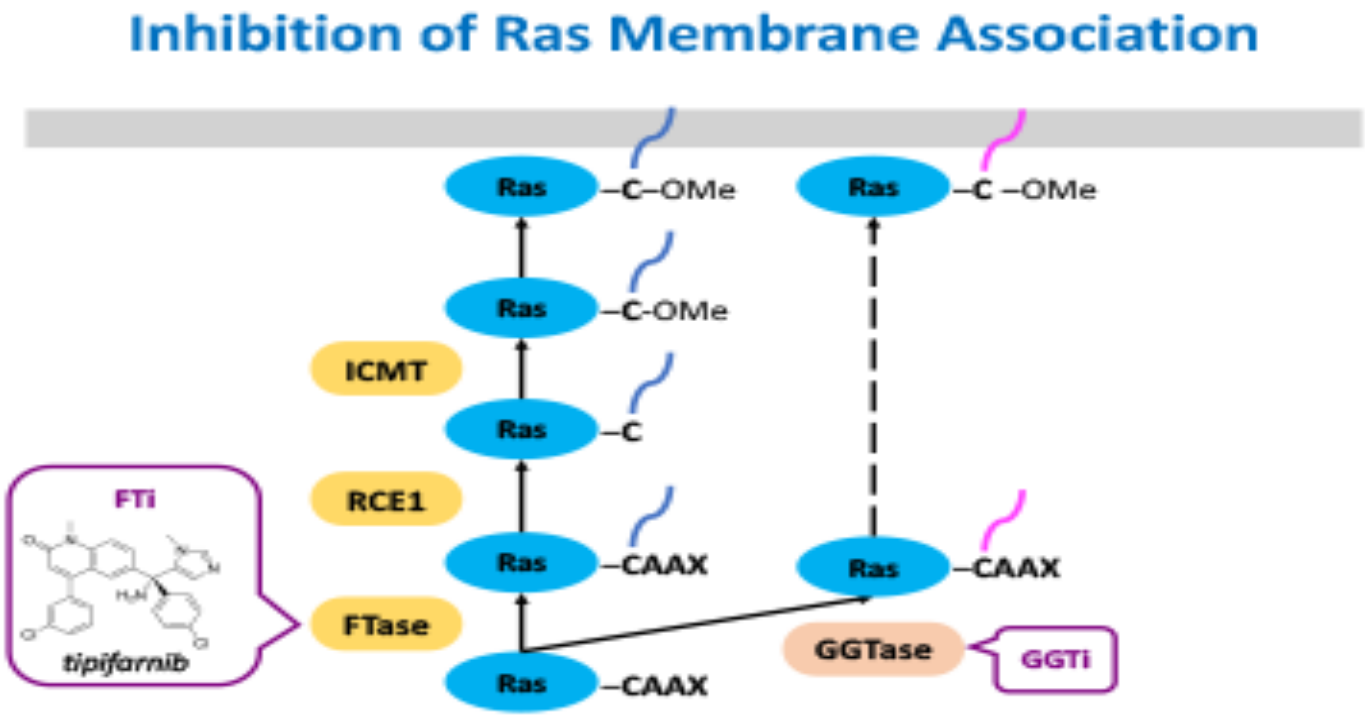
Neoplastic transformation



Ras signaling

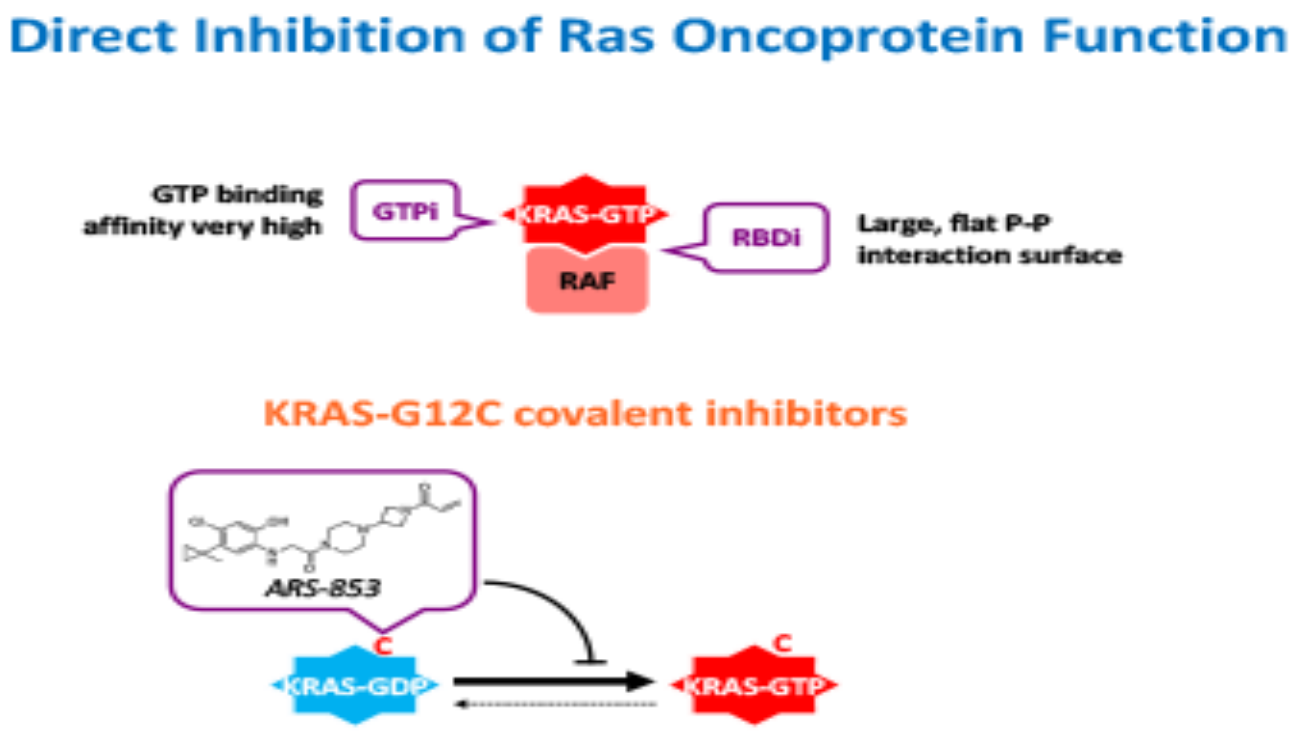


Ras membrane association



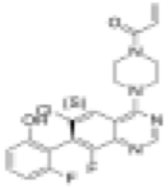
- Alternative membrane localization pathway
- Lack of KRAS selectivity – pathway shared by other small GTPases

Ras inhibition



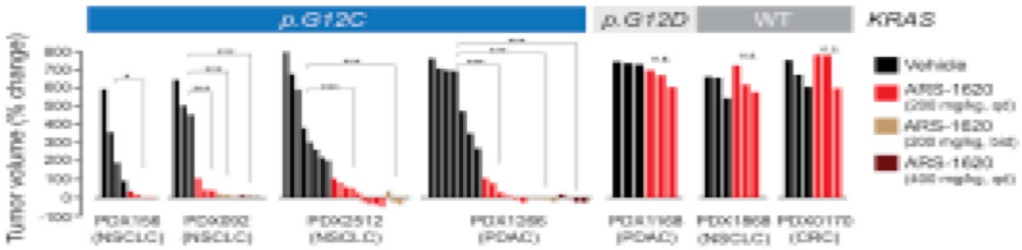
Direct inhibition

Direct Inhibition of Ras Oncoprotein Function

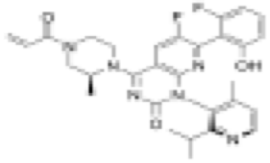


ARS-1620

Mouse PDX lung tumors



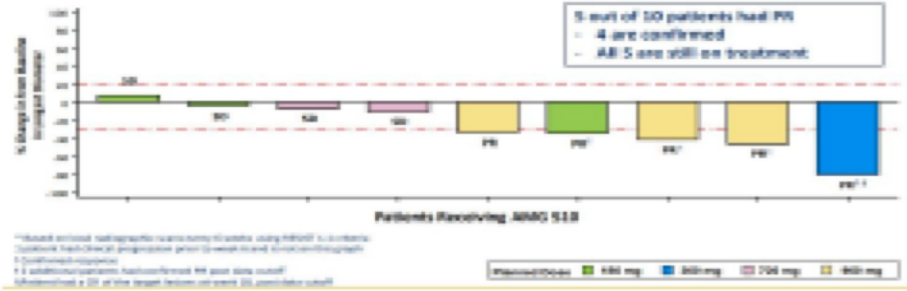
(Jones et al. Cell 2018)



AMG 510

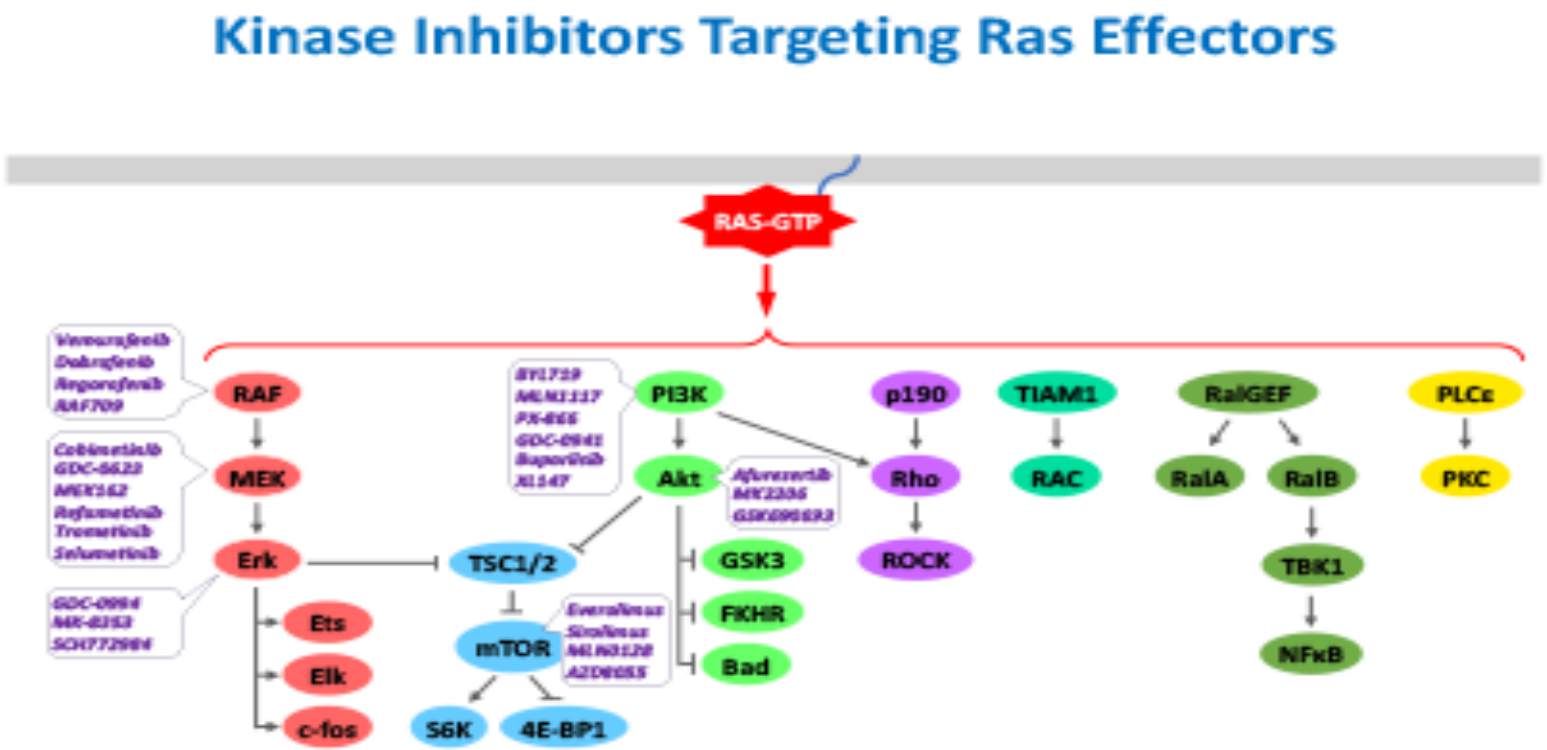
Human G12C lung tumors Phase I trial

NSCLC: Best Tumor Response* (n=10)

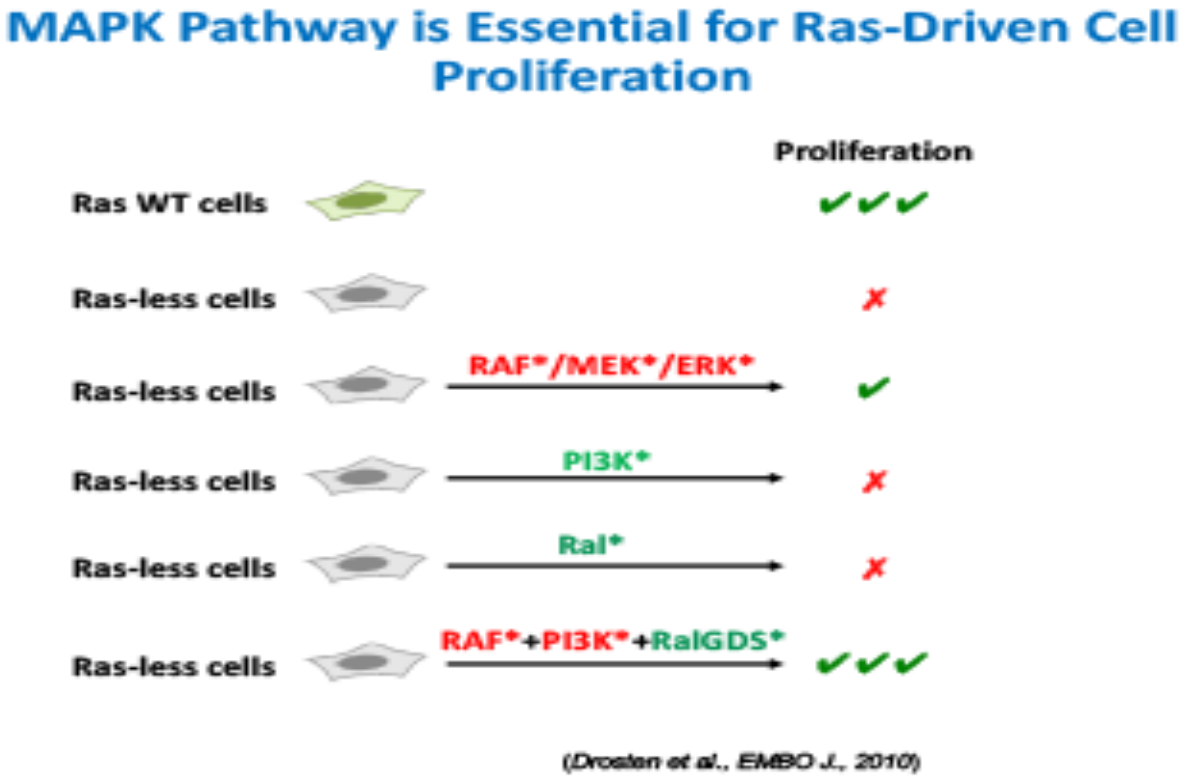


(Amgen, ASCO 2019)

Kinase inhibitors

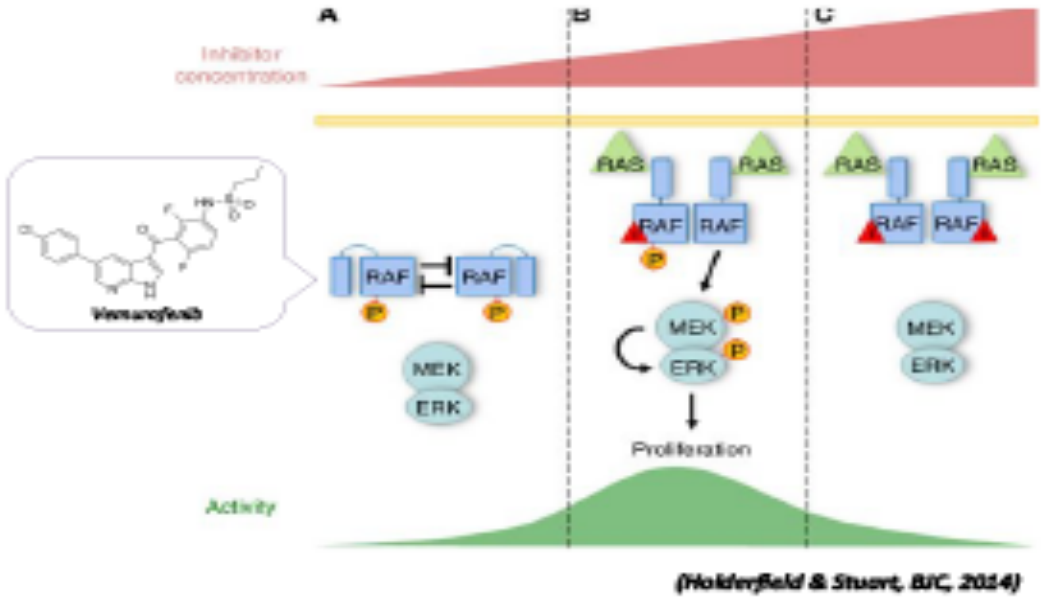


MAPK pathway



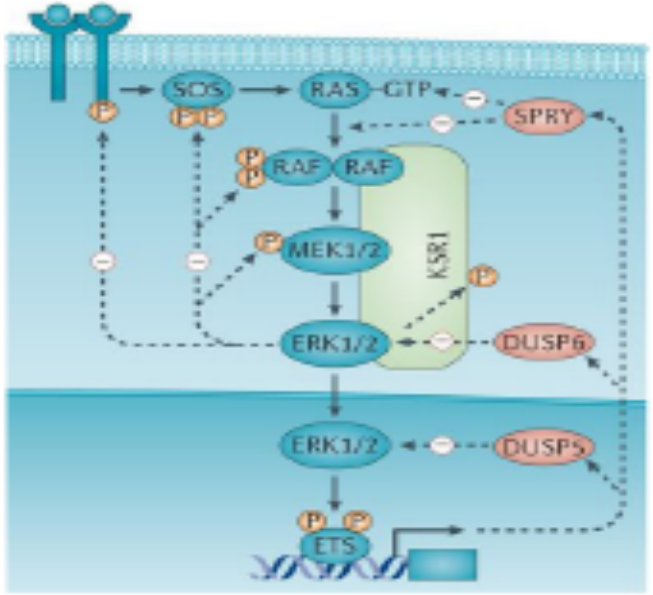
MAPK pathway

RAF Inhibitors Activates MAPK Pathway in Ras-Mutant Cells

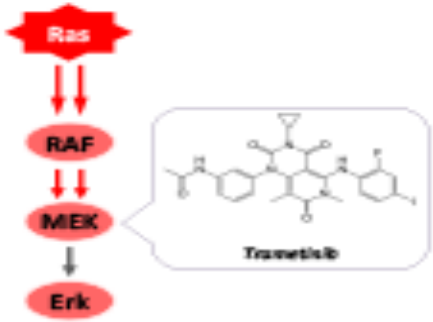


Feedback activation

MEK Inhibitors Leads to Feedback Activation of the MAPK Pathway



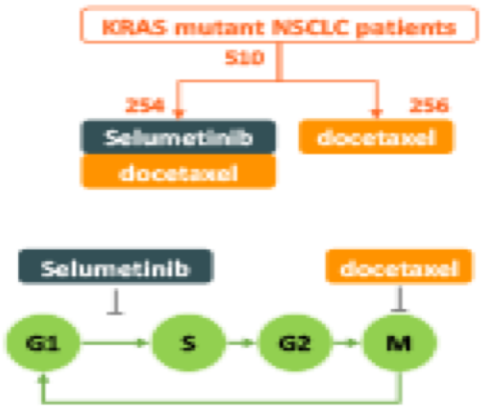
(Count et al., Nat Rev. Cancer, 2015)

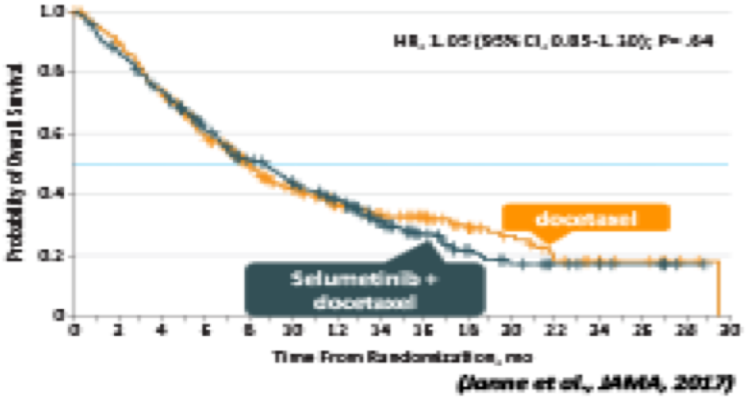


MEKi combinations

Current MEKi Combinations Are Ineffective in KRAS Mutant Cancer

SELECT-1 Trial: advanced NSCLC Tumors with KRAS mutation





(Janne et al., JAMA, 2017)

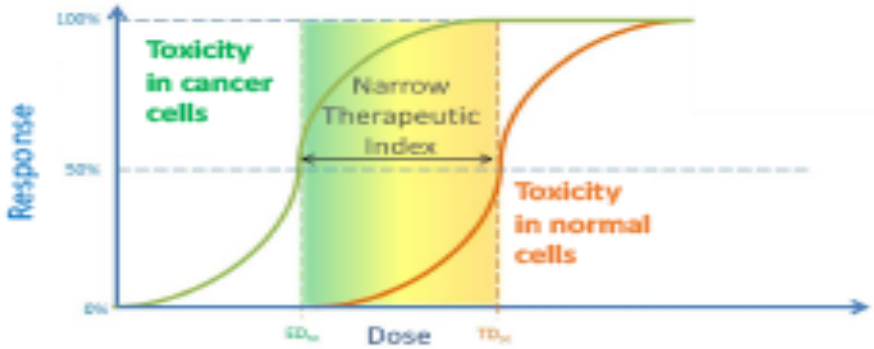
Combination of targeted therapy

- MEKi + PI3Ki
- MEKi + EGFRi
- MEKi + mTOR
- MEKi + CDK4/6

Toxicity?

Therapeutic window

Therapeutic Window is Key to Effective Combination Therapy in Cancer



Good combination therapies

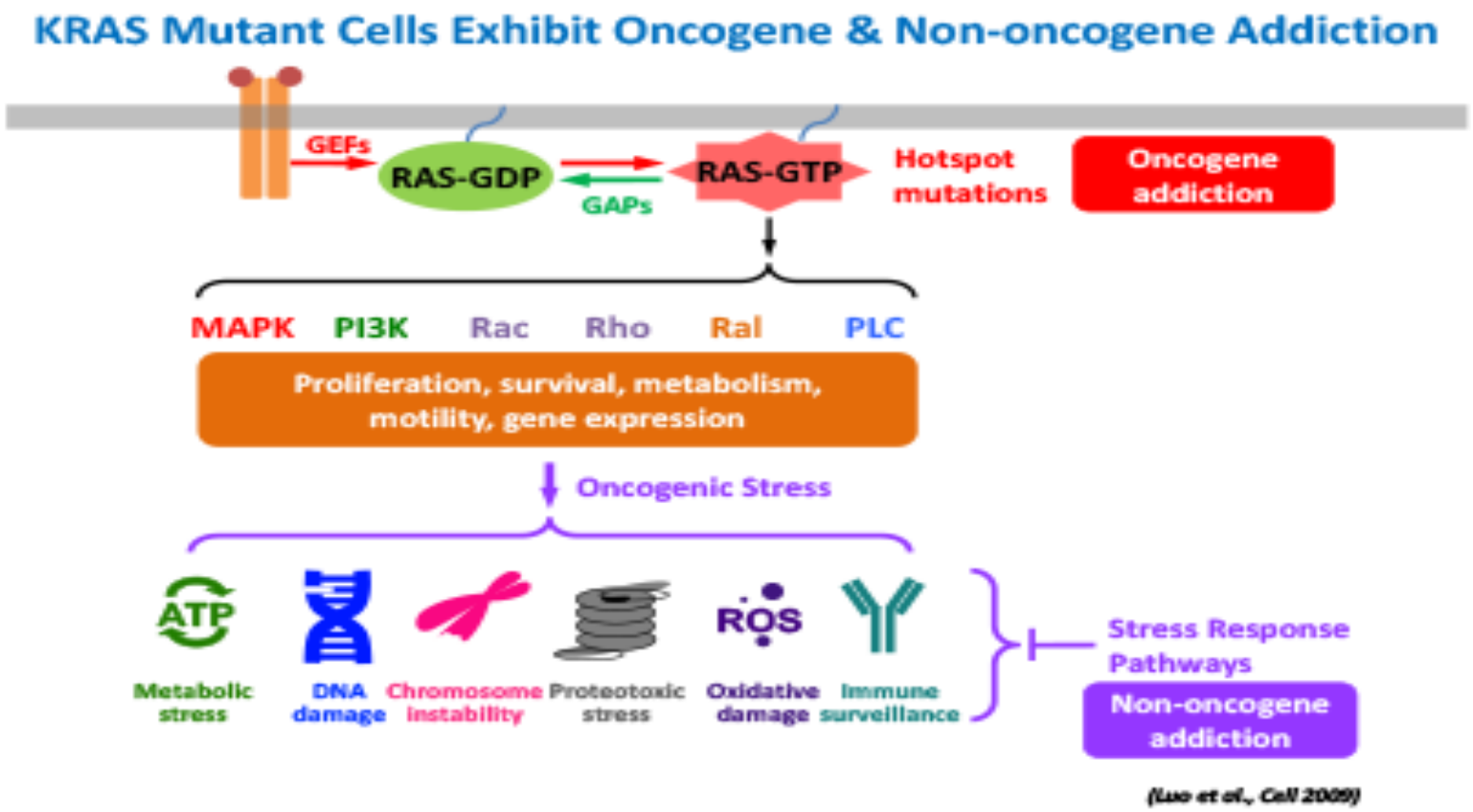
- Confer genotype-specific synergy
- Widens the therapeutic window
- Delays on-set of drug resistance

Synthetic lethality

Synthetic Lethality Reflects Genetic Buffering and Pathway Redundancy

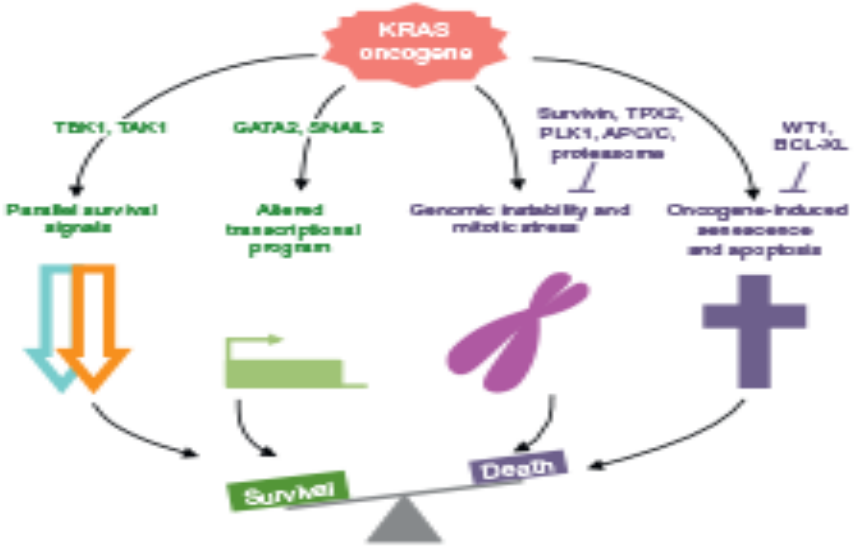
Gene A	Gene B	Viability
WT	WT	✔
WT	Loss	✔
Mutant	WT	✔
Mutant	Loss	✗

Oncogene addiction



Synthetic lethal interactions

Synthetic Lethal Interactions in KRAS Mutant Cells



- Tissue- and genetic context-driven synthetic lethal interactions
- Cooperate with KRAS oncogenic signaling pathways

(Ni & Luo, The Enzymes 2014)

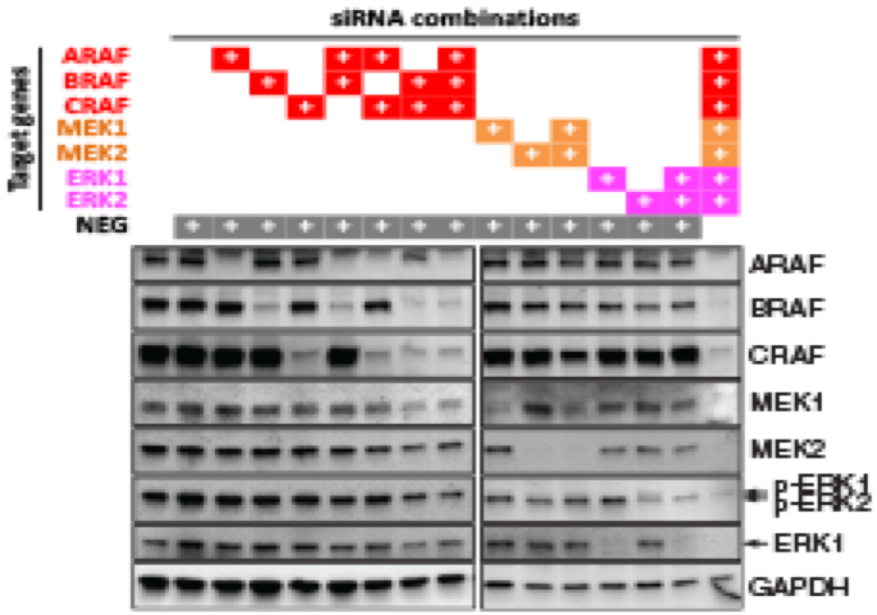
Dissecting the contribution

Dissecting The Contribution of Oncogene and Non-oncogene Addiction in KRAS Mutant Cancer

- What are the critical onco-effectors for mutant KRAS?
  - Distinguishing oncogenic and physiological Ras signaling
  - Critical for mutant KRAS signaling, dispensable in normal cells
- How is KRAS addiction communicated through its effector network?
  - Partitioning of KRAS dependency among pathways
  - Interaction and cooperation among pathways
- What are the critical stress-response pathways in KRAS mutant cells
  - Activated by oncogenic stress, dispensable in normal cells
- What are the rational target combinations downstream of mutant KRAS
  - Genotype selectivity
  - Orthogonal mechanisms of action

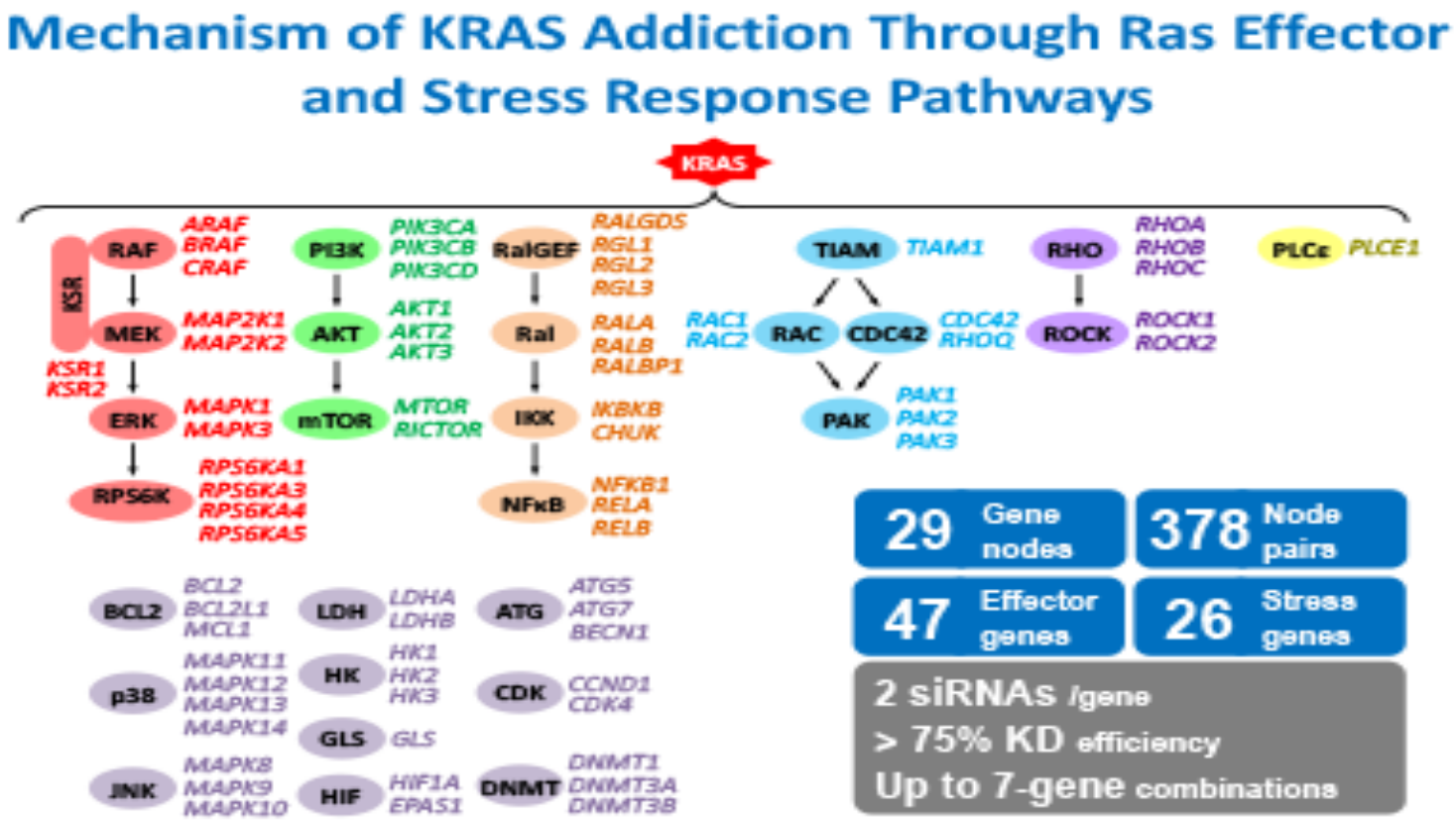
siRNA platform

A Combinatorial siRNA Platform to Co-targeting Multiple Genes And Evaluate Target Combinations

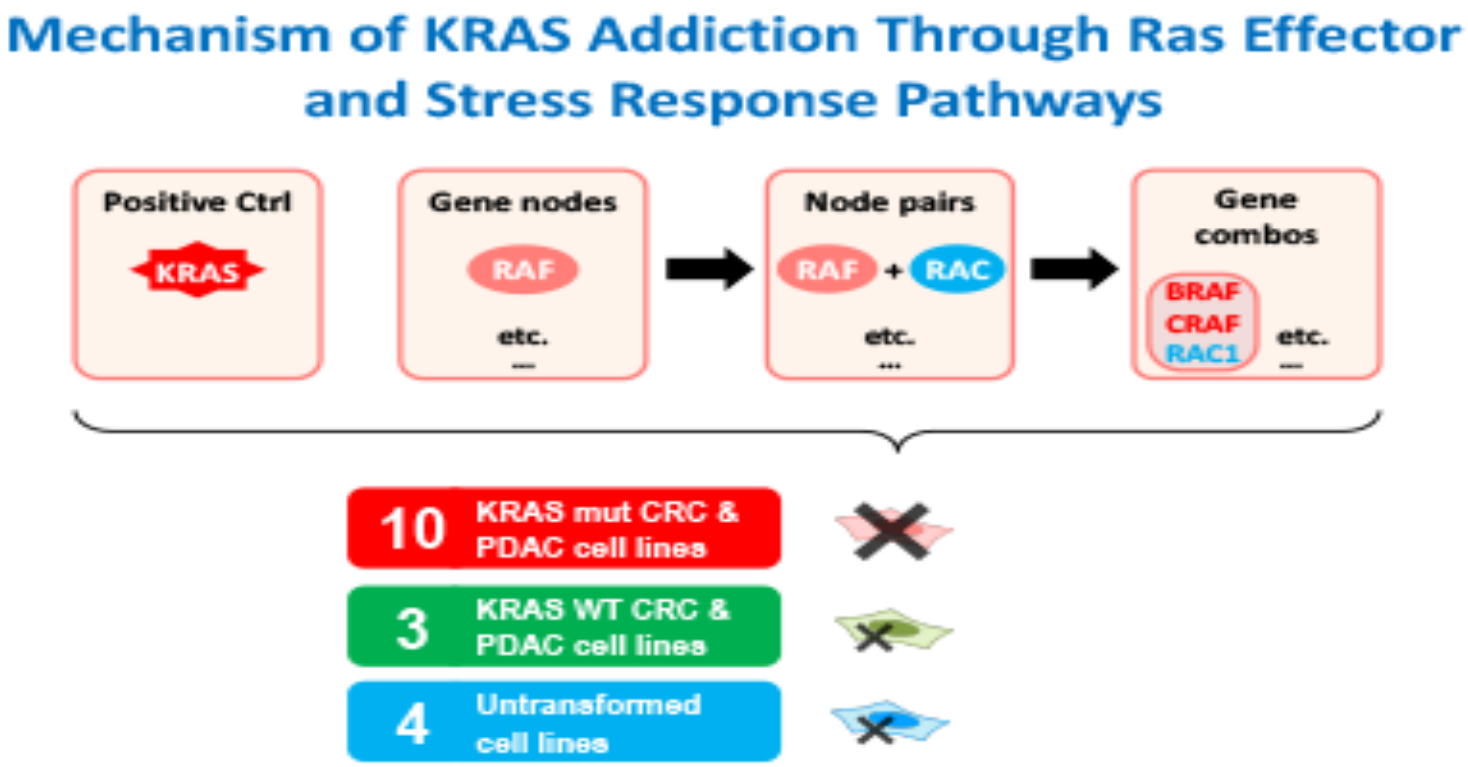


(Yuan et al. Cancer Discovery 2014)

KRAS addiction



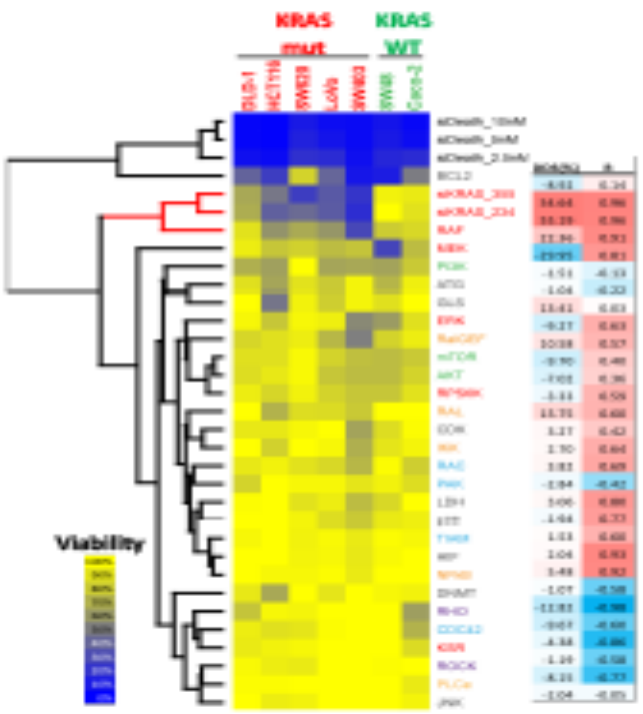
Ras effector and stress response



(Lee et al. PNAS 2019)

Single node dependency

Single Node Dependency Profile in KRAS Mutant Cells

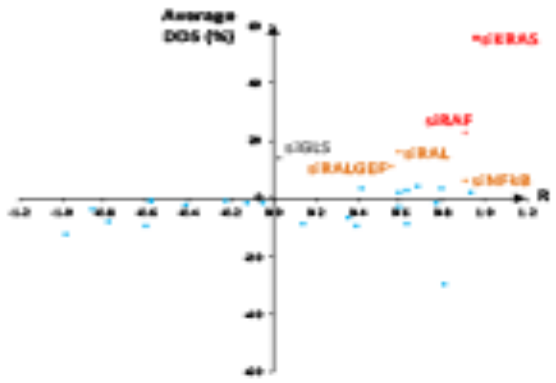


Differential Dependency Score (%)

$DDS = \bar{V}_{WT} - V_{mut}$

Pearson correlation

$R = corr(siKRAS_{mut}, siComb_{mut})$



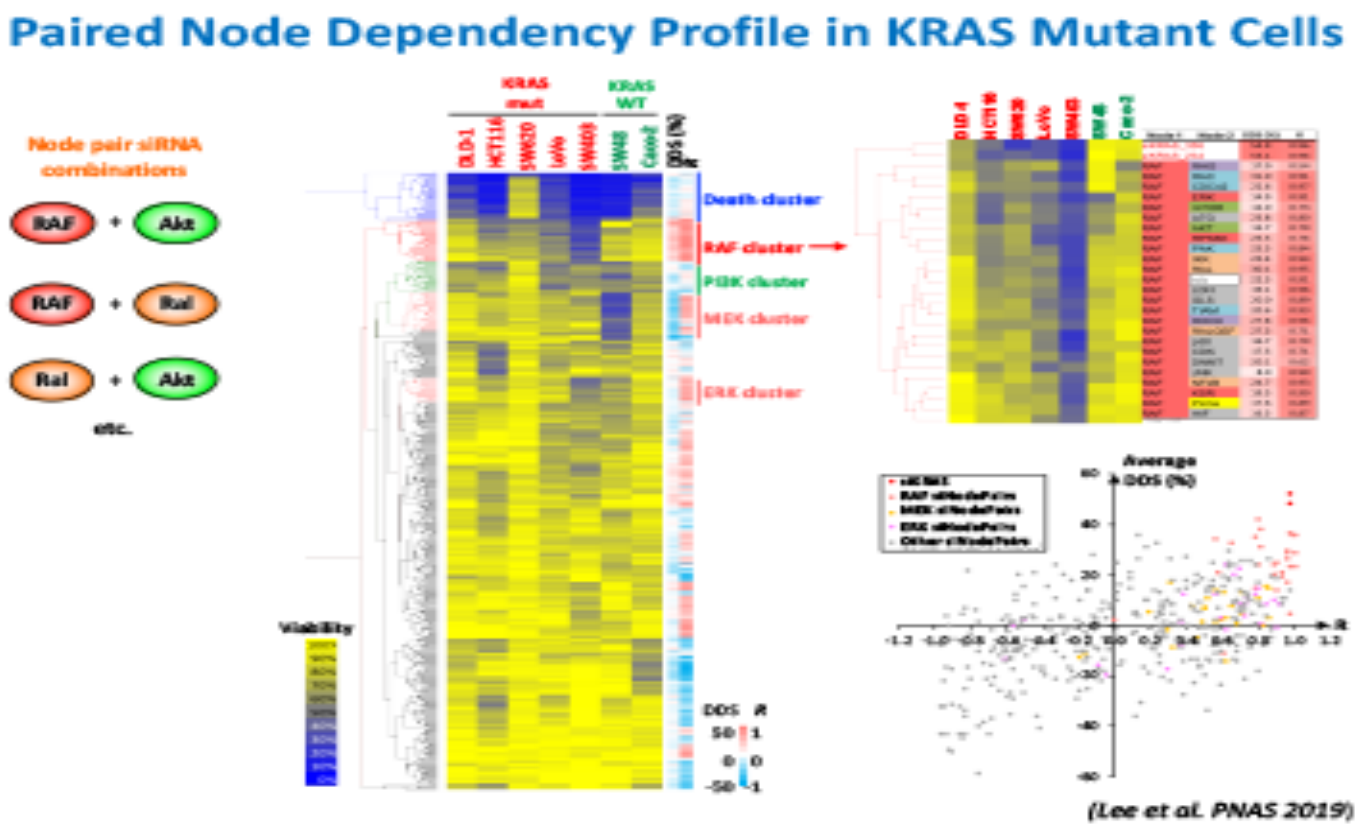
(Lee et al. PNAS 2019)

Private node dependency

Private Node Dependency Reveals Heterogeneity in Pathway Utilization for Supporting KRAS Addiction

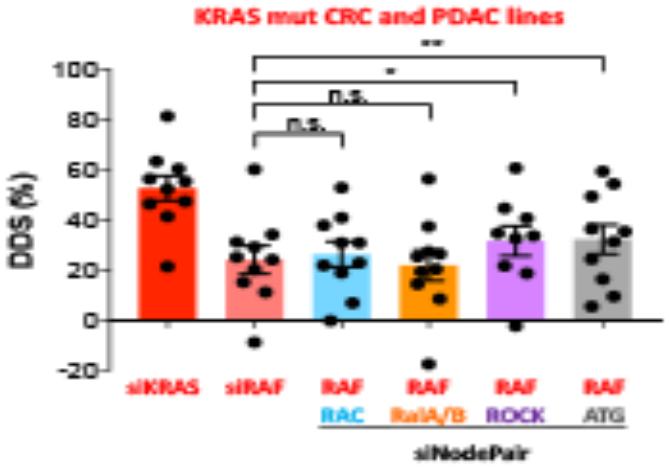
(Lee et al. PNAS 2019)

Paired node dependency



Paired node pair dependencies

Public Node Pair Dependencies in KRAS Mutant Cell Lines



(Lee et al. PNAS 2019)

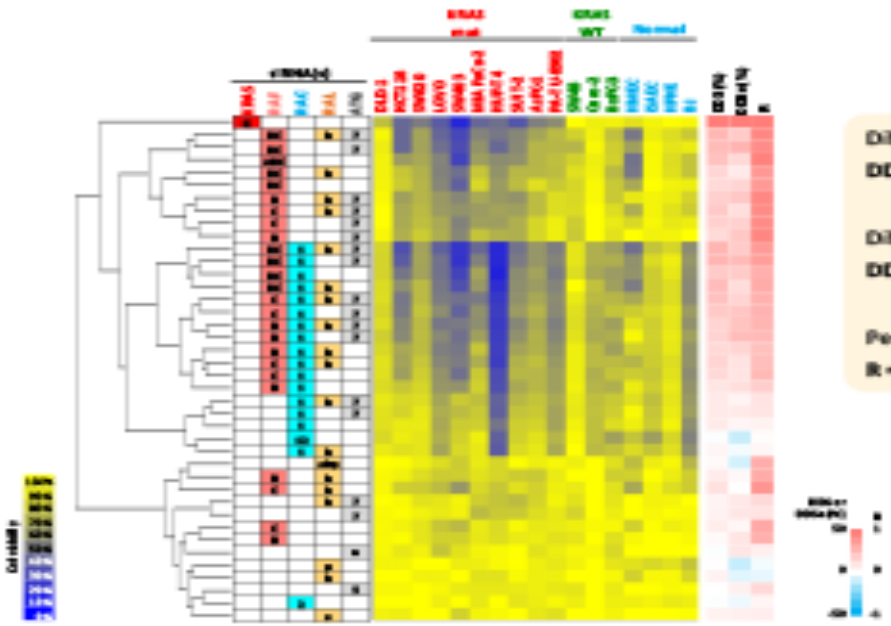
Private node pair dependency

Private Node Pair Dependency Is Dictated by Private Single Node Dependency

(Lee et al. PNAS 2019)

Gene paralog combinations

Deconvolution of Gene Paralog Combinations



Differential Dependency Score (%)

DDS = $\bar{V}_{wt} - V_{mut}$

Differential Dependency Score **Normal Cells** (%)

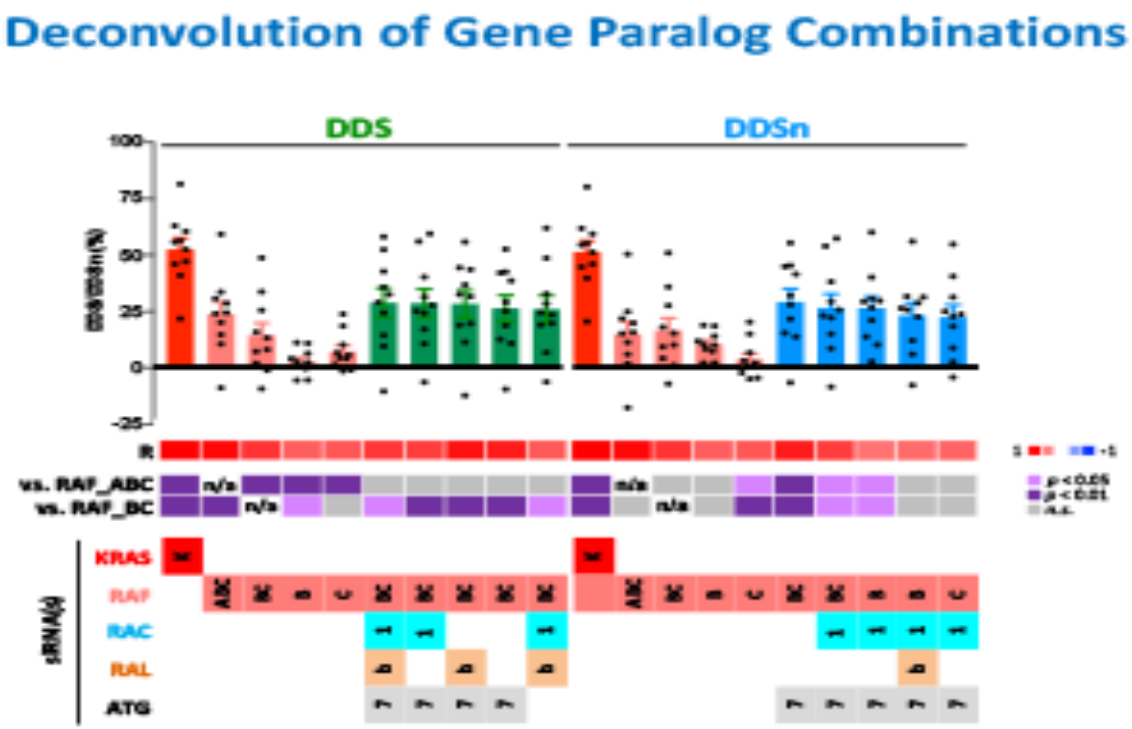
DDSn = $\bar{V}_{normal} - V_{mut}$

Pearson correlation

R = corr (siKRAS$_{mut}$, siComb$_{mut}$)

(Lee et al. PNAS 2019)

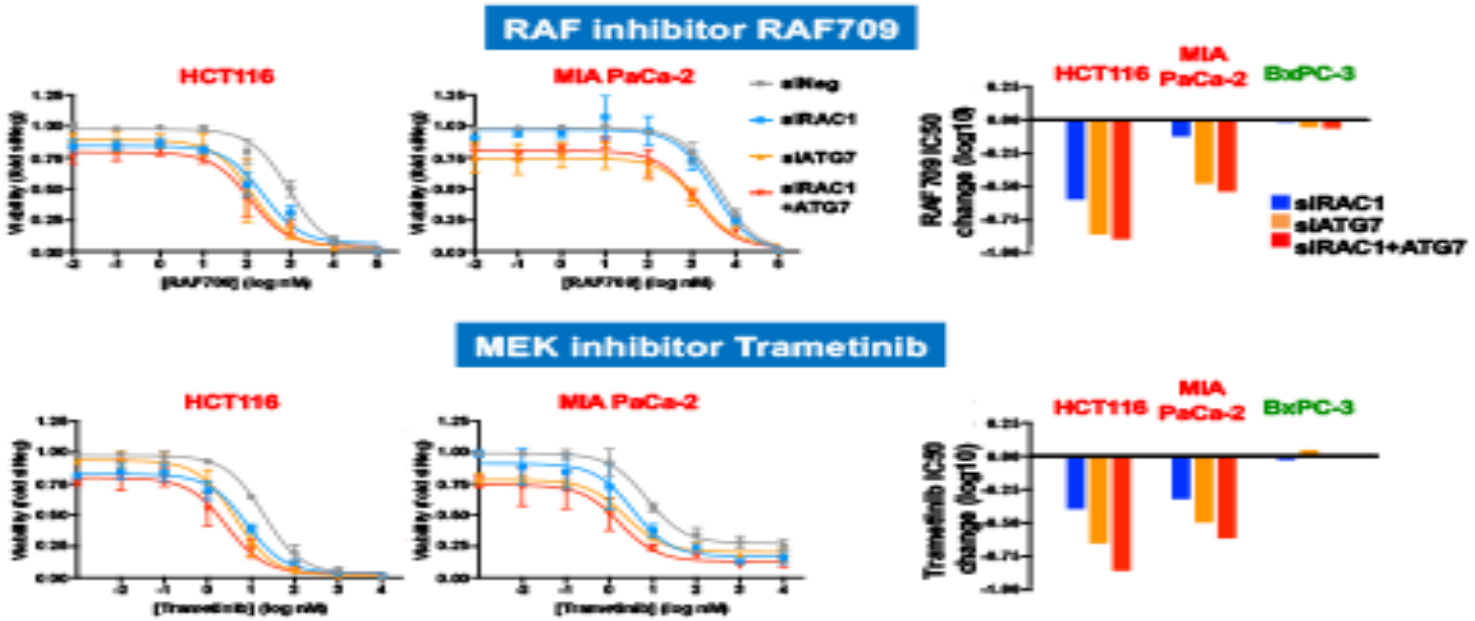
Deconvolution



(Lee et al. PNAS 2019)

Knockdown

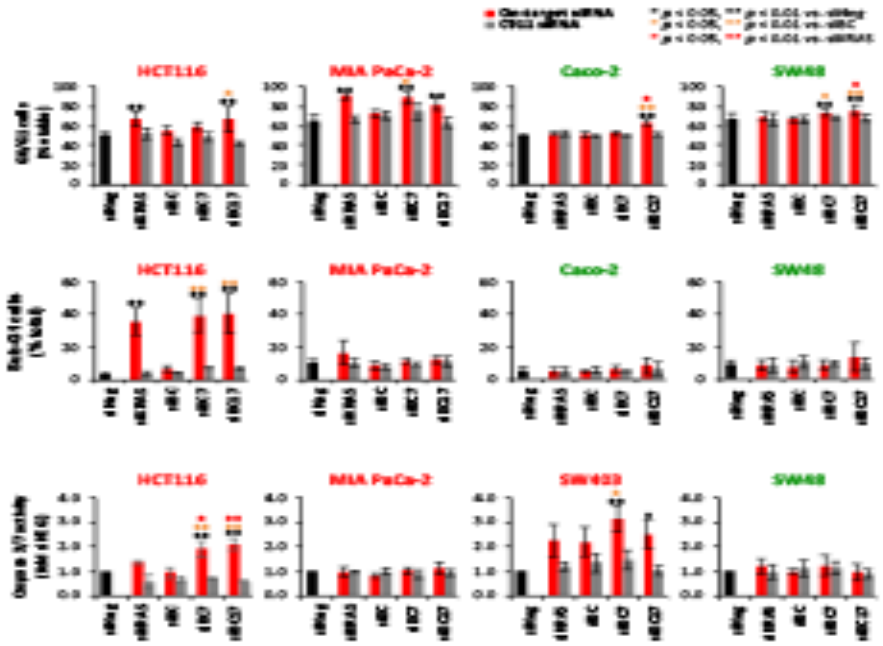
RAC1 and ATG7 Knockdown Sensitizes KRAS Mutant Cells Towards MAPK Pathway Inhibitors



(Lee et al. PNAS 2019)

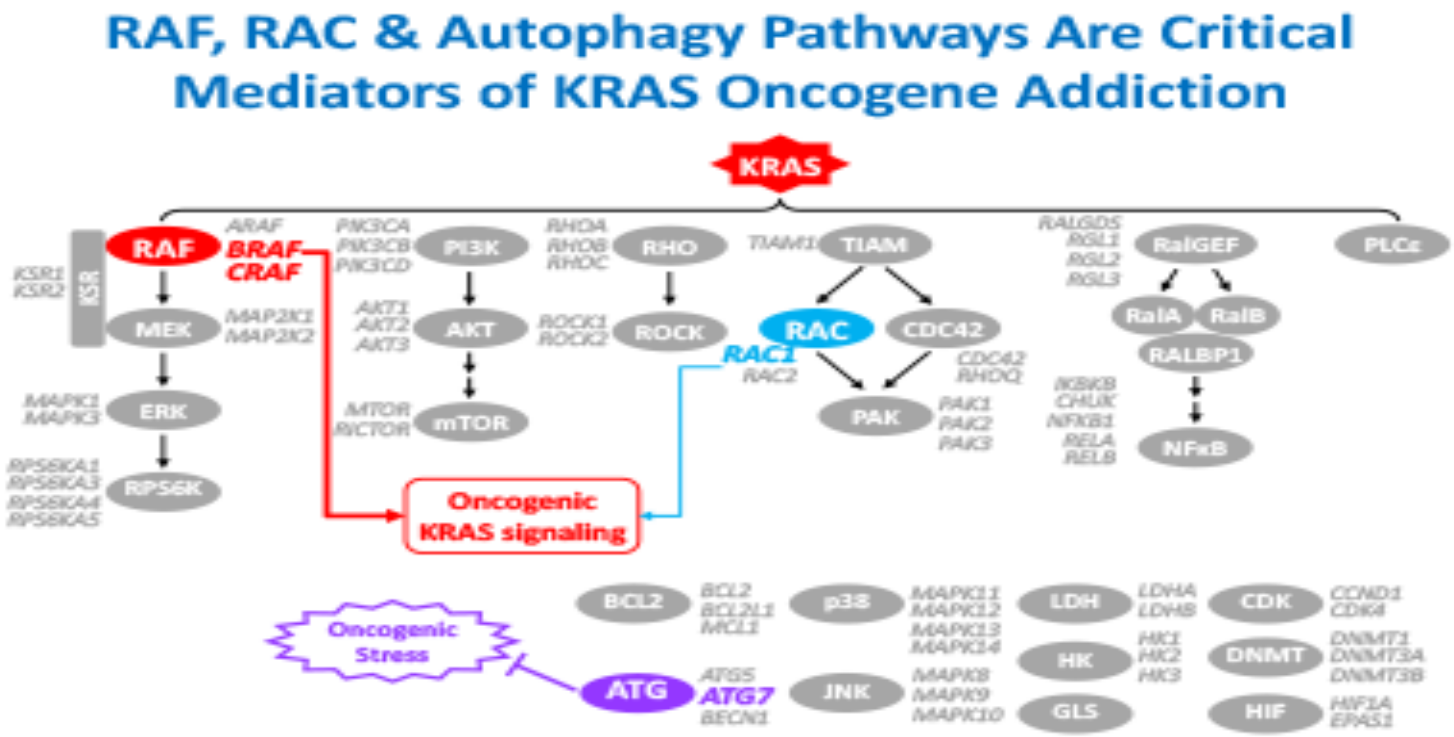
Cell cycle arrest

BRAF, CRAF and ATG7 Co-depletion Enhances Cell Cycle arrest and Cell death in KRAS Mutant Cells

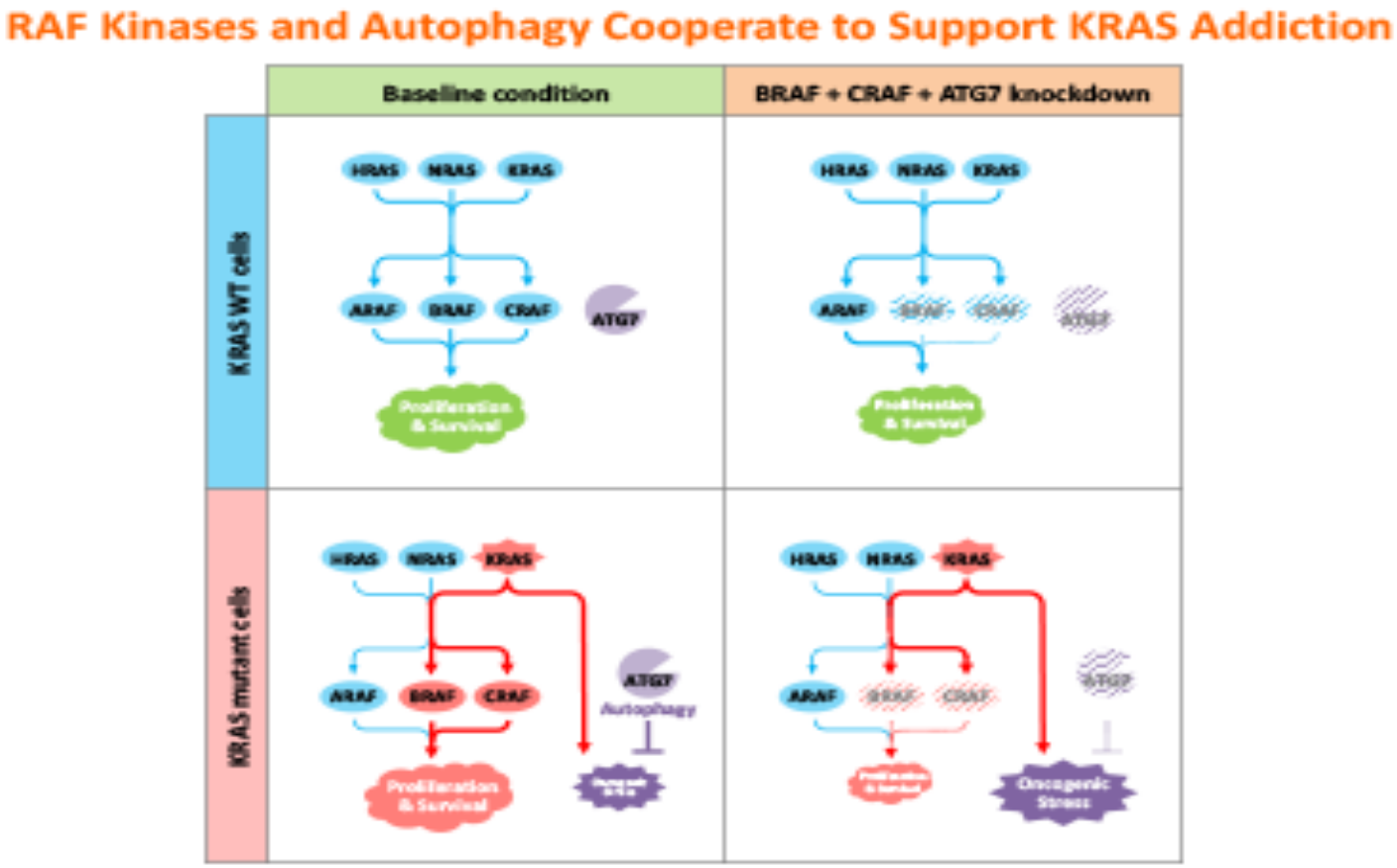


(Lee et al. PNAS 2019)

Autophagy pathways



RAF kinase and autophagy



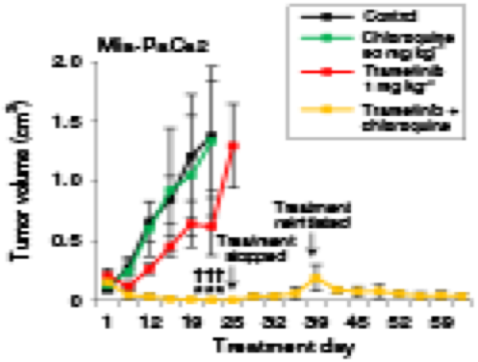
Co-targeting

Co-targeting the MAPK and Autophagy Pathway In KRAS Mutant Pancreatic Cancer Cells

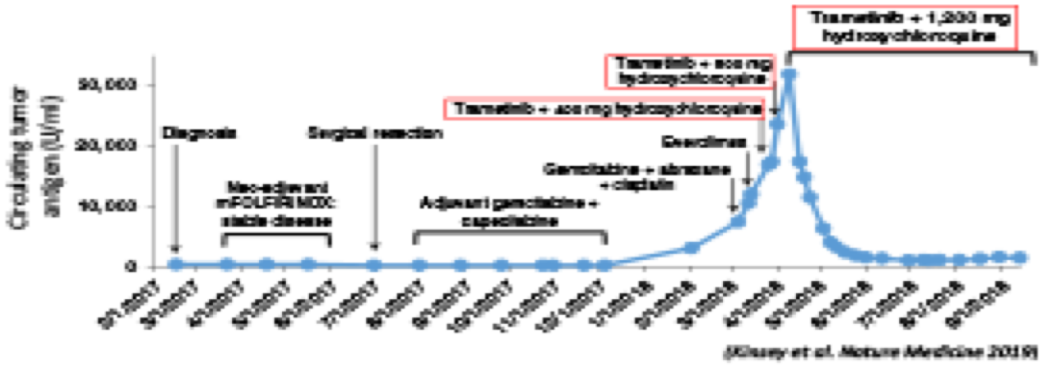
Kinsey … McMahon (Nature Medicine 2019)
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

Bryant … Der, (Nature Medicine 2019)
Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer

Mia-PaCa2 xenograft tumor



KRAS mutant pancreatic cancer patient



Phase I Trial: Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer

MAPK and autophagy

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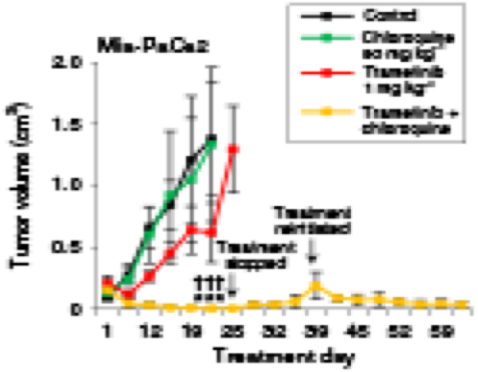

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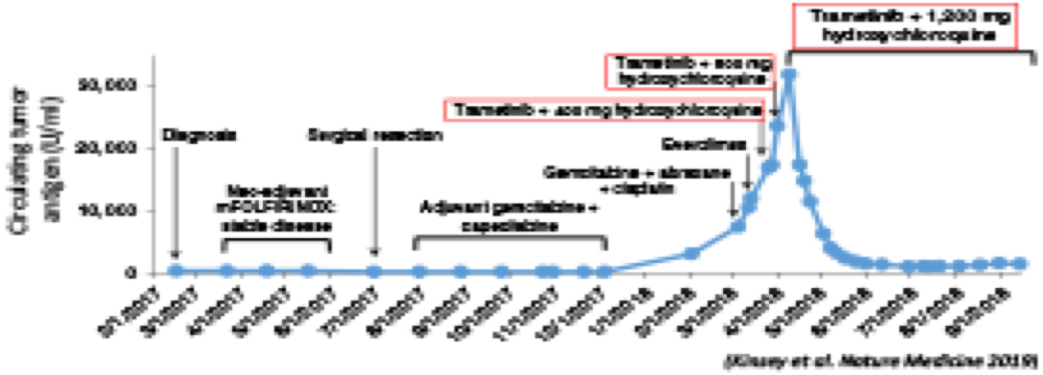

(Kinsey et al. Nature Medicine 2019)

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Acknowledgment

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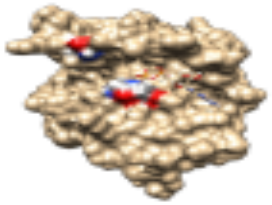

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