

Ras oncogene

The Ras Oncogene

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TRACO Lecture
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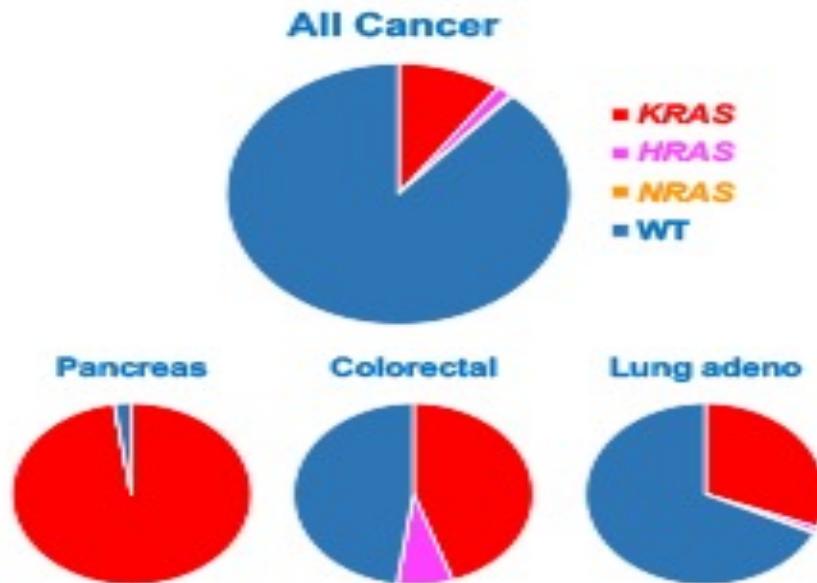


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Center for Cancer Research

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

Content

Content

- **History of the Ras oncogene**
- **Ras signaling pathways**
- **Ras mutations in human cancer**
- **Development of Ras-directed inhibitors**
- **Targeting Ras and its signaling network**
- **Synthetic lethal partners of the KRAS oncogene**
- **Optimizing target combinations for KRAS mutant cancer**
- **Concluding thoughts**

RAS oncogene

History of the Ras oncogene

RAS oncogene

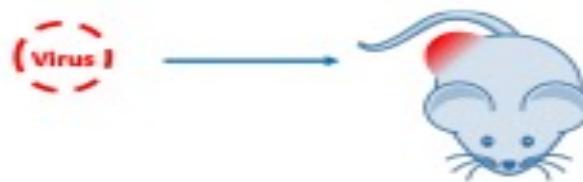
Discovery of the Ras Oncogene in Murine Tumor Viruses

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

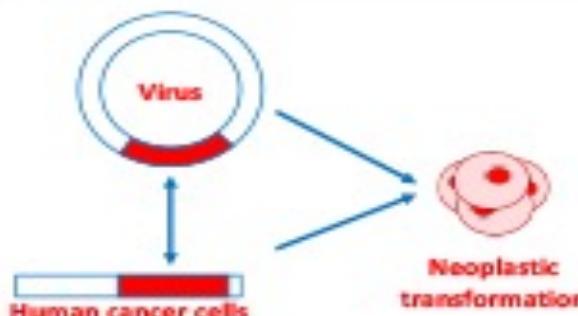
Chang ... Lowy (Nature, 1982) Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus

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

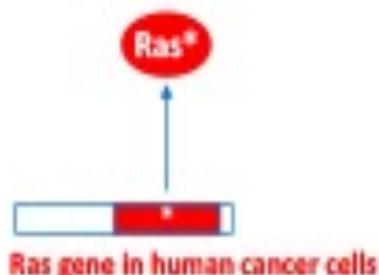
1982

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

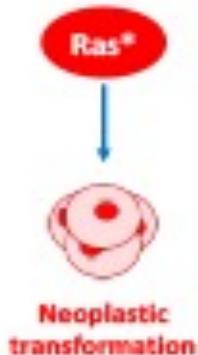
Human Ras Oncogene Encodes a Transforming Oncoprotein

1984

Stacey ... Kung (Nature, 1984) Transformation 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

1985

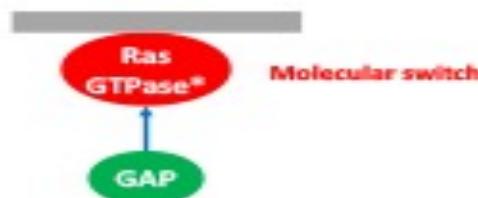
Mulcahy ... Stacey (Nature, 1985) Requirement for *ras* proto-oncogene function during serum-stimulated growth of NIH 3T3 cells



Biochemical properties

Biochemical Properties of the Ras Protein

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

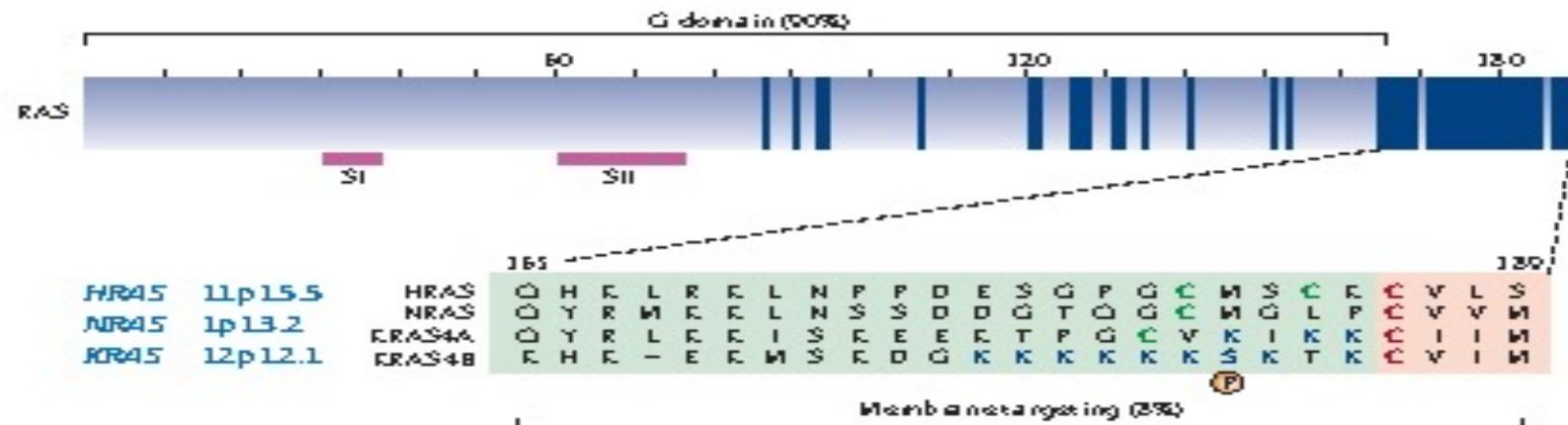
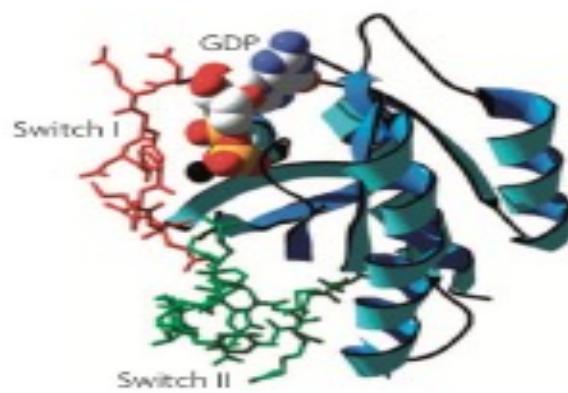


RAS signaling

Ras signaling pathway

RAS GTPases

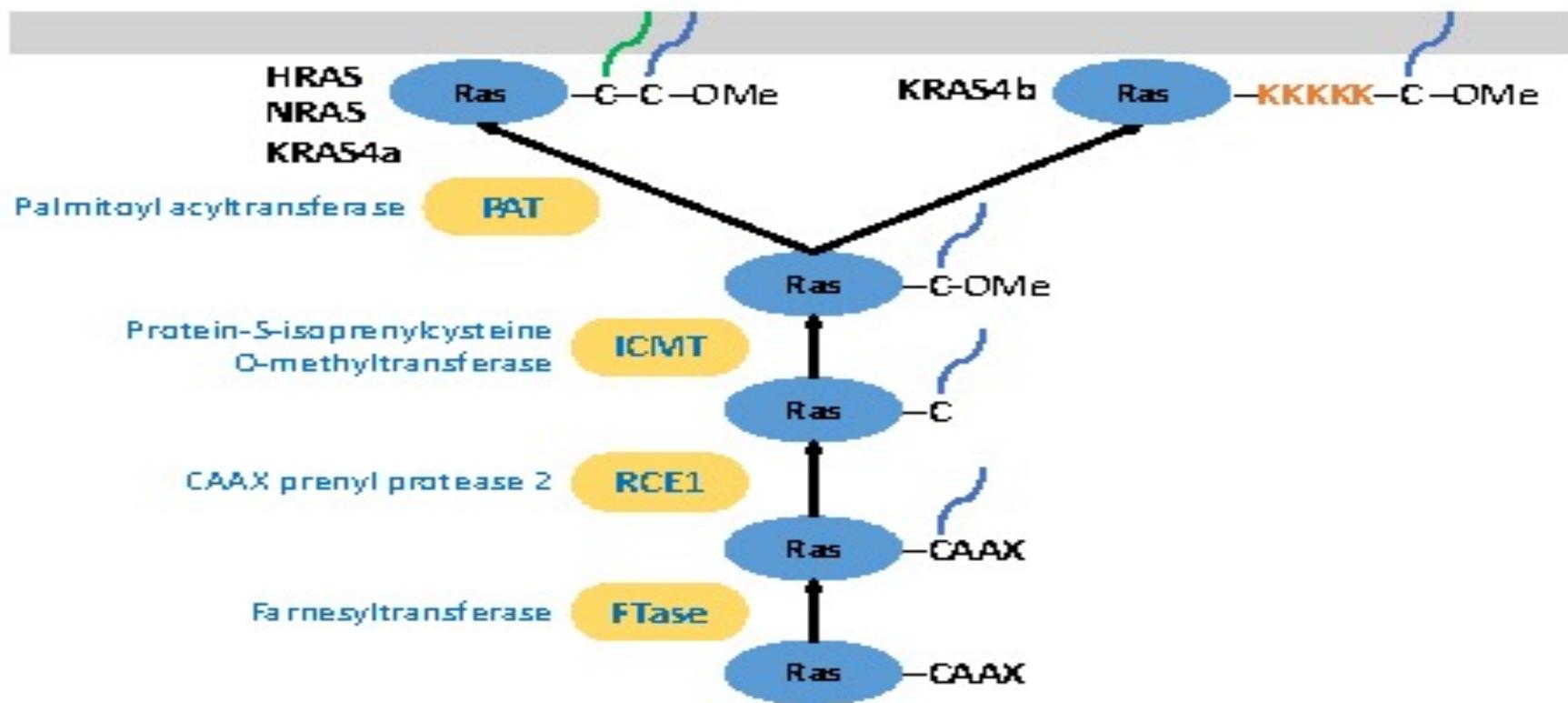
The Ras family of small GTPases



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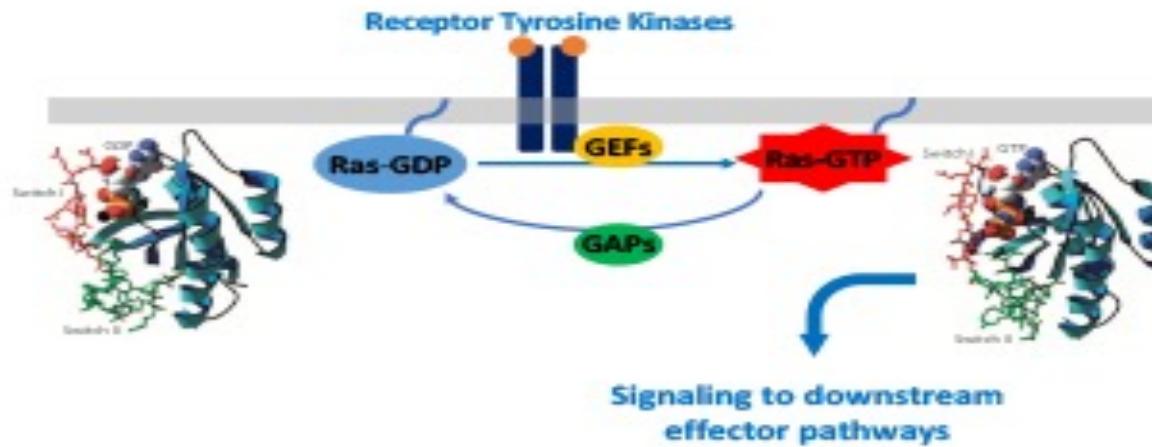
Ras membrane localization

Enzymatic steps for Ras membrane localization



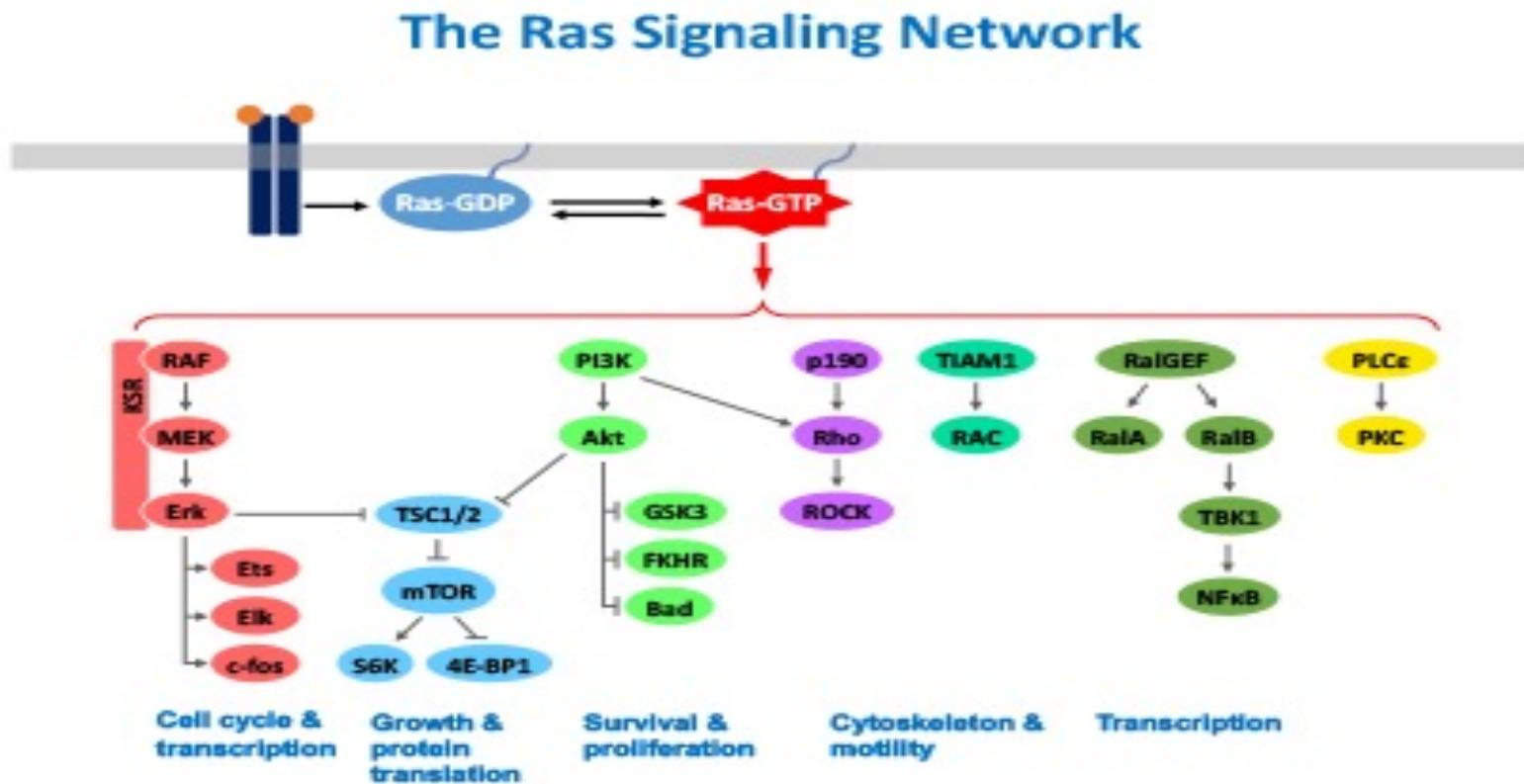
Ras GDP-GTP Cycle

Ras GDP-GTP Cycle



(Modified from Kamen & Weinberg, 2000)

Ras signaling network

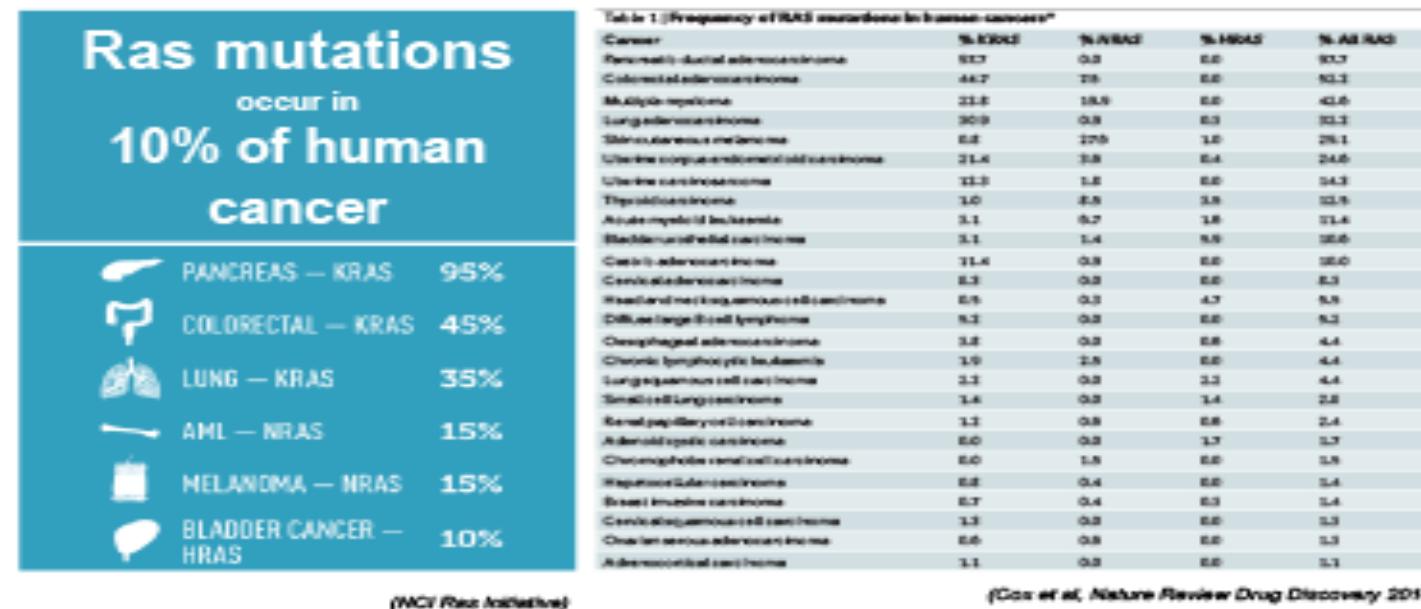


RAS mutations

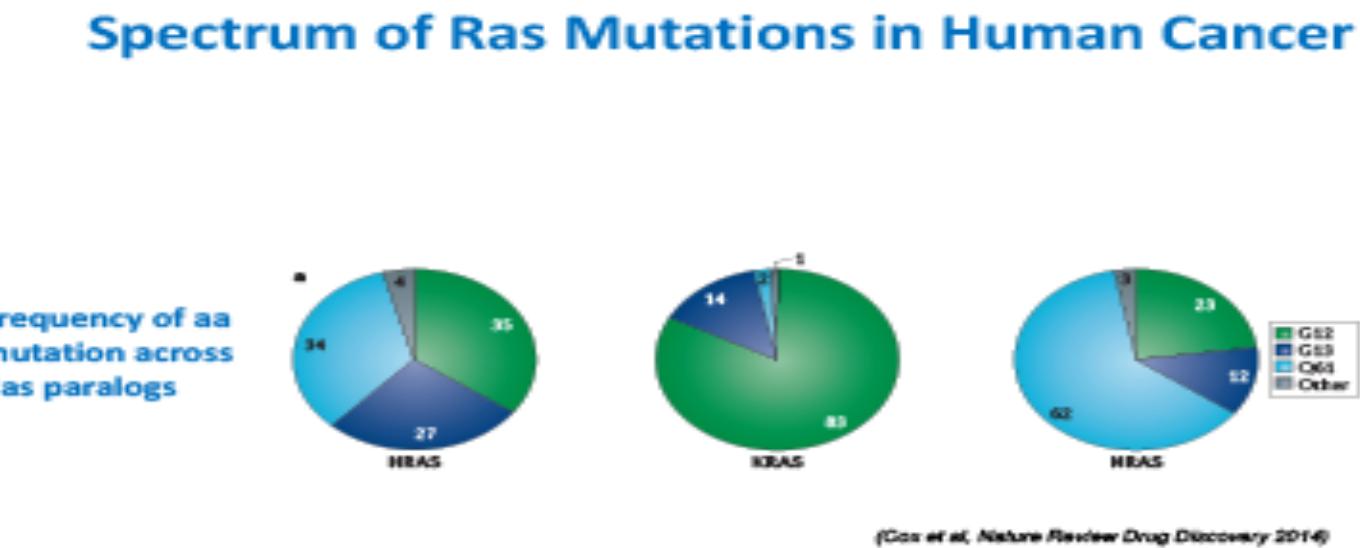
Ras mutations in human cancer

Ras mutations

Incidence of Ras mutations in human cancer

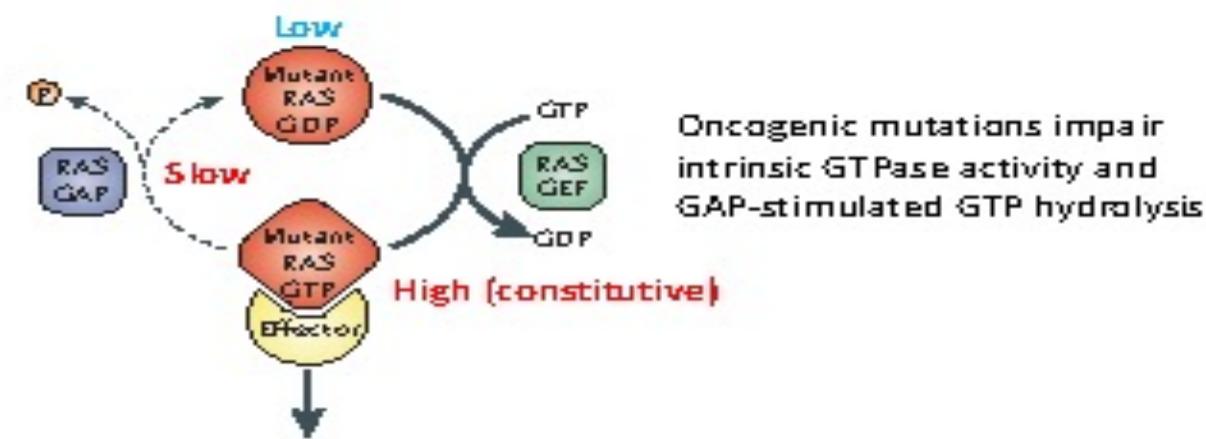
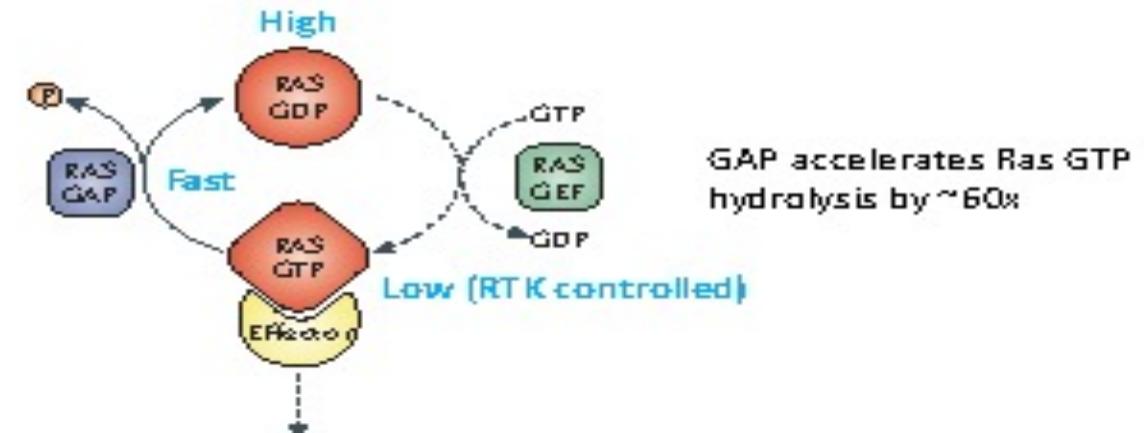
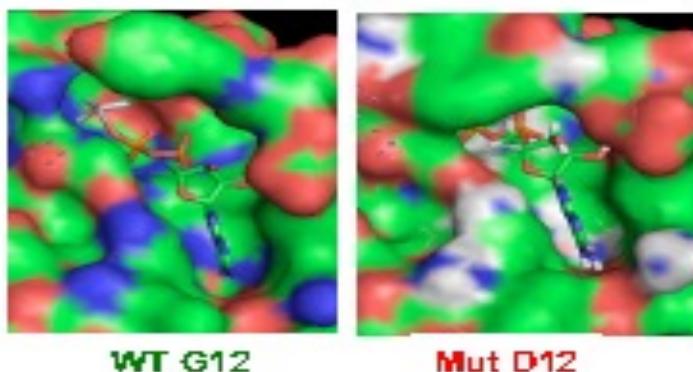
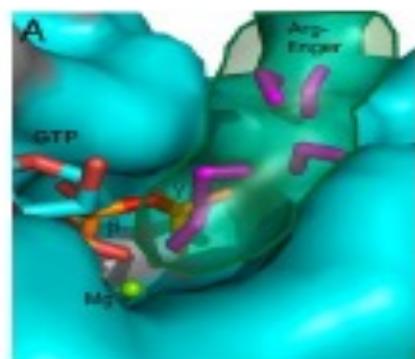


Mutation spectrum



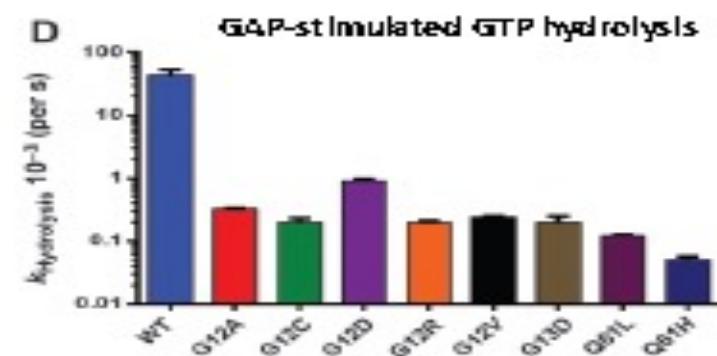
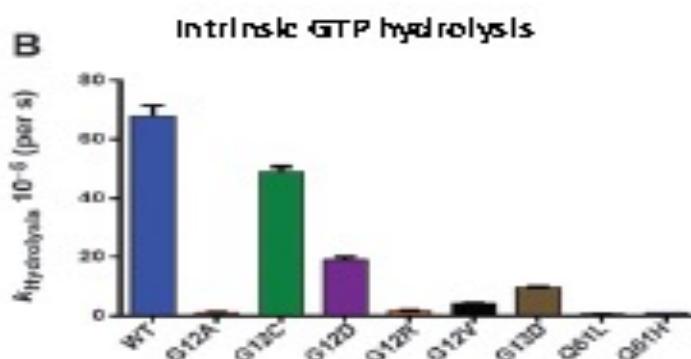
GTP hydrolysis impairment

Oncogenic Ras mutations impair GTP hydrolysis



Ras mutations

Oncogenic Ras mutations impair GTP hydrolysis



(-Cuper et al. Mol Cell Biol 2010)

- KRAS mutants differ in their impact on intrinsic GTPase activity
- KRAS mutant all strongly impair GAP-stimulated GTP hydrolysis

RAS inhibitors

Development of Ras-directed inhibitors

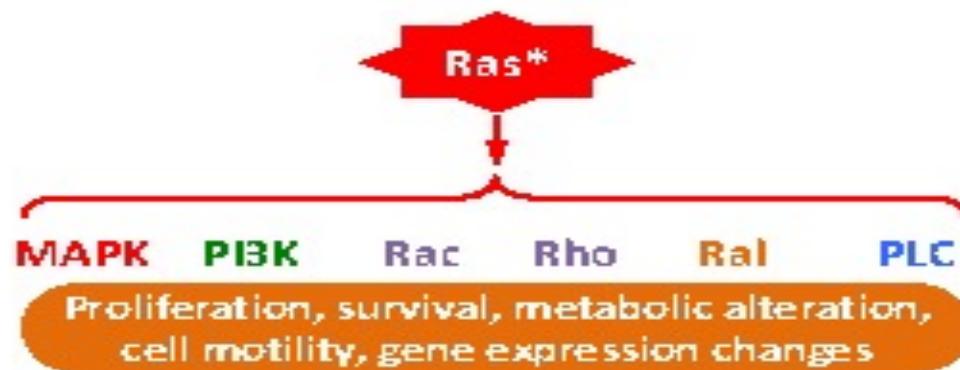
KRAS and adenocarcinomas

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 ...

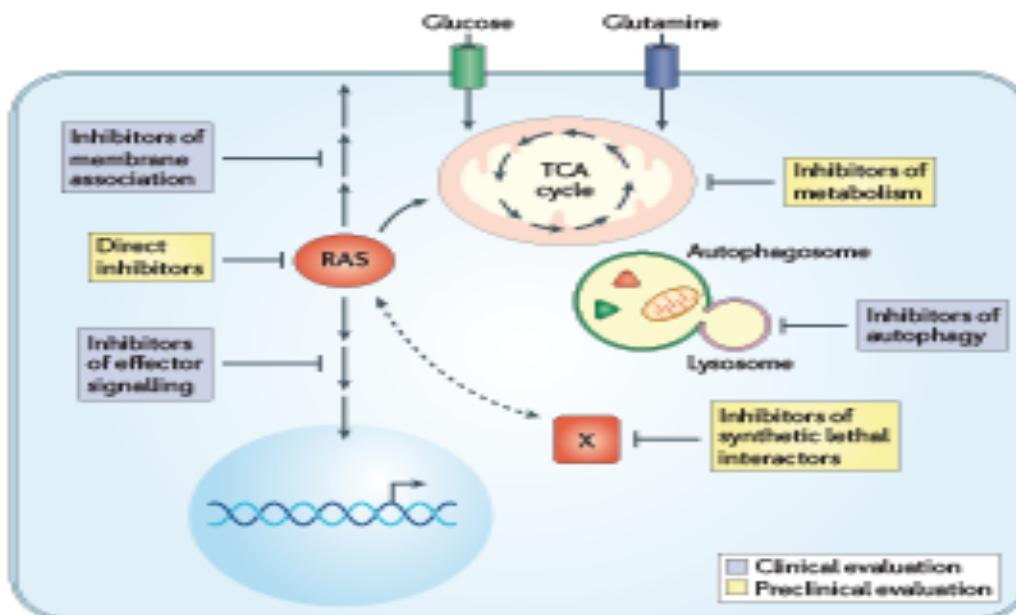
Oncogene addiction

Oncogenic Ras signaling leads to neoplastic transformation and oncogene addiction



Ras signaling

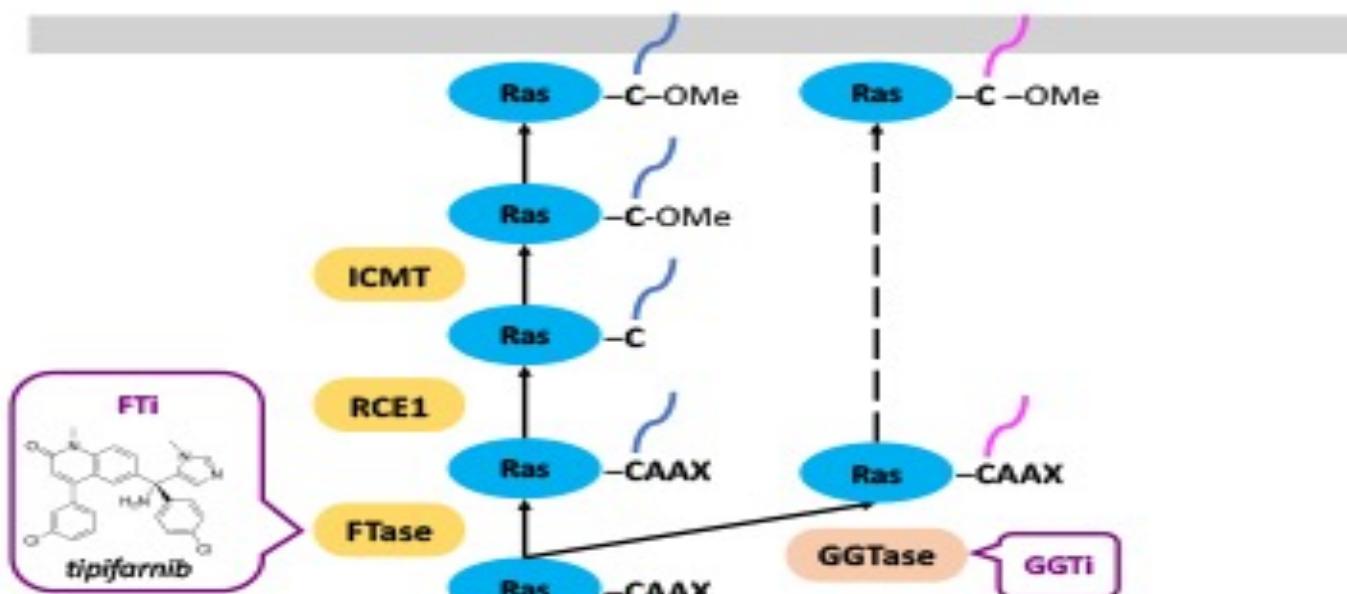
Strategies to Target Oncogenic Ras Signaling



(Cox et al., 2016)

Ras membrane association

Inhibition of Ras Membrane Association



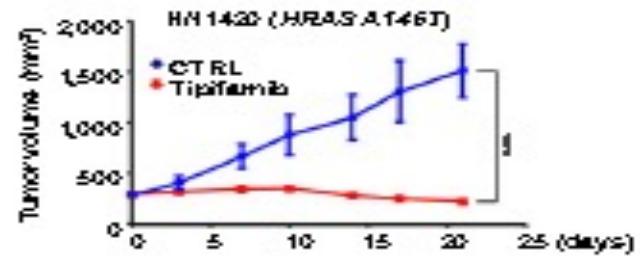
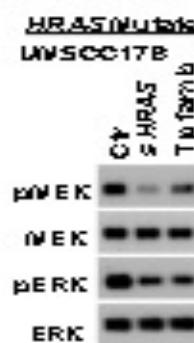
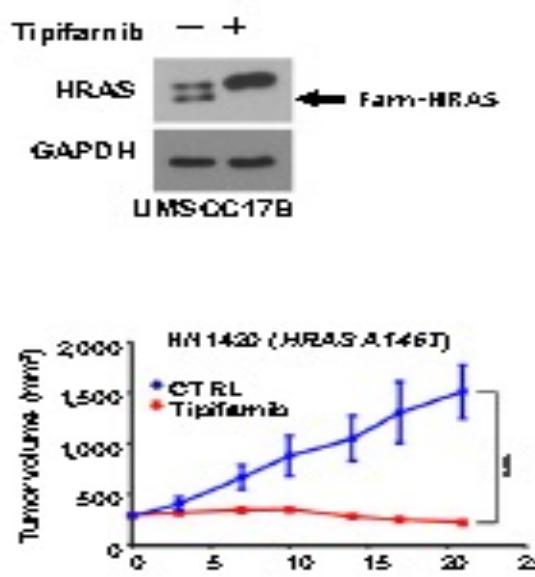
- Alternative membrane localization pathway
- Lack of KRAS selectivity – pathway shared by other small GTPases

Ftase inhibitor

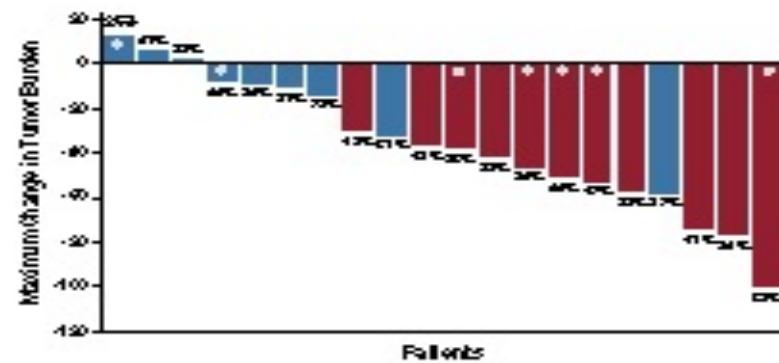
FTase inhibitor in HRAS mutant head and neck cancer

FTase inhibitor (tipifarnib)

Head and neck squamous cell carcinomas with HRAS mutation



(Giancotti et al. Mol Cancer Ther 2020)



Median PFS = 5.6 mo

Median OS = 15.4 mo

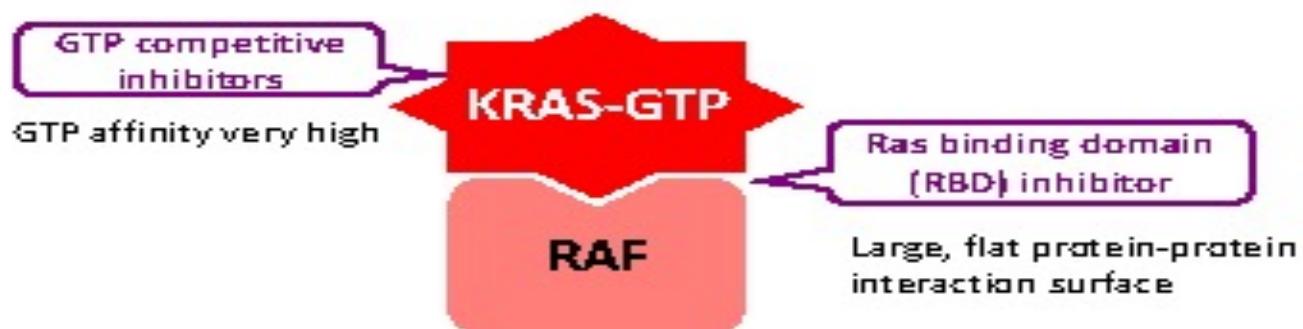
(-o- ctrl. J Clin Oncol 2021)

- FDA granted Breakthrough Therapy Designation (Feb 2021)
- Phase III trial on-going

Direct inhibition

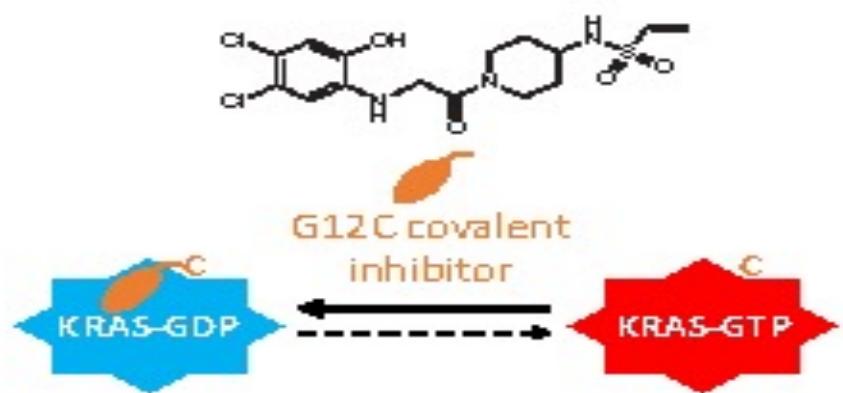
Direct inhibition of Ras oncoprotein function

Early attempts at directly blocking Ras oncoprotein activity



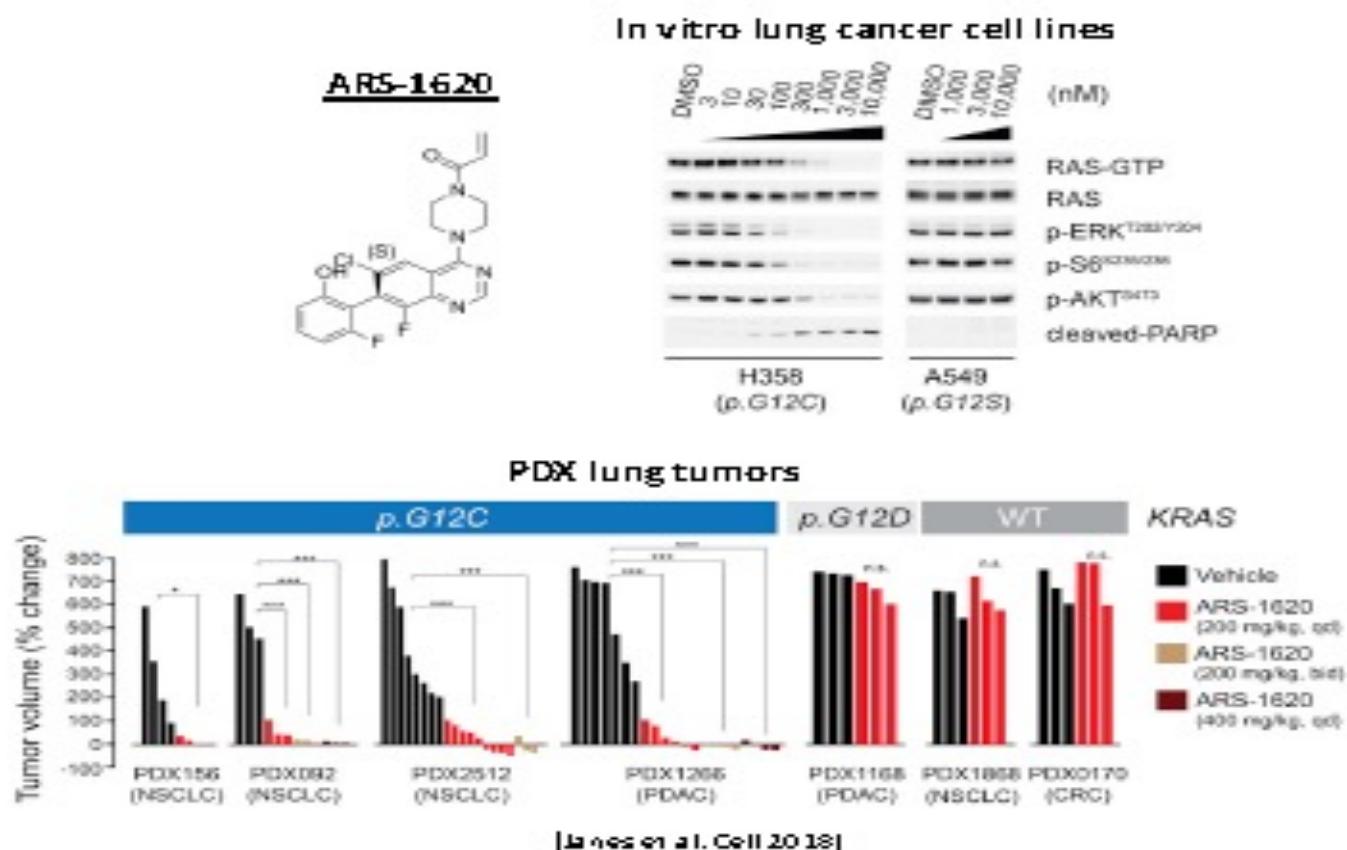
Covalent inhibitors

Development of covalent inhibitors against the KRAS-G12C mutant



[Snoek Lab, Ossenkoppele et al. Nature 2013]

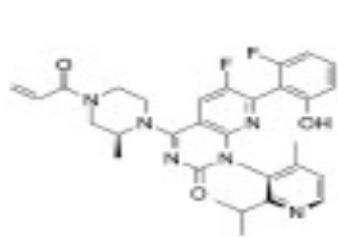
- Moderately active cysteine cross-linking warhead
- KRAS binding scaffold that directs the specificity of the warhead
- Preferential binding to the GDP-bound form of KRAS
- Inhibitor binding leads to an induced fit in the switch II pocket



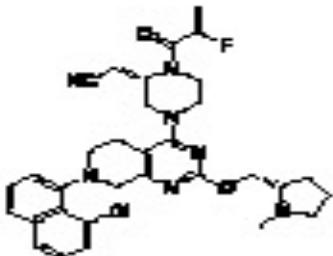
Clinical efficacy

Clinical efficacy of KRAS-G12C inhibitors

Cancer Type	KRAS frequency among KRAS mutations
Lung	40%
Pancreatic	8%
Colorectal	3%

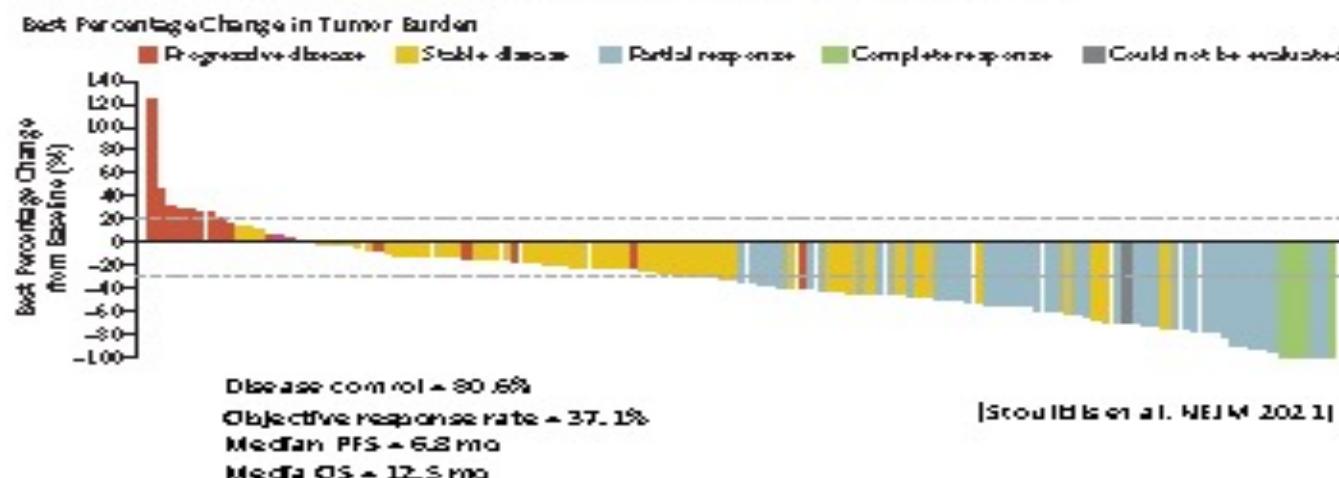


AMG-510
(Sotorasib)



MRTX849
(Adagrasib)

Phase 2 Trial: Patients with KRAS-G12C mutated advanced NSCLC previously treated with standard therapies (CodeBreak 100)

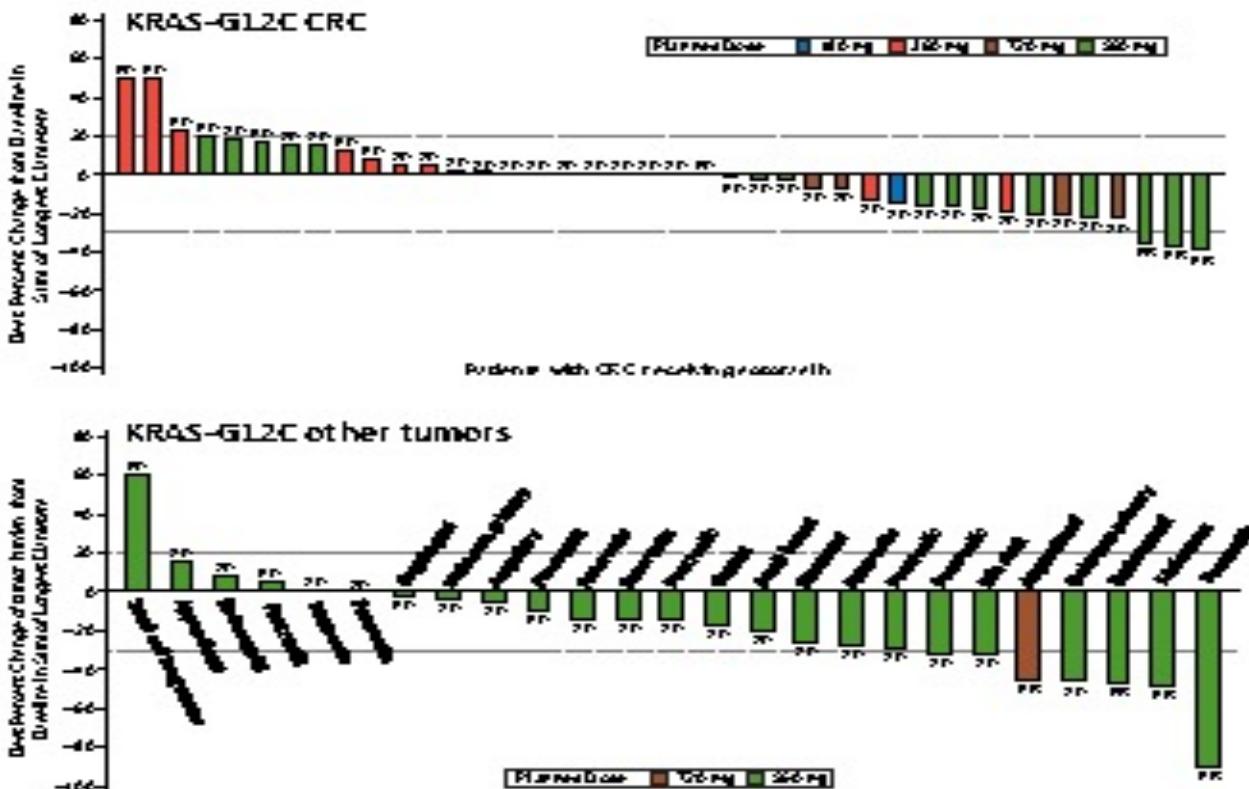


FDA granted accelerated approval of sotorasib for locally advanced or metastatic NSCLC with KRAS-G12C mutation (May 2021)

Clinical efficacy

Clinical efficacy of KRAS-G12C inhibitors

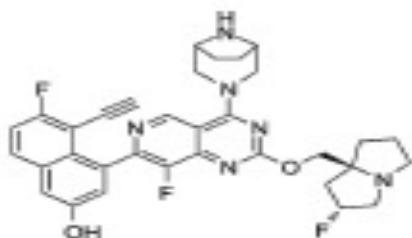
- Other types of tumors with KRAS-G12C mutation likely to benefit from G12C inhibitors
- Response rate likely differ in different cancer types



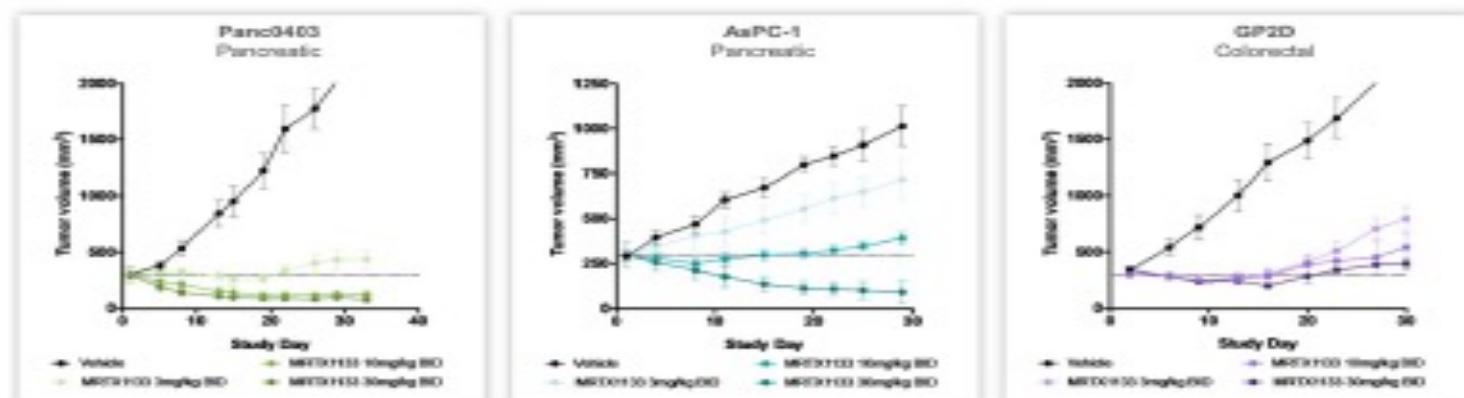
Covalent inhibitors

Covalent inhibitors targeting KRAS-G12D mutant

Cancer Type	KRAS G12C frequency among KRAS mutations
Lung	18%
Pancreatic	48%
Colorectal	34%



MRTX1133: Anti-Tumor Activity Observed in Pancreatic and CRC In-Vivo Models

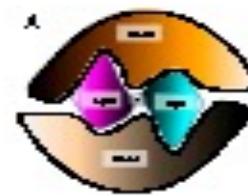
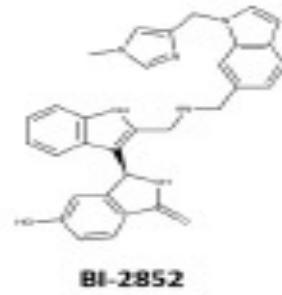


MRTX1133 demonstrates dose-dependent inhibition of KRAS-dependent signalling, demonstrating tumor regression in G12D mutant tumor models

[MTRX1133 Therapeutic data presentation]

Non-covalent inhibitor

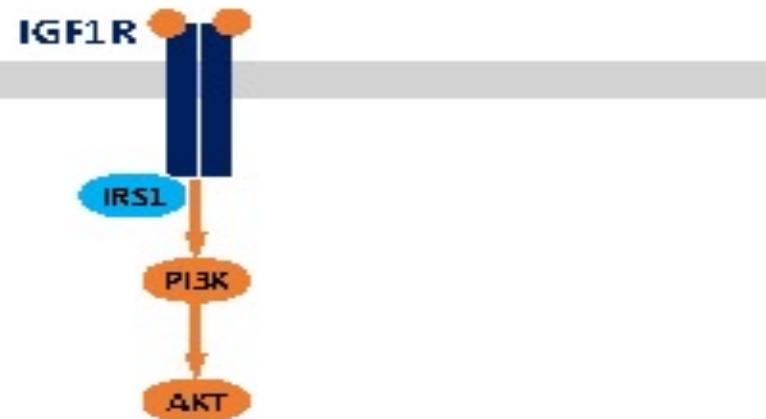
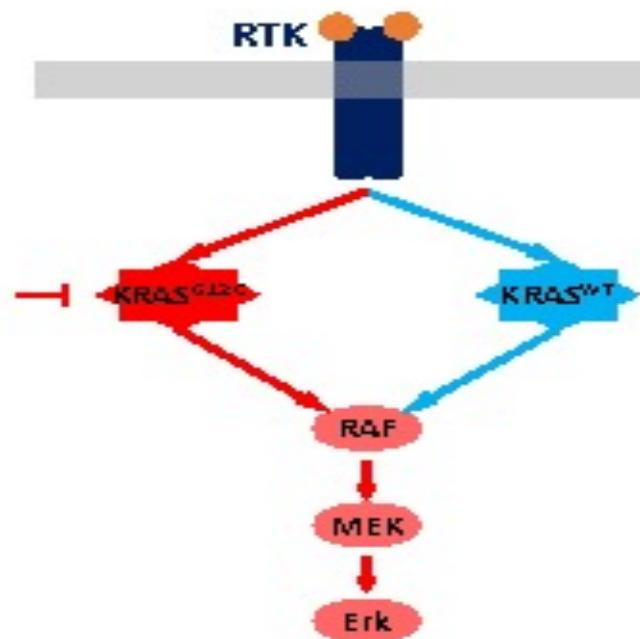
Non-covalent KRAS inhibitors



- Induces non-productive KRAS dimer formation
- Potency is relatively weak

Resistance

Resistance to KRAS-G12C inhibitors: adaptive signaling changes



Cell state change: EMT

- Increased IGF1R-PI3K signaling
- Reduced addiction to KRAS oncogenic

[Adachi et al, Clin Can Res 2020]

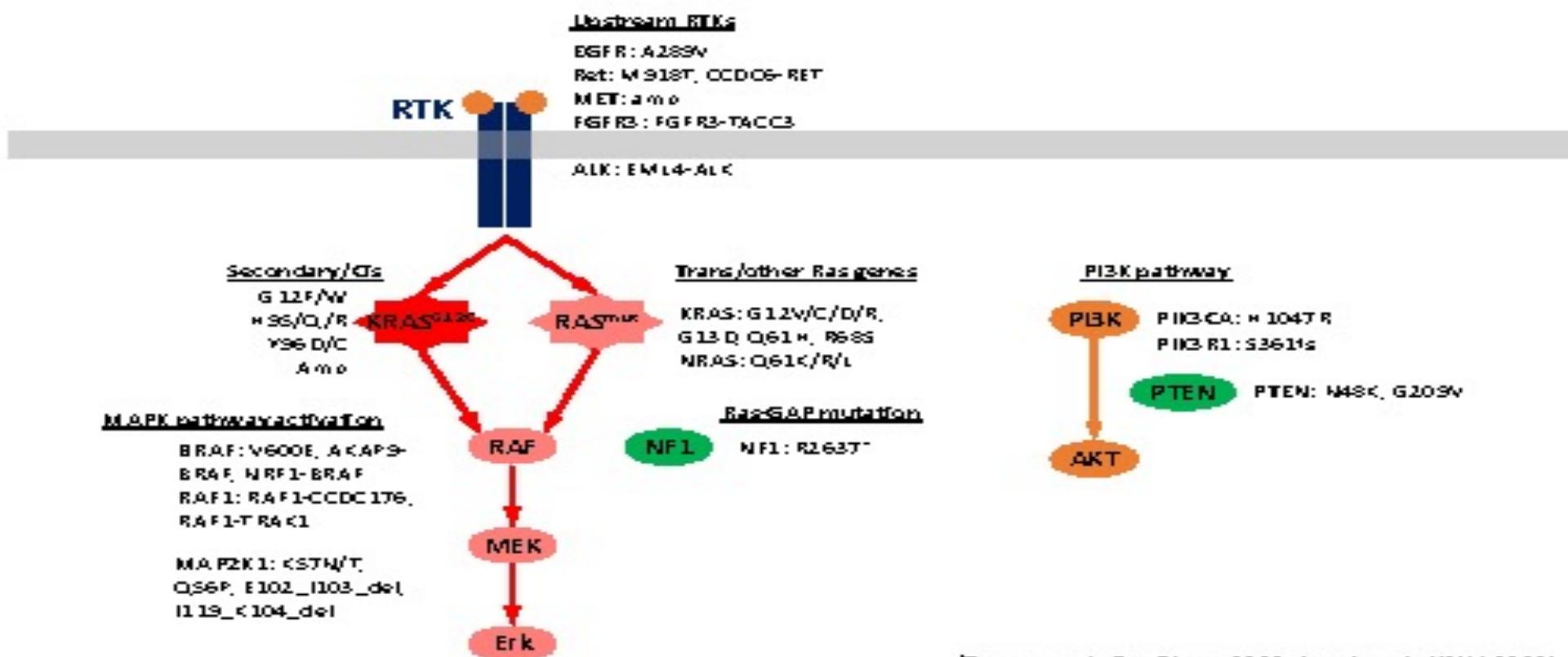
Feedback activation of RTK-Ras signaling

- Increased RTK signaling to WT KRAS protein
- Increased level of KRAS-G12C protein that are maintained in the GTP-bound state

[Ryan et al, Clin Can Res 2020; Xue et al, Nature 2019]

Resistance

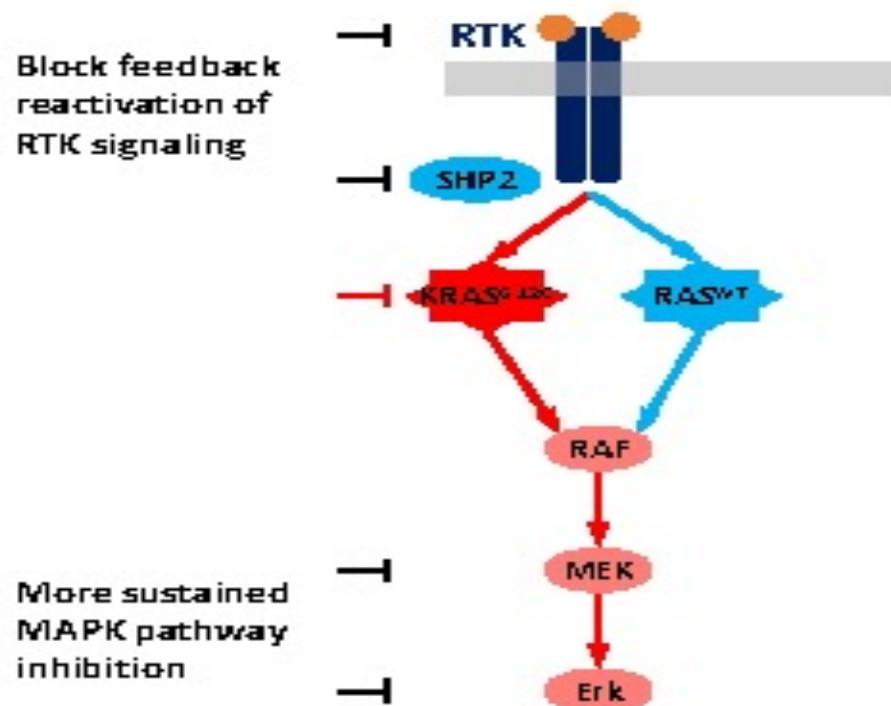
Resistance to KRAS-G12C inhibitors: clinically acquired mutations



[Tanaka et al., *Cancer Discovery* 2021; Awad et al., *NEJM* 2021]

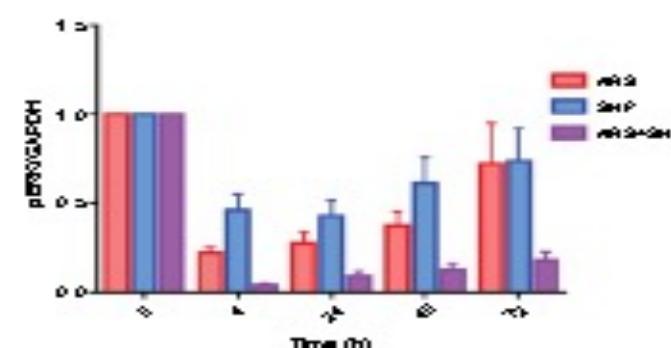
Combination therapy

Combination therapy to improve KRAS-G12C inhibitor efficacy



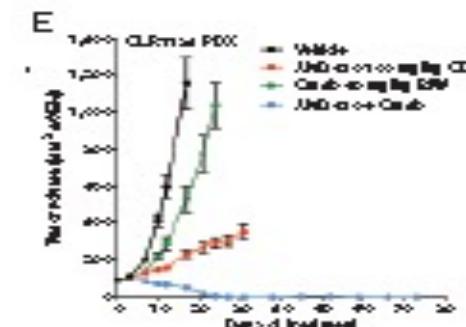
KRAS^{G12C} [ARS1620] + SHIP2I [SHIP099]

[Ryan et al., Clin Can Res 2019]



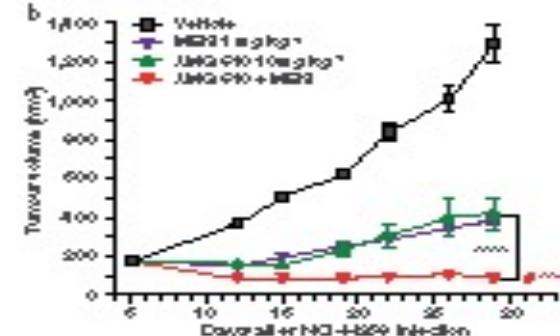
KRAS^{G12C} [AMG510] + EGFRi (cetuximab)

[Amodio et al., Can Devov 2021]



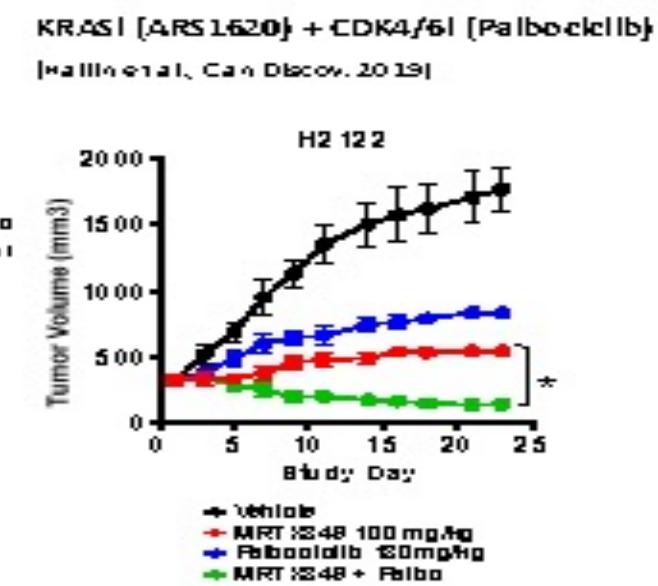
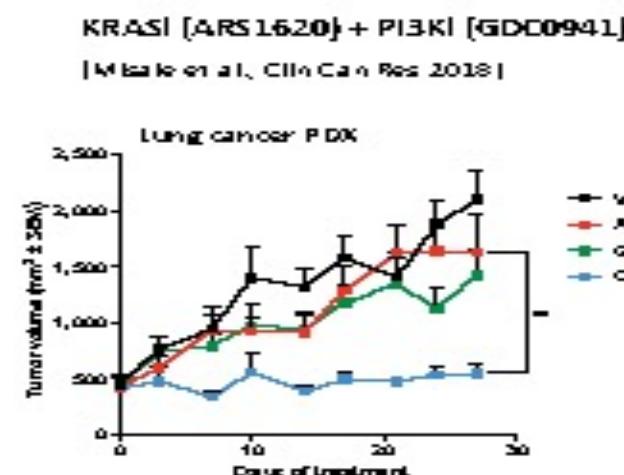
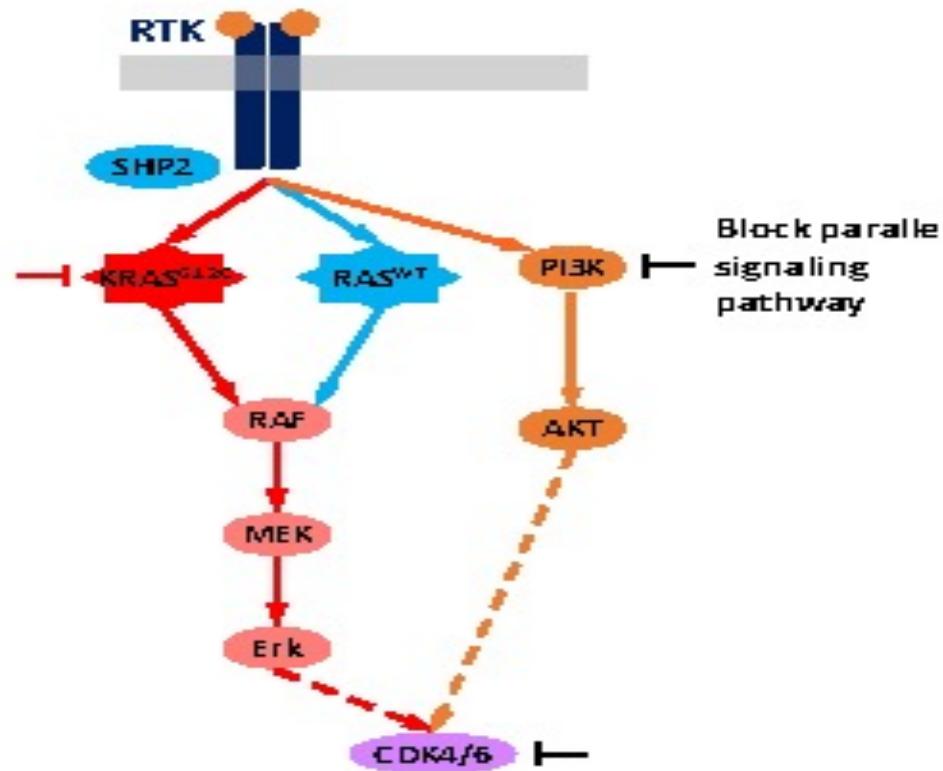
KRAS^{G12C} [ARS1620] + MEK1 (PD-0325901)

[Kanon et al., Nature 2019]



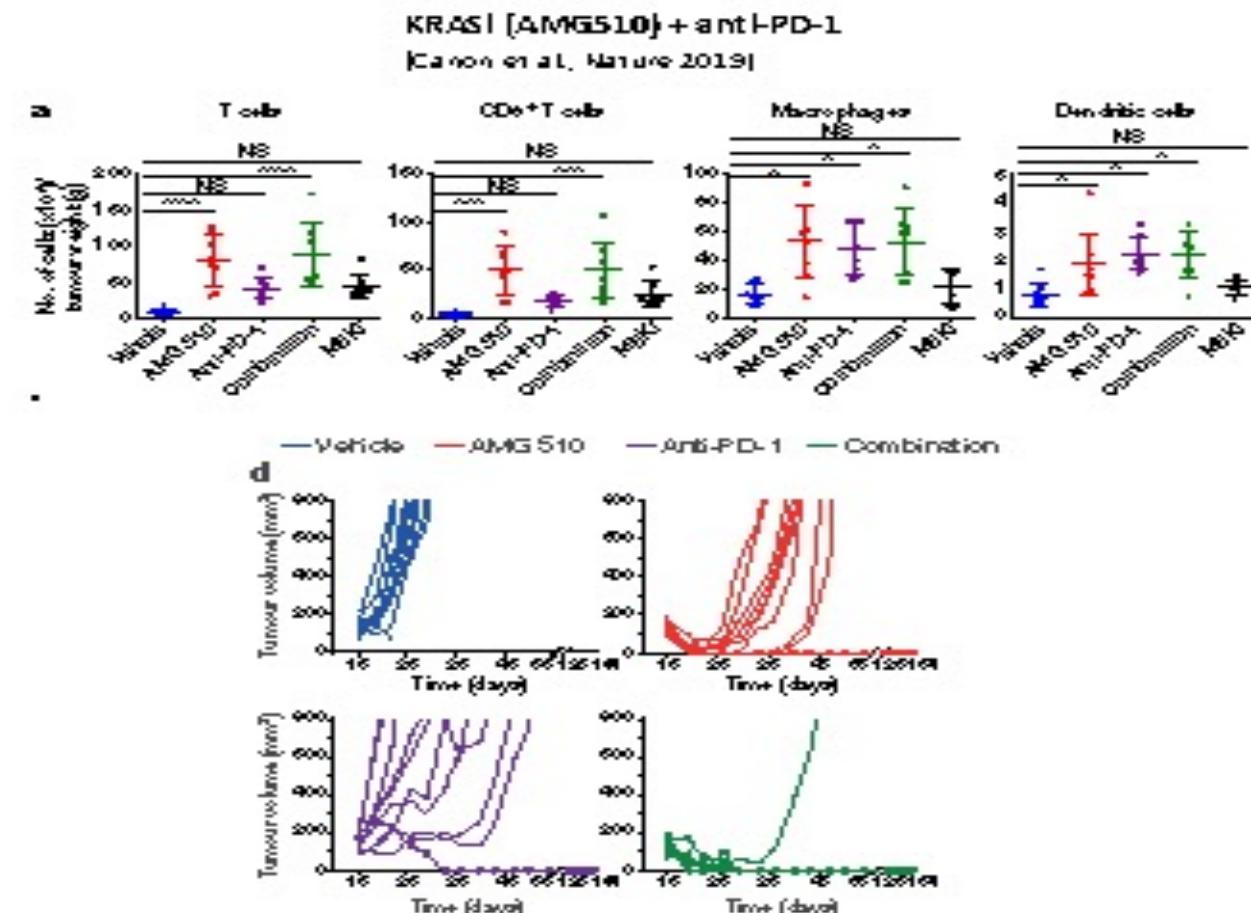
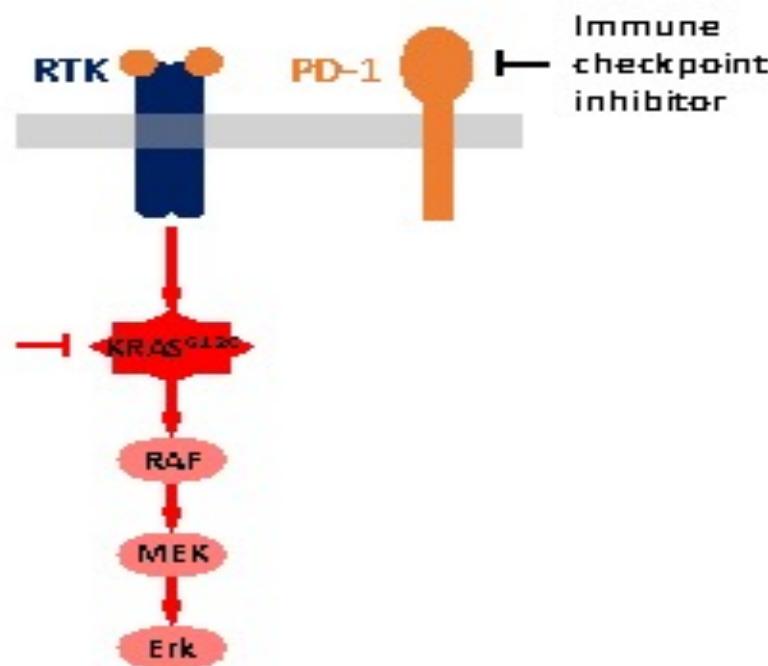
Combination therapy

Combination therapy to improve KRAS-G12C inhibitor efficacy



Combination therapy

Combination therapy to improve KRAS-G12C inhibitor efficacy

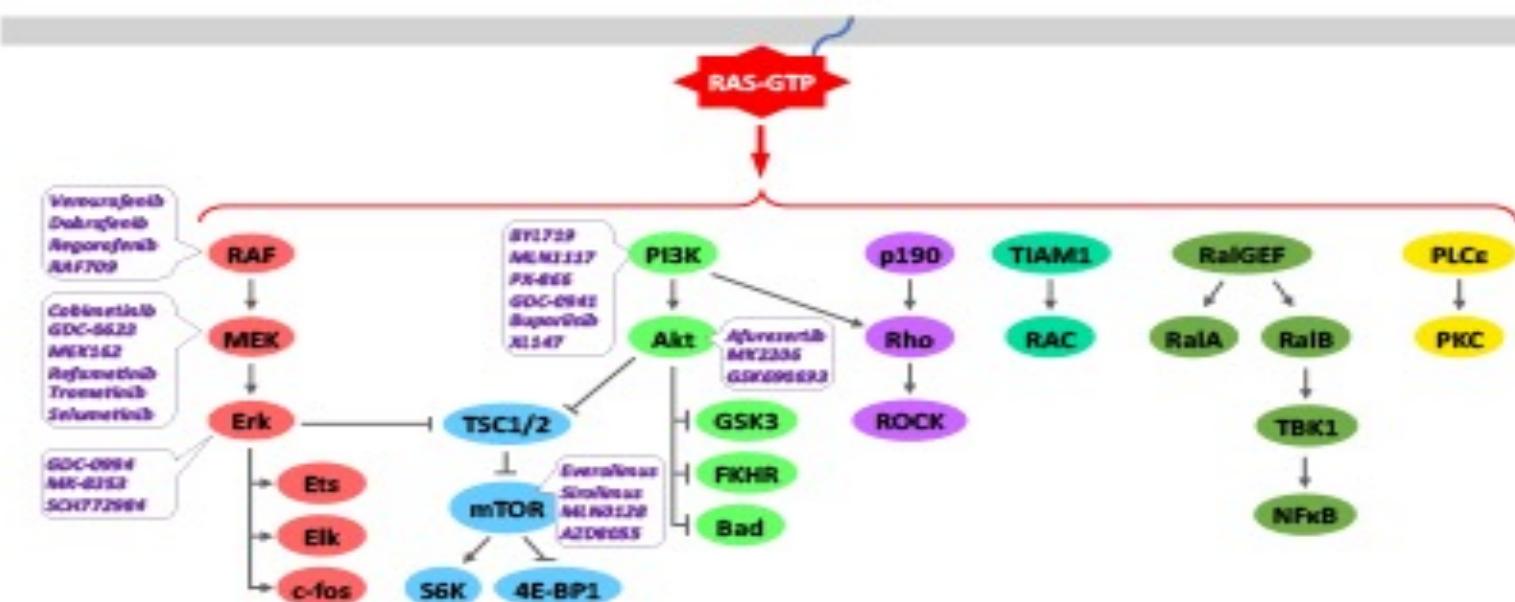


RAS signaling network

Targeting Ras and its signaling network

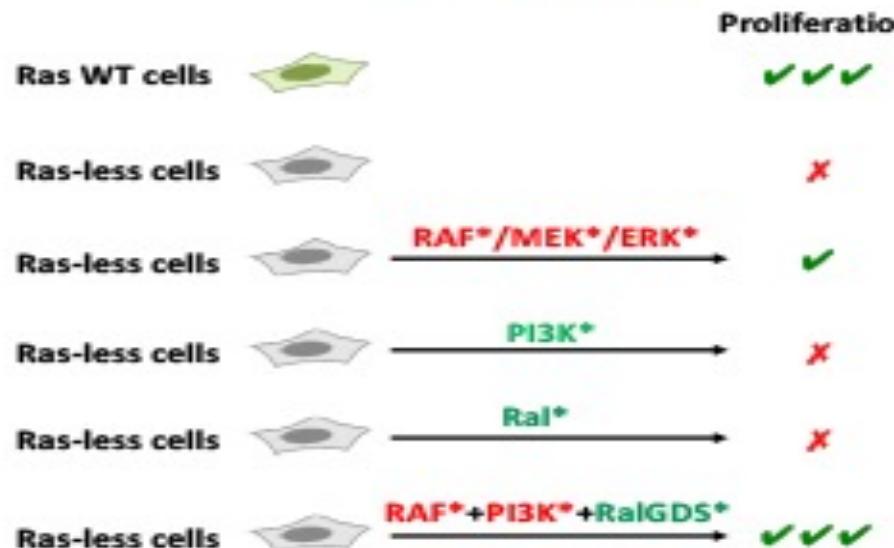
Kinase inhibitors

Kinase Inhibitors Targeting Ras Effectors



MAPK pathway

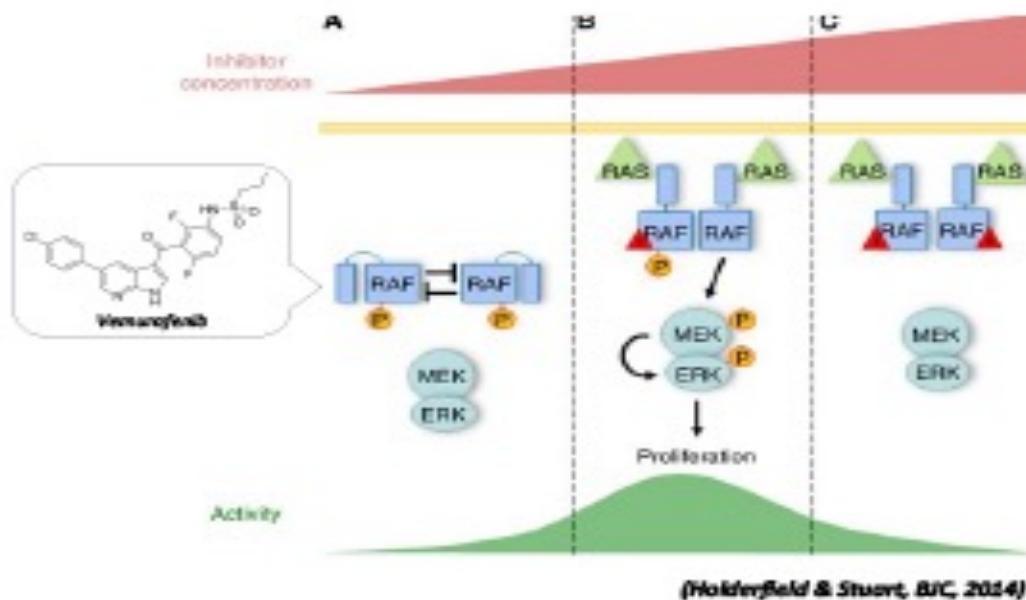
MAPK Pathway is Essential for Ras-Driven Cell Proliferation



(Drost et al., EMBO J., 2010)

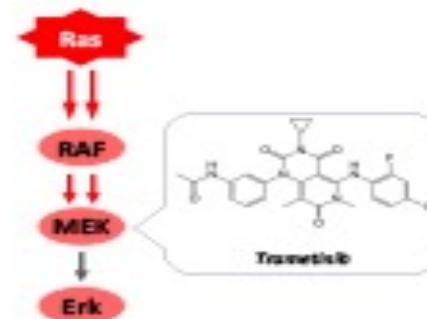
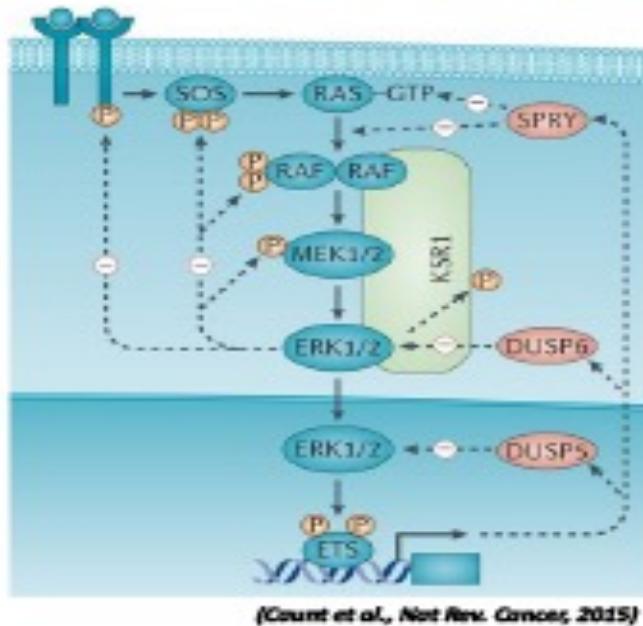
MAPK pathway

RAF Inhibitors Activates MAPK Pathway in Ras-Mutant Cells



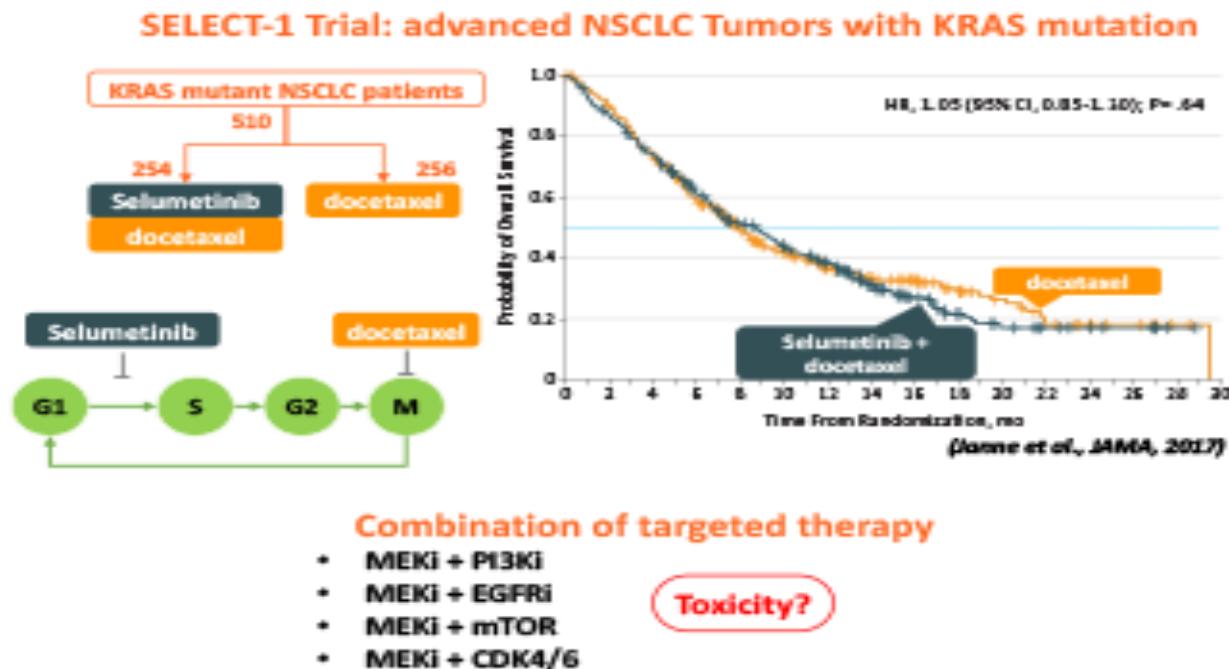
Feedback activation

MEK Inhibitors Leads to Feedback Activation of the MAPK Pathway



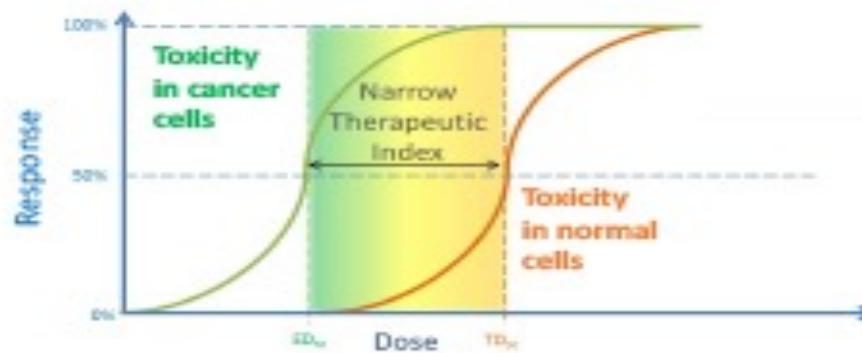
MEKi combinations

Current MEKi Combinations Are Ineffective in KRAS Mutant Cancer



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 lethal partners

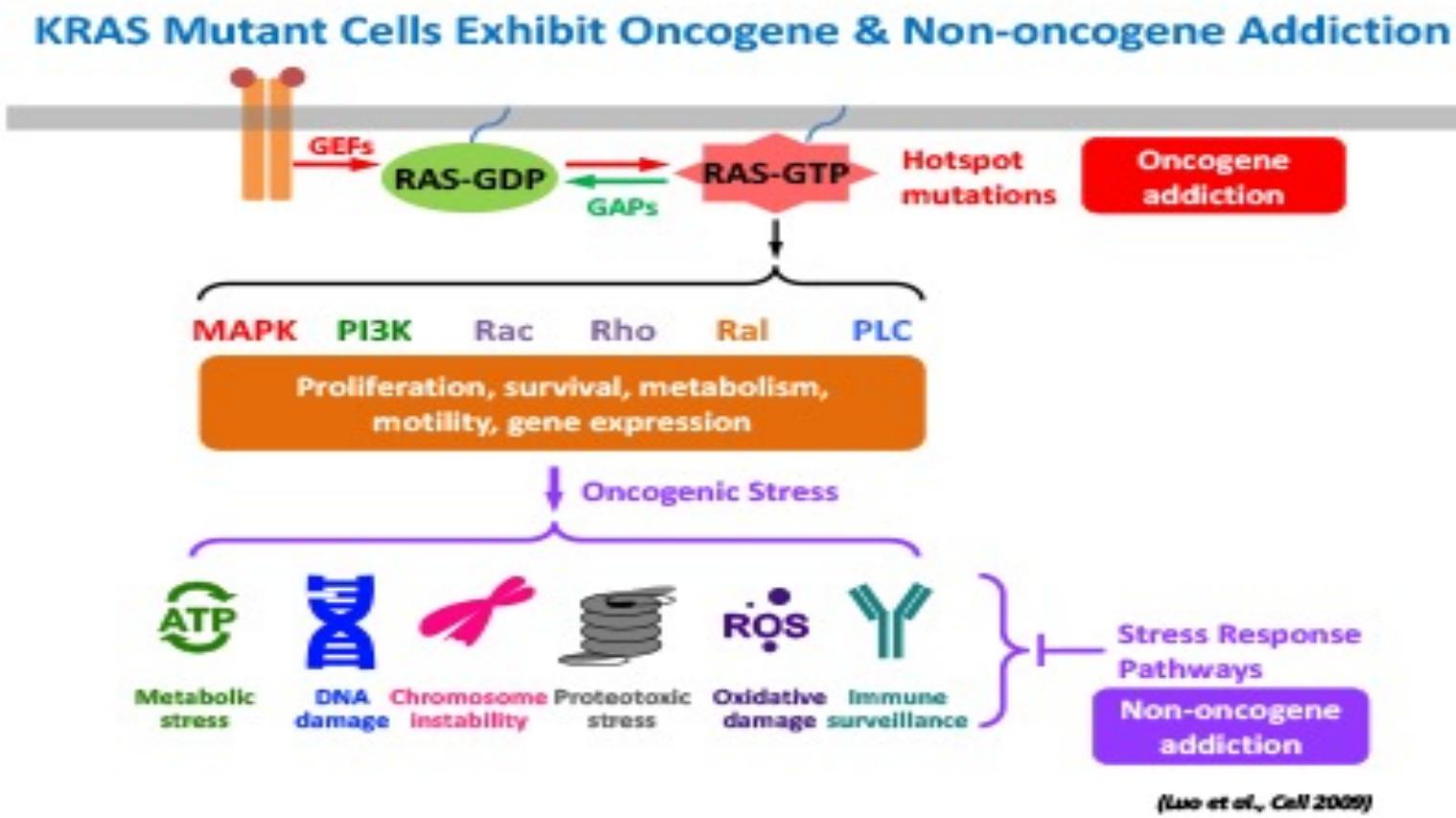
**Synthetic lethal partners of the KRAS oncogene
Optimizing target combinations for KRAS mutant cancer**

Synthetic lethality

Synthetic Lethality Reflects Genetic Buffering and Pathway Redundancy

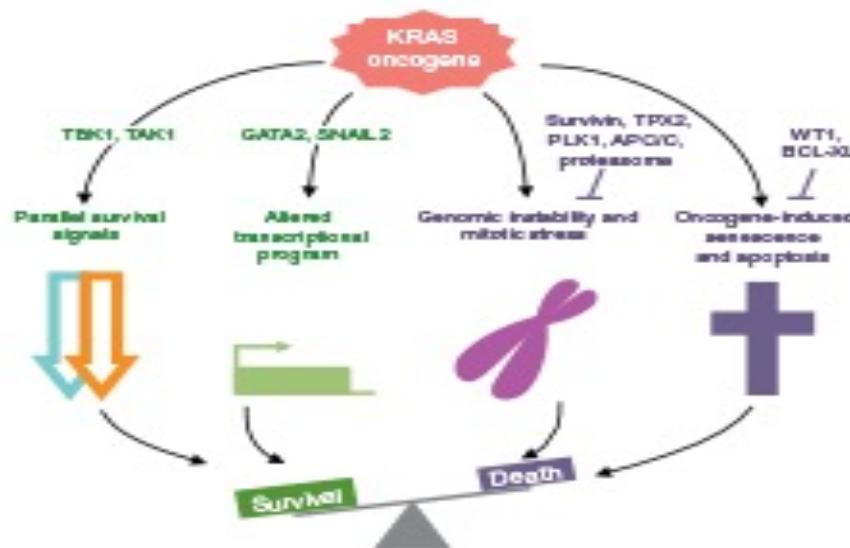
Gene A	Gene B	Viability
WT	WT	✓
WT	Loss	✓
Mutant	WT	✓
Mutant	Loss	✗

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

(Hu & Luo, *The Enzymes* 2014)

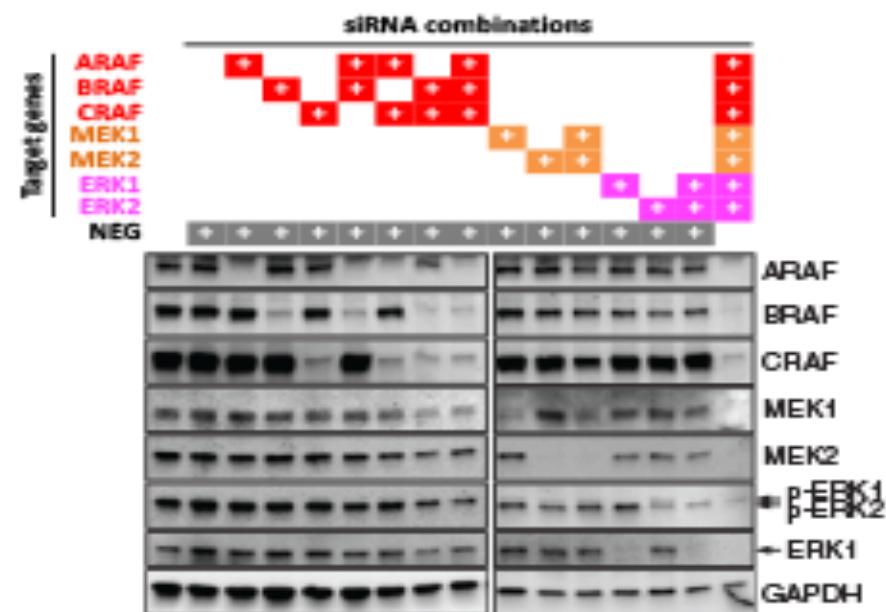
Dissecting the contribution

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

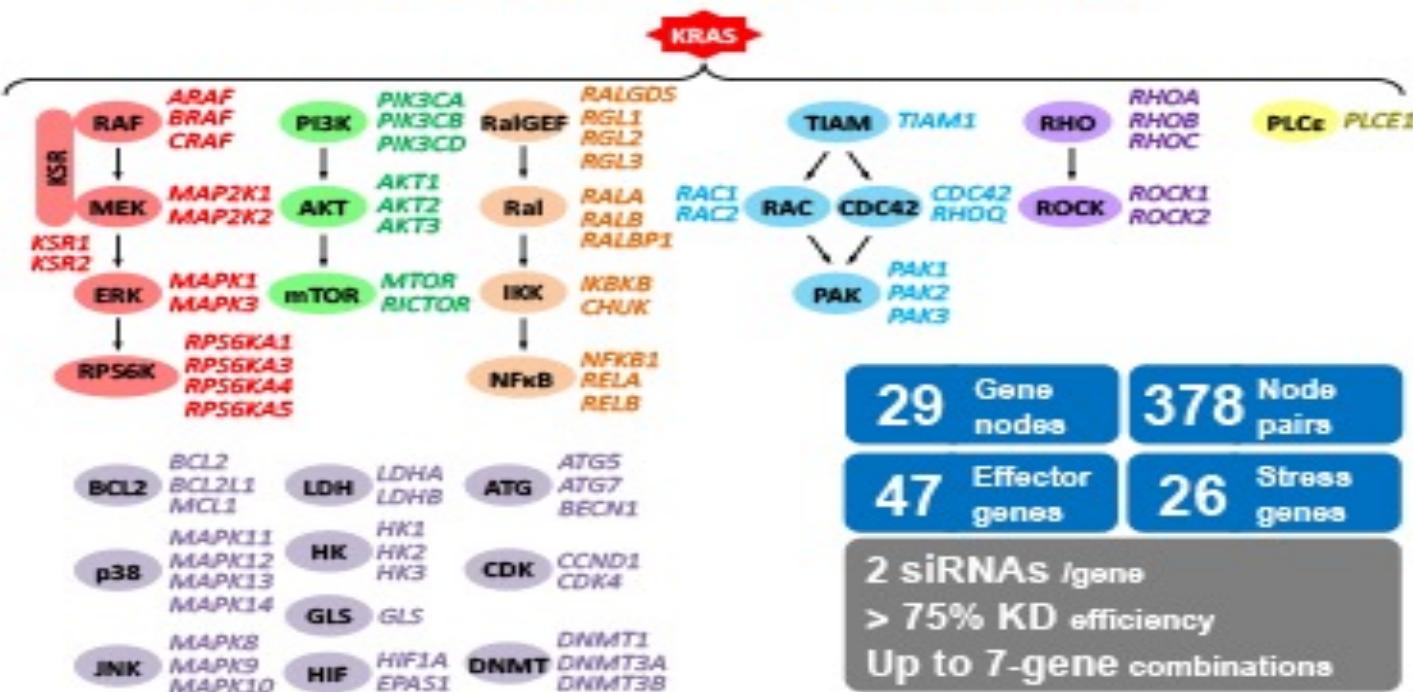
A Combinatorial siRNA Platform to Co-targeting Multiple Genes And Evaluate Target Combinations



(Yuan et al. Cancer Discovery 2014)

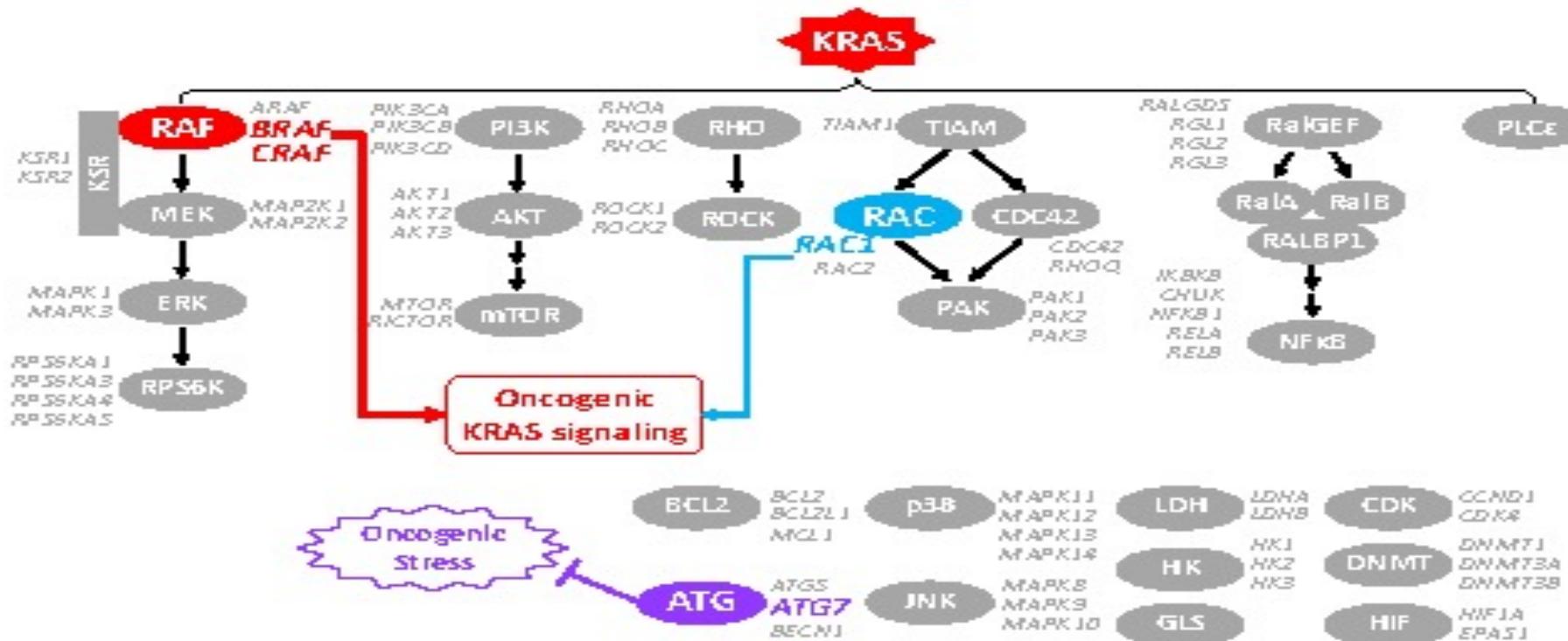
KRAS addiction

Mechanism of KRAS Addiction Through Ras Effector and Stress Response Pathways



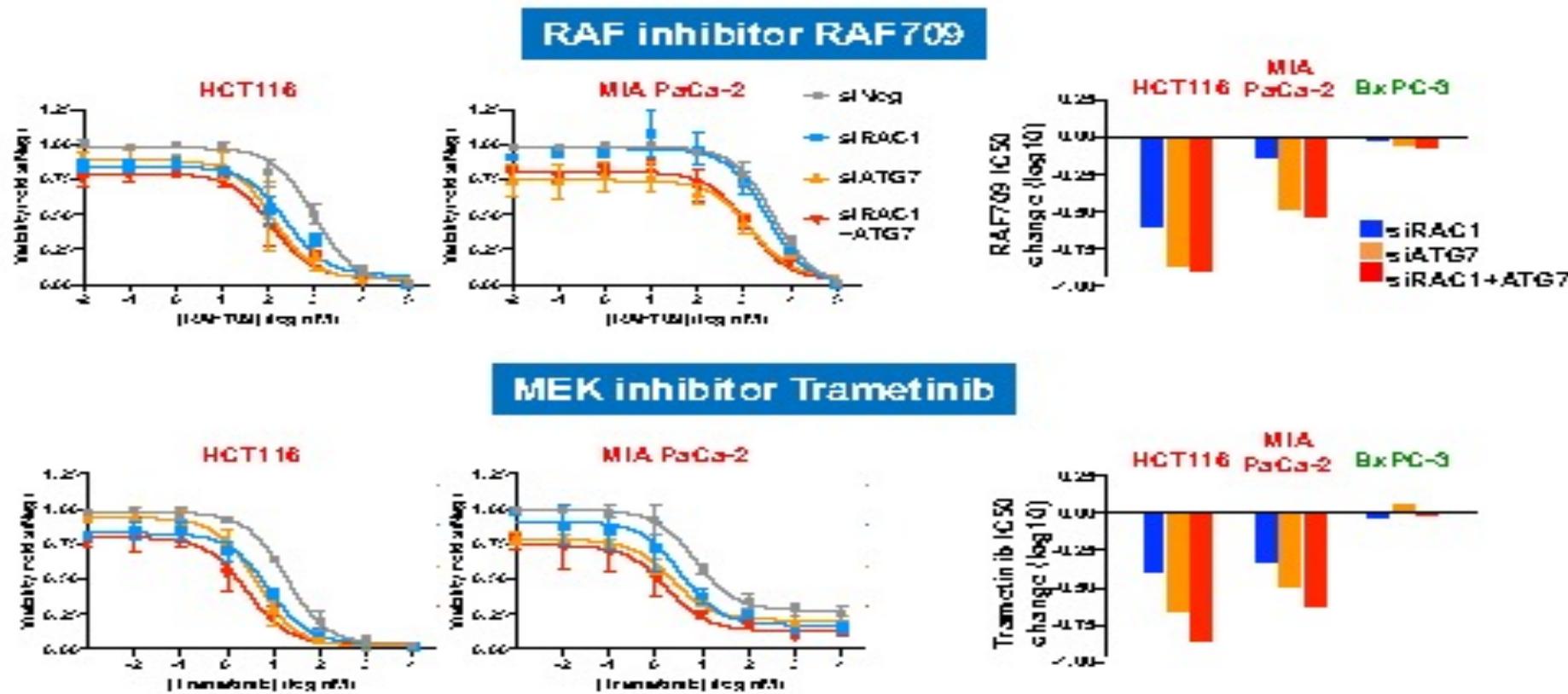
Autophagy pathways

RAF, RAC & autophagy pathways are critical mediators of KRAS oncogene addiction



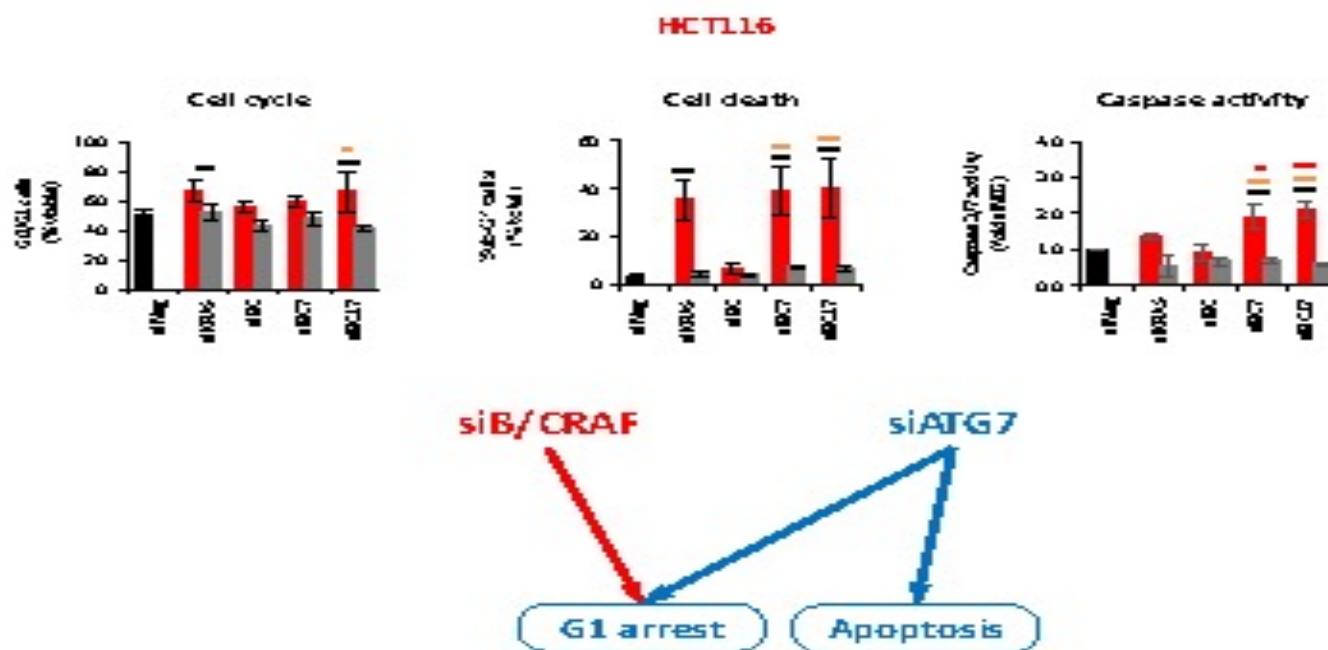
MAPK inhibitors

RAC1 and ATG7 knockdown sensitizes KRAS mutant cells towards MAPK pathway inhibitors



Cell cycle arrest

RAF and ATG7 co-depletion enhances cell cycle arrest and cell death in KRAS mutant cells



Co-targeting

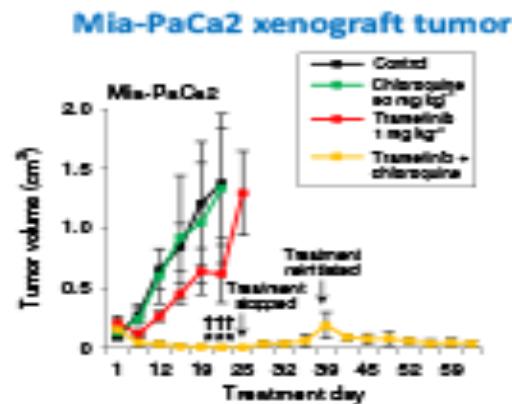
Co-targeting the MAPK and Autophagy Pathway In KRAS Mutant Pancreatic Cancer Cells

Kinsey ... McMahon (*Nature Medicine* 2019)

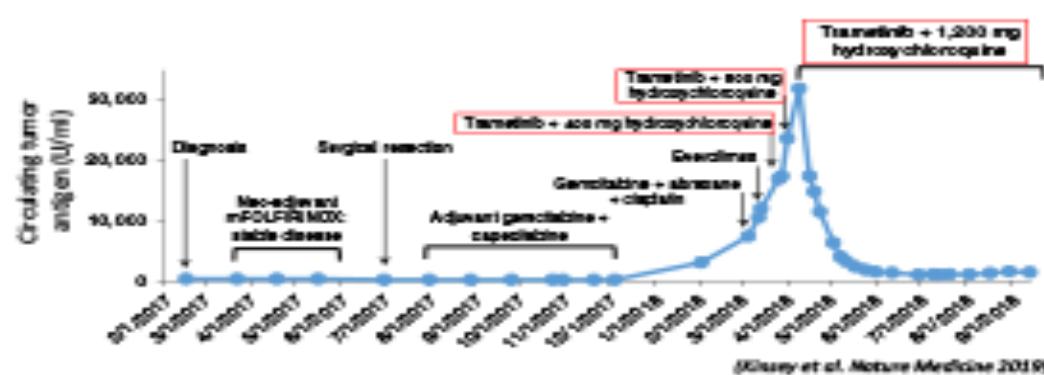
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

Bryant ... Der, (*Nature Medicine* 2019)

Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer



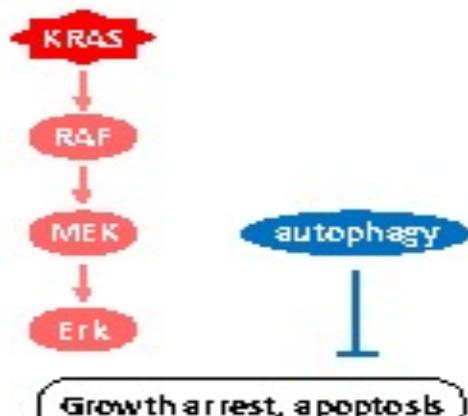
KRAS mutant pancreatic cancer patient



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

Clinical trials

Pharmacological inhibition of MEK and autophagy in clinical trials for patients with KRAS mutant cancer



Trial ID	Disease	Drug combination
NCT04132505 [Phase 1]	KRAS mutant metastatic pancreatic cancer	Biimetinib (MEK1) Hydroxychloroquine (autophagy)
NCT04566133 [Phase 2]	KRAS mutation refractory glioblastoma carcinoma	Tafamidis (MEK1) Hydroxychloroquine (autophagy)
NCT04214418 [Phase 1/2]	KRAS mutant advanced malignancies	Coelenteritinib (MEK1) Hydroxychloroquine (autophagy) Anezoltumab (ICB)
NCT03979661 [Phase 1]	NRAS mutant melanoma	Tafamidis (MEK1) Hydroxychloroquine (autophagy)
NCT03825289 [Phase 1]	Pancreatic cancer	Tafamidis (MEK1) Hydroxychloroquine (autophagy)
NCT04386057 [Phase 2]	Pancreatic cancer	LY3214996 (ERK1) Hydroxychloroquine (autophagy)

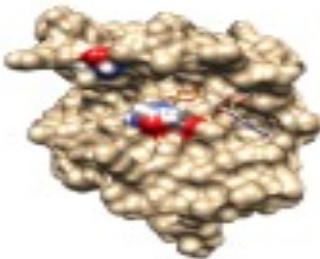
Orthogonal drug combinations

Orthogonal drug combinations for KRAS mutant cancer



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