Ras oncogene

The Ras Oncogene

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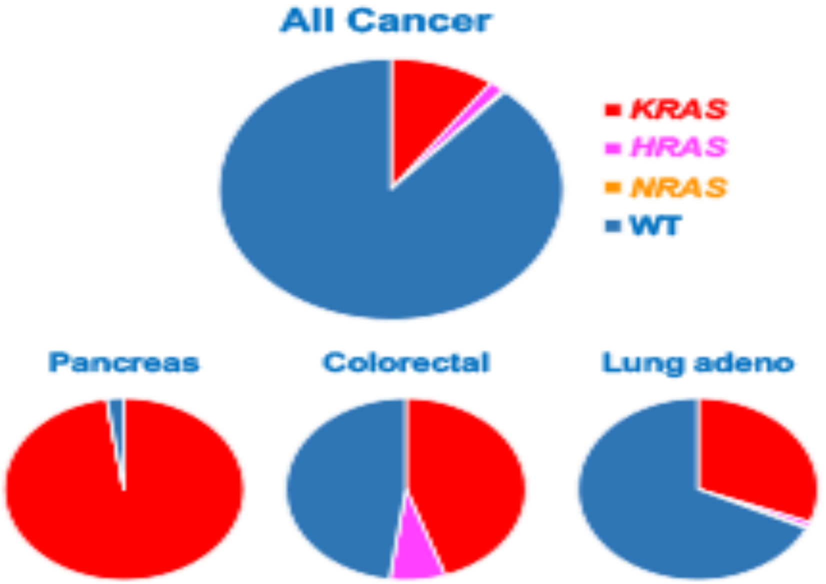
Research goals

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

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

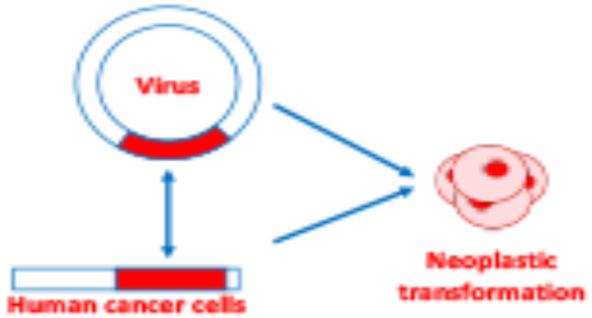
Molecular Characterization of the Viral Ras Protein

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

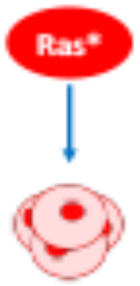
Human Ras Oncogene Has a Point Mutation

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 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

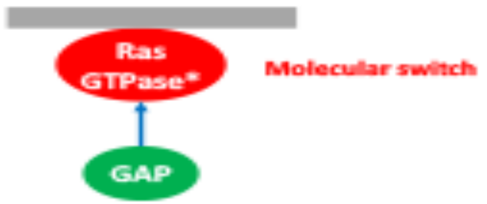


Neoplastic transformation

Biochemical properties

Biochemical Properties of the Ras Protein

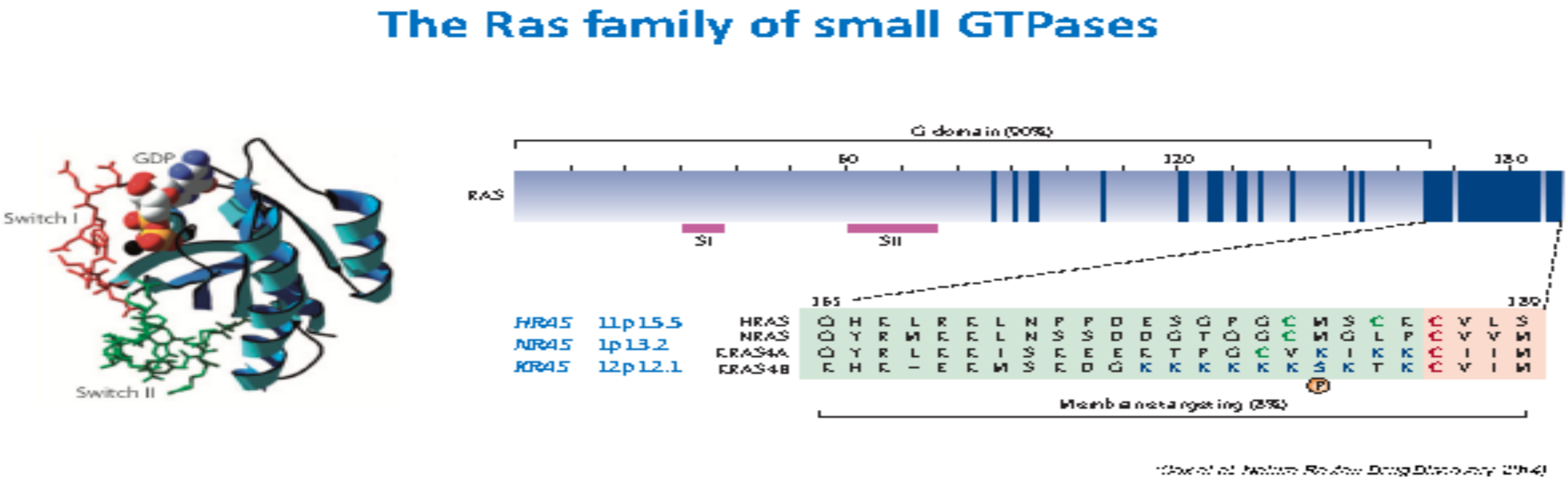
1984	***Willumsen & Lowy (Nature, 1984)*** The p21 ras C-terminus is required for transformation and membrane association
1984 1985	***Sweet ... Lowy (Nature, 1984)*** The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity ***Gibbs ... Scolnick (PNAS,1984)*** Intrinsic 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 signaling

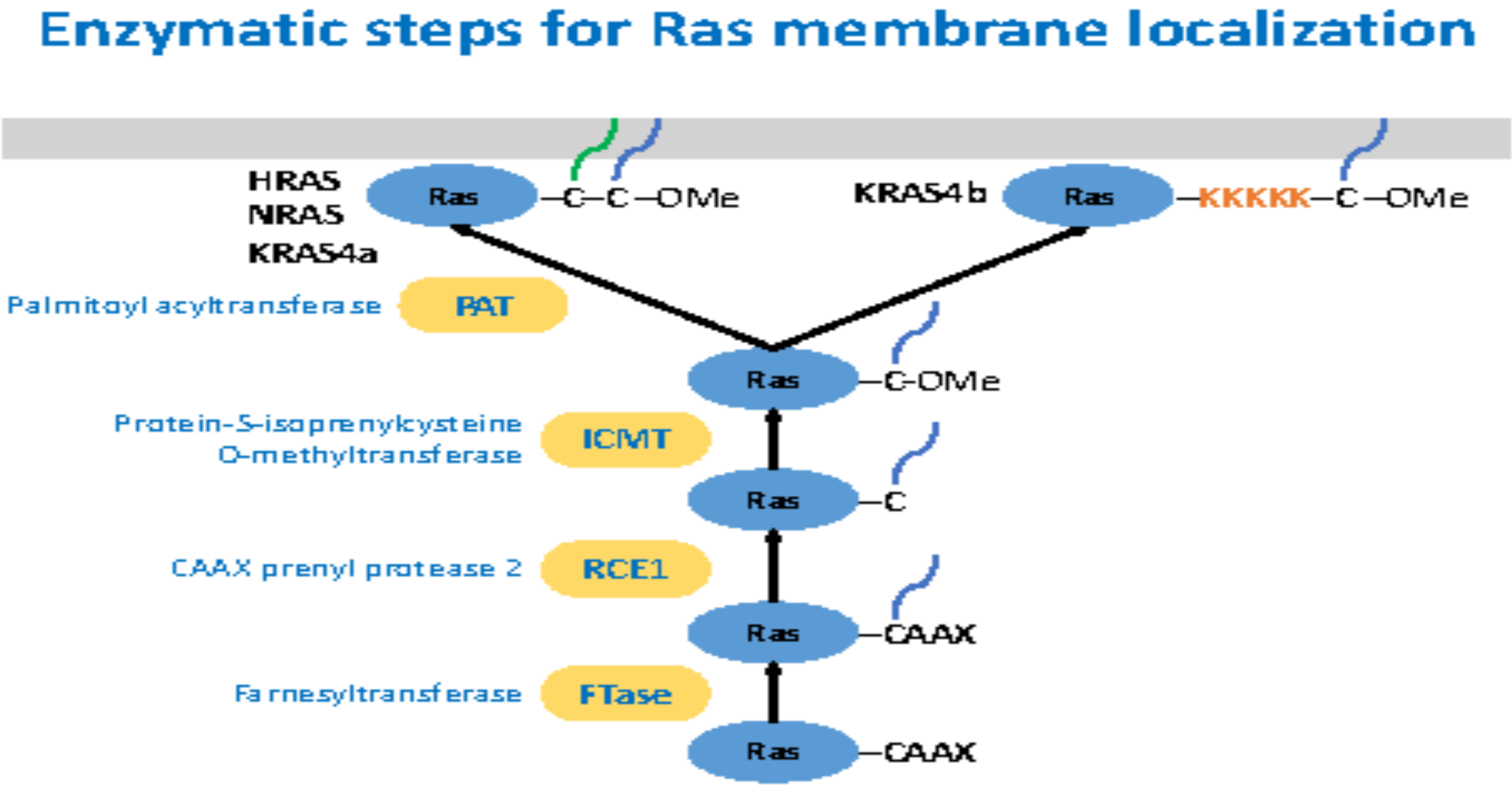
Ras signaling pathway

RAS GTPases

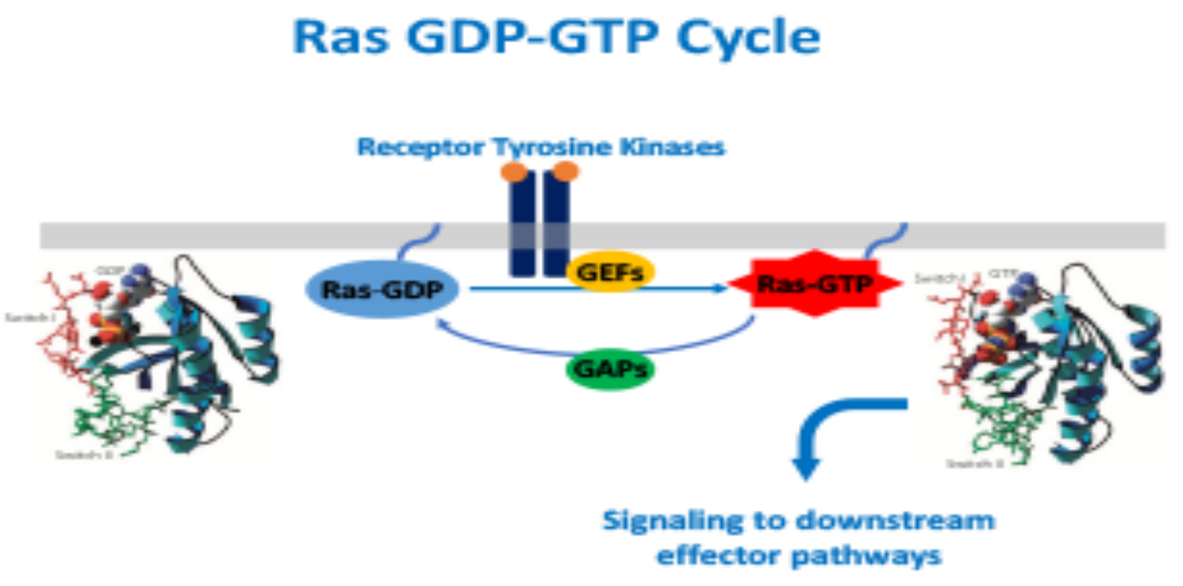


The Ras family of small GTPases
GDP
Switch I
Switch II
60
120
180
RAS
SI
SII
HRAS 11p15.5
NRAS 1p13.2
KRAS 12p12.1
HRAS
NRAS
KRAS4A
KRAS4B

Ras membrane localization

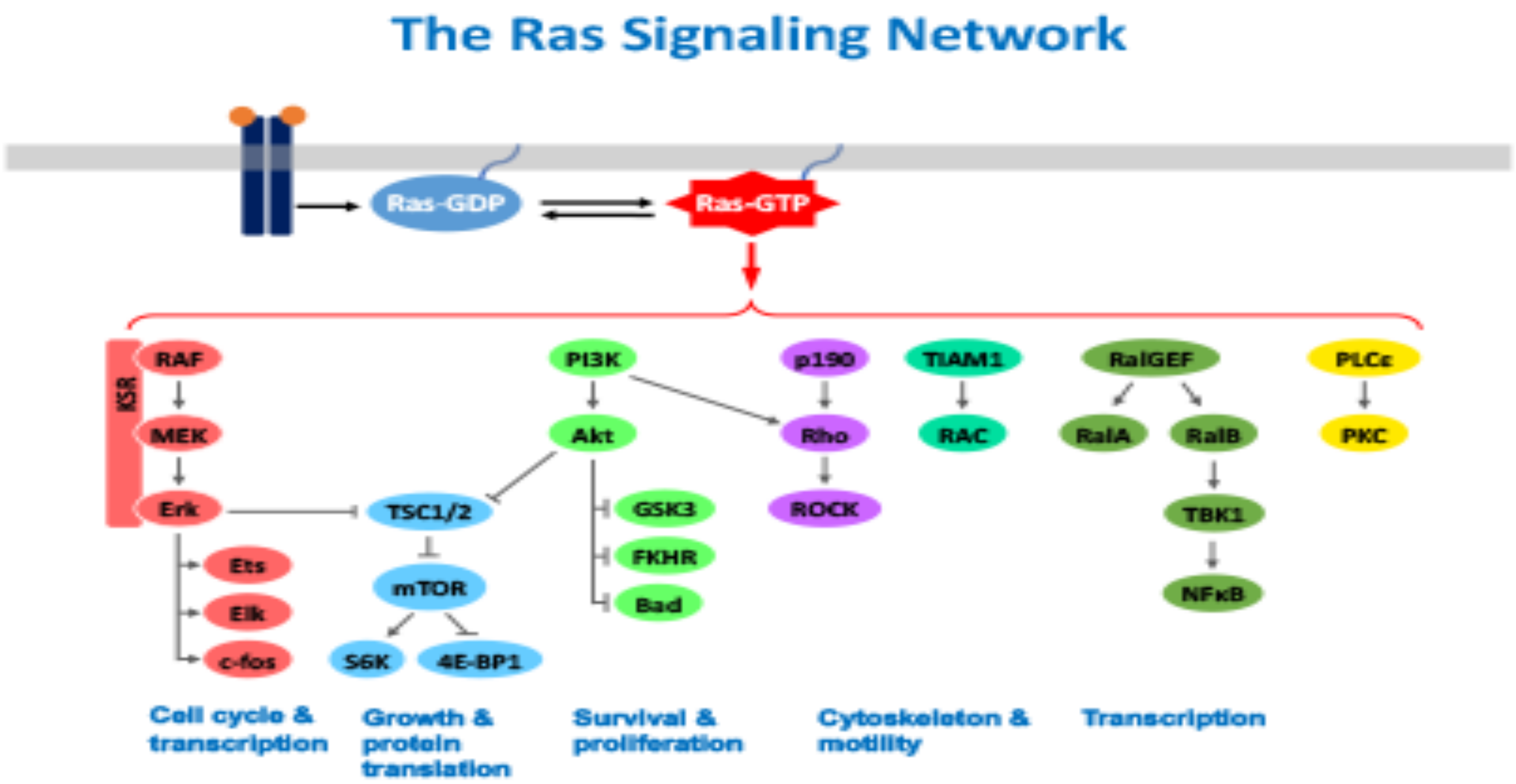


Ras GDP-GTP Cycle



(Modified from Karnoub & Weinberg, 2008)

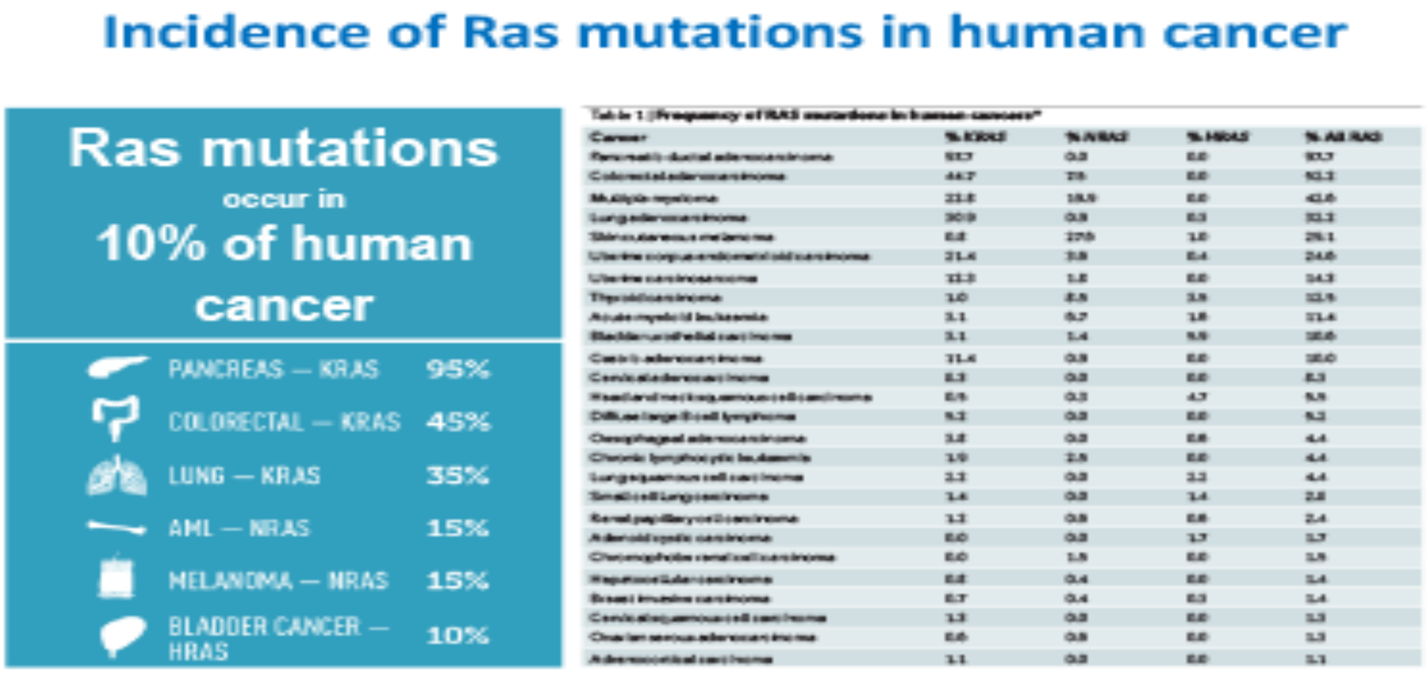
Ras signaling network



RAS mutations

Ras mutations in human cancer

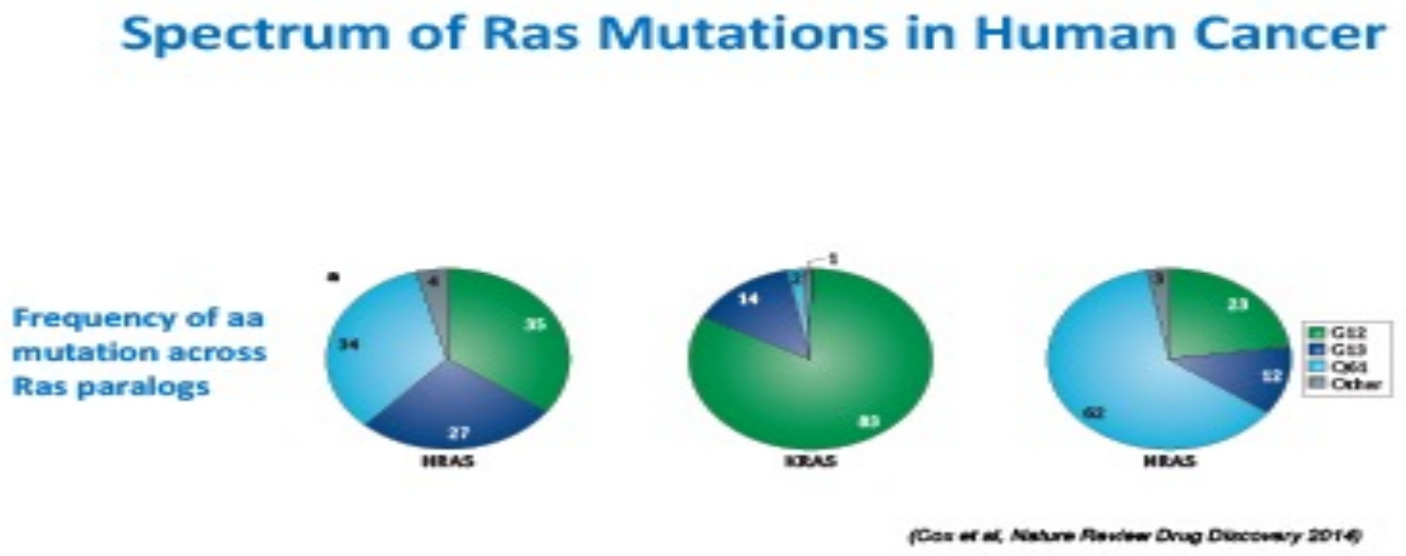
Ras mutations



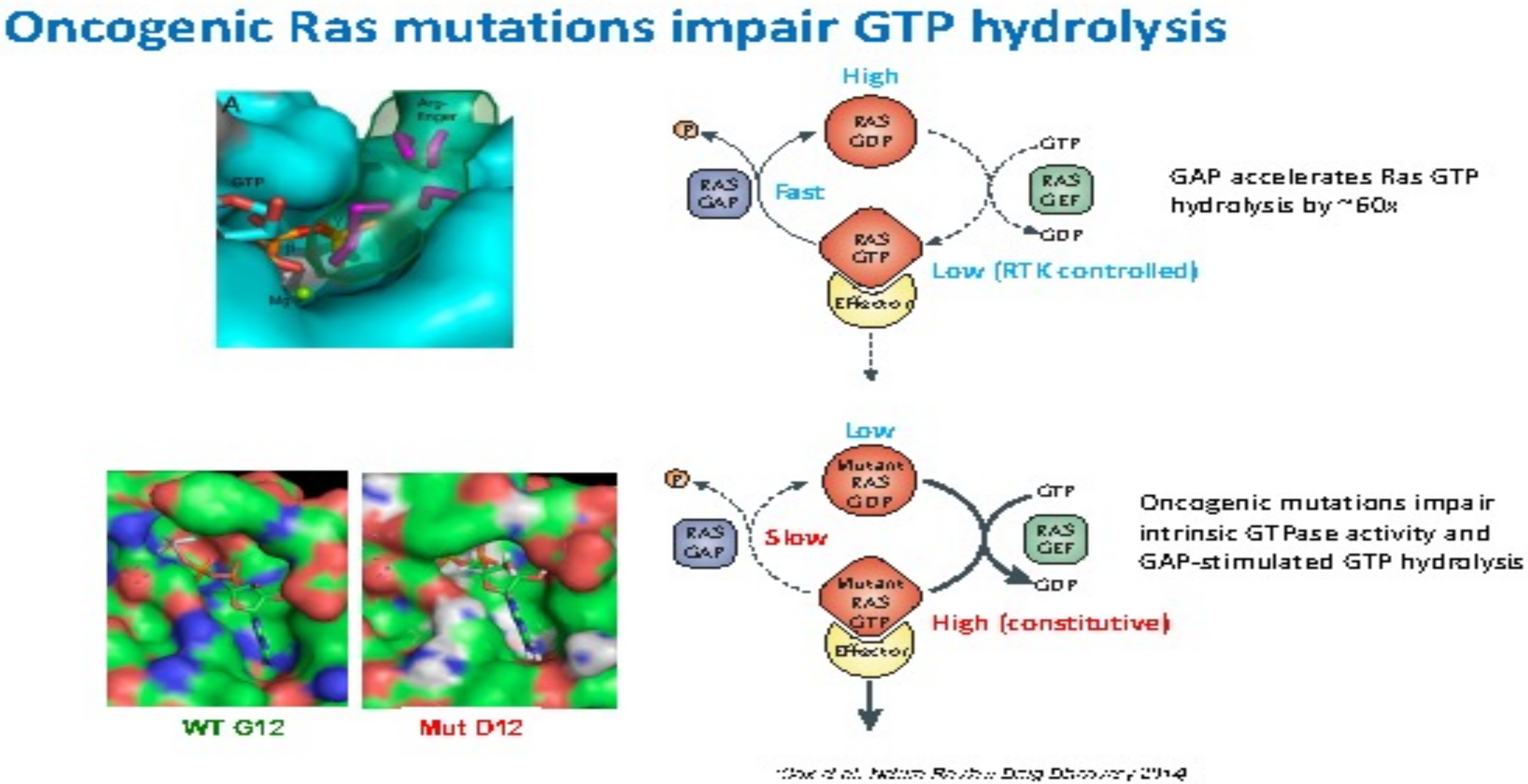
(NCI Ras Initiative)

(Cox et al, Nature Review Drug Discovery 2014)

Mutation spectrum

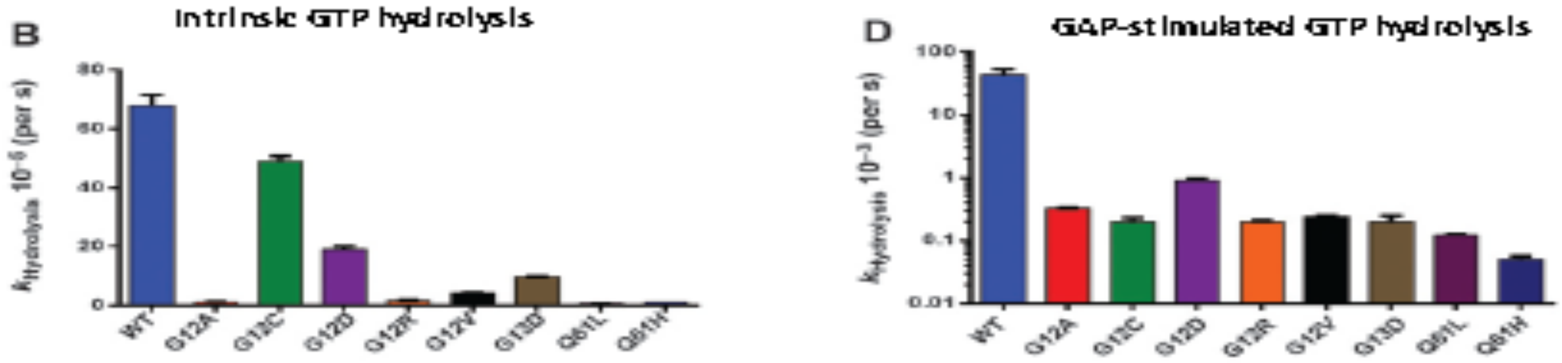


GTP hydrolysis impairment



Ras mutations

Oncogenic Ras mutations impair GTP hydrolysis



(Hunter et al. Mol Can Res 2015)

- 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

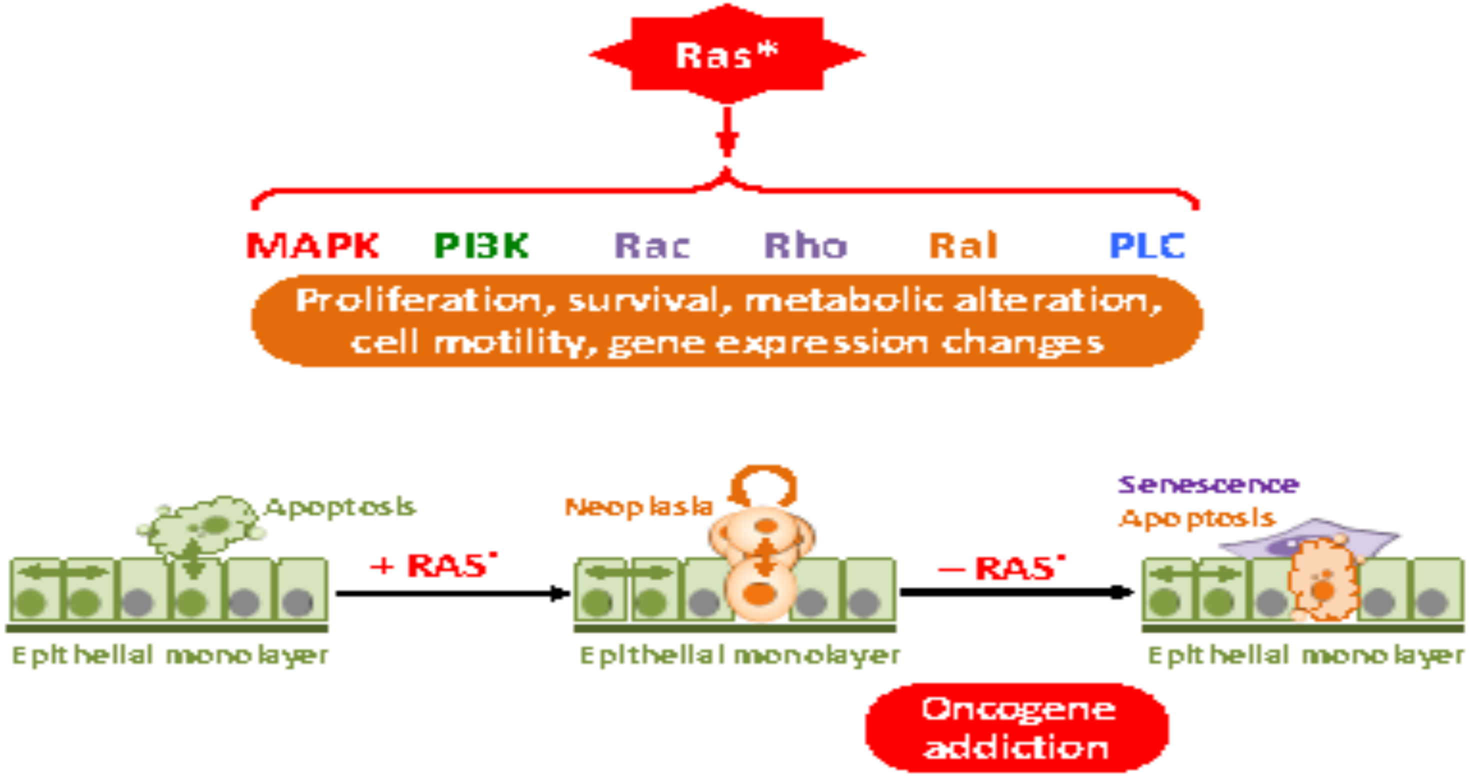
KRAS and adenocarcinomas

KRAS is a major oncogenic driver in adenocarcinomas

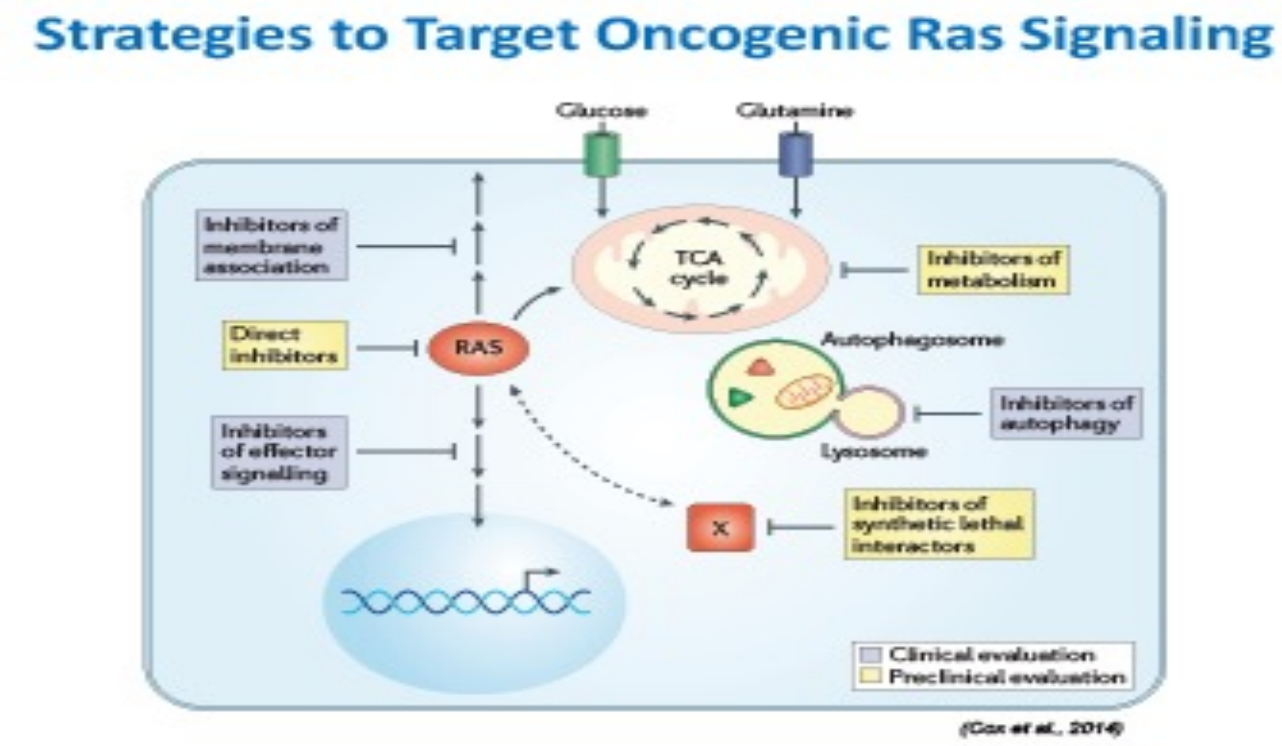
	Early Neoplasia	Adenoma	Adeno-carcinoma
Lung	**KRAS**	**KRAS** TP53	**KRAS** TP53 LKB1 ...
Pancreas	**KRAS**	**KRAS** CDKN2A	**KRAS** CDKN2A TP53 ...
Colon	APC	APC **KRAS**	APC **KRAS** TP53 ...

Oncogene addiction

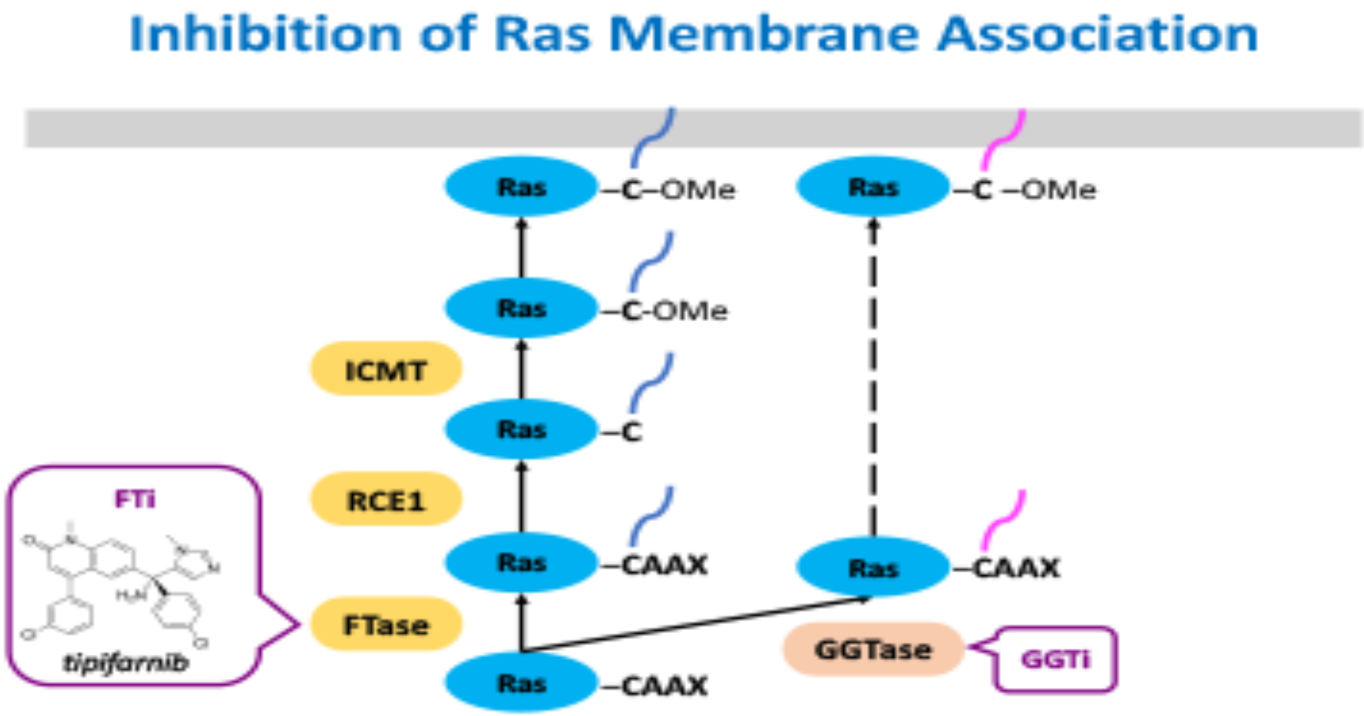
Oncogenic Ras signaling leads to neoplastic transformation and oncogene addiction



Ras signaling



Ras membrane association

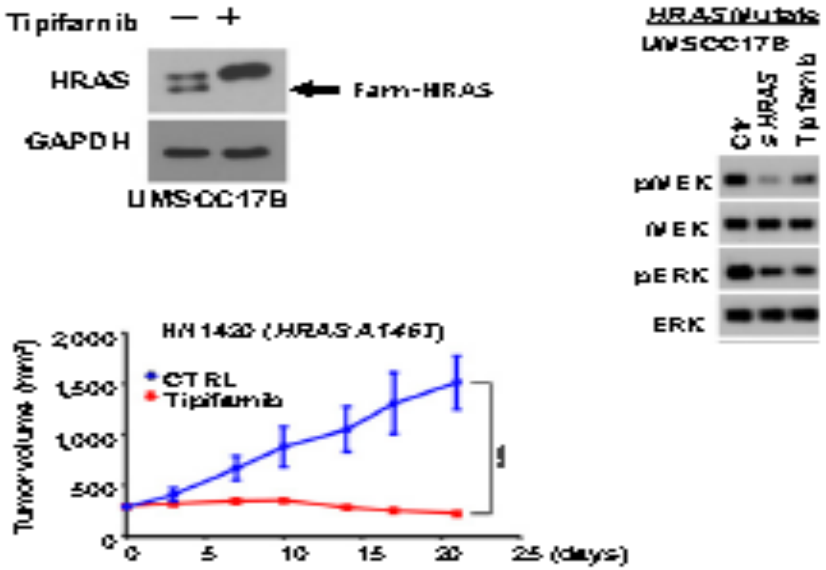


- Alternative membrane localization pathway
- Lack of KRAS selectivity – pathway shared by other small GTPases

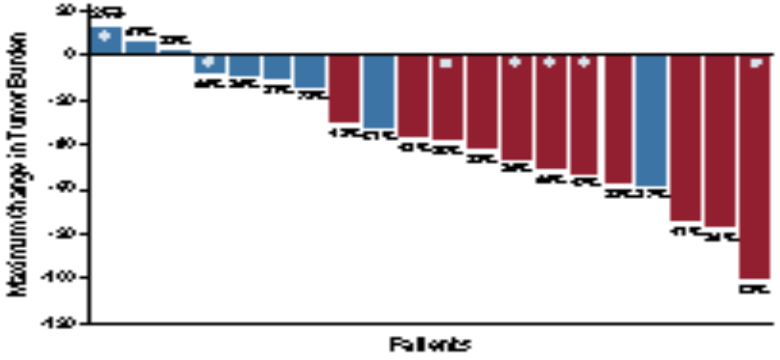
Ftase inhibitor

FTase inhibitor in HRAS mutant head and neck cancer

FTase inhibitor (tipifarnib)
Head and neck squamous cell carcinomas with HRAS mutation



(Gilardi et al., Mol Cancer Ther 2020)

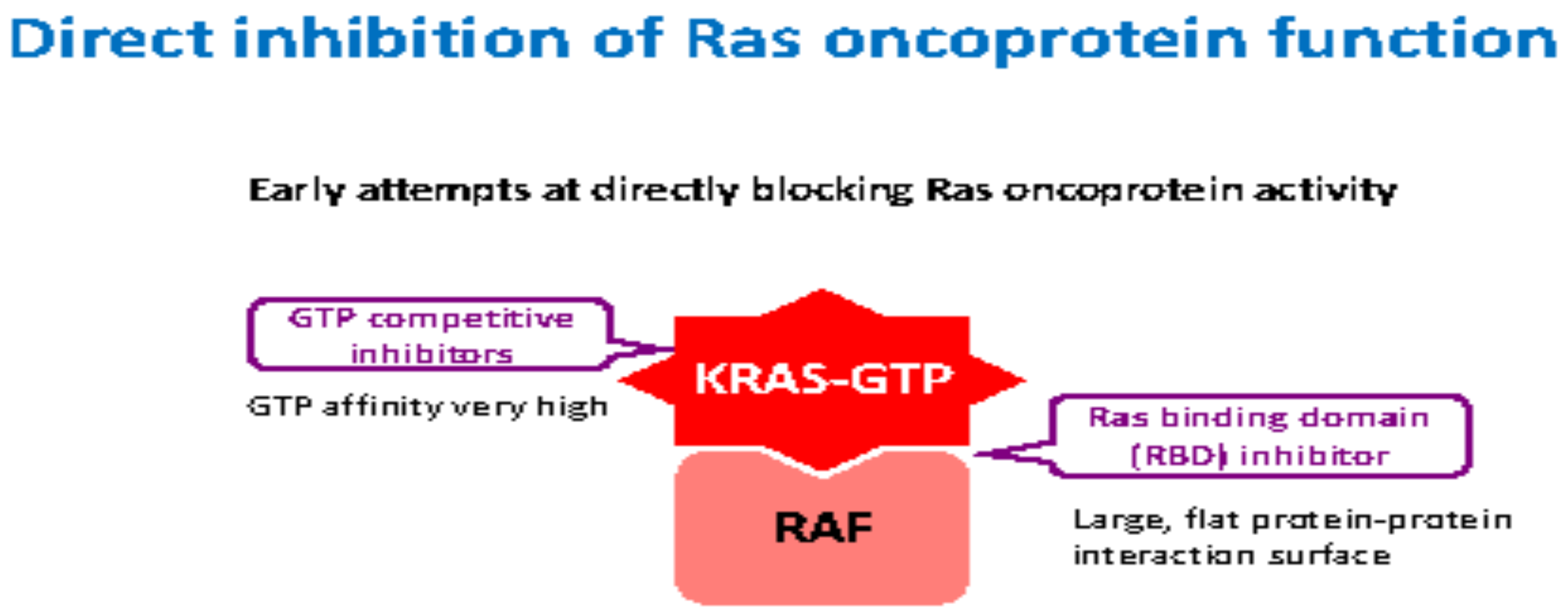


Median PFS = 5.6 mo
Median OS = 15.4 mo

(Ho et al., J Clin Oncol 2021)

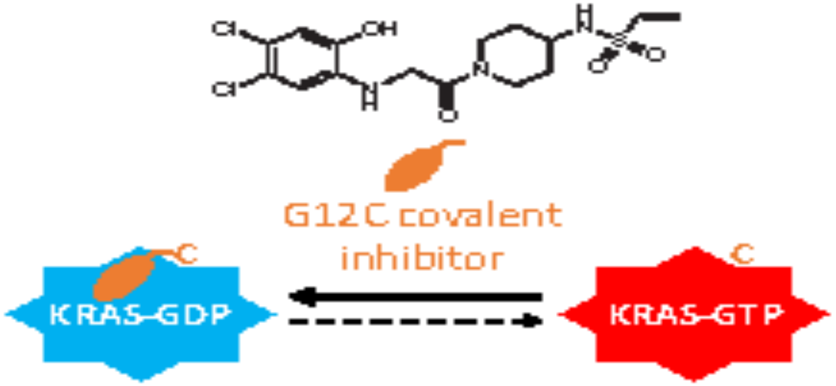
- FDA granted Breakthrough Therapy Designation (Feb 2021)
- Phase III trial on-going

Direct inhibition



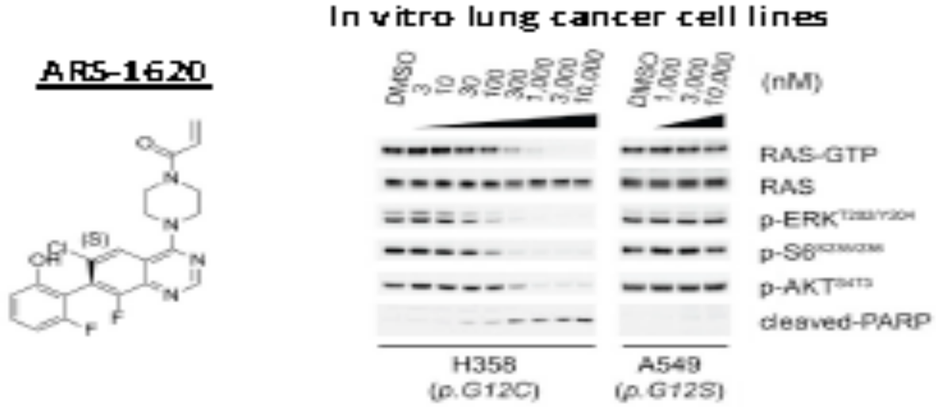
Covalent inhibitors

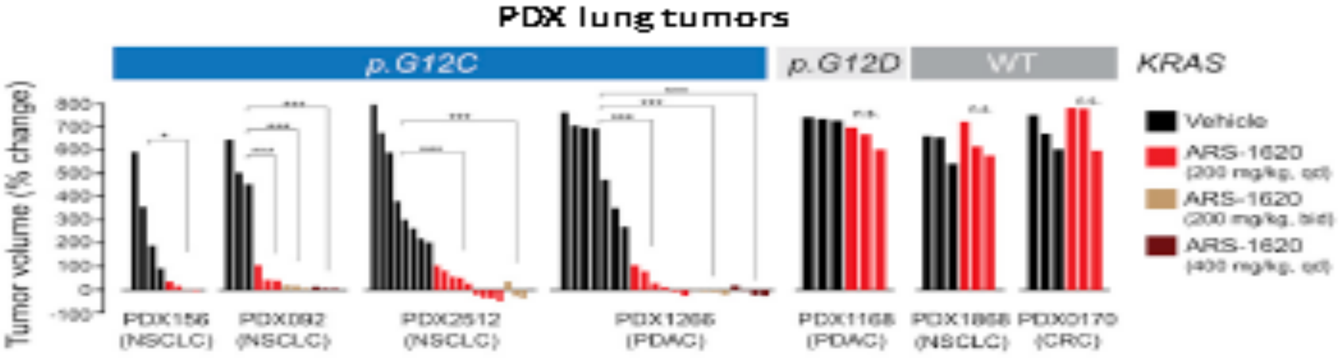
Development of covalent inhibitors against the KRAS-G12C mutant



[Shokat lab, Ostrem 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



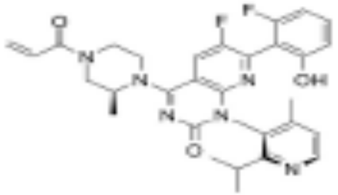


[Janes et al. Cell 2018]

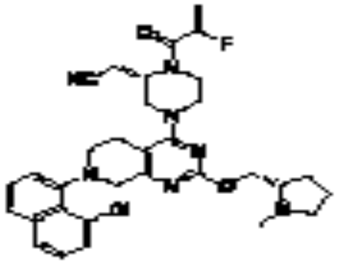
Clinical efficacy

Clinical efficacy of KRAS-G12C inhibitors

Cancer Type	G12C frequency among KRAS mutations
Lung	40%
Pancreatic	8%
Colorectal	3%

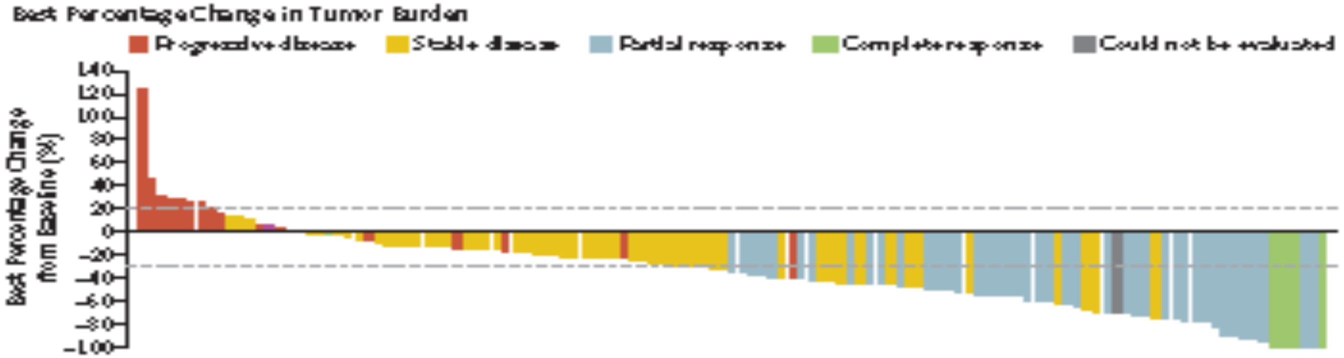


AMG-510
(Sotorasib)



MRTX849
(Adagrasib)

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



Disease control = 80.6%
Objective response rate = 37.1%
Median PFS = 6.8 mo
Media OS = 12.5 mo

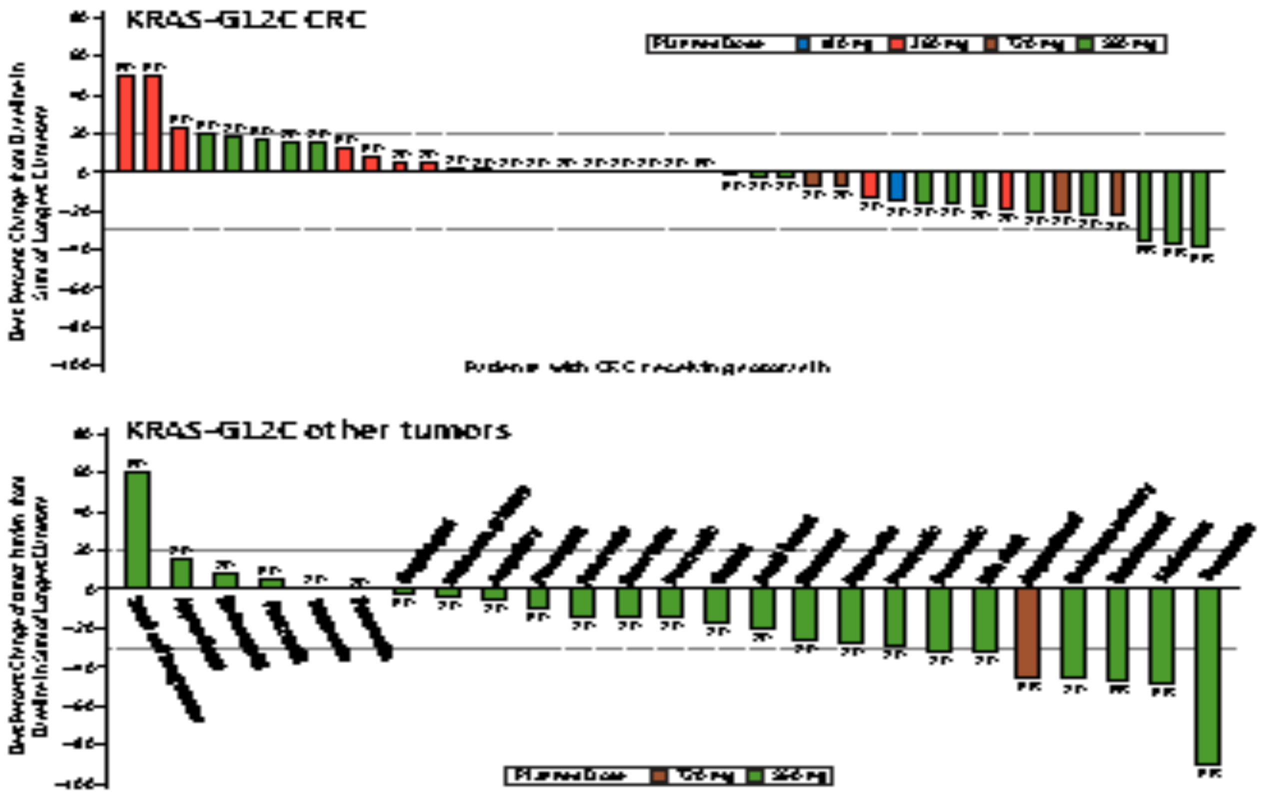
(Skoulidis et al. NEJM 2021)

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

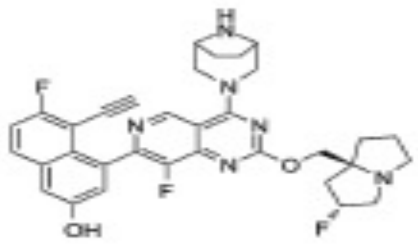


(Canon et al. Nature 2019, Hallin et al. Cancer Discov. 2019, Hong et al. NEJM 2020)

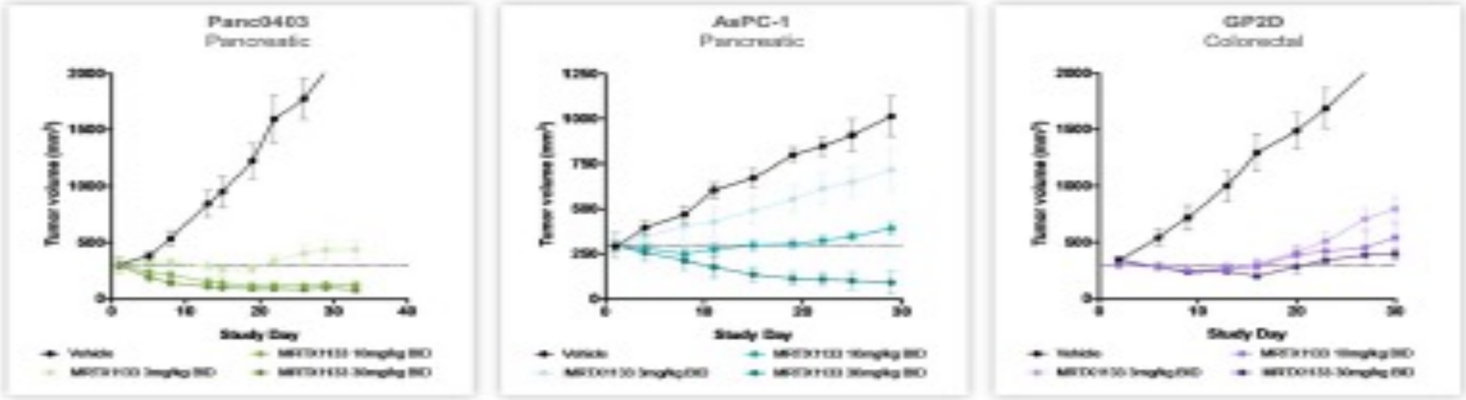
Covalent inhibitors

Covalent inhibitors targeting KRAS-G12D mutant

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



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

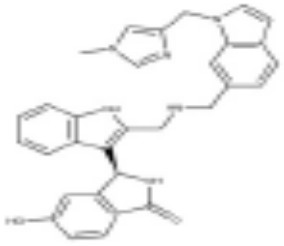


- MRTX1133 demonstrates dose-dependent inhibition of KRAS-dependent signaling, demonstrating tumor regression in G12D mutant tumor models

[Mirati Therapeutics data presentation]

Non-covalent inhibitor

Non-covalent KRAS inhibitors



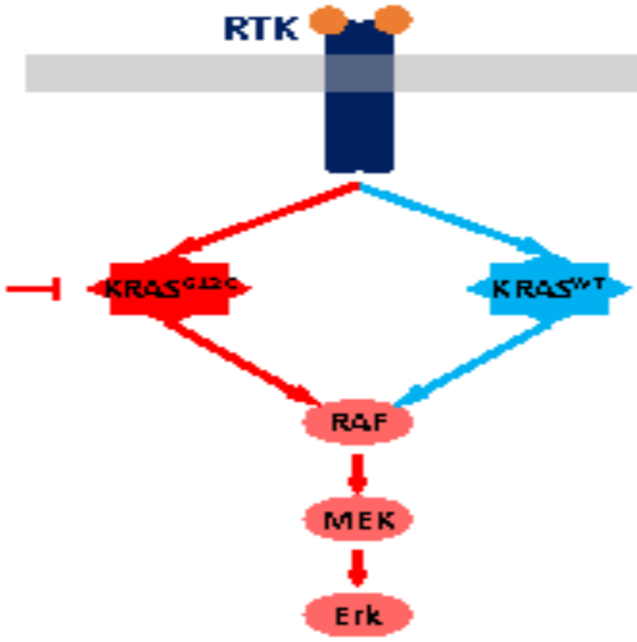
BI-2852

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

[Kessler et al. PNAS 2019, Tran et al. PNAS 2020]

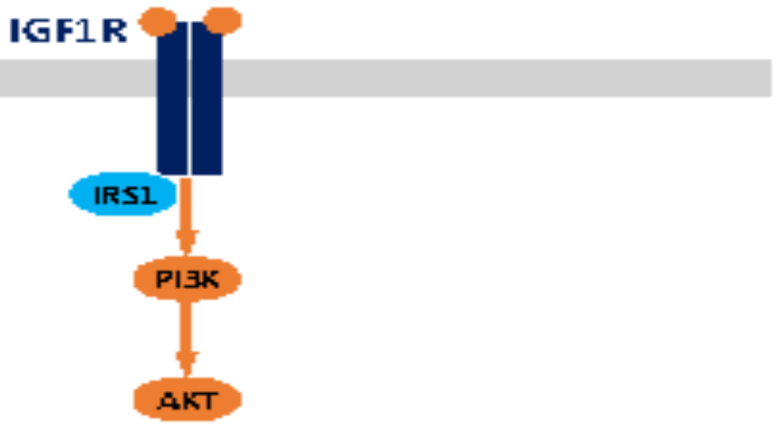
Resistance

Resistance to KRAS-G12C inhibitors: adaptive signaling changes



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]

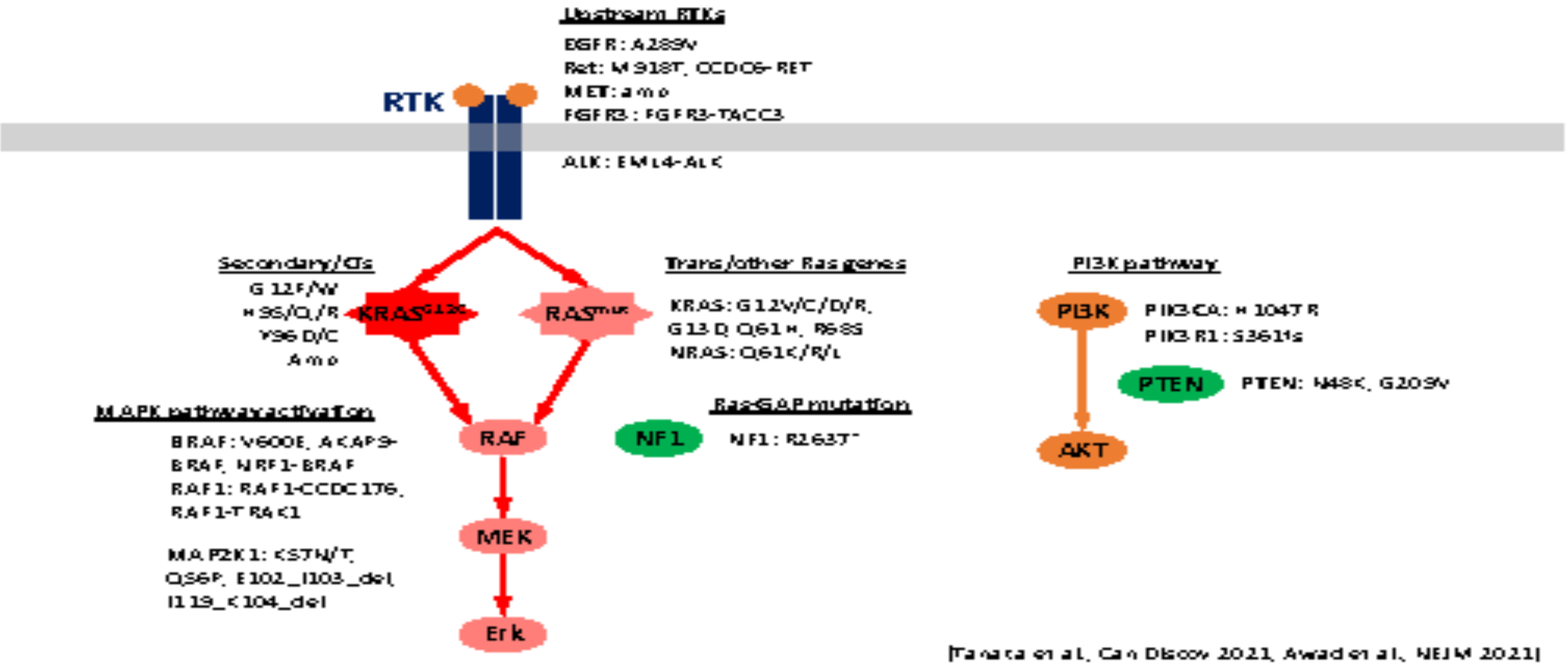


Cell state change: EMT
- Increased IGF1R-PI3K signaling
- Reduced addiction to KRAS oncogenic

[Adachi et al., Clin Can Res 2020]

Resistance

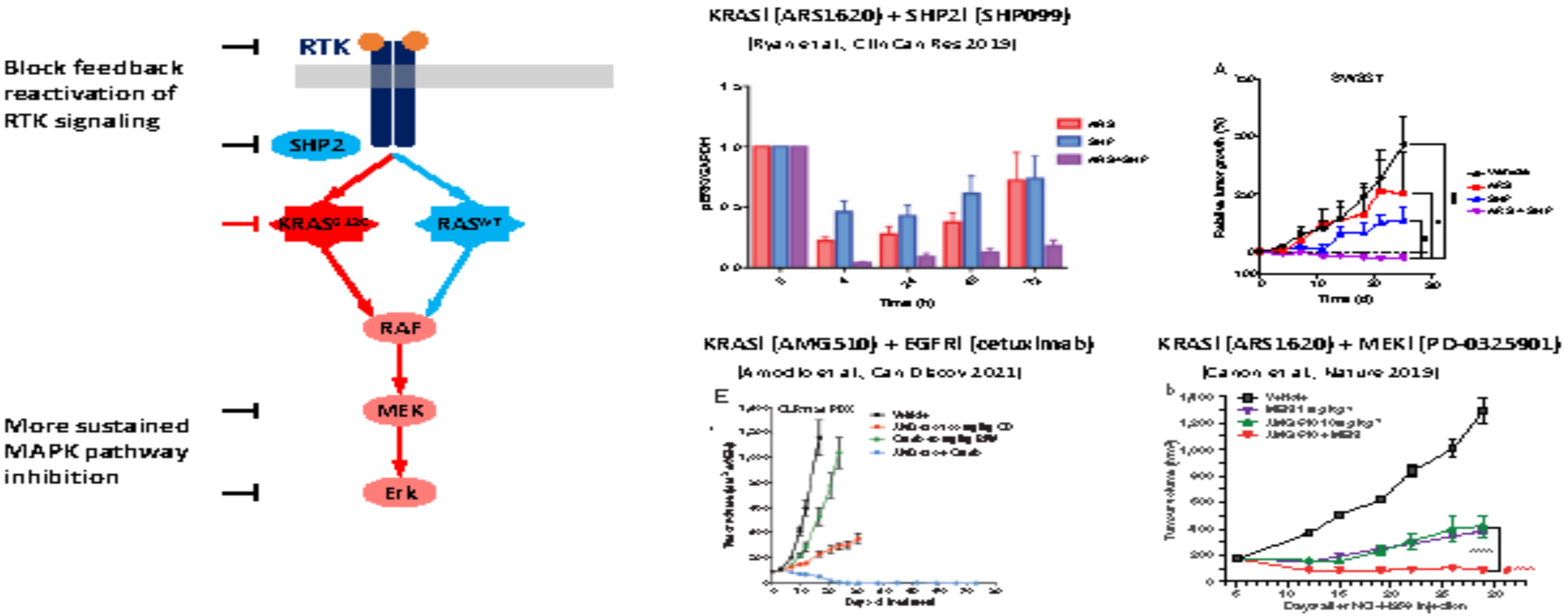
Resistance to KRAS-G12C inhibitors: clinically acquired mutations



[Tanaka et al., Can Discov 2021, Awad et al., NEJM 2021]

Combination therapy

Combination therapy to improve KRAS-G12C inhibitor efficacy



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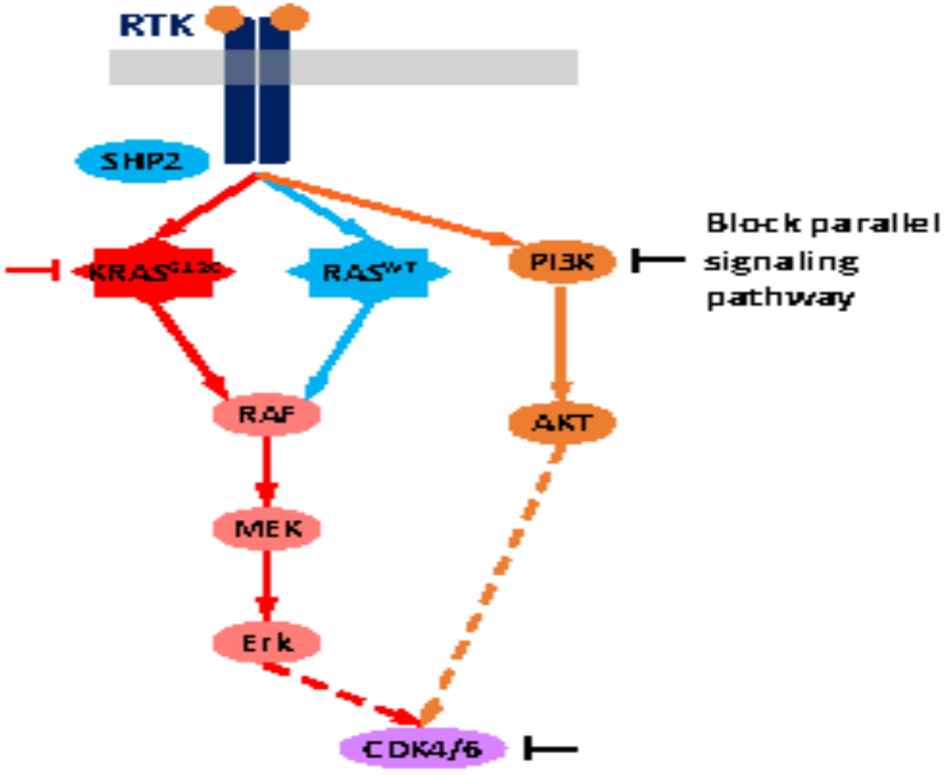
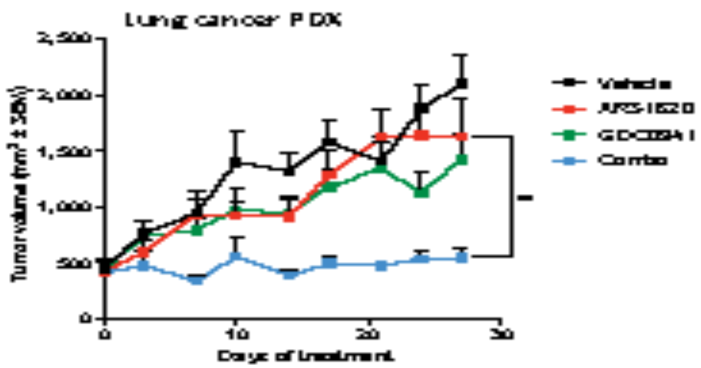
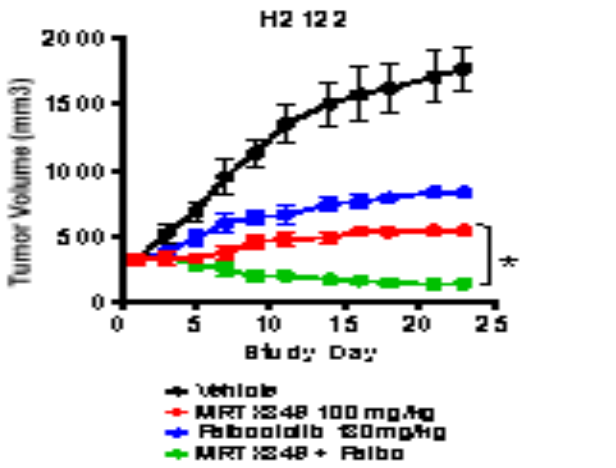


KRASi (ARS1620) + PI3Ki (GDC0941)

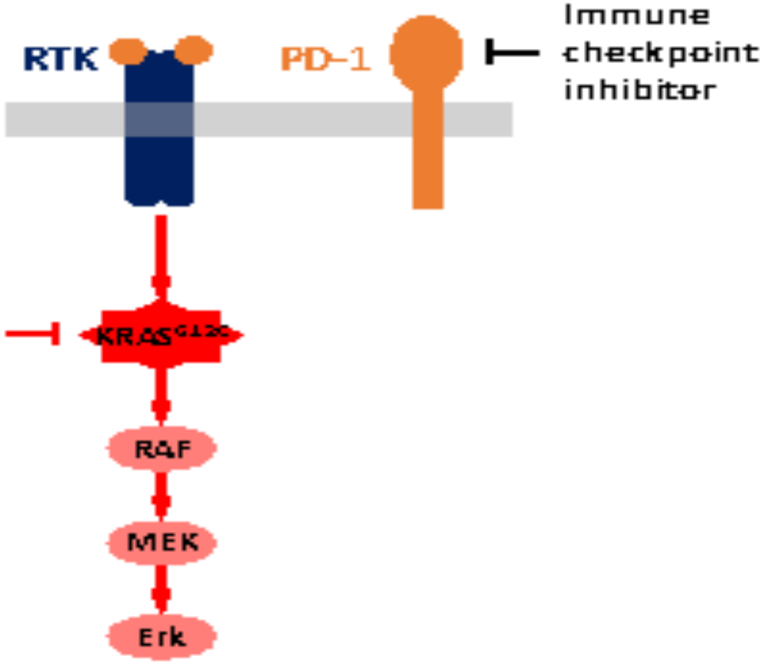


KRASi (ARS1620) + CDK4/6i (Palbociclib)

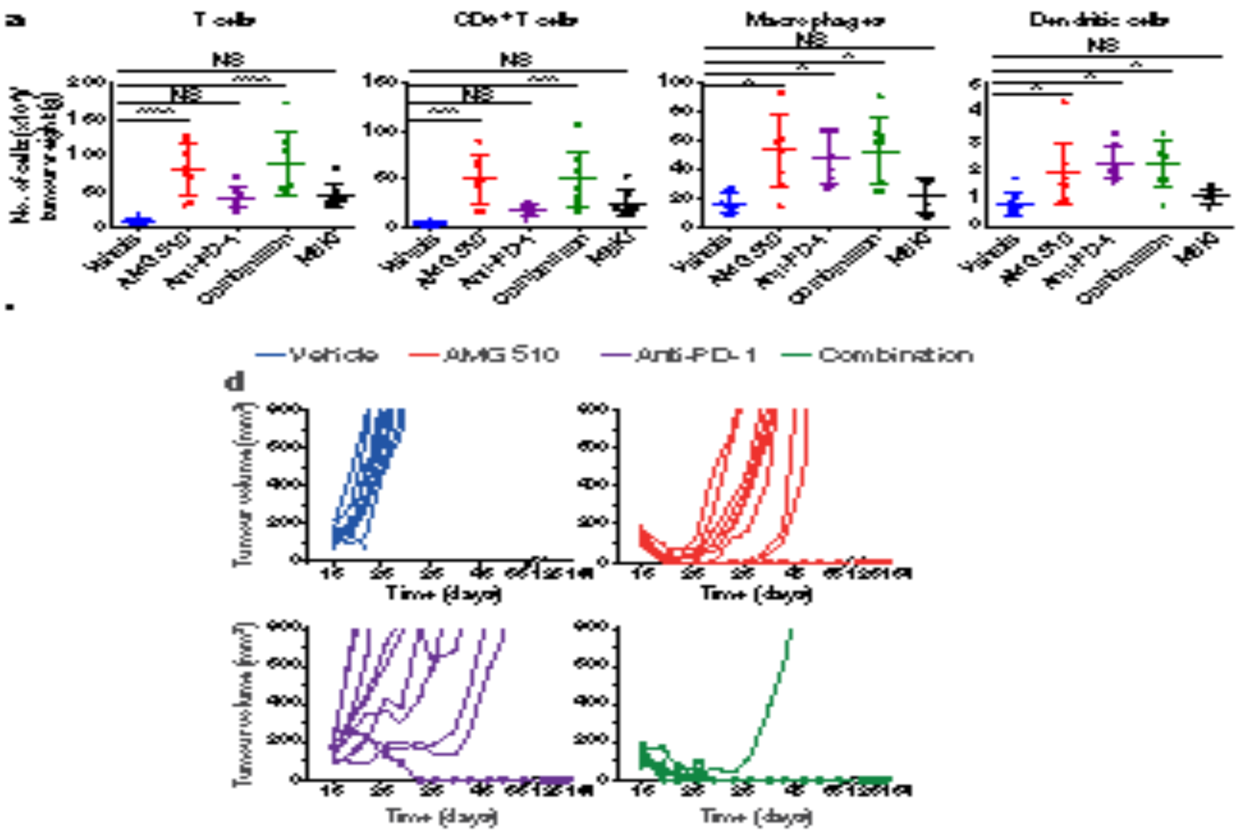


Combination therapy

Combination therapy to improve KRAS-G12C inhibitor efficacy



KRASi (AMG510) + anti-PD-1
(Canon et al., Nature 2019)



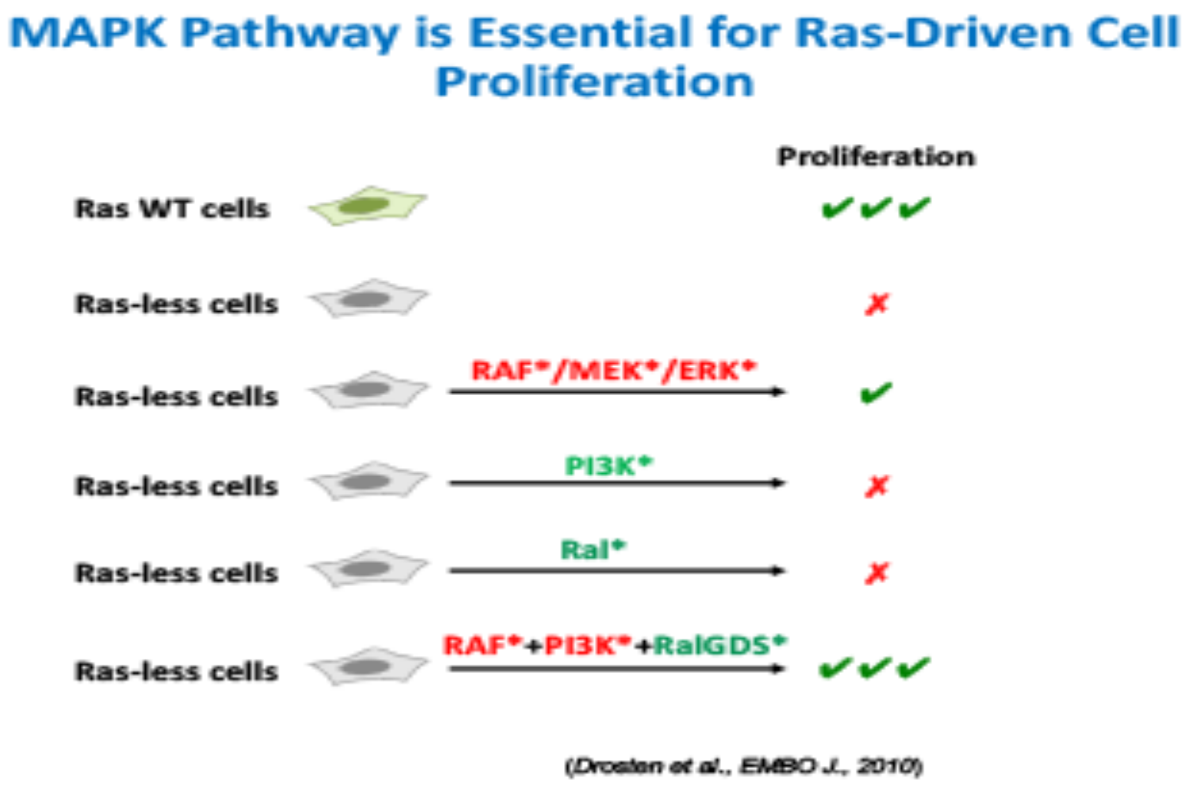
RAS signaling network

Targeting Ras and its signaling network

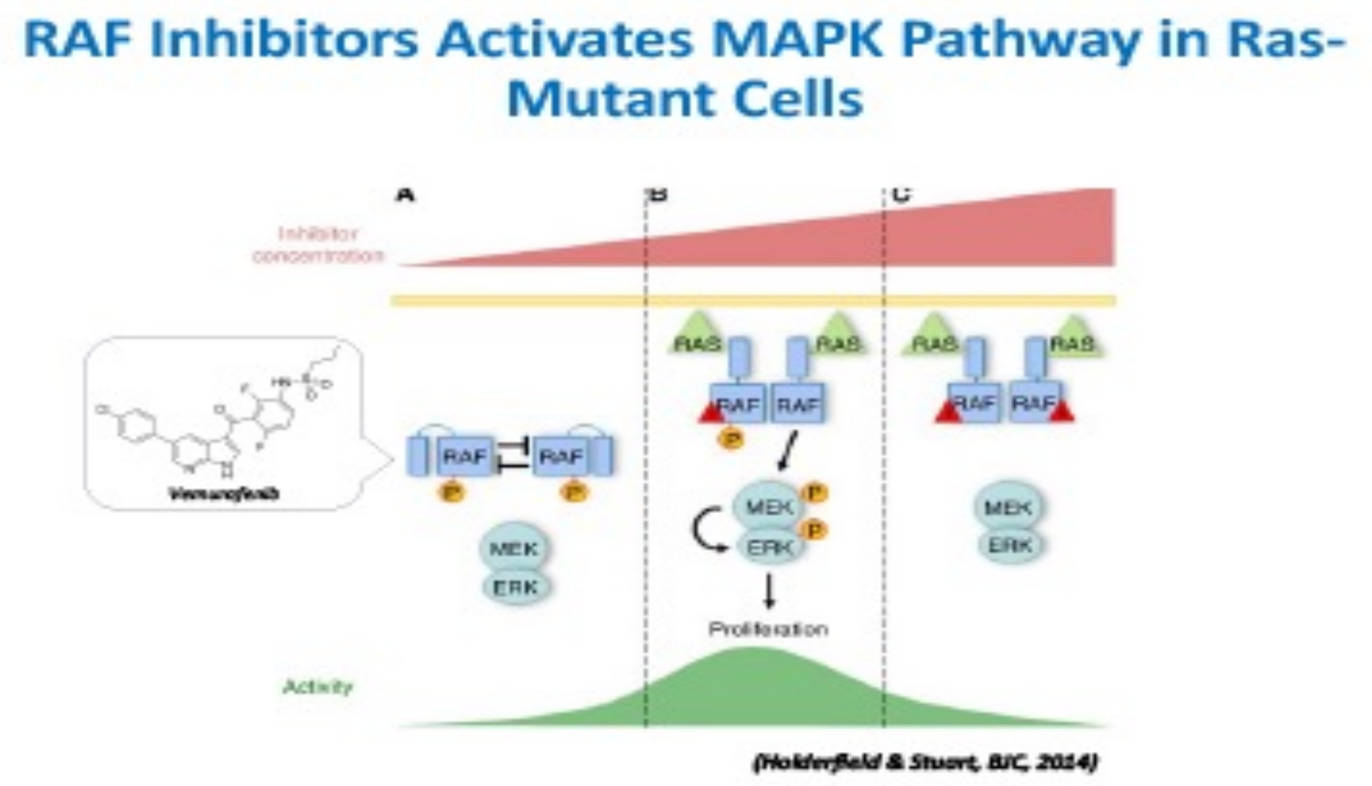
Kinase inhibitors

Kinase Inhibitors Targeting Ras Effectors

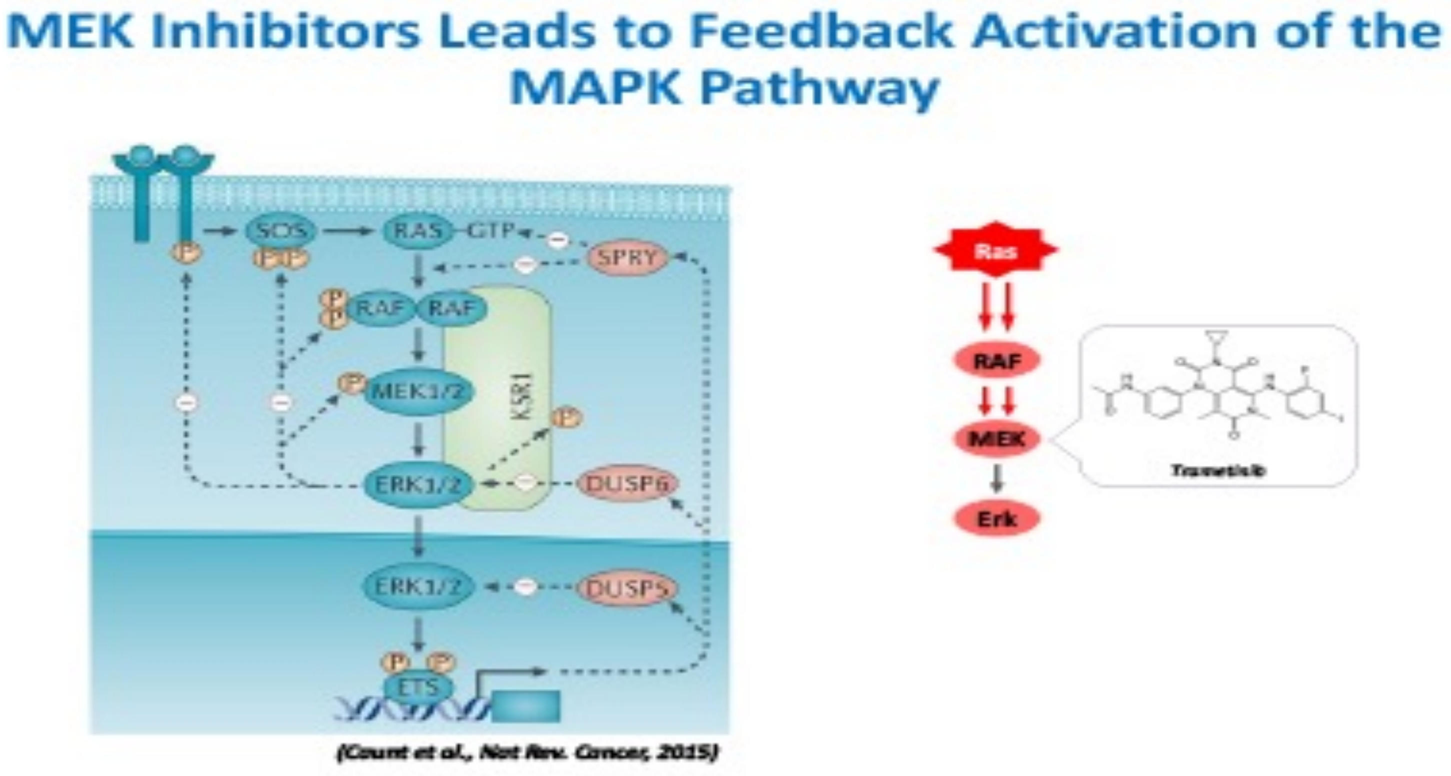
MAPK pathway



MAPK pathway



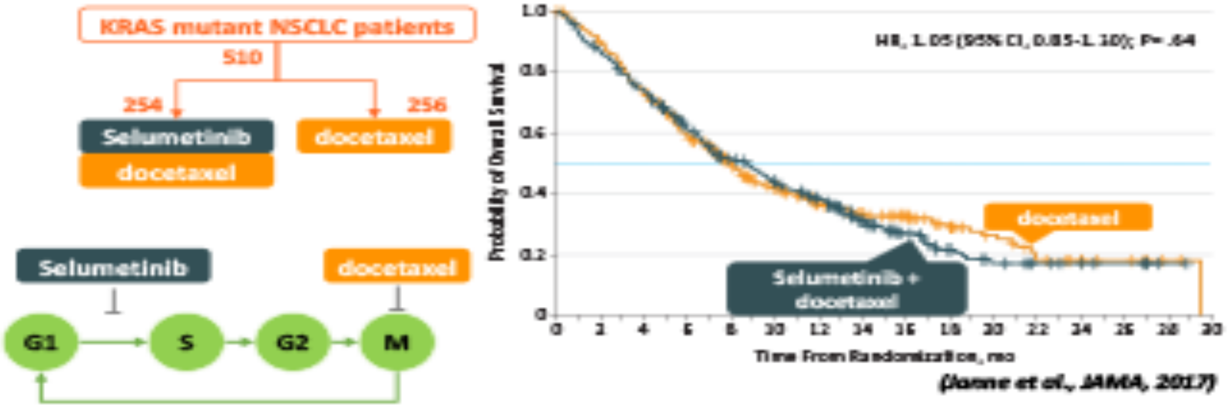
Feedback activation



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)

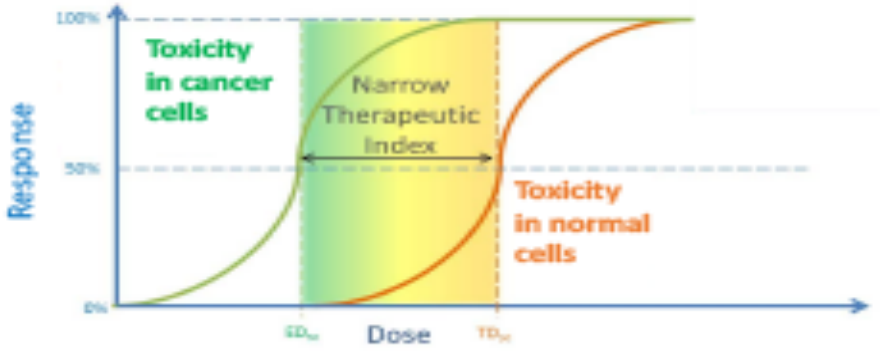
Combination of targeted therapy

- 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 lethal partners

Synthetic lethal partners of the KRAS oncogene

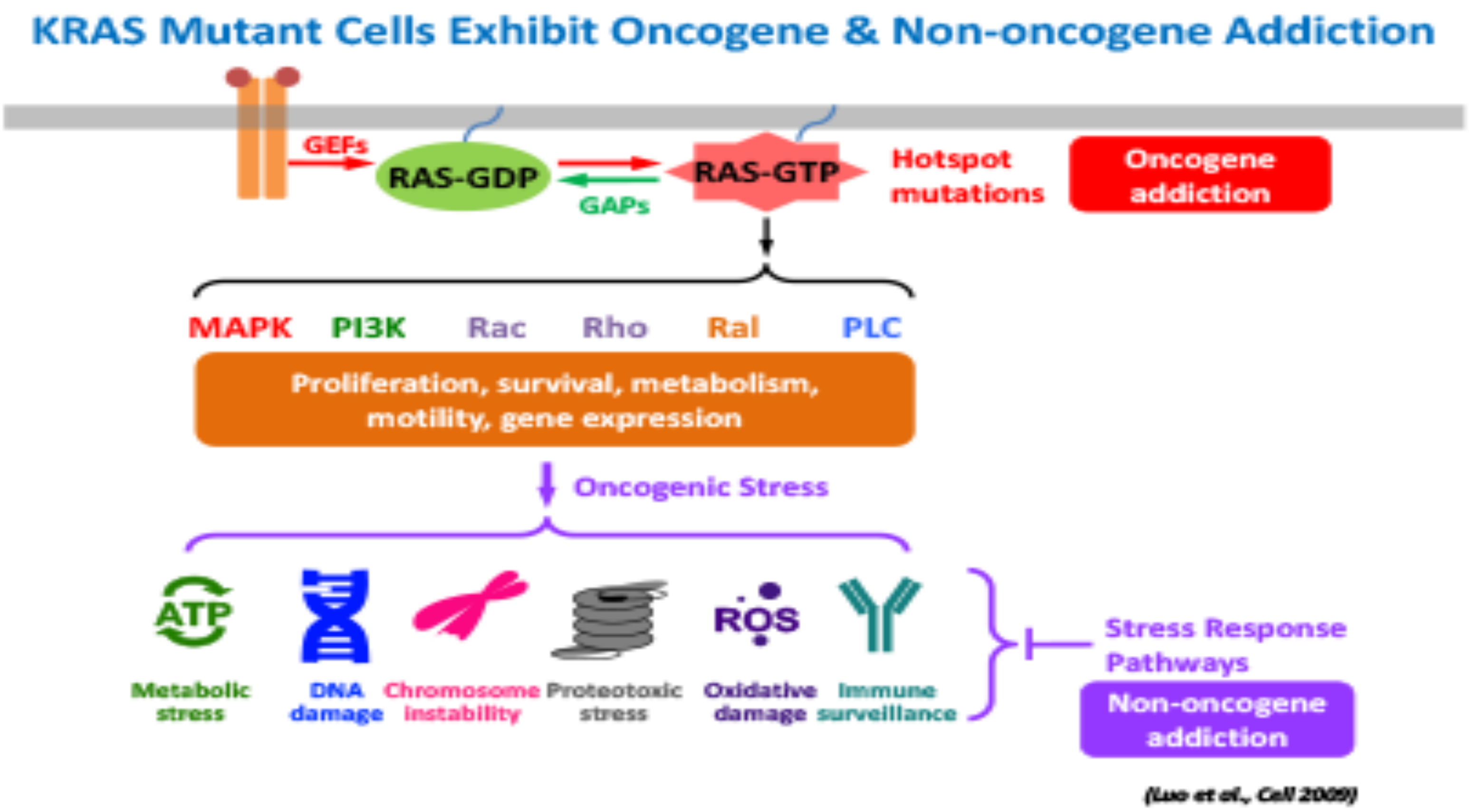
Optimizing target combinations for KRAS mutant cancer

Synthetic lethality

Synthetic Lethality Reflects Genetic Buffering and Pathway Redundancy

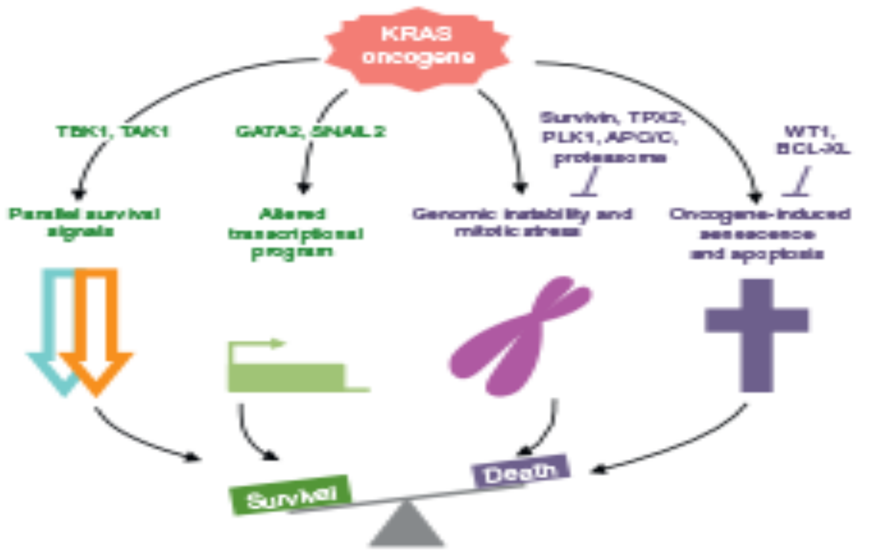
Gene A	Gene B	Viability
WT	WT	✔
WT	Loss	✔
Mutant	WT	✔
Mutant	Loss	✗

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

(Yu & Luo, The Enzymes 2014)

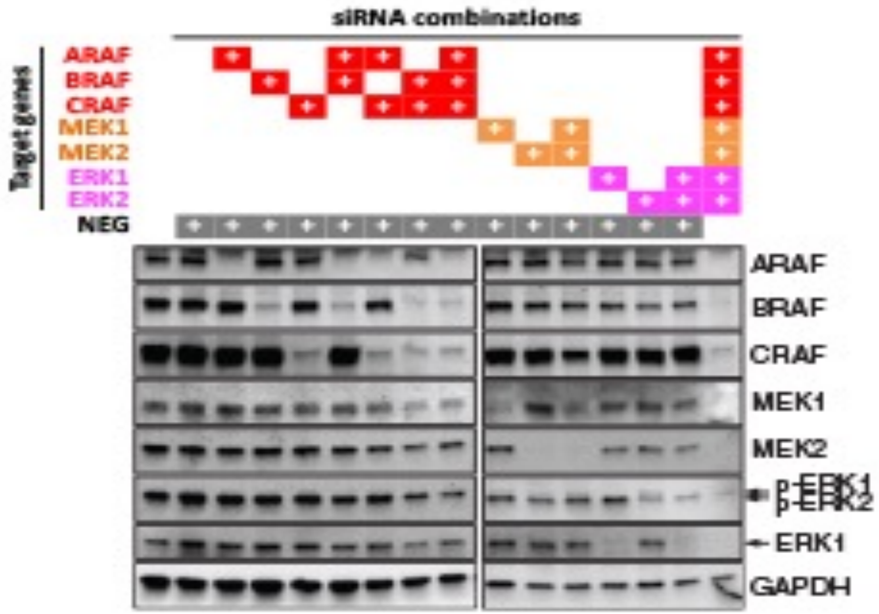
Dissecting the contribution

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

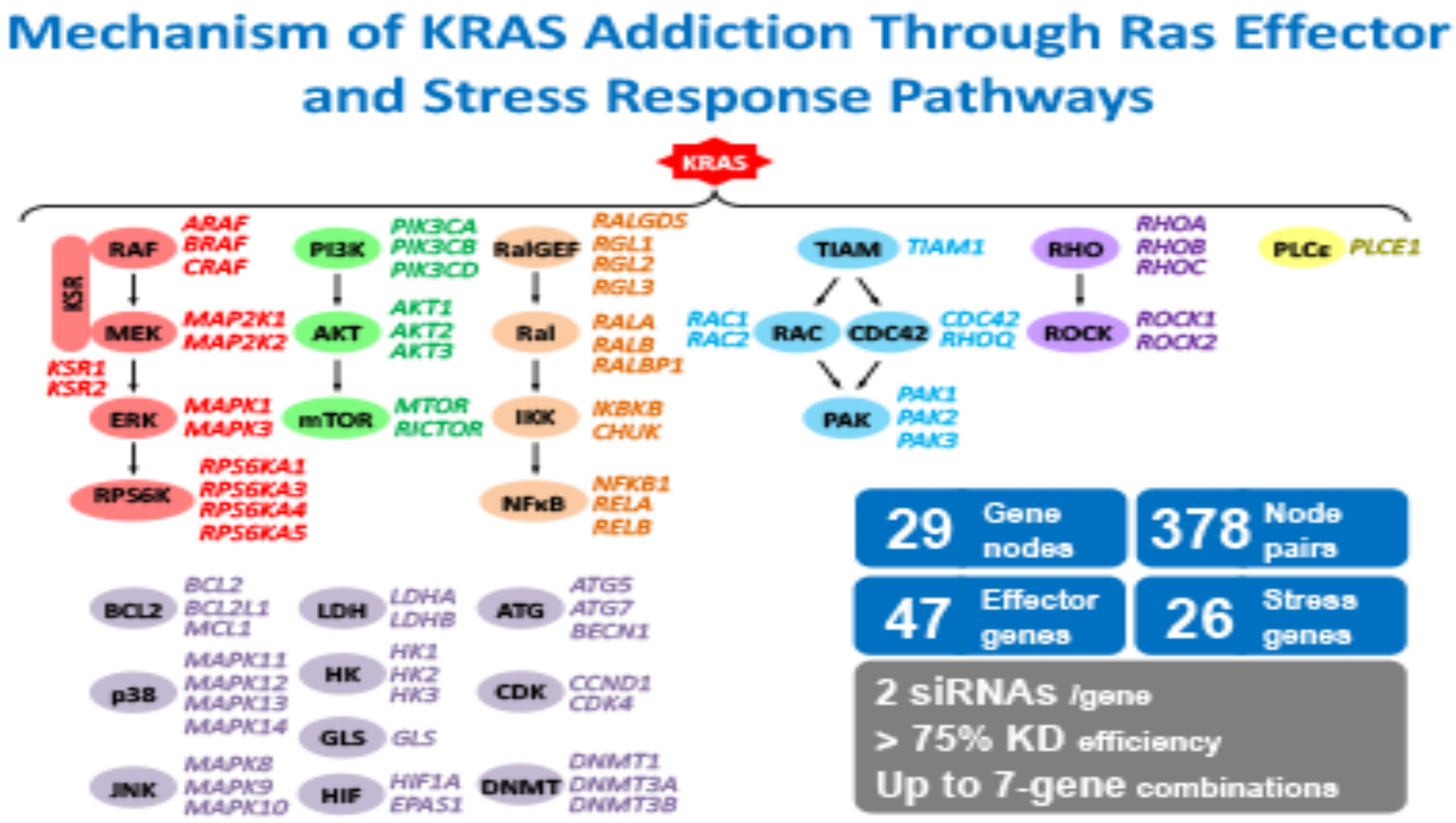
siRNA platform

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

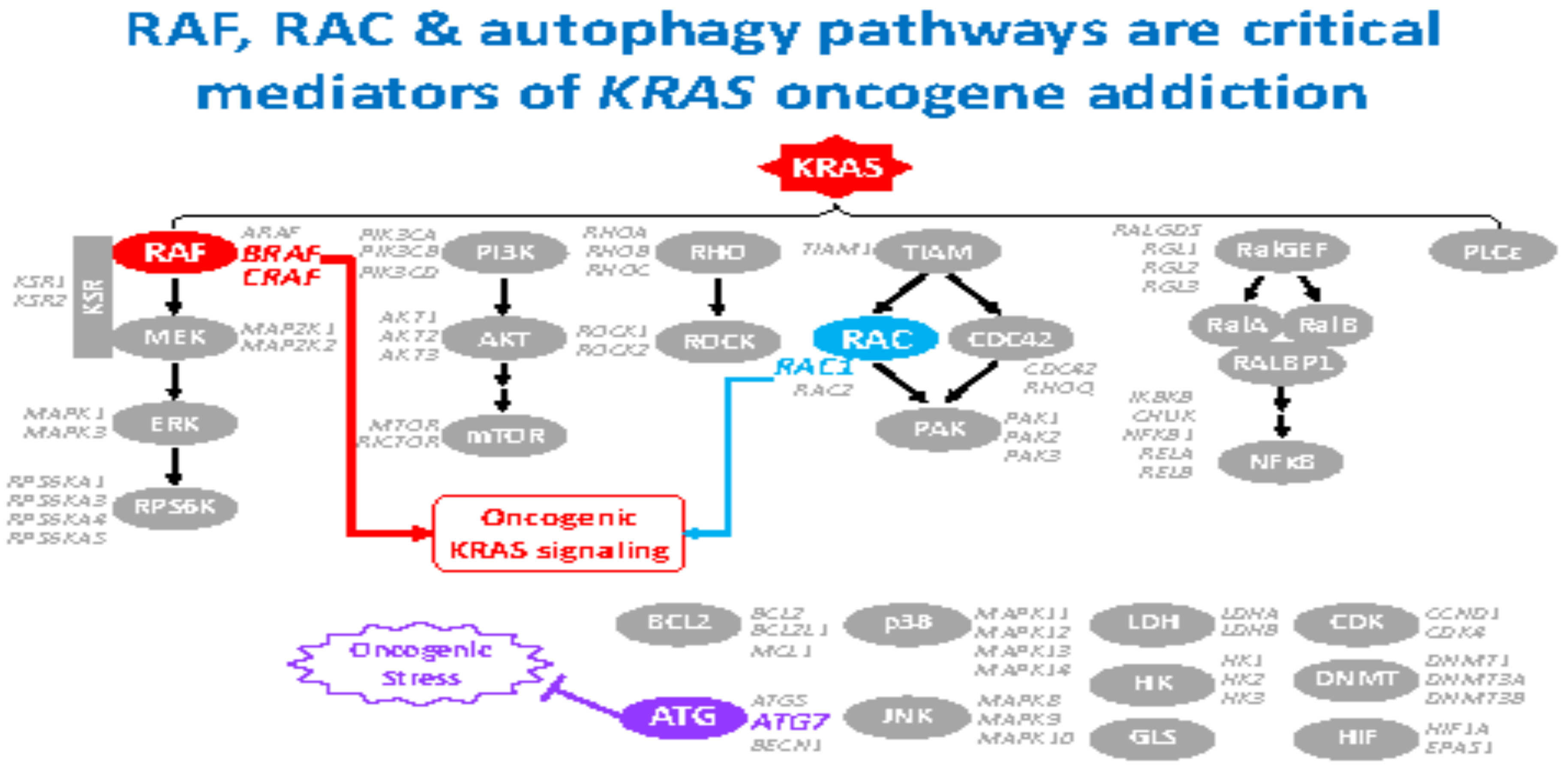


(Yuan et al. Cancer Discovery 2014)

KRAS addiction

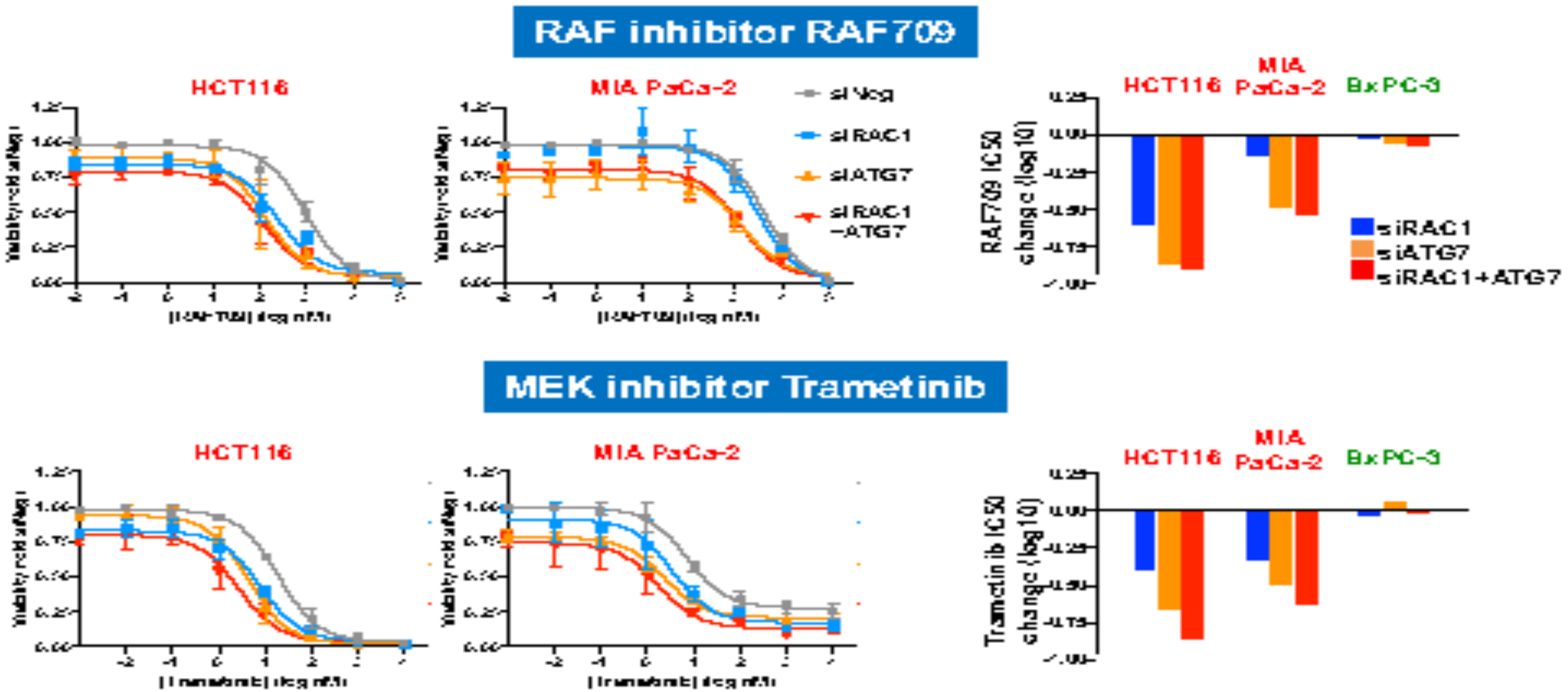


Autophagy pathways



MAPK inhibitors

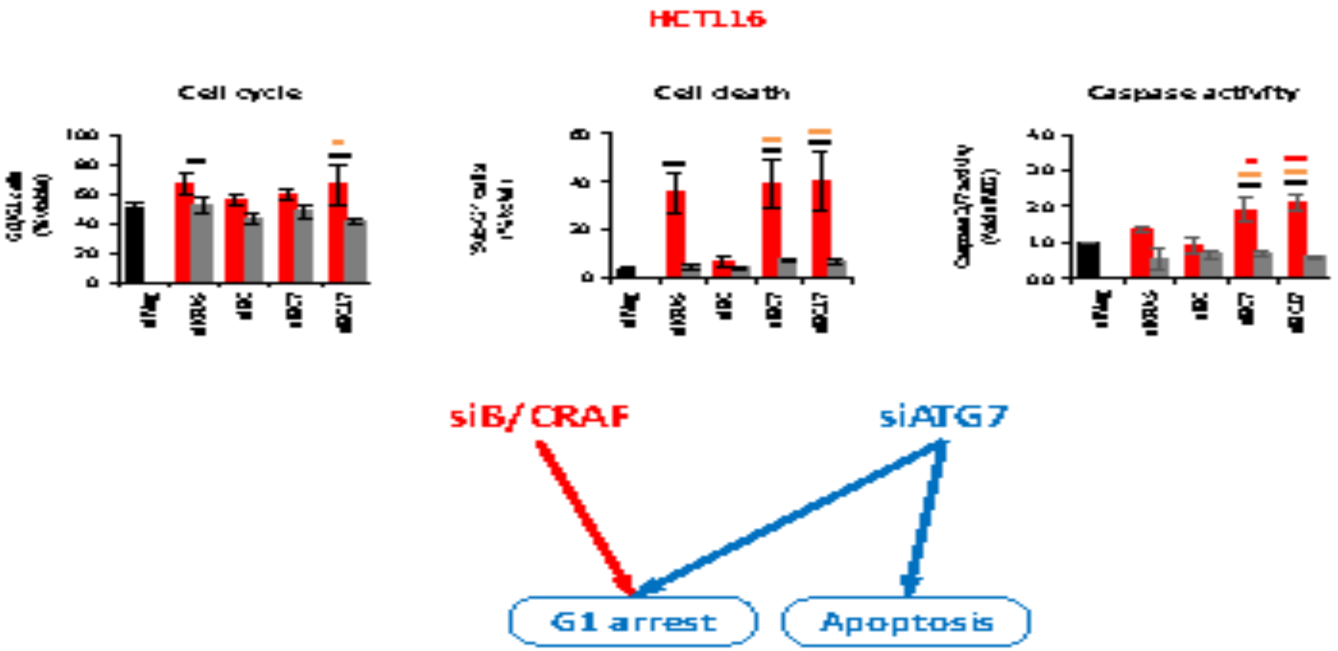
RAC1 and ATG7 knockdown sensitizes KRAS mutant cells towards MAPK pathway inhibitors



[Lee et al. PNAS 2019]

Cell cycle arrest

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



[Lee et al. PNAS 2019]

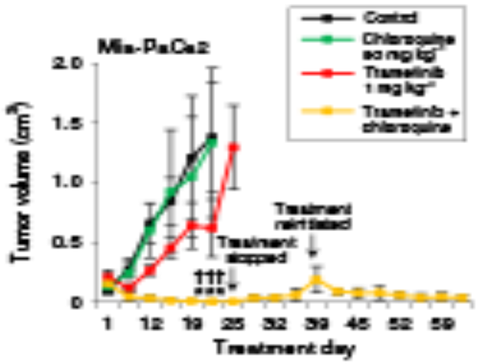
Co-targeting

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

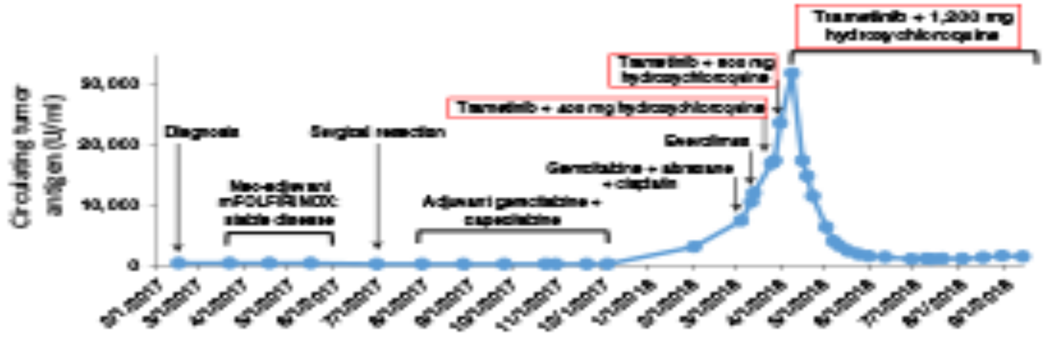
Kinsey … McMahon (Nature Medicine 2019)
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

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

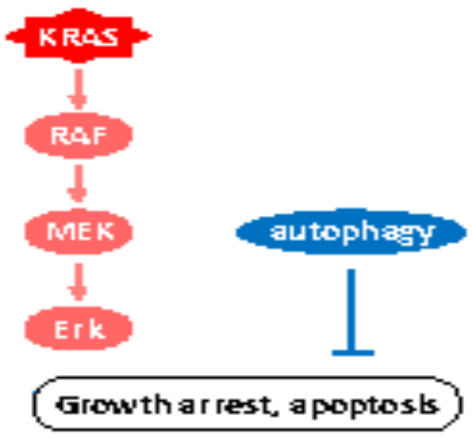


(Kinsey et al. Nature Medicine 2019)

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

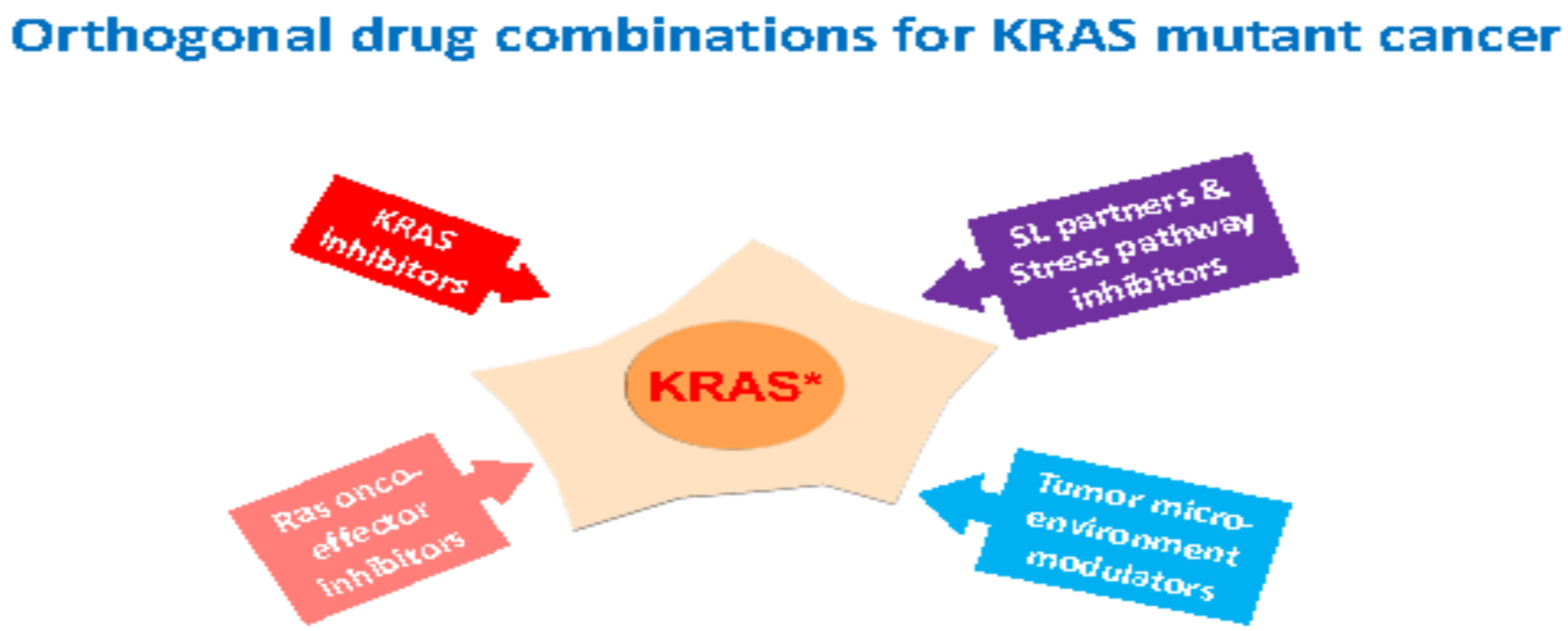
Clinical trials

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



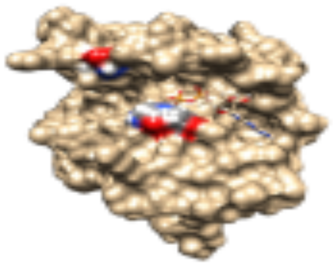
Trial ID	Disease	Drug combination
NCT04132505 (Phase 1)	KRAS mutant metastatic pancreatic cancer	Binimetinib (MEKi) Hydroxychloroquine (autophagyi)
NCT04566133 (Phase 2)	KRAS mutation refractory bile tract carcinoma	Trametinib (MEKi) Hydroxychloroquine (autophagyi)
NCT04214418 (Phase 1/2)	KRAS mutant advanced malignancies	Cobimetinib (MEKi) Hydroxychloroquine (autophagyi) Atezolizumab (ICB)
NCT03979651 (Phase 1)	NRAS mutant melanoma	Trametinib (MEKi) Hydroxychloroquine (autophagyi)
NCT03825289 (Phase 1)	Pancreatic cancer	Trametinib (MEKi) Hydroxychloroquine (autophagyi)
NCT04386057 (Phase 2)	Pancreatic cancer	LY3214996 (ERKi) Hydroxychloroquine (autophagyi)

Orthogonal drug combinations



Acknowledgements

Acknowledgement



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