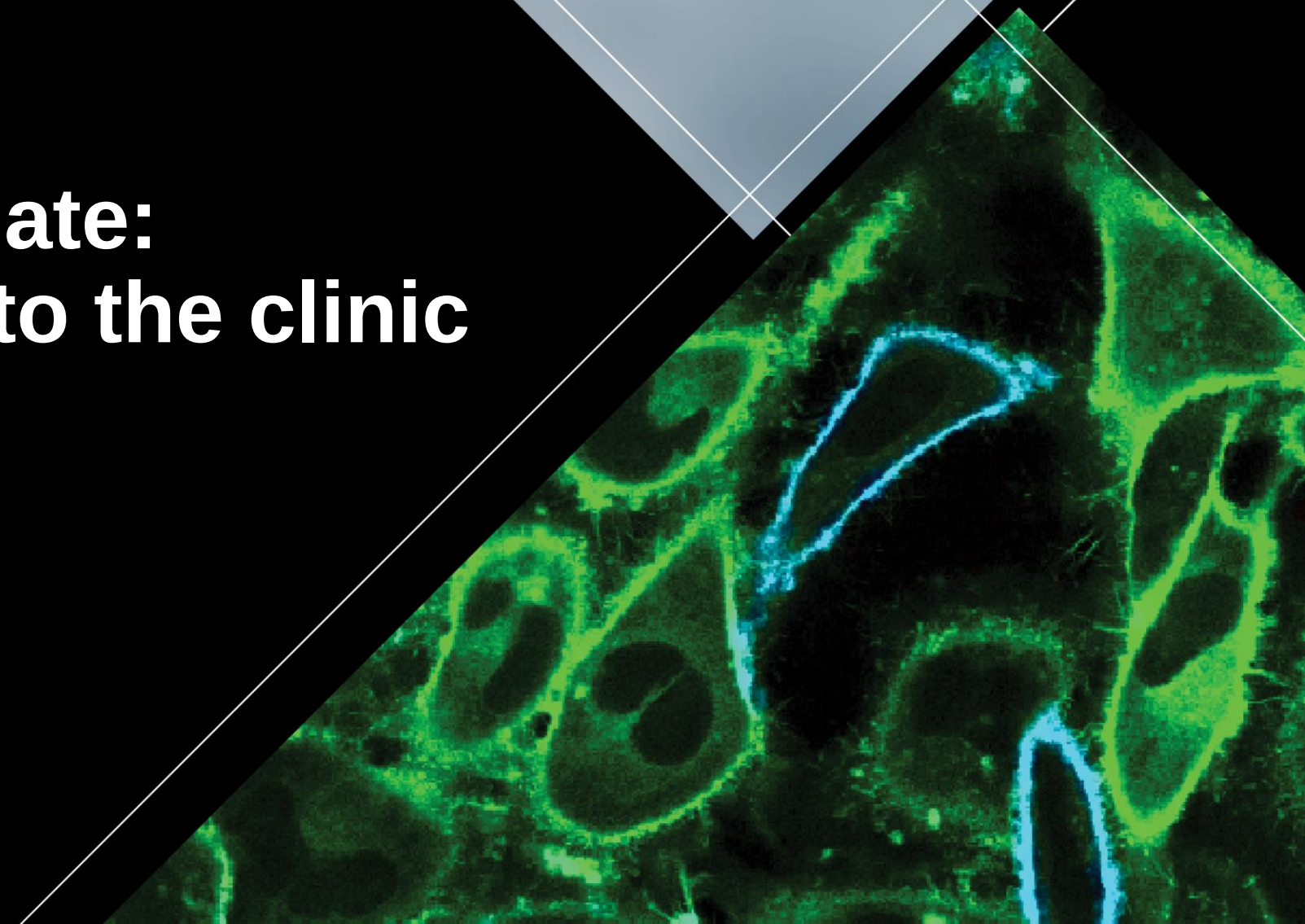
Frederick National Laboratory for Cancer Research

RAS INITIATIVE

RAS Initiative update: Taking our drugs to the clinic

July 10 2024 FNLAC meeting



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Growth factors

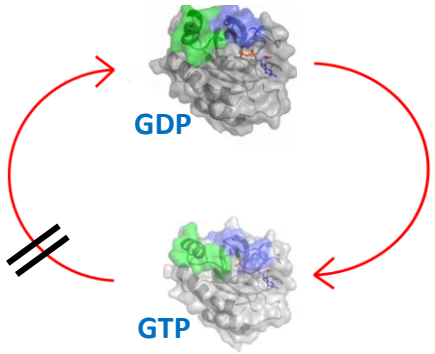
↓

Receptor Tyrosine Kinases

↓

Grb2/Shp2/Sos

↓



Neurofibromin,
other GAPs

↓

RAF

↓

MEK

↓

ERK

Transcription factors

Cell proliferation, migration and survival

Growth factors

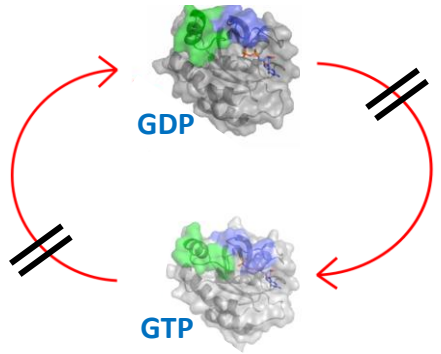
↓

Receptor Tyrosine Kinases

↓

Grb2/Shp2/Sos

↓



Sotorasib, Agarasib, *et al*

Neurofibromin,
other GAPs

↓

RAF

↓

MEK

↓

ERK

↓

Transcription factors

Cell proliferation, migration and survival

Growth factors

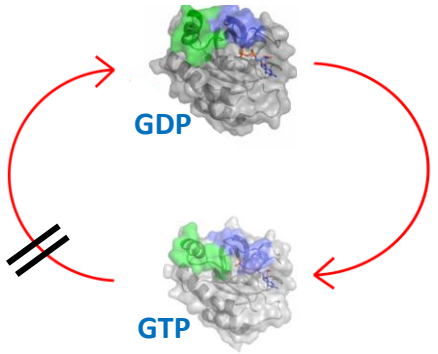
↓

Receptor Tyrosine Kinases

↓

Grb2/Shp2/Sos

↓



Neurofibromin,
other GAPs

BBO-8520

↓

RAF

↓

MEK

↓

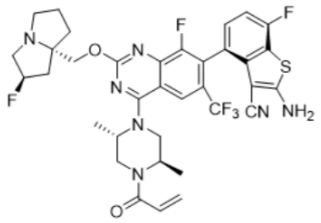
ERK

↓

Transcription factors

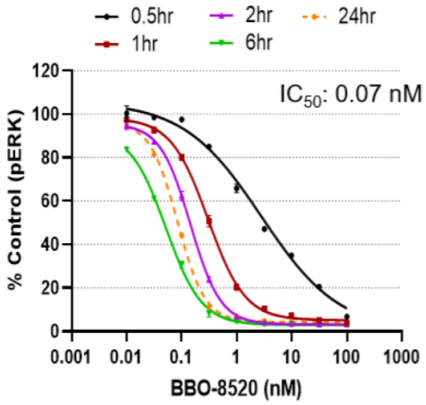
Cell proliferation, migration and survival

BBO-8520: A covalent G12C inhibitor that binds the GTP state



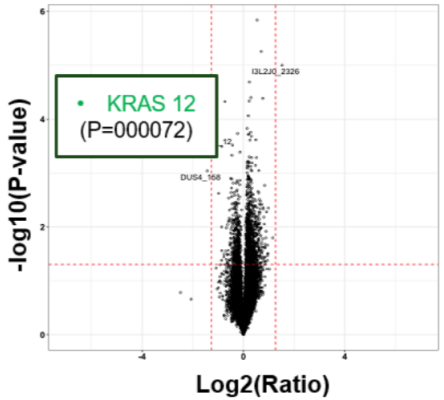
Assay NCI-H358	BBO-8520
Ki/Kinact	43,000 $M^{-1}S^{-1}$
pERK	0.07 nM
3D viability	0.04 nM
$ED_{50/90}$	0.6 / 1.6 mg/kg
>50% CR	10 mg/kg

Cellular Potency



Cellular Selectivity

Global Cysteine Proteomics (20 nM)



Frederick National Lab

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Marcin Dyba	Monalisa Swain
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Roger Ma	Frank McCormick

bridgebio

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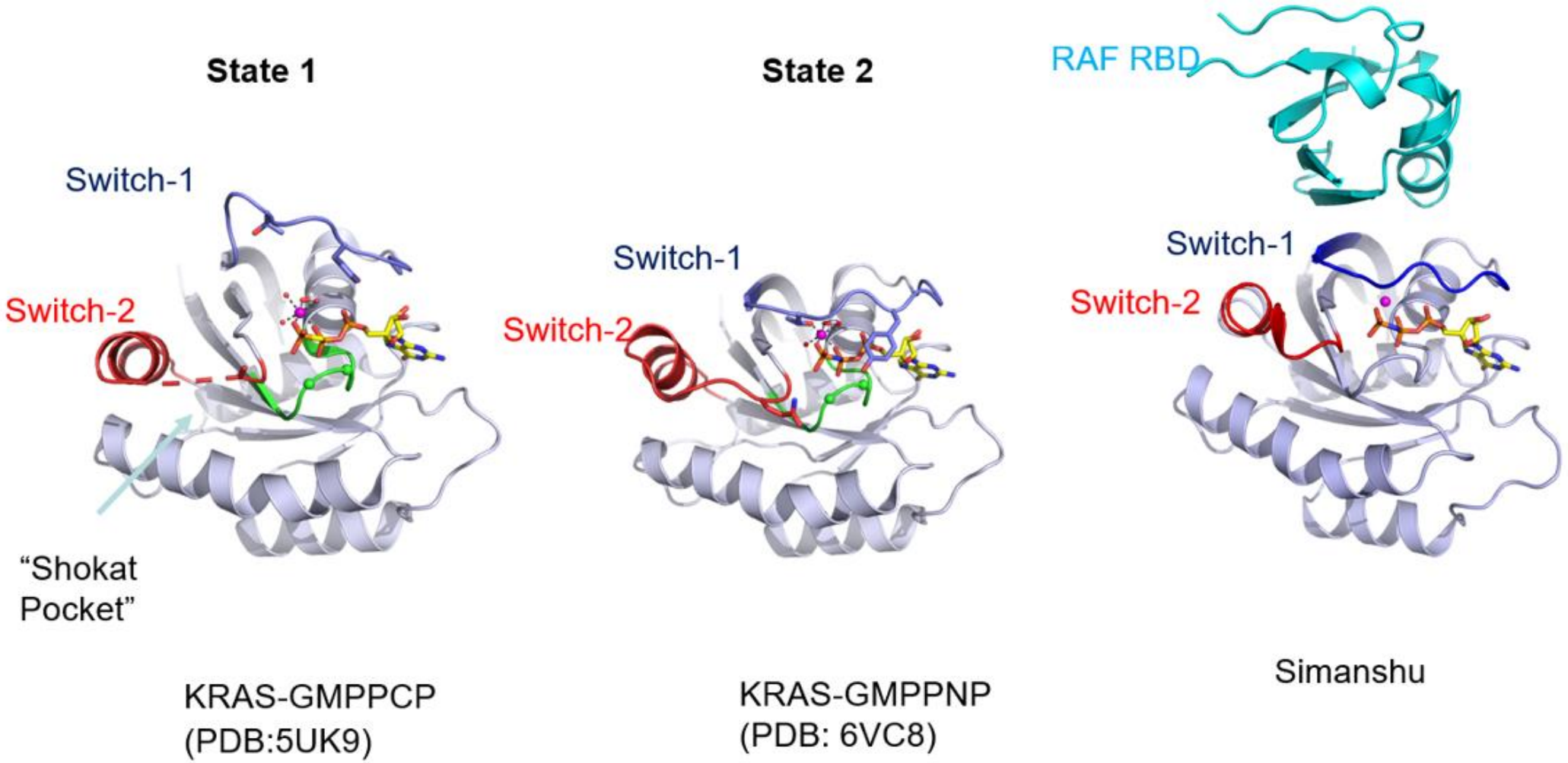
Jin Ju	James Stice	

Lawrence Livermore National Laboratory

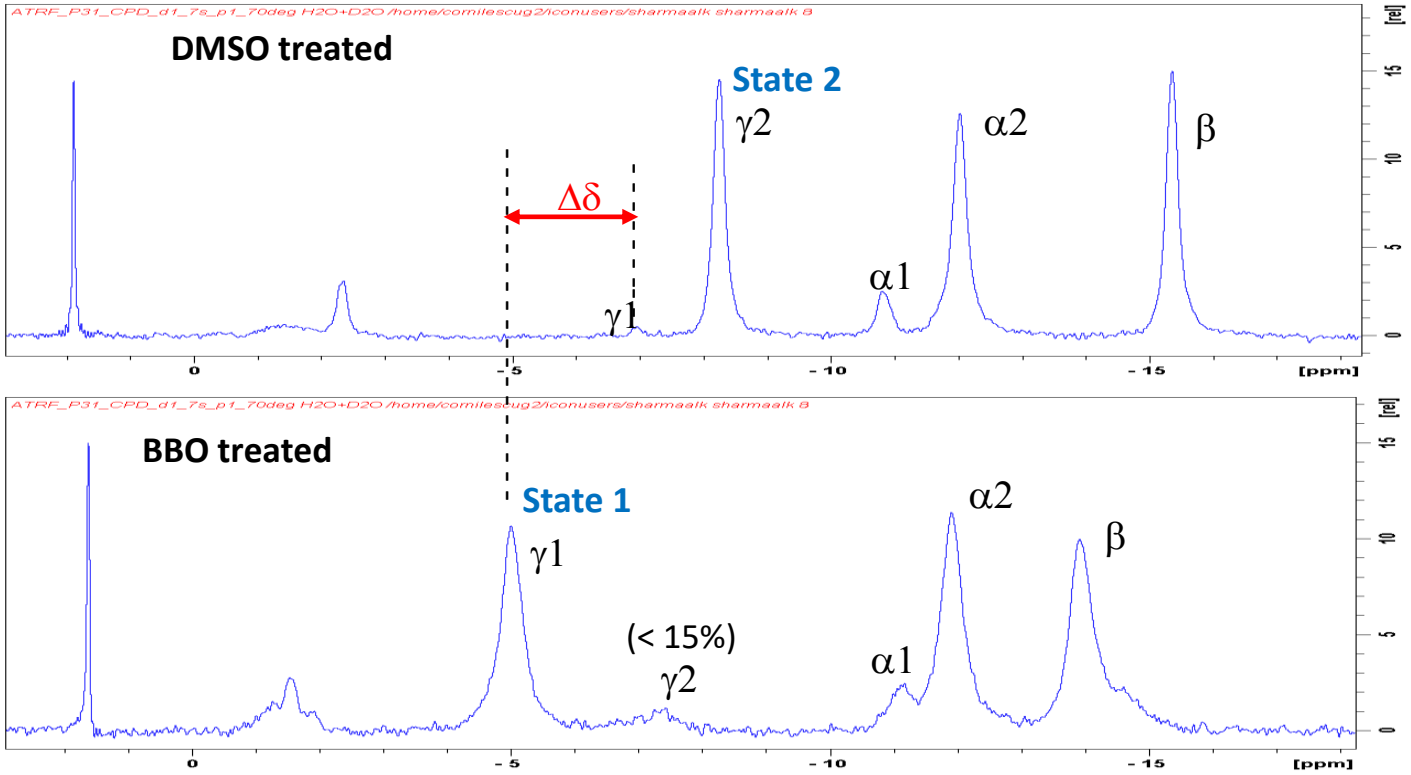
Felice Lightstone

Yue Yang

GTP-bound RAS proteins can exist in 2 states



Trapping KRAS.GTP in State 1



^{31}P NMR spectrum @ 5 ^{o}C

γ1 population increases from < 5% to > 85% upon binding

ARTICLE IN PRESS

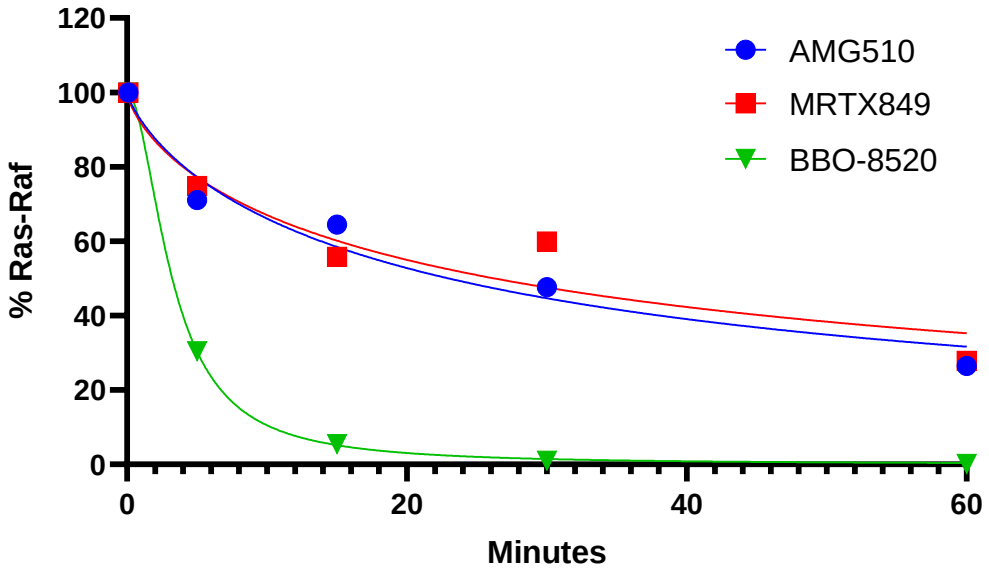
JBC COMMUNICATION

Revealing the mechanism of action of a first-in-class covalent inhibitor of KRASG12C (ON) and other functional properties of oncogenic KRAS by ^{31}P NMR

Received for publication, September 18, 2023, and in revised form, December 27, 2023 Published, Papers in Press, xxx,
https://doi.org/10.1016/j.jbc.2024.105650

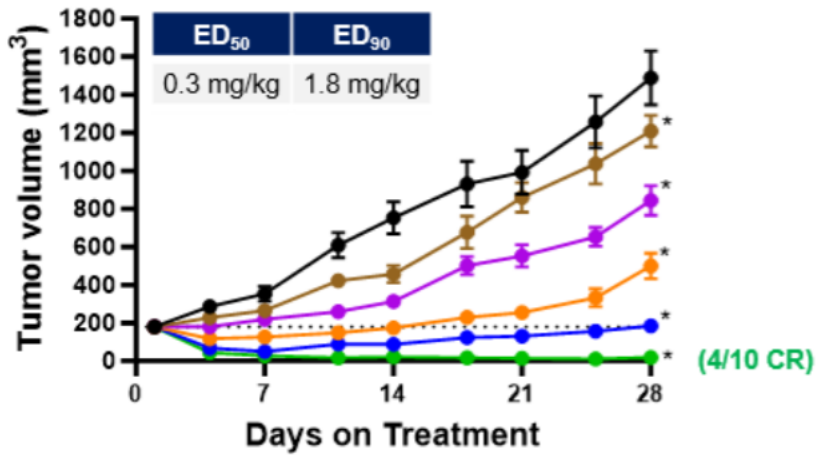
Alok K. Sharma[1,*], Jun Pei[2], Yue Yang[2], Marcin Dyba[1], Brian Smith[1], Dana Rabara[1], Erik Larsen[1], Felice C. Lightstone[2], Dominic Esposito[2], Andrew G. Stephen[1], Bin Wang[1], Pedro J. Beltran[2], Eli Wallace[3], Dwight V. Nissley[1], Frank McCormick[1,3,4], and Anna E. Maciag[1,*]

Rapid target engagement in cells



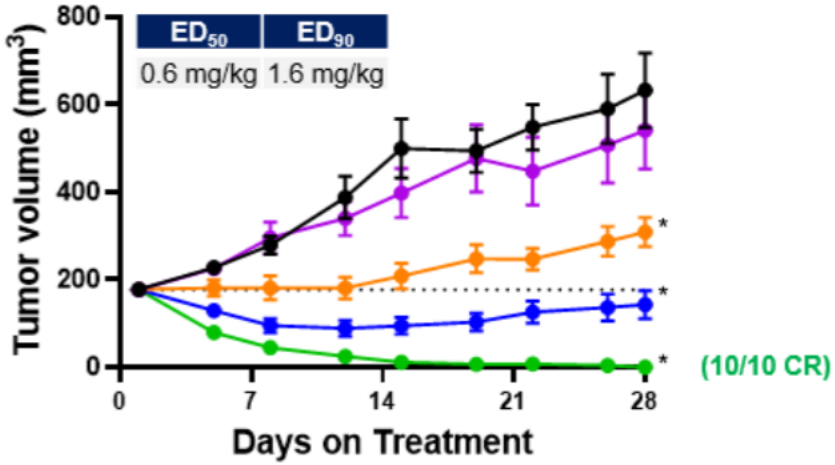
Complete responses in xenograft models

MIA PaCa-2 KRAS^{G12C} PDAC



- Vehicle (QD, po)
- BBO-8520 (0.1 mg/kg)
- BBO-8520 (0.3 mg/kg)
- BBO-8520 (1 mg/kg)
- BBO-8520 (3 mg/kg)
- BBO-8520 (10 mg/kg)

H358 KRAS^{G12C} NSCLC

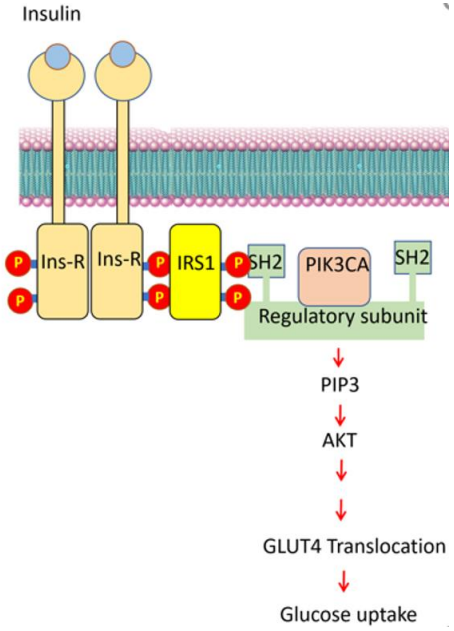


- Vehicle (QD, po)
- BBO-8520 (0.3 mg/kg)
- BBO-8520 (1 mg/kg)
- BBO-8520 (3 mg/kg)
- BBO-8520 (10 mg/kg)

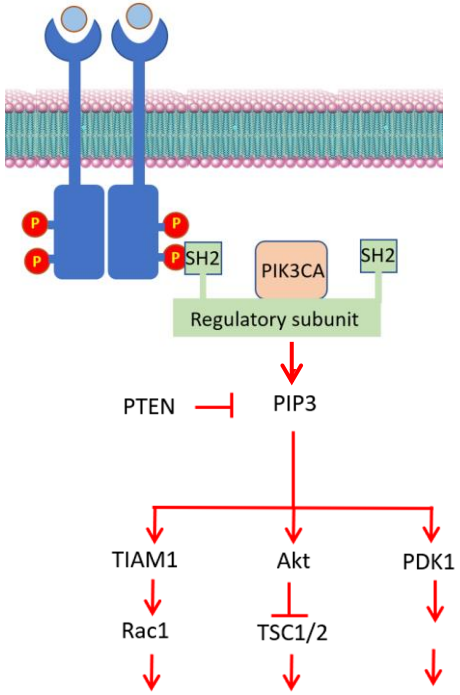
*RMANOVA, p<0.01 vs Vehicle

Activation of PI 3’ kinase by growth factors and RAS

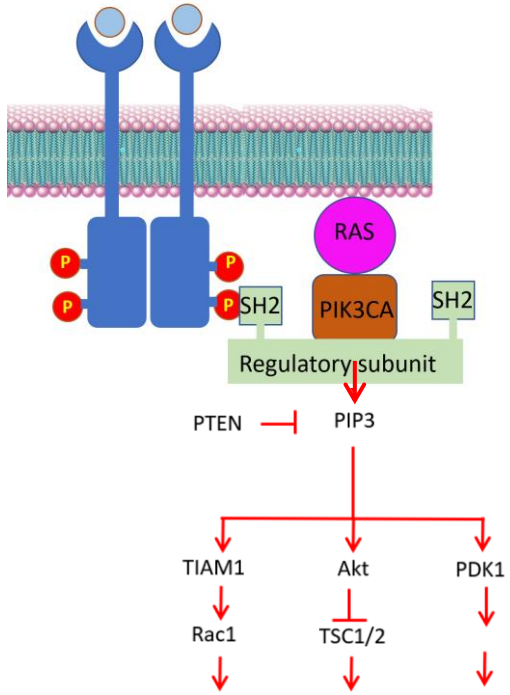
Insulin Receptor



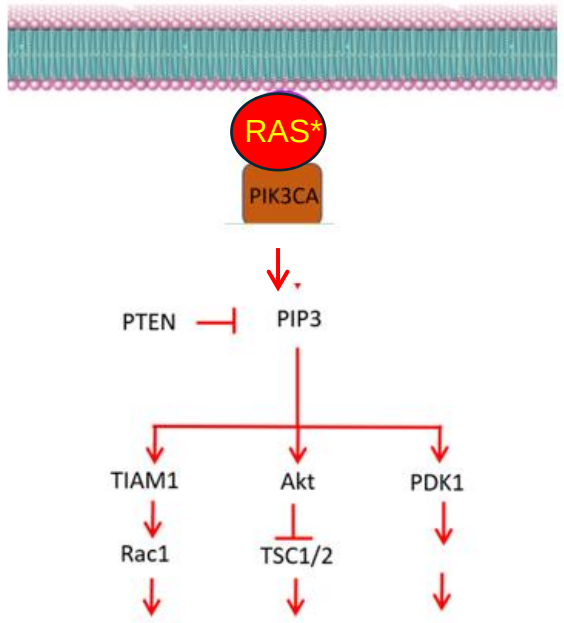
Normal RTK



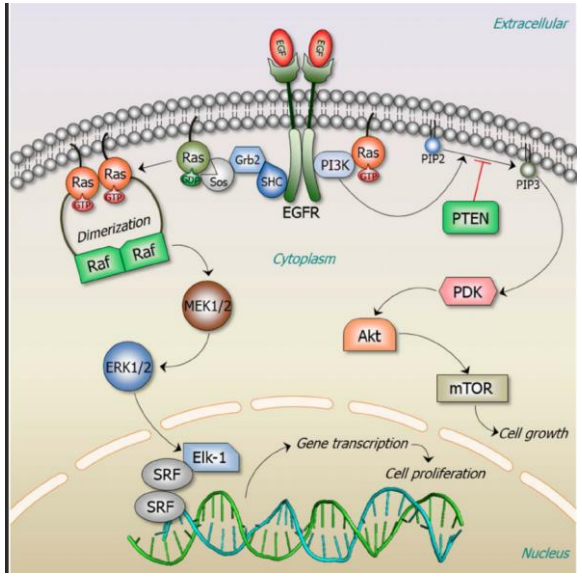
Development, Angiogenesis, Oncogenesis

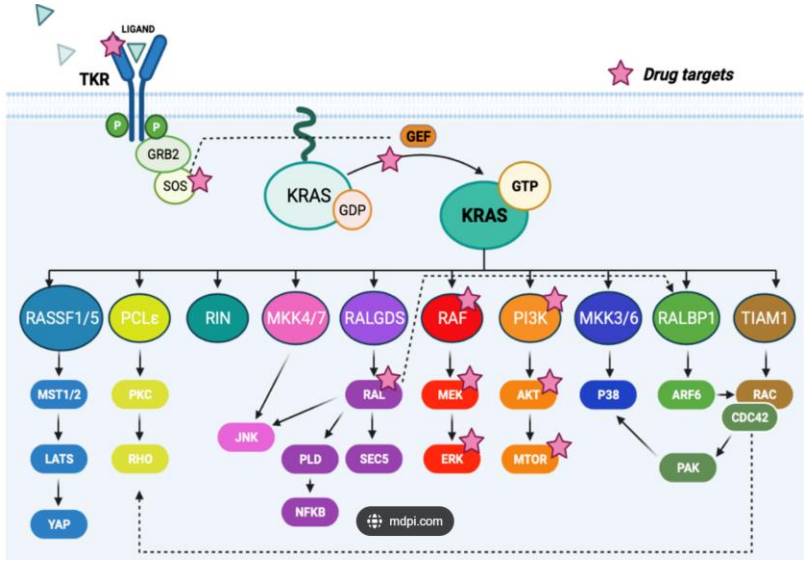


Oncogenic KRAS

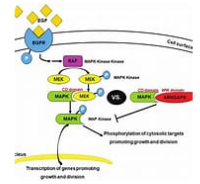


Is PI3' kinase a KRAS effector?





MAPK and PI3K/AKT pathways



KRAS is a GTPase that functions as an on/off switch that alternates between an active GTP-bound and an inactive GDP-bound state, regulated by GAPs and GEFs, such as Sos1. This oncogene regulates several effector pathways, including the **MAPK and PI3K/AKT pathways**.

ChatGPT

Oncogenic RAS proteins can activate PI 3’ kinases

Tc21 = RRAS2
R-RAS3 = MRAS

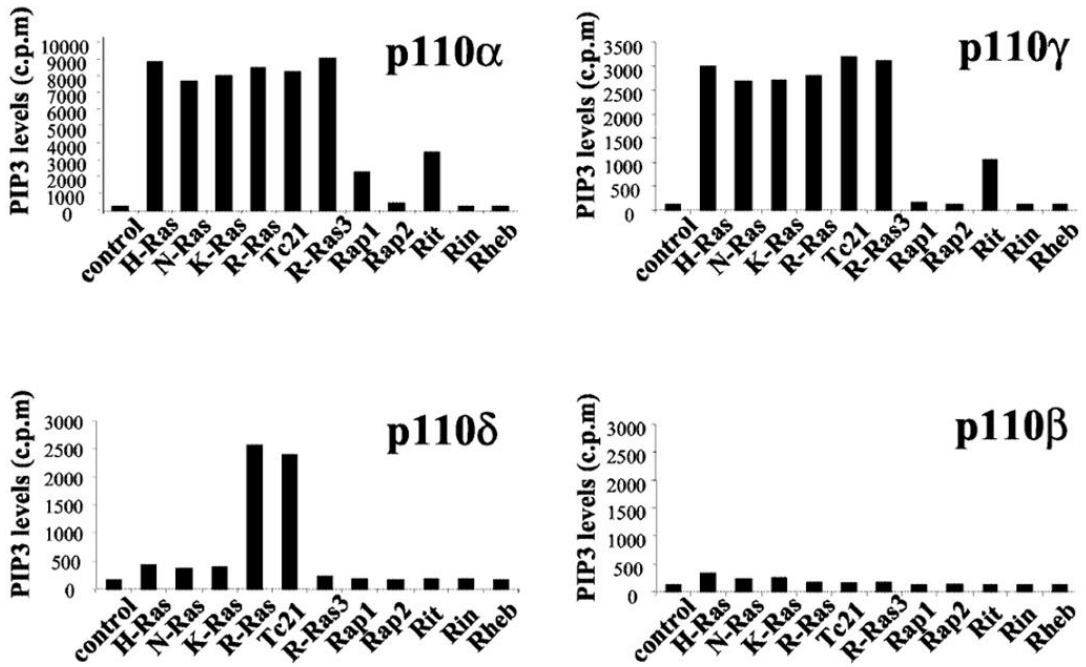


FIG. 3. Activation of class I PI3Ks by RFGs. Constitutively active RFGs were cotransfected into 293T cells with PI3K isoforms. Two days after transfection, cells were labeled with [^{32}P]orthophosphate, and total cellular PIP3 levels were measured by high-pressure liquid chromatography. The levels of PI(4,5)P2 were standardized to 200,000 cpm. Results shown are representative of at least three independent experiments.

MOLECULAR AND CELLULAR BIOLOGY, June 2004, p. 4943–4954 Vol. 24, No. 11
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.11.4943–4954.2004

Signaling Specificity by Ras Family GTPases Is Determined by the Full Spectrum of Effectors They Regulate

Pablo Rodriguez-Viciana, Celine Sabatier, and Frank McCormick*
Cancer Research Institute and Comprehensive Cancer Center, University of California,

The Role of RAS-PI 3' kinase in cancer

Binding of Ras to Phosphoinositide 3-Kinase p110α Is Required for Ras-Driven Tumorigenesis in Mice

Surbhi Gupta,[1,4] Antoine R. Ramjaun,[1,4] Paula Haiko,[3] Yihua Wang,[1] Patricia H. Warne,[1] Barbara Nicke,[1] Emma Nye,[2] Gordon Stamp,[2] Kari Alitalo,[3] and Julian Downward[1,*]

Cell Reports
Report

Disruption of the Interaction of RAS with PI 3-Kinase Induces Regression of EGFR-Mutant-Driven Lung Cancer

Miguel M. Murillo,[1,2] Sareena Rana,[1] Bradley Spencer-Dene,[2] Emma Nye,[2] Gordon Stamp,[2] and Julian Downward[1,2,3,*]
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[2]Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK

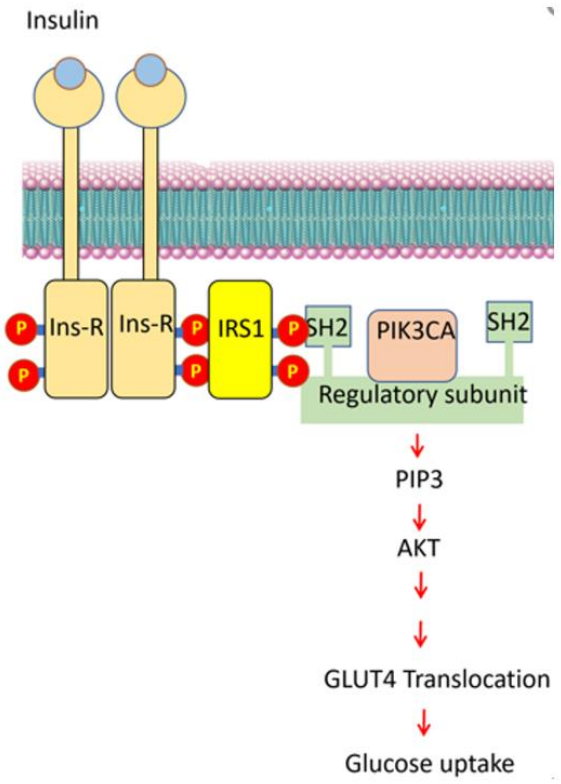
LETTERS

nature cell biology

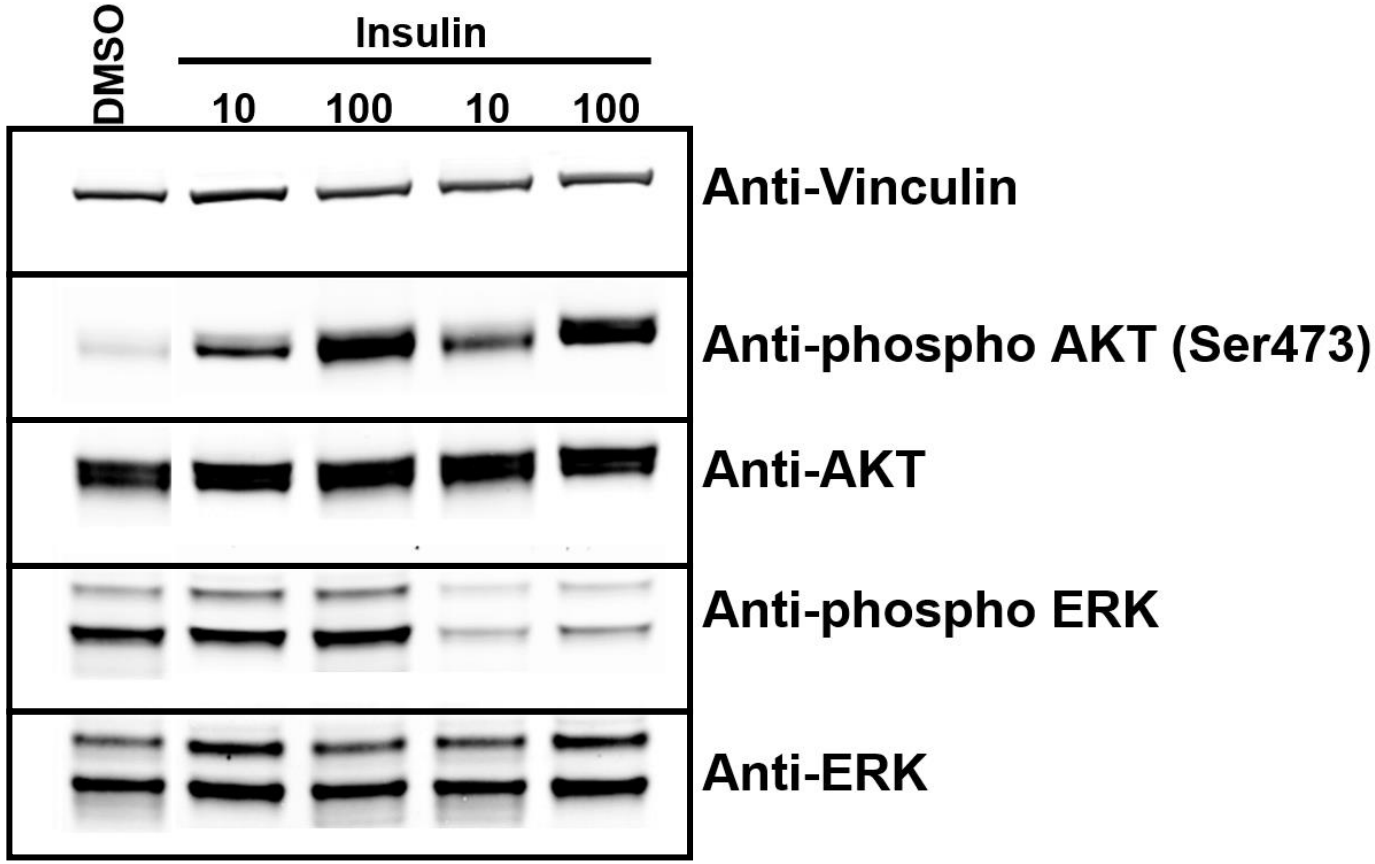
Input from Ras is required for maximal PI(3)K signalling in *Drosophila*

Mariam H. Orme[1], Saif Alrubaie[1], Gemma L. Bradley[1], Cherryl D. Walker[1] and Sally J. Leevers[1,2]

Insulin does not depend on canonical RAS proteins to activate PI 3’ kinase



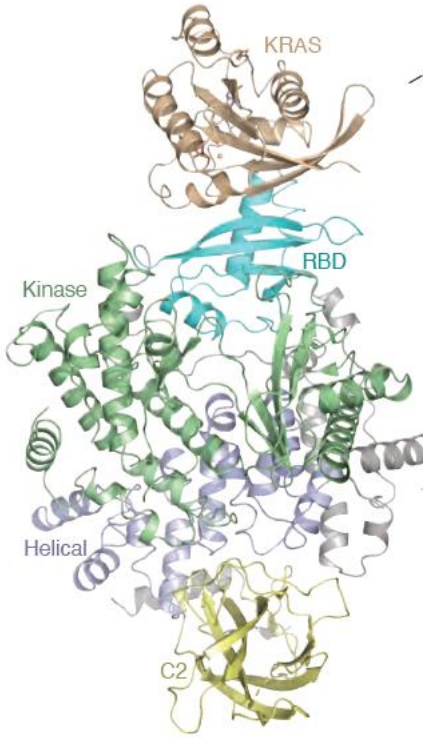
RMC-6236 (Pan-RAS inhibitor: N, H, N, K and MRAS)



Differentiated L6 myocytes

Nikki Fer

BBIO-10203 (The Breaker) prevents RAS proteins binding to PI 3' kinase α



Assay		BBO-10203
PI3Kα MALDI-TOF MS (% modified)	15 / 30 / 120 / 240 min	94 / 97 / 100 / 100
ITC PI3Kα RBD	KRAS/HRAS/NRAS	No binding
BRET IC_{50} (nM)	PI3Kα:KRAS / $PI3K\alpha^{c/s}$:KRAS	3 / 2000
Target Engagement IC_{50} (nM)	BT474	3
pAKT IC_{50} (nM)	BT474 / KYSE-410*	6 / 4
pAKT IC_{50} (nM) inactive isomer	BT474 / KYSE-410	2600 /2300
Kinact/KI (M^{-1}/S^{-1})	BT474	7122
3D Viability IC_{50} (nM)	BT474/KYSE-410*	141/345

Dhirendra K. Simanshu[1*], Rui Xu[2*], James Stice[2], Daniel J. Czyzyk[1], Siyu Feng[2], John-Paul Denson[1], , Erin Riegler[2], Yue Yang[1], Ming Chen[2], Sofia Donovan[2], Brian P. Smith[1], Maria Abreu-Blanco[1], Cindy Feng[2], Lijuan Fu[2], Dana Rabara[1], Lucy C Young[4], Marcin Dyba[1], Wupeng Yan[1], Ken Lin[2], Samar Ghorbanpoorvalukolaie[5], Erik K. Larsen[1], Wafa Malik[3], Allison Champagne[1], Katie Parker[3], Cathy Zhang[2], Dominic Esposito[1], David M. Turner[1], Felice C. Lightstone[3], Bin Wang[2], Paul M. Wehn[2], Keshi Wang[2], Andrew G. Stpehen[1], Anna E. Maciag[1], Aaron N. Hata[3], Kerstin Sinkevicius[2], Dwight V. Nissley[1], Eli M. Wallace[2], Frank McCormick[1,4], Pedro J. Beltran[2]

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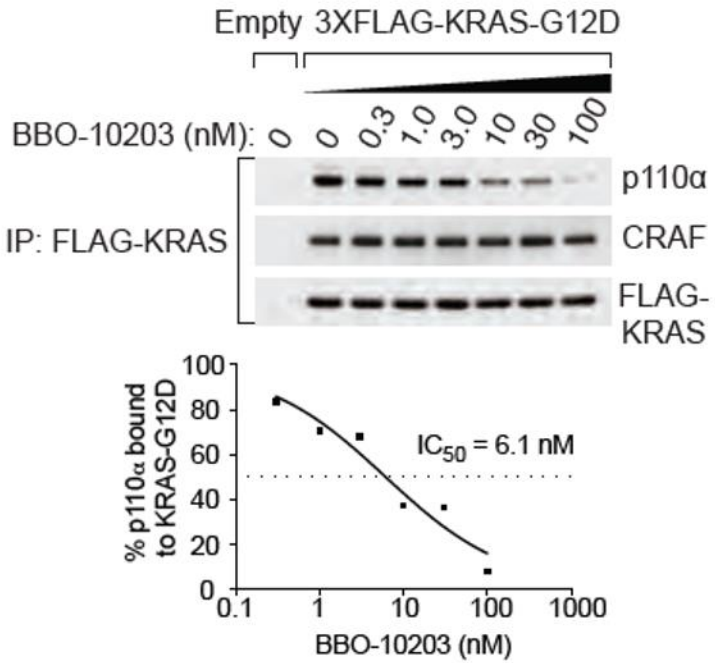
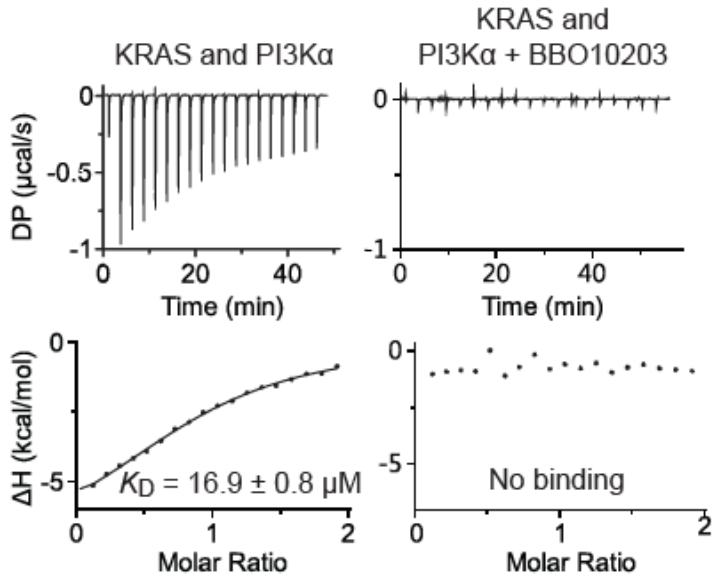
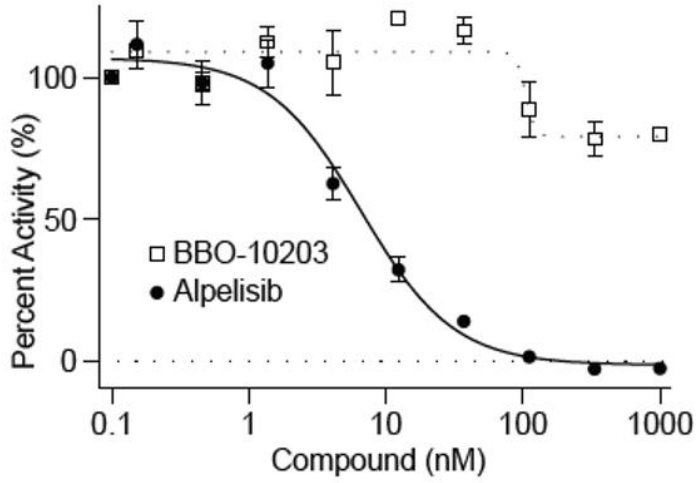
*Co-first authors.

Dhirendra Simanshu

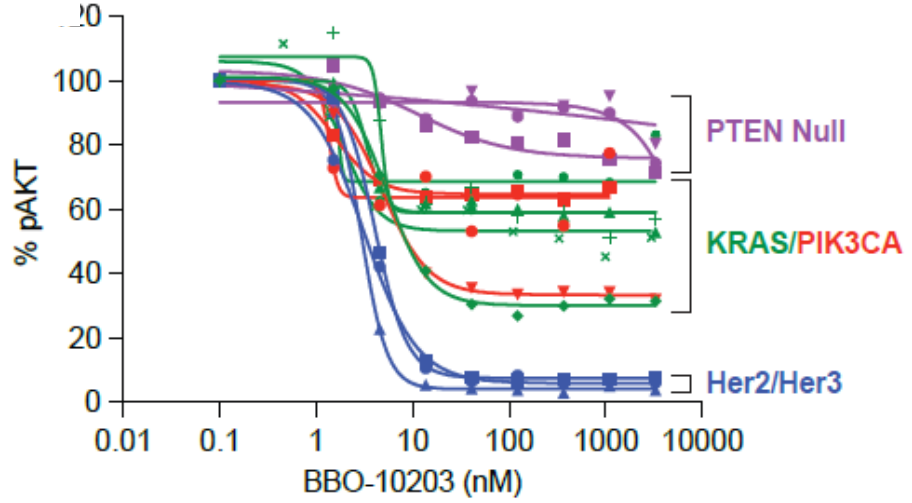
The Breaker blocks RAS binding to PI3Ka, but does not inhibit kinase activity



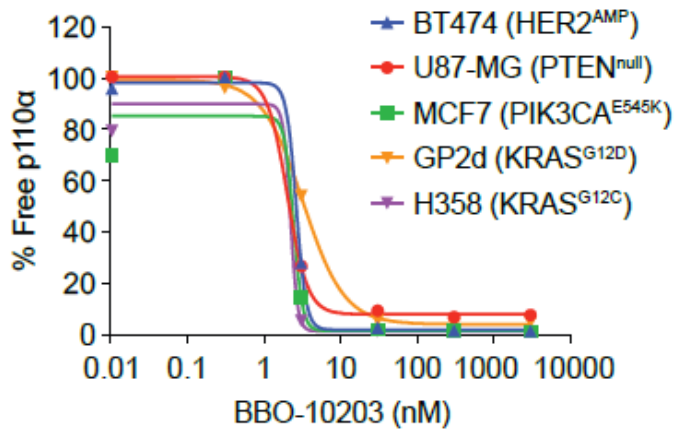


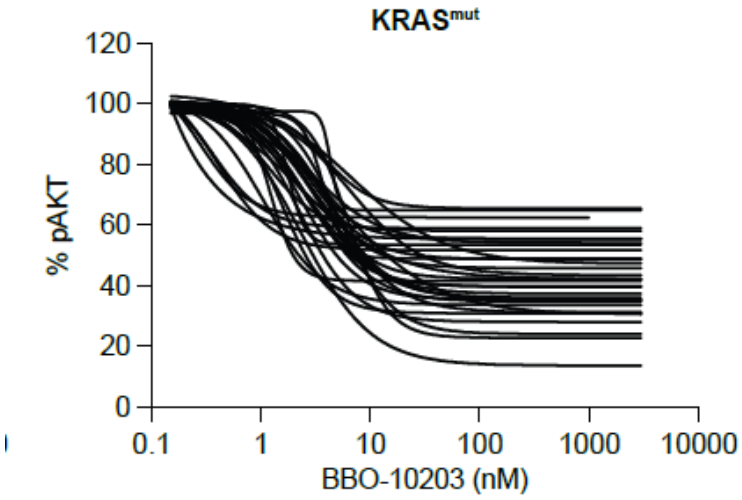


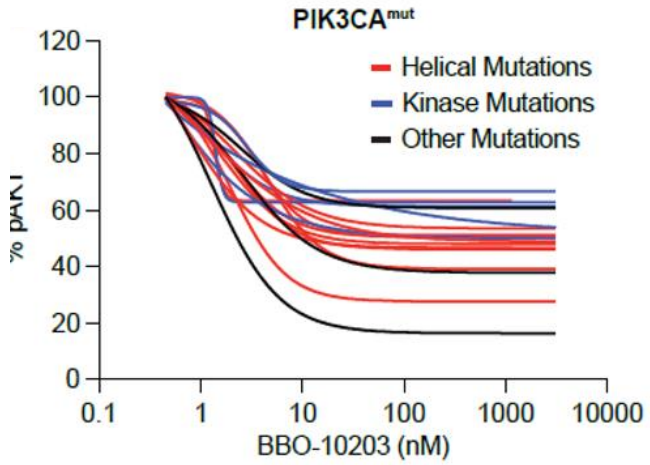
Effects of The Breaker on different genotypes

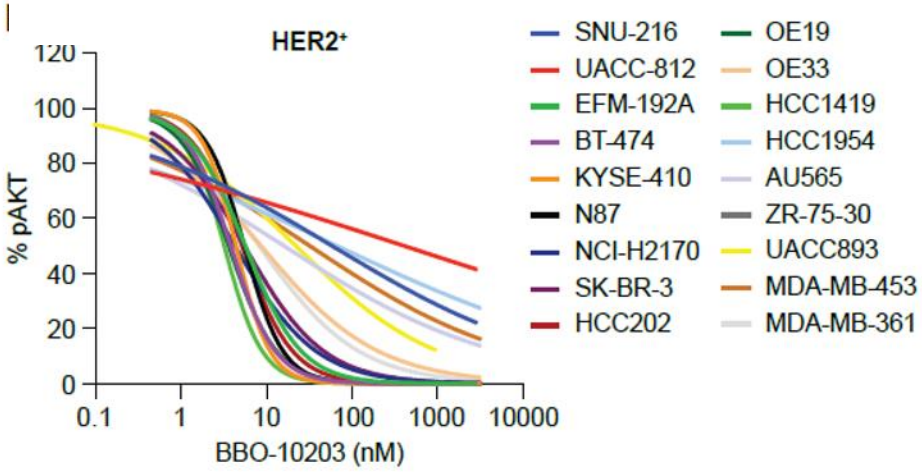


Target engagement assay

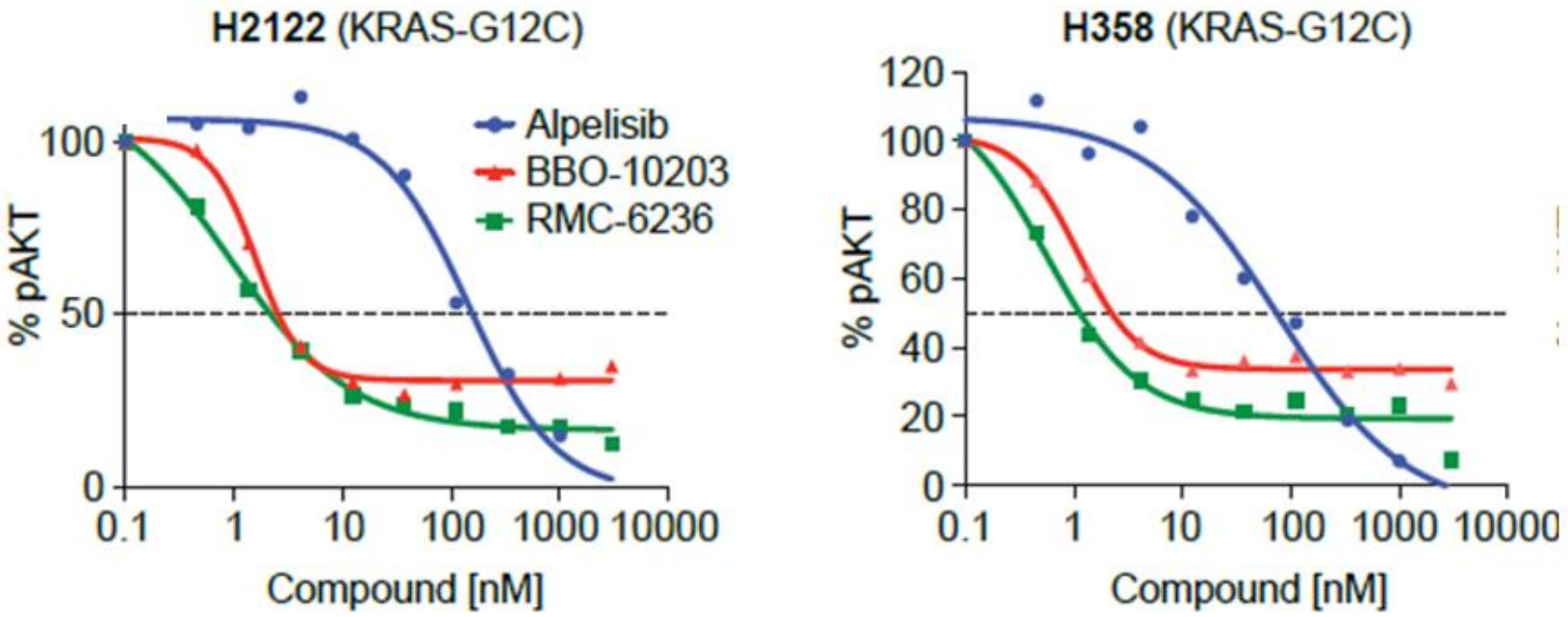






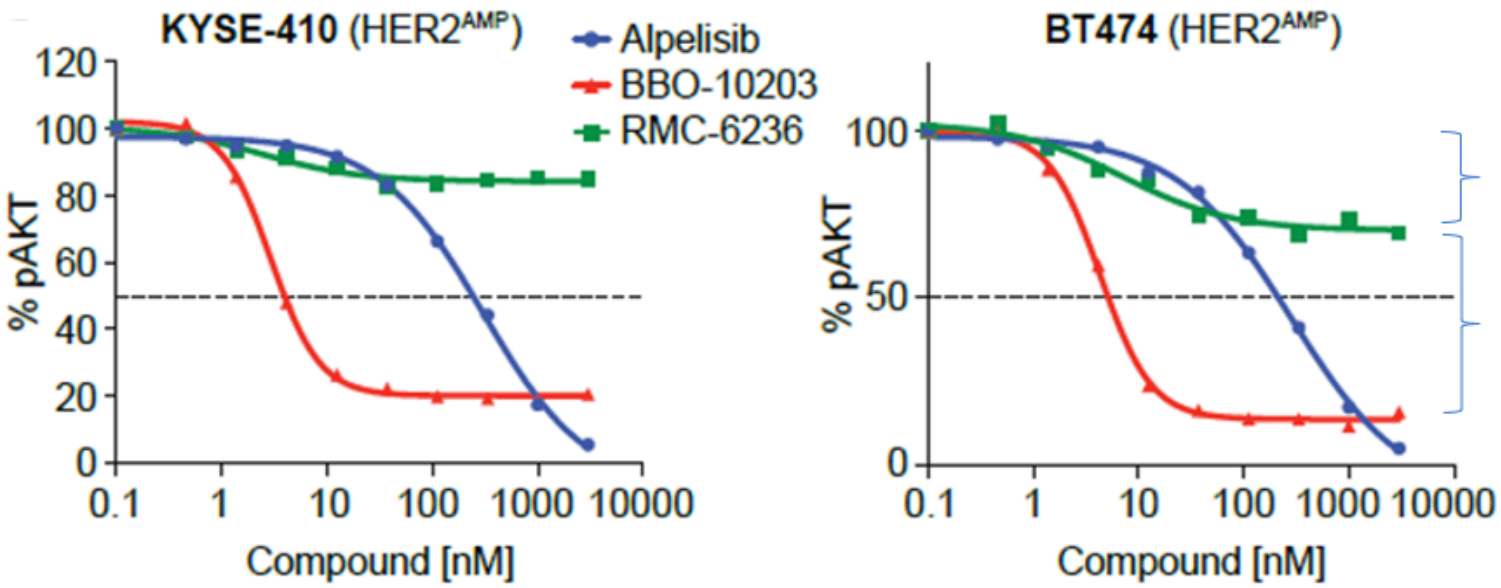


Most of the PI3' kinase α activity in KRAS mutant cells comes from RAS



RMC-6236 inhibits HRAS, NRAS, KRAS and MRAS

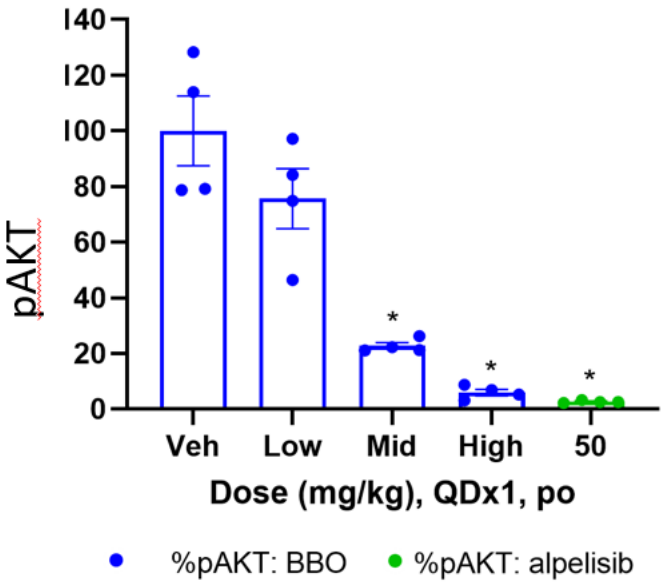
Most of the PI3' kinase α activity in HER2+ cells comes from something else



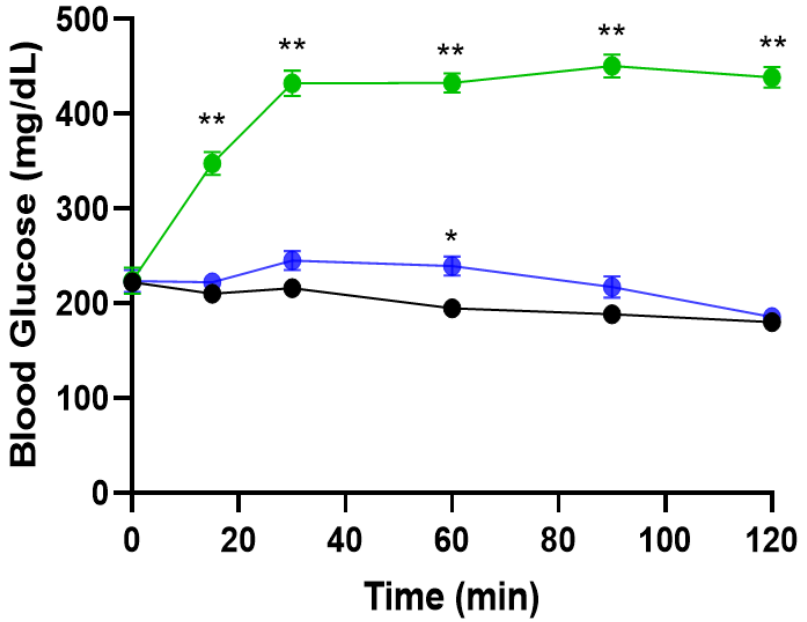
PI3Kα from H, N, K or MRAS

PI3Kα from ????

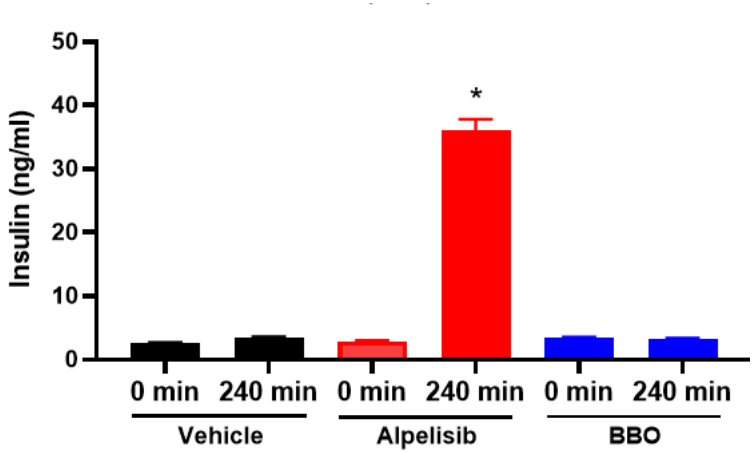
pAKT inhibition in vivo without induction of hyperglycemia



Blood glucose levels



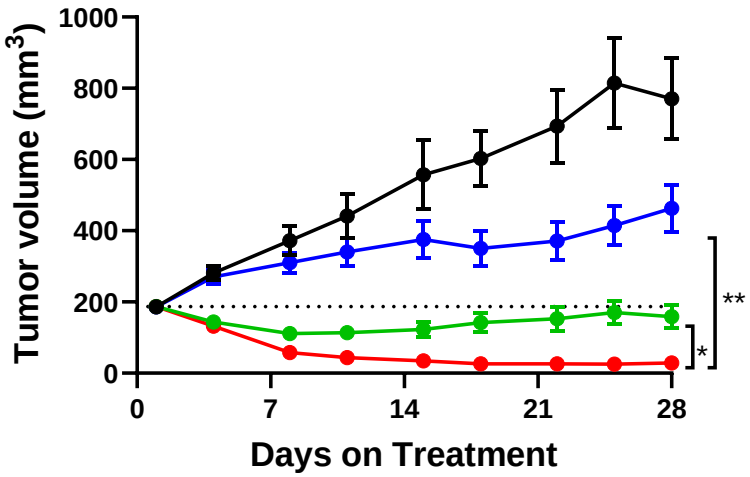
Insulin levels



Dose response: One-way ANOVA with Tukey's test vs vehicle *p<0.0001. Blood glucose levels: One-way ANOVA with Dunnett's multiple comparisons test vs vehicle: *p<0.01, **p<0.0001

Combining Breaker with KRAS G12Ci

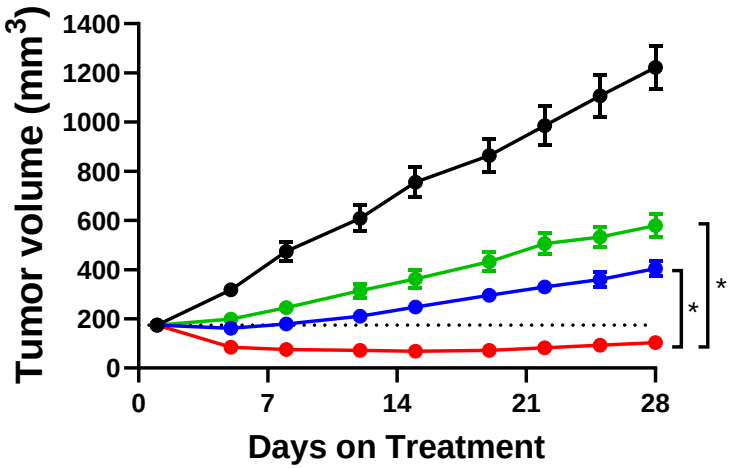
‘Sensitive’ model
H358 ($KRAS^{G12C}$) NSCLC



*p<0.01, **p<0.0001 compared to monotherapy group

Vehicle (QD, po)
BBO-8520 (3 mg/kg)
BBO-10203 (100 mg/kg)
BBO-8520 + BBO-10203

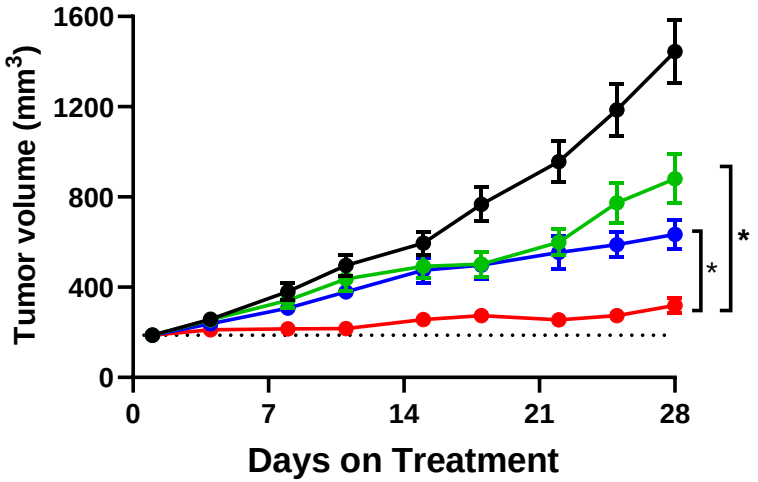
G12Ci resistant model 1
H2122 ($KRAS^{G12C}$ / $KEAP1_{mut}$ / $STK11_{mut}$) NSCLC



*p<0.0001 compared to monotherapy group

Vehicle (QD, po)
Breaker (100 mg/kg)
BBO-8520 (30 mg/kg)
Breaker + BBO-8520

G12Ci resistant model 2
SW1573 ($KRAS^{G12C}$ and $PIK3CA^{K111E}$) NSCLC

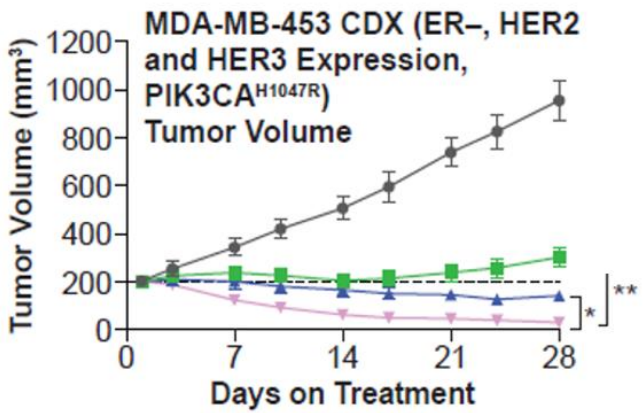


*p<0.0005 compared to monotherapy group

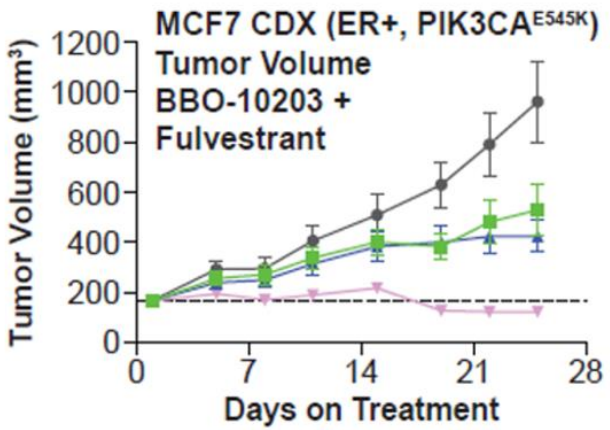
Vehicle (QD, po)
Breaker (30 mg/kg)
BBO-8520 (30 mg/kg)
Breaker + BBO-8520

Note: BBO-8520: Direct $KRAS^{G12C}$ (ON) inhibitor, *RMANOVA

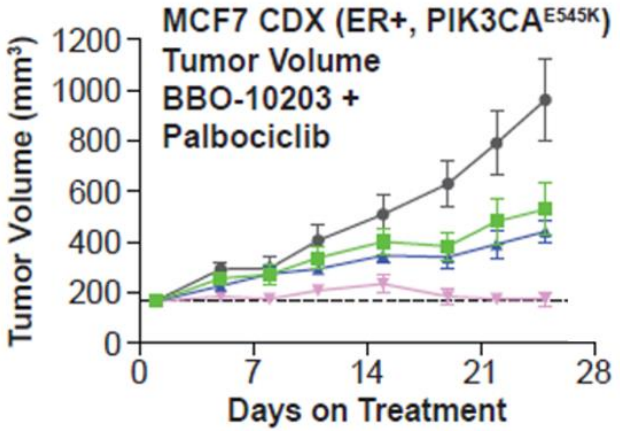
Potential Breaker combinations…



- Vehicle (QD, po)
- BBO-10203 (30 mg/kg, QD, po)
- Trastuzumab (4 mg/kg, Q7Dx4, ip)
- BBO-10203 (30 mg/kg, QD, po) + Trastuzumab (4 mg/kg, Q7Dx4, ip)



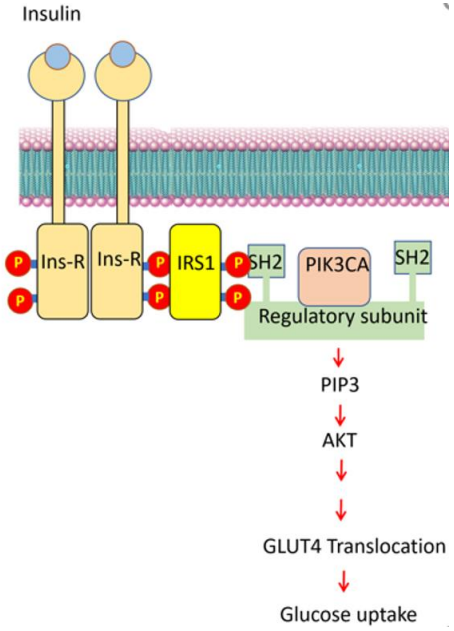
- Vehicle (QD, po)
- BBO-10203 (100 mg/kg, QD, po)
- Fulvestrant (25 mg/kg, Q7D, sc)
- BBO-10203 (100 mg/kg, QD, po) + Fulvestrant (25 mg/kg, Q7D, sc)



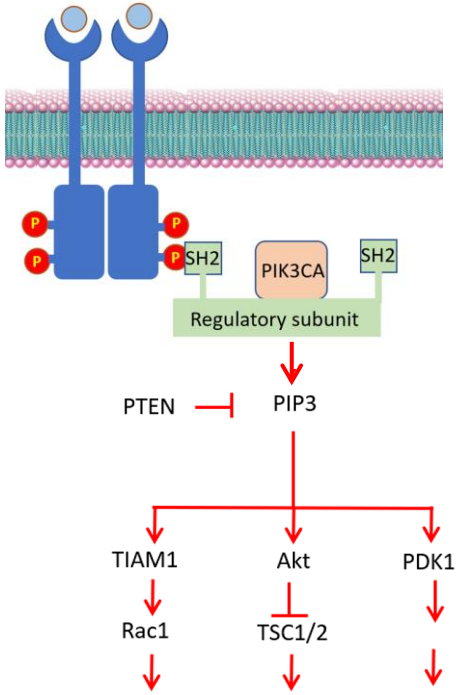
- Vehicle (QD, po)
- BBO-10203 (100 mg/kg, QD, po)
- Palbociclib (10 mg/kg, BID, po)
- BBO-10203 (100 mg/kg, QD, po) + Palbociclib (10 mg/kg, BID, po)

Activation of PI 3’ kinase by growth factors and RAS

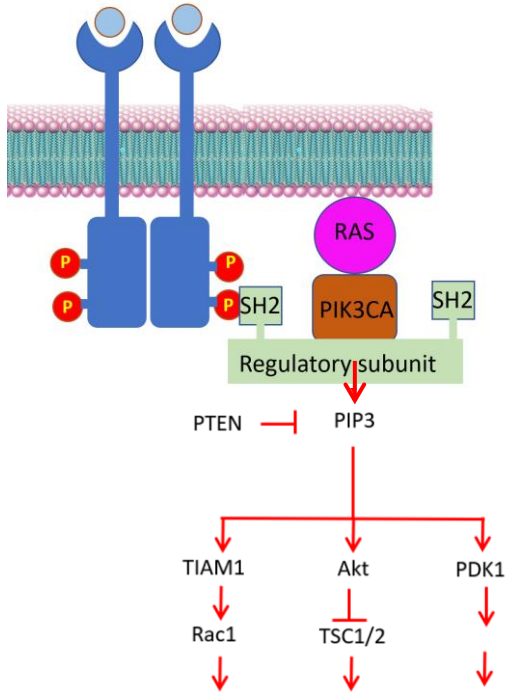
Insulin Receptor



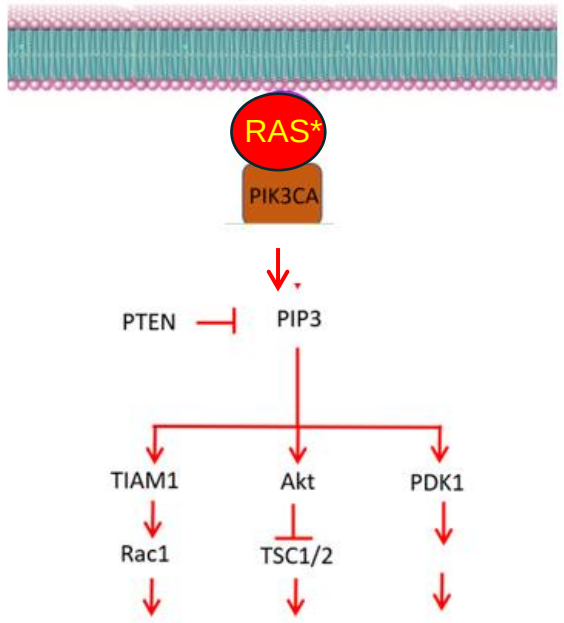
Normal RTK



Development, Angiogenesis, Oncogenesis



Oncogenic KRAS



Thanks!!

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