Progress in Targeting KRAS through the Frederick RAS Initiative

Frank McCormick

- **Disclosures: Advisor to BridgeBio, Leidos Biomedical, Frontier Medicines, Quanta Therapeutics, Amgen, Pfizer**

Goals

Test direct inhibitors of the active forms of KRAS in the clinic

Clinical testing of compounds that prevent RAS binding to PI 3’ kinase α

Molecular description of RAS activation of Raf-1

- Biochemical and biophysical analysis
- In silico modeling, in collaboration with Dept of Defense/Lawrence Livermore National Lab, *et al*
- Develop inhibitors of this process

Structural analysis of protein complexes to facilitate new approaches to drug discovery

Mechanisms of drug resistance

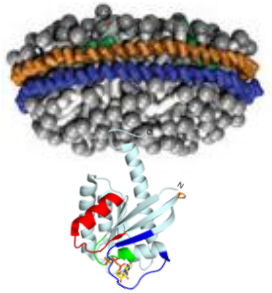
Develop drugs that inhibit NRAS, other GTPases

ID of RAS proteoforms in cancer cells

Determine how the NF1 protein neurofibromin is regulated

Hub and Spoke Model

RAS Initiative



"The Hub"

cCRADAs

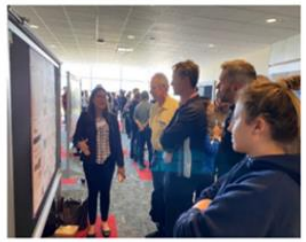
Strategic Collaborations

Industry Collaborations

Academic Collaborations

Community Engagement

Scientific Presentations

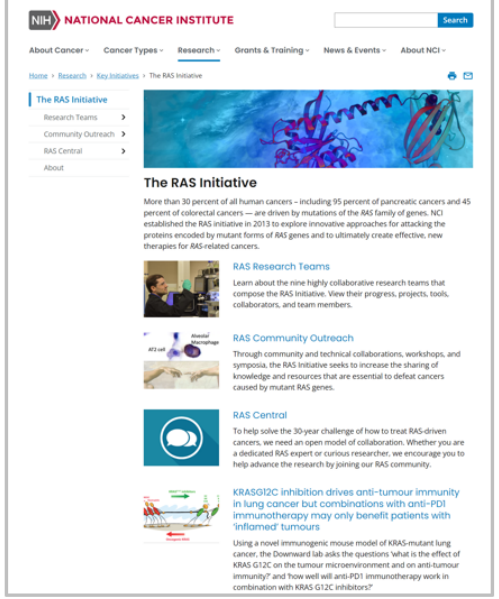


Mentoring from Experts

Community Networking

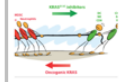
RAS Initiative Website

11,000+ monthly visitors



RAS Dialogue Blog

18,742 subscribers



KRASG12C inhibition drives anti-tumour immunity in lung cancer but combinations with anti-PD1 immunotherapy may only benefit patients with 'inflamed' tumours

November 14, 2022, by Jesse Boumelha, Edurne Mugarza, Sophie de Carné Trécesson, Febe van Maldegem, Miriam Molina and Julian Downward Francis Crick Institute, London

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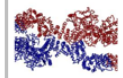
The use of Molecular Docking as a ligand discovery tool; Can machine learning help the pursuit for ligands?

July 6, 2022, by Trent E. Balius and Megan Rigby

Continue Reading >



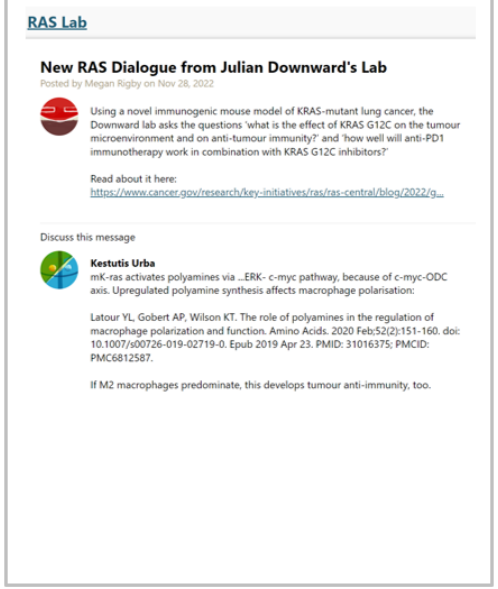
Could CryoEM structures of neurofibromin lead the way to better therapeutic approaches for Neurofibromatosis type 1?

May 18, 2022, by Dom Esposito

Continue Reading >

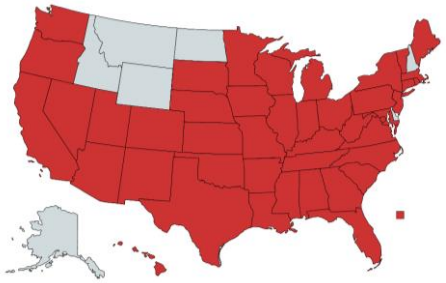
RAS Lab Discussion Forum

1,255 members

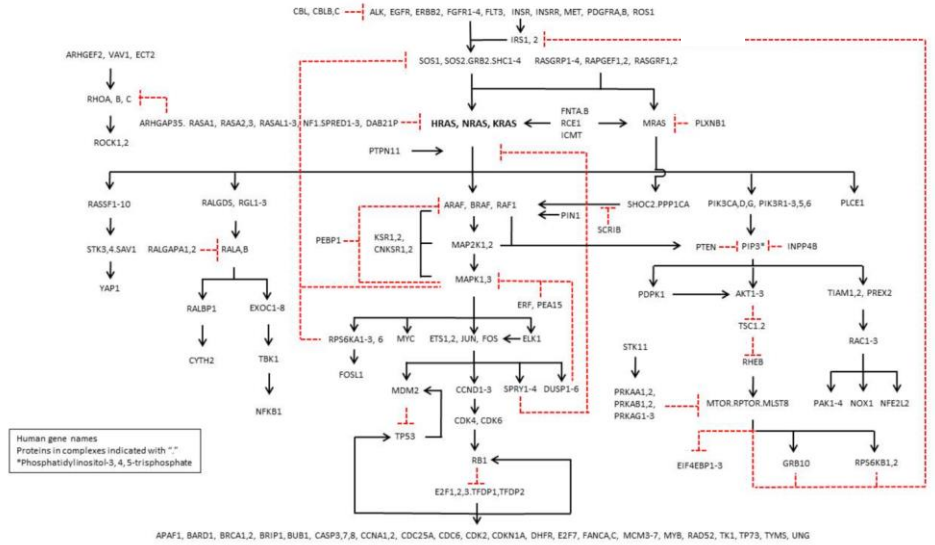


Distribution of RAS reagents

- Reagents widely distributed across the world
  - Materials sent to 623 Universities and NPOs
  - 43 states, 45 countries, 6 continents



- 13,127 plasmids & vectors distributed through Addgene
  - More than 3,000 individual RAS and RAS pathway plasmids
  - At least 1 request for each of the 180 genes
  - 21 complete RAS pathway kits (360 plasmids each)
  - 23 complete RAS mutant kits (61 plasmids each)



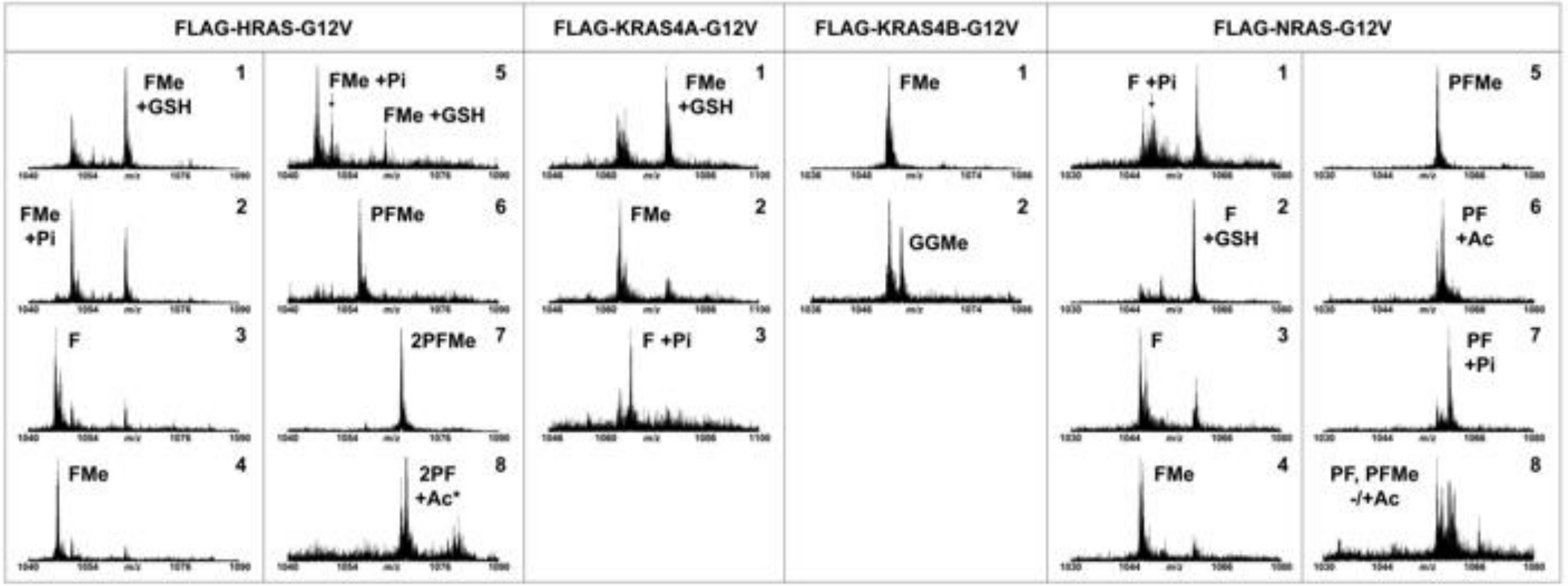
- 1,503 cell lines distributed from FNLCR

- RAS-dependent MEFs licensed to 23 companies and distributed to 97 academic groups

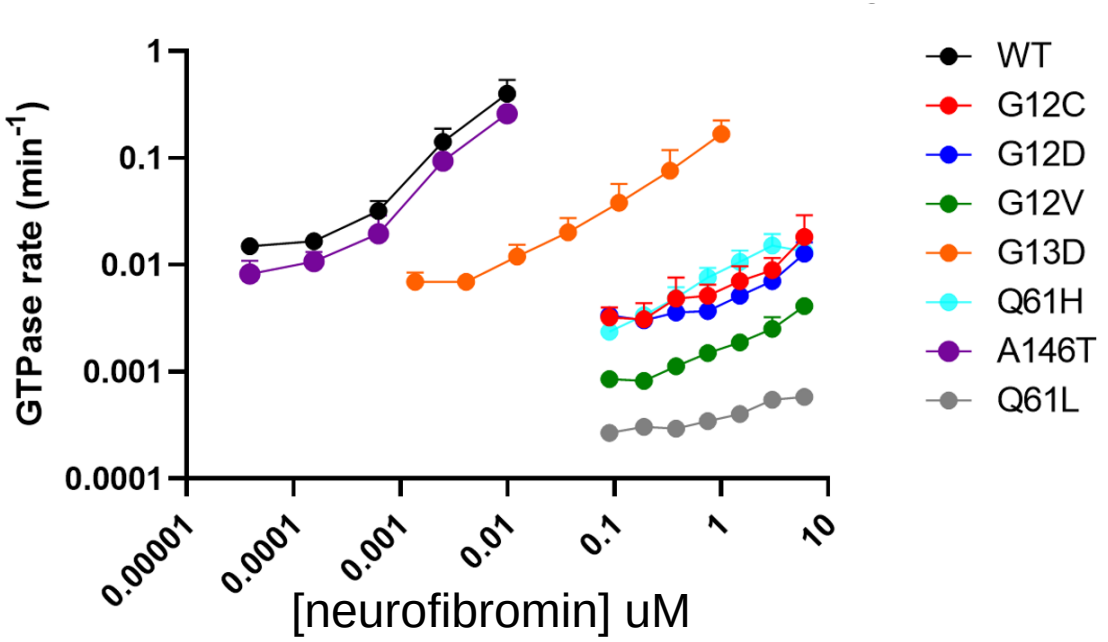
- KRAS-FMe materials licensed to 7 companies

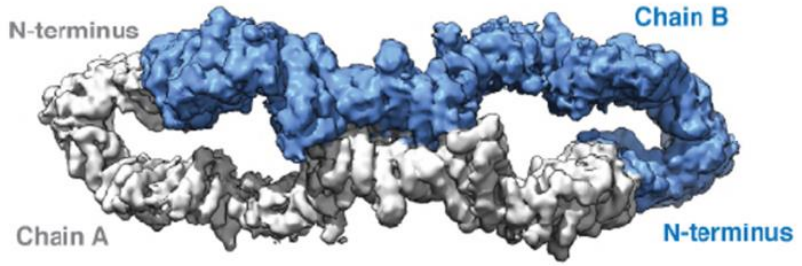
Analysis of RAS isoforms in cancer cells

Abundant FLAG-RAS-G12V proteoforms identified within a Panc1 cell line model by optimized IP-TDMS analysis



Biochemical and structural analysis of KRAS mutants



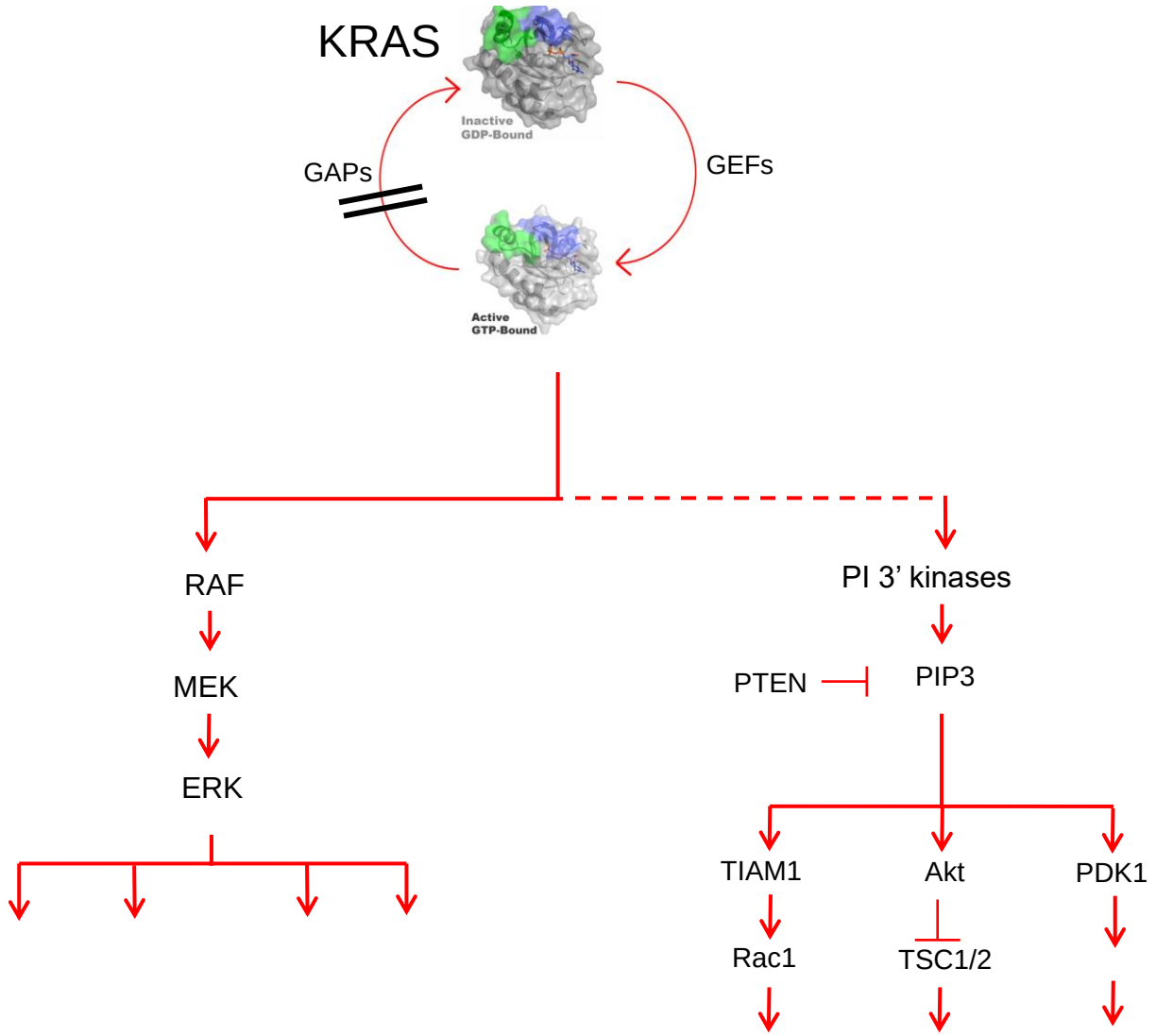


KRAS G13D sensitivity to neurofibromin-mediated GTP hydrolysis

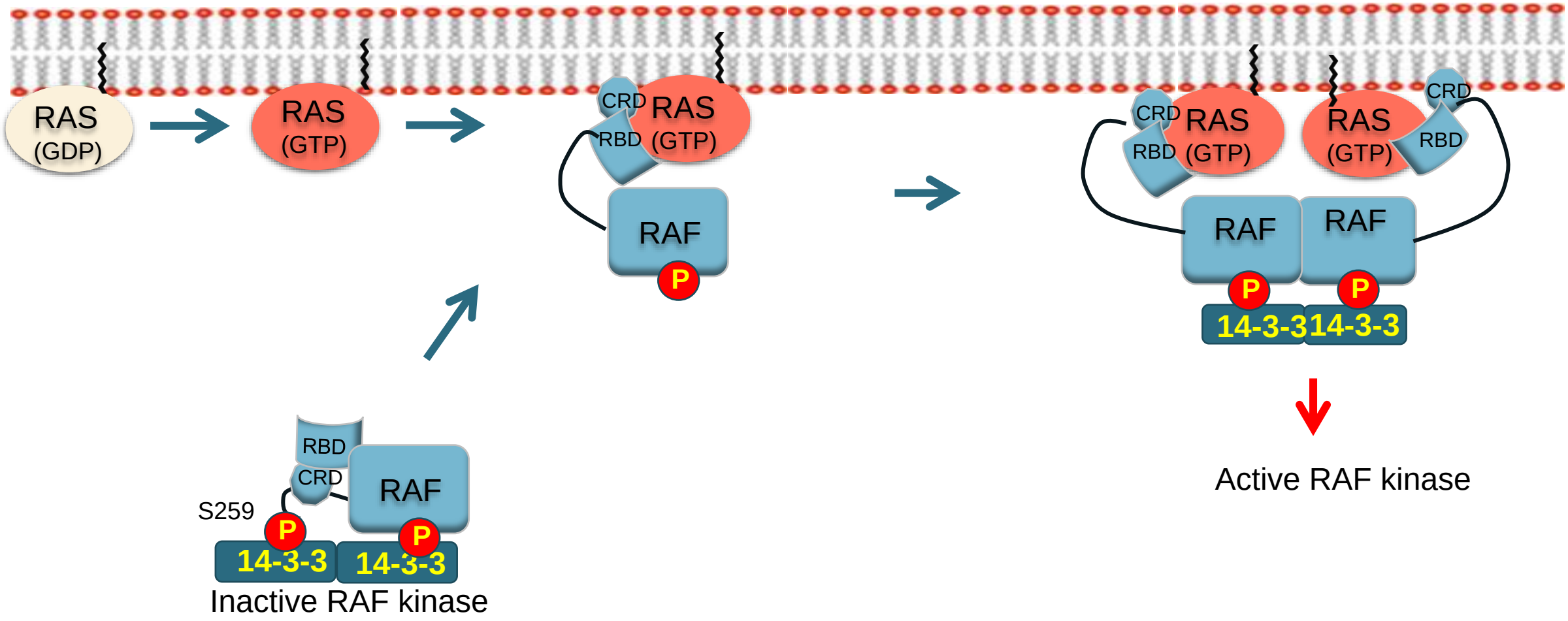
Dana Rabara[a,1], Timothy H. Tran[a,1], Srisathiyanarayanan Dharmaiah[a], Robert M. Stephens[a], Frank McCormick[a,b,2], Dhirendra K. Simanshu[a,2], and Matthew Holderfield[a,2,3]

Dana Rabara, Andy Stephen

Targeting KRAS directly, and through activation of RAF and PIK3CA

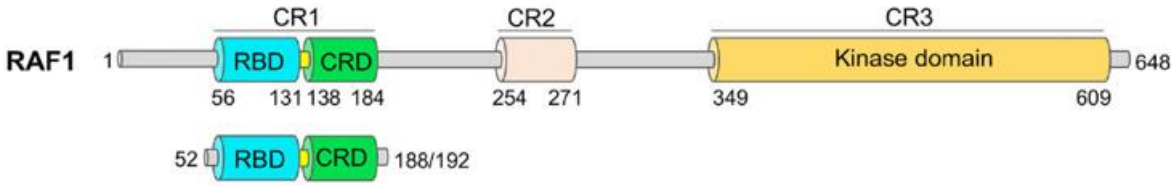


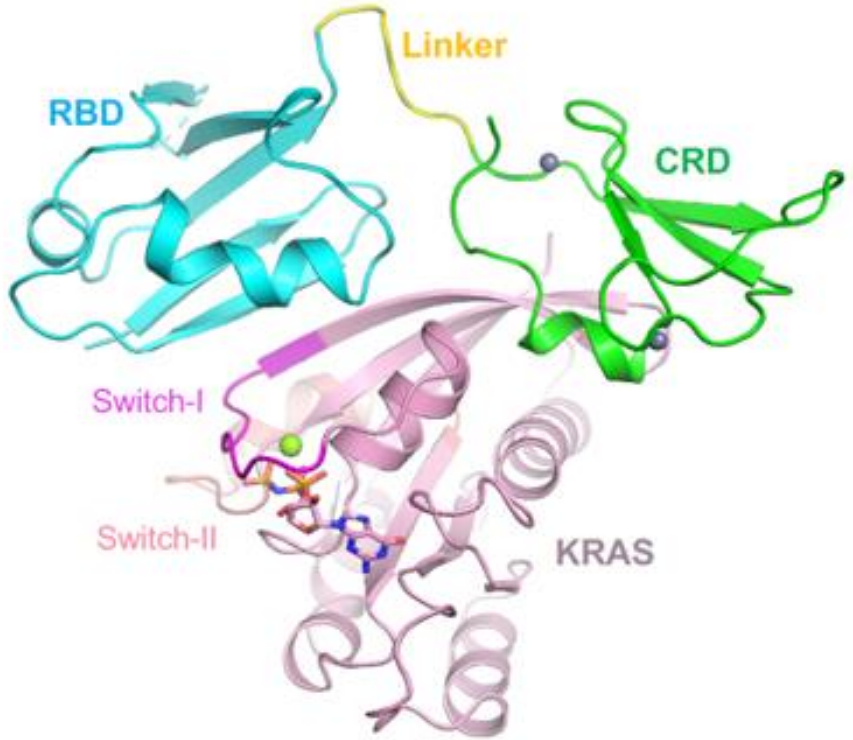
Targeting RAS-dependent activation of RAF kinase



The RAS-RAF interface

RBD: RAS binding domain
CRD: Cysteine rich domain

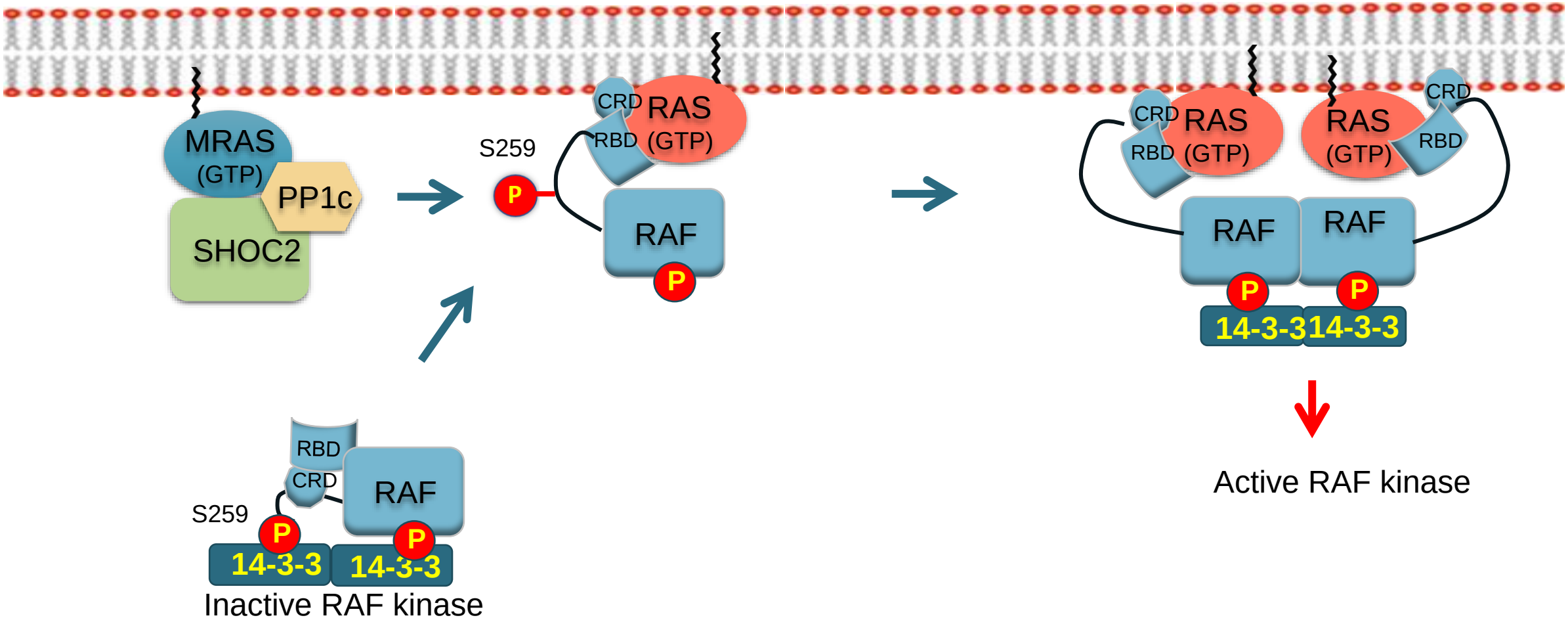




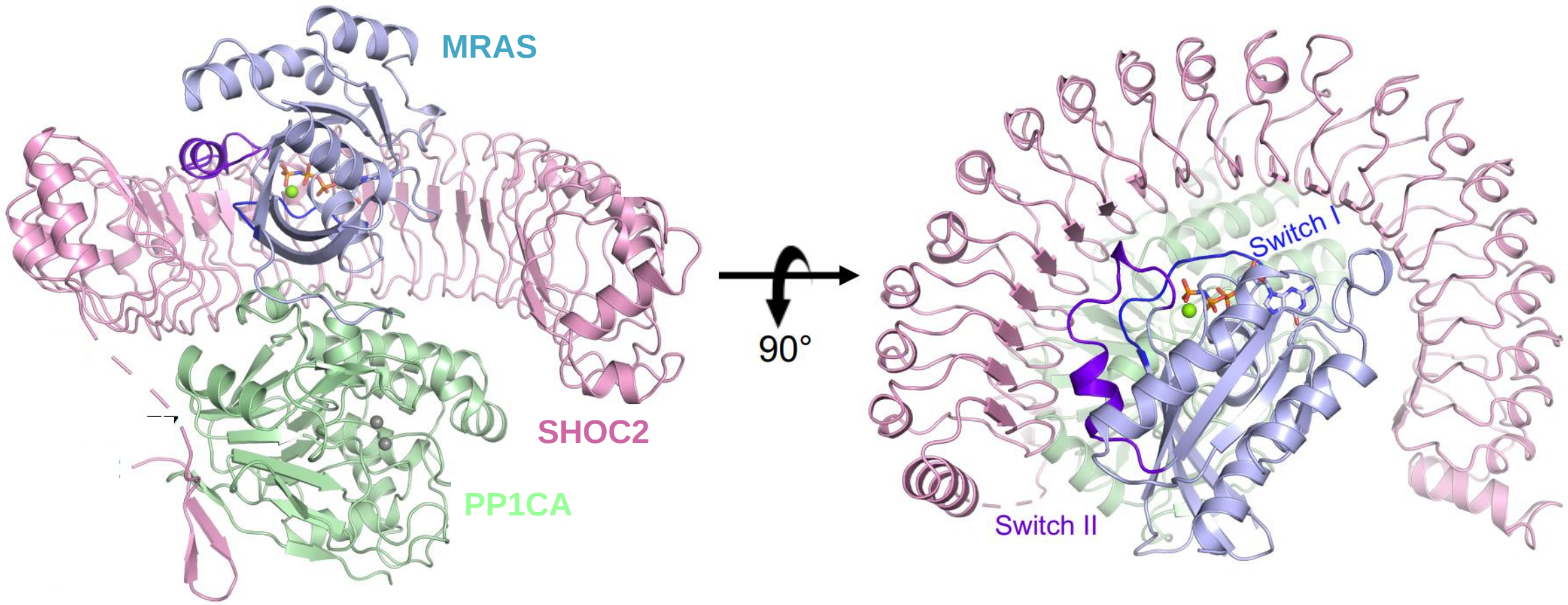
KRAS interaction with RAF1 RAS-binding domain and cysteine-rich domain provides insights into RAS-mediated RAF activation

Timothy H. Tran, Albert H. Chan, Lucy C. Young, Lakshman Bindu, Chris Neale, Simon Messing, Srisathiyanarayanan Dharmaiah, Troy Taylor, John-Paul Denson, Dominic Esposito, Dwight V. Nissley, Andrew G. Stephen, Frank McCormick, Dhirendra K. Simanshu

MRAS-SHOC2-PP1C is essential in RAS Cancers



Structure of the MRAS.SHOC2.PP1C complex



nature structural & molecular biology

Article

https://doi.org/10.1038/s41594-022-00841-4

Structure of the SHOC2–MRAS–PP1C complex provides insights into RAF activation and Noonan syndrome

Received: 17 February 2022

Accepted: 12 August 2022

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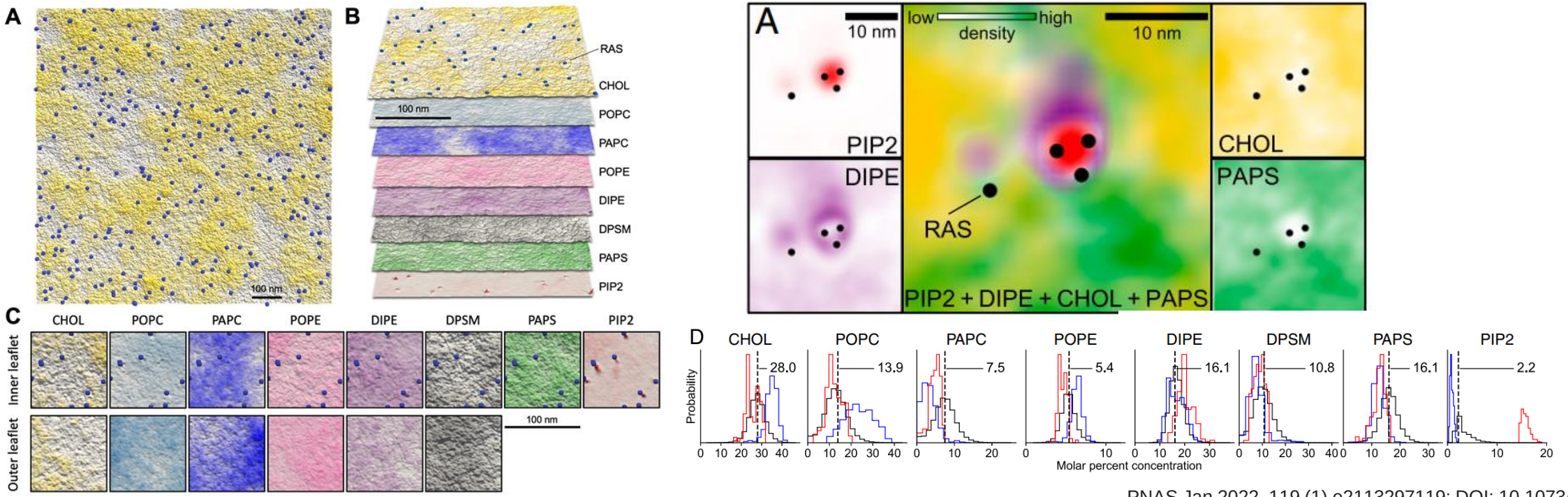
Daniel A. Bonsor[1], Patrick Alexander[1], Kelly Snead[1], Nicole Hartig[1], Matthew Drew[1], Simon Messing[1], Lorenzo I. Finci[1], Dwight V. Nissley[1], Frank McCormick[1,2], Dominic Esposito[1], Pablo Rodriguez-Viciana[1], Andrew G. Stephen[1] and Dhirendra K. Simanshu[1]

Modeling RAS and RAF using machine learning

Machine learning–driven multiscale modeling reveals lipid-dependent dynamics of RAS signaling proteins

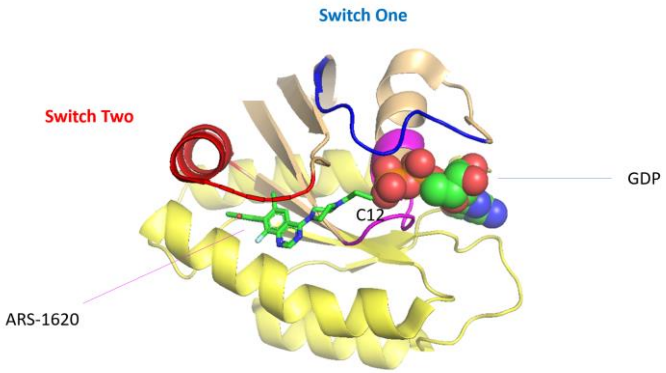
Helgi I. Ingólfsson[a], Chris Neale[b], Timothy S. Carpenter[a], Rebika Shrestha[c], Cesar A. López[b], Timothy H. Tran[c], Tomas Oppelstrup[a], Harsh Bhatia[d], Liam G. Stanton[a], Xiaohua Zhang[a], Shiv Sundram[a], Francesco Di Natale[d], Animesh Agarwal[b], Gautham Dharuman[a], Sara I. L. Kokkila Schumacher[f], Thomas Turbyville[c], Gulcin Gulten[c], Que N. Van[c], Debanjan Goswami[c], Frantz Jean-Francois[c], Constance Agamasu[c], De Chen[c], Jeevapani J. Hettige[b], Timothy Travers[b], Sumantra Sarkar[a], Michael P. Surh[a], Yue Yang[a], Adam Moody[d], Shusen Liu[d], Brian C. Van Essen[d], Arthur F. Voter[h], Arvind Ramanathan[i], Nicolas W. Hengartner[g], Dhirendra K. Simanshu[c], Andrew G. Stephen[c], Peer-Timo Bremer[d], S. Gnanakaran[b], James N. Glosli[a], Felice C. Lightstone[a], Frank McCormick[c,1], Dwight V. Nissley[c,1], and Frederick H. Streitz[a,1]

[a]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550; [b]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545; [c]RAS Initiative, The Cancer Research Technology Program, Frederick National Laboratory, Frederick, MD 21701; [d]Computing Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550; [e]Department of Mathematics and Statistics, San José State University, San José, CA 95192; [f]Data Centric Systems, IBM T. J. Watson Research Center, Yorktown Heights, NY 10598; [g]Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM 87545; [h]Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545; [i]Computing, Environment & Life Sciences Directorate, Argonne National Laboratory, Lemont, IL 60439; and [j]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94115



PNAS Jan 2022, 119 (1) e2113297119; DOI: 10.1073/pnas.2113297119

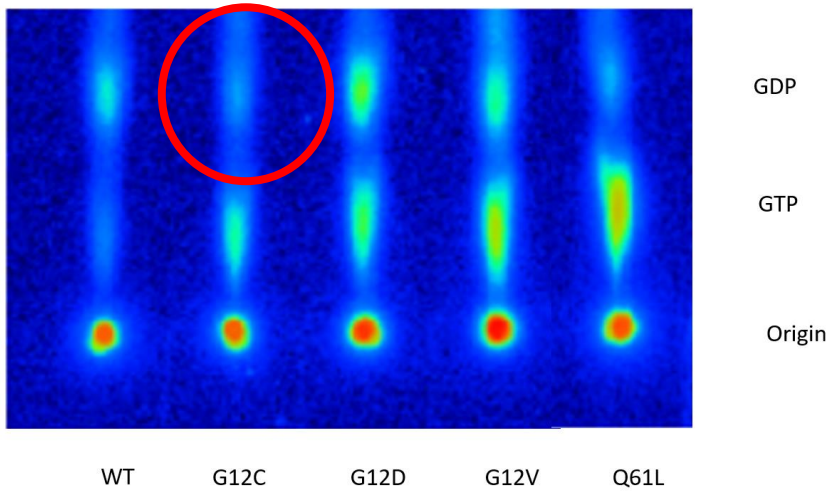
Targeting KRAS G12C with direct, covalent inhibitors

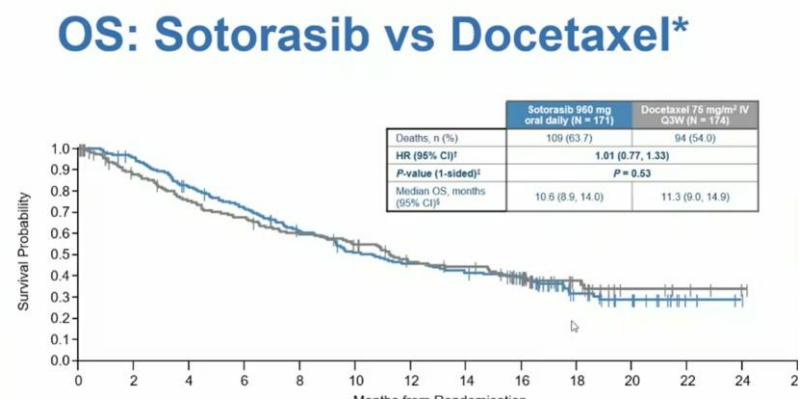


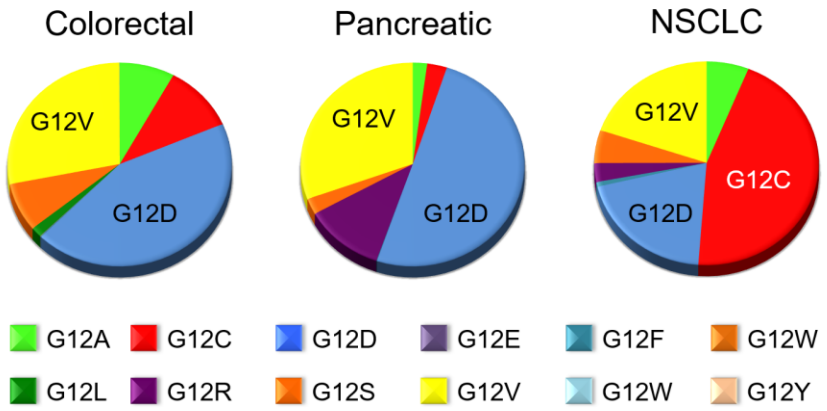
K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions

Jonathan M. Ostrem[1]*, Ulf Peters[1]*, Martin L. Sos[2], James A. Wells[2] & Kevan M. Shokat[1]

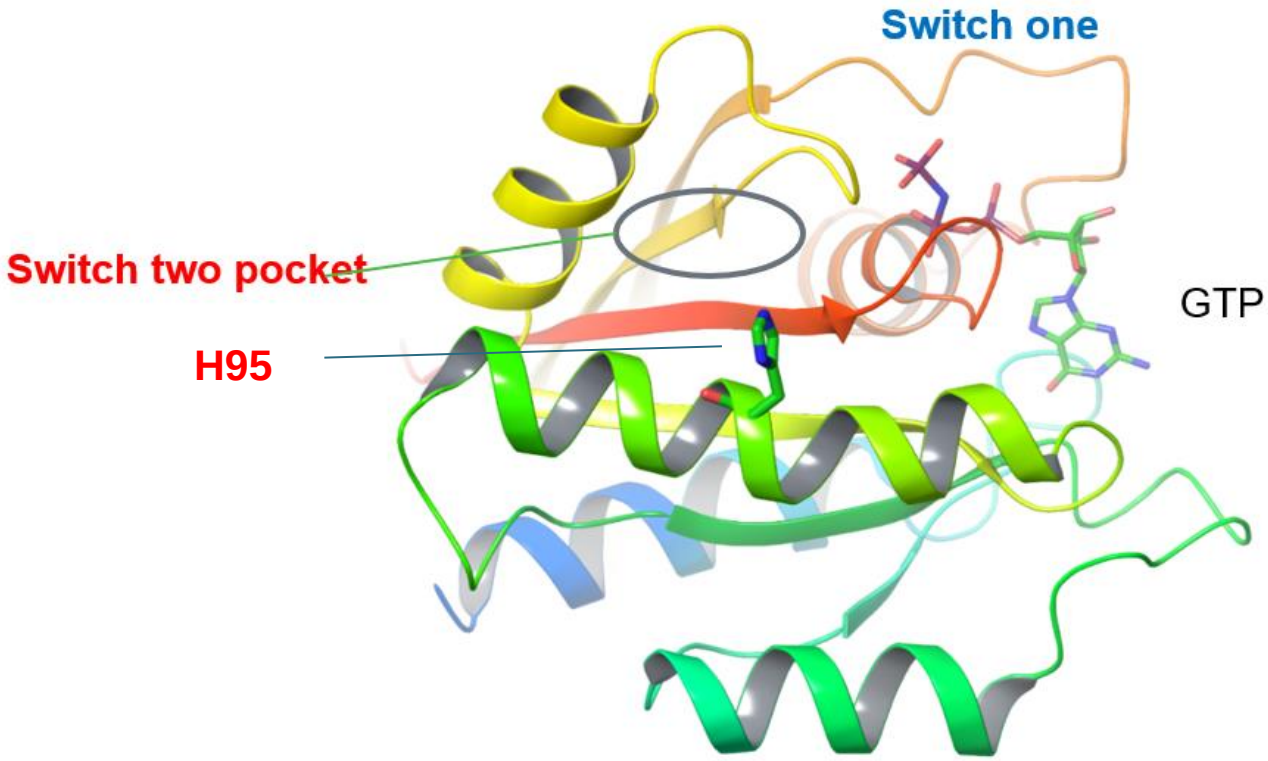
Levels of GDP and GTP on RAS oncogenic mutants





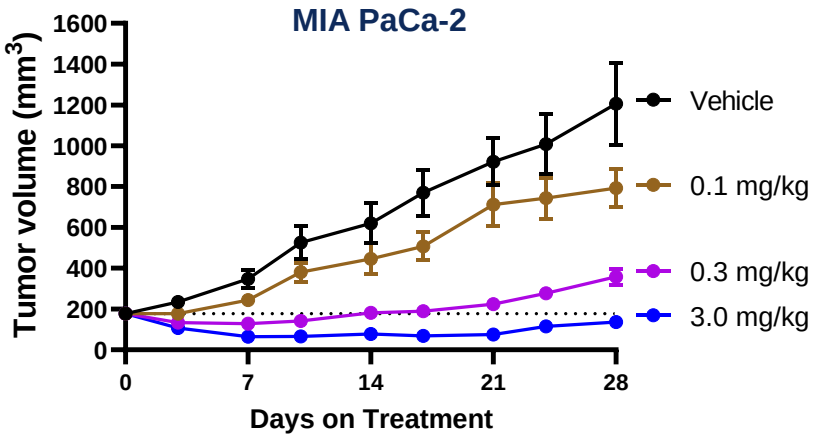


Targeting the GTP-bound forms of KRAS G12C, G12D, G12V, et al

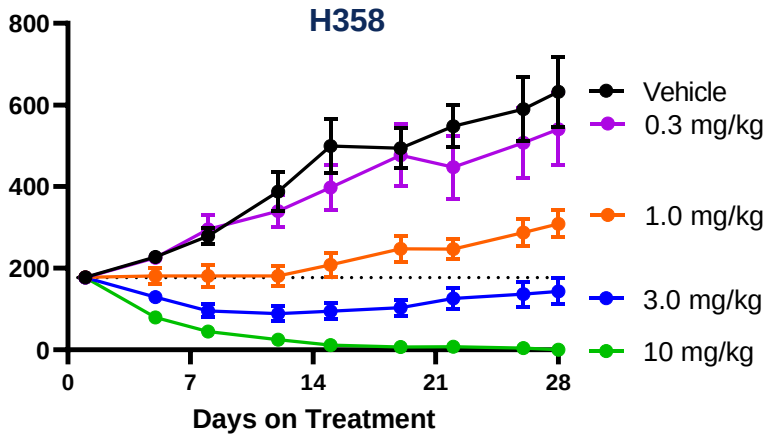


Anna Maciag and colleagues

Targeting active, GTP-forms of KRAS G12C in vivo

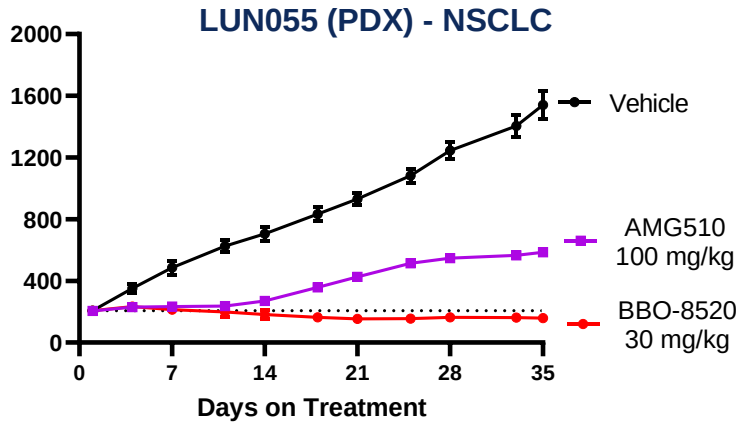


ED_{50}	ED_{90}
0.13 mg/kg	0.40 mg/kg
EC_{50}	EC_{90}
4.6 nM	9.9 nM



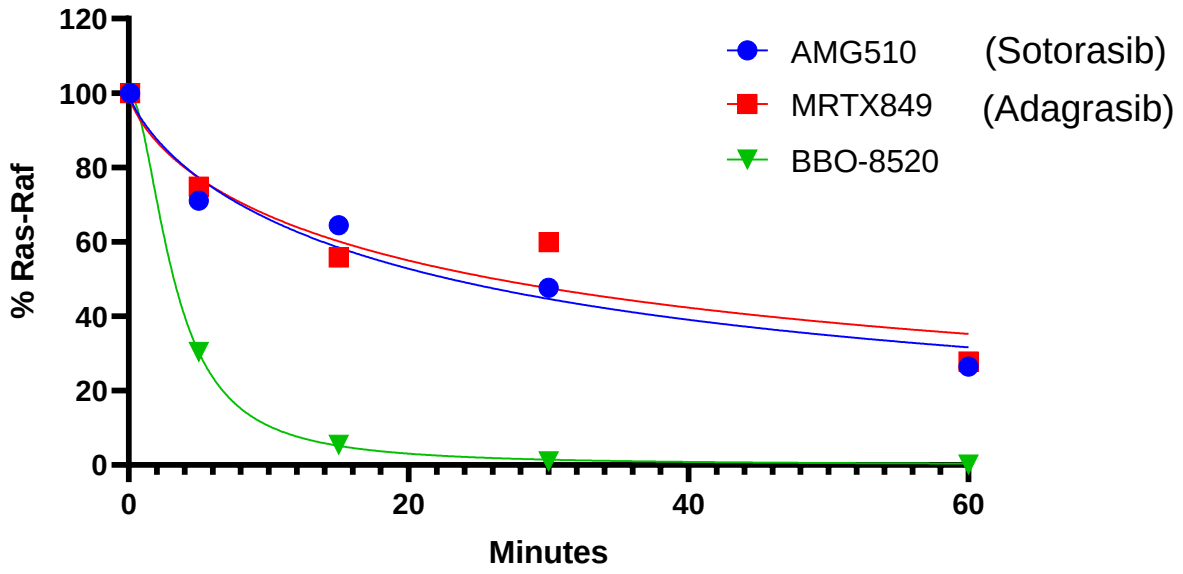
10/10 CRs at 10 mg/kg

ED_{50}	ED_{90}
0.61 mg/kg	1.6 mg/kg
EC_{50}	EC_{90}
14 nM	34 nM



Group (n=10)	Day 35		
	TGI	Regression	FF AUC_{0-24} (ng*hr/ml)
BBO-8520	100%	23% (7/10)	59
AMG510	71%	- (1/10)	1563

Rapid inhibition of $KRAS^{G12C}$ binding to RAF in cells

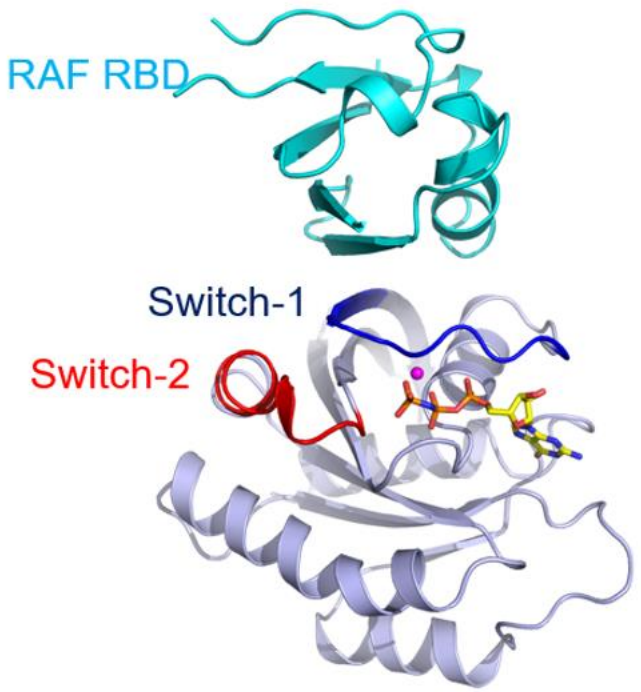


We expect to begin clinical testing later this year

First in class compound that directly targets the active, GTP-bound state of KRAS G12C

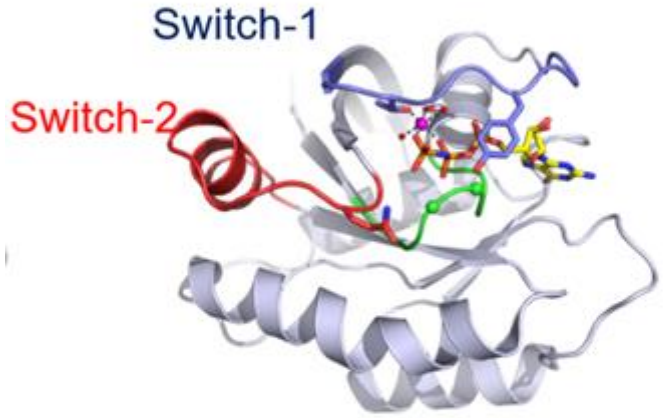
Drugs targeting active states of KRAS G12D and KRAS G12V to follow

RAS.GTP can exist in 2 states



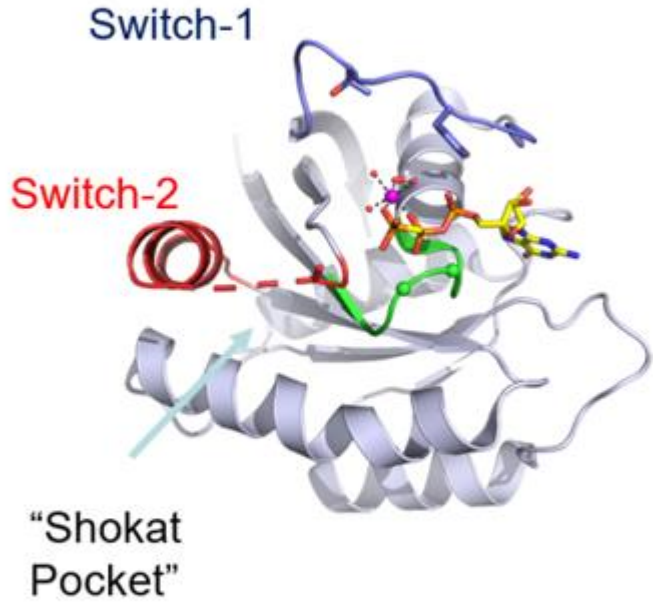
Simanshu

State 2



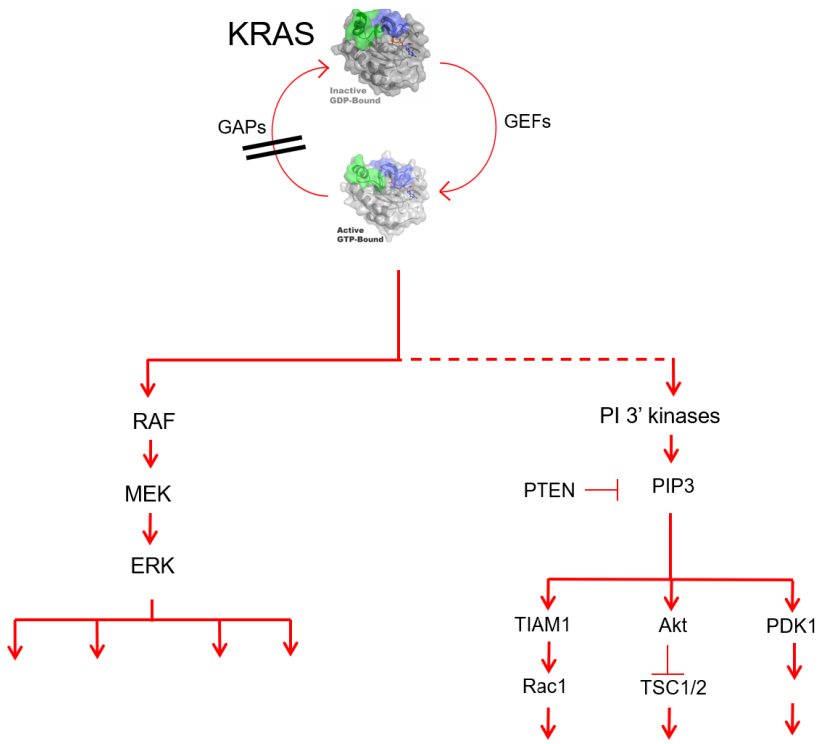
KRAS-GMPPNP
(PDB: 6VC8)

State 1



KRAS-GMPPCP
(PDB:5UK9)

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,*]
[1]Signal Transduction Laboratory
[2]Experimental Pathology Laboratory
Cancer Research UK London Research Institute, 44 Lincoln’s Inn Fields, London WC2A 3PX, UK
[3]Molecular/Cancer Biology, Biomedicum Helsinki, University of Helsinki, P.O.B. 63 (Haartmaninkatu 8), FIN-00014 Helsinki, Finland
[4]These authors contributed equally to this work.
*Correspondence: downward@cancer.org.uk
DOI 10.1016/j.cell.2007.03.051

Cell
Cancer Cell
Previews

Killing Tumors by Keeping Ras and PI3′ Kinase Apart

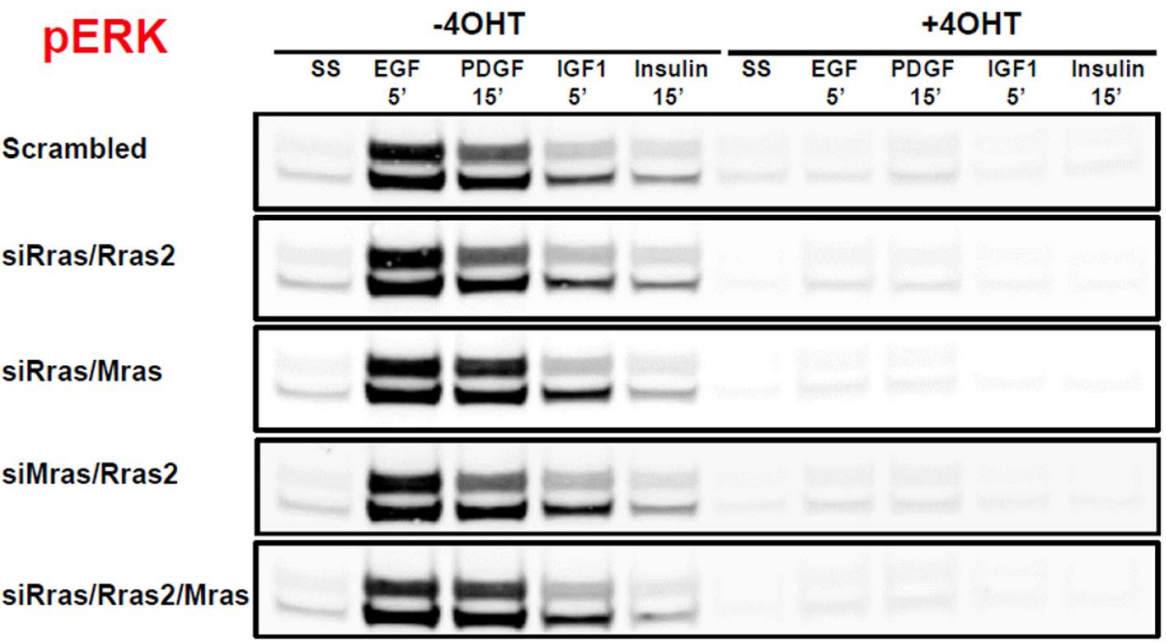
Tina L. Yuan[1] and Frank McCormick[1,*]
[1]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94158, USA
*Correspondence: mccormic@cc.ucsf.edu
http://dx.doi.org/10.1016/j.ccr.2013.10.015

Requirement for Interaction of PI3-Kinase p110α with RAS in Lung Tumor Maintenance

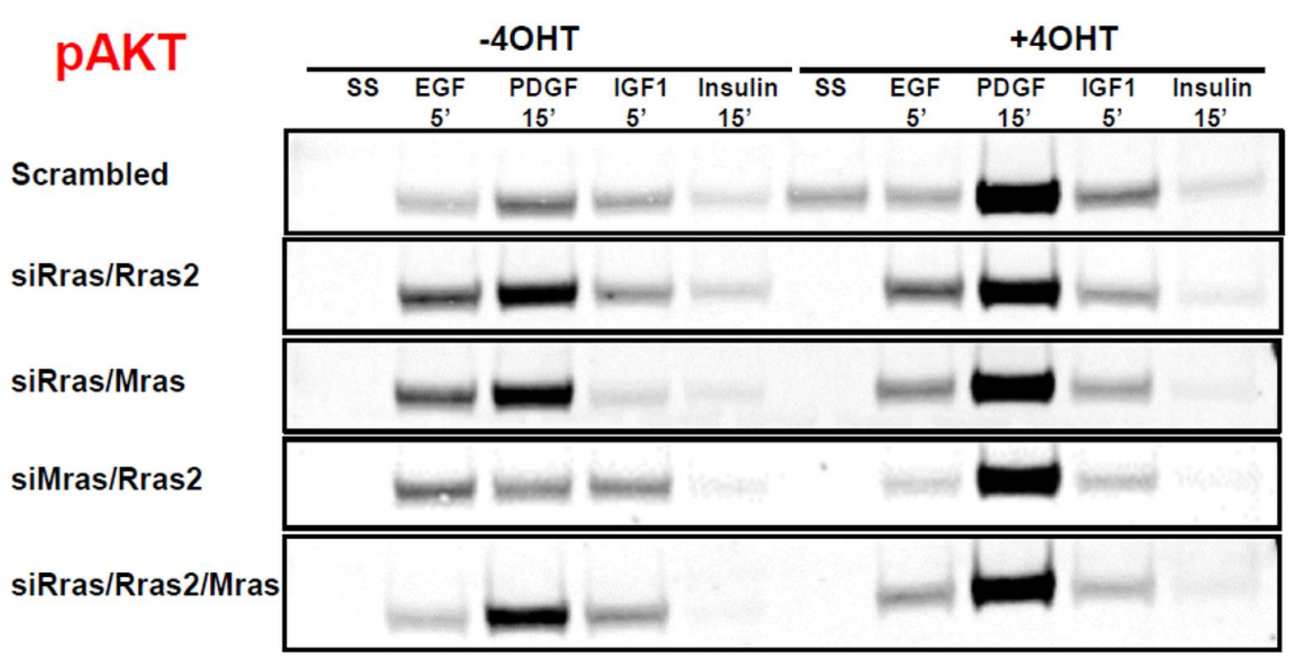
Esther Castellano,[1,7] Clare Sheridan,[1,7] May Zaw Thin,[2] Emma Nye,[3] Bradley Spencer-Dene,[3] Markus E. Diefenbacher,[4] Christopher Moore,[1] Madhu S. Kumar,[1] Miguel M. Murillo,[1,6] Eva Grönroos,[5] Francois Lassailly,[2] Gordon Stamp,[3] and Julian Downward[1,6,*]

RAS proteins are not needed to activate PI 3’ kinase in normal cells

RAS-less cells



RAS-less cells

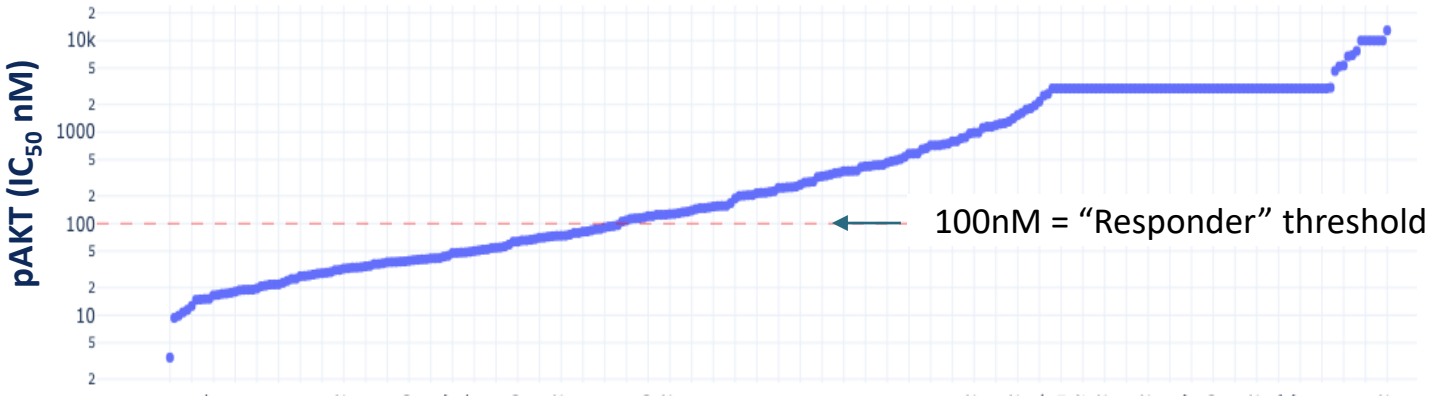


Nikki Fer

We have developed a potent, orally available compound that binds to PIK3CA and prevents RAS activation

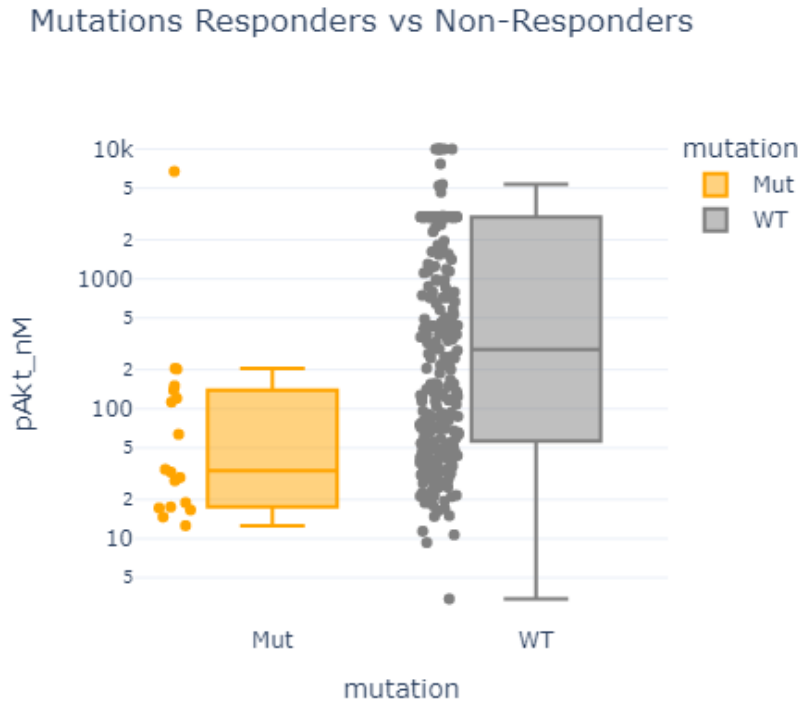
One third of all cancer cell lines depend on PI3Kα:RAS interaction for activation of AKT signaling

pAKT cell line screen



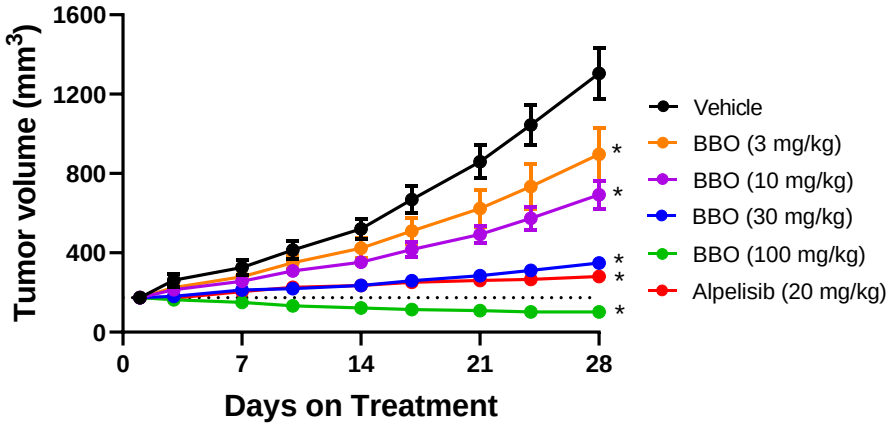
- **105/282 (37%) of screened cell lines are responders**
- **29/50 (58%) of screened KRAS^{G12X} cell lines are responders**

PIK3CA helical mutants are highly sensitive

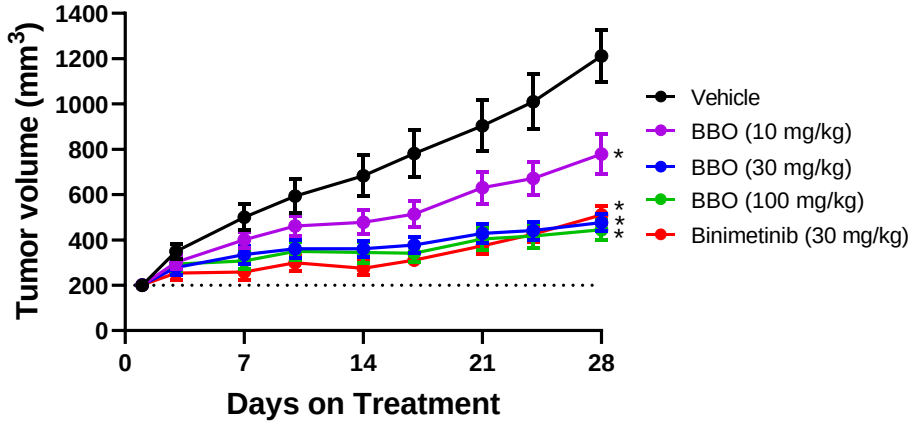


Efficacy in mouse models

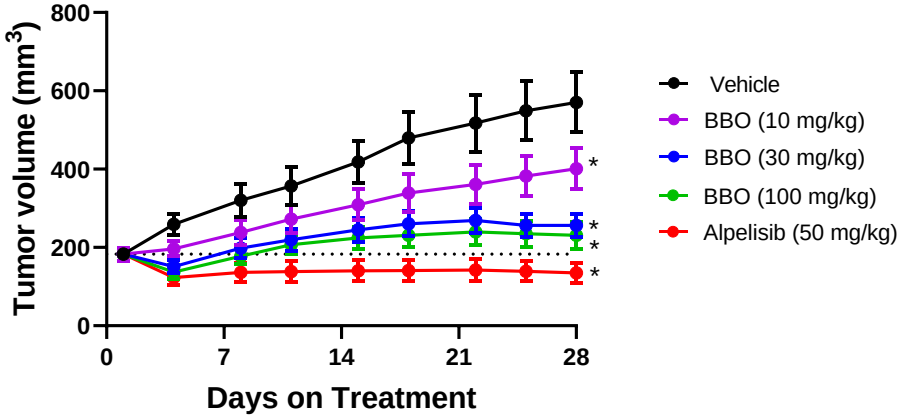
KYSE-410 CDX
- KRAS^{G12C}
- HER2^{amp}



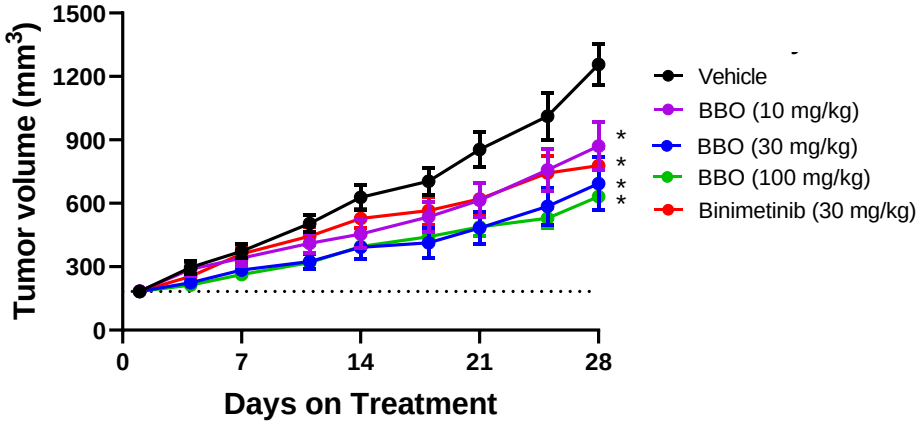
GP2d CDX
- KRAS^{G12D}
- PIK3CA^{H1047L}



SNU-601 CDX
- KRAS^{G12D}
- PIK3CA^{E542K}



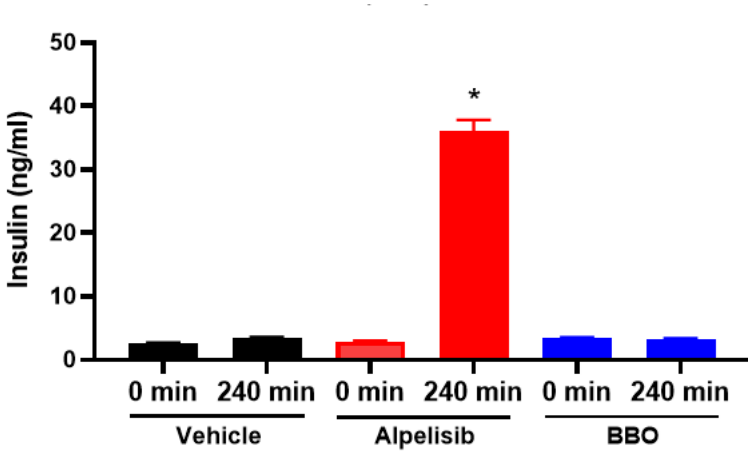
SNU-16 CDX
- KRAS^{G12D}



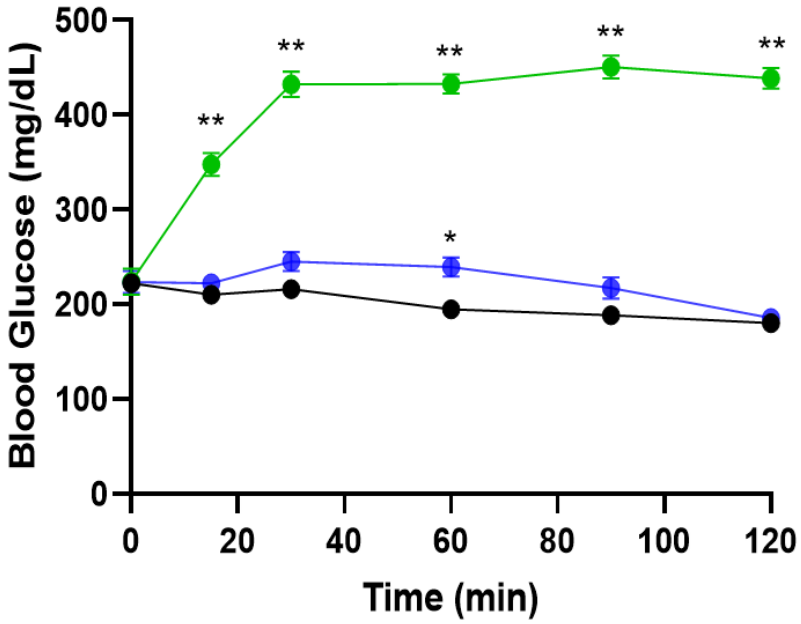
All groups dosed PO, QD, *p<0.0005 RM ANOVA vs vehicle

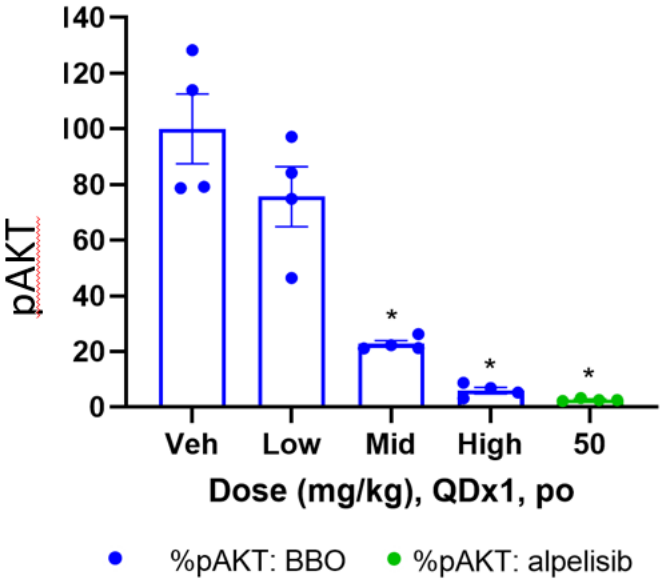
pAKT inhibition in vivo without induction of hyperglycemia

Insulin levels



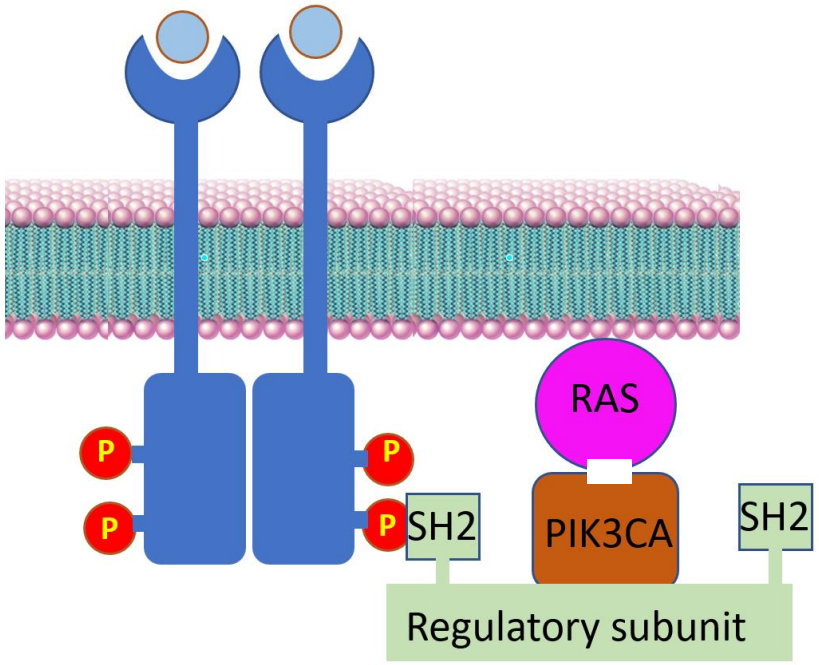
Blood glucose levels

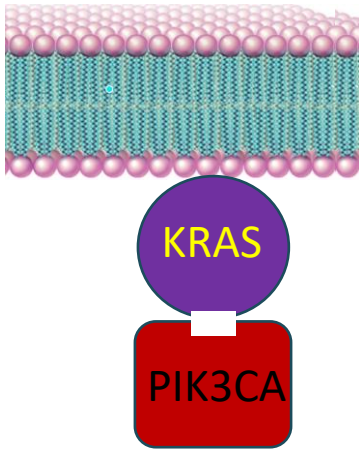


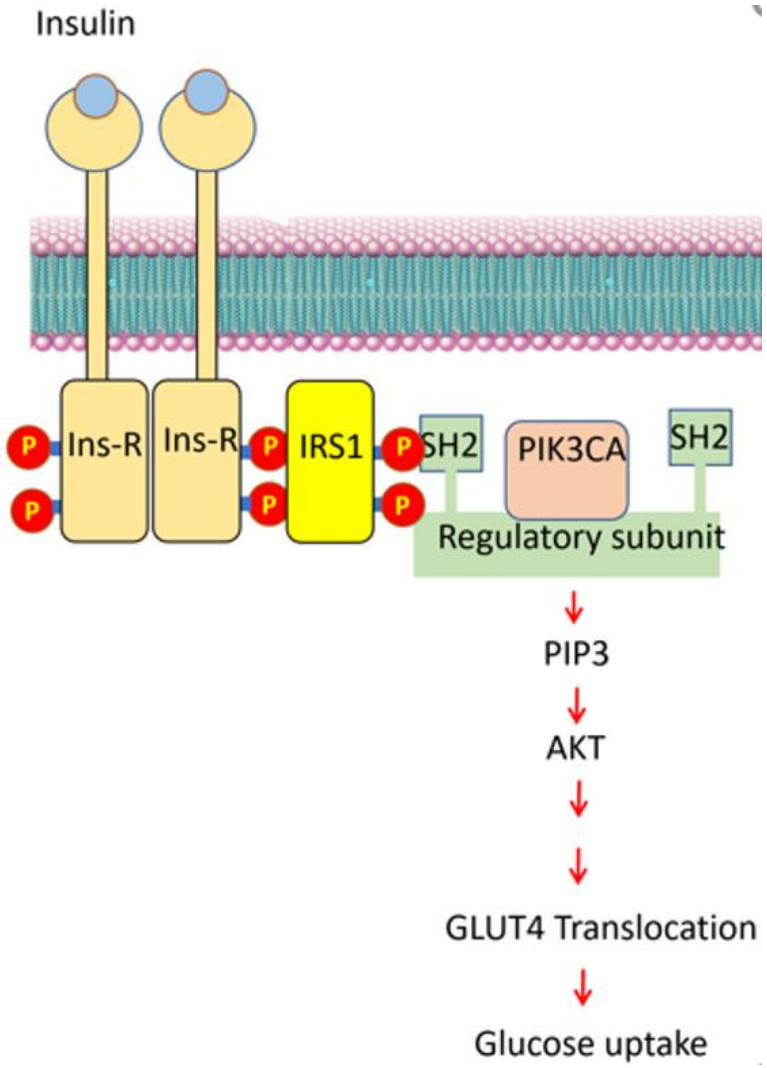


Dose response: One-way ANOVA with Tukey's test vs vehicle *p<0.0001. Blood glucose levels: One-way ANOVA with Dunnett's multiple comparisons test vs vehicle: *p<0.01, **p<0.0001

Compounds that bind PIK3CA and prevent RAS binding; Inhibition in tumor cells without affecting glucose uptake







Anticipating Drug Resistance in the clinic

First in class direct KRAS G12 ON inhibitors

- Point mutations that prevent drug binding
- Activation of other RAS genes, other proteins in the pathway
- Differentiation state changes
- Activation of YAP signaling
- Others?

First in class RAS-PIK3CA Breakers

- Point mutations that prevent drug binding
- Loss of PTEN?
- Activation of other PI kinases?
- Alternative pathways??

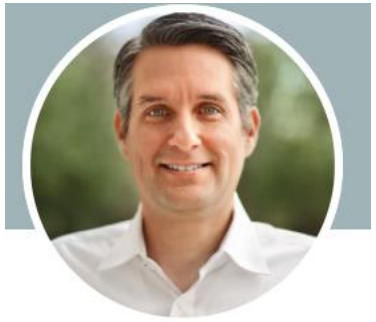
Frederick National Lab

Anna Maciag

Dhirendra Simanshu

Bridge Bio

Eli Wallace



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