ICR The Institute of Cancer Research

THE ROYAL MARSDEN

Direct targeting of RAS: small molecules

Dr Udai Banerji FRCP, PhD

Reader in Molecular Cancer Pharmacology

The Institute of Cancer Research / The Royal Marsden Hospital

ESMO symposium on signalling pathways in cancer

Targeting the MAPK pathway: From RAS to MEK

13th March 2015

NHS National Institute for Health Research

ECMC

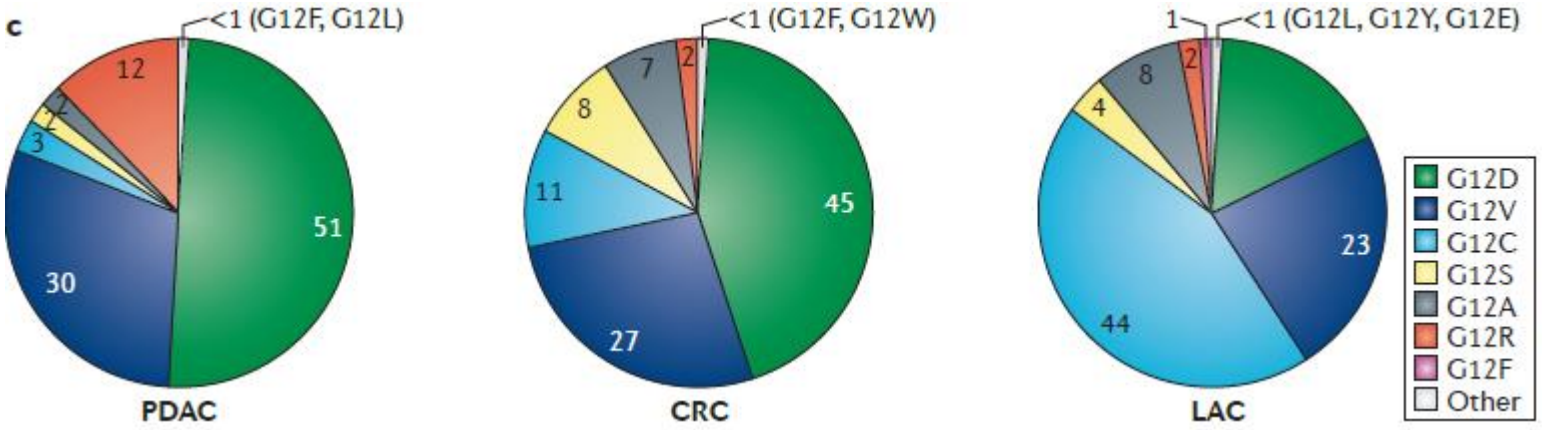
CANCER RESEARCH UK

Conflicts of interest

- I have no conflict of interest related to the subject matter discussed in this talk

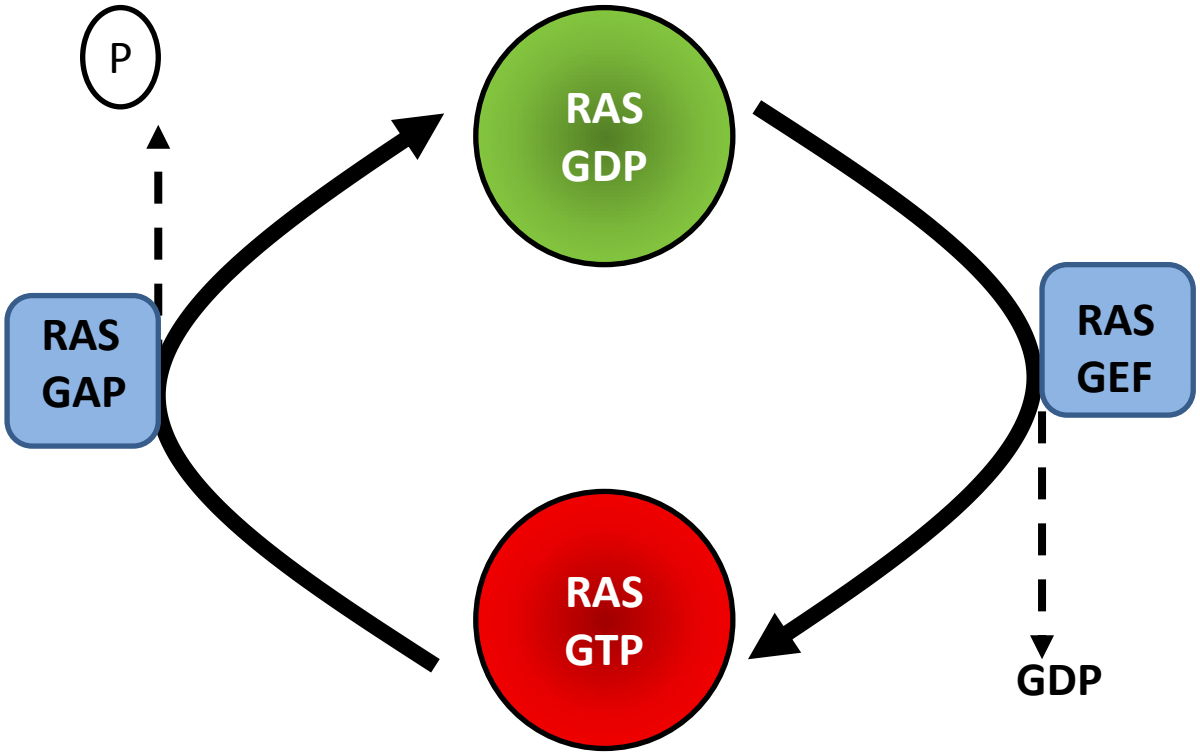
The usual suspects

Tumour	% KRAS	% NRAS	% HRAS	% RAS
Pancreatic	97.7	0	0	97.7
Colorectal	44.7	7.5	0	52.2
Multiple Myeloma	22.8	19.9	0	42.6
Lung adenocarcinoma	30.9	0.9	0.3	32.2
Melanoma	0.8	27.6	1	29.1



- **The frequency and distribution of RAS gene mutations are not uniform**
- **Often single base missense mutations on residues G12, G13 and Q61**
- **Drugs targeting RAS will have different effects on different isoforms and thus different effects on tumours**

RAS- The physiological problem



- While ATP binds to protein kinases at low µM affinity, GTP binds to RAS with pM affinity
- GTP/GDP present in µM concentrations in cells, including cancer cells

RAS- The pathological problem

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5704–5708, September 1984
Biochemistry

Intrinsic GTPase activity distinguishes normal and oncogenic *ras* p21 molecules

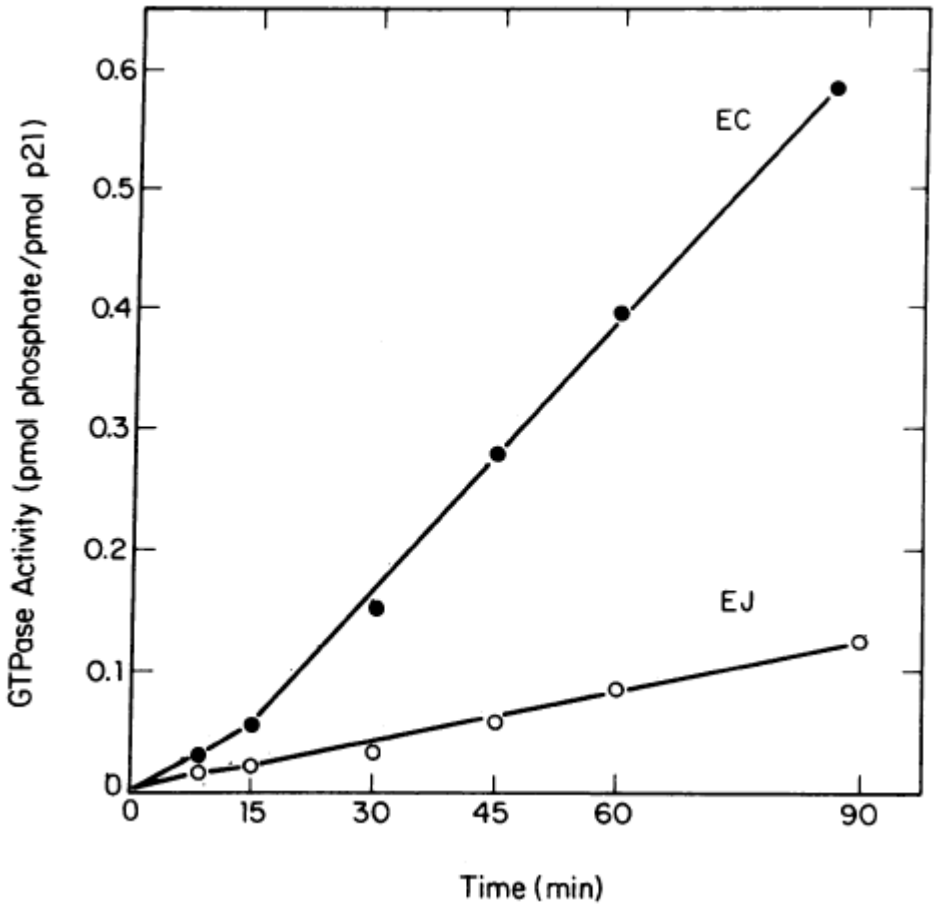
(p21 purification/oncogenic mutation/valine/threonine/autophosphorylating activity)

JACKSON B. GIBBS*, IRVING S. SIGAL*, MARTIN POE†, AND EDWARD M. SCOLNICK*

*Virus and Cell Biology Research, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486; and †Department of Biophysics, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

Contributed by Edward M. Scolnick, June 11, 1984

p21 preparation	GTPase
1. EC	596 ± 170 (22)
2. EC	481 ± 42 (7)
3. EC	562 ± 190 (5)
4. EJ	129 ± 50 (13)
5. EJ	169 ± 14 (4)
6. EC/v-Ha	153 ± 110 (4)
7. EC/v-Ha	118 ± 20 (5)
8. EJ/v-Ha	18 ± 10 (4)



- **Mutant RAS protein (RAS G12V) has a lower GTPase activity than wild type RAS, suggesting mutant RAS stays in an activated GTP bound form**

Gibbs JB, PNAS 1984, 81:5704-5708

Early low affinity inhibitors

Pergamon

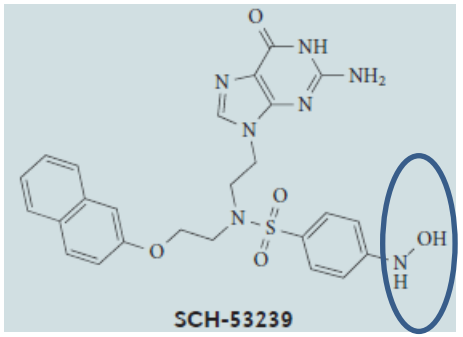
Bioorganic & Medicinal Chemistry, Vol. 5, No. 1, pp 125–133, 1997

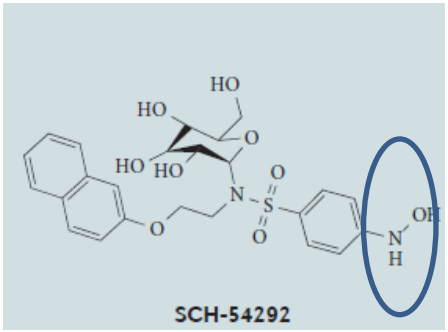
PII: S0968-0896(96)00202-7
0968-0896/97 $17.00 + 0.00

Ras Oncoprotein Inhibitors: The Discovery of Potent, Ras Nucleotide Exchange Inhibitors and the Structural Determination of a Drug–Protein Complex

A. G. Taveras,[a] S. W. Remiszewski,[a] R. J. Doll[a,*], D. Cesarz,[a] E. C. Huang,[a] P. Kirschmeier,[a] B. N. Pramanik,[a] M. E. Snow,[a] Y.-S. Wang,[a] J. D. del Rosario,[a] B. Vibulbhan,[a] B. B. Bauer,[a] J. E. Brown,[a] D. Carr,[a] J. Catino,[a] C. A. Evans,[a] V. Girijavallabhan,[a] L. Heimark,[a] L. James,[a] S. Liberles,[a] C. Nash,[a] L. Perkins,[a] M. M. Senior,[a] A. Tsarbopoulos,[a] A. K. Ganguly,[a] R. Aust,[b] E. Brown,[b] D. Delisle,[b] S. Fuhrman,[b] T. Hendrickson,[b] C. Kissinger,[b] R. Love,[b] W. Sisson[b], E. Villafranca[b] and S. E. Webber[b]

[a]*Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, U.S.A.*
[b]*Agouron Pharmaceuticals, San Diego, CA, 92121, U.S.A.*





- **Designed to bind nucleotide binding site but subsequently found to bind hydrophobic pocket near SII effector region**
- **Low affinity**
- **Contained hydroxylamine moiety which has toxicity and poor metabolic stability**

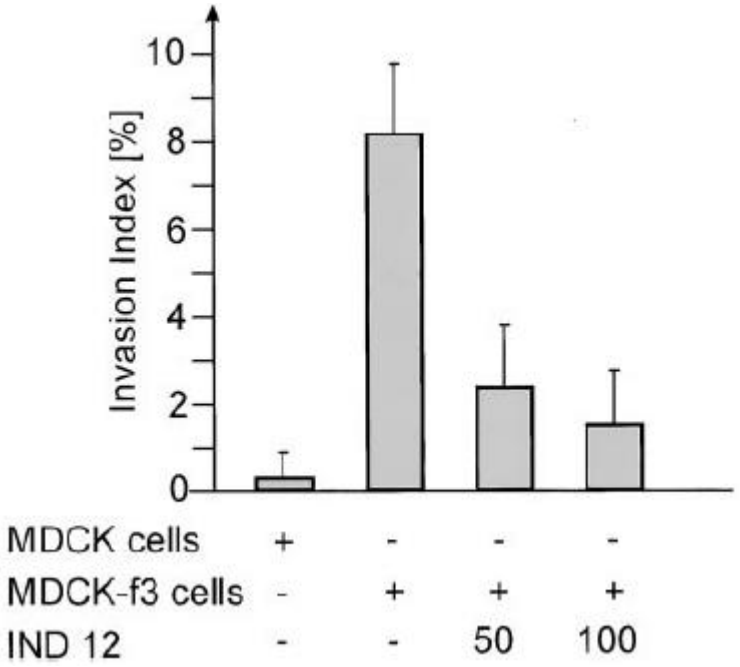
Taveras AG et al Bioorganic and Med Chem 1997, 5:125-133

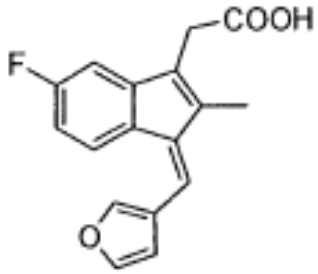
Early low affinity inhibitors

[CANCER RESEARCH 62, 1718–1723, March 15, 2002]

The New Sulindac Derivative IND 12 Reverses Ras-induced Cell Transformation[1]

Ioanna-Maria Karaguni, Peter Herter, Philip Debruyne, Slava Chtarbova, Alice Kasprzynski, Ulrike Herbrand, M-Reza Ahmadian, Karl-Heinz Glüsenkamp, Günther Winde, Marc Mareel, Tarik Möröy, and Oliver Müller[2]





- **Non covalently bind RAS to inhibit the formation of the RAS-RAF complex**
- **Not very potent compounds**
- **Multiple other off target effects**

Karaguni IM et al Bioorg and Med Chem letters 2002, 12:709-713

RAS- GEF targeted inhibitors

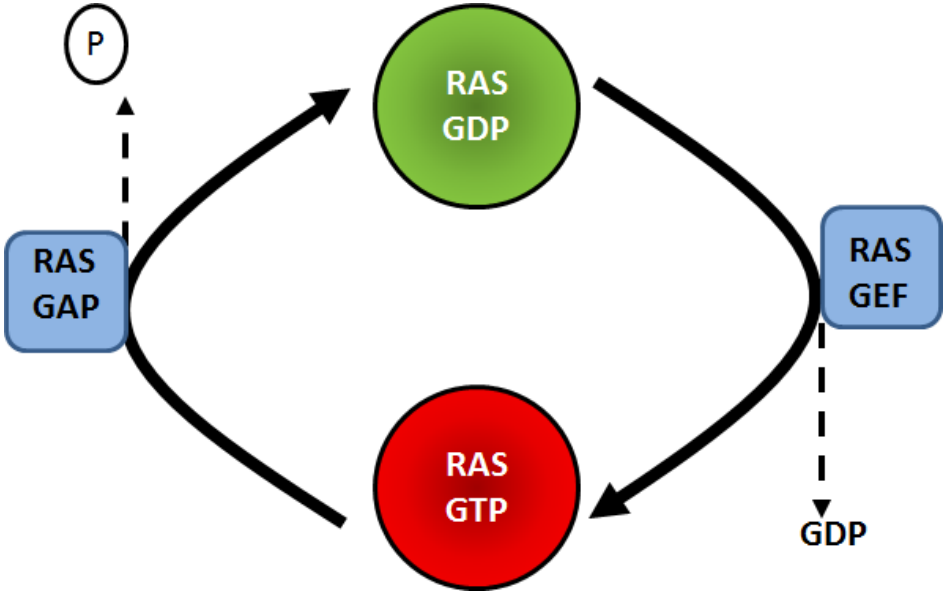
PNAS

Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity

Till Maurer[a,1], Lindsay S. Garrenton[b,1], Angela Oh[a], Keith Pitts[c], Daniel J. Anderson[b], Nicholas J. Skelton[d], Benjamin P. Fauber[d], Borlan Pan[a], Shiva Malek[c], David Stokoe[b], Mary J. C. Ludlam[b], Krista K. Bowman[a], Jiansheng Wu[e], Anthony M. Giannetti[f], Melissa A. Starovasnik[a], Ira Mellman[b], Peter K. Jackson[b], Joachim Rudolph[d], Weiru Wang[a,2], and Guowei Fang[b,2]

[a]Structural Biology, [b]Research Oncology, [c]Biochemical and Cellular Pharmacology, [d]Discovery Chemistry, and [e]Protein Chemistry, Genentech, Inc., One DNA Way, South San Francisco, CA 94080

Edited by* Sung-Hou Kim, University of California, Berkeley, CA, and approved February 2, 2012 (received for review October 6, 2011)



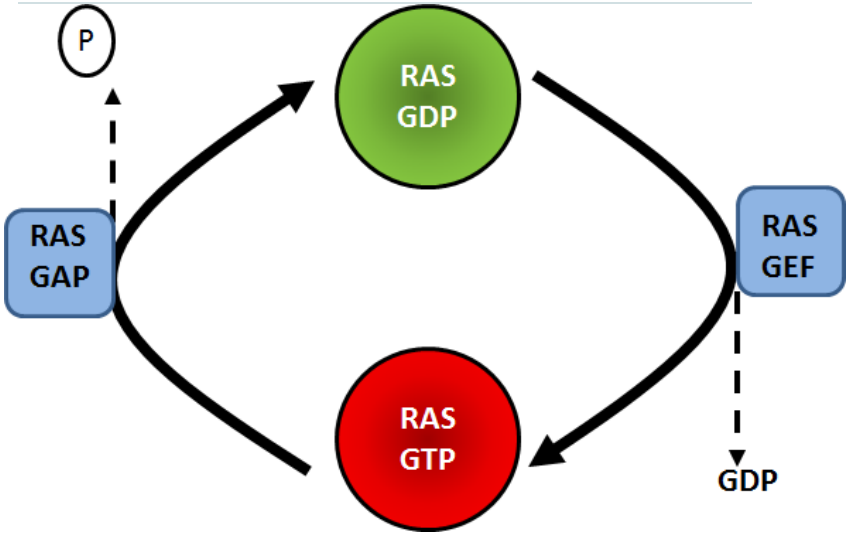
Angewandte Communications

DOI: 10.1002/anie.201201358

Fragment-Based Screening

Discovery of Small Molecules that Bind to K-Ras and Inhibit Sos-Mediated Activation**

*Qi Sun, Jason P. Burke, Jason Phan, Michael C. Burns, Edward T. Olejniczak, Alex G. Waterson, Taekyu Lee, Olivia W. Rossanese, and Stephen W. Fesik**



- **Fragment based approaches have identified compounds such as DACI and VU0460009 which binds to KRAS and inhibited RAS-SOS1 mediated nucleotide exchange**
- **Only weak binding to RAS and possibility that they may not work in the setting of mutationally active RAS**

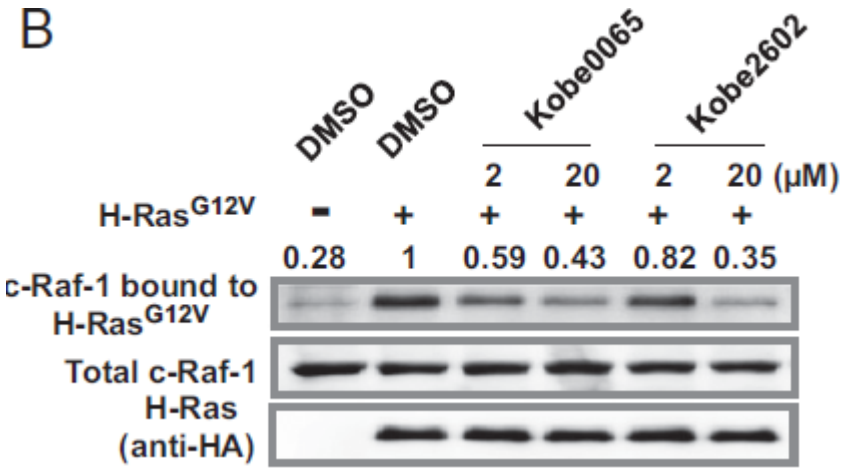
Maurer T et al PNAS 1012, 109:5299-5304 Sun Q et al Angew Chem Int Ed 2012,51:6140-6143

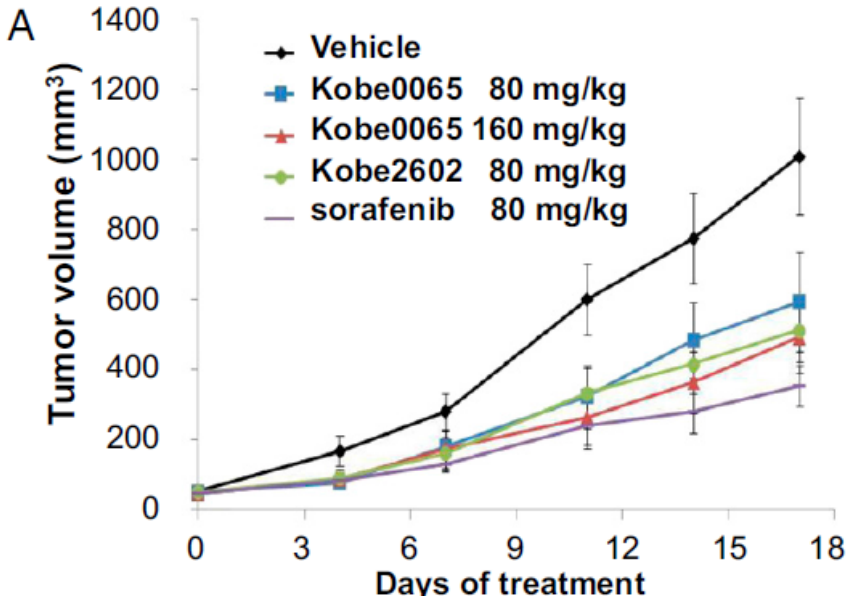
RAS-RAF interaction

PNAS

In silico discovery of small-molecule Ras inhibitors that display antitumor activity by blocking the Ras–effector interaction

Fumi Shima[a,1,2], Yoko Yoshikawa[a,1], Min Ye[a], Mitsugu Araki[a,b], Shigeyuki Matsumoto[a], Jingling Liao[a], Lizhi Hu[a], Takeshi Sugimoto[a], Yuichi Ijiri[a], Azusa Takeda[a], Yuko Nishiyama[a], Chie Sato[a], Shin Muraoka[a], Atsuo Tamura[b], Tsutomu Osoda[c], Ken-ichiro Tsuda[d], Tomoya Miyakawa[c], Hiroaki Fukunishi[e], Jiro Shimada[e], Takashi Kumasaka[f], Masaki Yamamoto[g], and Tohru Kataoka[a,2]





- **Kobe 0065 was identified with a computer docking screen using a virtual library and was selected for its ability to inhibit HRAS-GTP binding to the RAF-RAS binding domain**
- **Binds to the SII region of RAS close to but in a distinct pocket from DACI and VU0460009**

Shima F PNAS, 2013, 110: 8182-8187

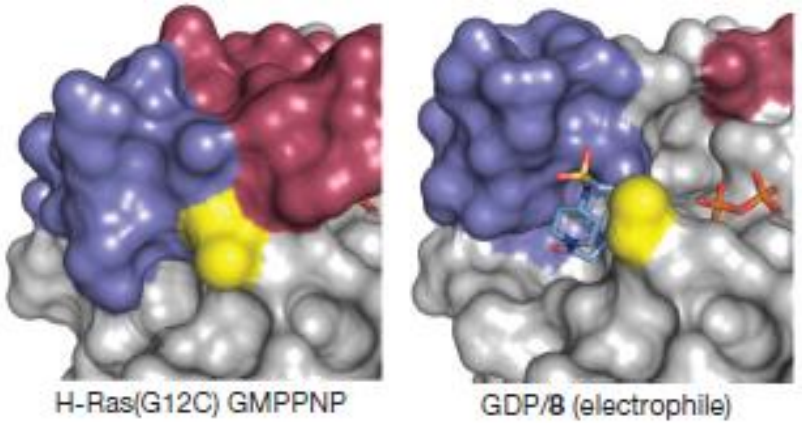
Mutant specific inhibitors

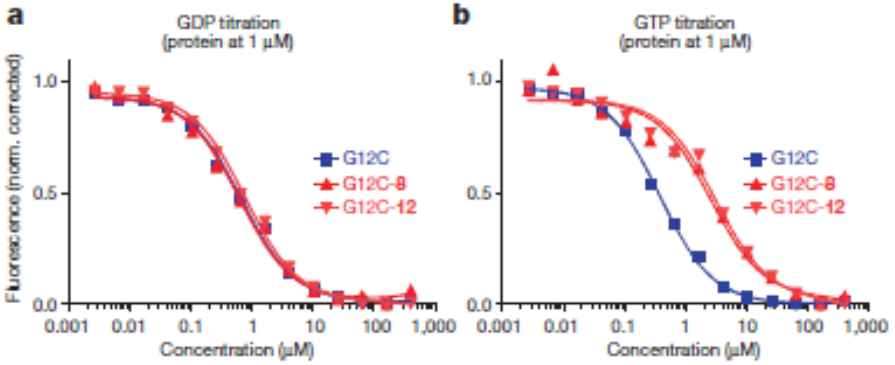
LETTER

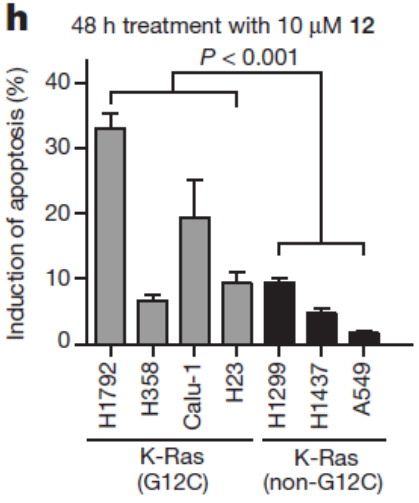
doi:10.1038/nature12796

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

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



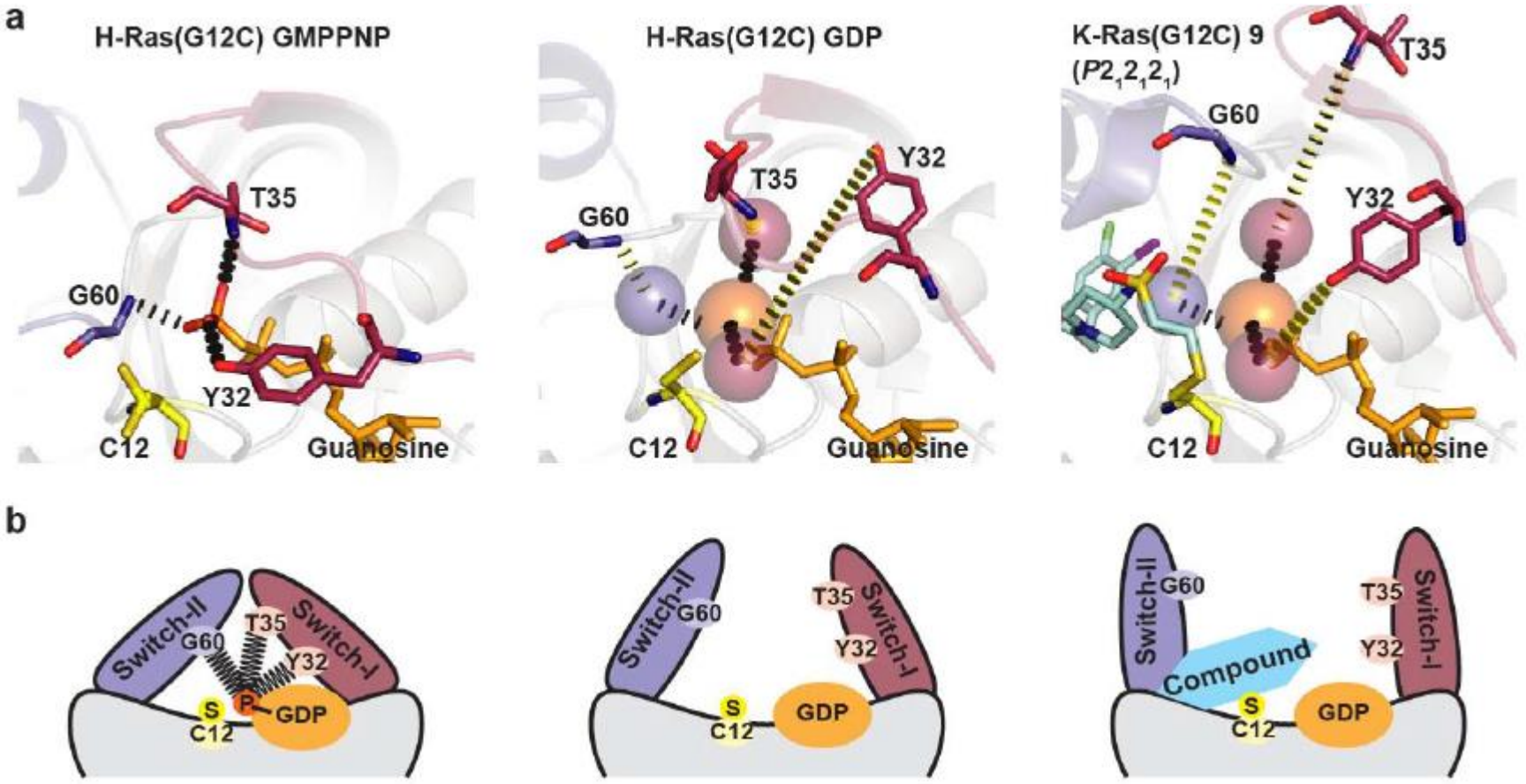




- **Compounds bind to pocket on the effector binding switch II region**
- **Allosteric binding makes mutant RAS favour GDP bound state**
- **Is more effective in mutations resulting in cysteine substitution rather than mutations that do not result in cysteine substitutions and wild type RAS**

Osterm JM et al Nature 2013, 503: 548-551

Mutant specific inhibitors



- ϒ phosphate interacts with Tyrosine 32, Threonine 30 and Glycine 60 to keep hold SII and SI in place
- Allosteric binding of compound leads to removal of ϒ phosphate leading to relaxation movement of SII

Osterm JM et al Nature 2013, 503: 548-551

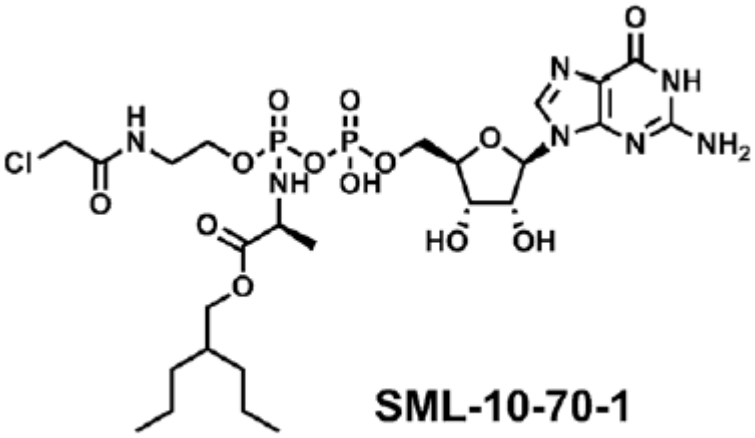
Mutant specific inhibitors

Drug Design Hot Paper

DOI: 10.1002/anie.201307387

Therapeutic Targeting of Oncogenic K-Ras by a Covalent Catalytic Site Inhibitor**

*Sang Min Lim, Kenneth D. Westover, Scott B. Ficarro, Rane A. Harrison, Hwan Geun Choi, Michael E. Pacold, Martin Carrasco, John Hunter, Nam Doo Kim, Ting Xie, Taebo Sim, Pasi A. Jänne, Matthew Meyerson, Jarrod A. Marto, John R. Engen, and Nathanael S. Gray**



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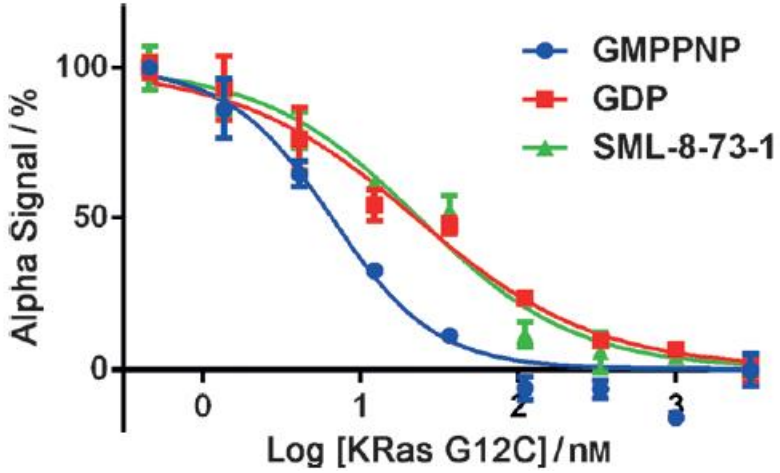
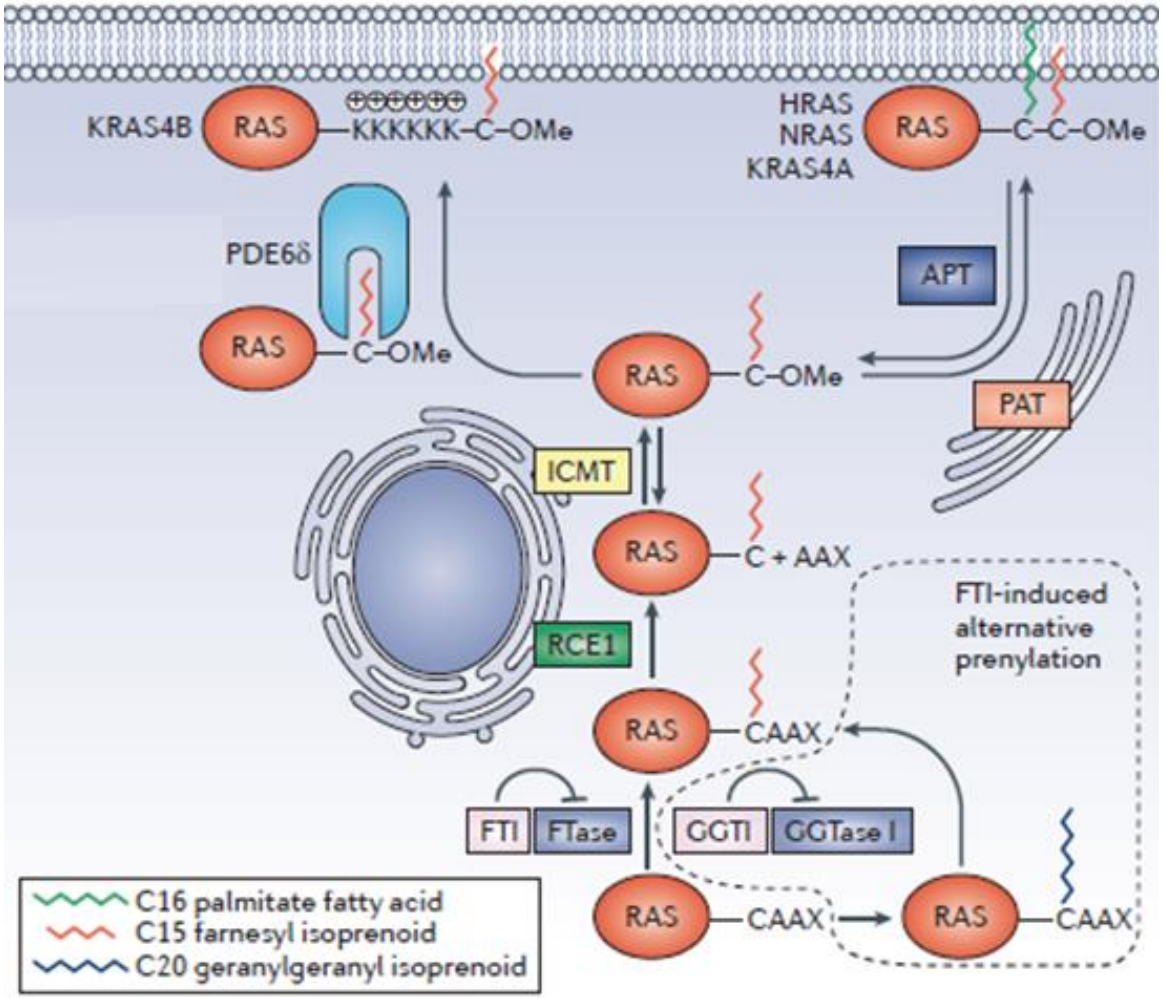


- Covalently binds to cysteine residue of G12C
- Does so in the presence of 1 mM concentration of GTP and GDP
- Not very cell permeable

Lim SM et al Angew Chem Int Ed 2014,53: 199-204

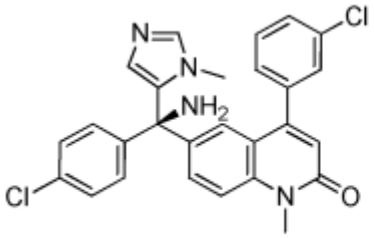
Post translational modification of RAS



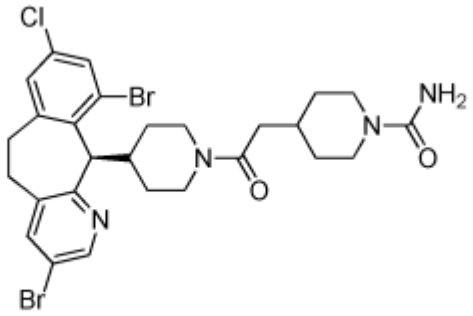
- All the 4 RAS proteins are synthesized as cytosolic inactive peptides
- HRAS and HREB is exclusively farnesylated
- RHOA, RHOC are exclusively geranylgeranlyated
- RHOA, RHOC are both farnesylated and geranylgeranlyated
- KRAS and NRAS are preferentially farnesylated but will be geranylgeranlyated in the presence of a FT inhibitor

Cox AD Nat Revs Drug Disov 2014, 13:828-850

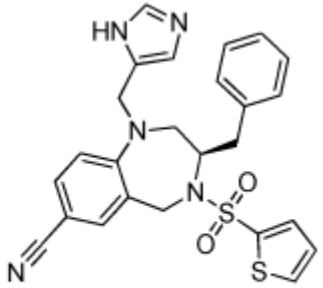
Farnesyl transferase inhibitors



24 (Tipifarnib)



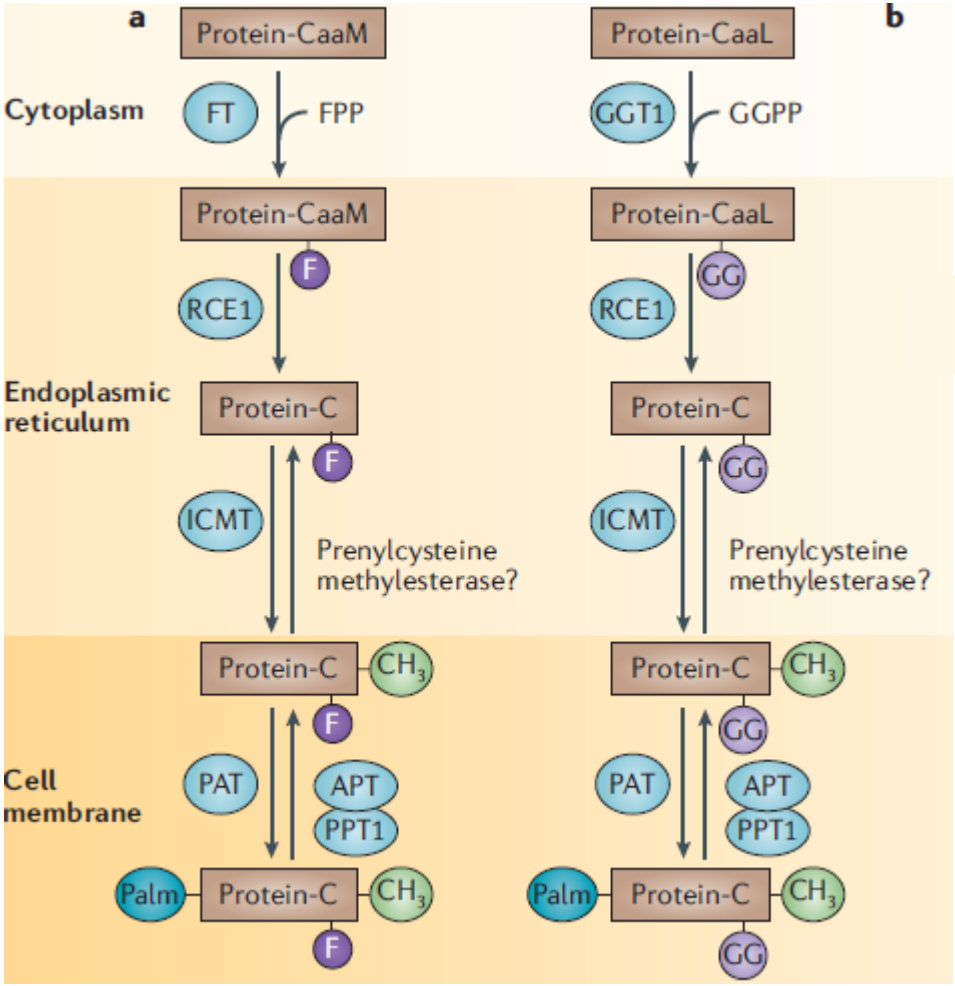
25 (Lonafarnib)



26 (BMS-214662)

- **Multiple phase I and phase II studies conducted**
- **Disappointing activity in solid tumours, due to the fact that most RAS mutations are KRAS or NRAS which can be phrenylated by geranylgeranly trasnferase upon FT inhibition**
- **Some activity seen in leukaemia's and haematological malignancies however these patients did not have RAS mutations, possibly due to the effects of farnesylation of other proteins**
- **Nausea, diarrhoea and fatigue are predominant toxicities and could be due to inhibition of farnesylation of multiple proteins.**

Other targets that effect post-translational modification of RAS

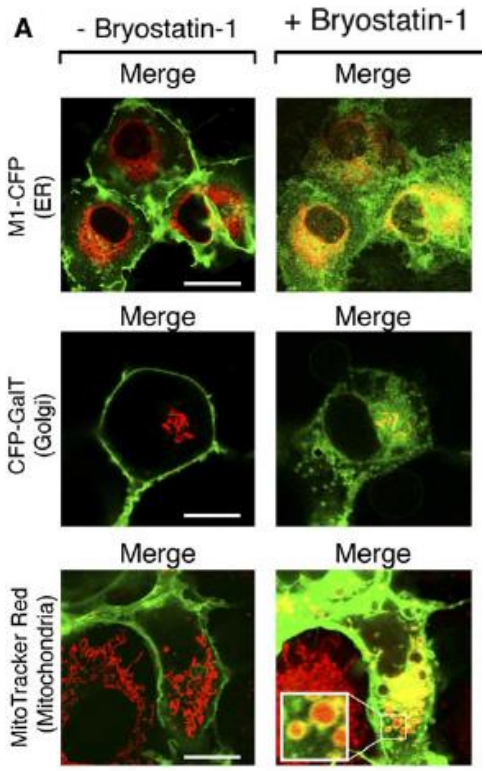


- Geranylgeranyl transferase adds a C-20 isoprenoid to selected RAS isoforms. Inhibitors include GGT1-2418
- RCE1 (RAS converting enzyme 1)
- ICMT (isoprenylcysteine-carboxymethyl transferase)
- Palmitoyation of KRAS4A, HRAS and NRAS is essential to their membrane association and function.
- One human pamytoyl aceltyl transferase (DHHC9-GCP16 complex) has activity against HRAS and NRAS

Berndt N Nat Revs Cancer 2011, 11:775-791

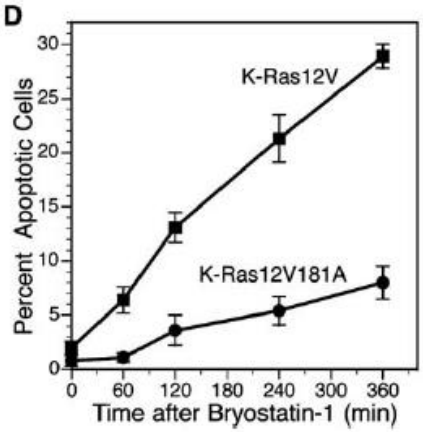
Cox AD Nat Revs Drug Disov 2014, 13:828-850

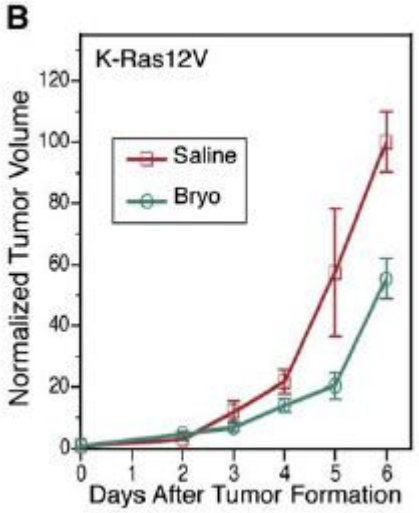
Other targets that effect post-translational modification of RAS: PKCα



Molecular Cell *21*, 481–493, February 17, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2006.01.012

PKC Regulates a Farnesyl-Electrostatic Switch on K-Ras that Promotes its Association with Bcl-X_L on Mitochondria and Induces Apoptosis





- **PKCα catalyses phosphorylation of KRAS4B at S181 within the C terminal.**
- **This phosphorylation causes the KRAS4 to disassociate from the plasma membrane and move to the endomembrane**
- **Bryostatin is a PKC agonist**

Bivona TG, Mol Cell 2006: 21:481-493

Other targets that effect post-translational modification of RAS: PDE6δ

LETTER

doi:10.1038/nature12205

Small molecule inhibition of the KRAS–PDEδ interaction impairs oncogenic KRAS signalling

Gunther Zimmermann[1]*, Björn Papke[2]*, Shehab Ismail[3]*, Nachiket Vartak[2], Anchal Chandra[2], Maike Hoffmann[4], Stephan A. Hahn[4], Gemma Triola[1], Alfred Wittinghofer[3], Philippe I. H. Bastiaens[2,5] & Herbert Waldmann[1,5]

LETTER

doi:10.1038/nature12205

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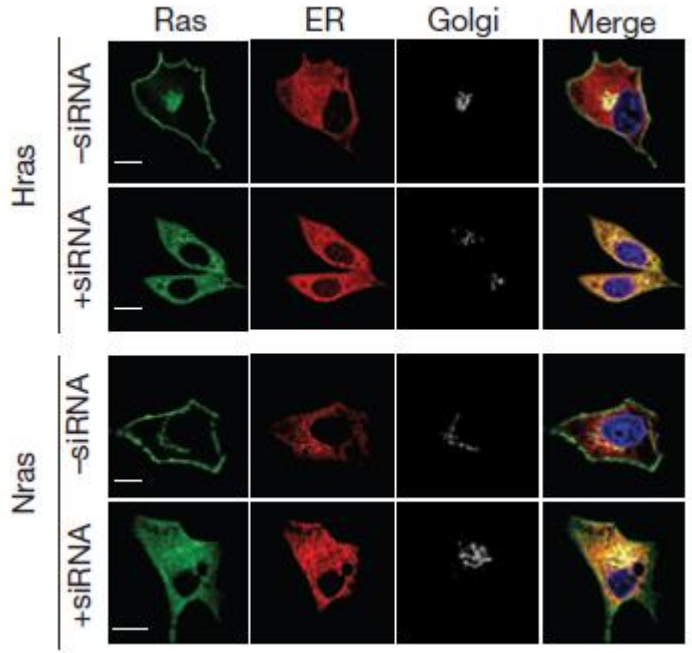
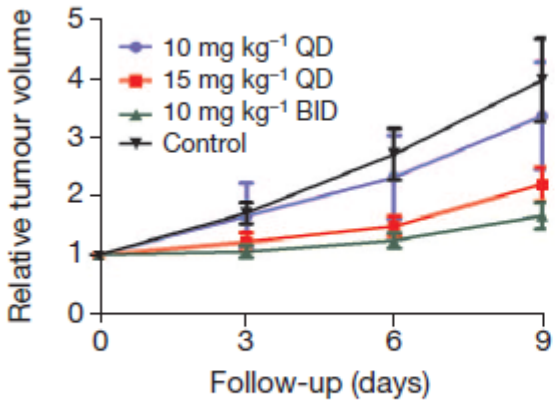
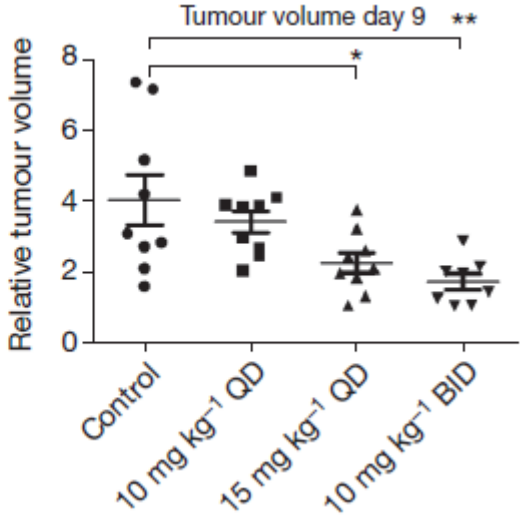
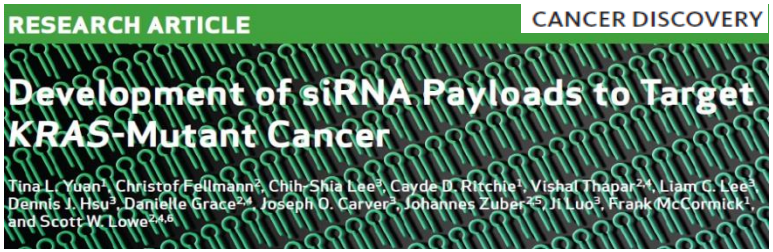


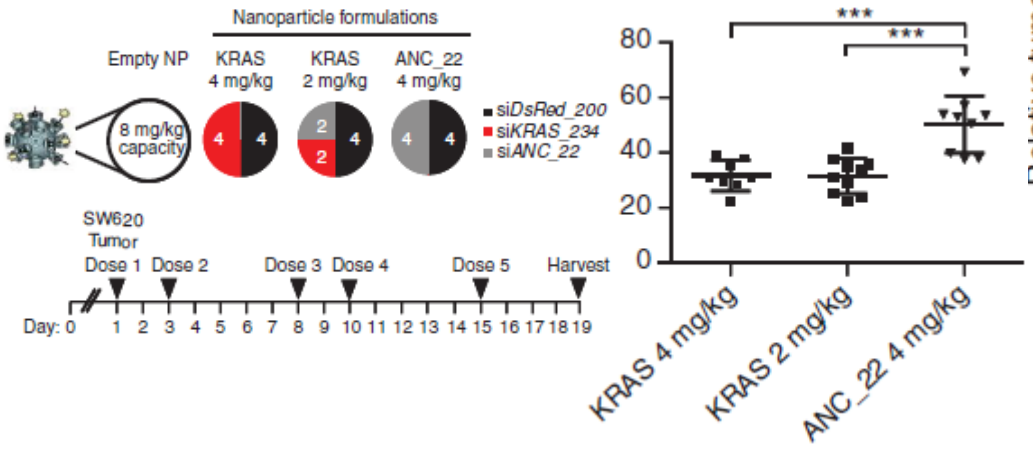


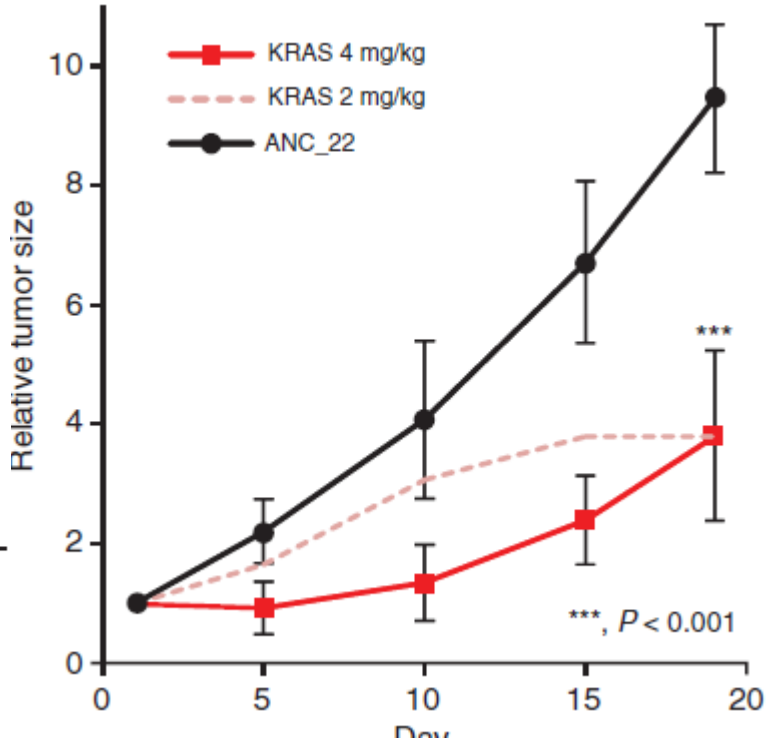


Chandra A et al Nature Cell Biology 2012, 14:148-58

siRNA payloads to target KRAS mutant cancers







- **Preclinical efforts to try and deliver SiRNA payloads with nanoparticles are improving**

Yuan TL et al Cancer Discovery 2014, 4:1182-97

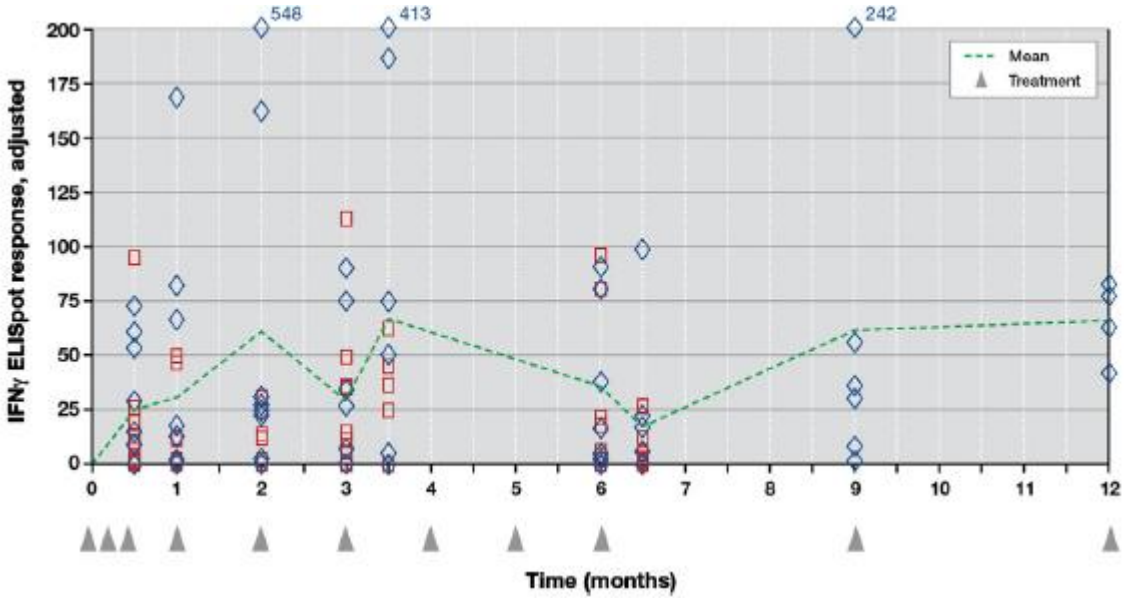
Nothing is complete without a mention of immunology – not even RAS!

Original Study

CrossMark

Phase II Study of the GI-4000 *KRAS* Vaccine After Curative Therapy in Patients With Stage I-III Lung Adenocarcinoma Harboring a *KRAS* G12C, G12D, or G12V Mutation

Jamie E. Chaft,[1] Anya Litvak,[1] Maria E. Arcila,[2] Payal Patel,[1] Sandra P. D'Angelo,[1] Lee M. Krug,[1] Valerie Rusch,[3] Alicia Mattson,[4] Claire Coeshott,[4] Bernard Park,[3] David M. Apelian,[4] Mark G. Kris,[1] Christopher G. Azzoli[1]



- **Vaccines usable only in a adjuvant setting**
- **Extracellular expression of mutant RAS not present however antigen presenting cells may display antigen**
- **Bystander effect on adjacent cancer cells**

Chaft JE et al Clinical Lung Cancer 2014, 15:405-10

Conclusions

- Physiological and pathological problems to directly target RAS still limiting
- Fragment based screens have made more active compounds but not very potent but this has lead to improved understanding of druggability of RAS
- Mutant specific compounds are of interest
- Early efforts of drugging post translational modification of RAS with FT inhibitors proved disappointing and may have limited efficacy in *HRAS* mutant tumours, however other emerging areas such as PDE6δ inhibitors are be interesting
- Combinations of downstream inhibitors have enabled some success
- Multiple synthetic lethal approaches are being pursed and have lead to interesting hypotheses that need new drugs or intelligent use of existing inhibitors to be tested in the clinic

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