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The genomic landscape of RAS-driven tumours

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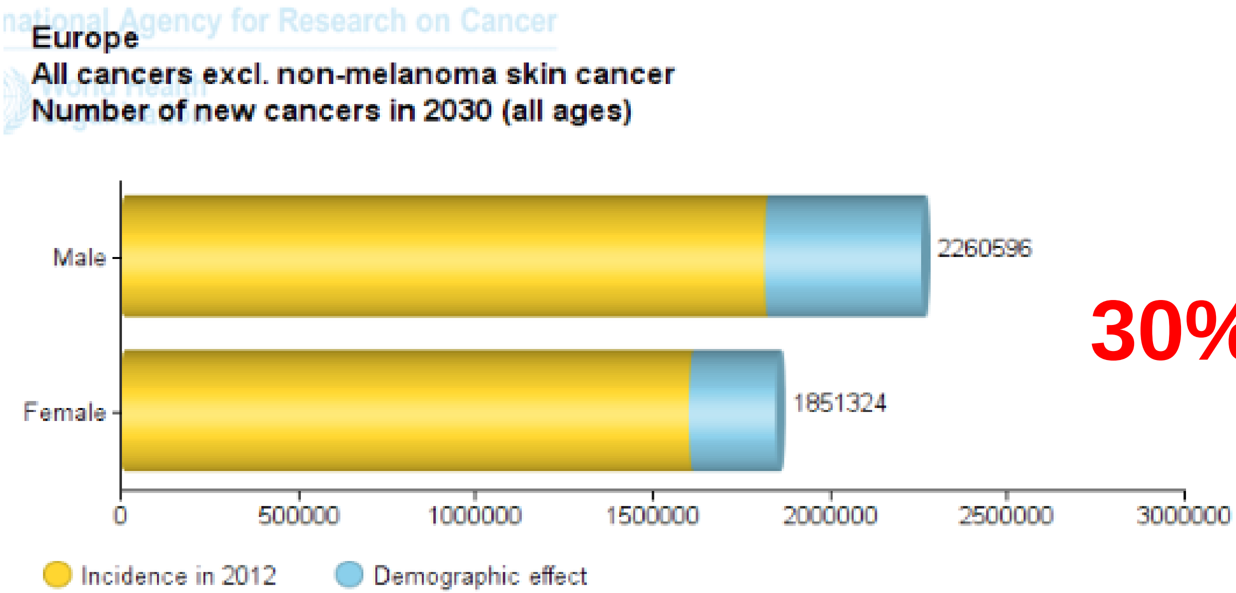
ARC NET APPLIED RESEARCH ON CANCER
RICERCA APPLICATA SUL CANCRO
'CENTRO MIRIAM CHERUBINI LORO'

Barcelona, Spain 13-14 March 2015

DISCLOSURE

No conflicts of interest to declare

GLOBOCAN Projection for 2030 in Europe



Incidence: 4,000,000 new cancer cases

30% *RAS*-mutated

1,200,000 cases *RAS*-mutated

GLOBOCAN 2012 (IARC)

OUTLINE

1. *RAS* family members and their mutations
2. Prognostic and predictive role of *RAS* mutations
3. Downstream to *RAS*
4. *RAS* is guilty but he is not the only one

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RAS-oncogenic mutations

from COSMIC catalogue

Incidence per year in USA of RAS mutations in human cancers

Primary Tissue	*KRAS* (%)	*HRAS* (%)	*NRAS* (%)	Total (%)	
Pancreas	71	0	<1	71	**95%**
Colon	35	1	6	42	**45%**
Small intestine	35	0	<1	35	
Biliary tract	26	0	2	28	**45%**
Endometrium	17	<1	5	22	
Lung	19	<1	1	20	**35%**
Skin (melanoma)	1	1	18	20	
Cervix	8	9	2	19	
Urinary tract	5	10	1	16	

Stephen AG, Cancer Cell 25, March 17, 2014

RAS family oncogenic mutations

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KRAS-oncogenic mutations

from COSMIC catalogue

Incidence per year in USA of *KRAS* mutations in human cancers

	All *KRAS*	G12C	G12D	G12V	G13D
Colorectal	60,000	5,700	25,000	15,700	13,600
Lung	45,600	23,000	9,200	11,900	1,500
Pancreas	32,200	1,000	19,500	11,500	200
Total new cases/year	137,800	29,700	53,700	39,100	15,300

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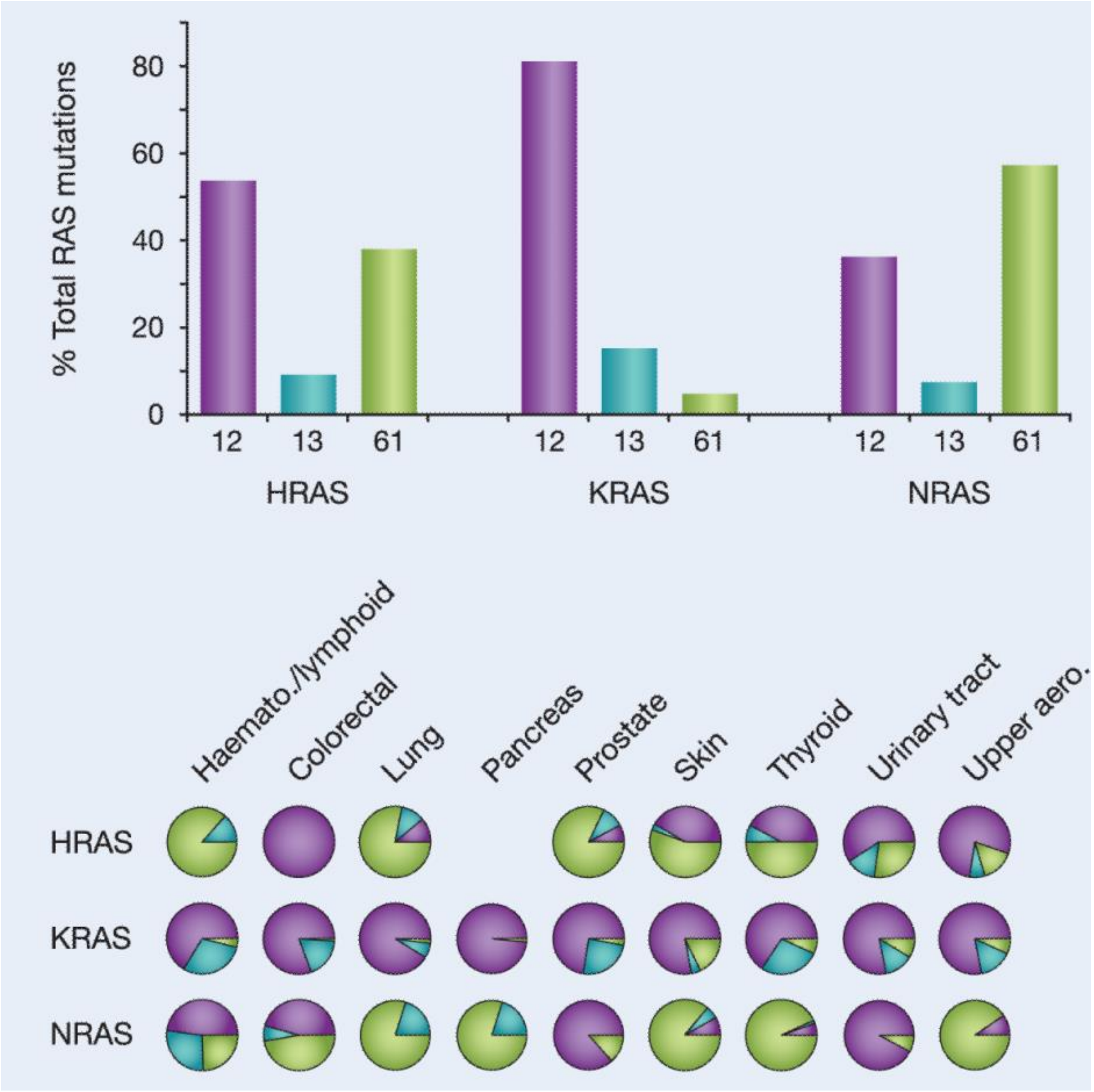
Stephen AG, Cancer Cell 25, March 17, 2014

RAS family codon mutations

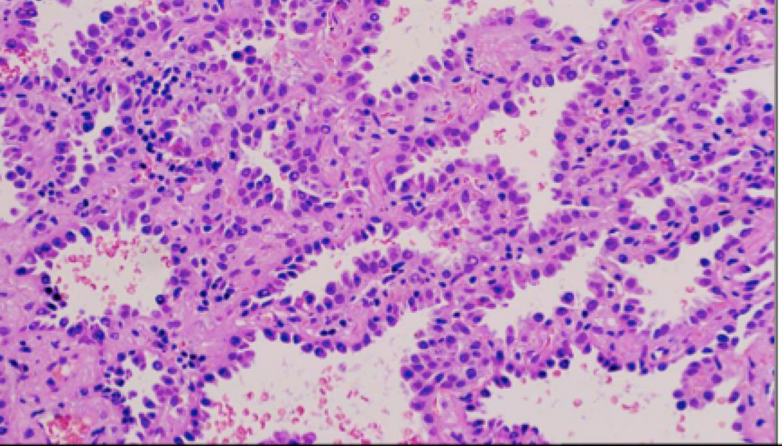
Isoform specific pattern

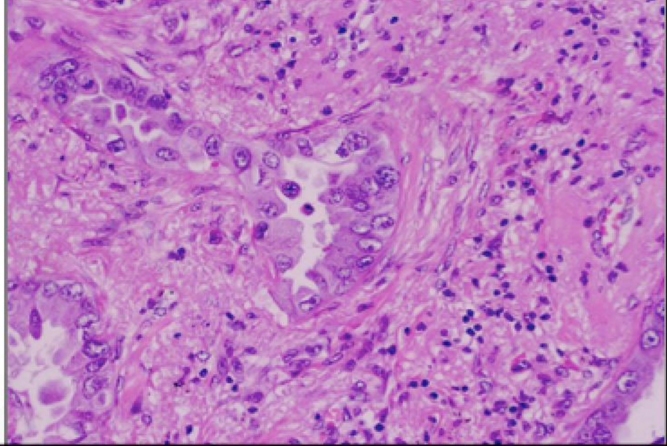
Cancer specific pattern

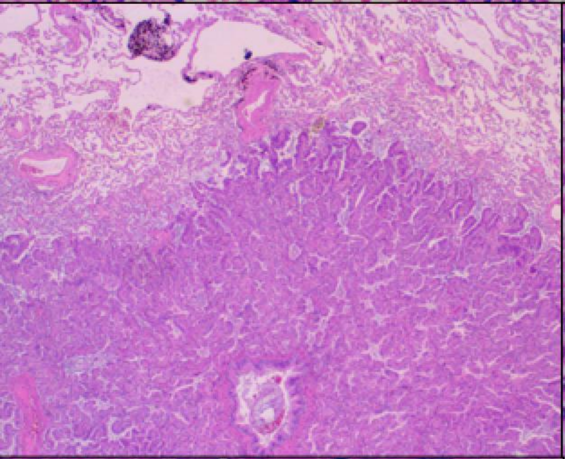


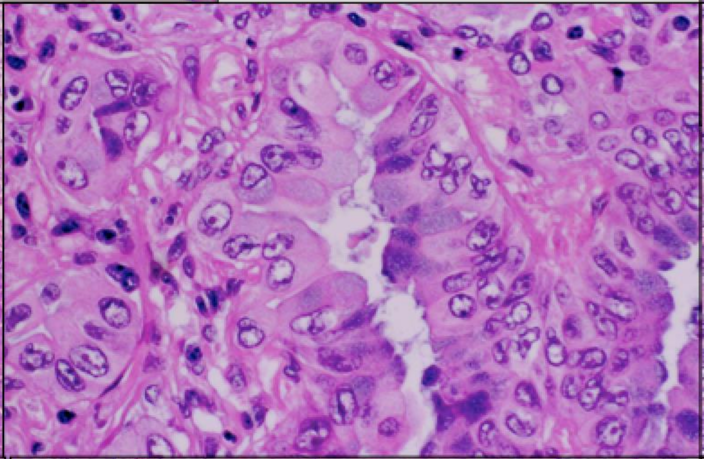
Prior IA, Cancer Res . 2012 May 15; 72(10)

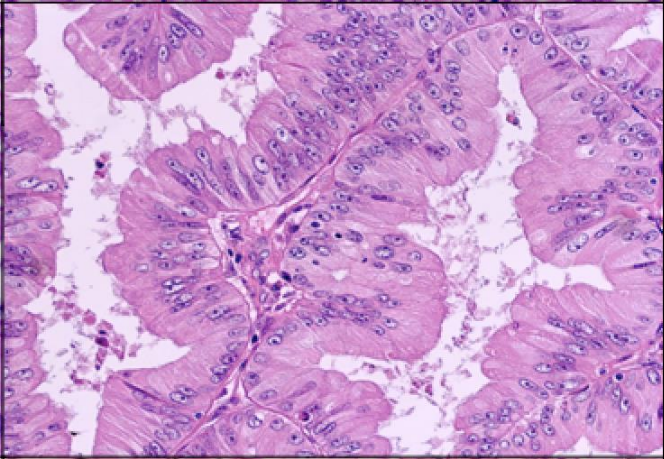
Lung Carcinoma

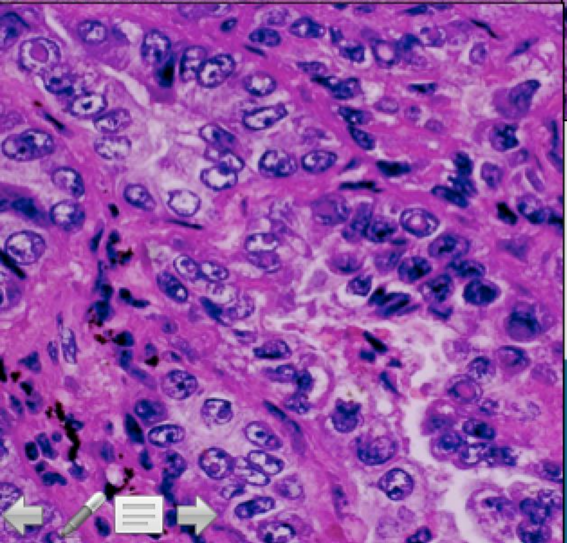


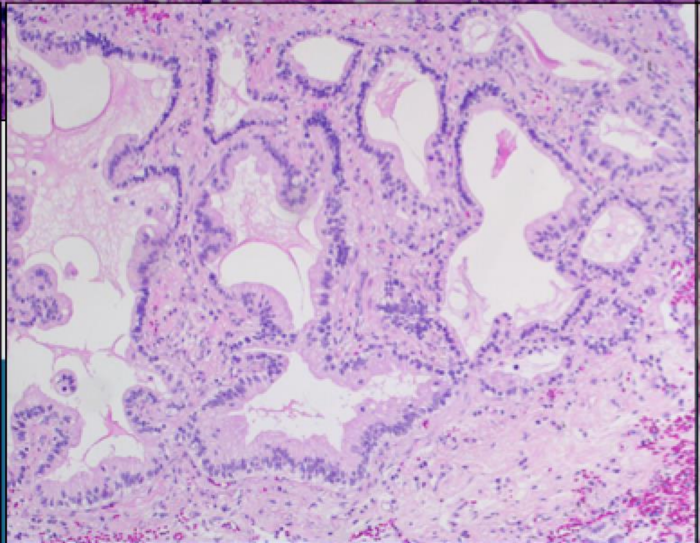


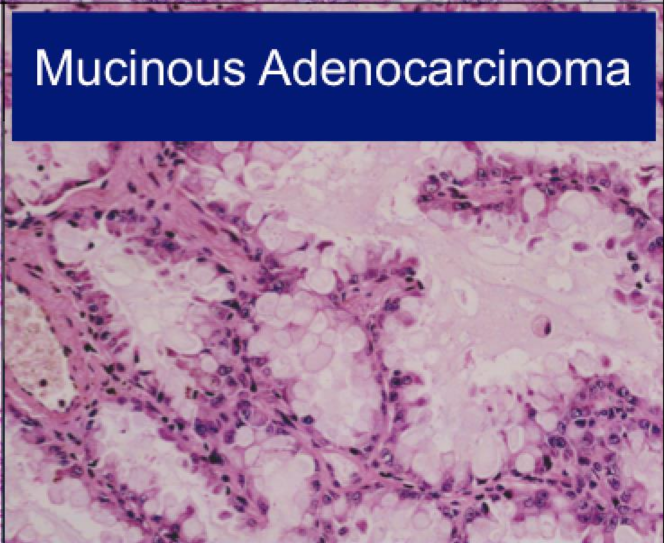






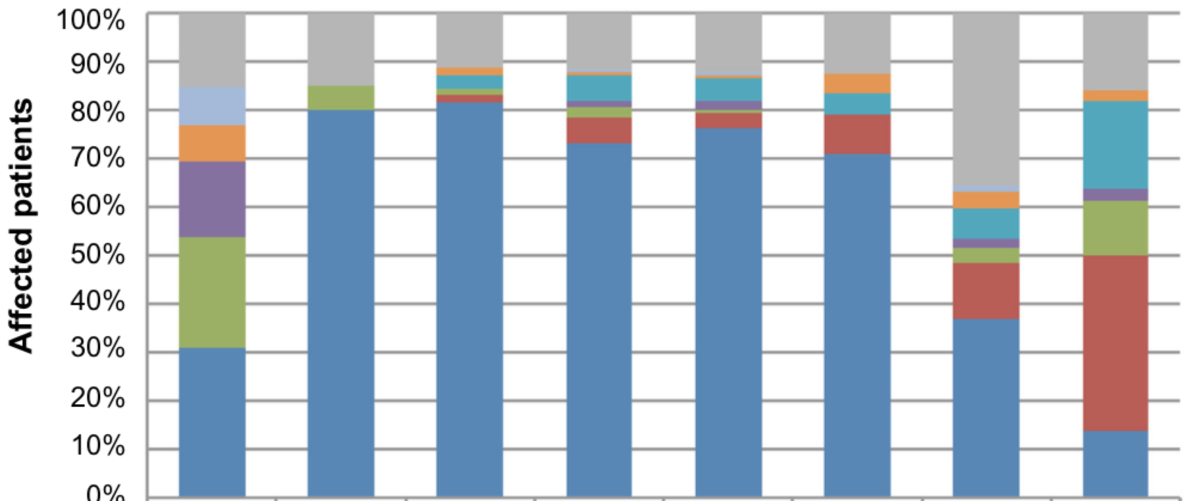






KRAS mutation: histotype association

East Asian Lung Carcinoma



	AAH/ AIS	MIA	LEP	ACN	PAP	MP	SLD	IMA
Pan-negative	2	3	8	57	19	3	58	7
*PIK3CA**	1	0	0	2	1	0	2	0
RET	1	0	1	3	1	1	6	1
ALK	0	0	2	27	7	1	10	8
BRAF	2	0	0	6	3	0	3	1
HER2	3	1	1	11	1	0	5	5
KRAS	0	0	1	26	5	2	19	16
EGFR	4	16	58	356	118	17	60	6

AAH/AIS: preinvasive lesions

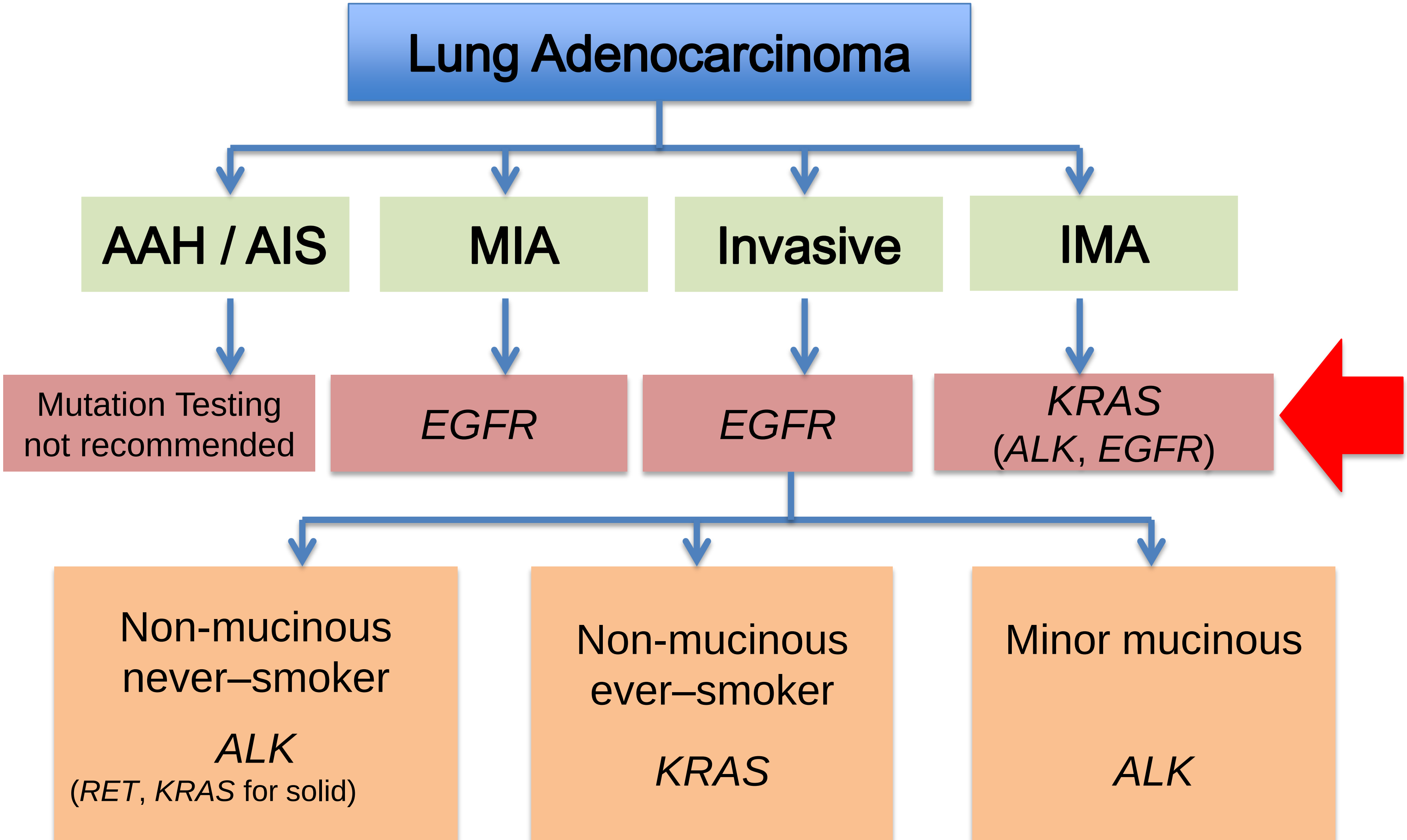
MIAs: minimally invasive adenocarcinomas

Invasive Carcinoma

- **LEP**: lepidic
- **ACN**: acinar
- **PAP**: papillary
- **MP**: micropapillary
- **SLD**: solid
- **IMA**: invasive mucinous adenocarcinoma

Hu H, Onco Targets Ther. 2014 Aug 13;7

KRAS mutation: histotype drives diagnostic workflow



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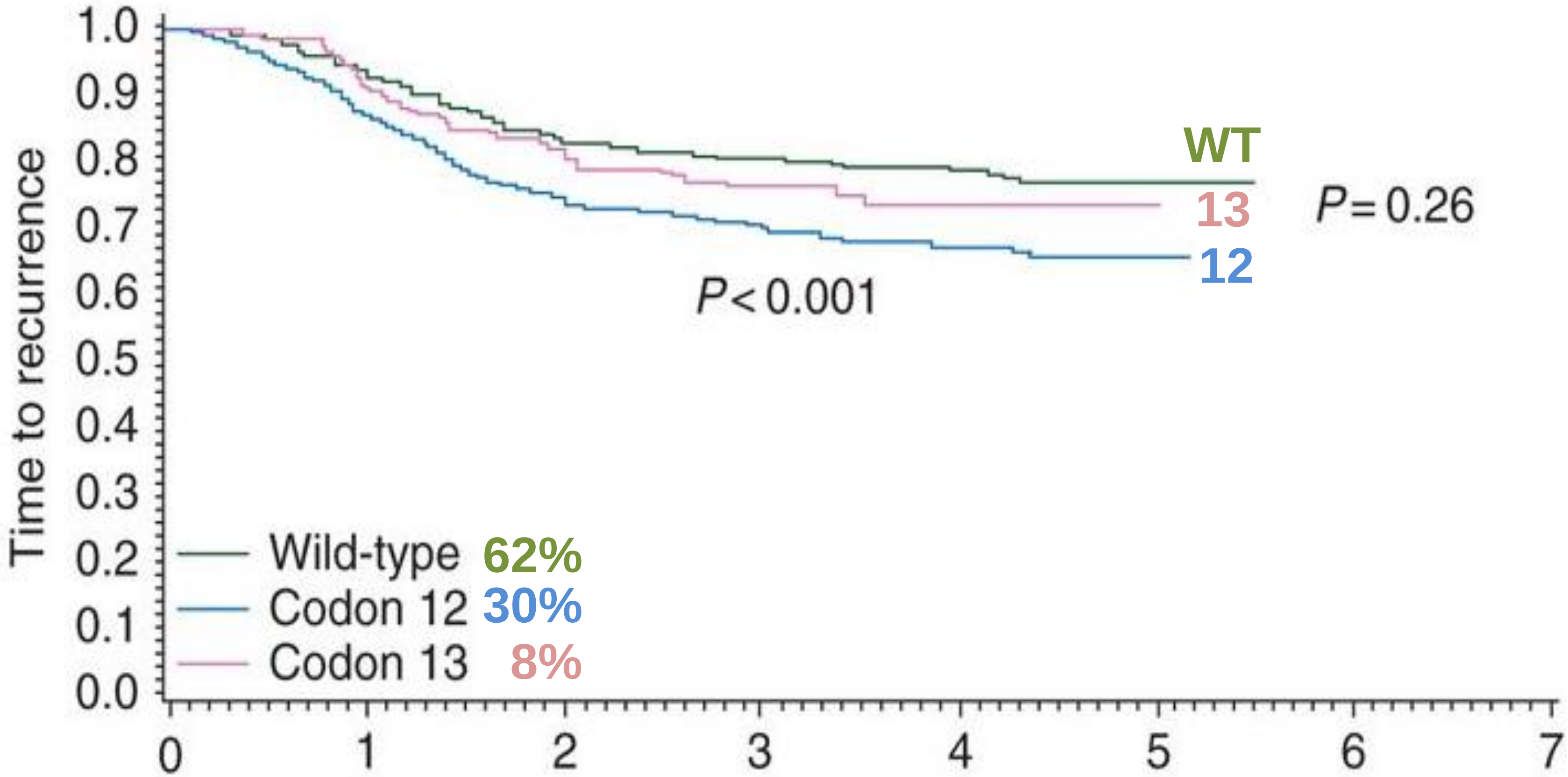
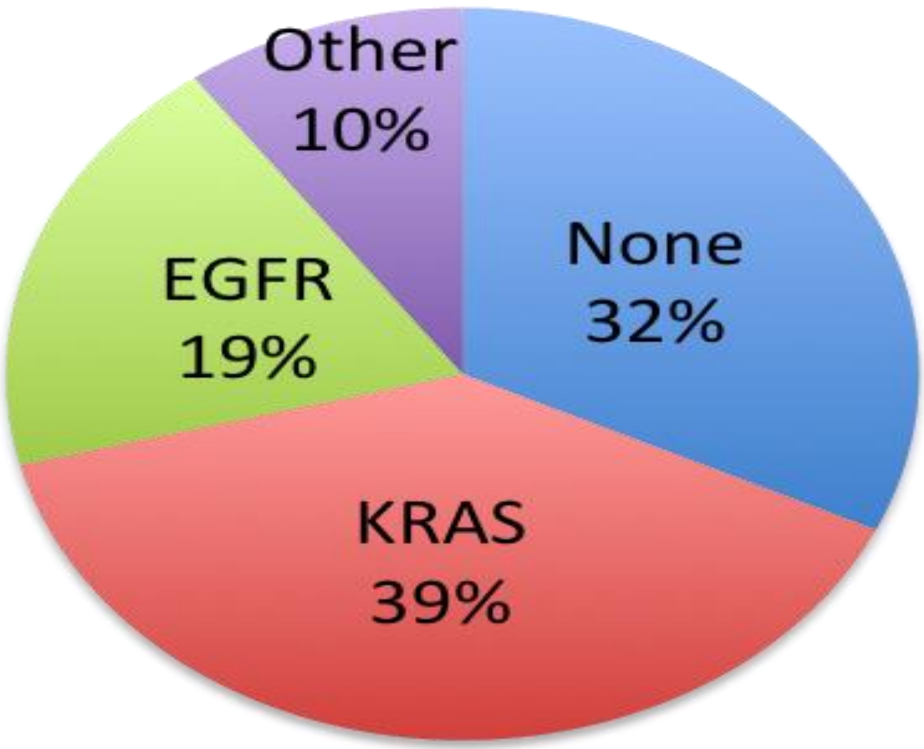
KRAS mutations: Prognostic significance Colorectal Cancer

Codon 12 mutations associated with shorter TTP



KRAS mutations: Prognostic significance Lung Cancer

312 resected stage I cancers



Izar B. J Thorac Oncol. 2014 Sep;9(9)

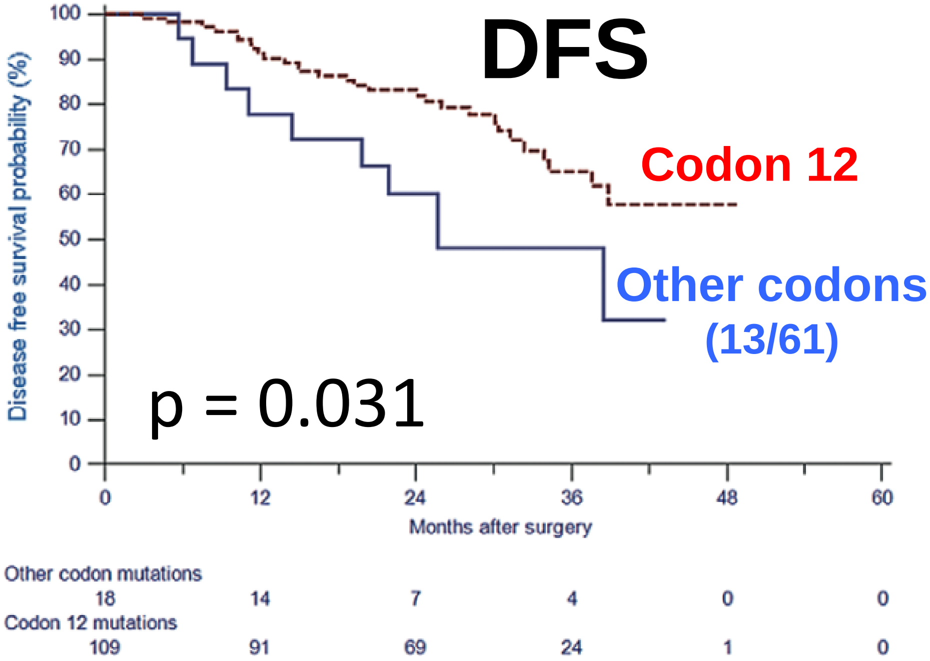
KRAS mutations: Prognostic significance Lung Cancer

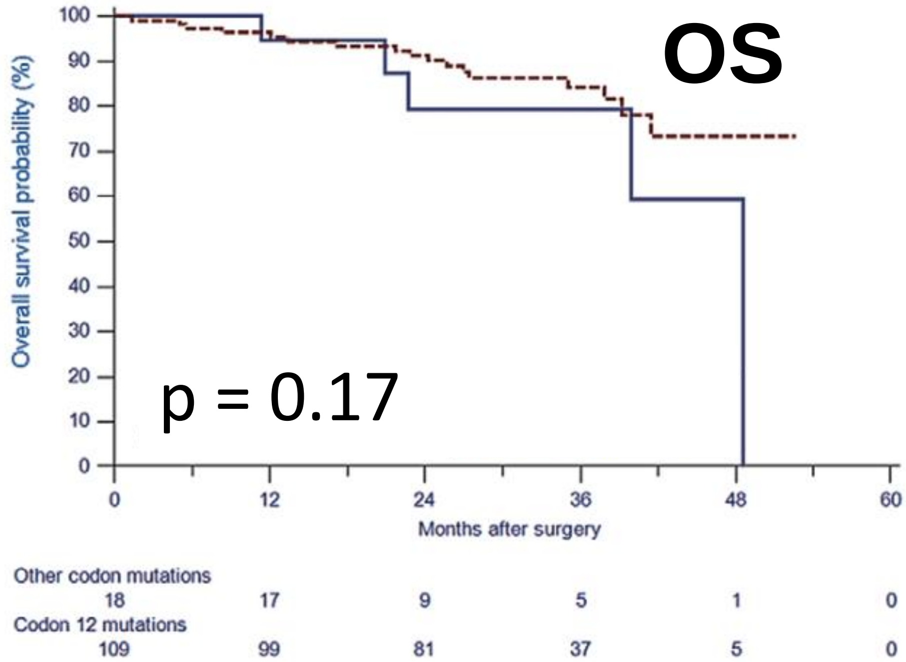
312 resected stage I cancers

KRAS mutation: the only independent predictor of shorter OS ($p = 0.001$) and DFS ($p < 0.0001$) at multivariate analysis.

KRAS mutations: Prognostic significance Lung Cancer

312 resected stage I cancers





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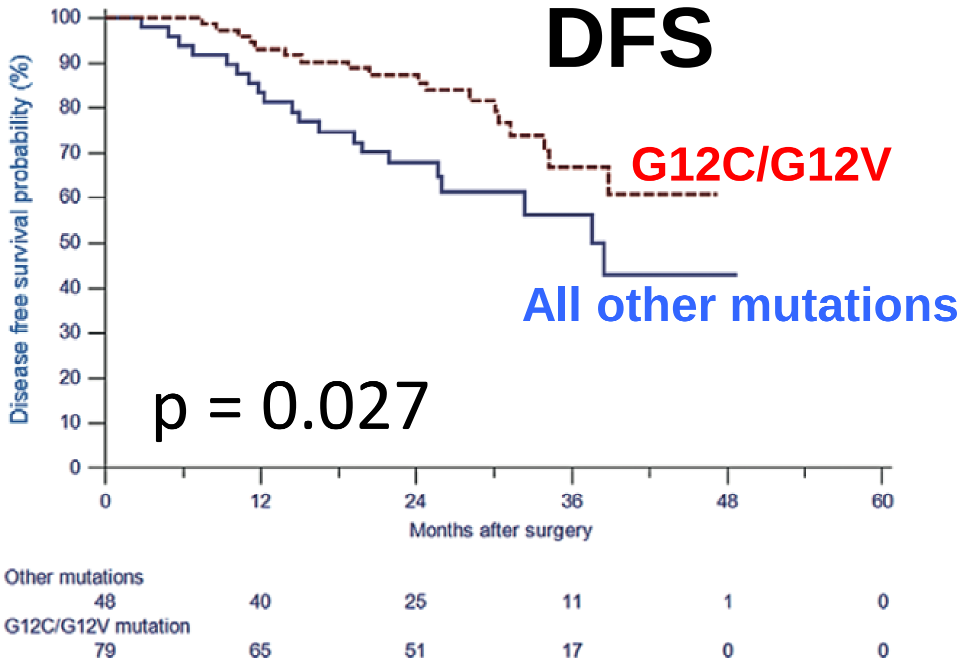

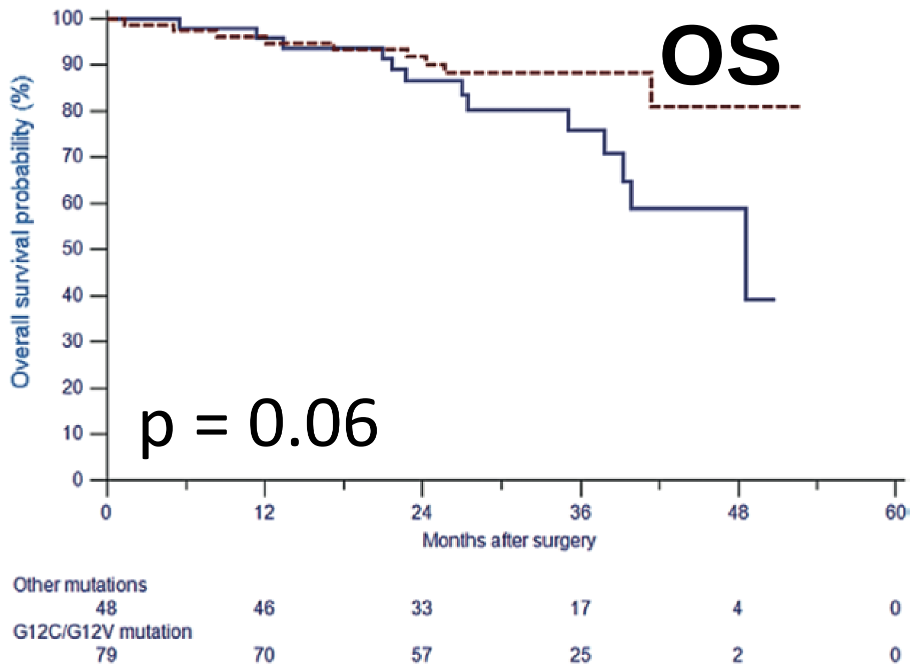

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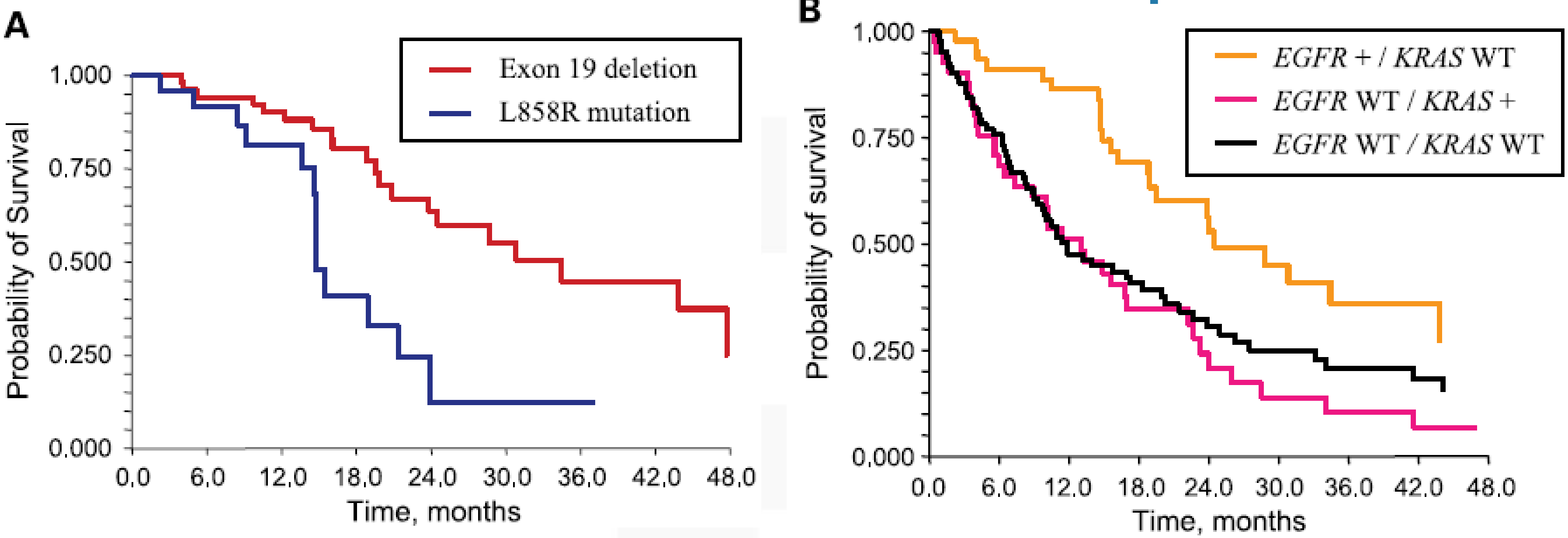
Advanced Lung Cancer: anti EGFR prediction

KRAS status does not affect response



	EGFR + / KRAS WT	*EGFR WT / KRAS +*	*EGFR WT / KRAS WT*	
N	47	41	83	P
RR	68%	0	5%	< ,001
Median TTP (months)	13,1	3,3	3,1	< ,0001
Median OS	24,5	13,0	11,8	,002

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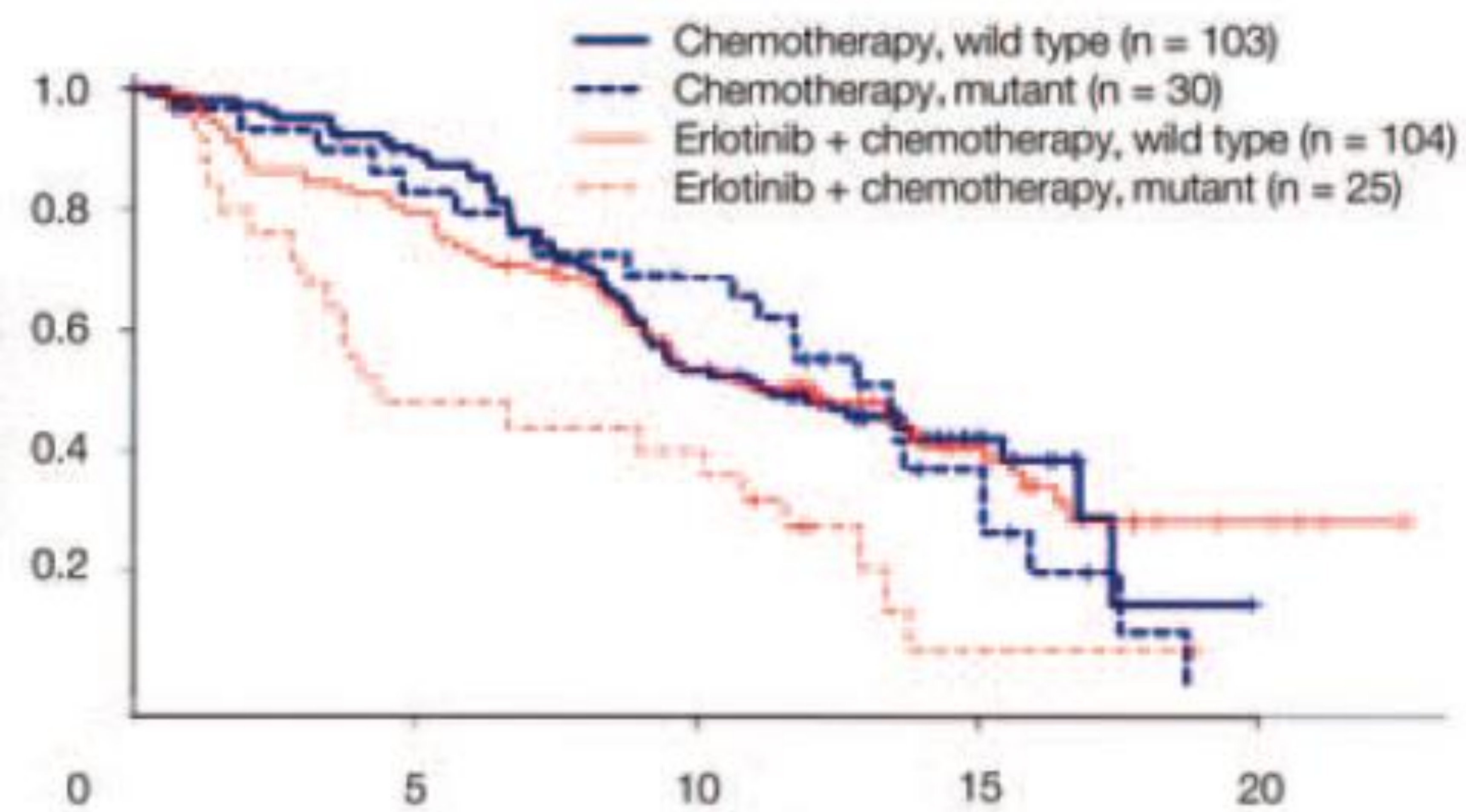
Jackman et al, Clin Cancer Res. 2009

Locally Advanced Lung Cancer: anti EGFR prediction

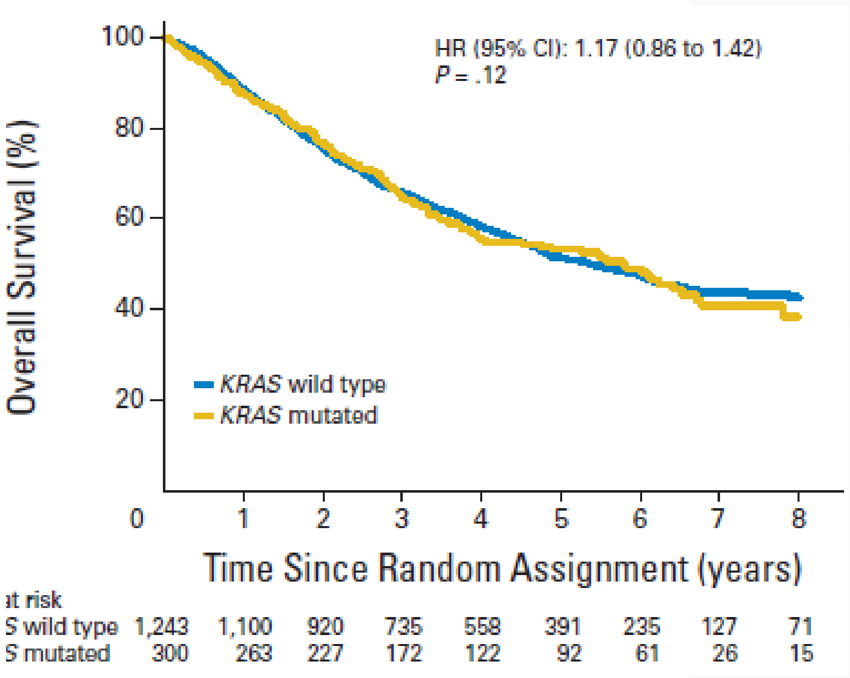
Erlotinib *is detrimental in KRAS* mutated

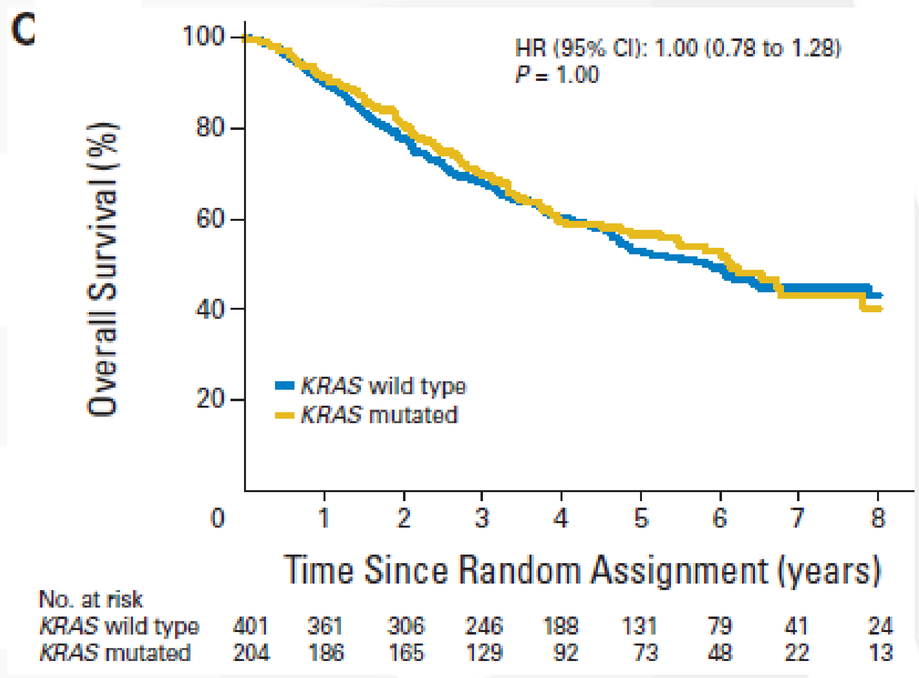


Eberhard, JCO 2005

Early Stage Resected Lung Cancer: anti EGFR prediction

KRAS status cannot be recommended to select patients for adjuvant chemotherapy





Shepherd JCO 2013

Colorectal Cancer: molecular classification

- *KRAS* mutated
- *NRAS* mutated
- *PIK3CA* mutated
- *RAF* mutated

- **Quadruple-negative**

Dienstmann et al. 2014 ASCO Educational Book

New scenarios in EGFR targeting in CRC

1022 tumours
treated with cetuximab

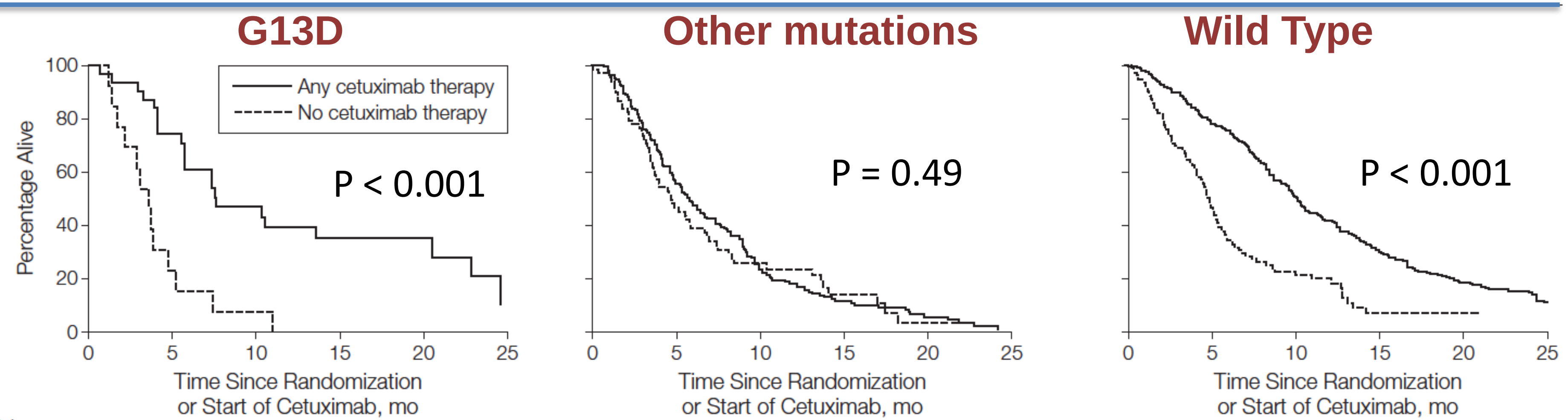
KRAS	*40%*
BRAF	*5%*
NRAS	*3%*
PIK3CA	*15% (4% ex 20)*

are significantly associated with a low response rate

Colorectal Cancer: anti EGFR prediction

KRAS-G13D respond to Cetuximab as Wild Type tumors

Figure 1. Overall Survival: Predictive Analysis by *KRAS* Status for Patients Receiving Any Cetuximab-Based Therapy vs No Cetuximab



No. at risk																		
Any cetuximab	32	22	13	6	6	1	195	105	40	16	6	1	345	261	141	70	37	16
No cetuximab	13	3	1	0	0	0	69	30	12	5	1	0	113	54	17	4	1	0

The no cetuximab group for all patients from the pooled data set is the best supportive care group from the CO.17 trial.

De Roock W, JAMA. 2010;304(16)

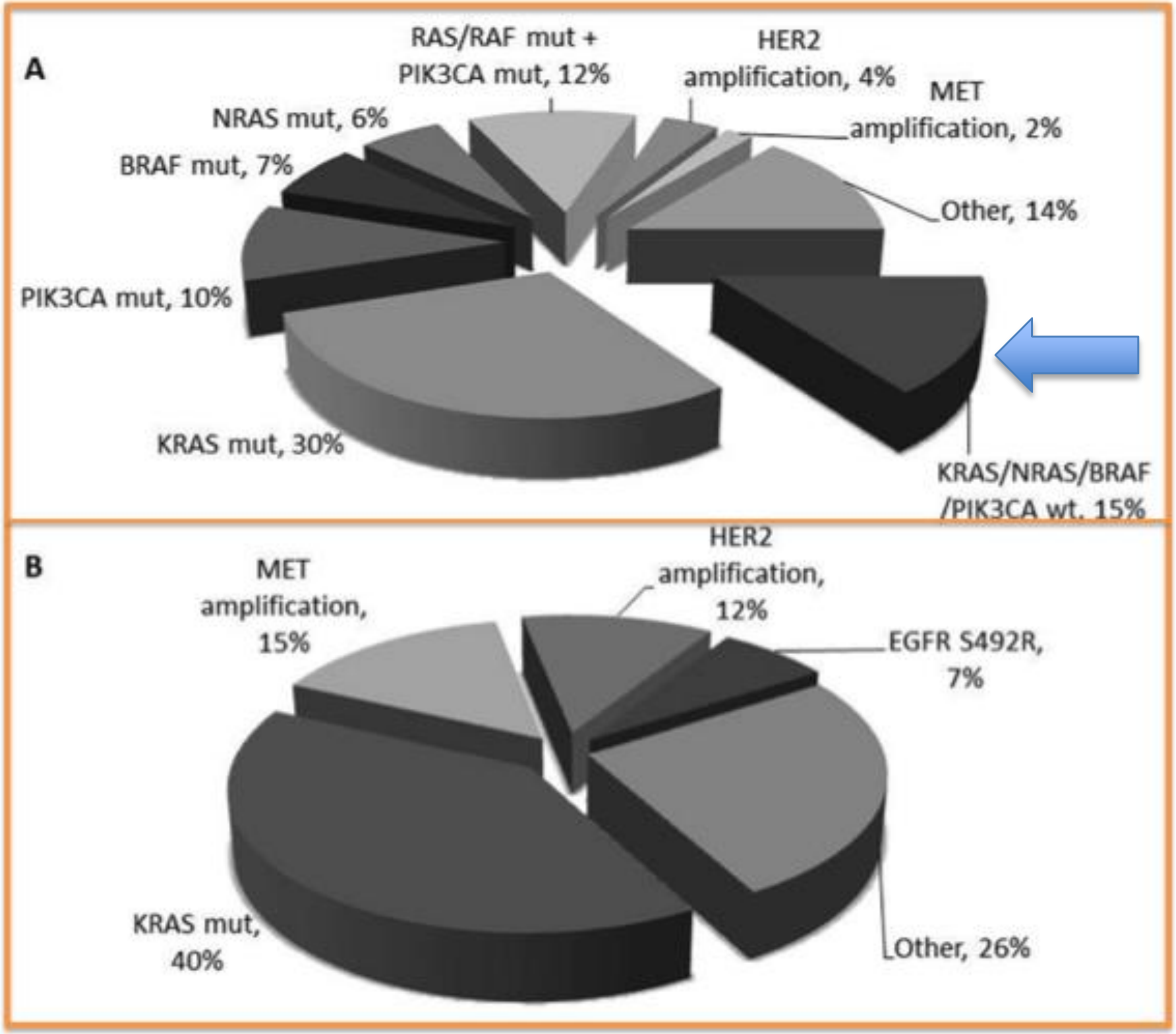
Colorectal Cancer: anti EGFR prediction

Resistance

Response

Primary

Acquired



Quadruple-negative

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Dienstmann et al. 2014 ASCO Educational Book

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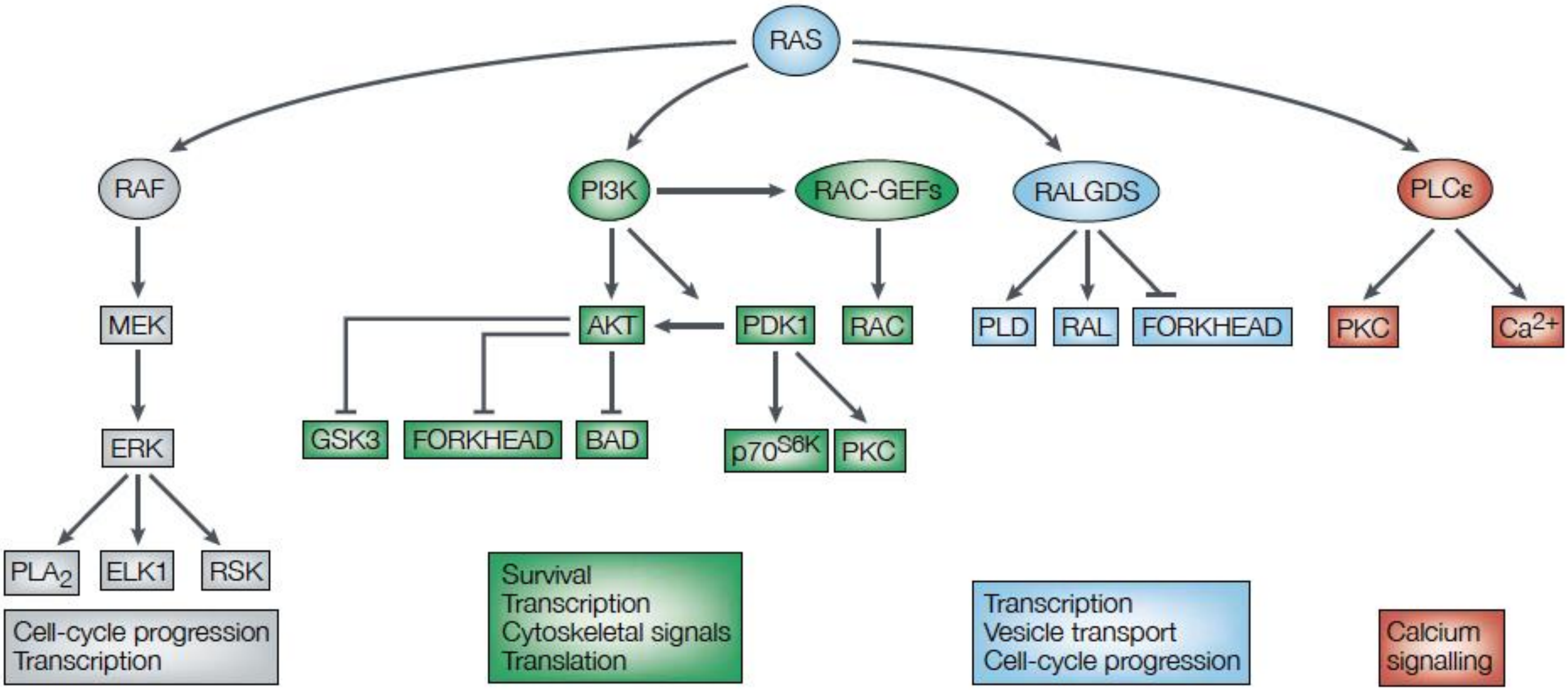
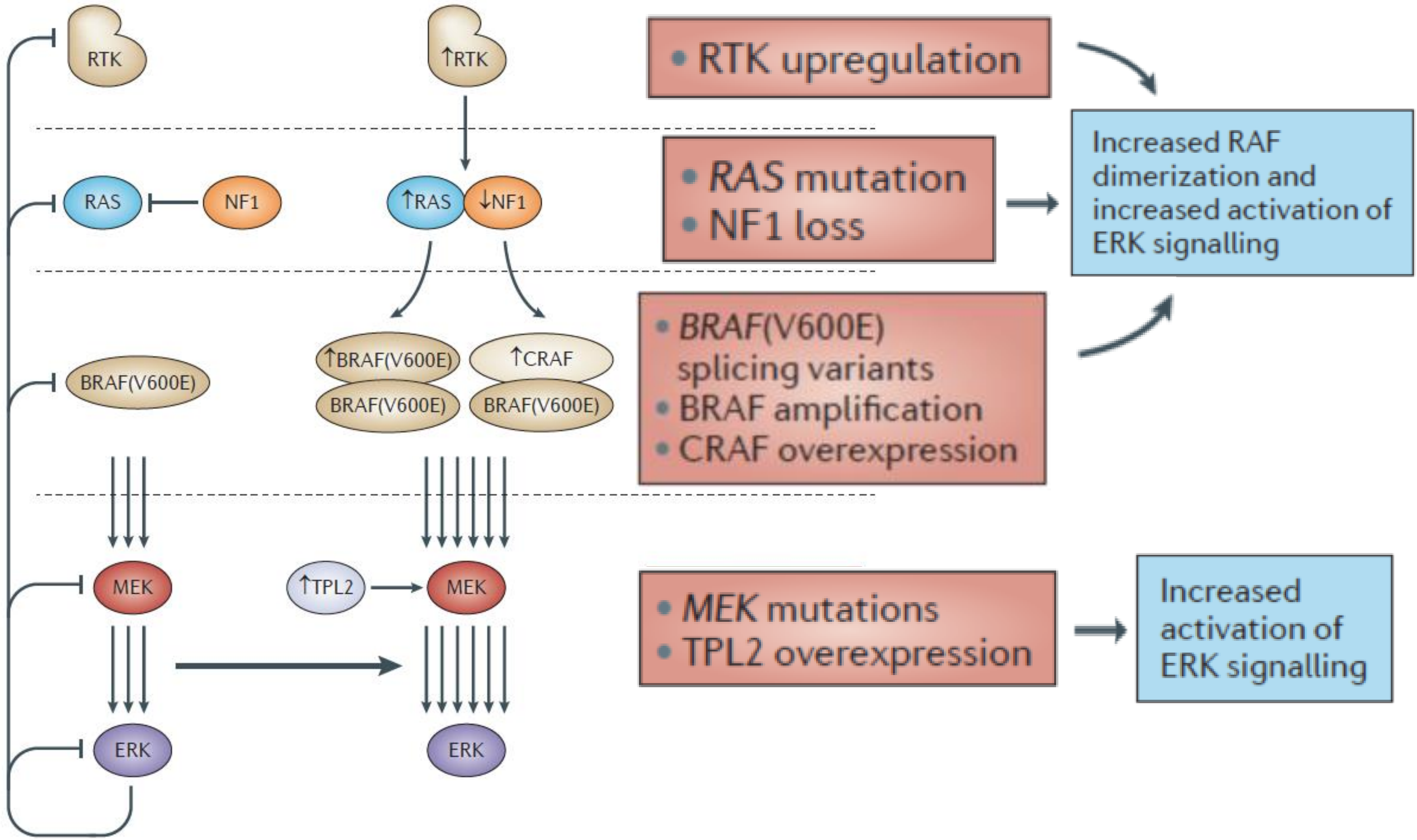
The landscape of RAS-driven pathway



Downward J., Nature Reviews, Cancer; Vol 3, January 2003

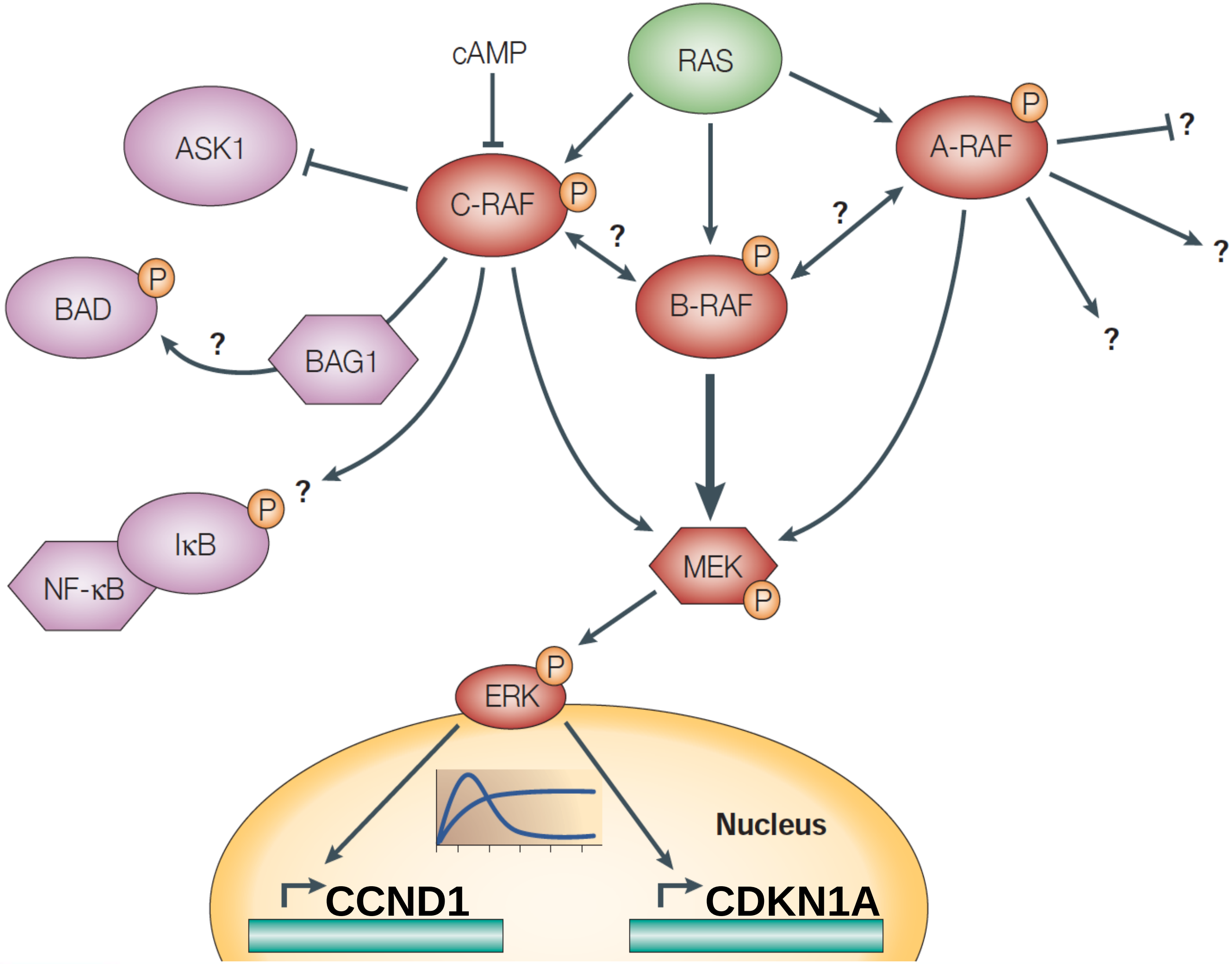
Mechanism of resistance

Sensitive state | **Resistant state** | **Mechanism**



Samatar AA, Nat Rev Drug Discov. 2014 Dec;13

RAF FAMILY



Wellbrock C, Nat Rev Mol Cell Biol. 2004 Nov;5(11)

BRAF

BRAF mutations:
- Melanoma
- Non-small cell lung cancer
- Colorectal cancer
- Bile duct cancer
- Hairy-cell leukemia

V600

Table 1 | **Correct numbering of the important amino acids in B-RAF**

Original assignment	Correct assignment	Comments
M116	M117	Mutated in cancer
R187	R188	Equivalent of R89 in C-RAF where it is required for RAS binding
I325	I326	Mutated in cancer
S364	S365	Phosphorylation site, possibly by AKT/PKB and PKA; forms core of 14-3-3 binding motif
S428	S429	AKT/PKB phosphorylation site
K438	K439	Mutated in cancer
T439	T440	AKT/PKB phosphorylation site; mutated in cancer
S445	S446	N-region phosphorylation site; equivalent to S338 of C-RAF
D448	D449	Equivalent of Y341 of C-RAF
V458	V459	Mutated in cancer
R461	R462	Mutated in cancer
I462	I463	Mutated in cancer
G463	G464	First glycine of the glycine-rich loop; mutated in cancer
G465	G466	Second glycine of the glycine-rich loop; mutated in cancer
F467	F468	Mutated in cancer
G468	G469	Third glycine of the glycine-rich loop; mutated in cancer
K474	K475	Mutated in cancer
N580	N581	Catalytic asparagine; mutated in cancer
E585	E586	Mutated in cancer
D586	D587	Mutated in cancer
D593	D594	Aspartic acid of the 'DFG' motif; mutated in cancer
F594	F595	Phenylalanine of the 'DFG' motif; mutated in cancer
G595	G596	Glycine of the 'DFG' motif; mutated in cancer
L596	L597	Mutated in cancer
T598	T599	Activation segment phosphorylation site; mutated in cancer
V599	V600	Most commonly mutated residue in cancer
K600	K601	Mutated in cancer
S601	S602	Activation segment phosphorylation site
R681	R682	Mutated in cancer
A727	A728	Located within C-terminal 14-3-3 binding motif; mutated in cancer
S728	S729	Phosphorylation site and core of 14-3-3 binding motif

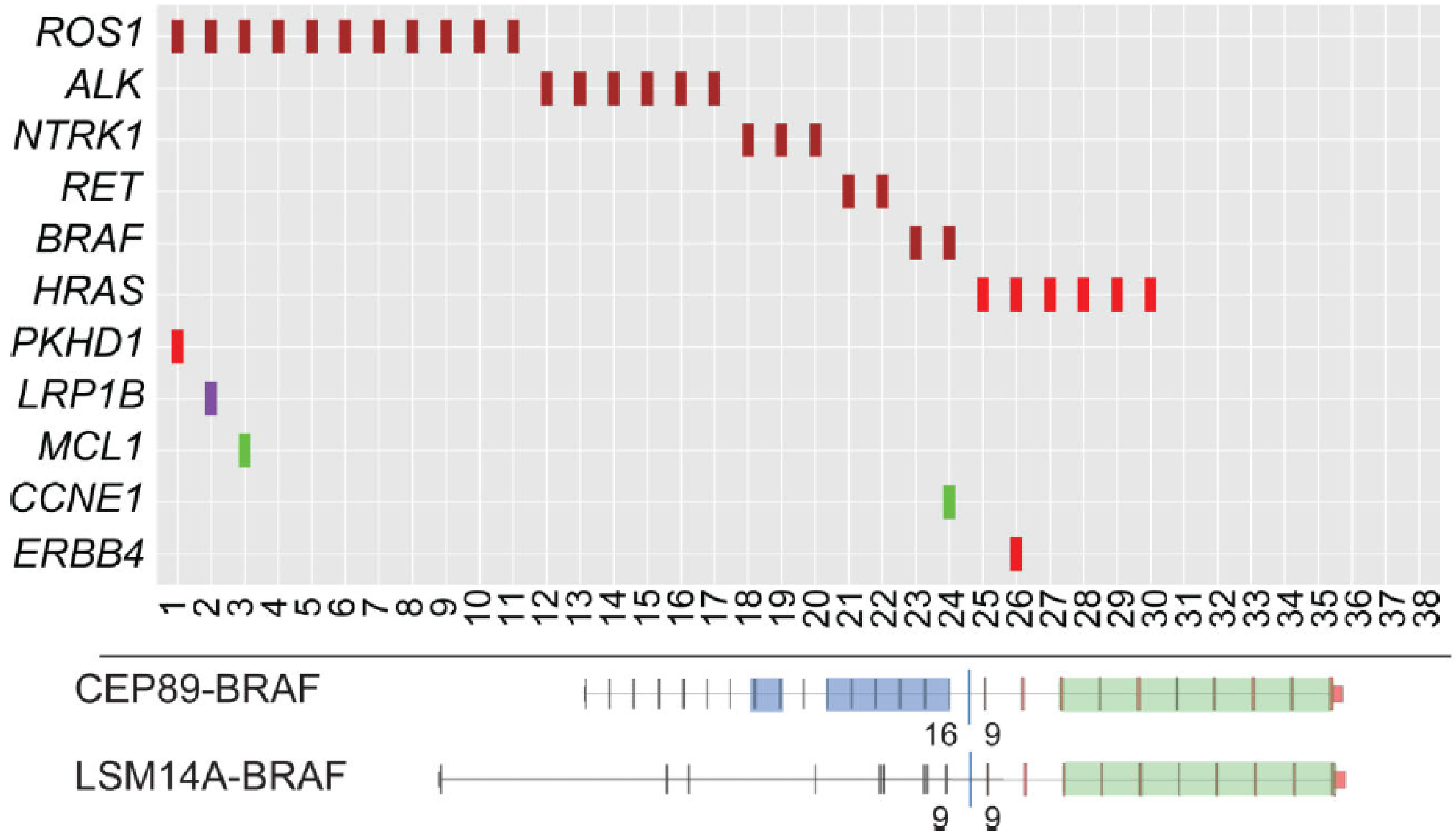
PKA, protein kinase A; PKB, protein kinase B.

Wellbrock C, Nat Rev Mol Cell Biol. 2004 Nov;5(11)

BRAF

Beyond BRAF mutations: translocations

38 cutaneous spitzoid lesions

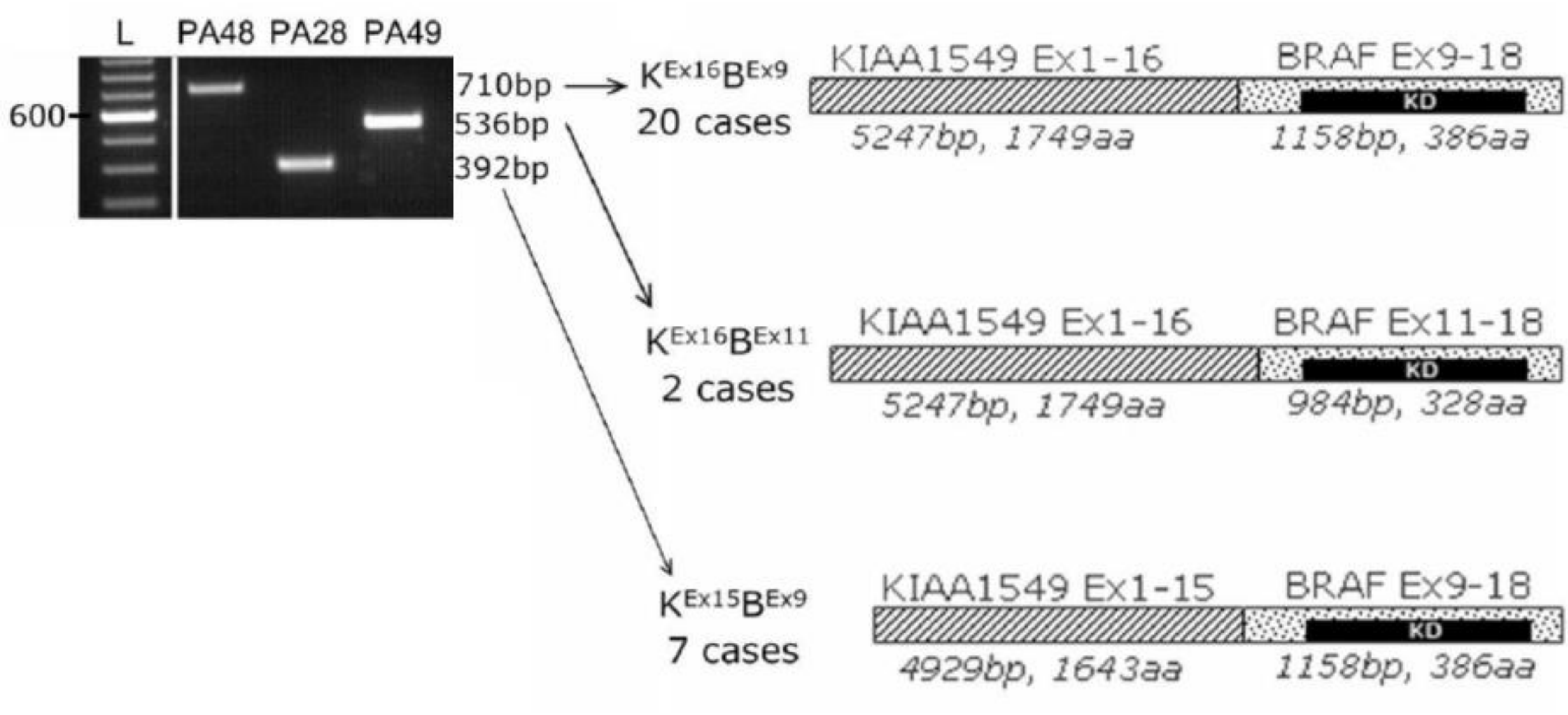


Wiesner T, Nat Commun. 2014;5:3116

BRAF

Beyond BRAF mutations: amplification/fusion genes

44 pilocytic astrocytomas



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Jones DTW, Cancer Res . 2008 November 1; 68(21)

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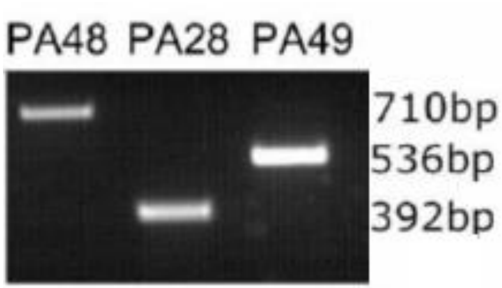

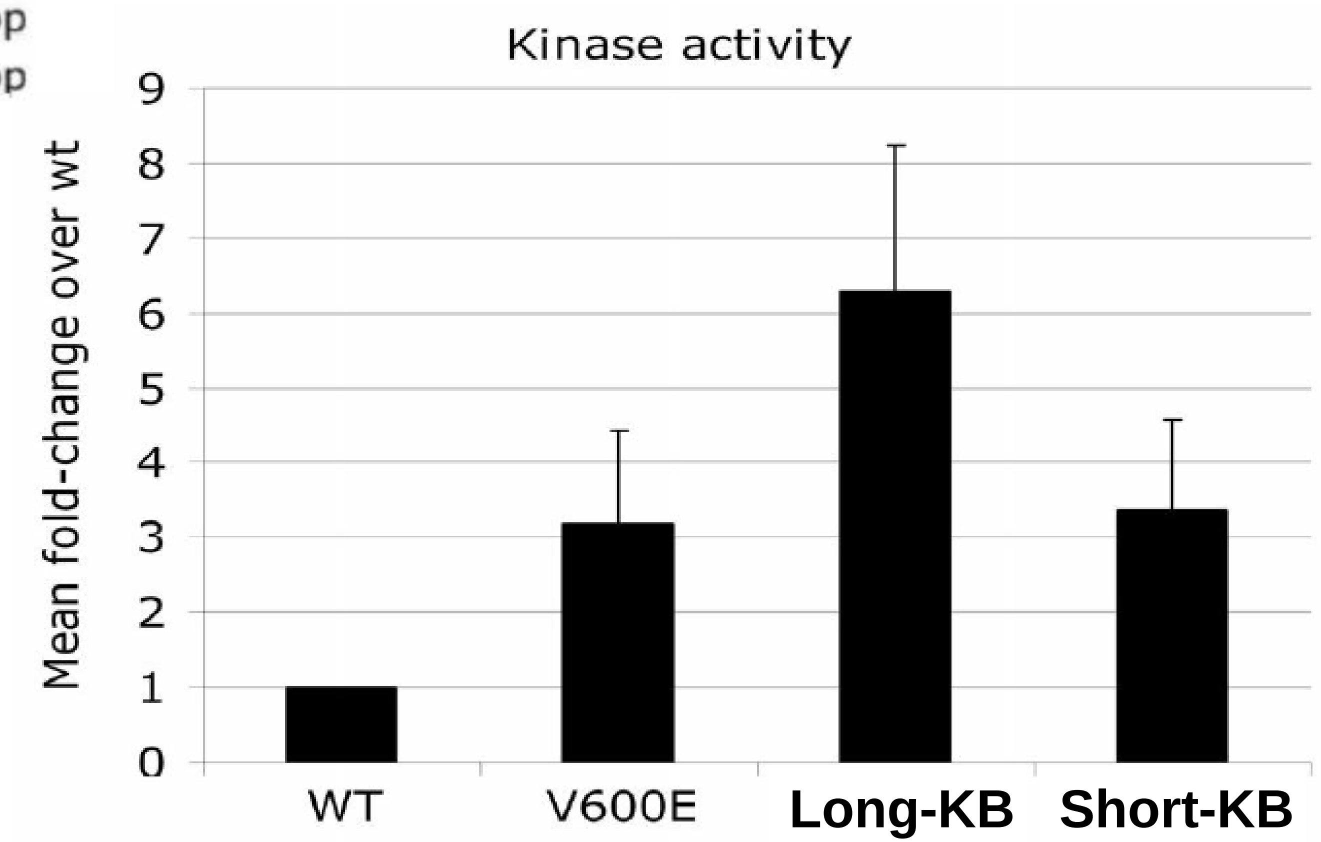

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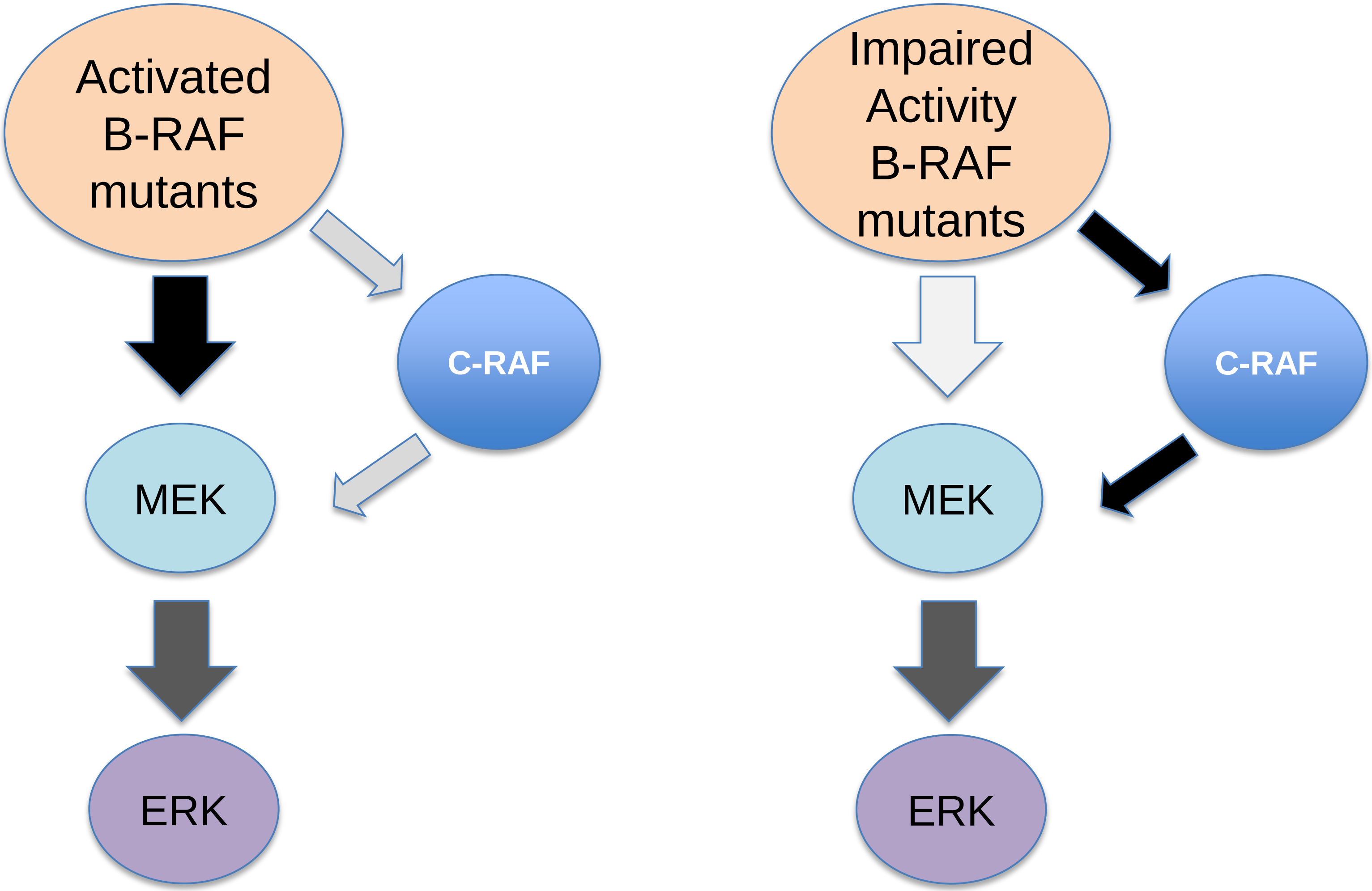

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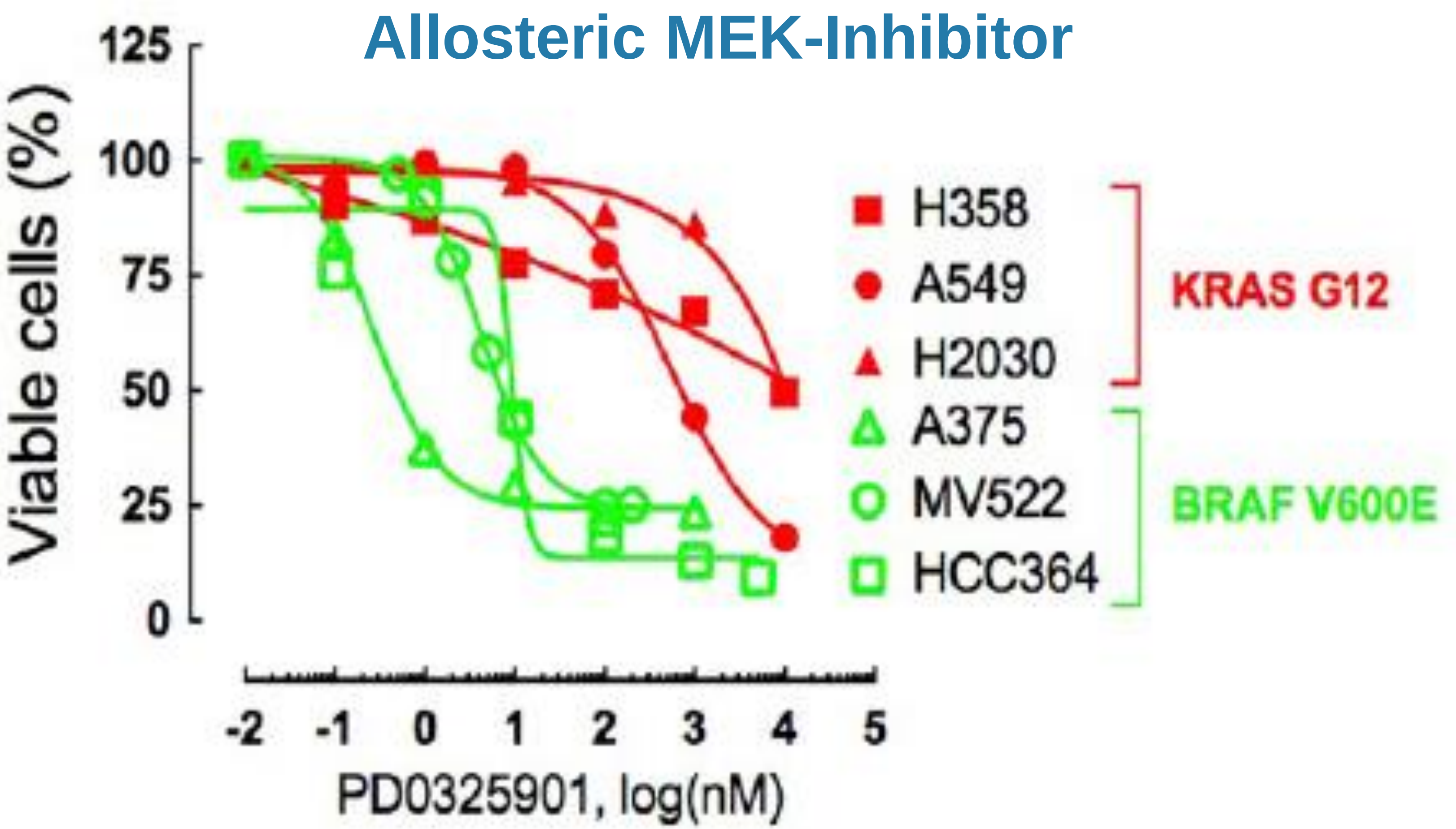
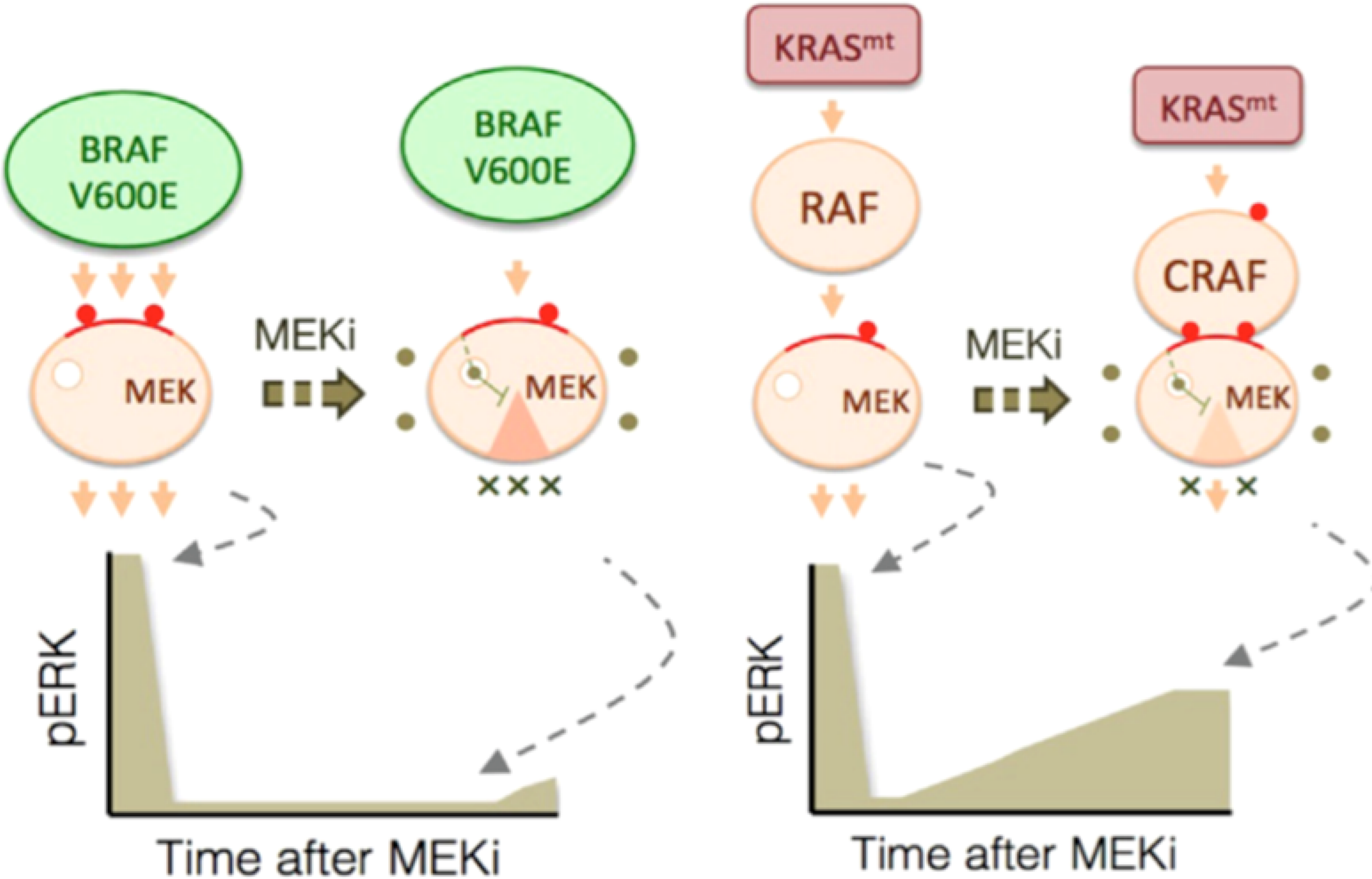
Wan PT, Cell. 2004 Mar 19

BRAF

C-RAF (Raf-1): there is more than MEK activation



Allosteric MEK-Inhibitor



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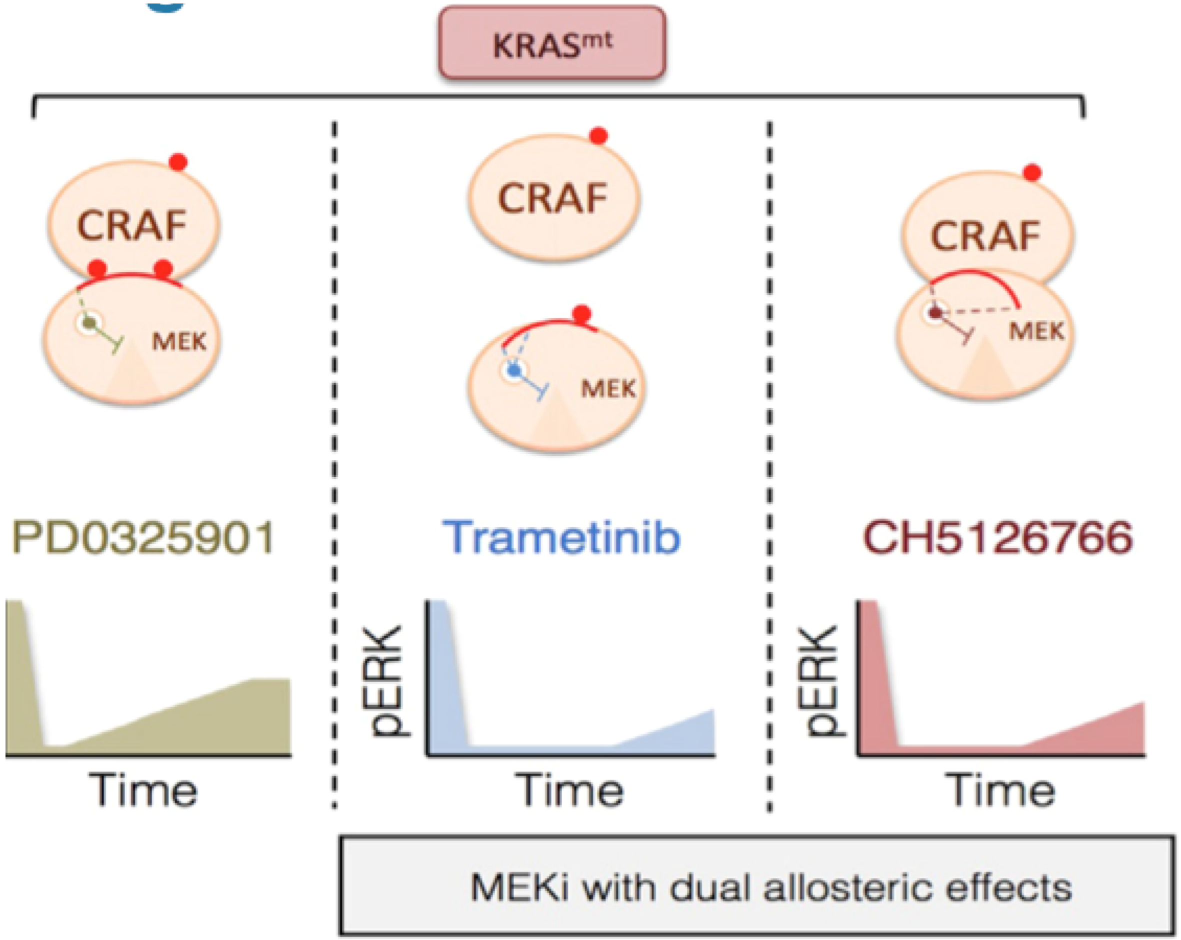
Lito P, Cancer Cell 25, 697–710, May 12, 2014

Stronger Allosteric MEK-Inhibitor



- Block MEK kinase activity
- Prevent RAF activation of MEK:
  - Disrupt RAF-MEK complexes
  - Block in-complex phosphorylation

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Lito P, Cancer Cell 25, 697–710, May 12, 2014

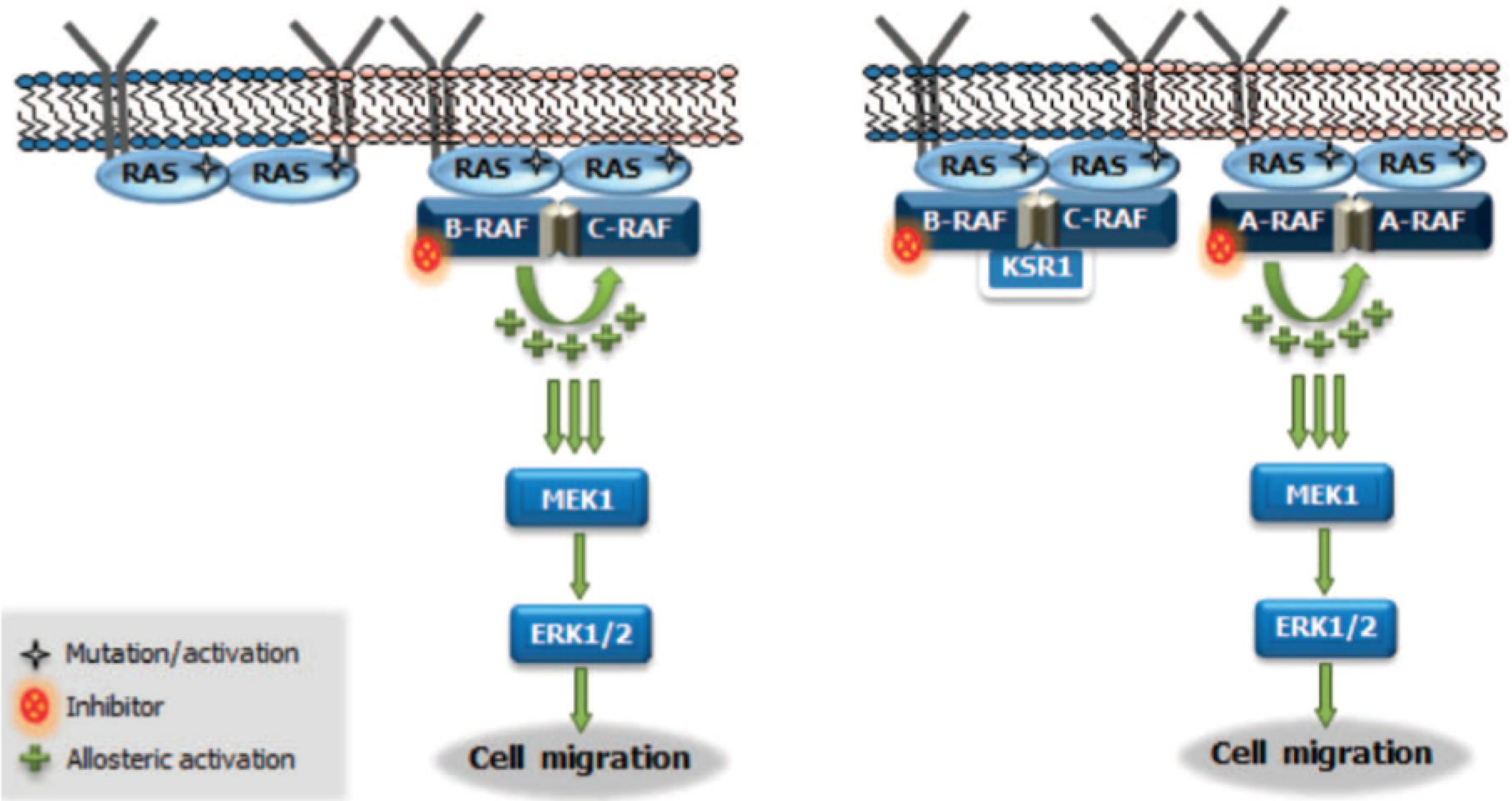
ARAF

ARAF independent

RAFi-mediated ERK1/2 activation needs CRAF in RAS mutated cells

ARAF dependent

RAFi-mediated ERK1/2 activation needs ARAF in a cell type–dependent manner



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Mooz J, Science Signaling 7 (337), ra73

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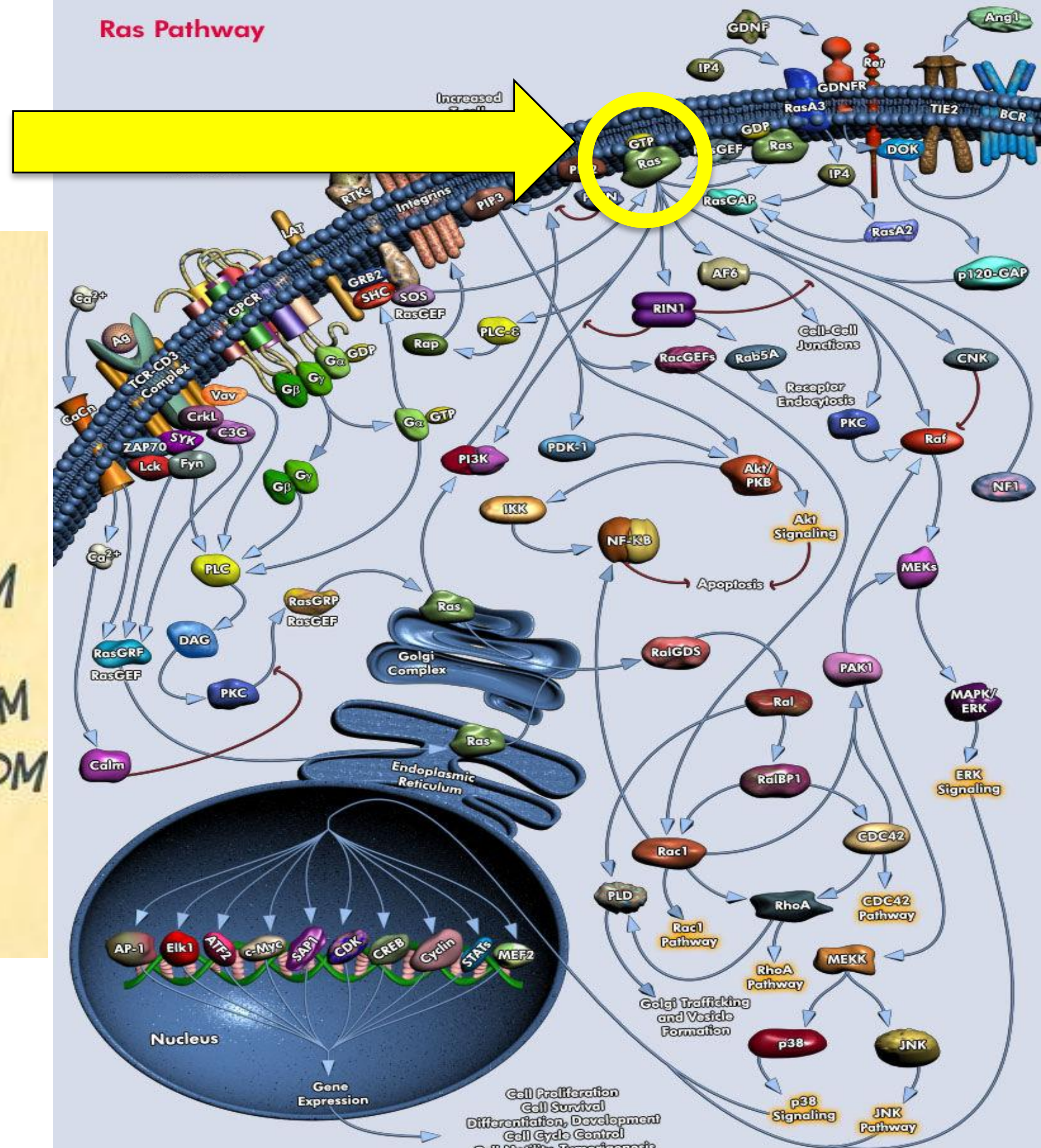
RAS-networking

Not a "one man band"

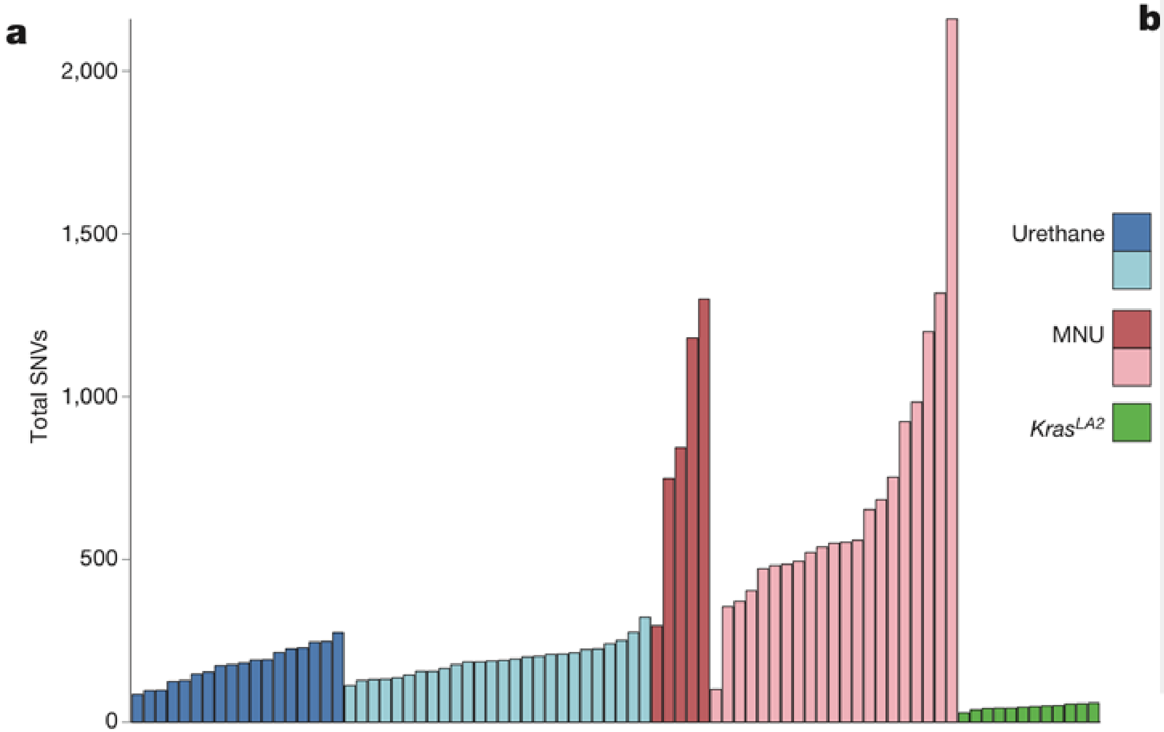


LETTER

doi:10.1038/nature13898

The mutational landscapes of genetic and chemical models of *Kras*-driven lung cancer

Peter M. K. Westcott[1,2], Kyle D. Halliwill[1,2], Minh D. To[1], Mamunur Rashid[3], Alistair G. Rust[3], Thomas M. Keane[3], Reyno Delrosario[1], Kuang-Yu Jen[4], Kay E. Gurley[5], Christopher J. Kemp[5], Erik Fredlund[6], David A. Quigley[1], David J. Adams[3] & Allan Balmain[1,7]



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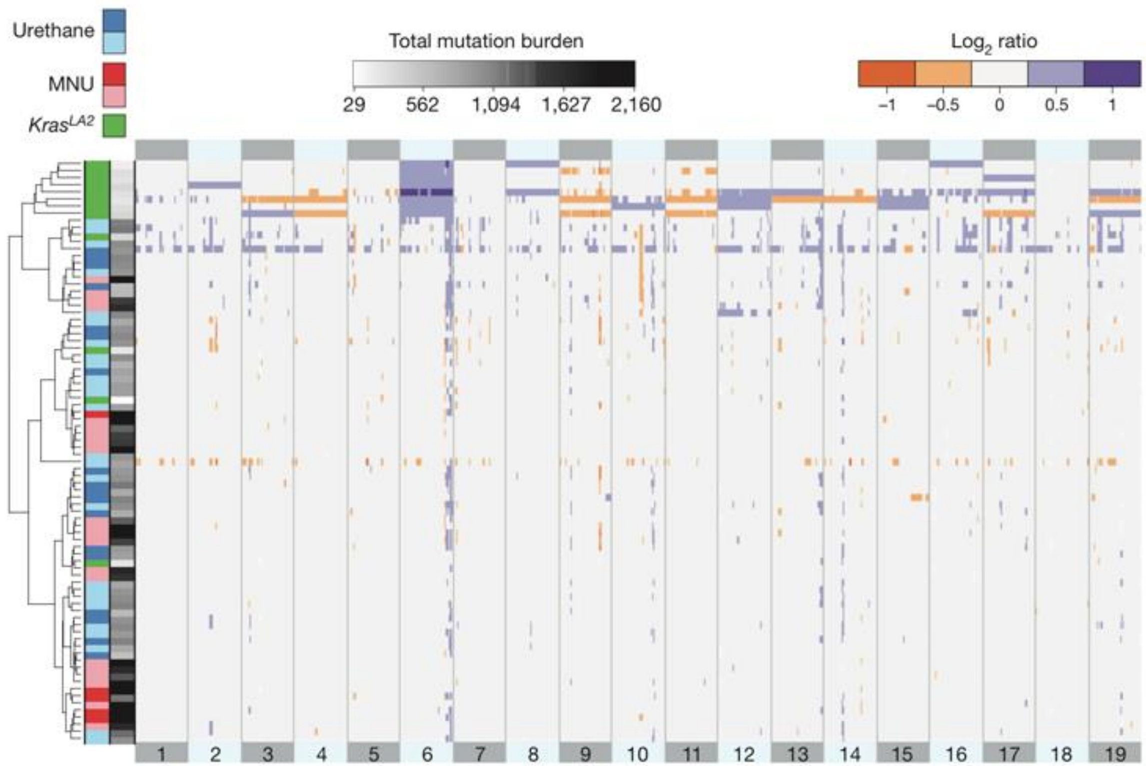


Lung cancer

Nature. 2008 October 23; 455(7216): 1069–1075. doi:10.1038/nature07423.

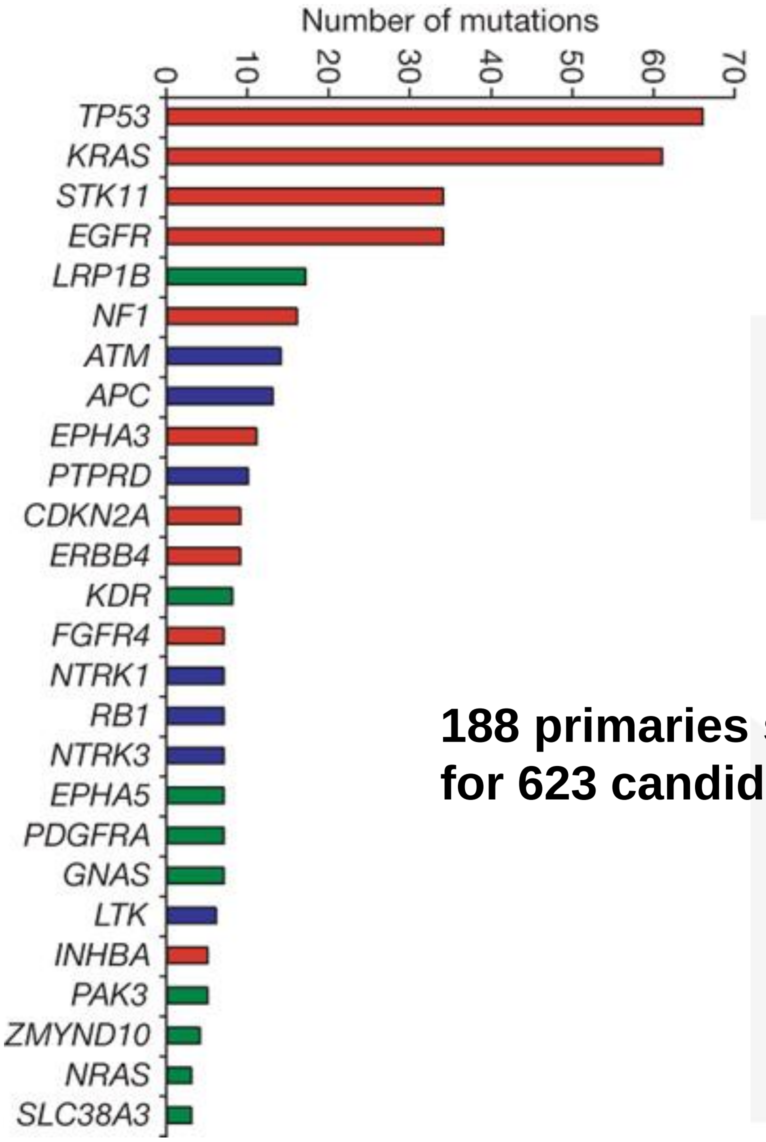
Somatic mutations affect key pathways in lung adenocarcinoma

Li Ding[1,*], Gad Getz[2,*], David A. Wheeler[3,*], Elaine R. Mardis[1], Michael D. McLellan[1], Kristian Cibulskis[2], Carrie Sougnez[2], Heidi Greulich[2,4], Donna M. Muzny[3], Margaret B. Morgan[3], Lucinda Fulton[1], Robert S. Fulton[1], Qunyuan Zhang[5], Michael C. Wendl[1], Michael S. Lawrence[2], David E. Larson[1], Ken Chen[1], David J. Dooling[1], Aniko Sabo[3], Alicia C. Hawes[3], Hua Shen[3], Shalini N. Jhangiani[3], Lora R. Lewis[3], Otis Hall[3], Yiming Zhu[3], Tittu Mathew[3], Yanru Ren[3], Jiqiang Yao[3], Steven E. Scherer[3], Kerstin Clerc[3], Ginger A. Metcalf[3], Brian Ng[3], Aleksandar Milosavljevic[3], Manuel L. Gonzalez-Garay[3], John R. Osborne[1], Rick Meyer[1], Xiaoqi Shi[1], Yuzhu Tang[1], Daniel C. Koboldt[1], Ling Lin[1], Rachel Abbott[1], Tracie L. Miner[1], Craig Pohl[1], Ginger Fewell[1], Carrie Haipek[1], Heather Schmidt[1], Brian H. Dunford-Shore[1], Aldi Kraja[5], Seth D. Crosby[1], Christopher S. Sawyer[1], Tammi Vickery[1], Sacha Sander[1], Jody Robinson[1], Wendy Winckler[2,4], Jennifer Baldwin[2], Lucian R. Chirieac[6,7], Amit Dutt[2,4], Tim Fennell[2], Megan Hanna[2,4], Bruce E. Johnson[4], Robert C. Onofrio[2], Roman K. Thomas[8,9], Giovanni Tonon[4], Barbara A. Weir[2,4], Xiaojun Zhao[2,4], Liuda Ziaugra[2], Michael C. Zody[2], Thomas Giordano[10], Mark B. Orringer[11], Jack A. Roth[12], Margaret R. Spitz[13], Ignacio I. Wistuba[12,14], Bradley Ozenberger[15], Peter J. Good[15], Andrew C. Chang[11], David G. Beer[11], Mark A. Watson[16], Marc Ladanyi[17,18], Stephen Broderick[17], Akihiko Yoshizawa[17], William D. Travis[17], William Pao[17,18], Michael A. Province[5], George M. Weinstock[1], Harold E. Varmus[19], Stacey B. Gabriel[2], Eric S. Lander[2], Richard A. Gibbs[3], Matthew Meyerson[2,4], and Richard K. Wilson[1]

Ding et al. Nature 2008, 455:1069-75

Lung cancer

26 significantly mutated genes in lung adenocarcinomas

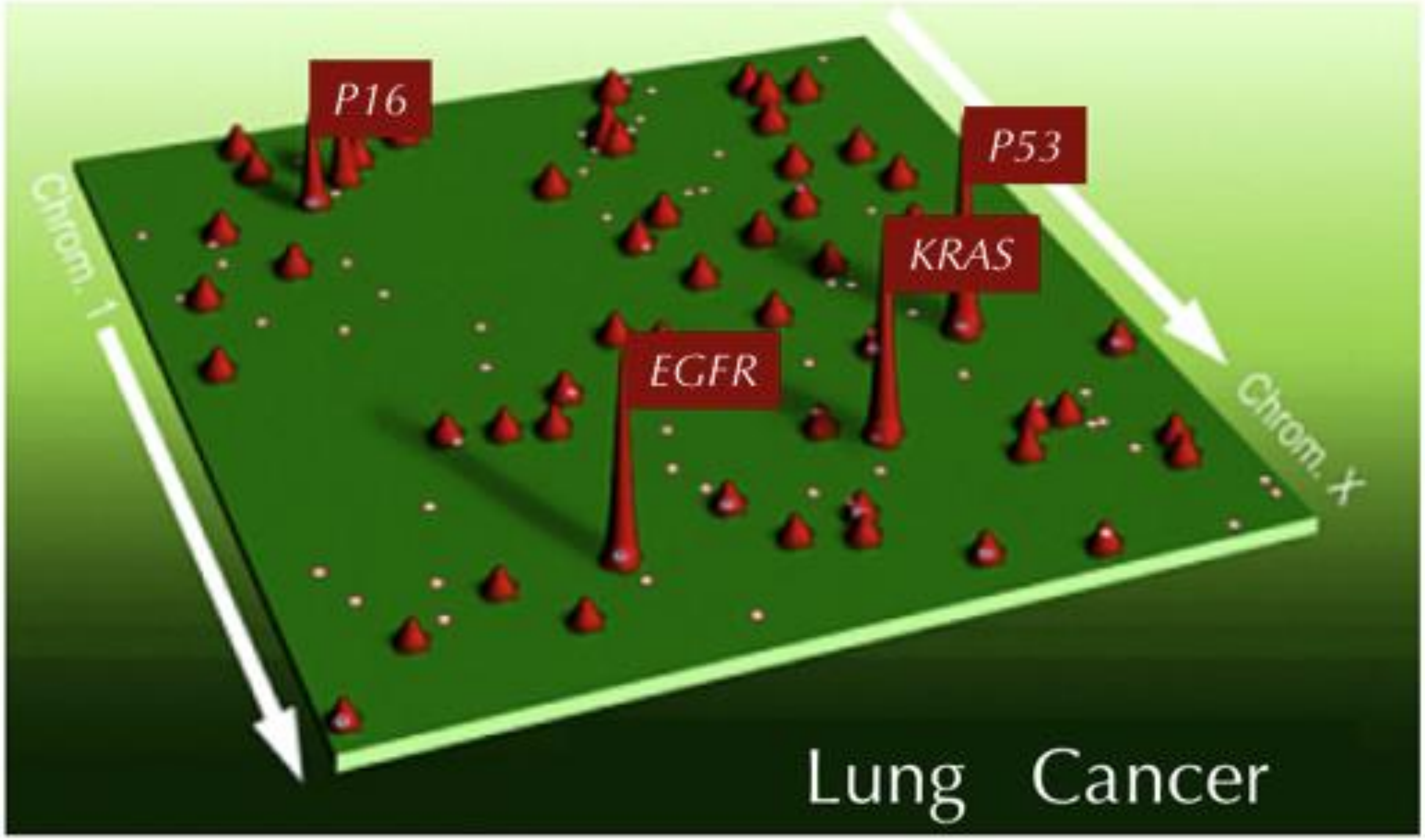


188 primaries screened for 623 candidate genes

L Ding *et al. Nature* **455**, 1069-1075 (2008)

Lung cancer

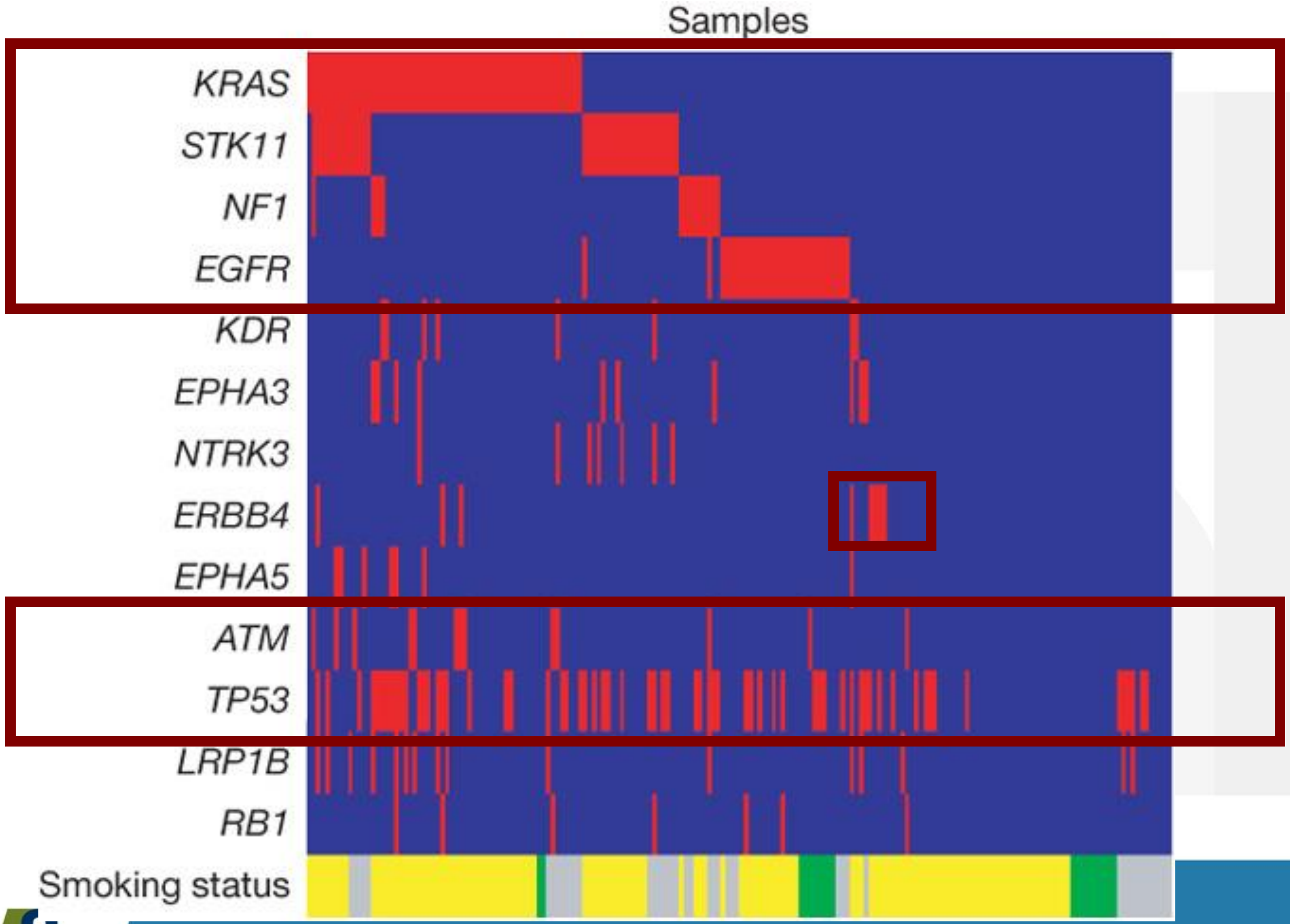
Each cancer holds from 4 to 29 mutated genes



Ding et al. Nature 2008, 455:1069-75

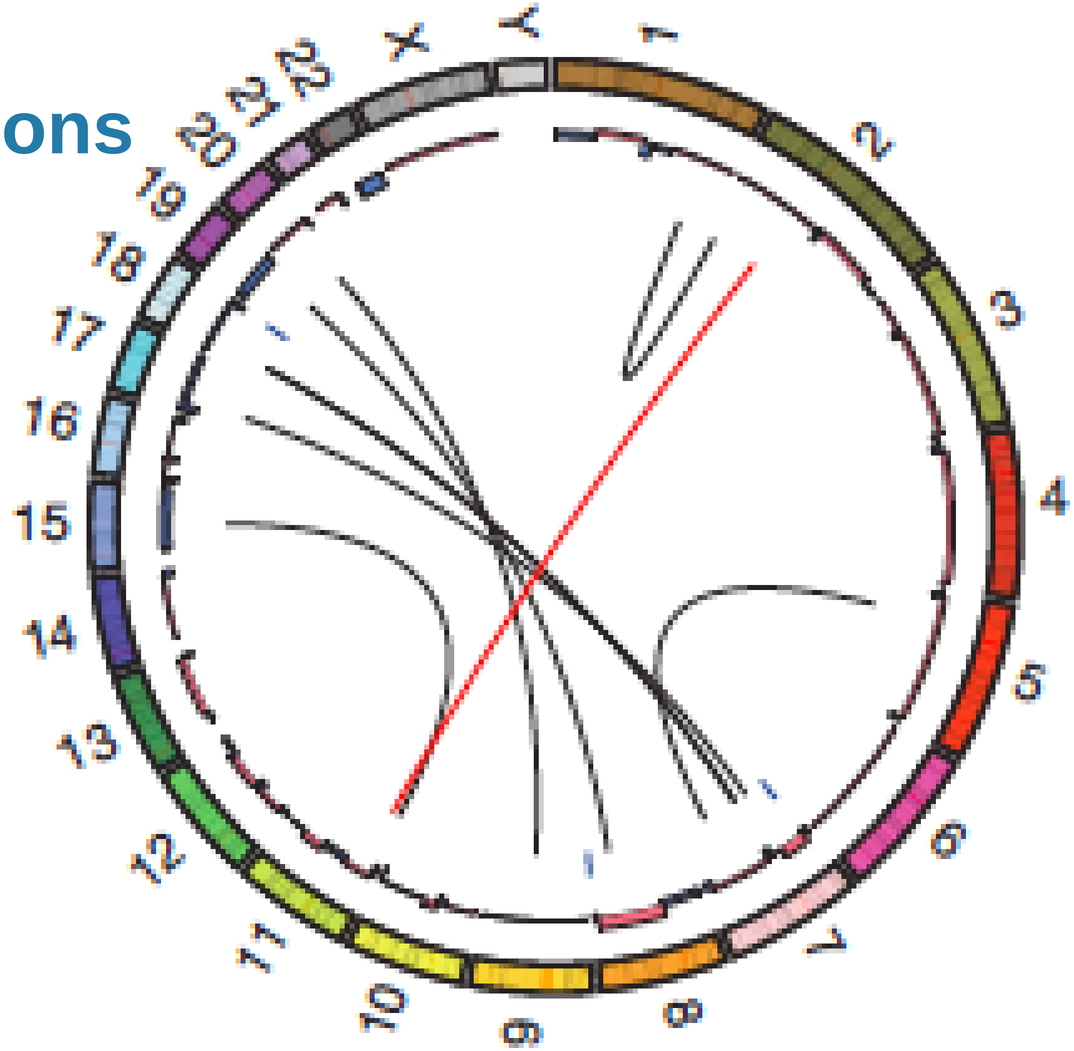
Lung cancer

Concurrent and mutual exclusion of mutations



Ding et al. Nature 2008, 455:1069-75

Mutations
Copy number alterations
Focal amplifications
Translocations

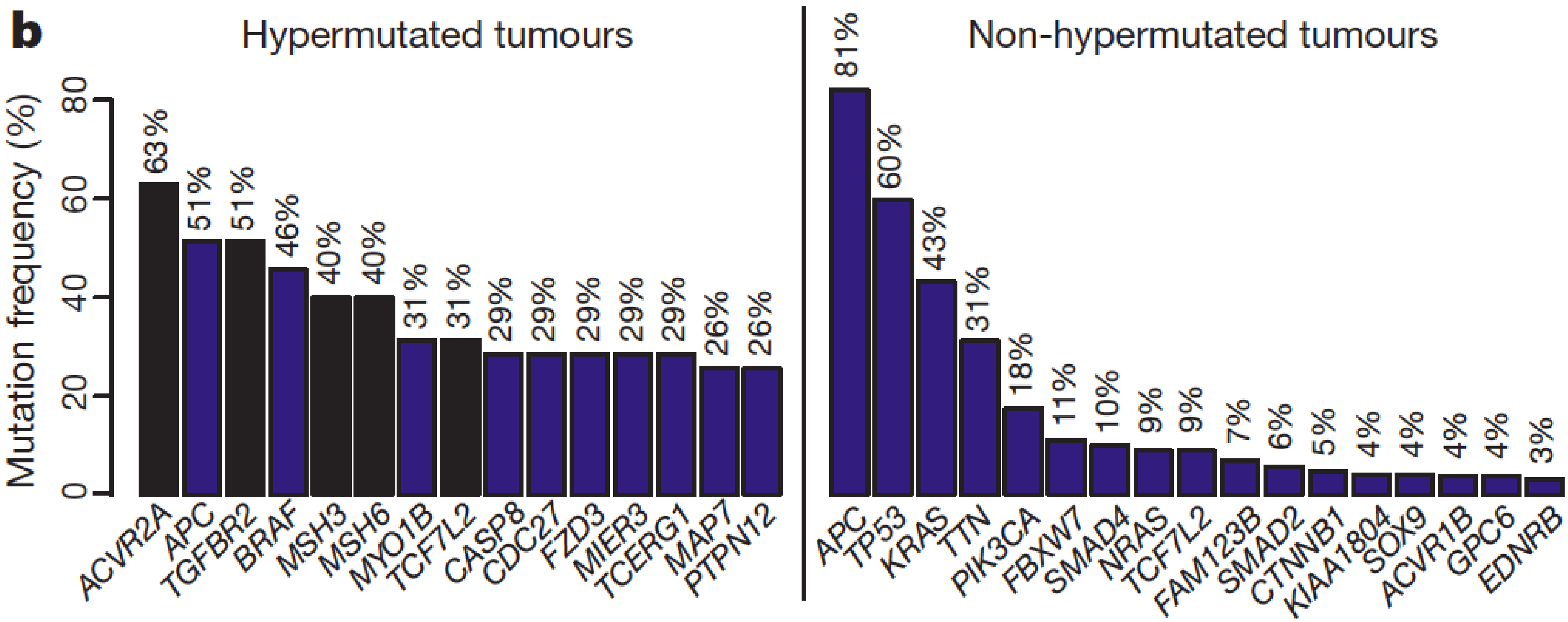


Comprehensive molecular characterization of human colon and rectal cancer. The Cancer Genome Atlas Network
Nature 2012, 487:330-7:

Colorectal cancer

32 somatic recurrently mutated genes

15 17



Comprehensive molecular characterization of human colon and rectal cancer. The Cancer Genome Atlas Network
Nature 2012, 487:330-7:

Lung cancer

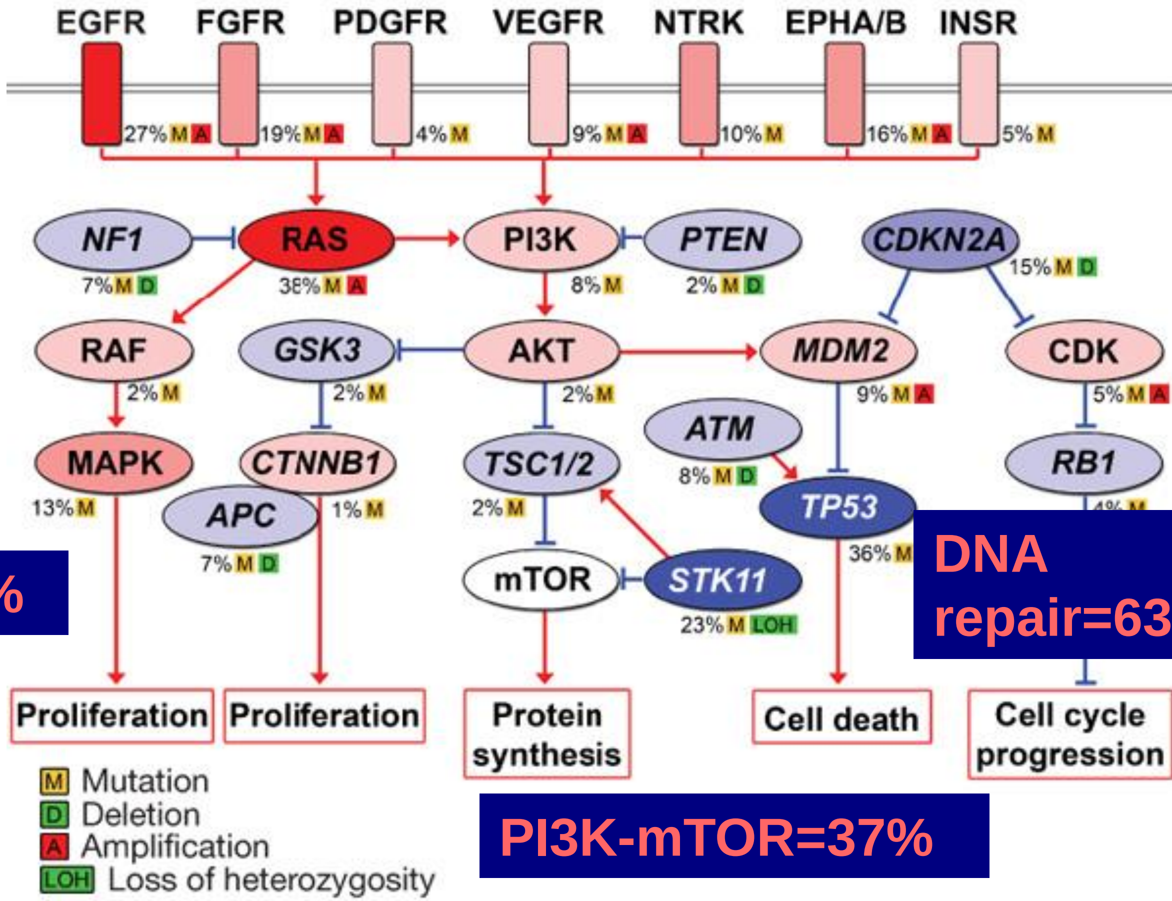
Mutated pathways in lung adenocarcinomas

RTK=90%

MAPK=60%

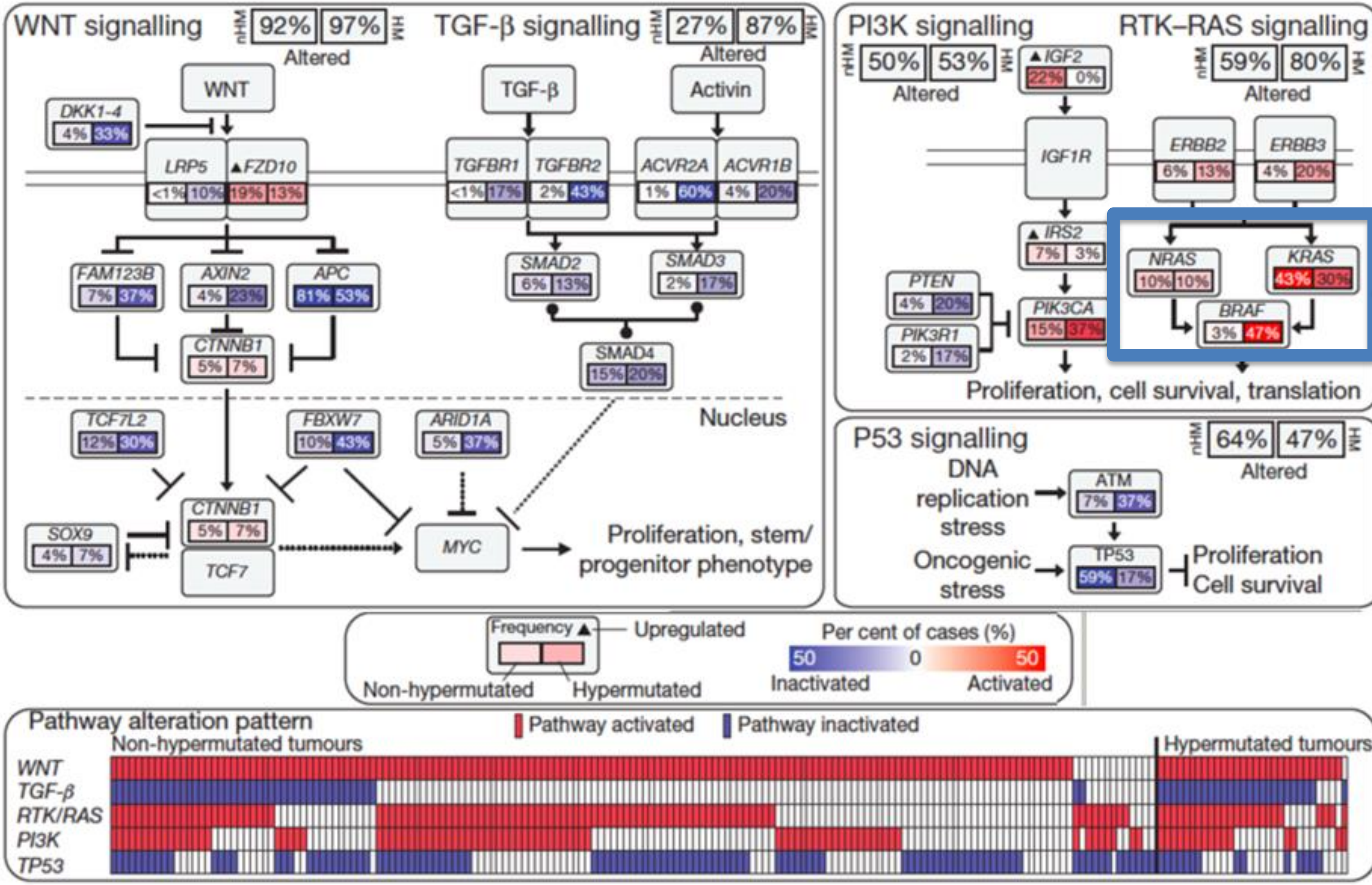
DNA repair=63%

PI3K-mTOR=37%



Ding et al. Nature 2008, 455:1069-75

Colorectal cancer



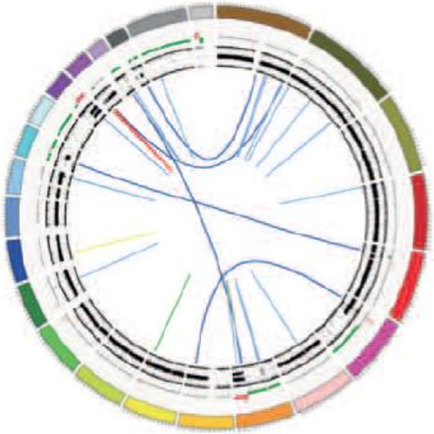
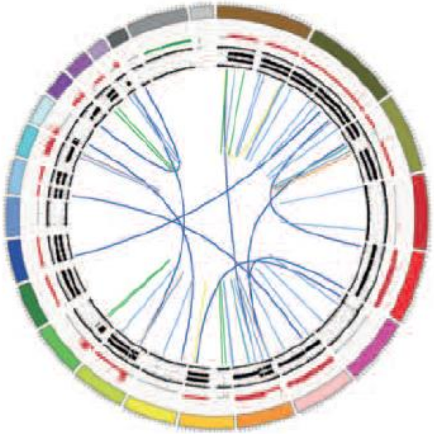
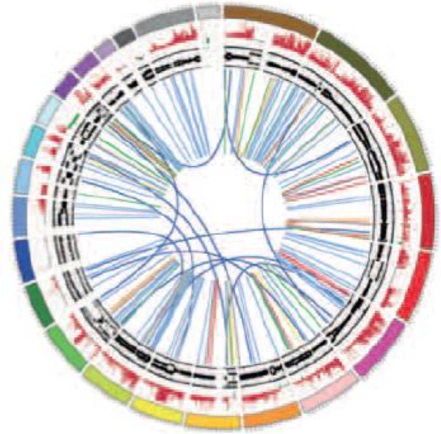
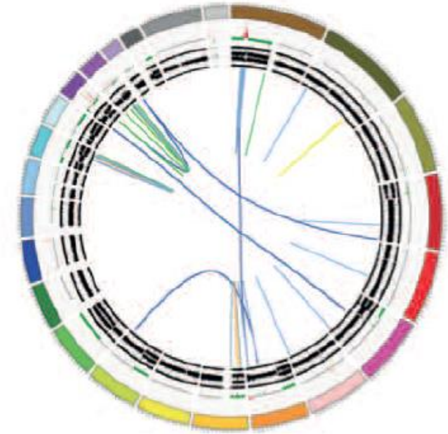
Comprehensive molecular characterization of human colon and rectal cancer. The Cancer Genome Atlas Network

Nature 2012, 487:330-7:

Pancreas cancer

Cowley MJ, J Hepatobiliary Pancreat Sci (2013) 20

Pancreas cancer

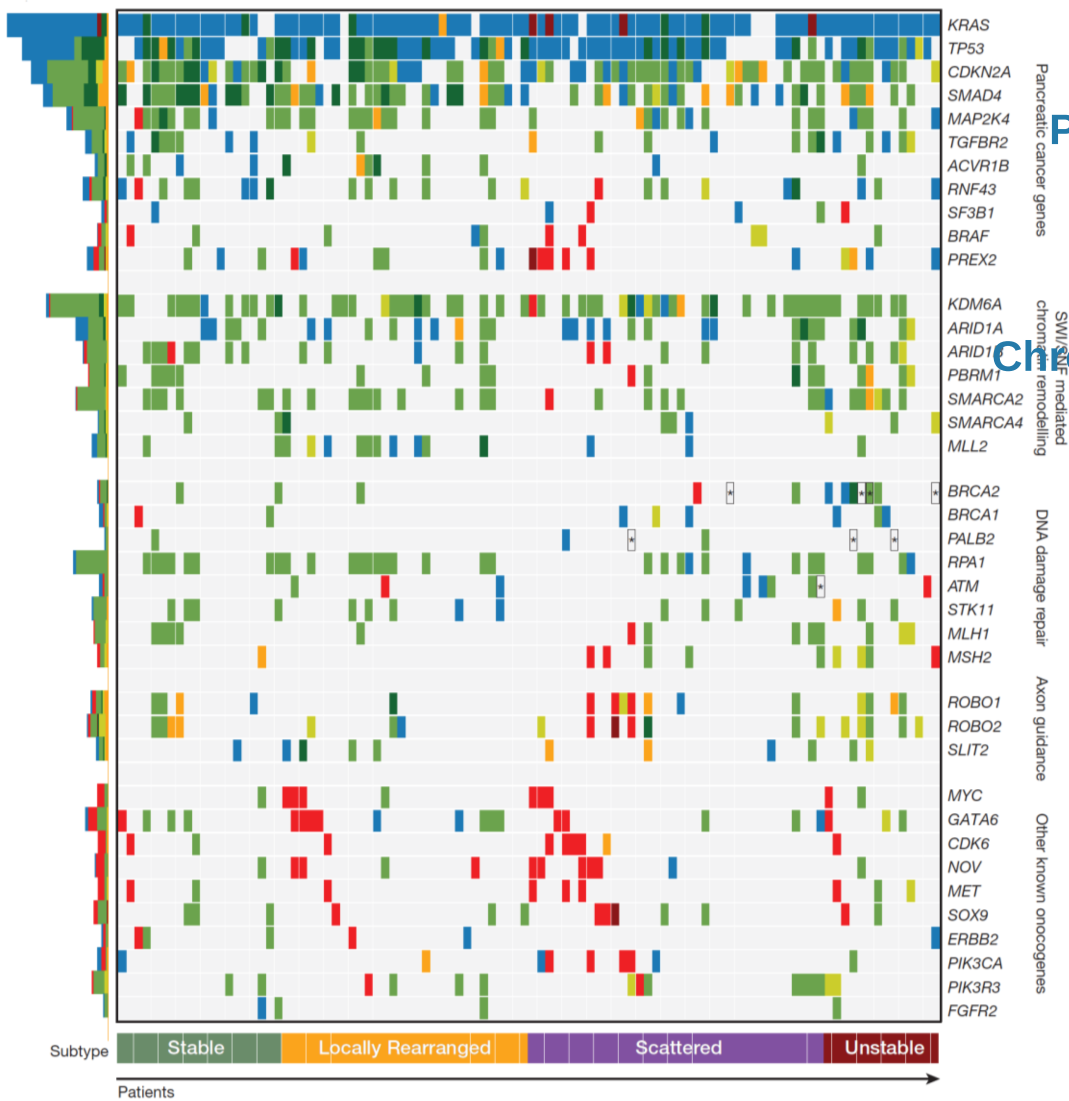
Stable	Scattered	Unstable	Locally rearranged
			
<50 events	50 – 200 events widespread	>200 events widespread	50-200 events 50% on 1 Chr
18%	**39%**	**18%**	**25%**

Intra-chromosomal rearrangement
Inter-chromosomal translocation
Duplication
Tandem duplication
Inversion
Foldback inversion
Deletion
Amplified inversion

Waddell N, Nature. 2015 Feb 26;518(7540)

Pancreas

Stable Locally rearranged Scattered Unstable



Pancreas cancer genes

Chromatin remodelling genes

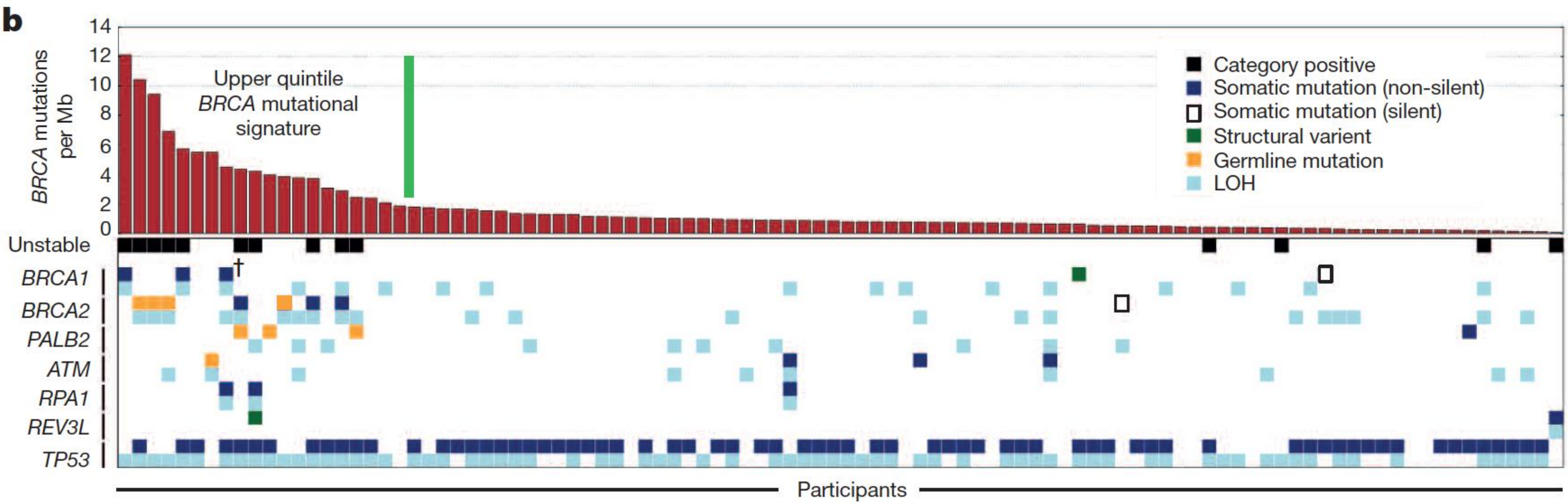
DNA damage repair

Axon guidance genes

Other genes

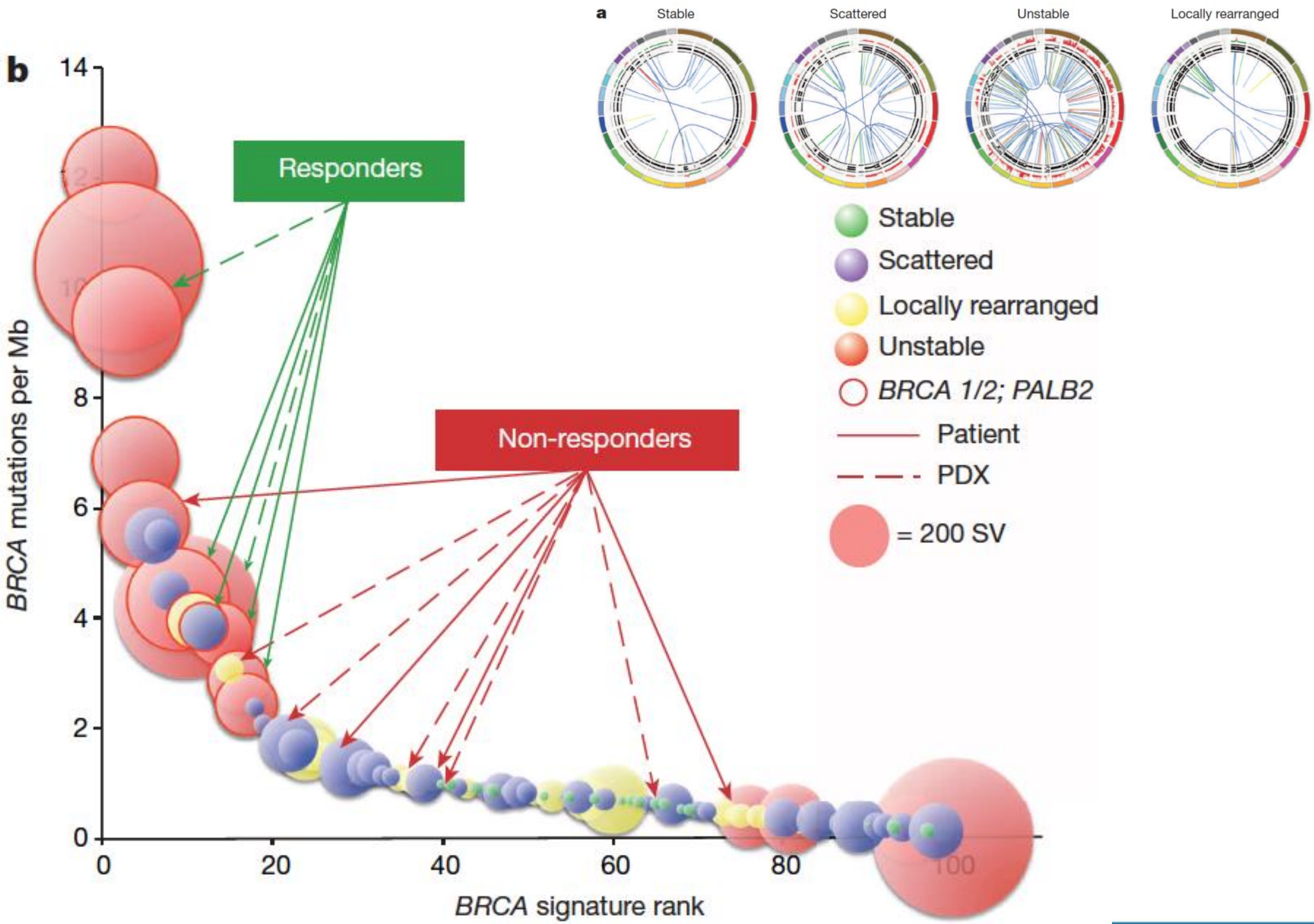
Waddell N, Nature. 2015 Feb 26;518(7540)

Pancreas cancer



Waddell N, Nature. 2015 Feb 26;518(7540)

Pancreas cancer



Waddell N, Nature. 2015 Feb 26;518(7540)

Targetting-Ras…dream or reality?