BRAF Mutant: What to do?
May need MSI

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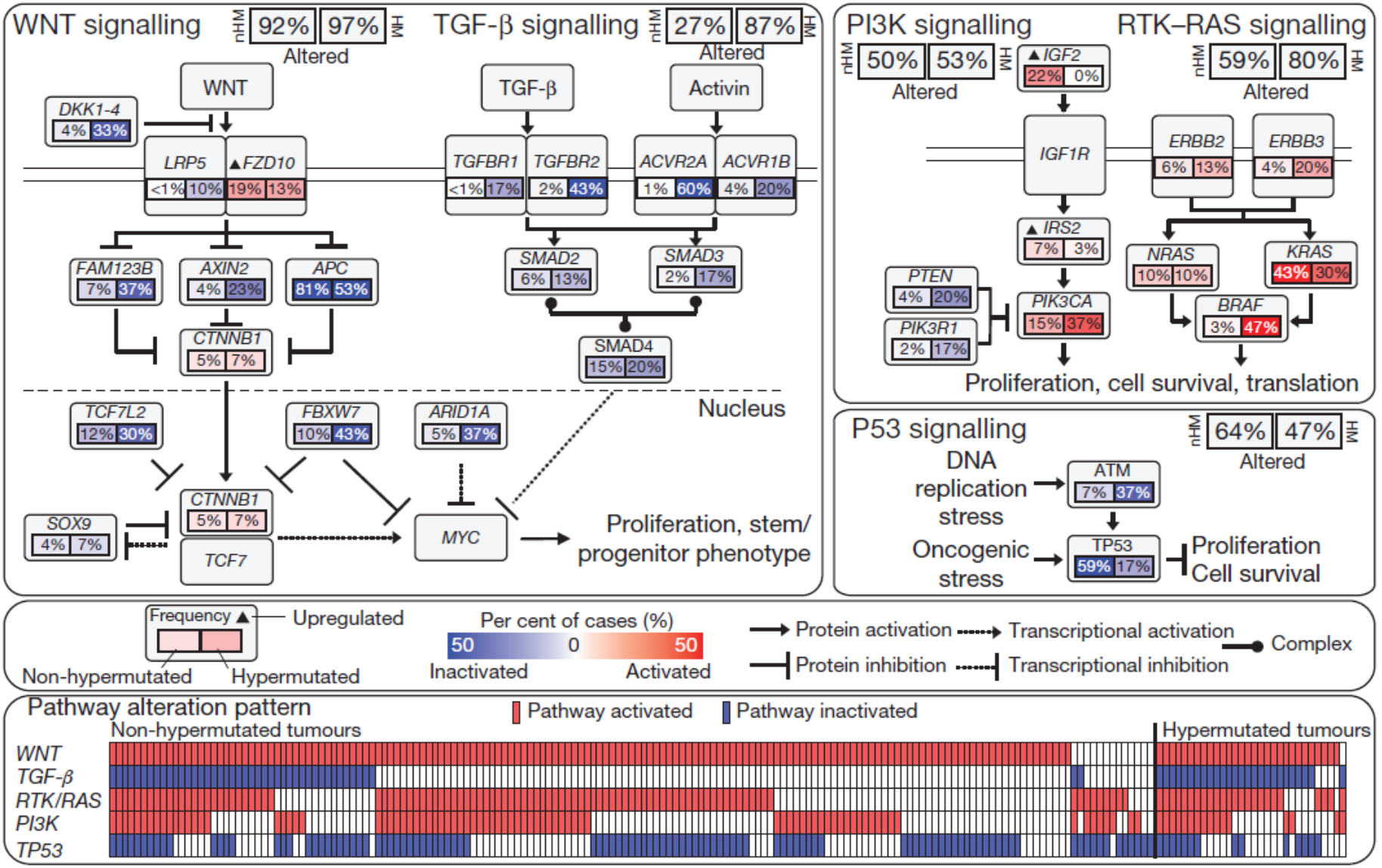
Genetic changes in CRC



Cancer Genome Atlas Network. Nature 2012;487:330–337

BRAF Background

- Overall, approximately 8% of all tumors have a BRAF mutation; in CRC it ranges from 5-10%
- The predominant mutation, similar to melanoma, is a single-base substitution of valine by glutamic acid at position 600 (V600E) within the activation segment.
- Signals through MEK/ERK activation pathway
- BRAF mutation is an early event in CRC and there is a high concordance between primary and metastatic tissue.
- Associated with:
  - R-sided tumors; high grade
  - Older age, female
  - MSI-high (due to epigenetic mechanisms, not HNPCC)
  - Serrated (as opposed to tubular) adenoma pathway

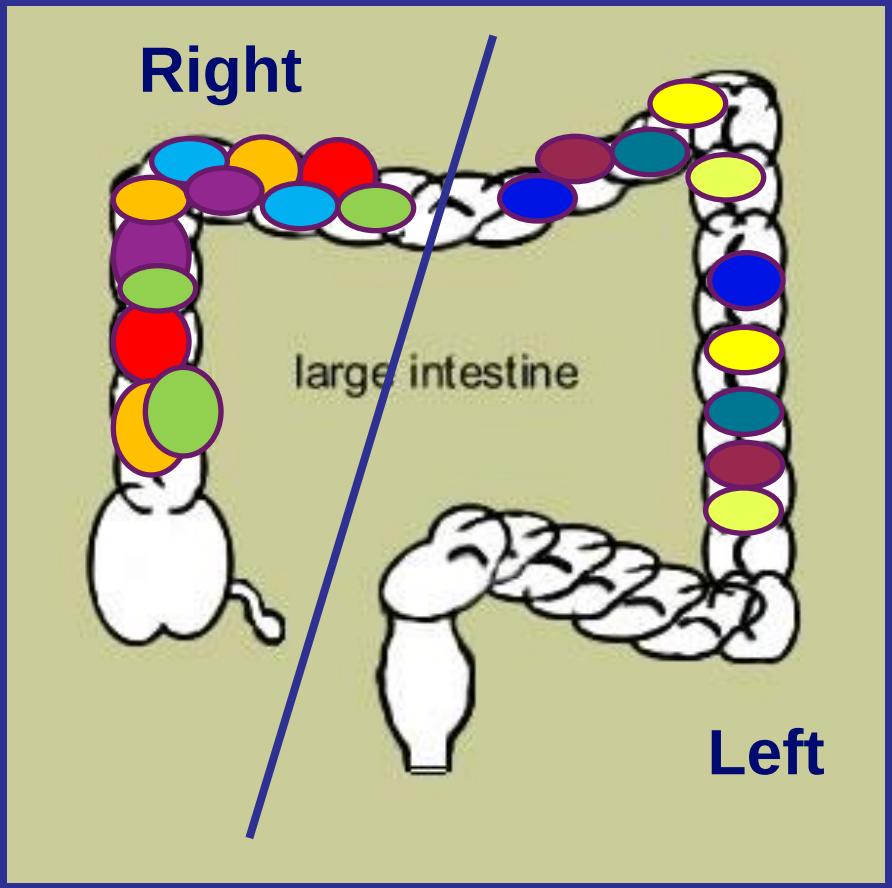
Questions we need to answer?

- 1. Why is braf mt associated with poor prognosis but not ras mt in metastatic disease?
- 2. Why is ras mt associated with efficacy of EGFR inhibitor but not braf mt?
- 2. How can MSI status reverse prognosis of braf?
- 3. Any immunophenotype in braf mutant tumors?

Distinct Biology of R v. L CRC

Analysis of PETACC-3 samples (n=2849)

- BRAF mut
- MSI
- KRAS
- PIK3CA
- Mucinous differentiation



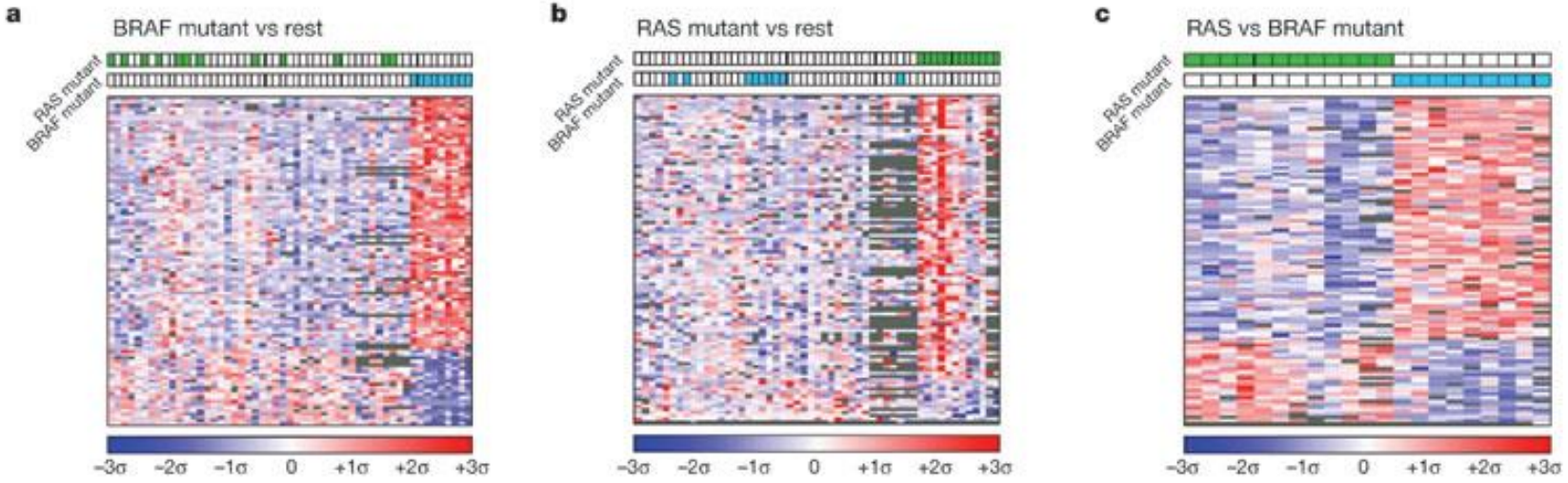
- EREG expression
- 18q loss
- 20q Gain
- EGFR gain
- HER2 gain

High mutation Frequency

Poor Prognosis

Sensitive to Cetuximab

Good Prognosis



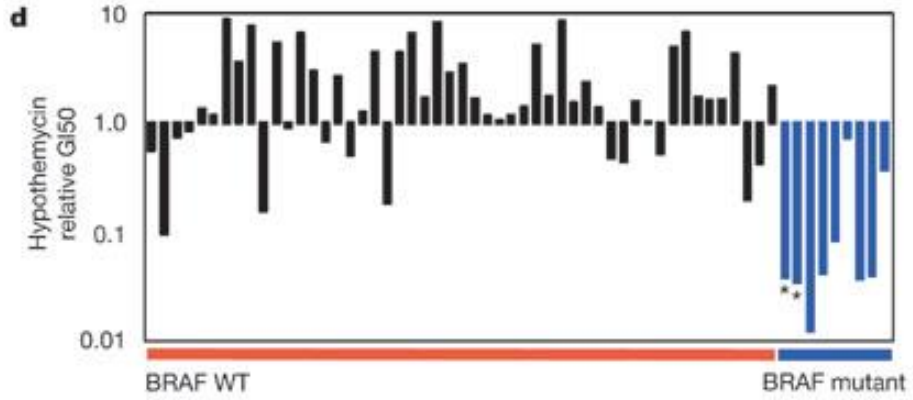
NSC no.	Name	Score	*P* value	NSC no.	Name	Score	*P* value
354462	Hypothemycin	8.61	1.66×10^{-11}	675984	–	3.28	1.99×10^{-3}
706829	Diamantane der.	8.24	8.10×10^{-11}	665564	–	3.25	2.02×10^{-3}
678518	Protein LF	6.47	1.17×10^{-7}	281272	AraA C	3.03	3.80×10^{-3}
658874	–	5.96	3.12×10^{-7}	218457	ICRF 202	2.98	4.94×10^{-3}
669995	–	5.88	3.12×10^{-7}	645827	–	2.97	4.49×10^{-3}
701104	–	5.76	5.07×10^{-7}	642290	–	2.89	6.12×10^{-3}

BRAF:

NSC no.	Name	Score	*P* value
354462	Hypothemycin	6.28	5.00×10^{-6}
706829	Diamantane der.	5.79	1.40×10^{-5}
669995	–	5.13	5.91×10^{-5}

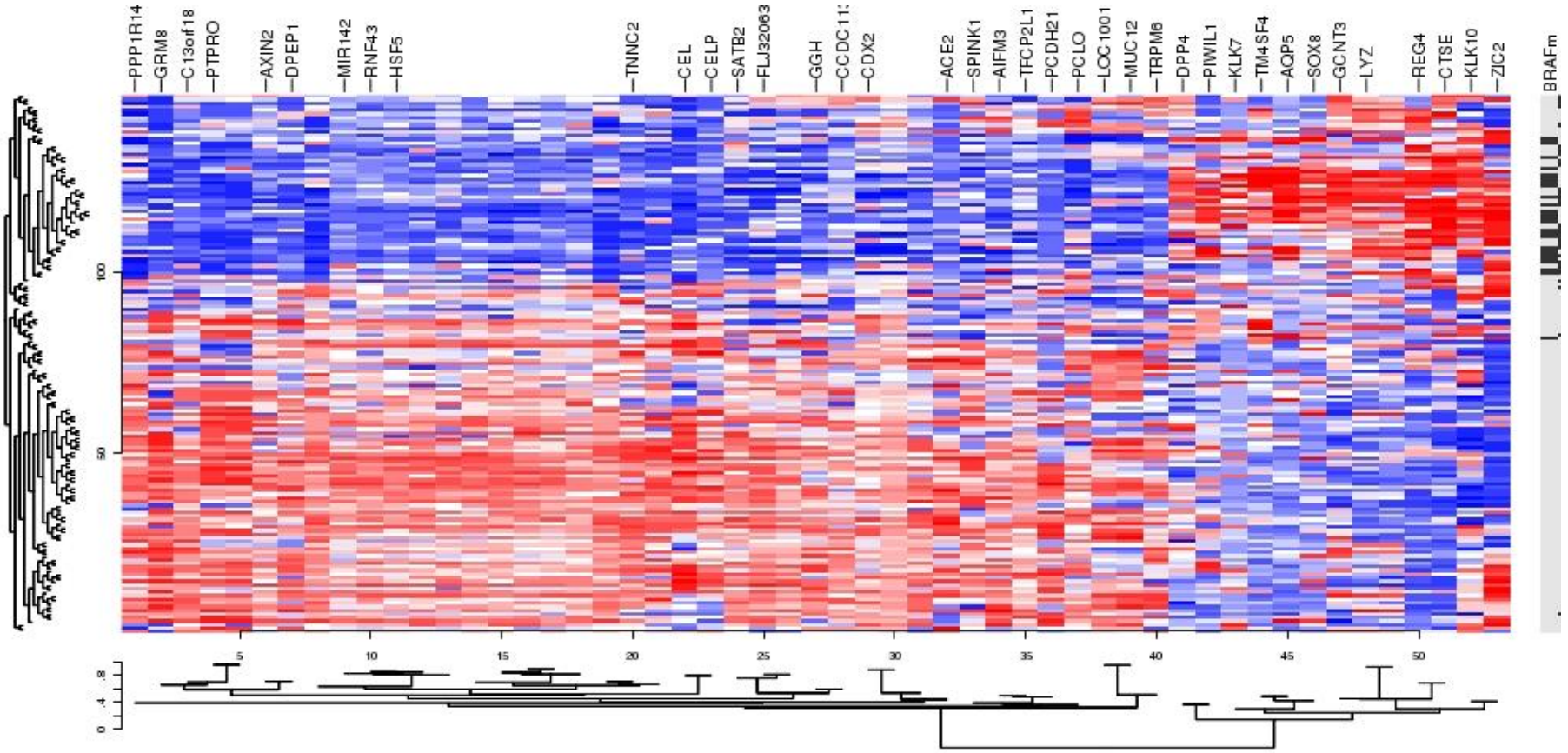
RAS:

NSC no.	Name	Score	*P* value
704408	–	4.01	8.13×10^{-4}
102029	–	3.82	2.13×10^{-3}
94889	Gardenin A	3.62	1.95×10^{-3}



David B. Solit, Levi A. Garraway, Christine A. Pratilas, Ayana Sawai, Gad Getz, Andrea Basso, Qing Ye, Jose M. Lobo, Yuhong She, Iman Osman, Todd R. Golub, Judith Sebolt-Leopold, William R. Sellers & Neal Rosen Nature 439, 358-362(19 January 2006)

BRAF mut vs WT 2: Differentially expressed probesets with fold change > 2 and <1% FDR (53 probesets)



Popovici JCO 2012

Adjusted for MSI status and BRAF/MSI interactions

VOLUME 30 · NUMBER 12 · APRIL 20 2012
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Identification of a Poor-Prognosis *BRAF*-Mutant–Like Population of Patients With Colon Cancer

Vlad Popovici, Eva Budinska, Sabine Tejpar, Scott Weinrich, Heather Estrella, Graeme Hodgson, Eric Van Cutsem, Tao Xie, Fred T. Bosman, Arnaud D. Roth, and Mauro Delorenzi

A combined oncogenic pathway signature of *BRAF*, *KRAS* and *PI3KCA* mutation improves colorectal cancer classification and cetuximab treatment prediction

Sun Tian,[1] Iris Simon,[1] Victor Moreno,[2,3] Paul Roepman,[1] Josep Tabernero,[4] Mireille Snel,[1] Laura van't Veer,[1] Ramon Salazar,[2] Rene Bernards,[1,5] Gabriel Capella[2]

The BRAF - signature identifies:

1) Tumors that carry the BRAF V600E gene mutation with 96% sensitivity and 86% specificity

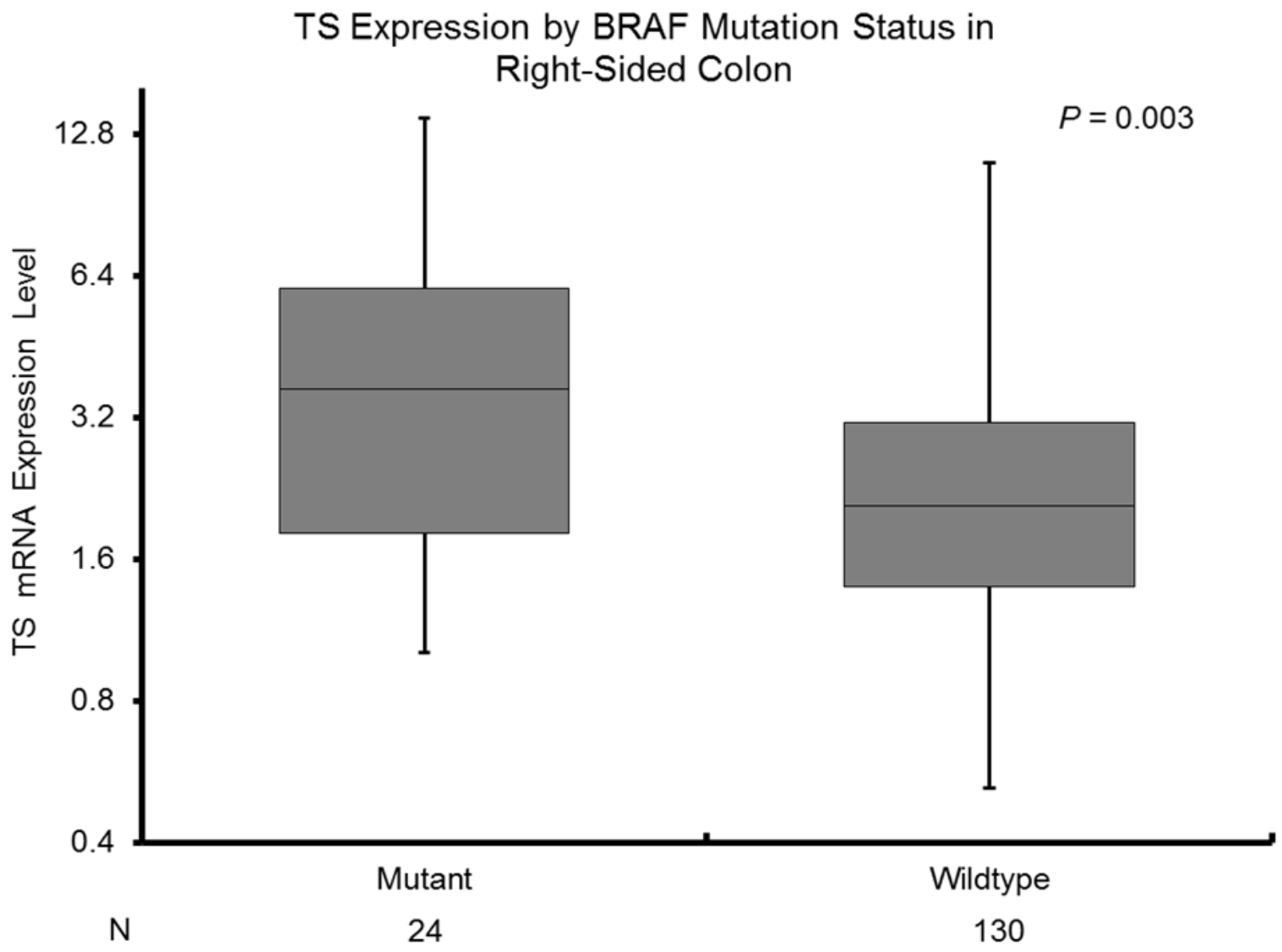
2) 30% of KRAS mutant CC
   13% of double wild type CC
   which carry the same gene expression profile as BRAF V600E

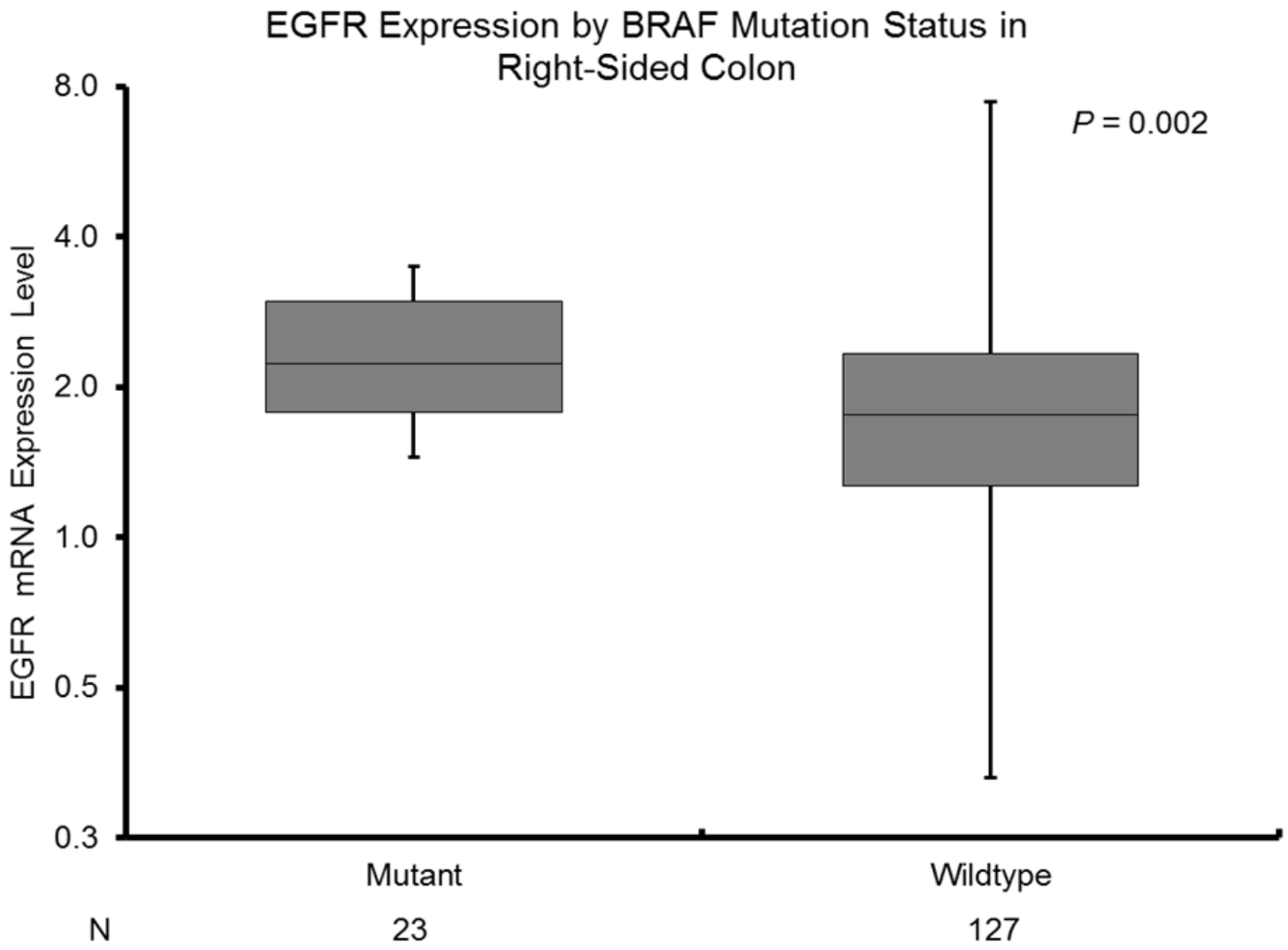
BRAF mut vs WT 2: Differentially expressed genes

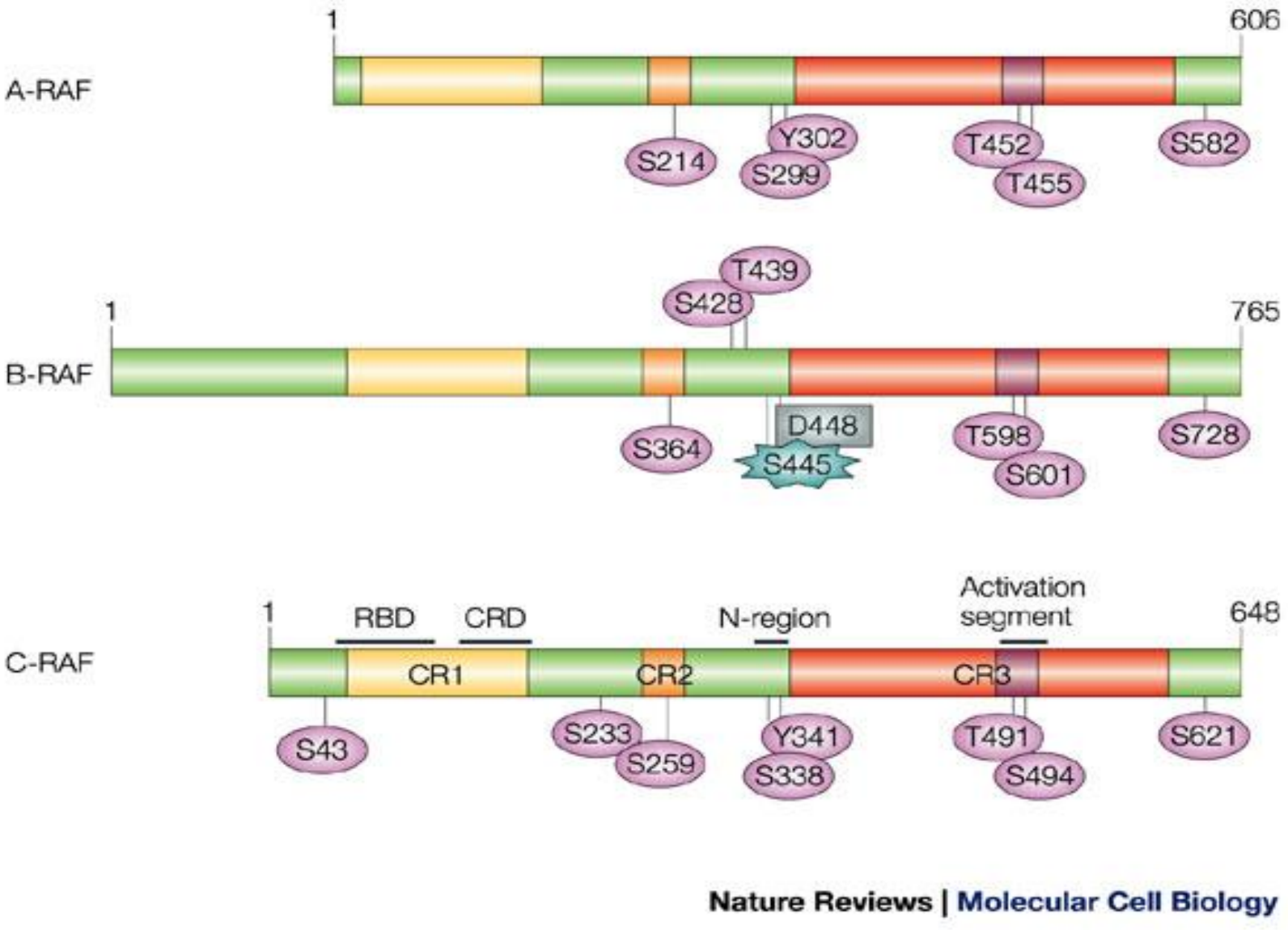
Gene	Gene name	Pathway	Fold.change	P.Adj
AQP5	Aquaporin 5	target of ESR1; promotes proliferation and inhibits apoptosis in chronic myelogenous leukemia; interacts with the MAPK and Rb pathway	5,5	9,7E-21
CTSE	cathepsin E	is found in highest concentration in the surface of epithelial mucus-producing cells of the stomach. Found in more than half of gastric cancers.	4,8	5,43E-11
SOX8	**SRY (sex determining region Y)-box 8**	**Wnt/beta-catenin signaling**	**2,6**	**0,000217**
REG4	regenerating islet-derived family, member 4	activator of the EGFR in CRC, involved in gastric and pancreatic cancers	3,7	1,68E-07
PIWIL1	piwi-like 1	intrinsic regulator of the self-renewal capacity of germline and hematopoietic stem cells.	2,5	9,43E-05
AXIN2	**Axin 2**	**Wnt/beta-catenin signaling**	**-2,1**	**5,65E-06**
CDX2	caudal type homeobox 2		-2,3	1,16E-10
HSF5	heat shock transcription factor		-3,1	5,79E-10
T				08
C				08
F				07
F				06
SPINK1	serine peptidase inhibitor, Kazal type 1	interacting with CTSB	-2,9	9,73E-06
SATB2	SATB homeobox 2	promotes growth and metastasis in breast cancer	-2,5	1,17E-05
DPEP1			-2,4	2,76E-05
TNNC2			-2,2	9,21E-05
PCLO			-2,5	0,00017

Analysis of differentially expressed genes between BRAF-mutant-like and the rest of KRAS mutants identified genes responsible for colon crypt differentiation, Wnt pathway activation and, more intriguingly, 50 other genes, all located on chromosome 20q, that were significantly down-regulated in BRAF-mutant-like tumors. Among these genes, there are a number of important tumor suppressors, like PLAGL2, TP53RK and POFUT1.

Popovici JCO 2012 Adjusted for MSI status and BRAF/MSI interactions

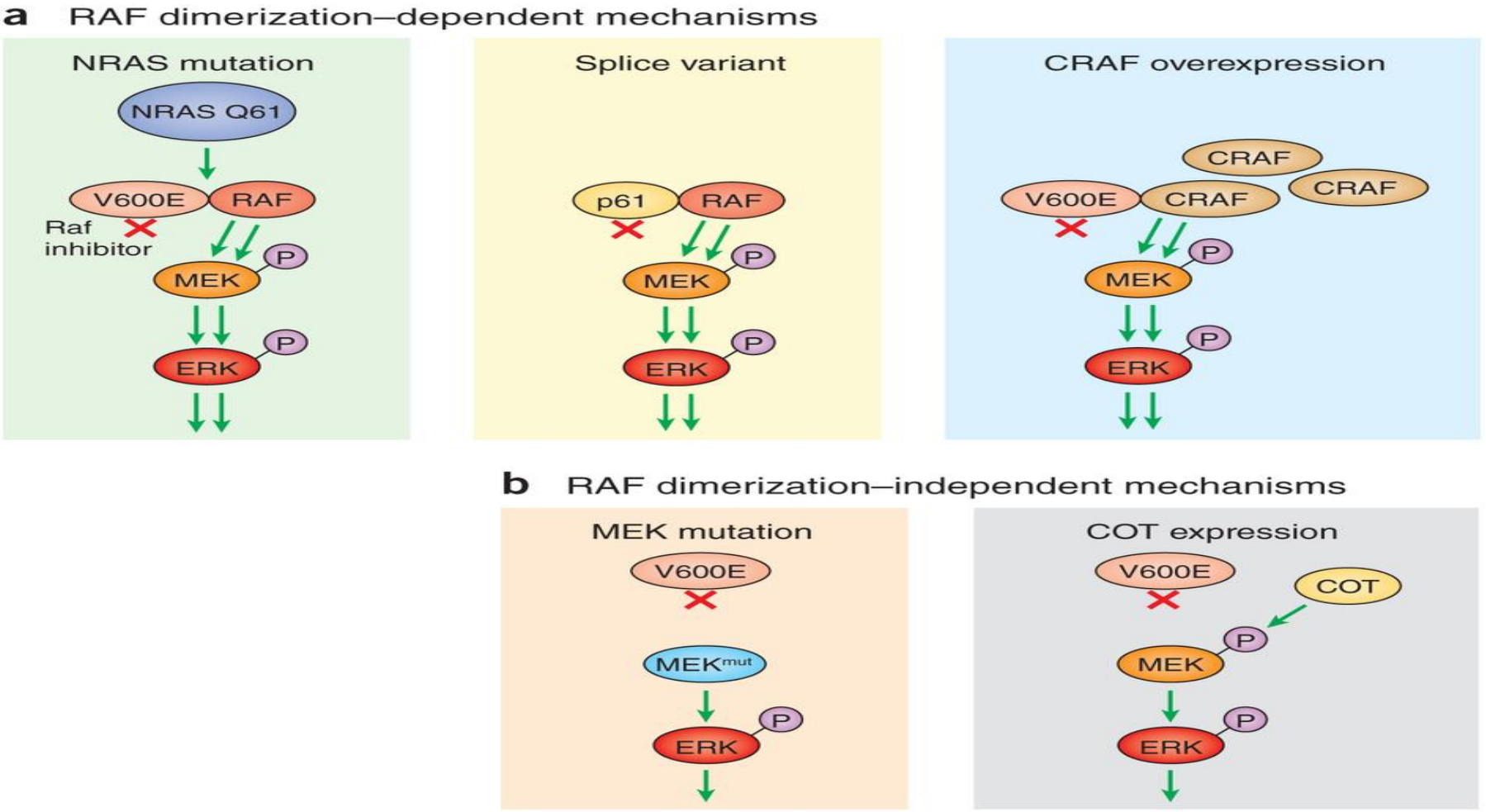

TS Expression by BRAF Mutation Status in Right-Sided Colon
P = 0.003
TS mRNA Expression Level
12.8
6.4
3.2
1.6
0.8
0.4
Mutant
Wildtype
N
24
130


EGFR Expression by BRAF Mutation Status in Right-Sided Colon
P = 0.002
EGFR mRNA Expression Level
8.0
4.0
2.0
1.0
0.5
0.3
Mutant
Wildtype
N
23
127



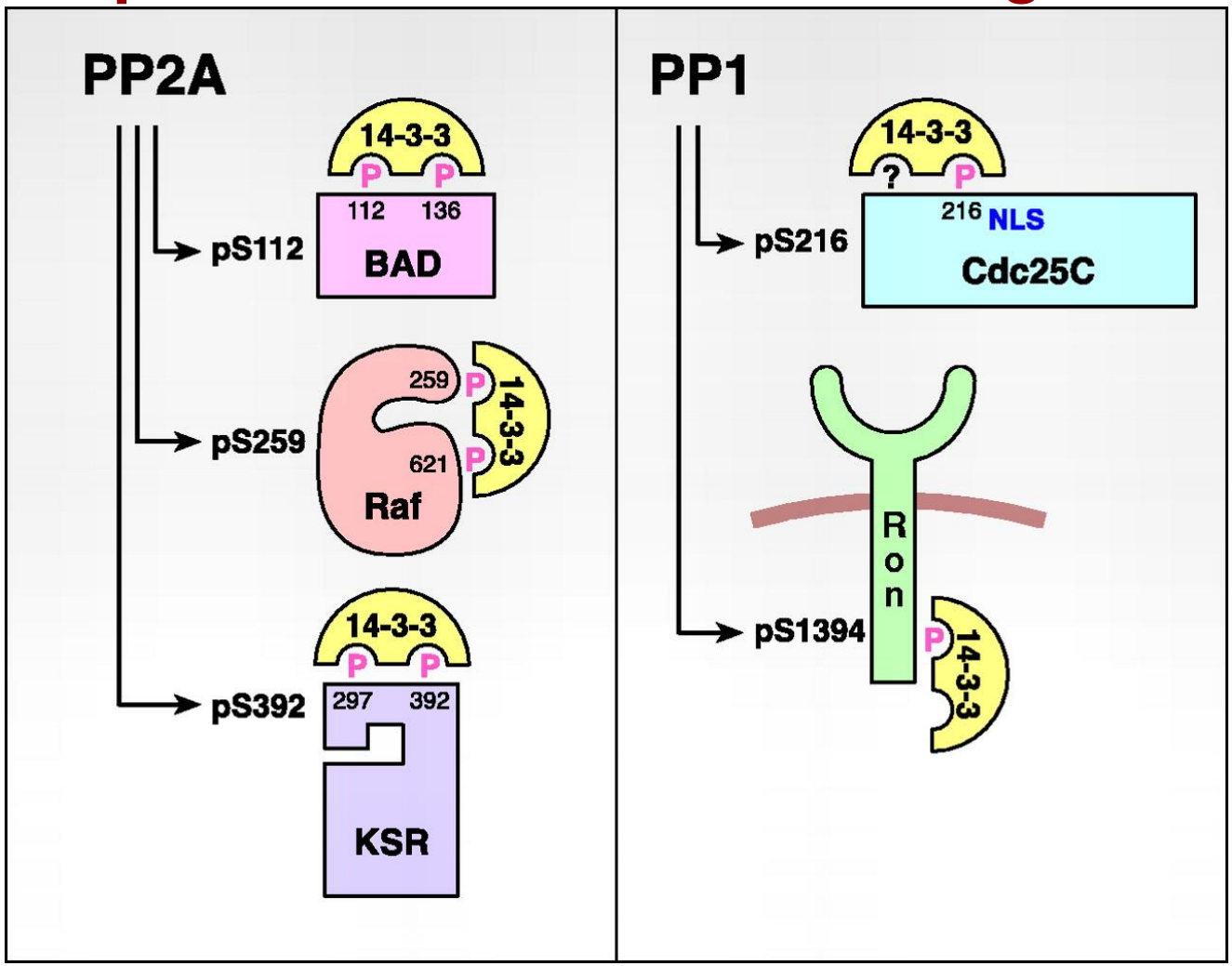
Nature Reviews Molecular Cell Biology 5, 875-885 (November 2004)

Mechanisms of Resistance to braf Inhibitors



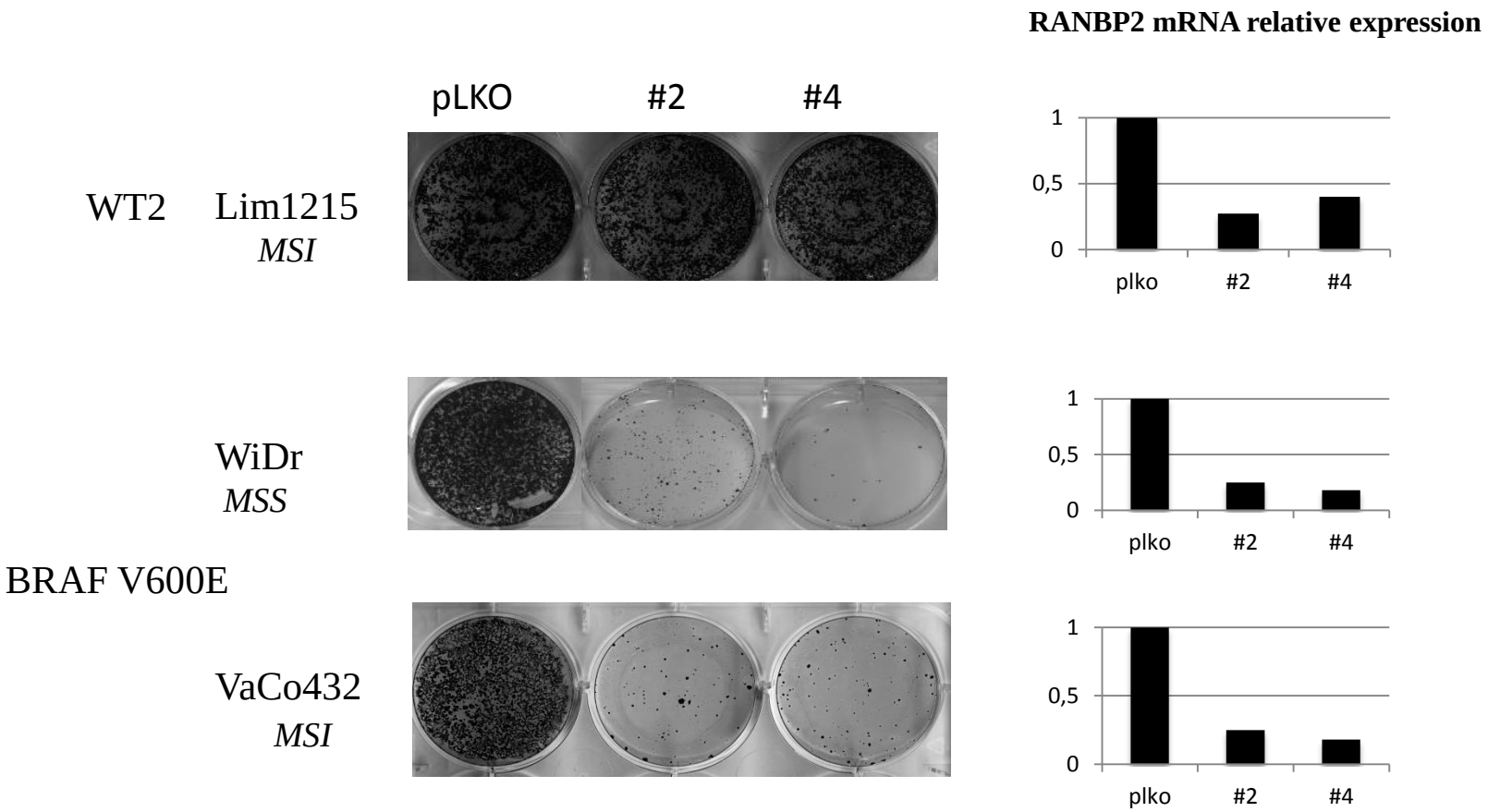
a) Alterations that promote enhanced RAF dimerization such as NRAS mutation (NRAS Q61), expression of RAF splice variants (p61), CRAF overexpression, NF1 loss and RTK activation (latter two mechanisms not shown) cause resistance to RAF inhibitors. (b) Reactivation of ERK signaling and resistance to RAF inhibitors may also occur in a dimerization-independent fashion as a result of downstream mutations in MEK or RAF bypass resulting from activation of COT (an ERK kinase kinase).

Regulation of 14-3-3 target binding by protein phosphatases. Protein Trafficing of Braf



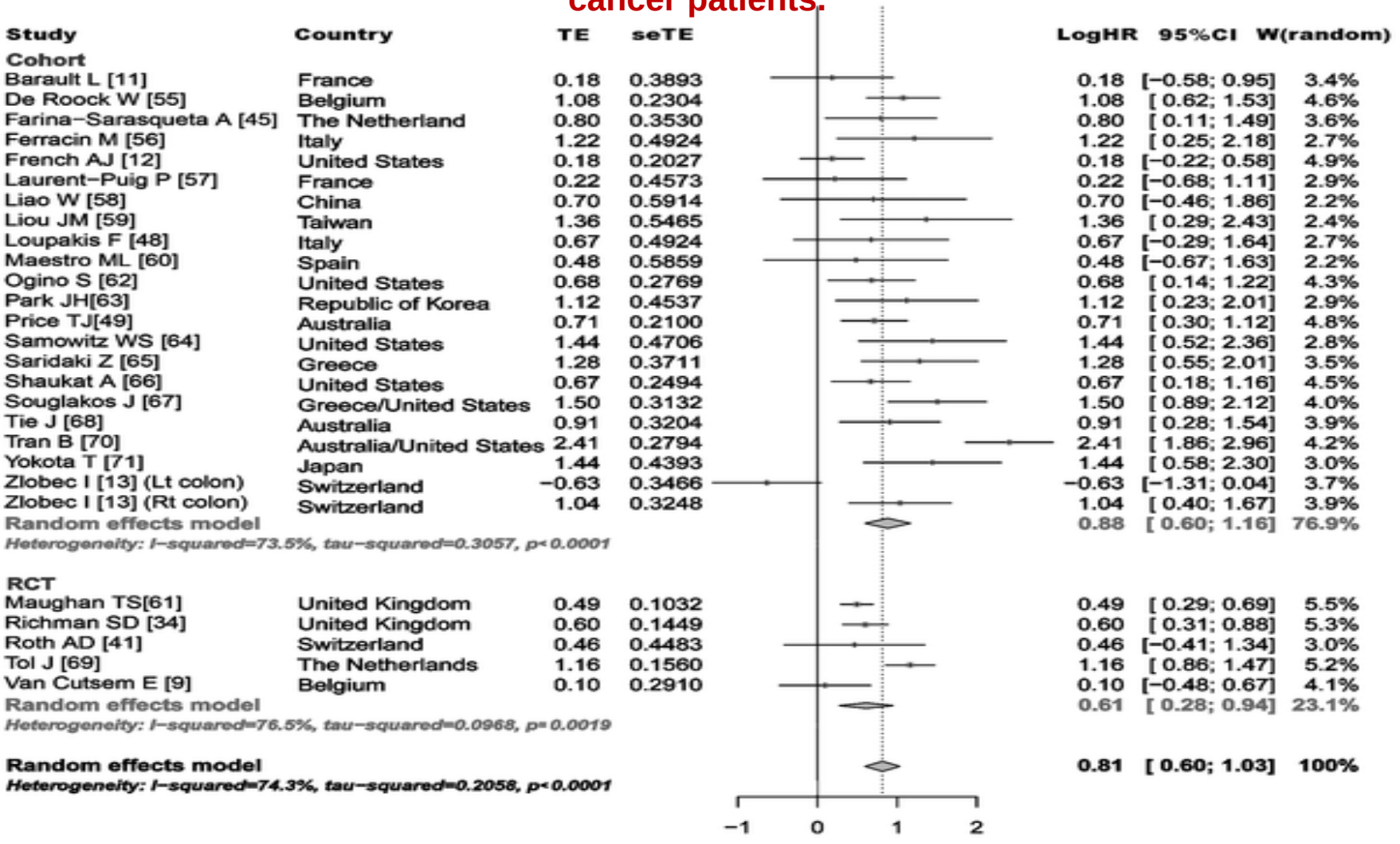
Dougherty M K , and Morrison D K J Cell Sci 2004;117:1875-1884

RANBP2 is selectively synthetic lethal with BRAF V600E in CC



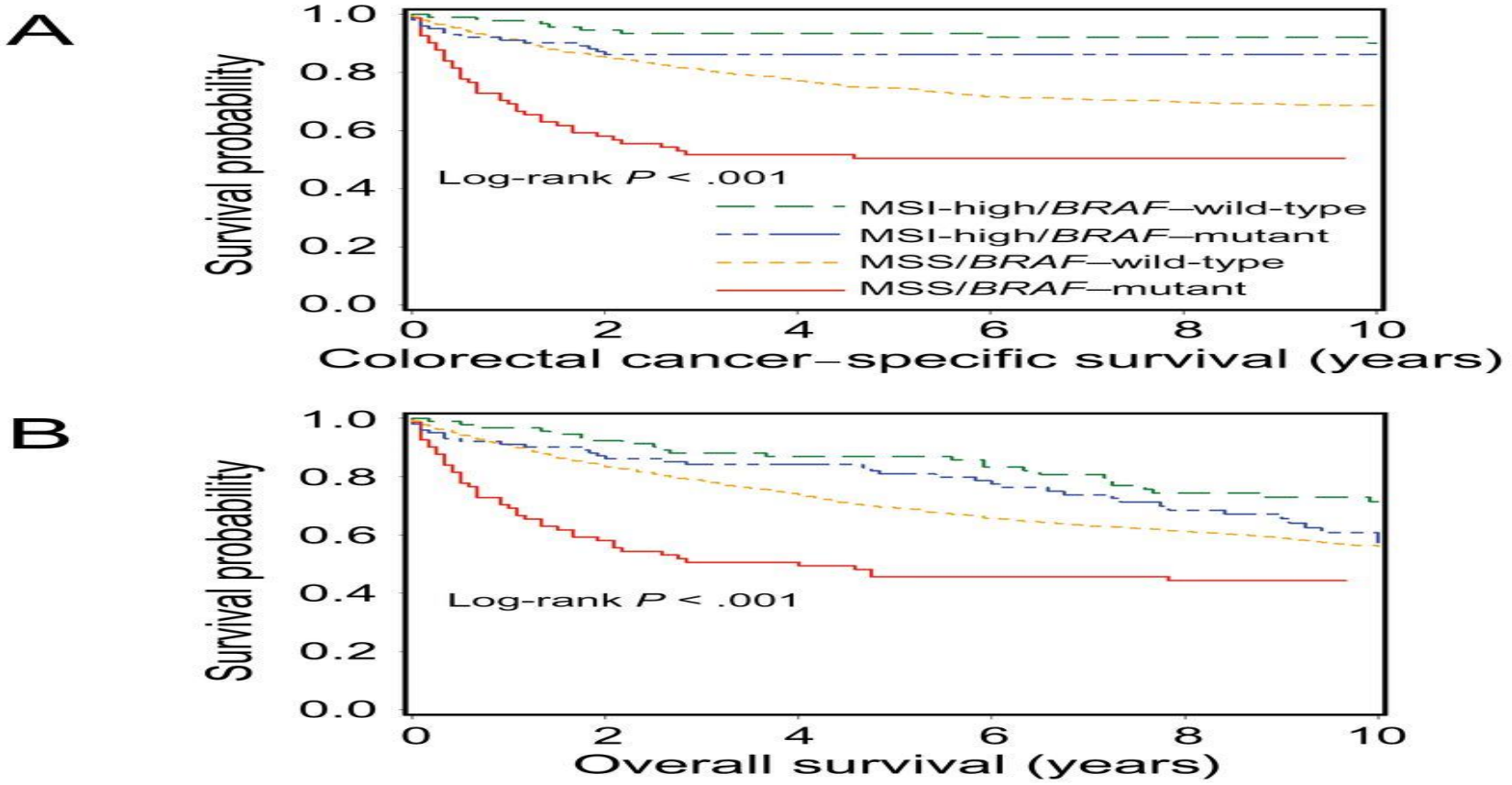
Vecchione WGI Barcelona 2014

Random effect model of Log hazard ratio (LogHR) with 95% confidence interval for studies comparing the effect of BRAF-V600E mutation on overall survival of colorectal cancer patients.



Safaee Ardekani G, Jafarnejad SM, Tan L, Saeedi A, et al. (2012) The Prognostic Value of BRAF Mutation in Colorectal Cancer and Melanoma: A Systematic Review and Meta-Analysis. PLoS ONE 7(10): e47054. doi:10.1371/journal.pone.0047054
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0047054

Kaplan–Meier survival plots for colorectal cancer according to combined MSI/BRAF subgroup.



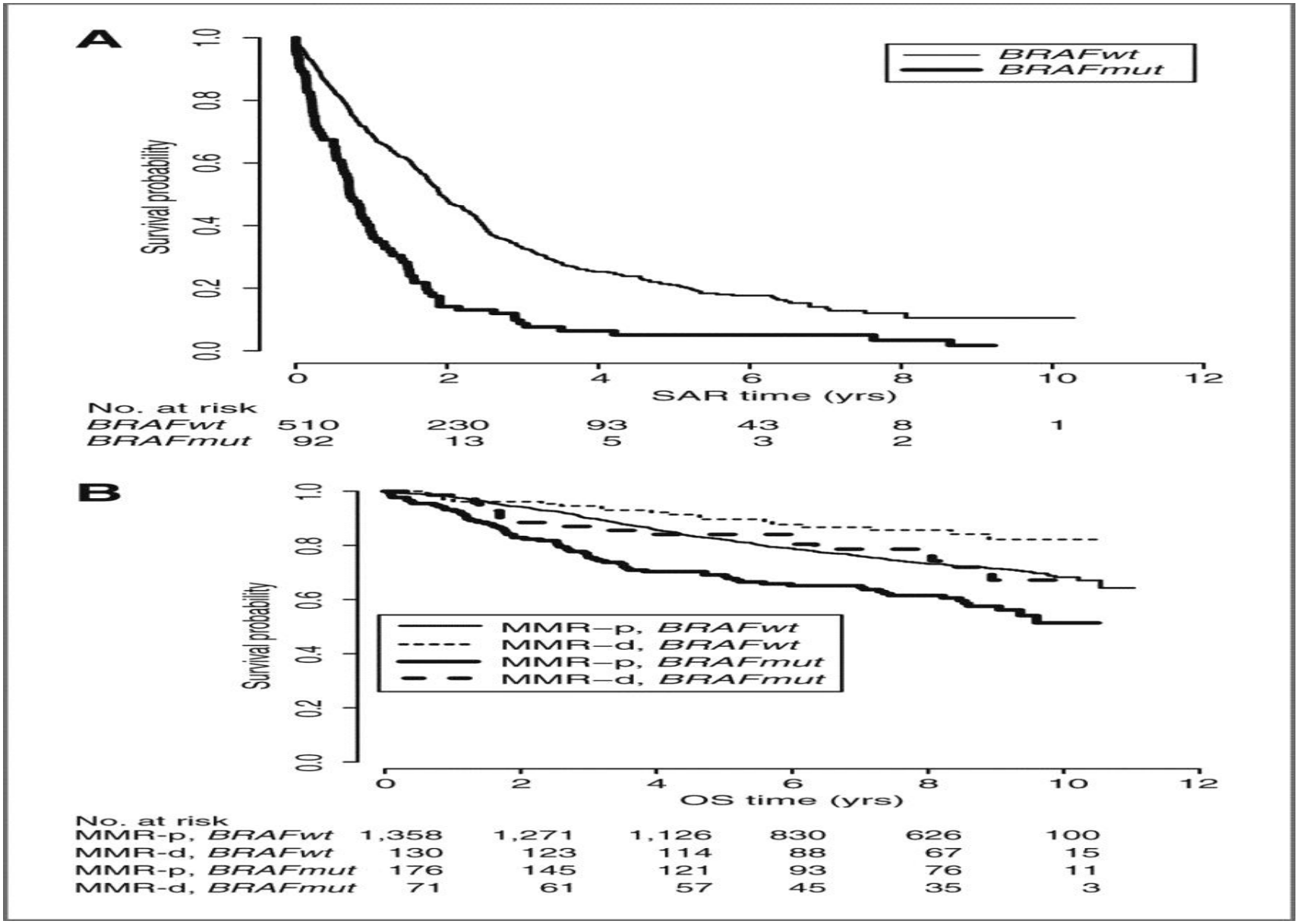
Number at risk

Year	0	2	4	6	8	10
MSI-high/*BRAF*–wild-type	92	85	76	68	56	43
MSI-high/*BRAF*–mutant	101	87	82	65	49	31
MSS/*BRAF*–wild-type	979	815	704	578	494	385
MSS/*BRAF*–mutant	81	47	40	37	32	29

Lochhead P et al. JNCI J Natl Cancer Inst 2013;105:1151-1156

JNCI

Kaplan–Meier plots of BRAF and MMR status

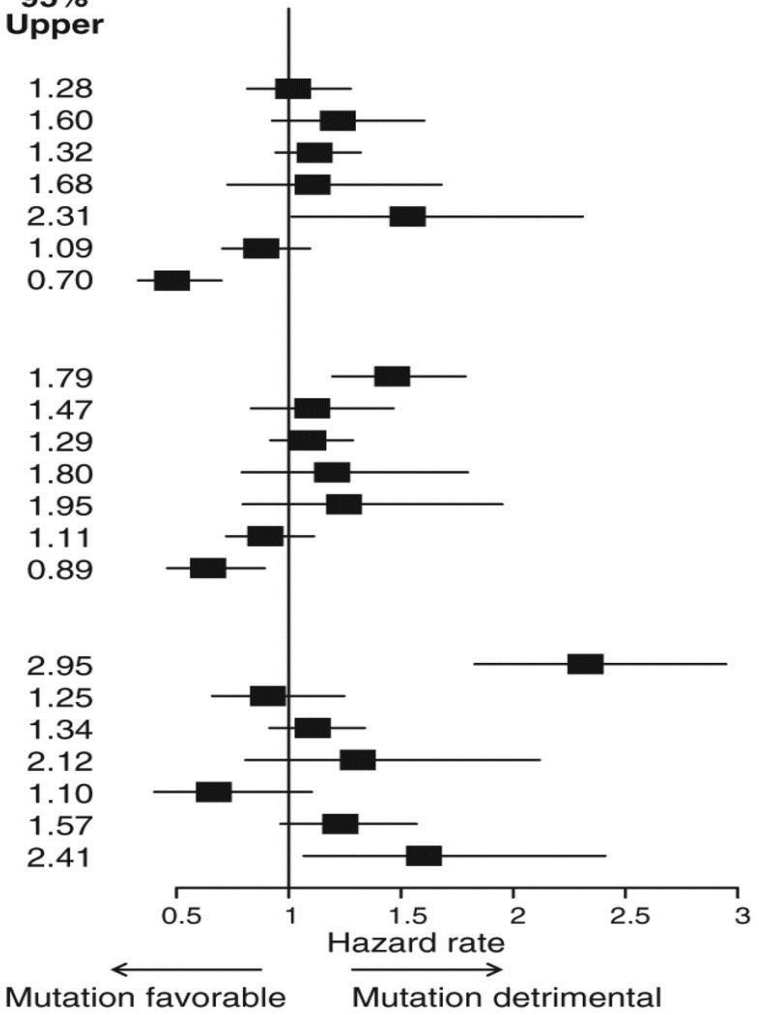


Gavin P G et al. Clin Cancer Res 2012;18:6531-6541

Clinical Cancer Research

Forest plot of mutations and MMR status and their association with recurrence, OS, and SAR. HR > 1 indicates detriment.

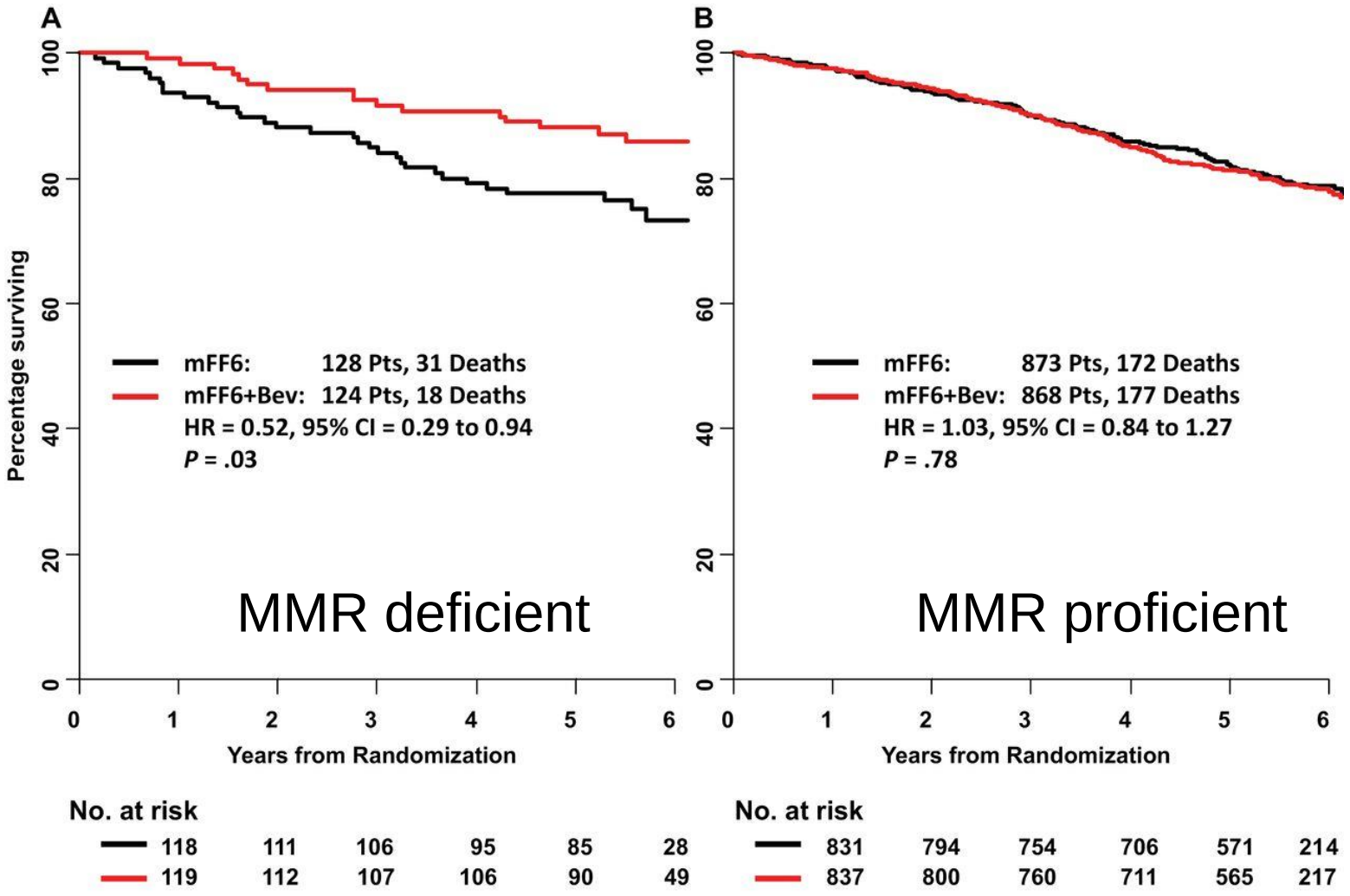
	P	HR	95% Lower	95% Upper
Time to recurrence				
BRAF	0.86	1.02	0.82	1.28
KRAS_G12V	0.16	1.22	0.93	1.60
KRAS	0.21	1.12	0.94	1.32
MET	0.64	1.11	0.73	1.68
NRAS	0.04	1.53	1.01	2.31
PIK3CA	0.25	0.88	0.71	1.09
MMR status	0.0001	0.48	0.33	0.70
OS				
BRAF	0.0002	1.46	1.20	1.79
KRAS_G12V	0.49	1.11	0.83	1.47
KRAS	0.33	1.09	0.92	1.29
MET	0.40	1.19	0.79	1.80
NRAS	0.34	1.25	0.80	1.95
PIK3CA	0.32	0.90	0.72	1.11
MMR status	0.0084	0.64	0.46	0.89
SAR				
BRAF	<0.0001	2.31	1.83	2.95
KRAS_G12V	0.55	0.91	0.66	1.25
KRAS	0.30	1.11	0.92	1.34
MET	0.27	1.31	0.81	2.12
NRAS	0.11	0.67	0.40	1.10
PIK3CA	0.10	1.23	0.96	1.57
MMR status	0.02	1.60	1.07	2.41



Gavin P G et al. Clin Cancer Res 2012;18:6531-6541

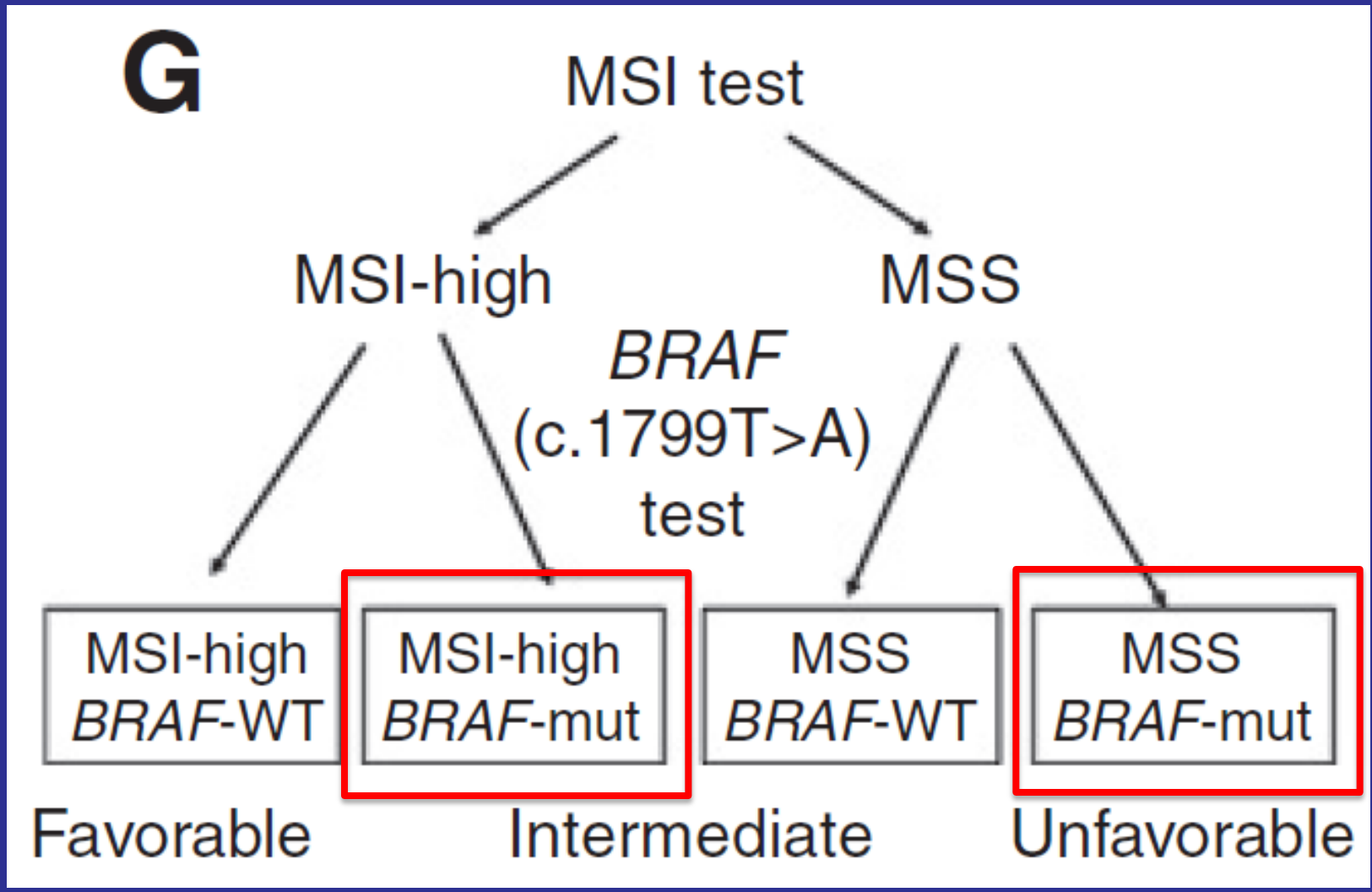
Clinical Cancer Research

The effect of bevacizumab (Bev) treatment on overall survival by mismatch repair (MMR) status for colon cancer: NSABP C-08.



Pogue-Geile K et al. JNCI J Natl Cancer Inst 2013;105:989-992

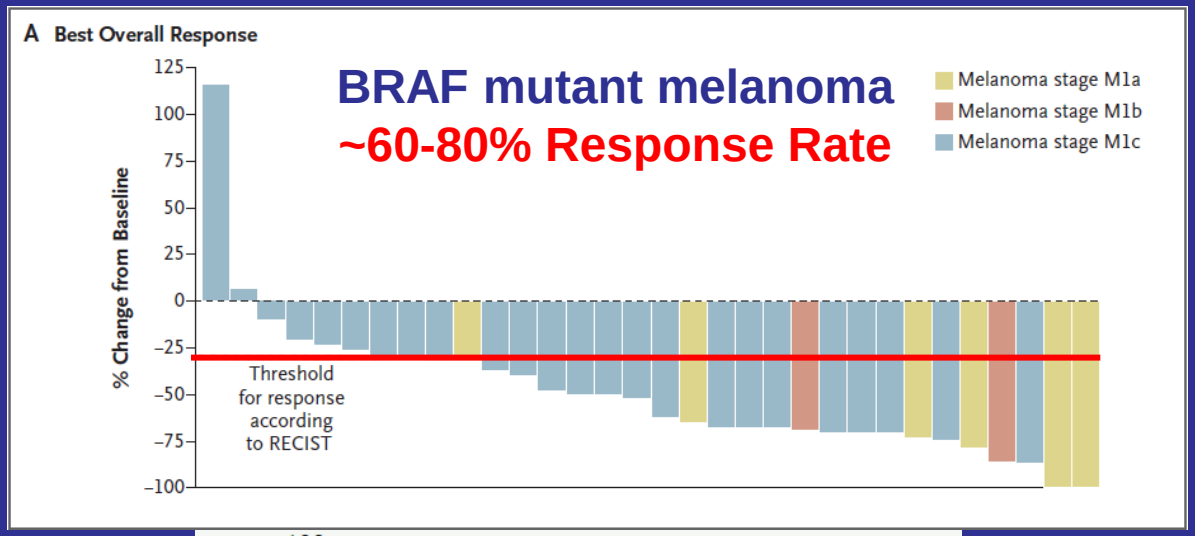
JNCI

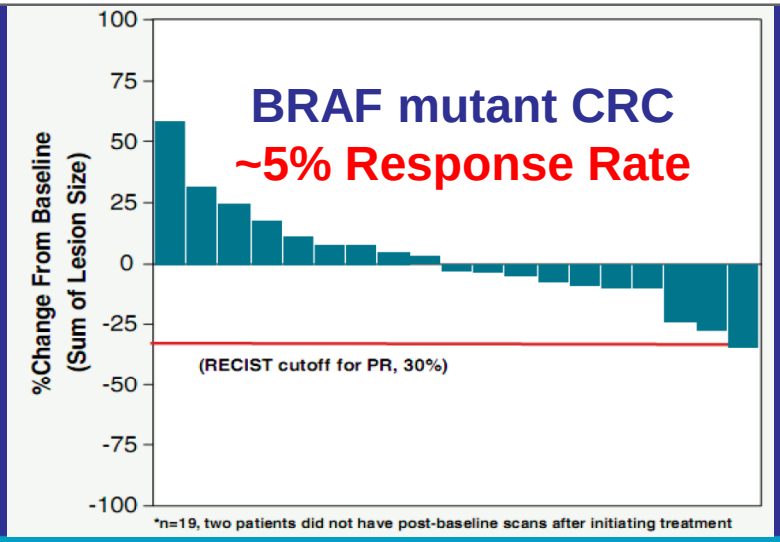


- Due to confounding effect of MSI status in BRAF MT patients, Ogino proposed this strategy for classification. Must split, rather than lump, BRAF MT patients

Ogino, Clin Can Res 2012

BRAF inhibition alone ineffective in BRAF-mutant CRC

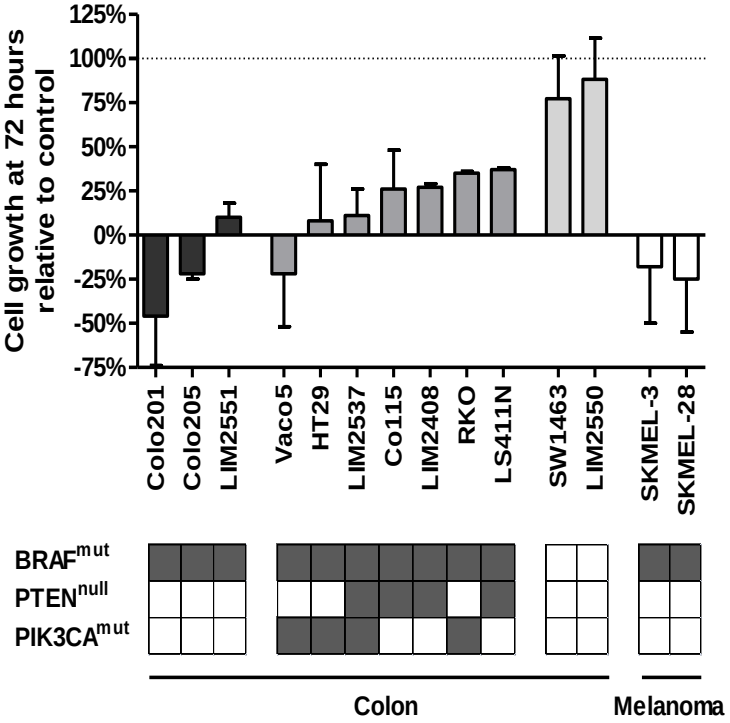


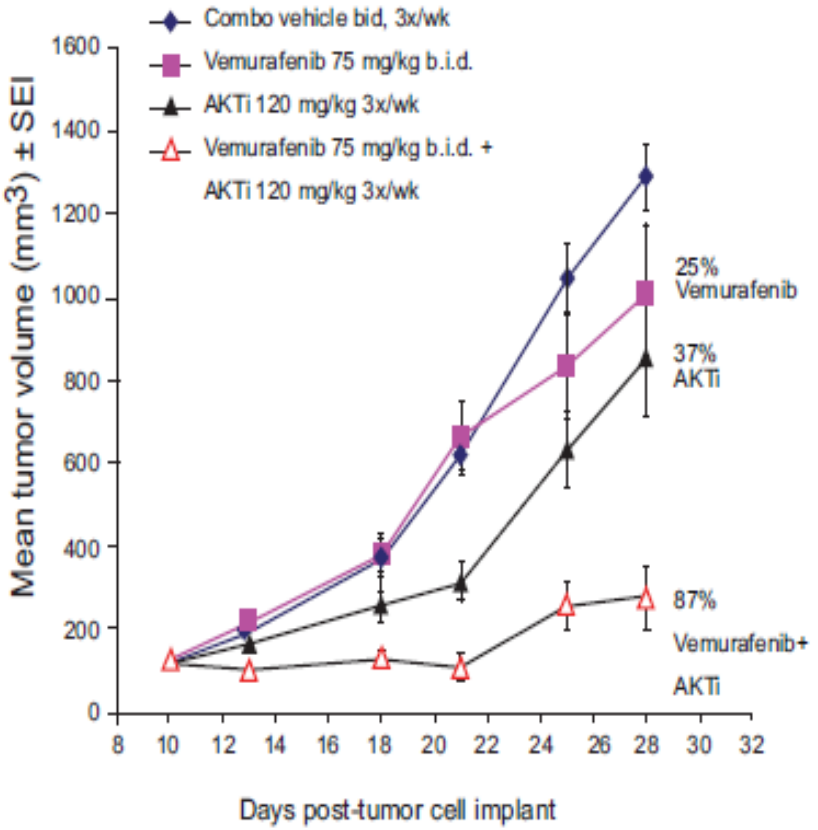


Flaherty *et al*, NEJM, 2010[1]
Kopetz *et al*, ASCO, 2010[2]

1. Flaherty *et al*., *N Engl J Med* 2010:**363**;809-19.
2. Kopetz *et al.*, presented at ASCO 2010 (abstract 3534).

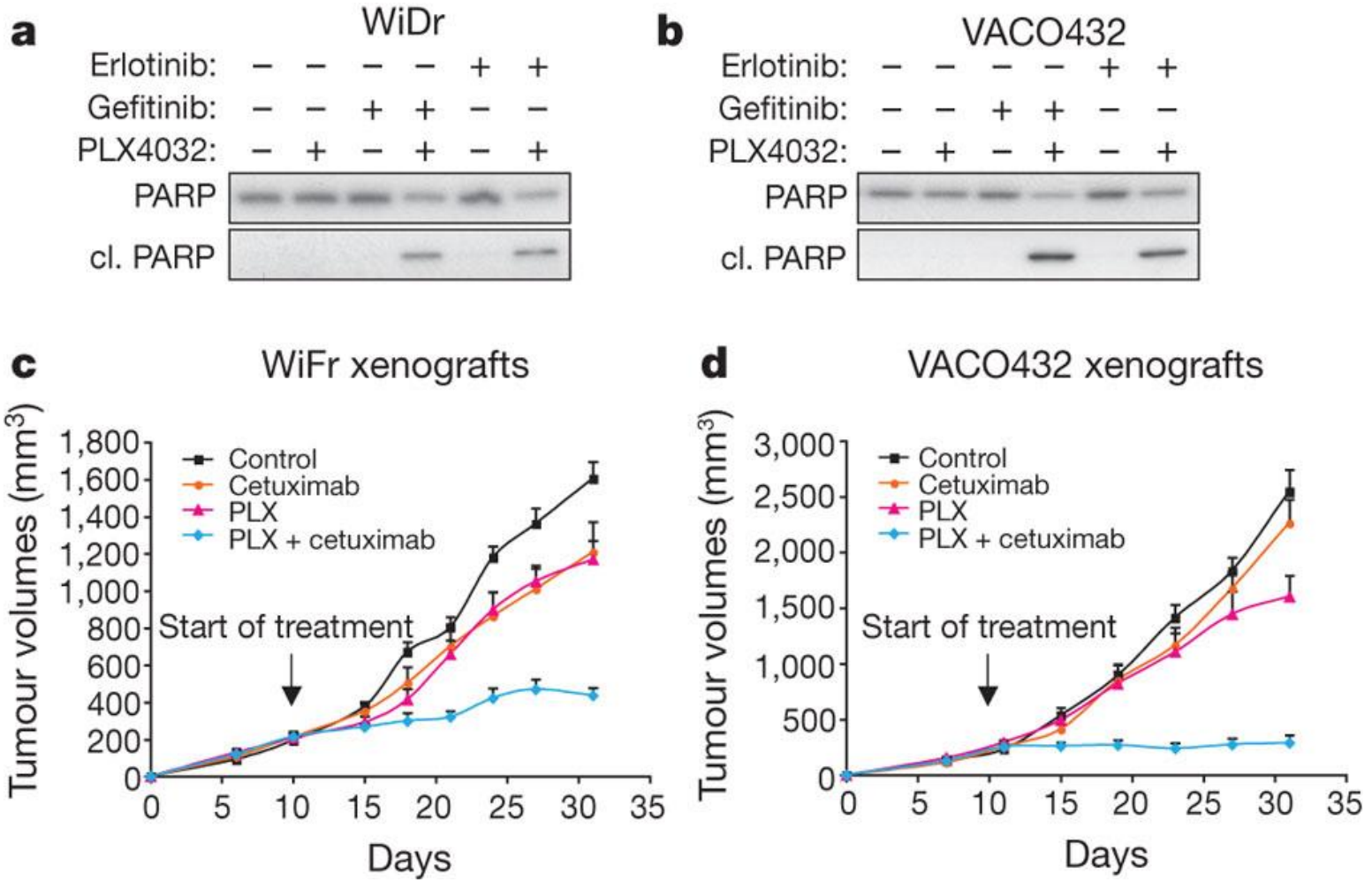
A murine study in a resistant BRAFmut CRC cell line combining venurafenib and an AKT inhibitor showed promising activity





Su et al CR 2012

EGFR and BRAF(V600E) inhibitors synergize to induce apoptosis of CRC cells and to suppress CRC tumour growth in a xenograft model.



nature

A Prahallad *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature10868

New studies in the $BRAF^{V600E}$ mutant CRC population

- As examples of clinical trials evaluating the combination of BRAFV600E inhibitors plus anti-EGFR inhibitors in the BRAF mutant population in CRC:
  - NCT01524978: Vemurafenib + Cetuximab (BASKET) – Phase Ib
  - NCT01750918: BRAF/MEK Inhibitors (dabrafenib + trametinib) + Panitumumab – Phase Ib ➔RP2
  - NCT01719380: LGX818 and Cetuximab or LGX818, BYL719, and Cetuximab – Phase Ib➔RP2

Pharmacodynamic and efficacy analysis of the BRAF inhibitor dabrafenib (GSK436) in combination with the MEK inhibitor trametinib (GSK212) in patients with BRAFV600 mutant colorectal cancer (CRC)

R. B. Corcoran[1], G. S. Falchook[2], J. R. Infante[3], O. Hamid[4], W. A. Messersmith[5], A. Daud[6], E. L. Kwak[1], D. P. Ryan[1], R. Kurzrock[2], C.E. Atreya[6], J. Luan[6], P. Sun[7], M. Schaeffer[7], M. Motwani[7], M. Bleam[7], C. Moy[7], K. Patel[7], K. Orford[7], S. Kopetz[8], A. P. Venook[6]

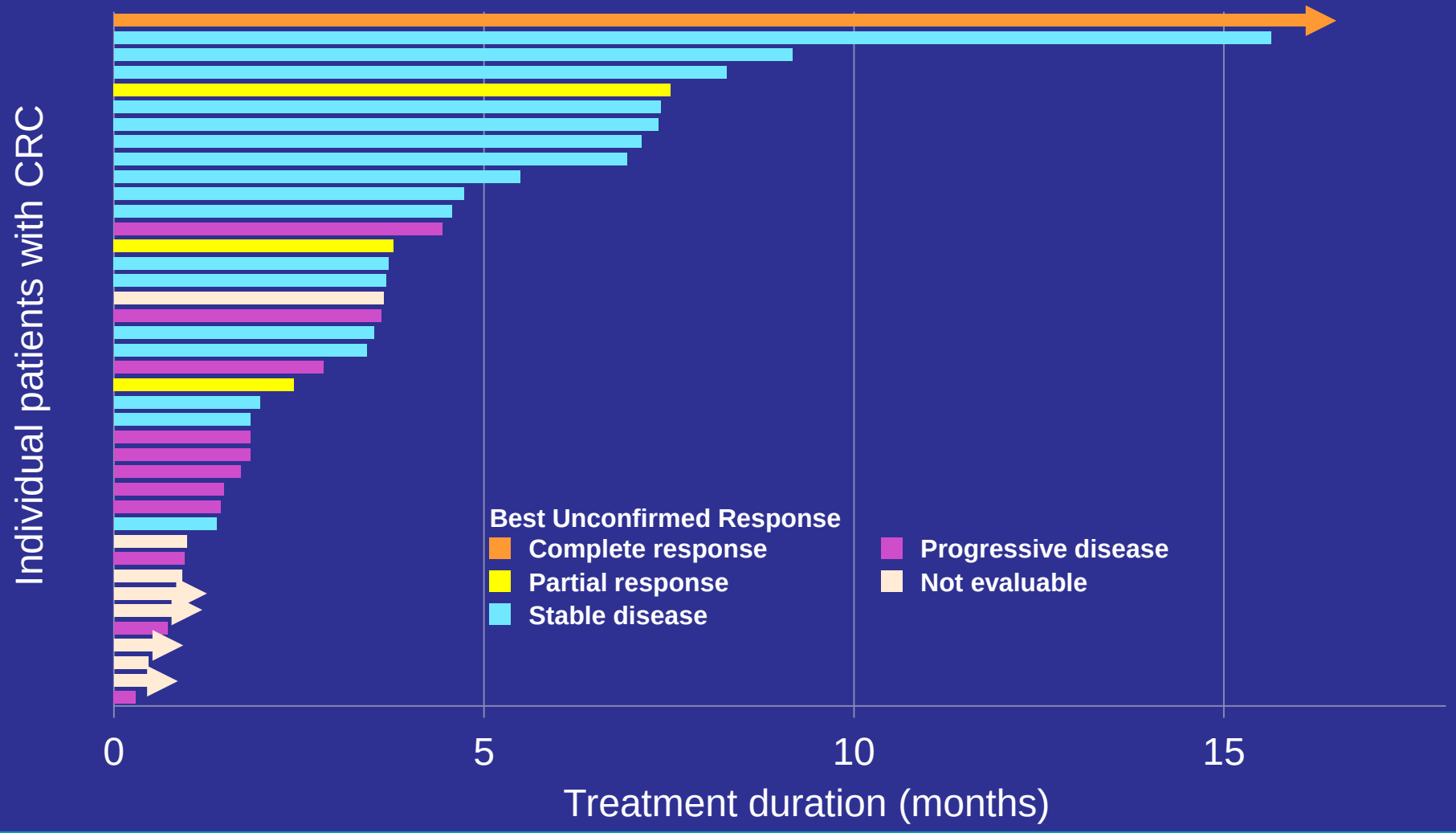
[1]Massachusetts General Hospital Cancer Center, Boston, MA, [2]Department of Investigational Cancer Therapeutics, The University of Texas, MD Anderson Cancer Center, Houston, TX, [3]Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN, [4]Department of Medical Oncology, The Angeles Clinic and Research Institute, Los Angeles, CA, [5]Department of Medical Oncology, University of Colorado, Aurora, CO, [6]University of California, San Francisco, San Francisco, CA, [7]GlaxoSmithKline, Collegeville, PA, [8]Department of Gastrointestinal Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, TX

Presented at the 2013 ASCO Annual Meeting.

ASCO Annual '13 Meeting

Results: Efficacy

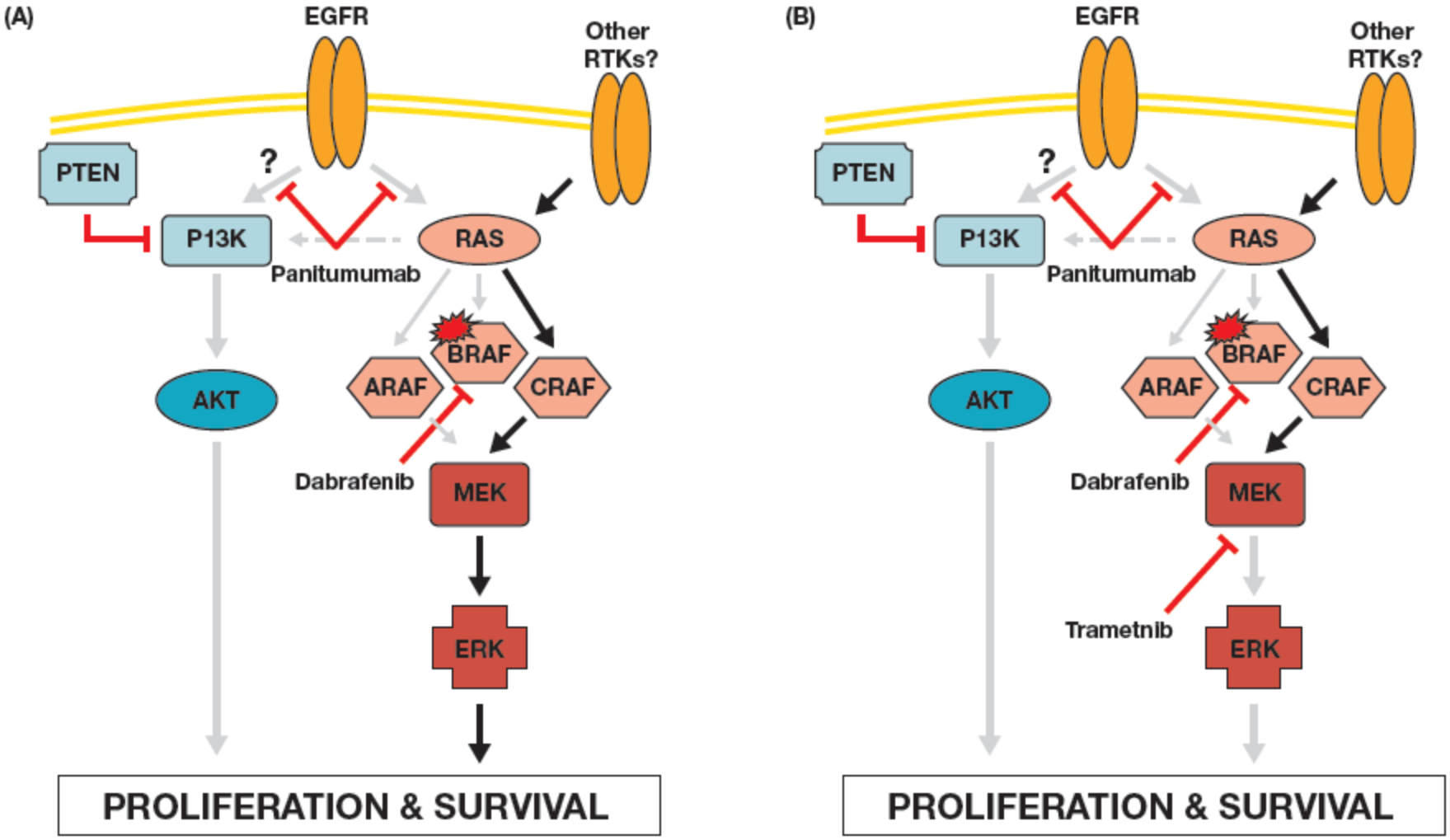
Duration on Study Treatment (40 patients)



Updated model

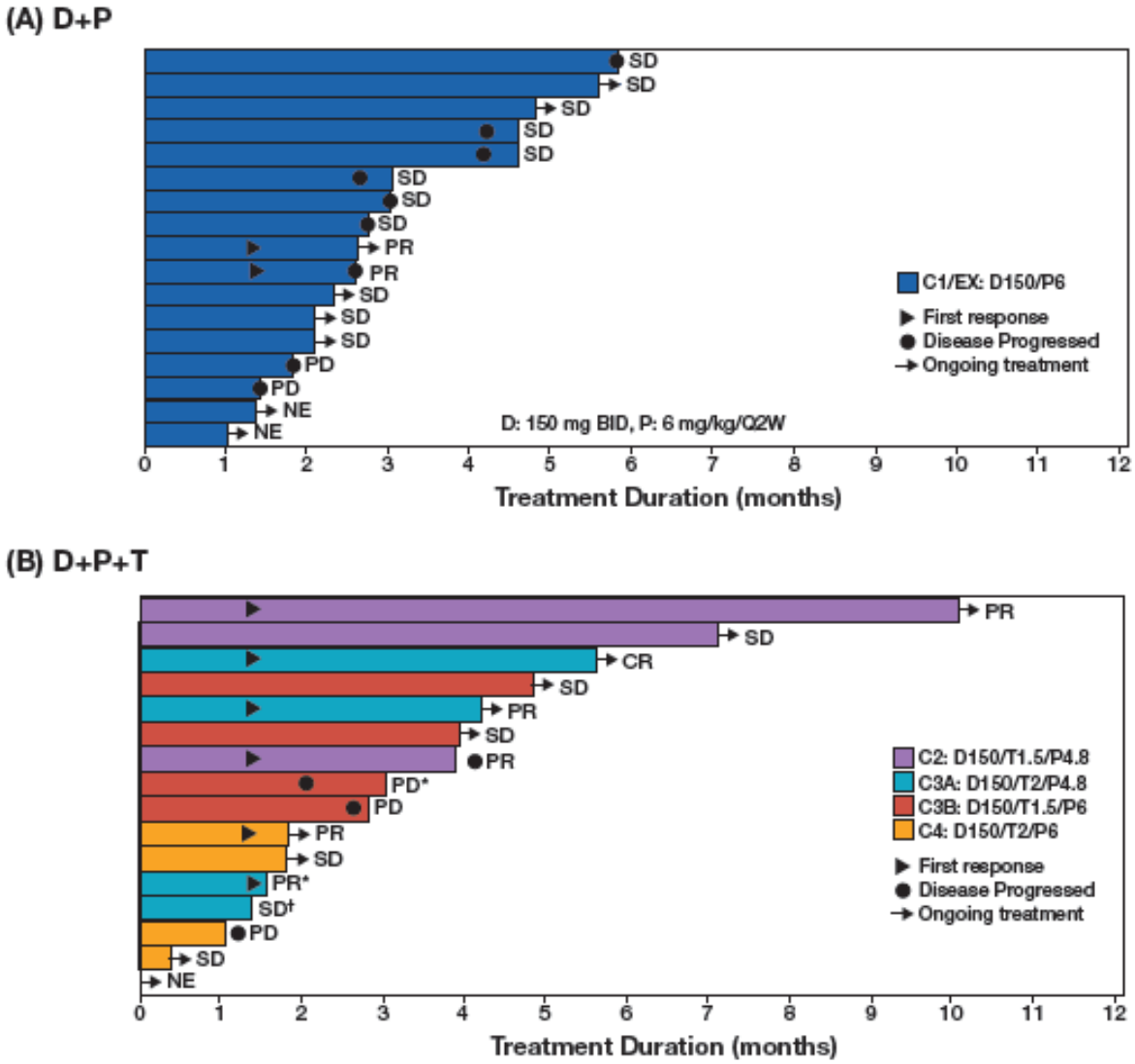
Partial inhibition of MAPK pathway signaling with inhibition of BRAF and EGFR

Robust inhibition of MAPK pathway signaling with inhibition of BRAF, MEK, EGFR



Johanna Bendell triple inhibitor EGFR/MEK and BRAF ASCO 2014

Preliminary Efficacy: Time on Study

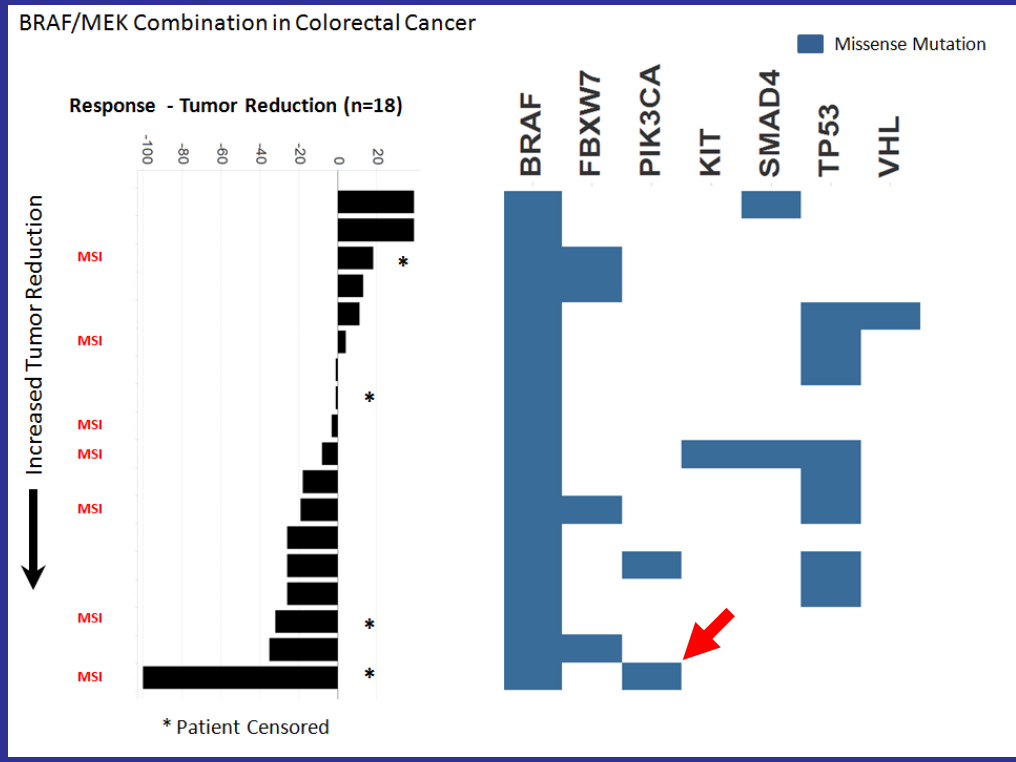


Plot represents duration on treatment by best unconfirmed response. The best response without confirmation is displayed at the end of the bar for each subject. * indicates that patient came off therapy for intercurrent illness. † indicates that the patient withdrew consent.

Potential Biomarkers

Mutational analysis by NGS

- NGS performed on archival tissues (n=17)
  - ION Torrent Ampliseq, 46 genes
  - PIK3CA mutations confirmed by Sanger Sequencing
- Results
  - Mutations were identified in a number of cancer-related genes
    - BRAF, APC, PIK3CA, TP53
  - No clear relationship between NGS results and clinical outcome
  - PIK3CA hotspot mutation present in tumor with CR



SWOG 1406 Braf driven Trial based on preclincal data (Kopetz/Lenz)

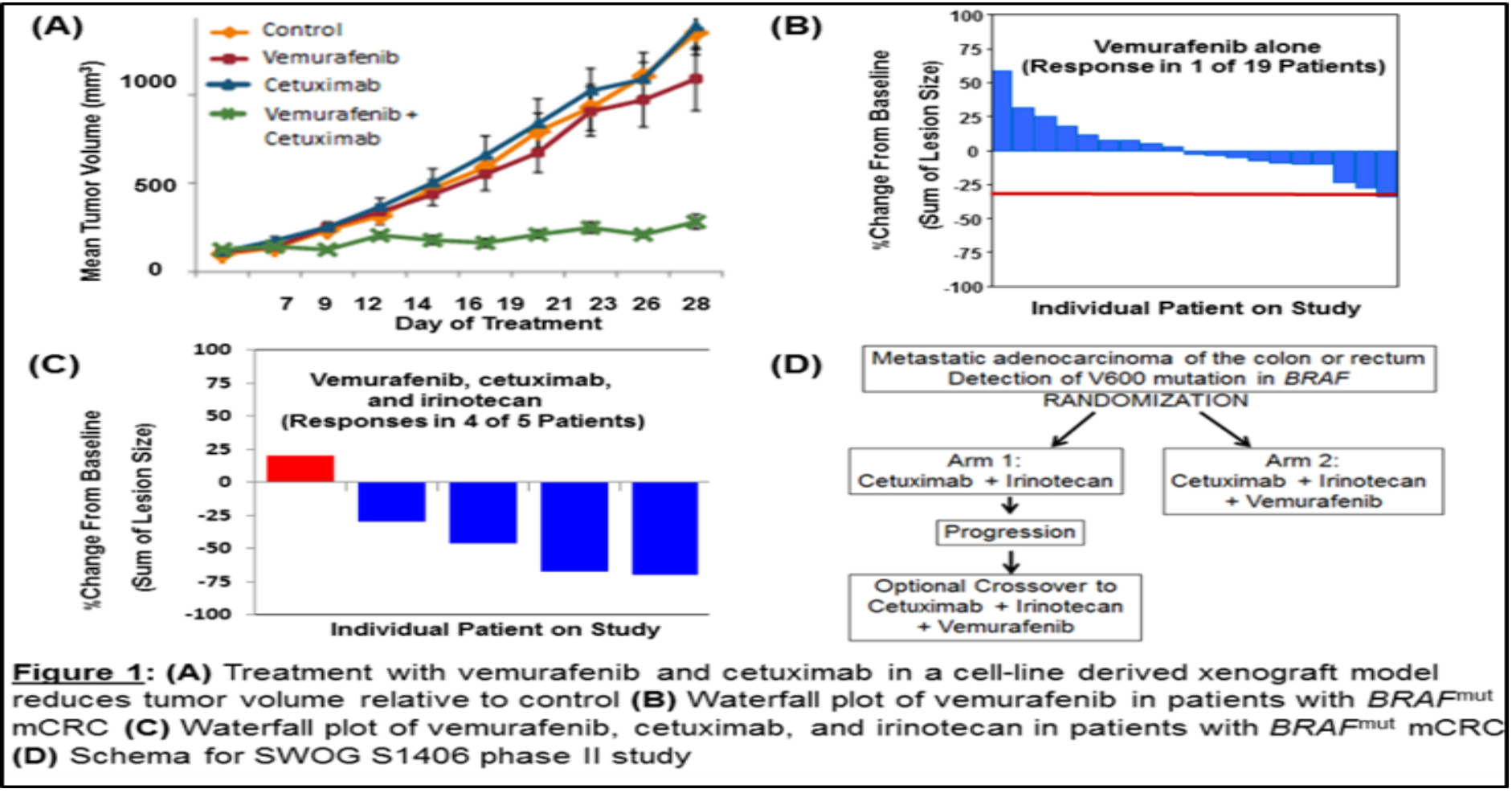


Figure 1: **(A)** Treatment with vemurafenib and cetuximab in a cell-line derived xenograft model reduces tumor volume relative to control **(B)** Waterfall plot of vemurafenib in patients with *BRAF*^{mut} mCRC **(C)** Waterfall plot of vemurafenib, cetuximab, and irinotecan in patients with *BRAF*^{mut} mCRC **(D)** Schema for SWOG S1406 phase II study

SWOG 1406 Tumor Model Program with JAX

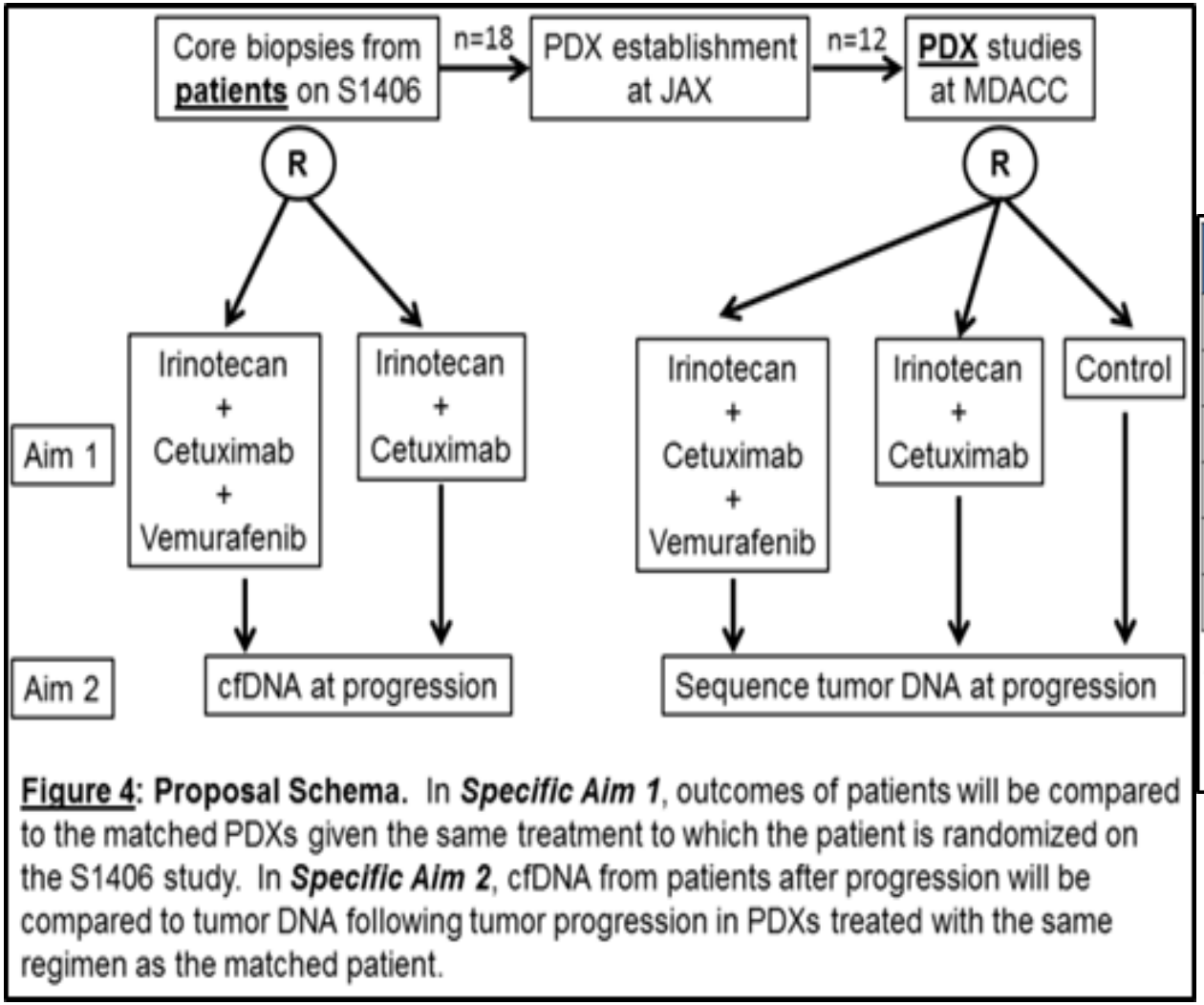


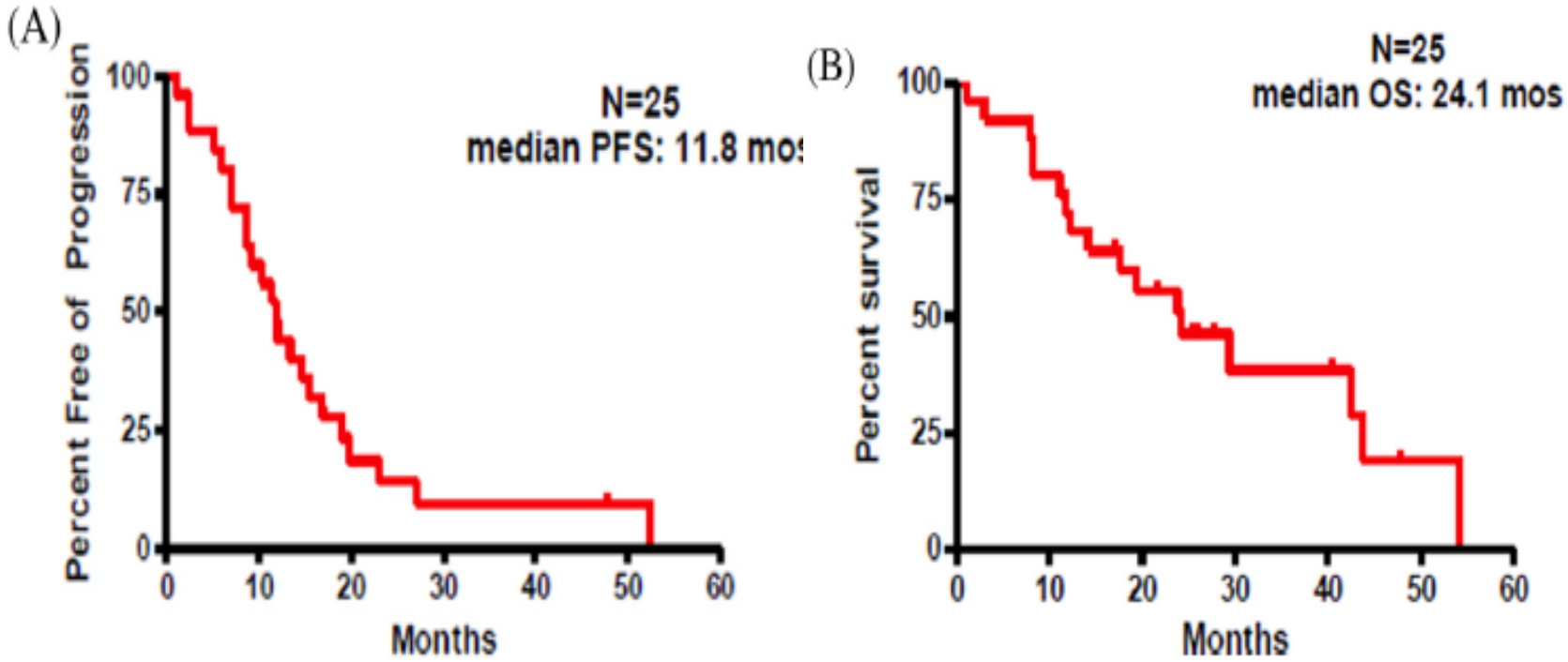
Figure 4: Proposal Schema. In ***Specific Aim 1***, outcomes of patients will be compared to the matched PDXs given the same treatment to which the patient is randomized on the S1406 study. In ***Specific Aim 2***, cfDNA from patients after progression will be compared to tumor DNA following tumor progression in PDXs treated with the same regimen as the matched patient.

PDX	Acquired Mutation
1	*MEK1* L42F
2	*EGFR* Y869C
3	*KRAS* G12D
4	*MEK1* G607A
5	*KRAS* G12R
6	*NRAS* G13D

Figure 3: Acquired mutations in a *BRAF*^{mut} mCRC PDX after resistance to vemurafenib not seen in untreated/control mice from the same parent tumor.

Treatment option for mCRC if no clinical trials available

FOLFOXIRI plus Bevacizumab



Loupakis F et al. Eur J Cancer. 2014 Jan;50(1):57-63.

Recommendations and Future Directions

- We recommend that routine screening for BRAF mutations and MSI should be considered early in ALL patients with metastatic CRC

- BRAFV600 mutant CRC patients have poor prognosis and typically do not respond well to standard therapy
- Early identification of BRAF mutations will enable CRC patients to consider several interesting and potentially effective therapies currently in clinical trials
- Consider clinical trials as an earlier line of therapy
- Ensure that patients do not miss window to consider clinical trial

- We hope that further studies will identify more effective treatments and will identify biomarkers to define the population of patients most likely to benefit
- FOLFOXIRI/BEV demonstrated promising PFS and OS data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