Carcinoma del colon-retto - RAS e BRAF

Dr. Francesca Negri

Oncologia Medica
Azienda Ospedaliero-Universitaria di Parma
Verona, 18 settembre 2015

Molecular Mechanisms of Resistance to Therapies Targeting the Epidermal Growth Factor Receptor

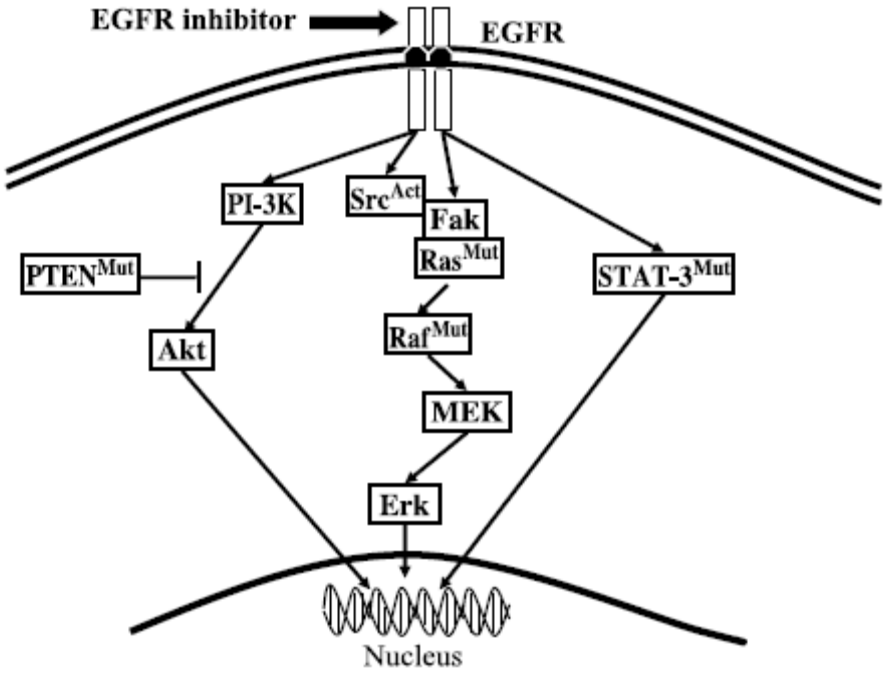


Fig. 2 Resistance based on constitutive activation of signaling pathways downstream of EGFR. Pathways potentially blocked by EGFR inhibitors can remain activated by mutations of downstream mediators. *Mut,* common mutations leading to up-regulation of the survival pathways; *Act,* increases in constitutive activation that are not usually due to mutations.

Camp ER, et al. Clin Cancer Res 2005

Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer

Table 2. Rates of Radiologic Response.*

Subgroup and Variable	Cetuximab plus Irinotecan	Cetuximab	P Value
Intention-to-treat population			
No. of patients	218	111	
Response — no. (%)			
Complete response	0	0	
Partial response	50 (22.9)	12 (10.8)	
Stable disease	71 (32.6)	24 (21.6)	
Progressive disease	68 (31.2)	59 (53.2)	
Could not be evaluated	29 (13.3)	16 (14.4)	
Overall response†	50 (22.9 [17.5–29.1])	12 (10.8 [5.7–18.1])	0.007
Disease control‡	121 (55.5 [48.6–62.2])	36 (32.4 [23.9–42.0])	<0.001
Subgroup with progression during or within 4 wk after prestudy irinotecan			
No. of patients	135	71	
Response — no. (%)	34 (25.2 [18.1–33.4])	10 (14.1 [7.0–24.4])	0.07
Subgroup with prior oxaliplatin therapy			
No. of patients	135	71	
Response — no. (%)	30 (22.2 [15.5–30.2])	6 (8.5 [3.2–17.5])	0.01

* Values in brackets are 95 percent confidence intervals.
† The overall response rate is the sum of the rate of complete response and the rate of partial response.
‡ The rate of disease control is the sum of the rates of complete response, partial response, and stable disease.

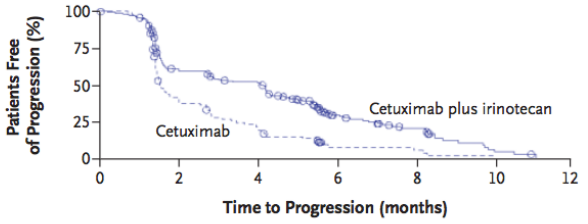


Figure 2. Time to Disease Progression in the Two Study Groups.
The hazard ratio for disease progression in the combination-therapy group as compared with the monotherapy group was 0.54 (95 percent confidence interval, 0.42 to 0.71) (P<0.001 by the log-rank test). The points on the curves represent the dates on which a patient's data were censored.

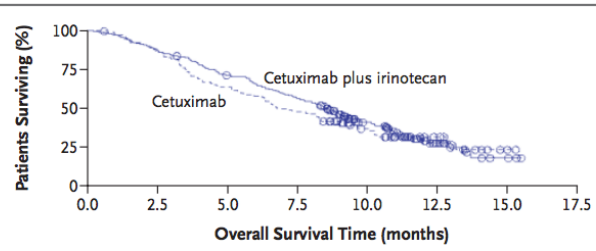


Figure 3. Overall Survival in the Two Study Groups.
The hazard ratio for death in the combination-therapy group as compared with the monotherapy group was 0.91 (95 percent confidence interval, 0.68 to 1.21) (P=0.48 by the log-rank test). The points on the curves represent the dates on which a patient's data were censored.

Cunningham D, et al. N Engl J Med 2004

Constitutive RAS Pathway Activation and Its Impact on EGFR mAb Therapy

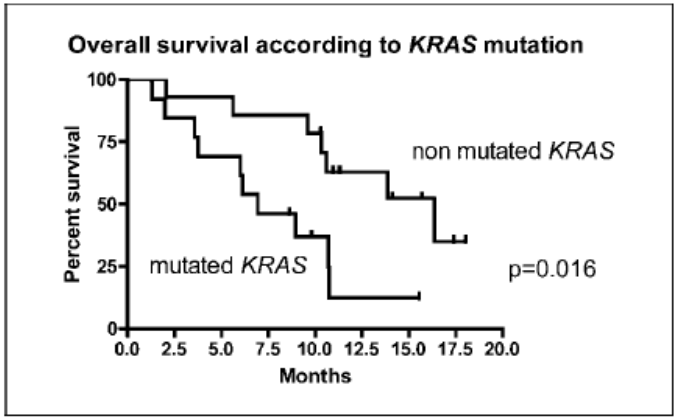
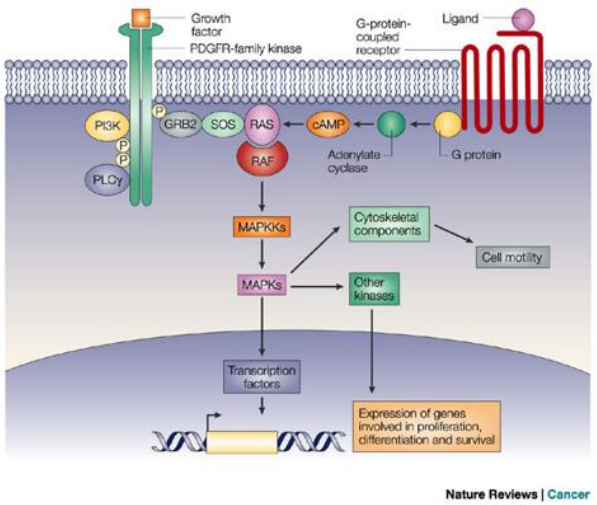


Figure 2. Overall survival curves of patients with a *KRAS*-mutated and nonmutated tumor.



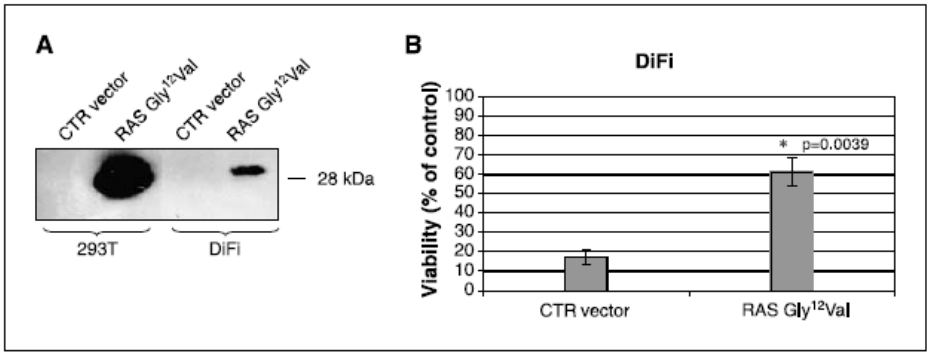


Figure 2. Activated K-RAS confers resistance to cetuximab in DiFi cell line. *A*, cells transfected with either empty vector (*CTR vector*) or RAS $Gly^{12}Val$ were lysed and subjected to a CRIB pull-down assay to check for RAS activity. *B*, DiFi cells were transfected with either empty vector (*CTR vector*) or RAS $Gly^{12}Val$ and then subjected to cetuximab treatment. Several concentrations, ranking from 5 to 20 nmol/L, were tested. Here, we show the results obtained with 20 nmol/L concentration. The graph shows the percentage of survival of the treated cells at day 9 posttransfection ($P = 0.0039$). The experiment was repeated two independent times and each time produced comparable results and *P* values.

Lievre A, et al. Cancer Res 2006
Benvenuti et al Cancer Res 2006

K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer

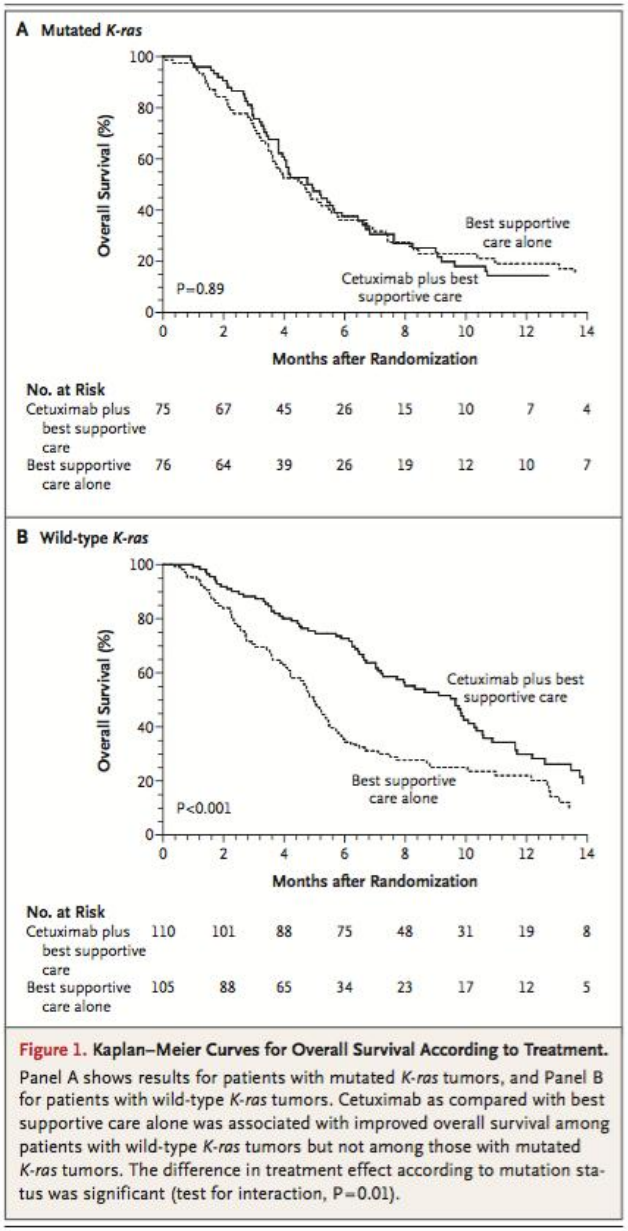


Figure 1. Kaplan–Meier Curves for Overall Survival According to Treatment.
Panel A shows results for patients with mutated *K-ras* tumors, and Panel B for patients with wild-type *K-ras* tumors. Cetuximab as compared with best supportive care alone was associated with improved overall survival among patients with wild-type *K-ras* tumors but not among those with mutated *K-ras* tumors. The difference in treatment effect according to mutation status was significant (test for interaction, P=0.01).

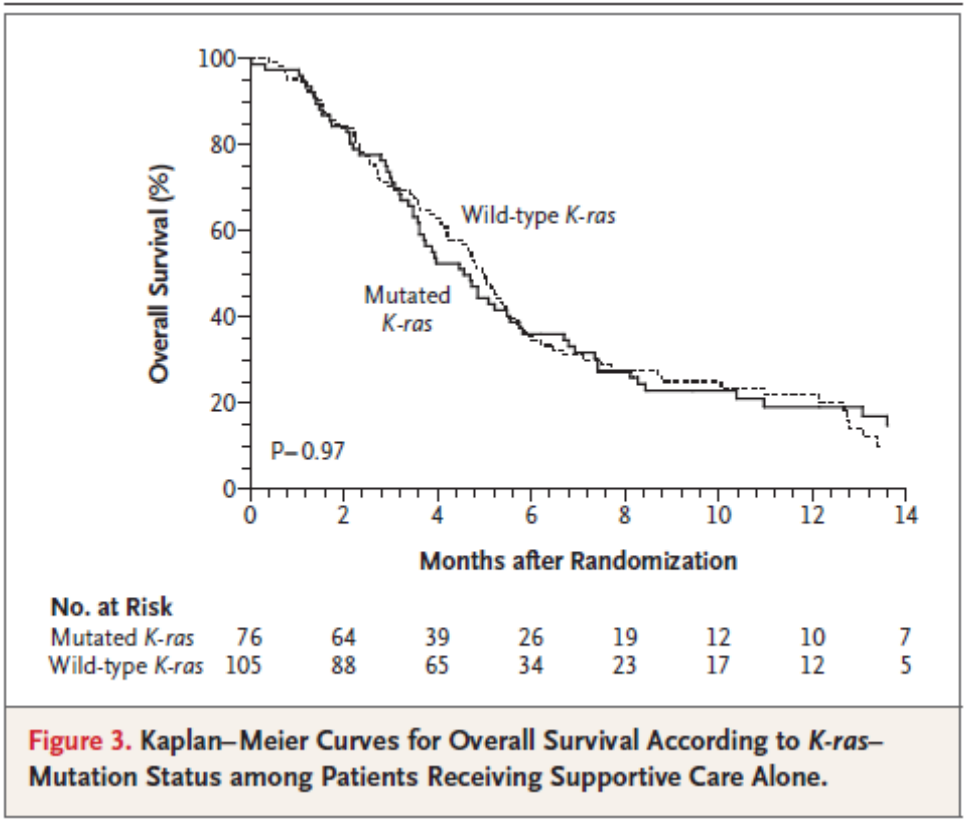


Figure 3. Kaplan–Meier Curves for Overall Survival According to *K-ras*–Mutation Status among Patients Receiving Supportive Care Alone.

Karapetis CS, et al. N Engl J Med 2008

Constitutive RAS Pathway Activation and Its Impact on EGFR mAb Therapy

Study	N. pts analyzed	KRAS mutations	Regimens	Overall survival (months)	
				KRAS mut	***KRAS WT***
Karapetis et al 2008	394/572 (68.9%)	**42.3%**	Cetuximab+ BSC/BSC	4.5 vs 4.6 HR=0.98 (p=0.89)	9.5 vs 4.8 HR=0.55 (p<0.001
Bokemeyer, et al 2009	233/337 (69.1%)	**42%**	FOLFOX+cetuximab/FOLFOX	5.5 vs 8.6 HR=1.83 (p=0.0192)	7.7 vs 7.2 HR=0.57 (p=0.0163)
Douillard, et al 2013	1060/1183 (90%)	**52%**	FOLFOX+Panitumumab /FOLFOX	15.5 vs 18.7 HR=1.21 (p=0.04)	25.8 vs 20.2 HR=0.77 (p=0.009)

Mutant *KRAS* Codon 12 and 13 Alleles in Patients With Metastatic Colorectal Cancer: Assessment As Prognostic and Predictive Biomarkers of Response to Panitumumab

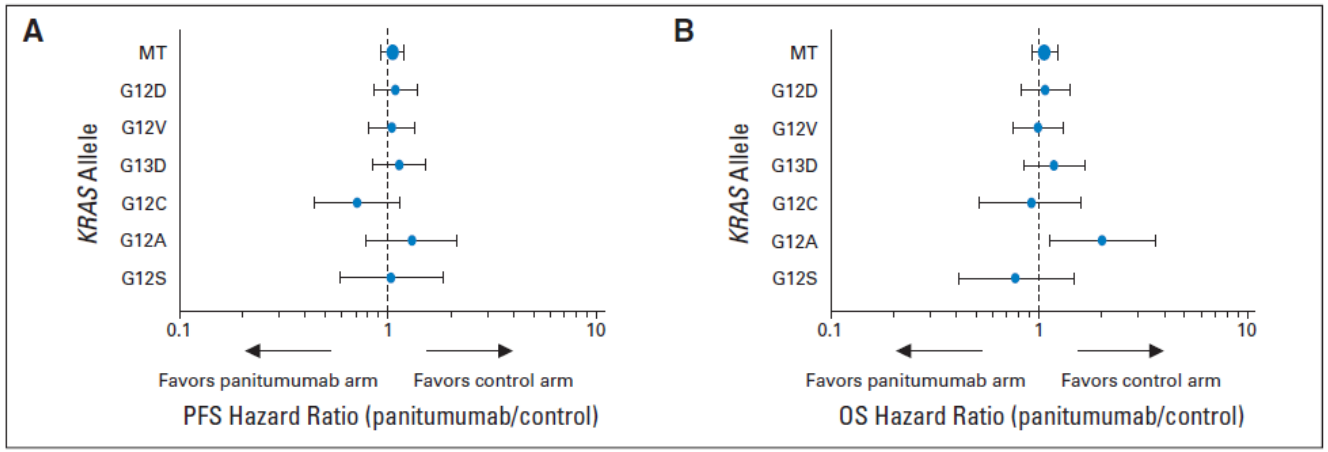


Fig 3. Pooled analysis of studies 20050203, 20050181, and 20020408: Predictive impact of mutant (MT) *KRAS* codon 12 and 13 alleles on (A) progression-free survival (PFS) and (B) overall survival (OS) in patients receiving either control (non–panitumumab-containing) or panitumumab-containing therapy. Point estimates for hazard ratios and their corresponding 95% CIs are plotted for the indicated mutant *KRAS* codon 12 and 13 alleles and are compared with the other mutant *KRAS* codon 12 and 13 alleles as a group.

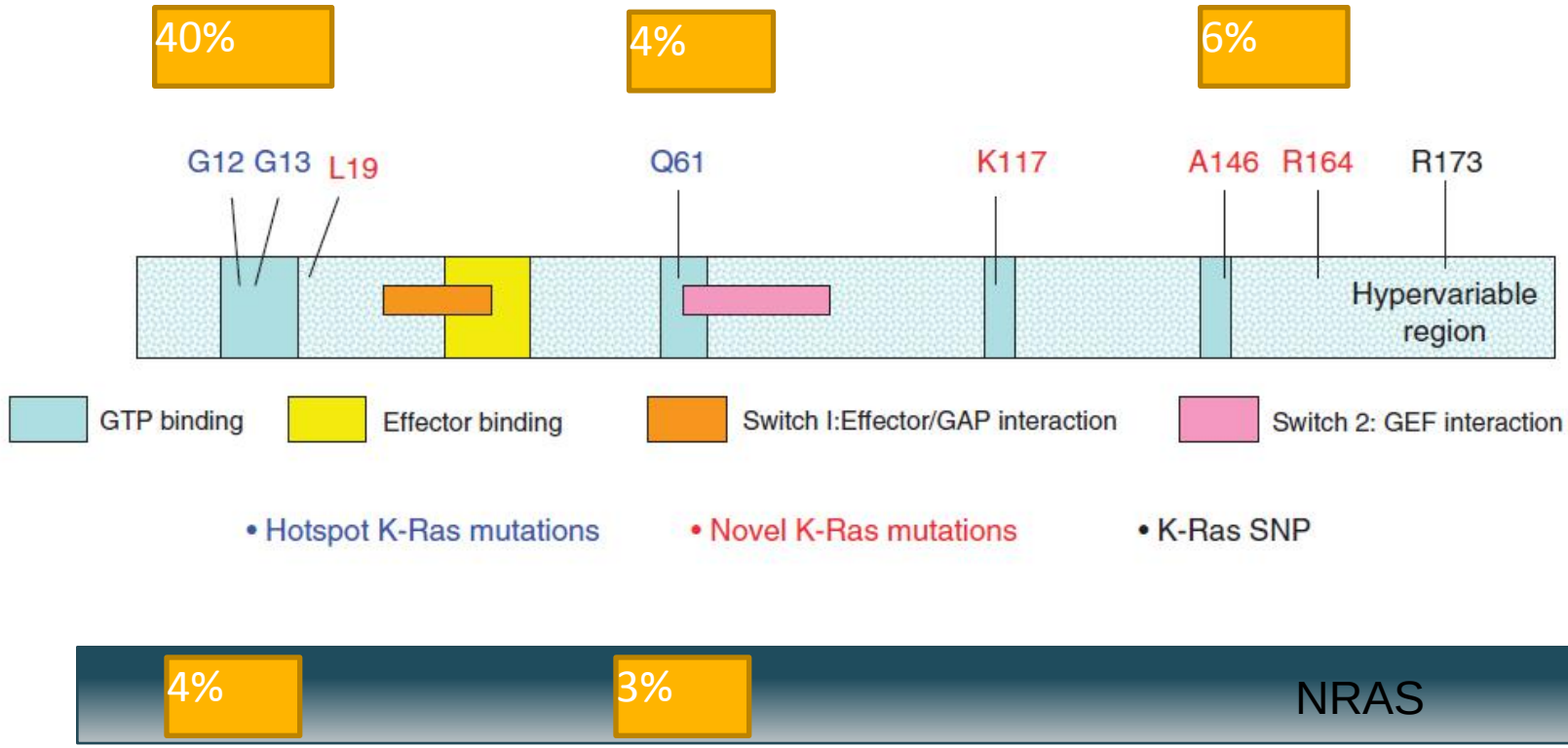
Table 2. *P* Values Determined From Quantitative Interaction Testing Exploring the Interaction Between the Specified Mutant *KRAS* Allele and Therapy on Either OS or PFS

KRAS Allele	Study 20050203		Study 20050181		Study 20020408	
	OS	PFS	OS	PFS	OS	PFS
G12D	.9870	.8692	.7351	.3658	.42	.41
G12V	.0369*	.4229	.2449	.7023	.48	.56
G13D	.0018*	.1609	.0665	.4736	.37	.90
G12C	.3005	.0590	.8457	.6291	N/D†	N/D†
G12A	.3362	.3279	.0974	.6547	N/D†	N/D†
G12S	.2866	.9641	.4437	.5878	N/D†	N/D†

Abbreviations: N/D, not determined; OS, overall survival; PFS, progression-free survival.
*Quantitative interaction tests with $P < .05$.
†Not performed because of limiting number of patients in these *KRAS* allele subgroups.

Peeters M, et al. JCO 2013

Exon 2, 3 and 4 KRAS and NRAS mutations



KRAS wild type ≈ 60%

RAS wild type ≈ 45%

Smith et al, Br J Cancer 2010

Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis

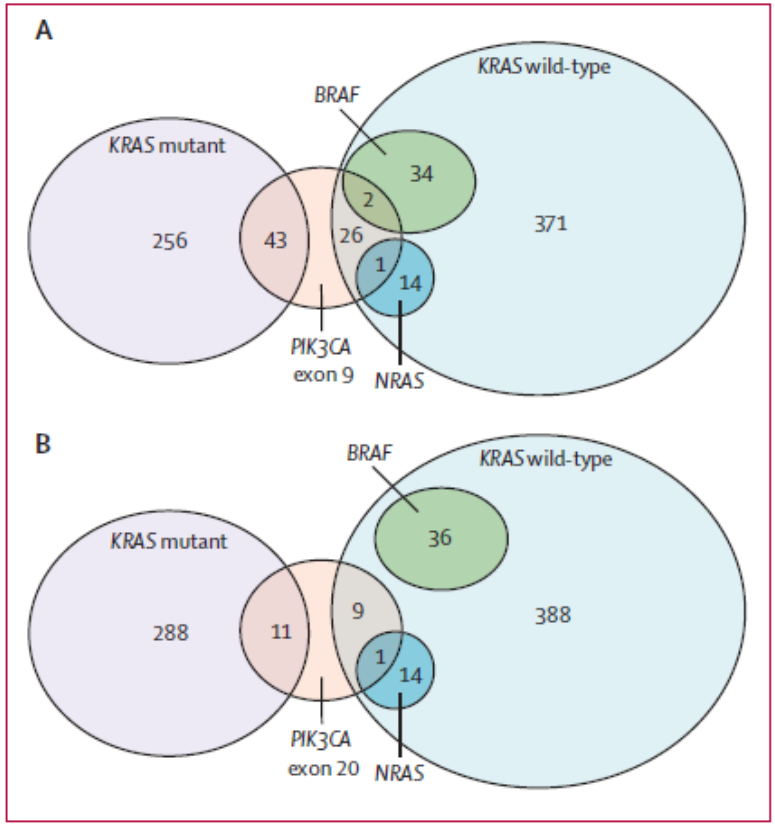


Figure 1: **Associations between mutations**
Absolute numbers of *KRAS* wild type, *KRAS* mutant, *BRAF* mutant, *NRAS* mutant, *PIK3CA* exon 9 mutant samples (A), and *PIK3CA* exon 20 mutant (B) samples are shown.

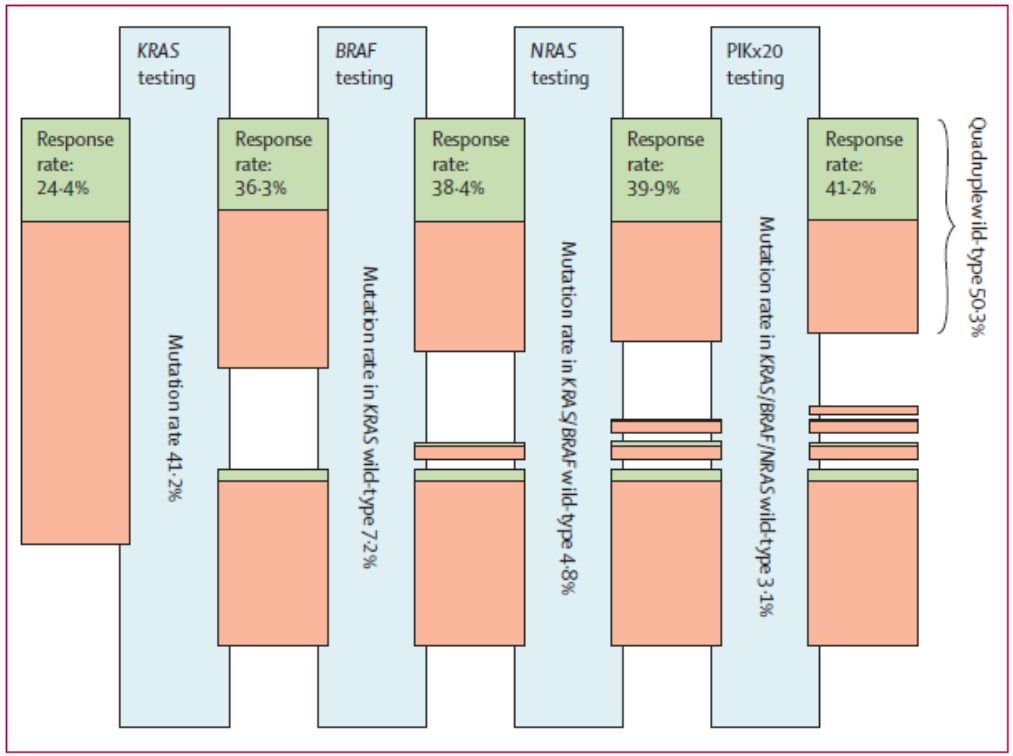


Figure 3: **The improvement in response prediction gained by assessing the mutation status of each gene**
Patients with missing data for any of the markers studied in this analysis were omitted from the start. The green bars represent responders; the orange bars non-responders. Bottom bars represent mutant tumours; upper bars wild-type tumours. The size of the bars is in agreement with the corresponding percentages. PIKx20=*PIK3CA* exon 20.

De Roock, et al. Lancet Oncol 2010

Massively Parallel Tumor Multigene Sequencing to Evaluate Response to Panitumumab in a Randomized Phase III Study of Metastatic Colorectal Cancer

Table 3. Response rates of patients with wild-type *KRAS* (codons 12/13/61) who were randomized to panitumumab plus BSC[a]

		Randomized phase III study panitumumab + BSC $n = 82$		Extension study panitumumab + BSC $n = 56$		Combined panitumumab + BSC $n = 138$	
Genotype		*n*	Response rate, % (95% CI)	*n*	Response rate, % (95% CI)	*n*	Response rate, % (95% CI)
NRAS	WT	76	13 (6–23)	50	24 (13–38)	126	17 (11–25)
	MT	4	0 (0–60)	5	0 (0–52)	9	0 (0–34)
EGFR	WT	82	12 (6–21)	52	23 (13–37)	134	16 (11–24)
	MT	0	NA	0	NA	0	NA
BRAF	WT	63	14 (7–25)	44	21 (10–35)	107	17 (10–25)
	MT	9	0 (0–34)	4	0 (0–60)	13	0 (0–25)
PTEN	WT	72	13 (6–22)	50	22 (12–36)	122	16 (10–24)
	MT	7	14 (0–58)	2	0 (0–84)	9	11 (0–48)
PIK3CA	WT	74	12 (6–22)	43	19 (8–33)	117	15 (9–22)
	MT	5	20 (1–72)	5	20 (1–72)	10	20 (3–56)
AKT1	WT	69	15 (7–25)	52	19 (10–33)	121	17 (10–24)
	MT	1	0 (0–98)	0	NA	1	0 (0–98)
TP53	WT	32	16 (5–33)	18	11 (1–35)	50	14 (6–27)
	MT	49	10 (3–22)	35	26 (13–43)	84	17 (9–26)
CTNNB1	WT	72	11 (5–21)	46	22 (11–36)	118	15 (9–23)
	MT	2	50 (1–99)	0	NA	2	50 (1–99)

NOTE: *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *CTNNB1*, catenin (cadherin-associated protein), β-1, 88 kDa; MT, mutant; NA, not available; *NRAS*, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphoinositide-3-kinase, catalytic, α-polypeptide; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53; WT, wild-type.

[a]Per local review.

Peeters M, et al. CCR 2013

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Panitumumab–FOLFOX4 Treatment and *RAS* Mutations in Colorectal Cancer

Table 2. Efficacy Results According to *RAS* Mutation Status.

Variable	Panitumumab–FOLFOX4	FOLFOX4 Alone	Hazard Ratio (95% CI)	P Value	P Value for Interaction Test*
No *KRAS* mutation in exon 2					
No. of patients	325	331			
Months of progression-free survival in primary analysis — median (95% CI)	9.6 (9.2–11.1)	8.0 (7.5–9.3)	0.80 (0.66–0.97)	0.02	
Months of overall survival — median (95% CI)					
Primary analysis	23.9 (20.3–28.3)	19.7 (17.6–22.6)	0.83 (0.67–1.02)	0.07	
Updated analysis	23.8 (20.0–27.7)	19.4 (17.4–22.6)	0.83 (0.70–0.98)	0.03	
***KRAS* mutation in exon 2**					
No. of patients	221	219			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (6.3–8.0)	8.8 (7.7–9.4)	1.29 (1.04–1.62)	0.02	
Months of overall survival — median (95% CI)					
Primary analysis	15.5 (13.1–17.6)	19.3 (16.5–21.8)	1.24 (0.98–1.57)	0.07	
Updated analysis	15.5 (13.1–17.6)	19.2 (16.2–21.5)	1.16 (0.94–1.41)	0.16	
No *RAS* mutation					
No. of patients	259	253			
Months of progression-free survival in primary analysis — median (95% CI)	10.1 (9.3–12.0)	7.9 (7.2–9.3)	0.72 (0.58–0.90)	0.004	
Months of overall survival — median (95% CI)					
Primary analysis	26.0 (21.7–30.4)	20.2 (17.7–23.1)	0.78 (0.62–0.99)	0.04	
Updated analysis	25.8 (21.7–29.7)	20.2 (17.6–23.6)	0.77 (0.64–0.94)	0.009	
No *KRAS* mutation in exon 2, other *RAS* mutation					
No. of patients	51	57			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (5.3–9.2)	8.0 (6.4–11.3)	1.28 (0.79–2.07)	0.33	0.04
Months of overall survival — median (95% CI)					
Primary analysis	17.1 (10.8–19.4)	18.3 (13.0–23.2)	1.29 (0.79–2.10)	0.31	0.07
Updated analysis	17.1 (10.8–19.4)	17.8 (13.0–23.2)	1.39 (0.91–2.13)	0.12	0.01
***RAS* mutation**					
No. of patients	272	276			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (6.3–7.9)	8.7 (7.6–9.4)	1.31 (1.07–1.60)	0.008	<0.001
Months of overall survival — median (95% CI)					
Primary analysis	15.6 (13.4–17.9)	19.2 (16.7–21.8)	1.25 (1.02–1.55)	0.03	0.004
Updated analysis	15.5 (13.4–17.9)	18.7 (16.5–21.5)	1.21 (1.01–1.45)	0.04	0.001

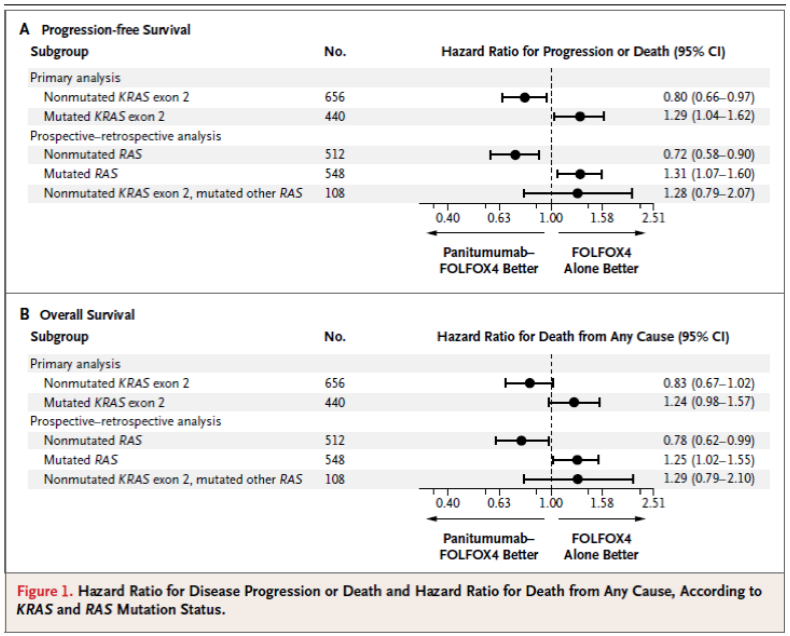


Figure 1. Hazard Ratio for Disease Progression or Death and Hazard Ratio for Death from Any Cause, According to *KRAS* and *RAS* Mutation Status.

17% other RAS mutations

Douillard JY, et al. N Engl J Med 2013

FDA Label Update

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use Vectibix safely and effectively. See full prescribing information for Vectibix.

Vectibix® (panitumumab)
Injection for intravenous infusion
Initial US Approval: 2006

WARNING: DERMATOLOGIC TOXICITY
See full prescribing information for complete boxed warning.
- **Dermatologic toxicities were reported in 90% of patients and were severe in 15% of patients receiving monotherapy. (2.3, 5.1, 6.1)**

----------RECENT MAJOR CHANGES----------

• Boxed Warning: infusion reactions	05/2014
• Indications and Usage (1)	05/2014
• Dosage and Administration (2)	05/2014
• Warnings and Precautions (5)	05/2014

----------INDICATIONS AND USAGE----------

Vectibix is an epidermal growth factor receptor (EGFR) antagonist indicated for the treatment of wild-type *KRAS* (exon 2) metastatic colorectal cancer (mCRC) as determined by an FDA-approved test for this use:
- In combination with FOLFOX for first-line treatment. (1.1, 14.2)
- As monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy. (1.1, 14.1)
- Limitation of Use: Vectibix is not indicated for the treatment of patients with *KRAS*-mutant mCRC or for whom *KRAS* mutation status is unknown. (1.2, 2.1, 5.2, 12.1)

----------DOSAGE AND ADMINISTRATION----------
- Administer 6 mg/kg every 14 days as an intravenous infusion over 60 minutes (≤ 1000 mg) or 90 minutes (> 1000 mg). (2)
- Infusion Reactions: Reduce infusion rate by 50% for mild reactions; terminate the infusion for severe infusion reactions. (2.3, 5.4)
- Dermatologic Toxicity: Withhold or discontinue for severe or intolerable toxicity; reduce dose for recurrent, grade 3 toxicity. (2.3, 5.1)

----------DOSAGE FORMS AND STRENGTHS----------
- Single-use vials (20 mg/mL): 100 mg/5 mL, 200 mg/10 mL, 400 mg/20 mL. (3)

----------CONTRAINDICATIONS----------
- None

----------WARNINGS AND PRECAUTIONS----------
- Dermatologic and Soft Tissue Toxicity: Monitor for dermatologic and soft tissue toxicities and withhold or discontinue Vectibix for severe or life-threatening complications. Limit sun exposure. (5.1, 5.7)
- Increased tumor progression, increased mortality, or lack of benefit in patients with *KRAS*-mutant mCRC: Determine *KRAS*-mutant tumor status in an experienced laboratory using an FDA-approved test. (5.2)
- Electrolyte Depletion/Monitoring: Monitor electrolytes and institute appropriate treatment. (5.3)
- Infusion Reactions: Terminate the infusion for severe infusion reactions. (5.4)
- Pulmonary Fibrosis/Interstitial Lung Disease (ILD): Permanently discontinue Vectibix in patients developing ILD. (5.6)
- Ocular Toxicities: Monitor for keratitis or ulcerative keratitis. Interrupt or discontinue Vectibix for acute or worsening keratitis. (5.8)

----------ADVERSE REACTIONS----------

Most common adverse reactions (≥ 20%) of Vectibix as monotherapy are skin rash with variable presentations, paronychia, fatigue, nausea, and diarrhea. (6.1)

Most common adverse reactions (≥ 20%) in clinical trials of Vectibix in combination with FOLFOX chemotherapy are diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, acneiform dermatitis, pruritus, and dry skin. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Amgen Inc. at 1-800-77-AMGEN (1-800-772-6436) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- USE IN SPECIFIC POPULATIONS ----------
- **Pregnancy:** Based on animal data, may cause fetal harm. (8.1) Physicians are encouraged to enroll pregnant patients in Amgen's Pregnancy Surveillance Program by calling 1-800-772-6436 (1-800-77-AMGEN). (8.1)
- **Nursing Mothers:** Discontinue nursing or discontinue drug, taking into account the importance of the drug to the mother. (8.3)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 05/2014

Extended *RAS* mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials

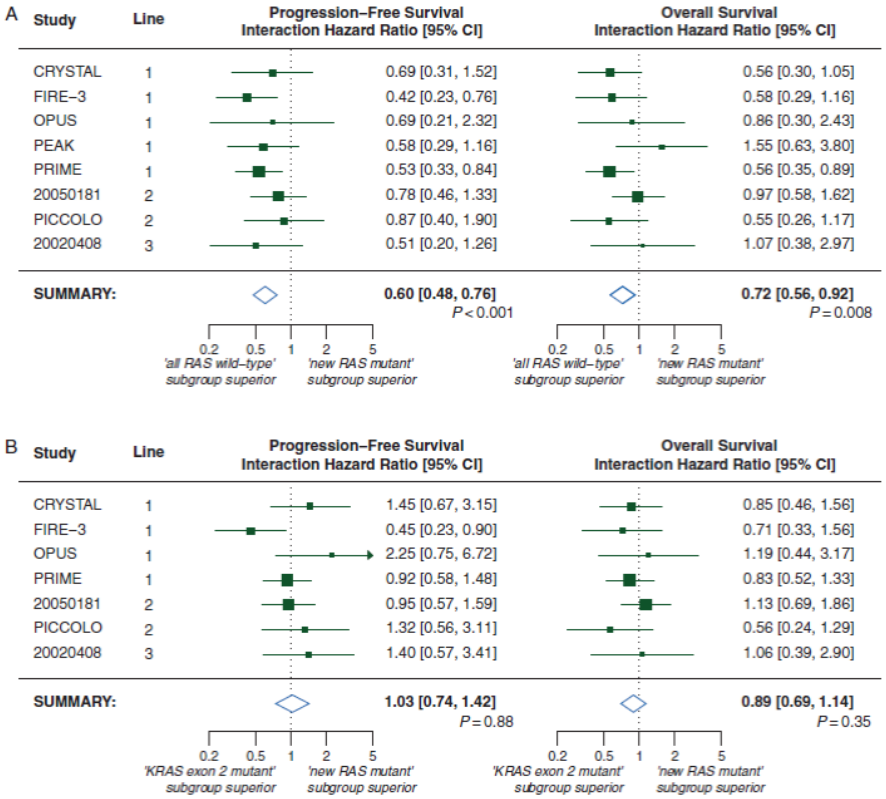


Figure 3. The relative size of the anti-EGFR treatment effect for tumors with one of the new *RAS* mutations compared with (A) tumors without any *RAS* mutations and (B) tumors with any *KRAS* exon 2 mutations.

	New RAS Total[a]	KRAS Exon 3[a]	KRAS Exon 4[a]	NRAS Exon 2[a]	NRAS Exon 3[a]	NRAS Exon 4[a]
		59 61	117 146	12 13	59 61	117 146
OPUS	26.3%	5.9%	9.3%	6.8%	5.1%	0.8%
PICCOLO	9.8%	NR[b]	3.7%[c]	6.3%[d]	NR[b]	NE
20020408	17.6%	4.8%[b]	5.0%	4.2%	3.0%[b]	1.1%
20050181	20.5%	4.6%	7.9%	2.3%	5.8%	0.0%
PRIME	17.4%	3.7%[b]	5.6%	3.4%	4.1%[b]	0.0%
FIRE-3	16.0%	4.3%[b]	4.9%[c]	3.8%	2.0%[b]	0.0%
PEAK	20.1%	4.1%	7.7%	5.4%	5.9%	0.0%
COIN	8.4%	2.1%[b]	NE	0.9%[e]	3.0%[b]	NE
CRYSTAL	14.7%	3.3%	5.6%	3.5%	2.8%	0.9%
SUMMARY[f]	19.9% (16.7%, 23.4%)	4.3% (3.3%, 5.5%)	6.7% (5.7%, 7.9%)	3.8% (3.0%, 4.8%)	4.8% (3.4%, 6.8%)	0.5% (0.2%, 1.2%)

Figure 2. Prevalence of new *RAS* mutations across studies. NA, not applicable; NE, not evaluated; NR, evaluated but not reported. [a]New *RAS* mutations are reported as a proportion of the *KRAS* exon 2 wild-type group. [b]*KRAS* and *NRAS* codon 59 mutation not evaluated. [c]*KRAS* codon 117 mutation not evaluated. [d]Exon 3 codon 61 mutations in addition to the exon 2 mutations. [e]Only *NRAS* mutation G12C evaluated. [f]Random-effects meta-analysis summary estimates (95% confidence interval) based on studies that have evaluated all relevant codons.

Sorich MJ, et al. Ann Oncol 2015

Sensitivity methods KRAS mutations

Table 6 | Laboratory analysis of *KRAS* mutations

Method for assessing gene status	Sensitivity (%)*
Direct dideoxy sequencing	20–30
Direct pyrosequencing	5
Allele specific probes	10
High-resolution melting analysis	5
ARMS/scorpion probes	1

*The lowest level of mutant DNA that can be detected, expressed as a percentage of total DNA in the tumor sample analyzed. Abbreviation: ARMS, amplification refractory mutation system.

Normanno N, et al. Nat Rev Clin Oncol 2009

Wild-Type *BRAF* Is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer

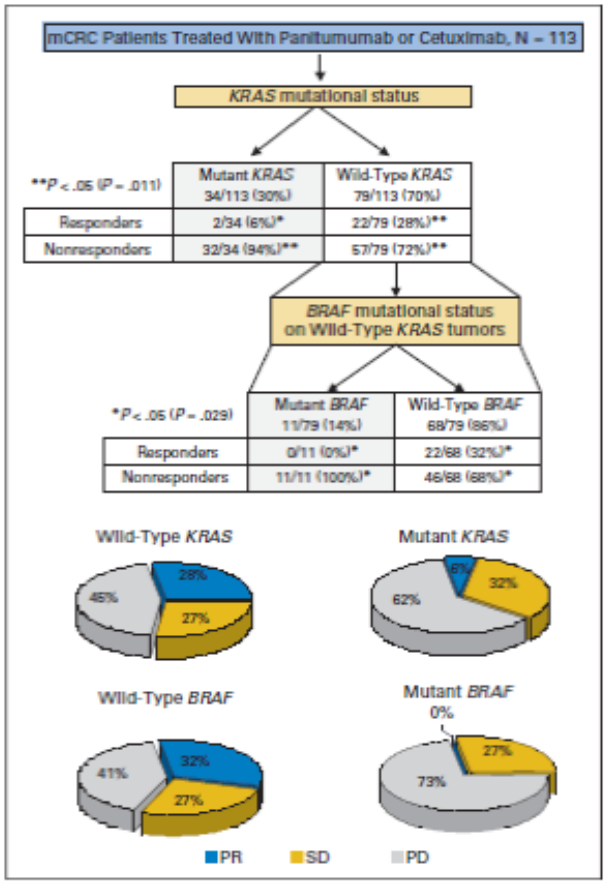


Fig 1. *KRAS* and *BRAF* mutations correlate with lack of response to treatment with monoclonal antibodies targeting epidermal growth factor receptor. The number of responders and nonresponders (stable disease [SD] + progressive disease [PD]) is indicated according to *KRAS* or *BRAF* mutational status. The percentage of patients displaying partial response (PR), SD, or PD is shown in the pie charts. mCRC, metastatic colorectal cancer.

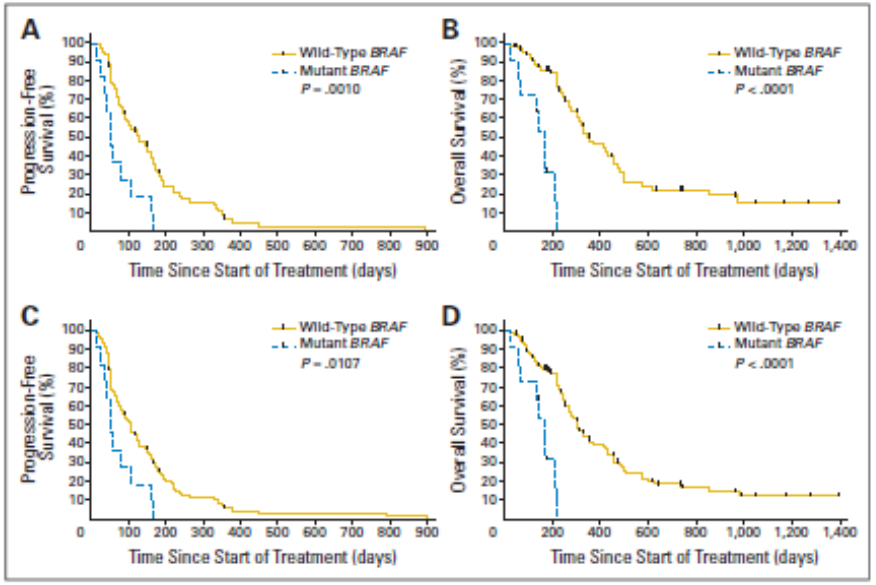


Fig 3. (A and B) In wild-type *KRAS* patients, those carrying a *BRAF*-mutated tumor had a shorter progression-free survival (PFS) and overall survival (OS) than wild-type *BRAF* patients (log-rank test, $P = .0010$ and $P < .0001$, respectively). (C and D) In the entire cohort of patients, individuals with wild-type *BRAF* tumors still displayed longer PFS and OS than patients with *BRAF*-mutated tumors ($P = .0107$ and $P < .0001$, respectively).

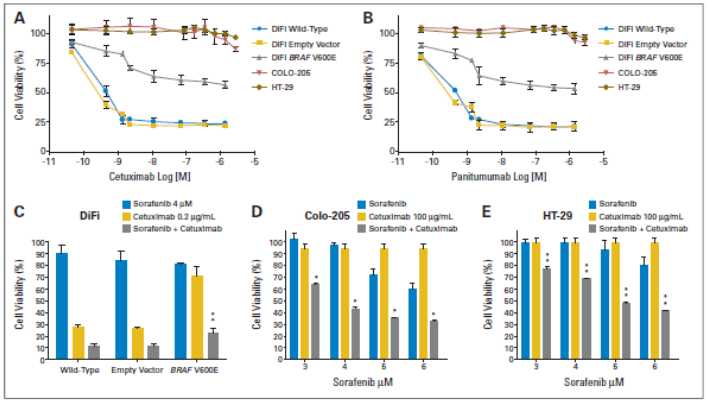


Fig 4. (A and B) The colorectal cancer cell line DiFi was transduced with either an empty vector or a *BRAF* V600E–encoding lentiviral vector. Cell viability was measured after treatment with either cetuximab or panitumumab. (C to E) Viability of DiFi, COLO-205, and HT-29 cells after combinatorial treatment with cetuximab plus sorafenib. Results (average ± standard deviation) were normalized to untreated cells. (*) $P < .05$ and (**) $P < .01$ by Bonferroni multiple comparison test.

Di Nicolantonio F, et al. JCO 2008

BRAF Mutations in mCRC

- 5% mCRC
- Phenotype
- Right colon
- More commonly in women
- Strongly associated with MSI

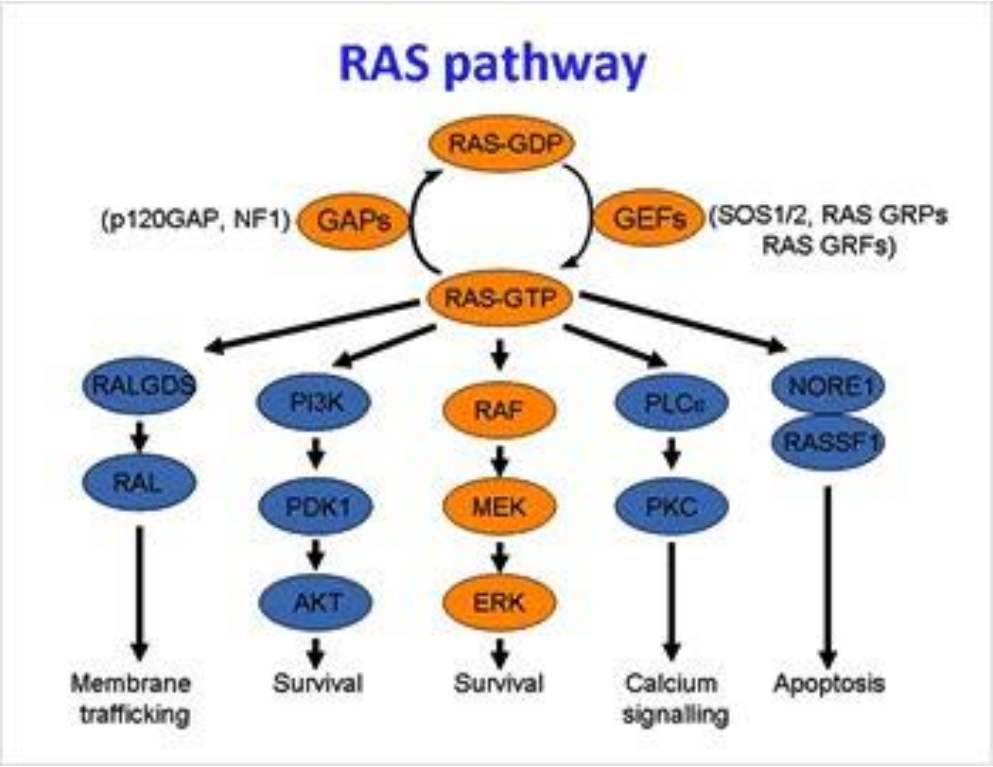


Table 3. Efficacy Results According to *RAS* and *BRAF* Mutation Status in the Primary-Analysis Population.*

Variable	Panitumumab–FOLFOX4	FOLFOX4 Alone	Hazard Ratio (95% CI)	P Value
No *RAS* or *BRAF* mutations				
No. of patients	228	218		
Months of progression-free survival — median (95% CI)	10.8 (9.4–12.4)	9.2 (7.4–9.6)	0.68 (0.54–0.87)	0.002
Months of overall survival — median (95% CI)	28.3 (23.7–NE)	20.9 (18.4–23.8)	0.74 (0.57–0.96)	0.02
No *RAS* mutation, *BRAF* mutation				
No. of patients	24	29		
Months of progression-free survival — median (95% CI)	6.1 (3.7–10.7)	5.4 (3.3–6.2)	0.58 (0.29–1.15)	0.12
Months of overall survival — median (95% CI)	10.5 (6.4–18.9)	9.2 (8.0–15.7)	0.90 (0.46–1.76)	0.76
***RAS* or *BRAF* mutation**				
No. of patients	296	305		
Months of progression-free survival — median (95% CI)	7.3 (6.3–7.7)	8.0 (7.5–9.0)	1.24 (1.02–1.49)	0.03
Months of overall survival — median (95% CI)	15.3 (12.7–17.6)	18.0 (15.9–20.8)	1.21 (0.99–1.47)	0.06
No *KRAS* mutation in exon 2, other *RAS* or *BRAF* mutation				
No. of patients	75	86		
Months of progression-free survival — median (95% CI)	6.7 (5.3–8.2)	7.3 (5.7–8.0)	1.05 (0.73–1.52)	0.80
Months of overall survival — median (95% CI)	14.5 (10.4–18.5)	15.8 (11.9–18.8)	1.14 (0.78–1.66)	0.51

* NE denotes not evaluated.

Douillard JY, et al. N Engl J Med 2013

Overall survival and BRAF status

	KRAS wt (months)	KRAS wt/BRAF mut (months)
	CRYSTAL	
FOLFIRI	20.0	10.3
FOLFIRI/Cetuximab	23.5	14.1
	CAIRO 2	
XELOX/bevacizumab	20.3	15.0
XELOX/bevacizumab/cetuximab	19.4	15.2
	OPUS	
FOLFOX	18.5	4.4
FOLFOX/cetuximab	22.8	20.7
	FIRE 3	
FOLFIRI/cetuximab	33.1	13.3

Meta-analysis of *BRAF* mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for *RAS* wild-type metastatic colorectal cancer

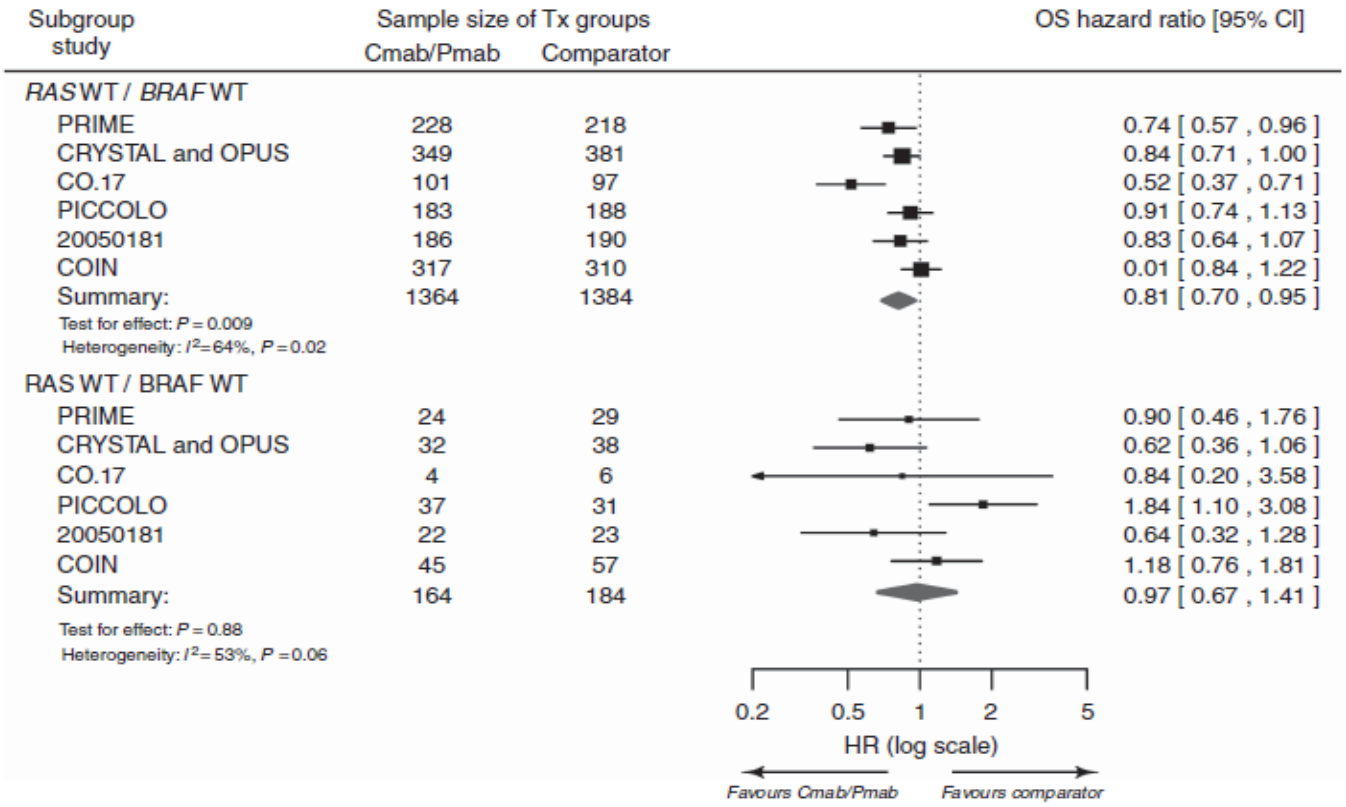


Figure 2. Forest plot of the overall survival benefit with anti-EGFR mAb therapy for subgroups defined by tumour *RAS* and *BRAF* mutations. Cmab = cetuximab; MT = mutant; Pmab = panitumumab; WT = wild type.

Rowland A, et al. BJC 2015

RAF inhibitor-based combinations

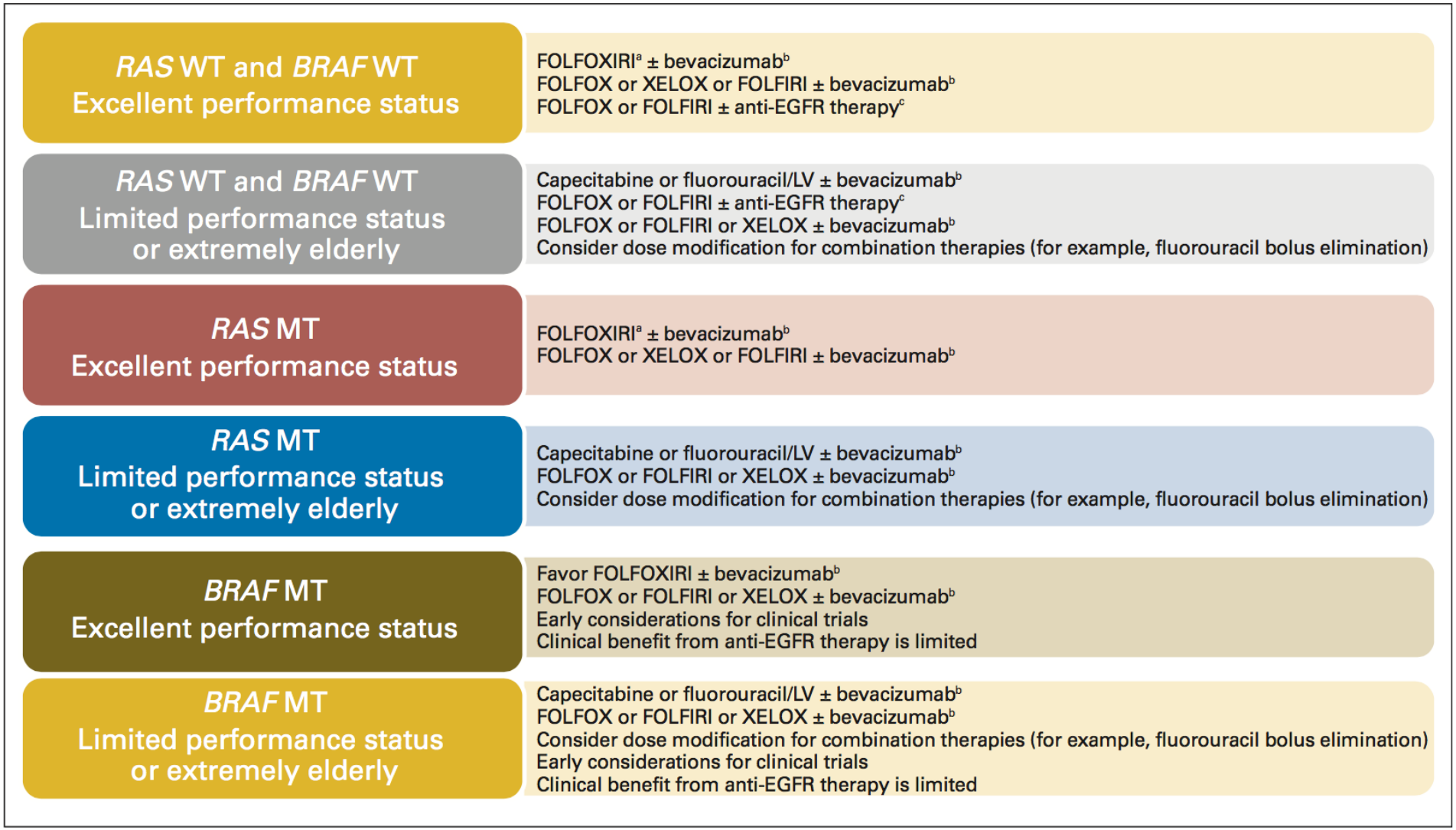
- Phase 1B study of vemurafenib in combination with irinotecan and cetuximab in patients with *BRAF*-mutated advanced cancers and metastatic colorectal cancer.
- In vitro data in CRC cell lines has shown that blockade of mutated BRAF by vemurafenib triggers compensatory activation of EGFR. Inhibition of EGFR combined with vemurafenib results in synergistic cytotoxicity in preclinical models, further augmented by irinotecan.
- Four of the 5 mCRC pts (80%) achieved a partial response. For the 5 mCRC pts, median best response was a reduction of -44% (range, 0% to -70%) with duration of responses of 5, 5+, 8+, 12+, and 14+ cycles.

Hong DS, et al. J Clin Oncol 32:5s, 2014 (suppl; abstr 3516)

Conclusions

- Expanded *RAS* mutation testing as part of the initial workup for mCRC, because this approach will identify an additional approximately 11% of patients with CRC who are unlikely to benefit from EGFR antibodies.
- Testing for *BRAF* V600 mutations because ongoing *BRAF*-directed clinical trials offer a promising alternative.
- The fundamentals learned from preclinical studies of signaling pathways.

Treatment algorithm for first-line metastatic colorectal cancer on the basis of RAS/BRAF status



Fakih MG. JCO 2015

Anti-EGFR vs Anti-VEGF in First-line MCRC
CALGB/SWOG 80405 Study

	FOLFOX CT	FOLFOX/Cet vs FOLFOX Beva	
	No.	**Months**	**HR**
KRAS	835	30.1 vs 26.9 (Δ=3.2)	0.9 (p=0.09)
RAS	390	32.5 vs 29.0 (Δ=3.5)	0.86 (p=0.2)

Lenz et al, ESMO 2014

Emergence of *KRAS* mutations and acquired resistance to anti EGFR therapy in colorectal cancer

a

Patient ID	Chemotherapy resistant tumors KRAS mutational status		
	Mutation	Percentage	Reads[a]/Events[b]
Patient #2	wt[a]	0%	0/30000
Patient #7	wt[a]	0%	0/11262
Patient #8	wt[b]	0.01%	5/76200
Patient #9	wt[b]	0%	0/89760
Patient #10	wt[b]	0%	0/34500
Patient #11	wt[b]	0%	0/190600
Patient #13	wt[a]	0%	0/18277
Patient #14	wt[a]	0%	0/27942
Patient #15	wt[a]	0%	0/43279
Patient #16	wt[a]	0%	0/41693
Patient #17	wt[a]	0%	0/30174
Patient #18	wt[a]	0%	0/16400
Patient #19	wt[a]	0%	0/29578
Patient #21	wt[a]	0%	0/18277

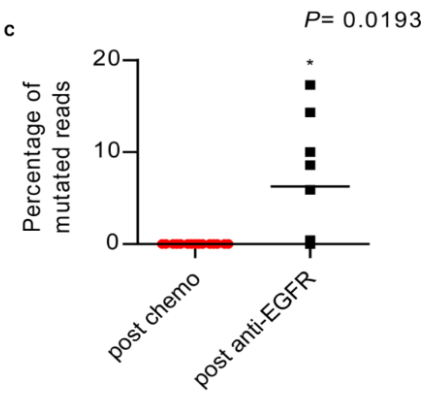
a: 454

b: BEAMing

b

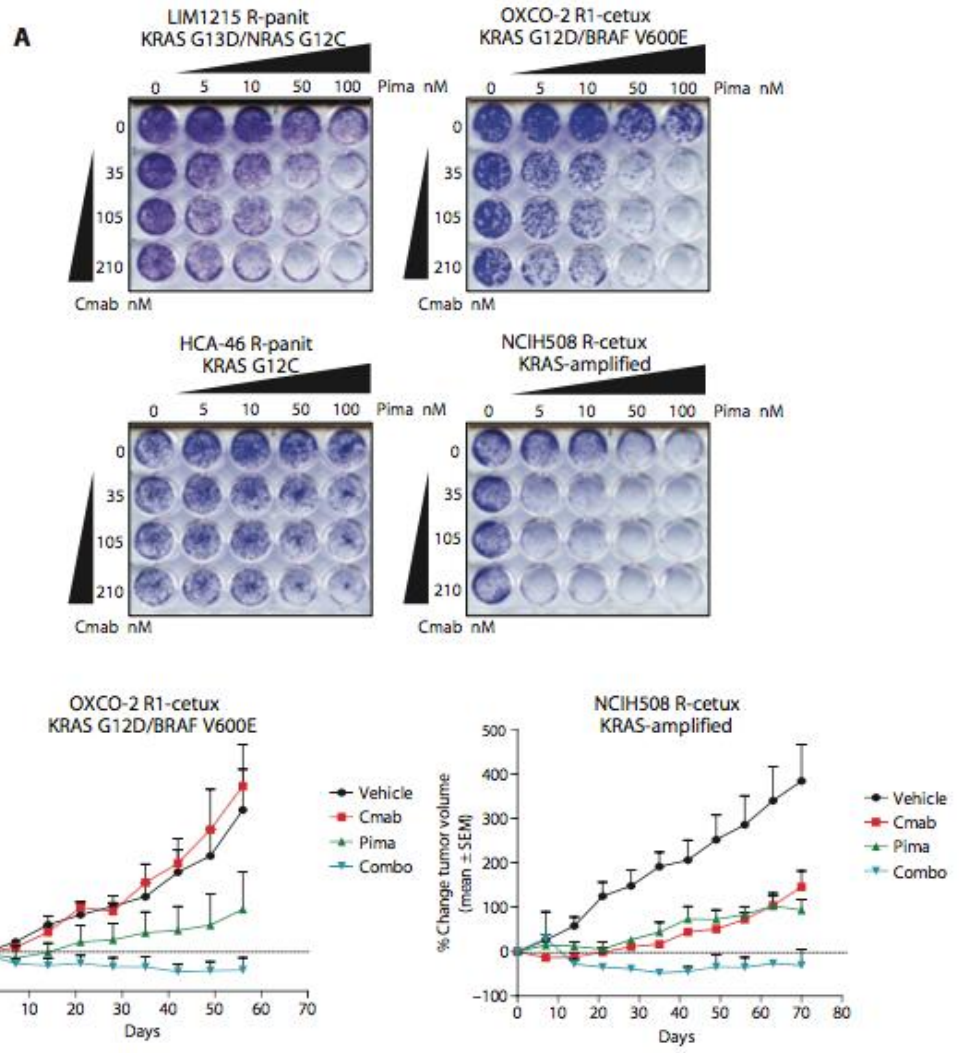
Patient ID	Anti-EGFR resistant tumors KRAS Mutational Status		
	Mutation	Percentage	Reads[a]/Events[b]
Patient #1	wt[a]	0%	0/12123
Patient #2	G13D[a]	10%	859/8556
Patient #4	G13D[a]	5.9%	461/7764
Patient #5	G13D[a]	14.3%	1037/7247
Patient #6	G13D[a]	8.6%	651/7577
Patient #7	wt[a]	0%	0/17142
Patient #8	Q61H[b]	17.3%	5960/190200
Patient #9	G12D[b] G13D[b]	0.04% 0.44%	17/40200 117/26400
Patient #10	wt[b]	0%	0/50300
Patient #11	wt (amplified)[b]	0%	0/30400

c



Misale S. Nature 2012

Blockade of EGFR and MEK Intercepts Heterogeneous Mechanisms of Acquired Resistance to Anti-EGFR Therapies in Colorectal Cancer



Misale S. Sci Trans Med 2014