The MEK/MAPK signalling pathway in the context of EGFR therapy in metastatic colorectal cancer

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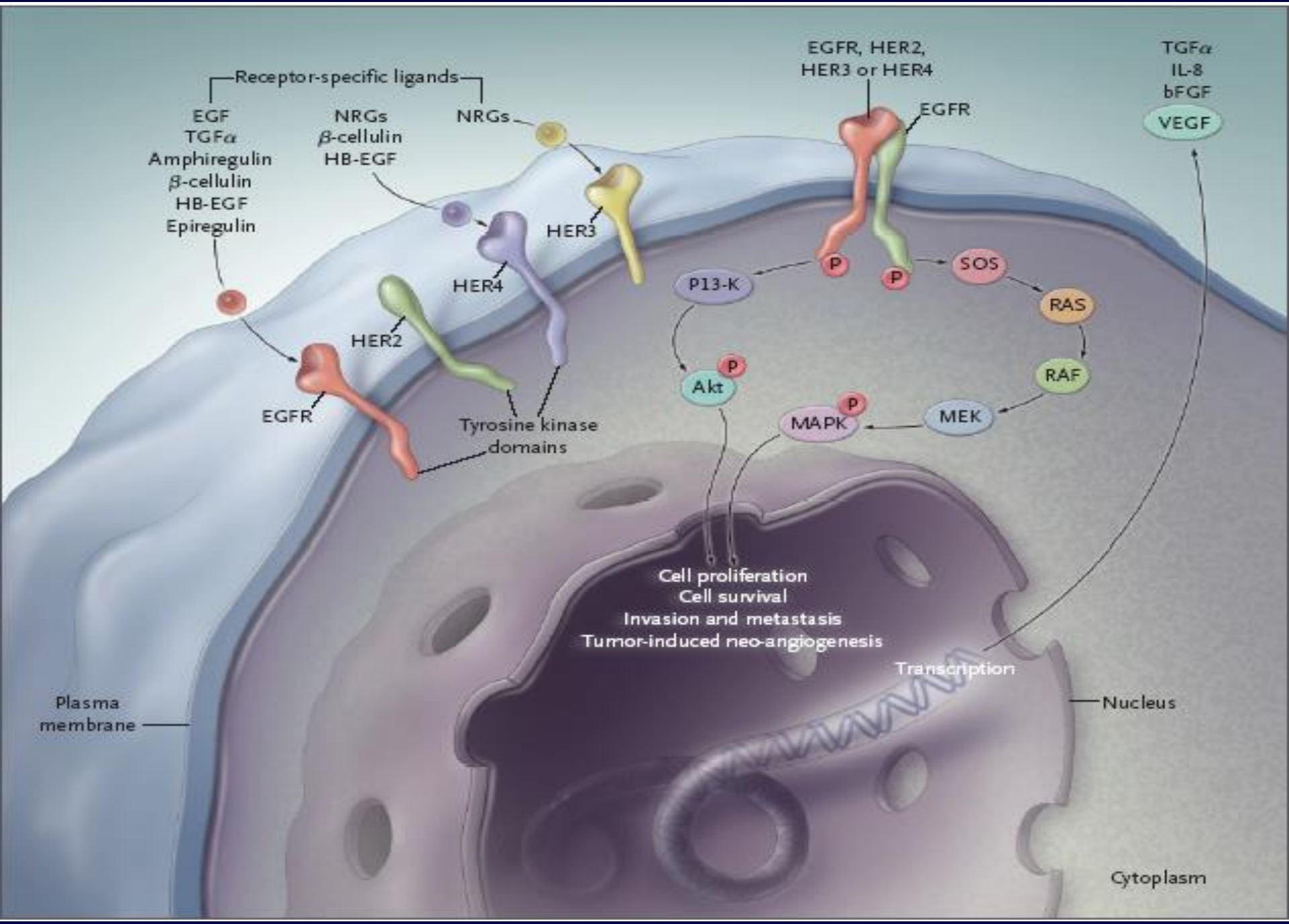
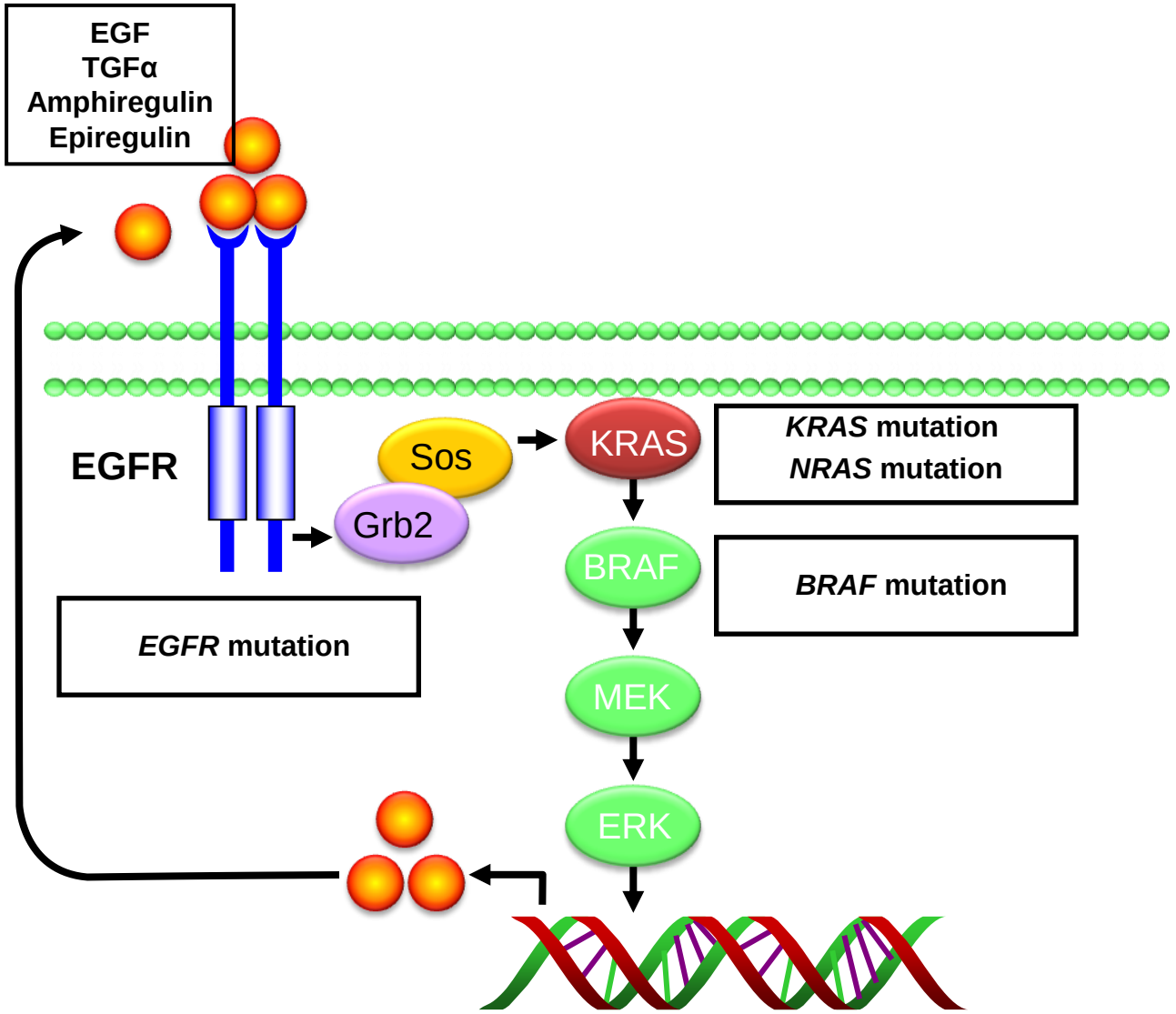
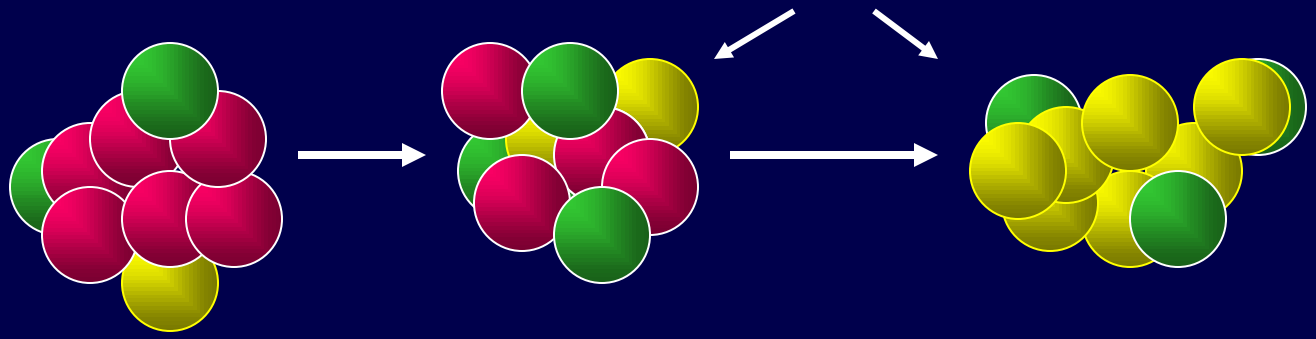


Ciardiello F and Tortora G. New Engl J Med 2008;358:1160–74.

Targeting the EGFR pathway in CRC



Anti-EGFR drugs as monotherapy in unselected chemorefractory metastatic CRC : clinical results



EGFR-dependent Growth

Non-EGFR-dependent Growth

Mechanisms of Intrinsic and Acquired Resistance to EGFR Inhibitors

- Target changes in cancer cells (selection of cancer cell clones with somatic EGFR gene mutations which confer resistance, i.e. the T790M mutation in lung adenocarcinoma, the S492R mutation in colon adenocarcinoma).

- Activation of downstream signaling pathways through EGFR-independent mechanisms:
  - Other cell membrane growth factor receptors (IGF1-R; ErbB2; ErbB3; MET);
  - PTEN-PI3K-AKT pathway;
  - **RAS-RAF-MEK-ERK pathway**;
  - Pro-angiogenic growth factors (VEGF) production;
  - Expression of VEGFRs in cancer cells.

- Epithelial to mesenchimal cancer cell transition (EMT).

Table 2 | Influence of *KRAS* status on cetuximab efficacy in single-arm studies of chemorefractory mCRC

Treatment regimen	Number of patients with *KRAS* mutation out of total number of patients (%)	ORR (CR+PR) in patients with *KRAS* mutations (%)	ORR (CR+PR) in patients with wild-type *KRAS* (%)	Comments
Cetuximab with or without chemotherapy or panitumumab	10 of 31 (32%)	2 of 10 (20%)	8 of 21 (38%)	First exploratory analysis[30]
Cetuximab and chemotherapy	13 of 30 (43%)	0 of 13 (0%)	11 of 17 (65%)	Better median OS in patients with wild-type *KRAS* (P=0.016)[31]
Cetuximab with or without chemotherapy or panitumumab	16 of 48 (33%)	1 of 16 (6%)	10 of 32 (31%)	Better median TTP in patients with wild-type vs mutant *KRAS* (P=0.044)[22]
Cetuximab and chemotherapy	10 of 27 (37%)	1 of 10 (10%)	9 of 17 (53%)	Wild-type *KRAS* correlated with ORR (P=0.05)[32]
Cetuximab and chemotherapy	22 of 59 (37%)	0 of 22 (0%)	12 of 37 (32%)	*KRAS* mutations associated with progressive disease (P=0.0005) and with worse TTP (3.0 vs 5.5 months, P<0.015)[33]
Cetuximab	30 of 80 (38%)	0 of 30 (%)	5 of 50 (10%)	Disease control rate (PR+ stable disease) higher in patients with wild-type vs mutant *KRAS* (10% vs 48%, P=0.0003)[43]
Cetuximab and chemotherapy	42 of 108 (39%)	0 of 42 (0%)	27 of 66 (40%)	Longer median OS in patients with wild-type vs *KRAS* mutations (43 vs 27.2 weeks, P=0.02)[35]
Cetuximab and chemotherapy	24 of 89 (27%)	0 of 24 (0%)	26 of 65 (40%)	Longer median DFS (31.4 vs 10.1 weeks, P=0.0001) and median OS (14.3 vs 10.1 months, P=0.0001) in patients with wild-type *KRAS* vs *KRAS* mutations[36]
Cetuximab and chemotherapy	27 of 64 (42%)	1 of 27 (4%)	10 of 37 (27%)	Wild-type *KRAS* correlates with improved ORR (P=0.02) and with longer PFS (5.3 vs 3.0 months, P=0.024)[37]
Cetuximab with or without chemotherapy or panitumumab: summary of the above studies	194 of 536 (36%)	5 of 194 (2.5%)	118 of 342 (34.5%)	Total numbers should be interpreted with caution as they derive from the sum of data from retrospective analyses of studies; however, all the studies show similar results

Abbreviations: CR, complete response; DFS, disease-free survival; mCRC, metastatic colorectal cancer; ORR, overall response rate; OS, overall survival; PR, partial response; PFS, progression-free survival; TTP, time to progression.

Normanno N et al. Nature Reviews Clinical Oncology, 6:519-27, 2009

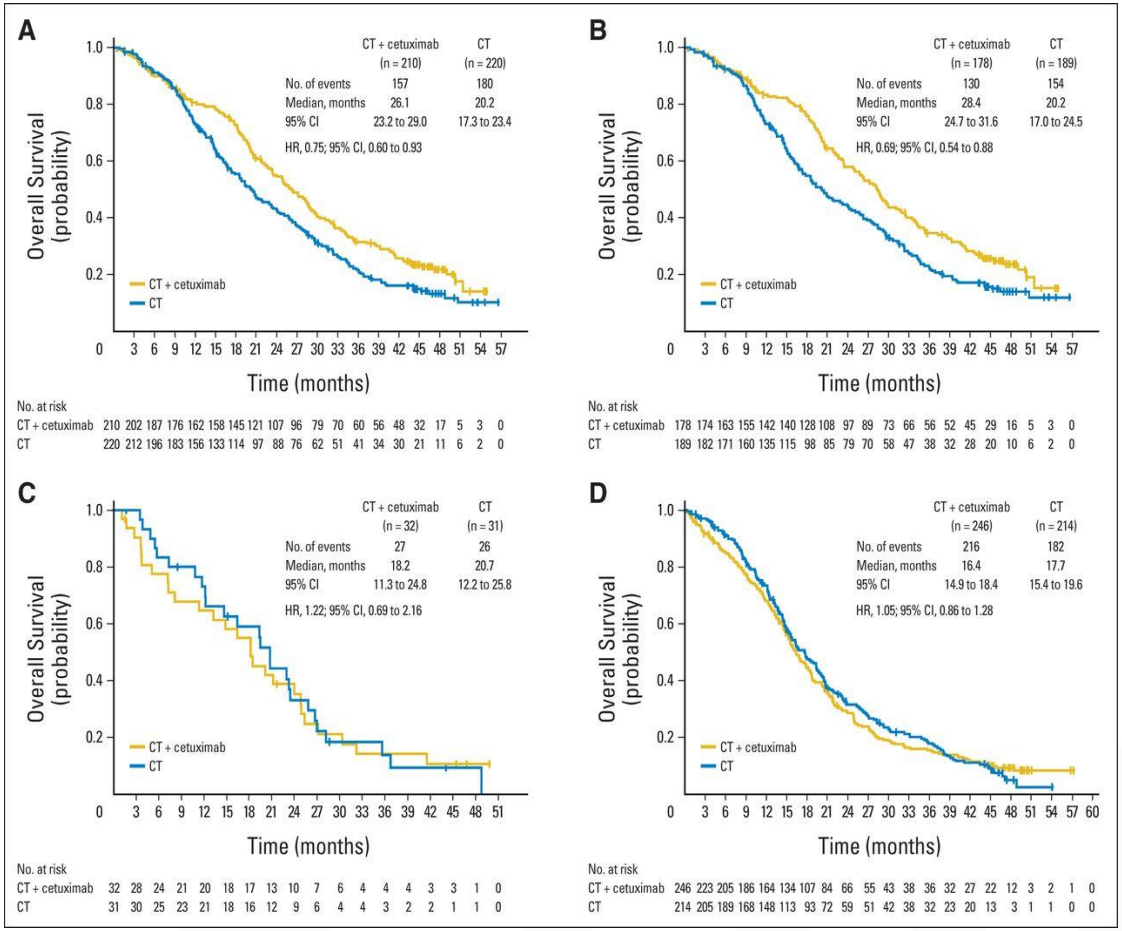
Cetuximab efficacy in all *RAS* wt patients

***KRAS* exon 2 wt**

All *RAS* wt

***KRAS* exon 2 wt**
Other *RAS* mut

RAS mut



CRYSTAL study	ITT	RAS ex 2 wt	All-RAS wt
Population	100%	60%	40-45%
OS HR	0.93	0.8	0.69
mOS (m)	19.9 vs 18.6	23.5 vs 20	28.4 vs 20.2

Van Cutsem et al. J Clin Oncol 2015

Pharmacogenomic and Pharmacoproteomic Studies of Cetuximab in Metastatic Colorectal Cancer: Biomarker Analysis of a Phase I Dose-Escalation Study

Josep Tabernero, Andres Cervantes, Fernando Rivera, Erika Martinelli, Federico Rojo, Anja von Heydebreck, Teresa Macarulla, Edith Rodriguez-Braun, Maria Eugenia Vega-Villegas, Stefanie Senger, Francisco Javier Ramos, Susana Roselló, Ilhan Celik, Christopher Stroh, José Baselga, and Fortunato Ciardiello

See accompanying article on page 1254

A B S T R A C T

Purpose
This study assessed biomarkers for cetuximab efficacy in tissue samples collected during a phase I dose-escalation study exploring every second week administration of cetuximab as first-line therapy in patients with metastatic colorectal cancer (mCRC).

Patients and Methods
Sixty-two patients received cetuximab monotherapy for 6 weeks, followed by cetuximab plus infusional fluorouracil, leucovorin, and irinotecan until disease progression. Patients in the control arm received cetuximab as a 400 mg/m^2 initial dose then 250 mg/m^2 per week; patients in the dose-escalation arms received 400 to 700 mg/m^2 every second week. Tumor and skin biopsies were taken for immunohistochemical and microarray expression analyses (tumor only) at baseline and week 4. Plasma was collected for proteomic analysis at baseline and week 4. *KRAS* tumor mutation status was assessed.

Results
In subsets of paired skin samples from 35 patients, cetuximab treatment was associated with substantial downregulation of phospho(p)-EGFR, p-MAPK and proliferation and substantial upregulation of p27^{Kip1} and p-STAT3 levels. No marked difference in these effects was noted for different schedules of administration and dose levels. In the cetuximab monotherapy phase, responses were seen only in patients whose tumors were wild-type for *KRAS* (eight of 29 *v* zero of 19 for *KRAS* mutant tumors; $P = .015$). Progression-free survival was longer for patients with *KRAS* wild-type compared with *KRAS* mutant tumors (log-rank $P = .048$). Genomics/proteomics analyses (42 and 45 patients, respectively) identified candidate biomarkers associated with response.

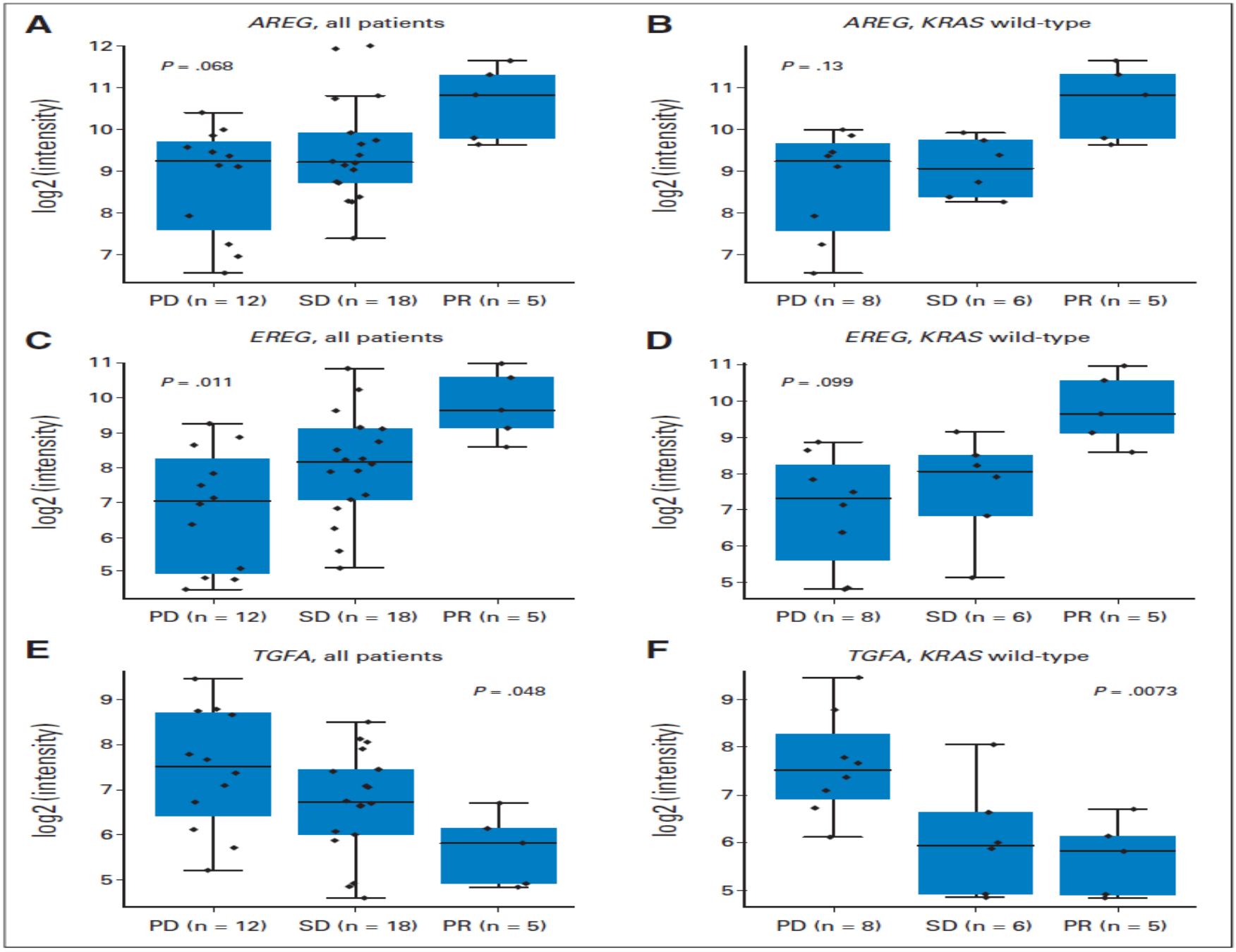
Conclusion
Biomarker analysis supported the functional equivalence of weekly and every second week administration of cetuximab and provided further confirmation that patients with *KRAS* wild-type mCRC were those most likely to benefit from cetuximab treatment.

J Clin Oncol 28:1181-1189. © 2010 by American Society of Clinical Oncology

Table 1. Correlation of *KRAS* Status With Response Rate and PFS

Parameter	Tumor *KRAS* Status							
	Cetuximab Monotherapy				Cetuximab Plus FOLFIRI			
	Wild-Type		Mutation		Wild-Type		Mutation	
	No.	%	No.	%	No.	%	No.	%
No. of patients	29		19		29		19	
Response								
Complete response	0		0		0		0	
Partial response	8	28	0		16	55	6	32
Stable disease	12	41	11	58	11	38	10	53
Progressive disease	9	31	8	42	0		3	16
Not evaluable	0		0		2	7	0	
Overall response rate	8	28	0	—	16	55	6	32
95% CI	13 to 47		0 to 18		36 to 74		13 to 57	
Fisher's exact test *P*	.015				.144			
Median PFS, months	—		—		9.4		5.6	
95% CI					7.0 to 11.3		3.3 to 12.2	
Hazard ratio					0.47			
Log-rank *P*					.0475			

Abbreviations: FOLFIRI, infusional fluorouracil, leucovorin, and irinotecan; PFS, progression-free survival.


A
AREG, all patients
P = .068
log2 (intensity)
PD (n = 12)
SD (n = 18)
PR (n = 5)
B
AREG, KRAS wild-type
P = .13
log2 (intensity)
PD (n = 8)
SD (n = 6)
PR (n = 5)
C
EREG, all patients
P = .011
log2 (intensity)
PD (n = 12)
SD (n = 18)
PR (n = 5)
D
EREG, KRAS wild-type
P = .099
log2 (intensity)
PD (n = 8)
SD (n = 6)
PR (n = 5)
E
TGFA, all patients
P = .048
log2 (intensity)
PD (n = 12)
SD (n = 18)
PR (n = 5)
F
TGFA, KRAS wild-type
P = .0073
log2 (intensity)
PD (n = 8)
SD (n = 6)
PR (n = 5)

Cancer Therapy: Preclinical

Clinical Cancer Research

Increased TGF-α as a Mechanism of Acquired Resistance to the Anti-EGFR Inhibitor Cetuximab through EGFR–MET Interaction and Activation of MET Signaling in Colon Cancer Cells

Teresa Troiani[1], Erika Martinelli[1], Stefania Napolitano[1], Donata Vitagliano[1], Loreta Pia Ciuffreda[3], Sara Costantino[3], Floriana Morgillo[1], Anna Capasso[1], Vincenzo Sforza[1], Anna Nappi[1], Raffaele De Palma[2], Elena D'Aiuto[2], Liberato Berrino[3], Roberto Bianco[4], and Fortunato Ciardiello[1]

Abstract

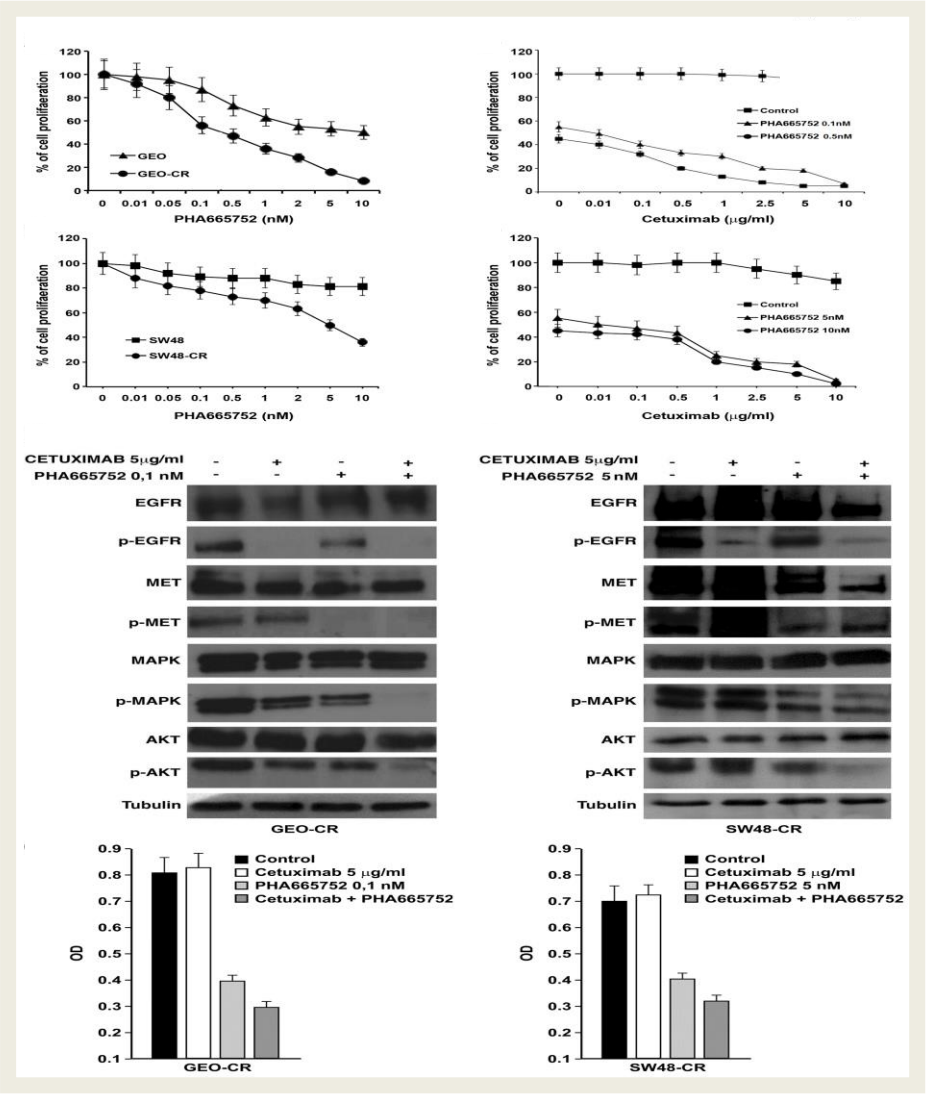
Purpose: Although cetuximab, an anti-EGF receptor (EGFR) monoclonal antibody, is an effective treatment for patients with *KRAS* wild-type metastatic colorectal cancer (mCRC), its clinical use is limited by onset of resistance.

Experimental Design: We characterized two colorectal cancer models to study the mechanisms of acquired resistance to cetuximab.

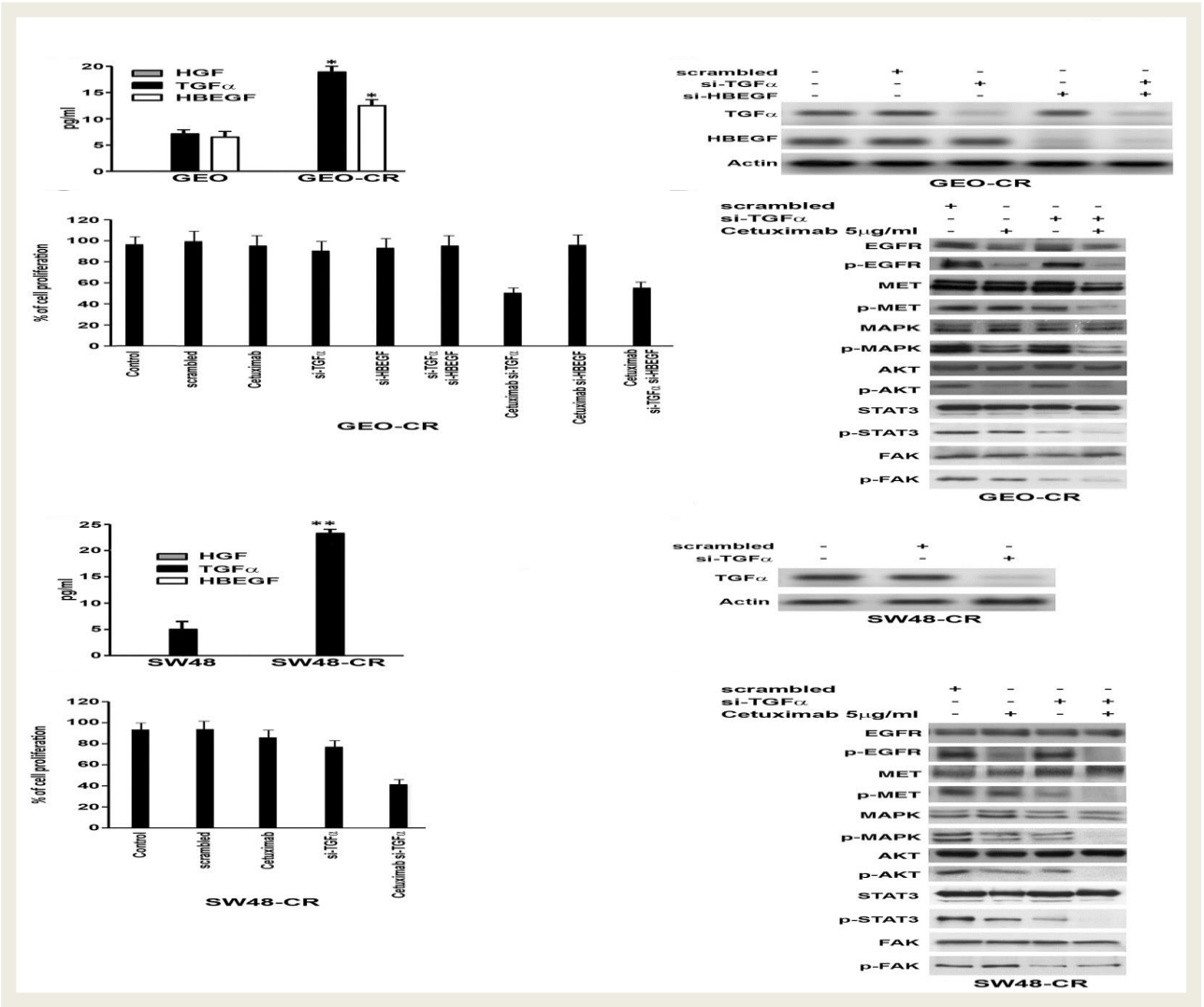
Results: Following chronic treatment of nude mice bearing cetuximab-sensitive human GEO colon xenografts, cetuximab-resistant GEO (GEO-CR) cells were obtained. In GEO-CR cells, proliferation and survival signals were constitutively active despite EGFR inhibition by cetuximab treatment. Whole gene expression profiling identified a series of genes involved in the hepatocyte growth factor (HGF)-MET–dependent pathways, which were upregulated in GEO-CR cells. Furthermore, activated, phosphorylated MET was detected in GEO-CR cells. A second colorectal cancer cell line with acquired resistance to cetuximab was obtained (SW48-CR). Inhibition of MET expression by siRNA restored cetuximab sensitivity in GEO-CR and SW48-CR cells, whereas exogenous activation of MET by HGF stimulation in cetuximab-sensitive GEO and SW48 cells induced resistance to cetuximab. Treatment of GEO-CR and SW48-CR cells with PHA665752, a selective MET inhibitor, inhibited cell growth, proliferation, and survival signals and impaired cancer cell migration. Overexpression of TGF-α, a specific EGFR ligand, was involved in the acquisition of cetuximab resistance in GEO-CR and SW48-CR cells. In fact, TGF-α overexpression induced the EGFR–MET interaction, with subsequent MET phosphorylation and activation of MET downstream effectors in GEO-CR and SW48-CR cells.

Conclusions: These results suggest that overexpression of TGF-α through induction of EGFR–MET interaction contributes to cetuximab resistance in colorectal cancer cells. The combined inhibition of EGFR and MET receptor could represent a strategy for preventing and/or overcoming cetuximab resistance in patients with colorectal cancer. *Clin Cancer Res; 19(24); 6751–65. ©2013 AACR.*

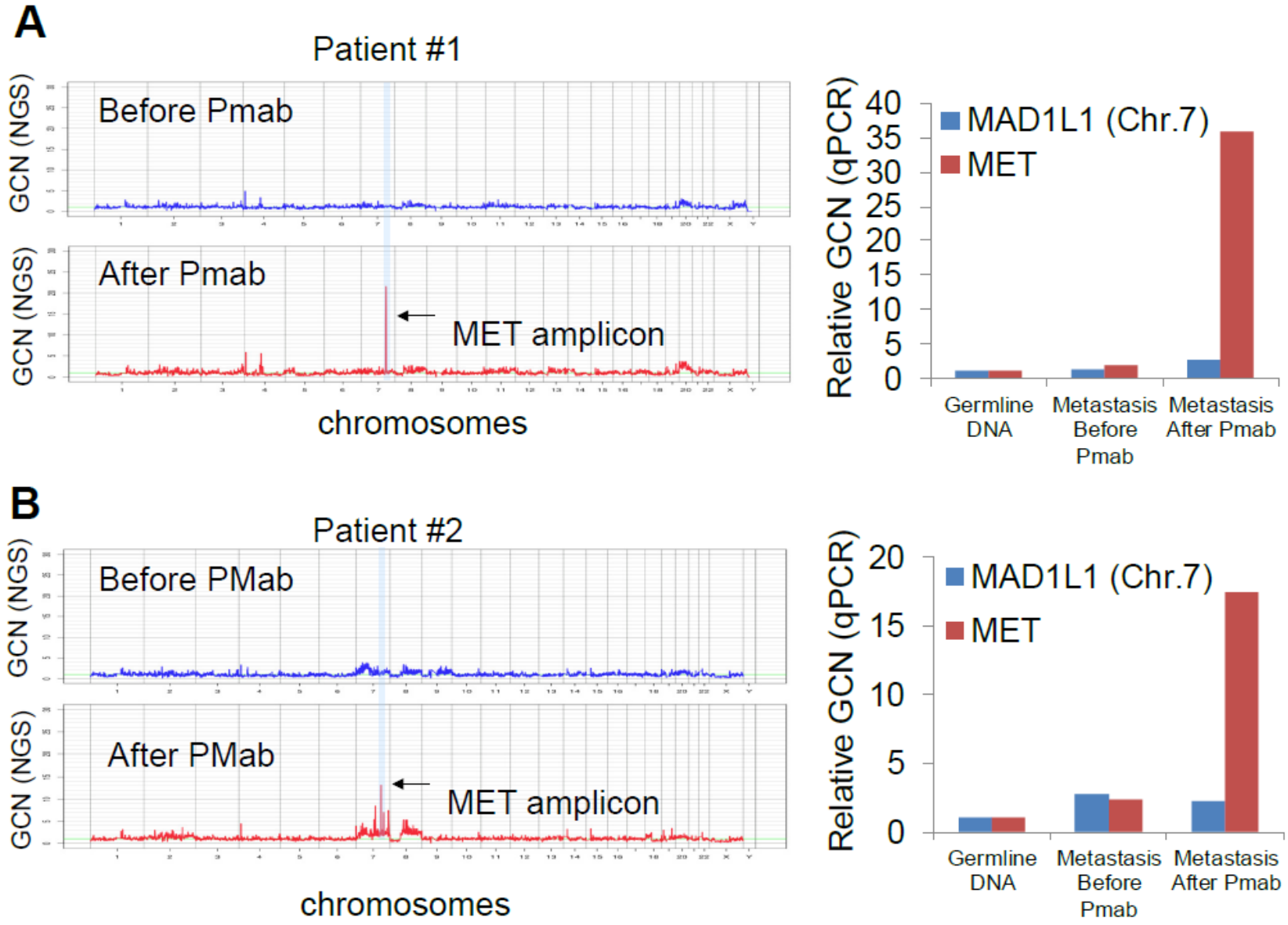
Treatment with a selective MET tyrosine kinase inhibitor sensitizes GEO-CR and SW48-CR cells to cetuximab



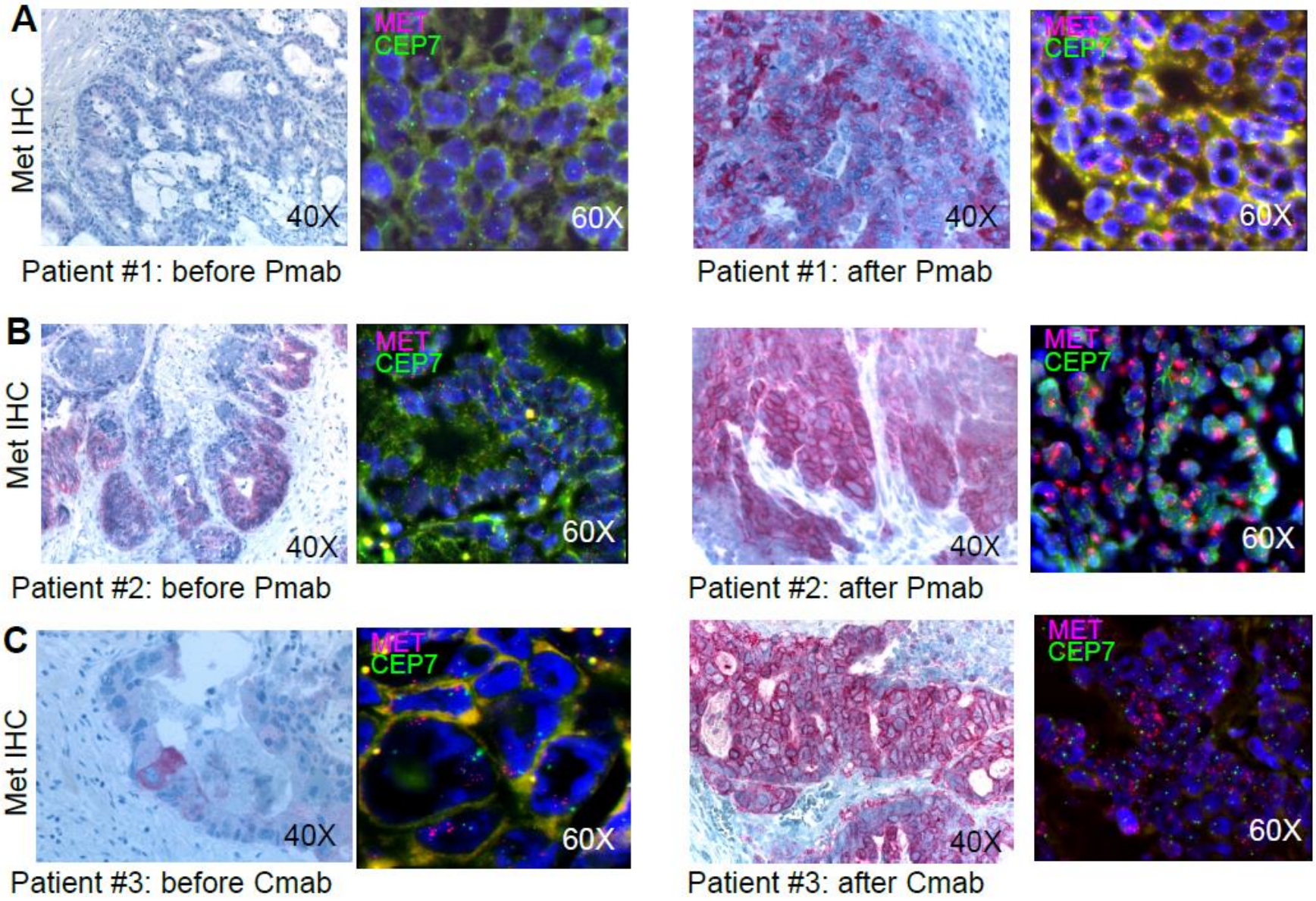
Inhibition of TGFα expression reverts cetuximab-resistance in GEO-CR and SW48-CR cells



Detection of MET amplification in patients treated with anti-EGFR MoAbs

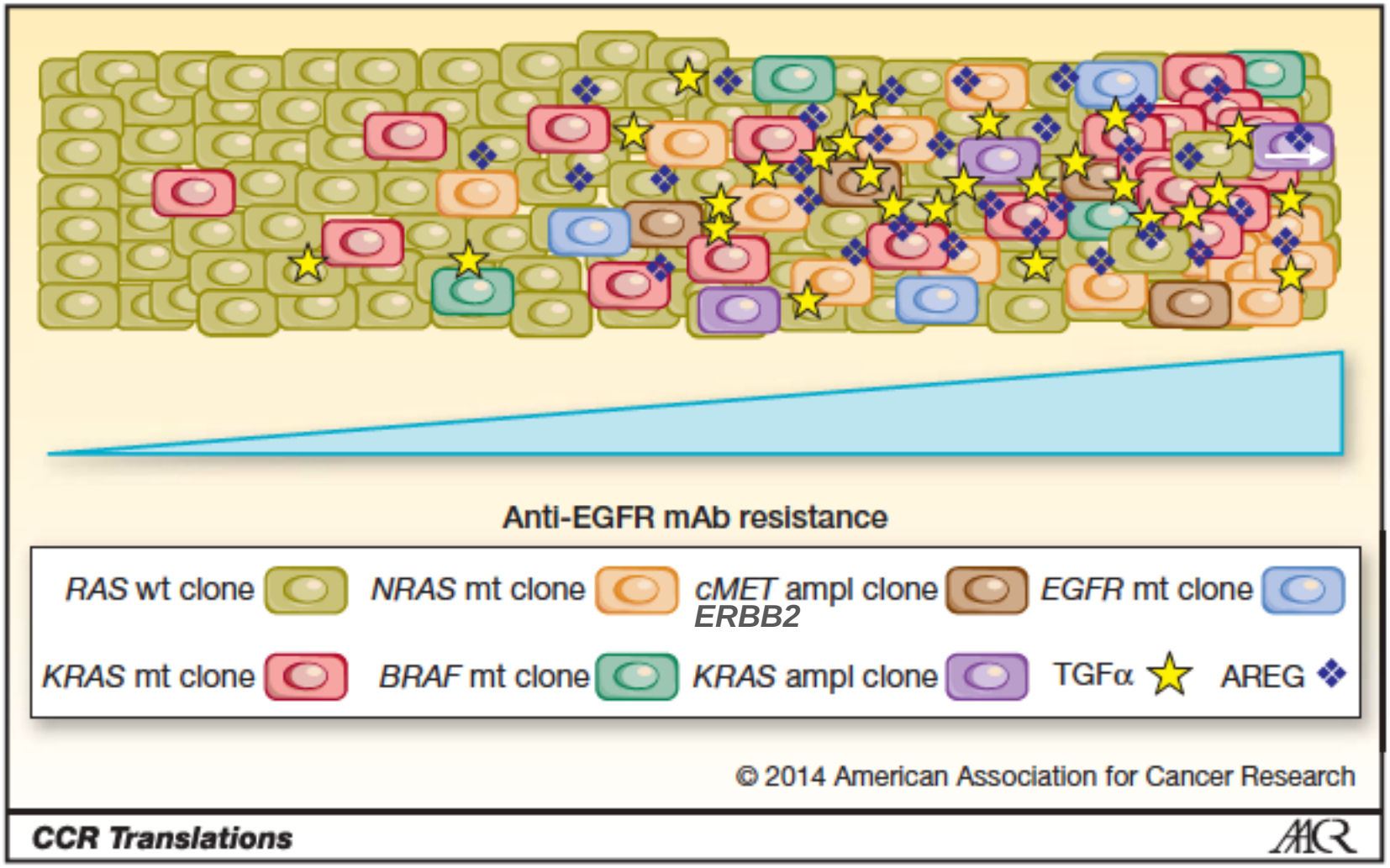


Bardelli. et al. Cancer Discovery 2013

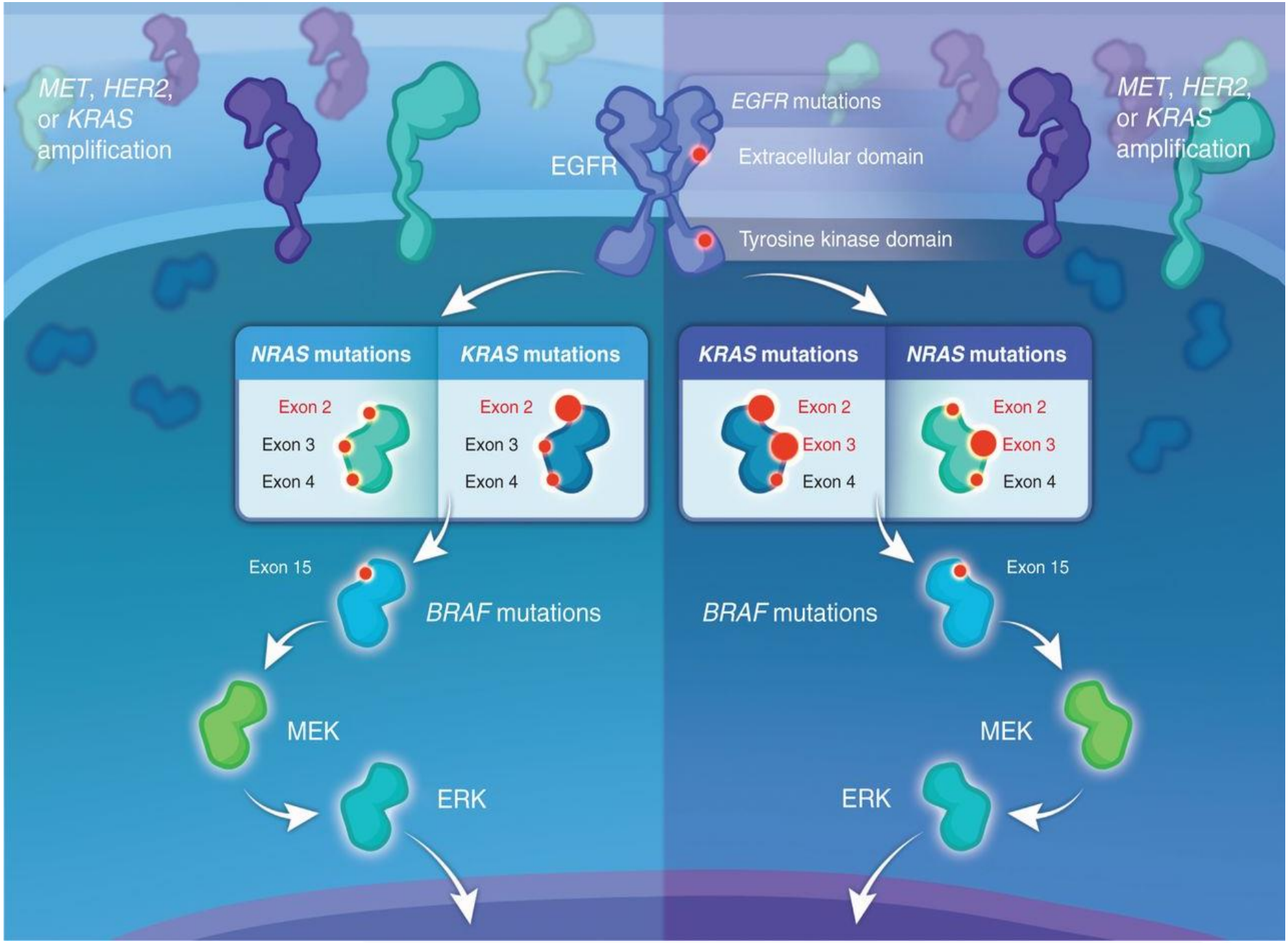


Bardelli. et al. Cancer Discovery 2013

Multiple mechanisms of acquired resistance to EGFR inhibition in CRC



Salazar R & Tabernero J, Clin Cancer Res 2014



Misale et al. Cancer Discov 2014

Cancer Therapy: Preclinical

Clinical Cancer Research

Primary and Acquired Resistance of Colorectal Cancer Cells to Anti-EGFR Antibodies Converge on MEK/ERK Pathway Activation and Can Be Overcome by Combined MEK/EGFR Inhibition

Teresa Troiani, Stefania Napolitano, Donata Vitagliano, Floriana Morgillo, Anna Capasso, Vincenzo Sforza, Anna Nappi, Davide Ciardiello, Fortunato Ciardiello, and Erika Martinelli

Abstract

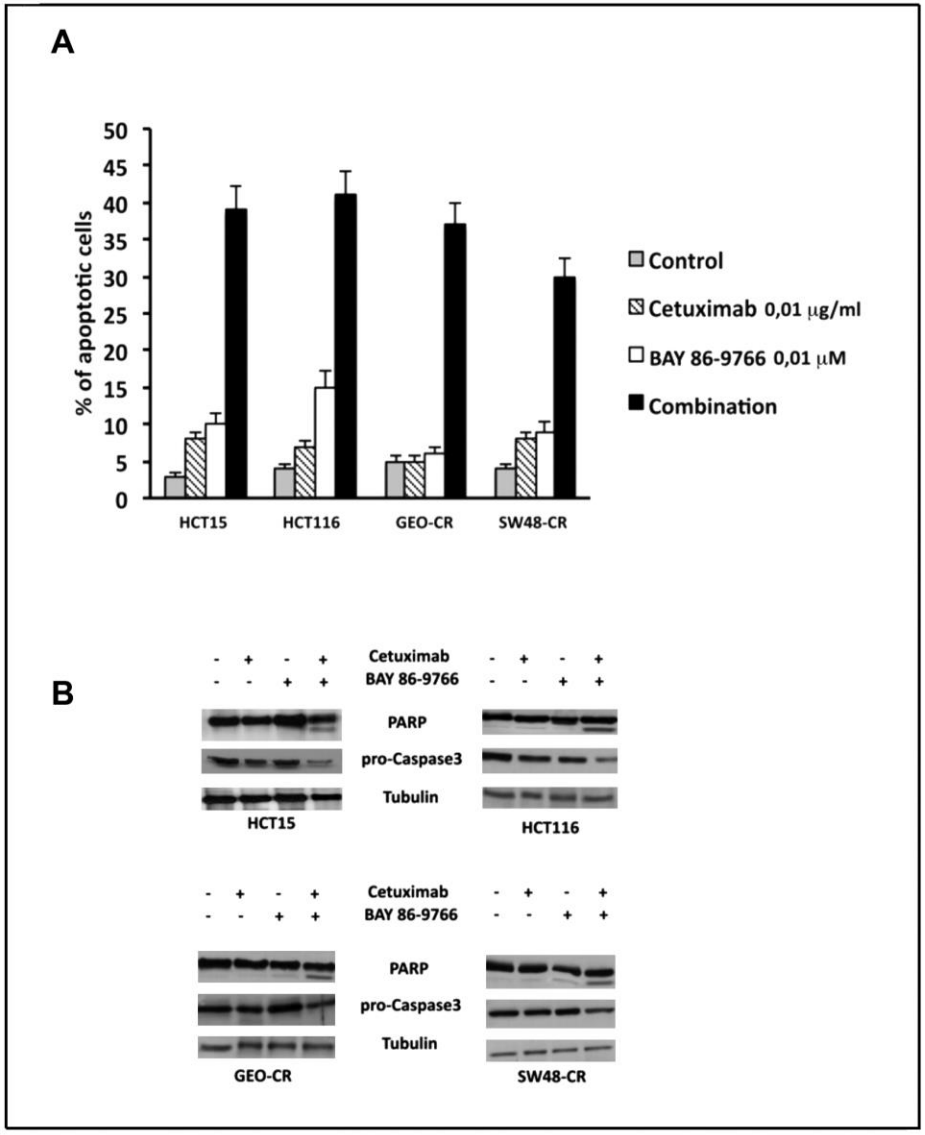
Purpose: The EGFR-independent activation of the RAS/RAF/MEK/MAPK pathway is one of the resistance mechanisms to cetuximab.

Experimental Design: We have evaluated, *in vitro* and *in vivo*, the effects of BAY 86-9766, a selective MEK1/2 inhibitor, in a panel of human colorectal cancer cell lines with primary or acquired resistance to cetuximab.

Results: Among the colorectal cancer cell lines, five with a *KRAS* mutation (LOVO, HCT116, HCT15, SW620, and SW480) and one with a *BRAF* mutation (HT29) were resistant to the antiproliferative effects of cetuximab, whereas two cells (GEO and SW48) were highly sensitive. Treatment with BAY 86-9766 determined dose-dependent growth inhibition in all cancer cells, including two human colorectal cancer cells with acquired resistance to cetuximab (GEO-CR and SW48-CR), with the exception of HCT15 cells. Combined treatment with cetuximab and BAY 86-9766 induced a synergistic antiproliferative and apoptotic effects with blockade in the MAPK and AKT pathway in cells with either primary or acquired resistance to cetuximab. The synergistic antiproliferative effects were confirmed using other two selective MEK1/2 inhibitors, selumetinib and pimasertib, in combination with cetuximab. Moreover, inhibition of MEK expression by siRNA restored cetuximab sensitivity in resistant cells. In nude mice bearing established human HCT15, HCT116, SW48-CR, and GEO-CR xenografts, the combined treatment with cetuximab and BAY 86-9766 caused significant tumor growth inhibition and increased mice survival.

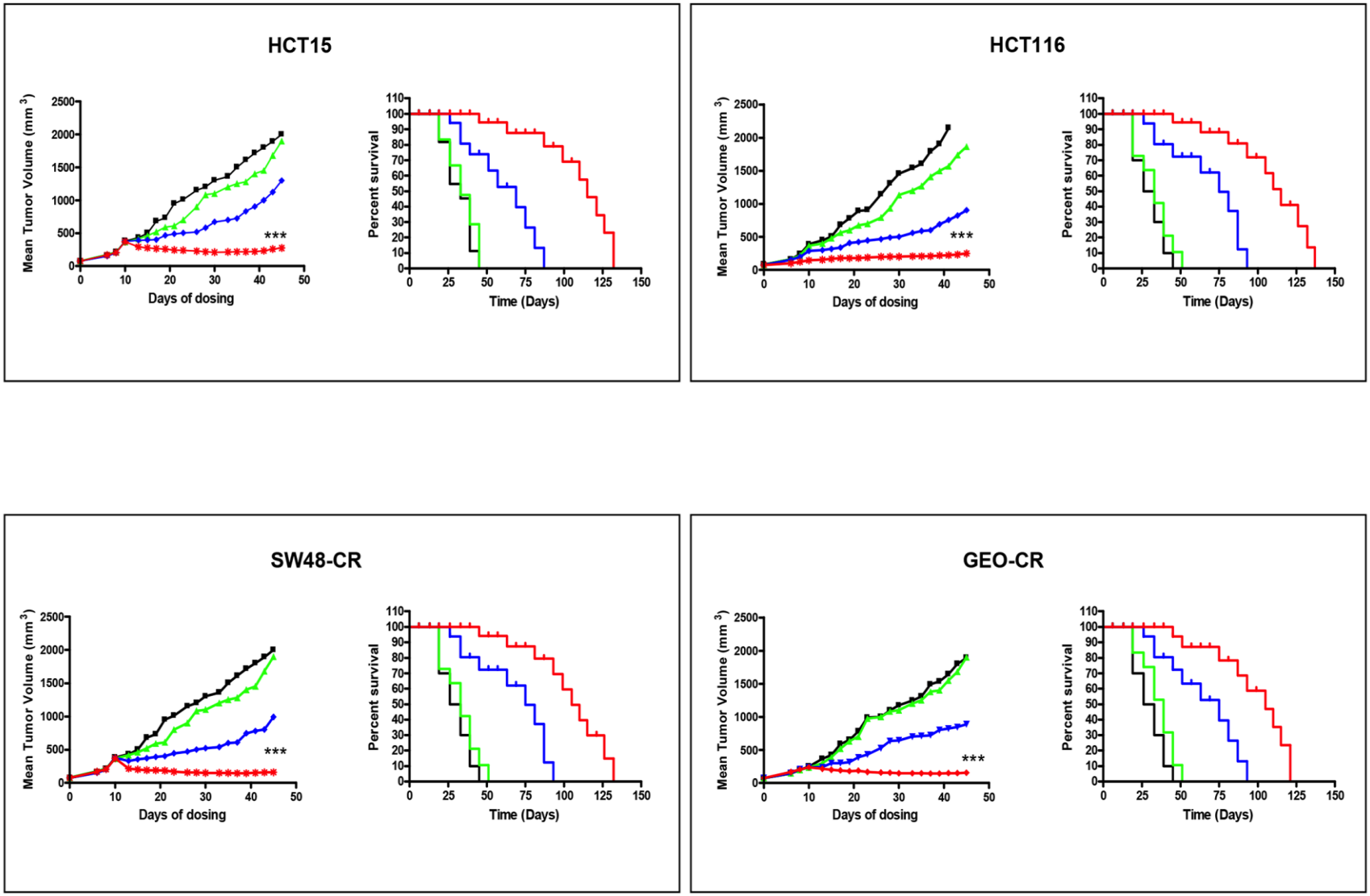
Conclusion: These results suggest that activation of MEK is involved in both primary and acquired resistance to cetuximab and the inhibition of EGFR and MEK could be a strategy for overcoming anti-EGFR resistance in patients with colorectal cancer. *Clin Cancer Res; 1–12. ©2014 AACR.*

Fig. 4

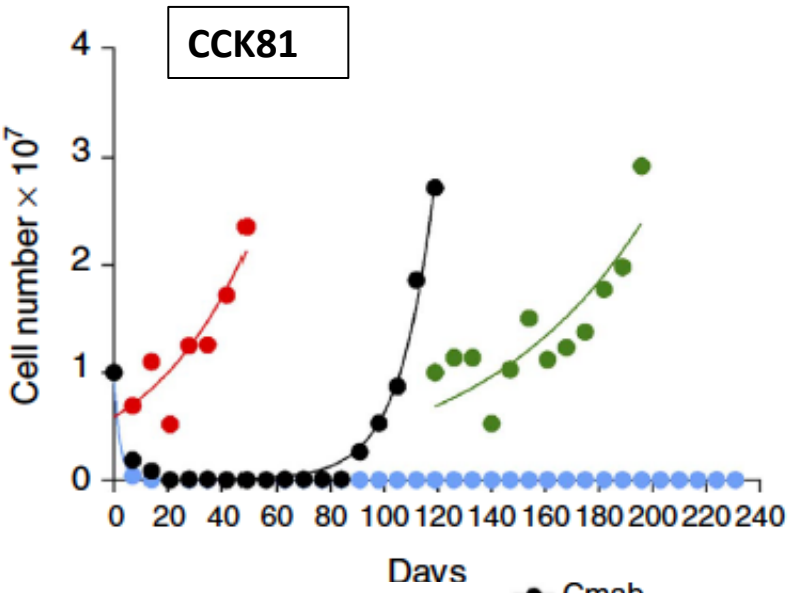

A
% of apoptotic cells
50
45
40
35
30
25
20
15
10
5
0
HCT15
HCT116
GEO-CR
SW48-CR
Control
Cetuximab 0,01 μg/ml
BAY 86-9766 0,01 μM
Combination
B
Cetuximab
BAY 86-9766
PARP
pro-Caspase3
Tubulin
HCT15
HCT116
GEO-CR
SW48-CR
C
Cetuximab
BAY 86-9766
p-EGFR
EGFR
p-MEK
MEK
p-AKT
AKT
p-MAPK
MAPK
Tubulin
HCT15
HCT116
D
SW48-CR
GEO-CR

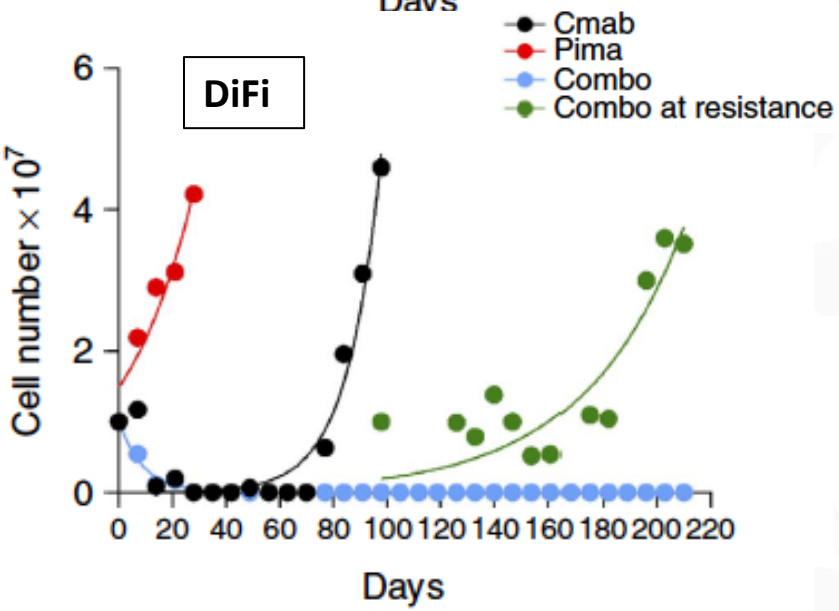
Control Cetuximab BAY 86-9766 Combination

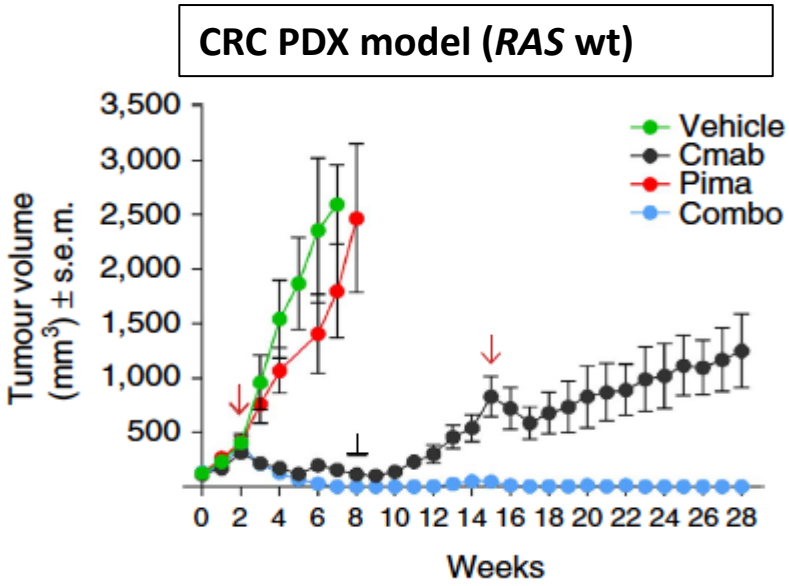
Fig.6

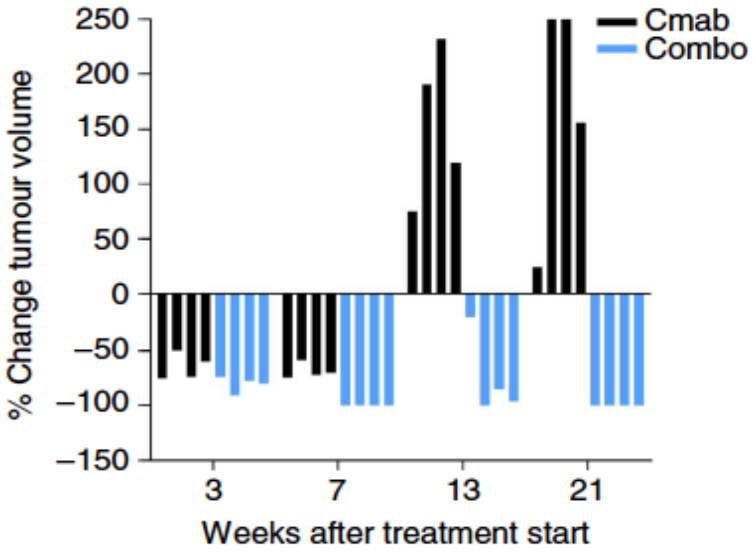


Preventing and/or delaying secondary resistance: EGFRi + MEKi









Misale S et al. Nature Commun 2015

A model of sequential treatment in EGFR inhibitor-sensitive human colon cancer xenografts: the role of MEK inhibition in preventing/delaying EGFR resistance development

Troiani T. et al., Clinical Cancer Research 2015

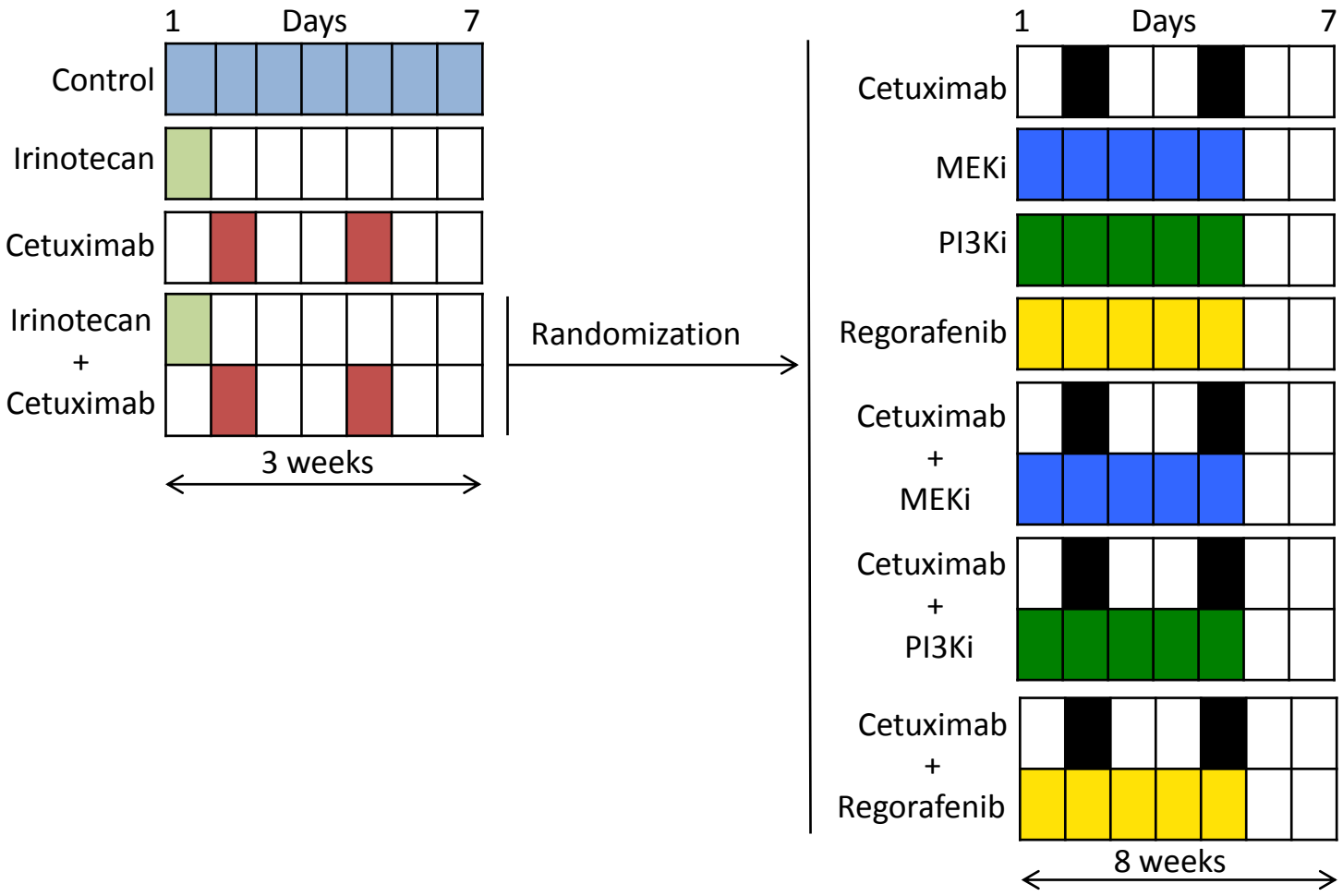
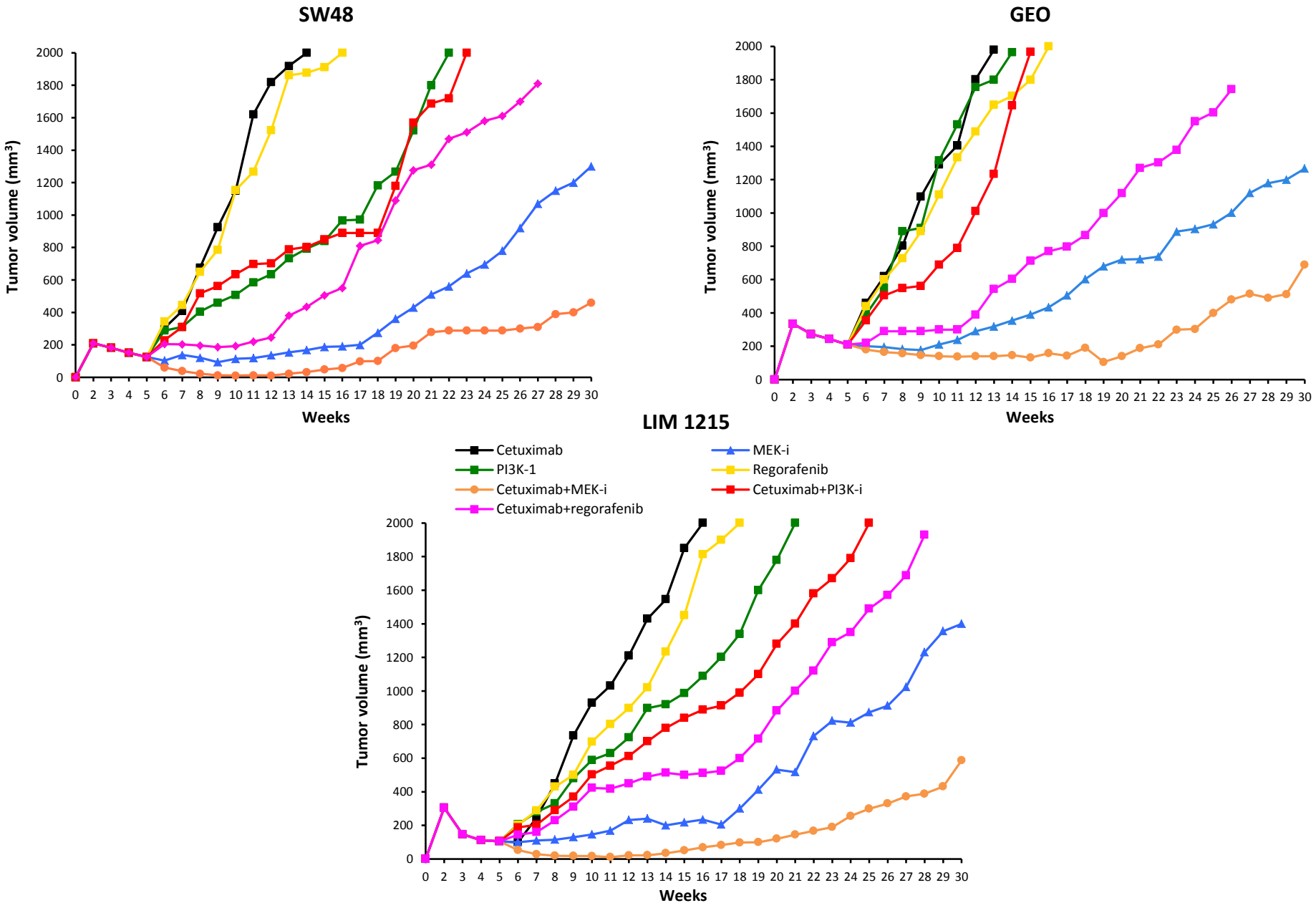
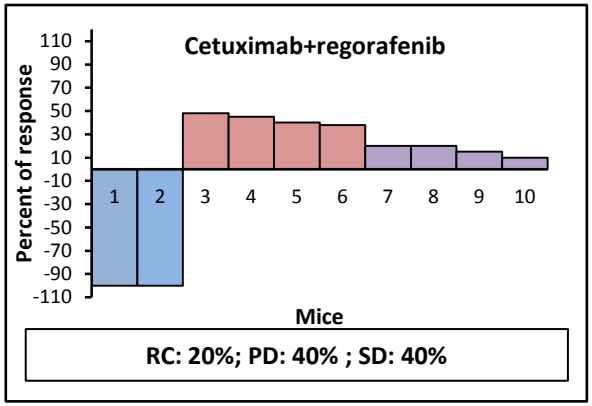

1
Days
7
Control
Irinotecan
Cetuximab
Irinotecan
+
Cetuximab
3 weeks
Randomization
1
Days
7
Cetuximab
MEKi
PI3Ki
Regorafenib
Cetuximab
+
MEKi
Cetuximab
+
PI3Ki
Cetuximab
+
Regorafenib
8 weeks

Figure 2


SW48
GEO
LIM 1215
Tumor volume (mm³)
Weeks
Cetuximab
PI3K-1
Cetuximab+MEK-i
Cetuximab+regorafenib
MEK-i
Regorafenib
Cetuximab+PI3K-i

SW48



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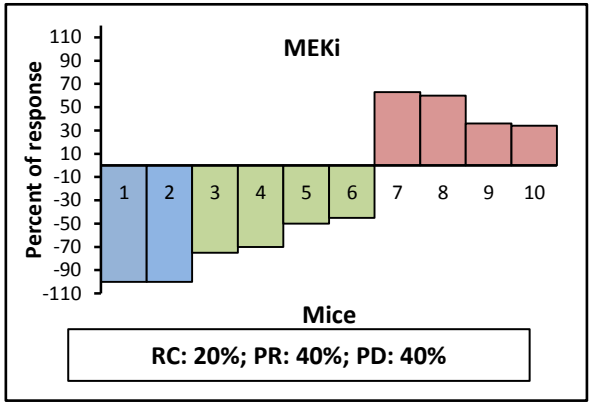

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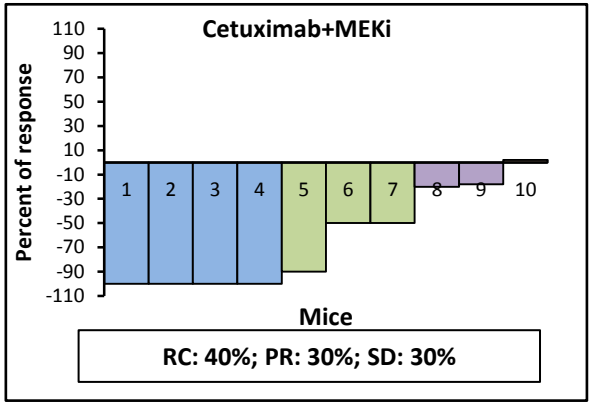
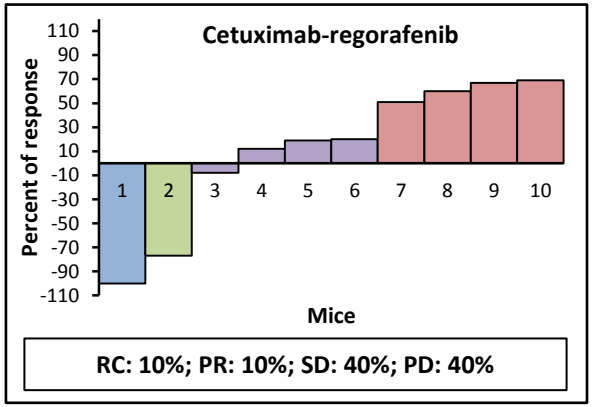


RC: 40%; PR: 30%; SD: 30%

GEO



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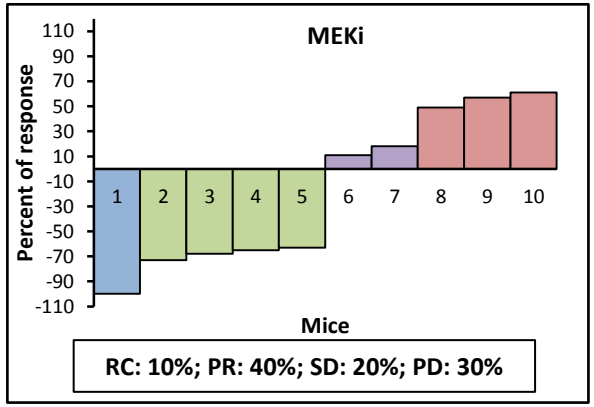

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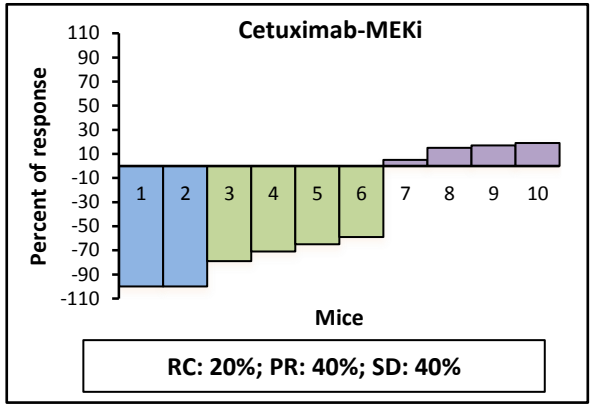
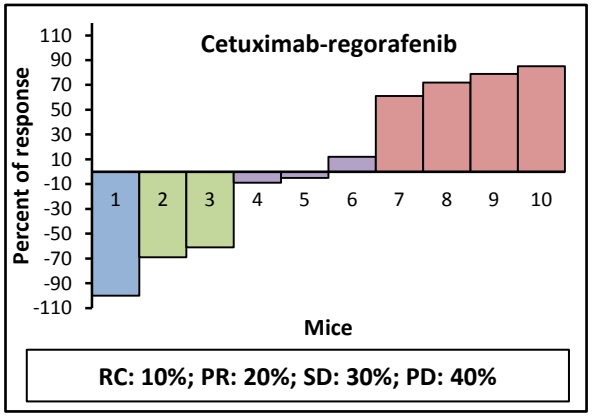


RC: 20%; PR: 40%; SD: 40%

LIM 1215



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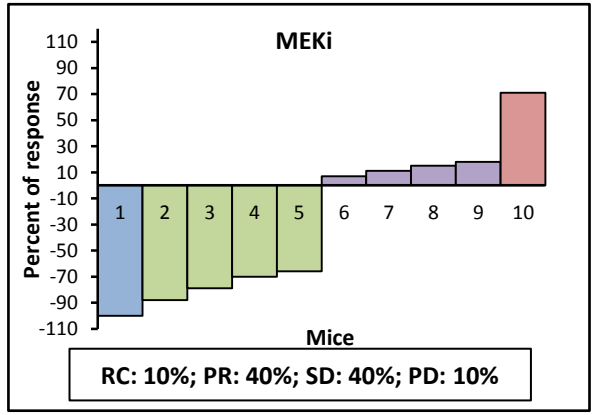
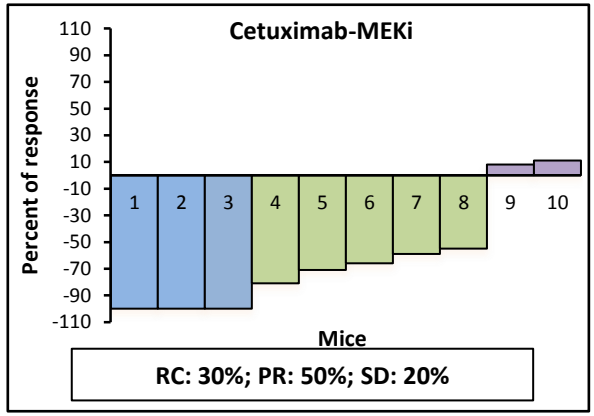


RC: 10%; PR: 40%; SD: 40%; PD: 10%



RC: 30%; PR: 50%; SD: 20%

Figure 3

SW48

Maintenance treatment groups	Week 2			Week 5			Week 13			Week 30		
	A	B	C	A	B	C	A	B	C	A	B	C
CTR	210	10/10	0	125	10/10	0	0	0/10	0	0	0/10	0
Cetuximab	210	10/10	0	125	10/10	0	1919	2/10	0	0	0/10	0
MEKi	210	10/10	0	125	10/10	0	153	10/10	2	1300	1/10	1
Pi3Ki	210	10/10	0	125	10/10	0	733	9/10	0	0	0/10	0
Regorafenib	210	10/10	0	125	10/10	0	1862	4/10	0	0	0/10	0
Cetuximab+ MEKi	210	10/10	0	125	10/10	0	22	10/10	4	460	5/10	2
Cetuximab+ Pi3Ki	210	10/10	0	125	10/10	0	788	3/10	0	0	0/10	0
Cetuximab + Regorafenib	210	10/10	0	125	10/10	0	380	10/10	2	1810	1/10	1

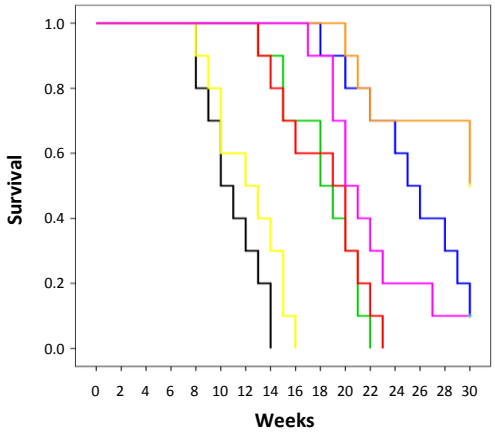
GEO

Maintenance treatment groups	Week 2			Week 5			Week 13			Week 30		
	A	B	C	A	B	C	A	B	C	A	B	C
CTR	335	10/10	0	210	10/10	0	0	0/10	0	0	0/10	0
Cetuximab	335	10/10	0	210	10/10	0	0	0/10	0	0	0/10	0
MEKi	335	10/10	0	210	10/10	0	318	9/10	1	1267	1/10	1
Pi3Ki	335	10/10	0	210	10/10	0	1800	3/10	0	0	0/10	0
Regorafenib	335	10/10	0	210	10/10	0	1400	5/10	0	0	0/10	0
Cetuximab+ MEKi	335	10/10	0	210	10/10	0	140	10/10	2	690	4/10	2
Cetuximab+ Pi3Ki	335	10/10	0	210	10/10	0	1234	6/10	0	0	0/10	0
Cetuximab + Regorafenib	335	10/10	0	210	10/10	0	543	10/10	1	1980	1/10	0

LIM 1215

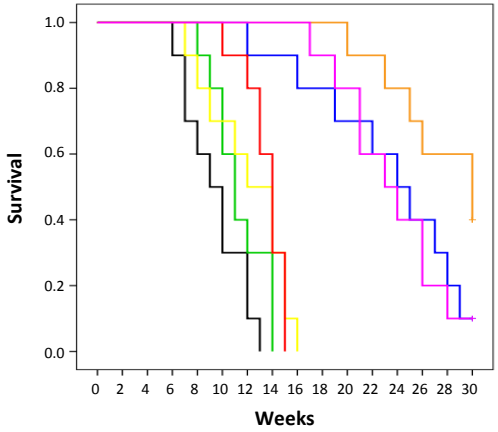
Maintenance treatment groups	Week 2			Week 5			Week 13			Week 30		
	A	B	C	A	B	C	A	B	C	A	B	C
CTR	305	10/10	0	105	10/10	0	0	0/10	0	0	0/10	0
Cetuximab	305	10/10	0	105	10/10	0	1430	3/10	0	0	0/10	0
MEKi	305	10/10	0	105	10/10	0	240	9/10	1	1400	1/10	0
Pi3Ki	305	10/10	0	105	10/10	0	898	7/10	0	0	0/10	0
Regorafenib	305	10/10	0	105	10/10	0	1031	4/10	0	0	0/10	0
Cetuximab+ MEKi	305	10/10	0	105	10/10	0	22	10/10	3	587	4/10	2
Cetuximab+ Pi3Ki	305	10/10	0	105	10/10	0	700	8/10	0	0	0/10	0
Cetuximab + Regorafenib	305	10/10	0	105	10/10	0	490	10/10	1	0	0/10	0

SW48



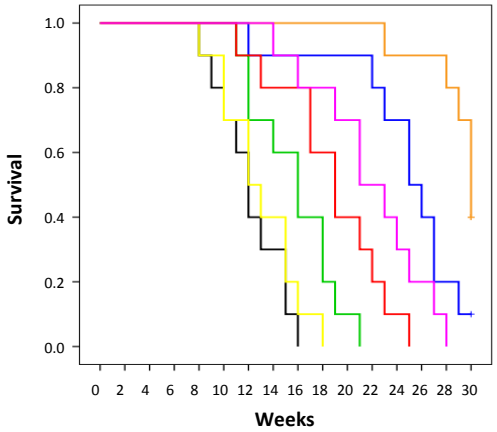
ARM	Median survival (95% CI) in weeks
Cetuximab	10 (7.9 - 12.1)
MEKi	25 (21.9 - 28.1)
PI3Ki	18 (13.9 – 22.1)
Regorafenib	12 (7.4 – 16.6)
Cetuximab+MEKi	30 (27.5 – 33.2)
Cetuximab+PI3Ki	19 (14.9 – 23.1)
Cetuximab+Regorafenib	20 (17.9 – 22.1)

GEO



ARM	Median survival (95% CI) in weeks
Cetuximab	9 (6.9 - 11.06)
MEKi	24 (19.3 - 28.6)
PI3Ki	11(9.4 – 12.5)
Regorafenib	12 (8.9 – 15.0)
Cetuximab+MEKi	30 (23.9-36.0)
Cetuximab+PI3Ki	14 (13.0 – 14.9)
Cetuximab+Regorafenib	23 (18.3– 27.6)

LIM 1215



ARM	Median survival (95% CI) in weeks
Cetuximab	12 (10.4 – 13.5)
MEKi	25 (21.9 – 28.0)
PI3Ki	16(12.9 – 19.0)
Regorafenib	12 (8.9 – 15.0)
Cetuximab+MEKi	30 (29.9-31.0)
Cetuximab+PI3Ki	19 (15.9 – 22.0)
Cetuximab+Regorafenib	21 (16.8– 25.1)

Cetuximab
MEKi
PI3Ki
Regorafenib
Cetuximab + MEKi
Cetuximab + PI3Ki
Cetuximab + Regorafenib

Treatment Group	Frequency of Genetic Alteration (percentage %)		
	KRAS	*BRAF*	*PIK3CA*
Control	0/4 (0%)	0/4 (0%)	0/4 (0%)
Cetuximab	0/4 (0%)	0/4 (0%)	0/4 (0%)
MEKi	3/4 (75%)	1/4 (25%)	0/4 (0%)
PI3Ki	0/4 (0%)	0/4 (0%)	1/4 (25%)
Regorafenib	0/4 (0%)	0/4 (0%)	1/4 (25%)
Cetuximab+MEKi	1/4 (25%)	0/4 (0%)	0/4 (0%)
Cetuximab+PI3Ki	0/4 (0%)	0/4 (0%)	0/4 (0%)
Cetuximab+Regorafenib	0/4 (0%)	0/4 (0%)	0/4 (0%)

Treatment Group	Type of Genetic Alteration (allele frequency %)
MEKi	KRAS p.G13D (43,5%)
MEKi	KRAS p.G13D (45,5%) + BRAF pV600K (32,8%)
MEKi	KRAS p.G13D(49,6%)
PI3Ki	PIK3CA p.H1047Y (2.1%)
Regorafenib	PIK3CA p.Q546R (1.8%)
Cetu+MEKi	KRAS pG13D(20.9%)

Conclusions

- Activation of the MEK/MAPK intracellular signalling pathway plays a major role in the intrinsic and in the acquired mechanims of resistance to EGFR inhibition in colorectal cancer.

- MEK/MAPK is a convergence mechanism of cancer cell escape to EGFR inhibition following activation of upstream signalling molecules (KRAS, NRAS, BRAF) as well as of constitutive activation of cell membrane growth factor receptors (EGFR mutations; HER2 amplification; MET amplification and/or activation).

- Combined treatment with EGFR inhibitors and selective MEK inhibitors could be a strategy to overcome and/or delay resistance to EGFR therapies.