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When to test for BRAF and what are the consequences?

by Dr. Chiara Cremolini, Dr. Armin Gerger
and Dr. Guillem Argilés

WHEN TO TEST FOR BRAF AND WHAT ARE THE CONSEQUENCES?

BY

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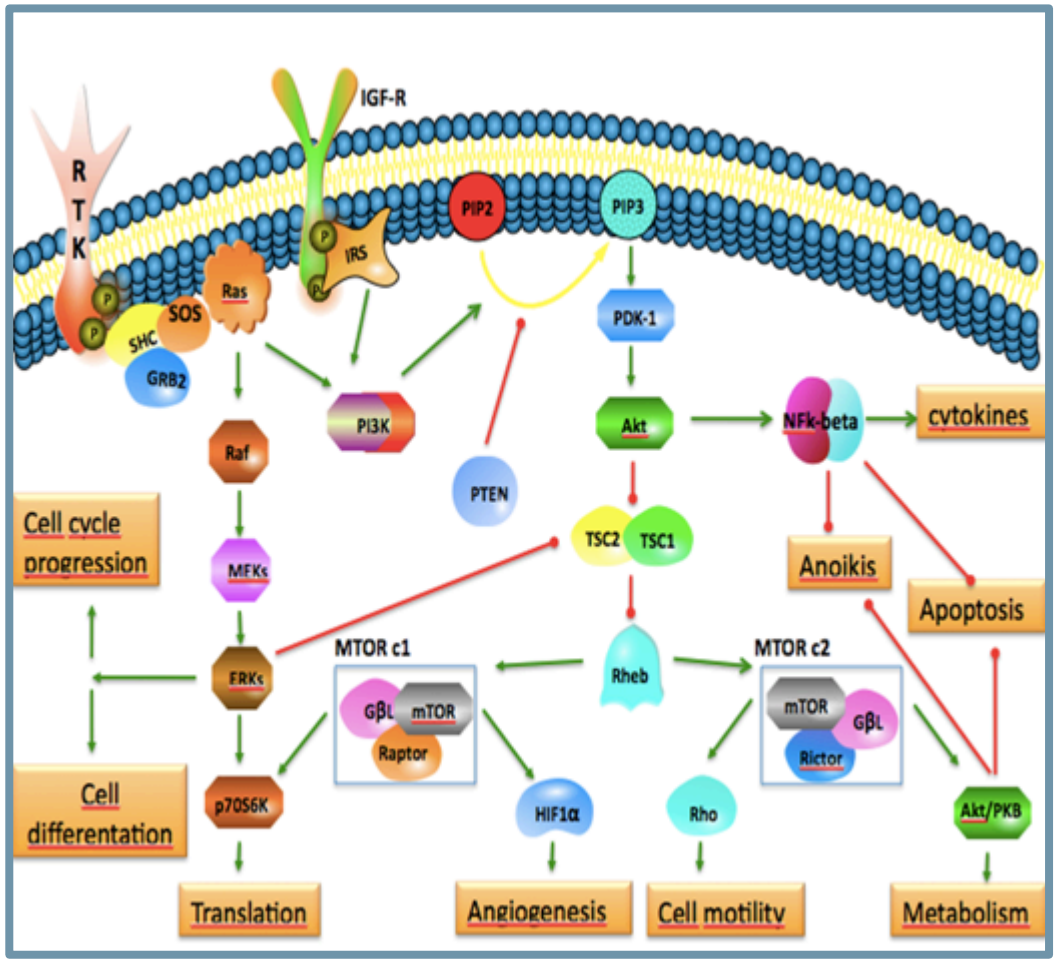
BRAF MUTATION, GENERAL CONSIDERATIONS



- BRAF V600E is found in approx. 5-10% of mCRC
  - 4% of non-hypermutat CRC
  - 46% of hypermutant CRC
- Driver mutation
- Considered mutually exclusive with RAS mutations

  (though ultra-sensitive NGS platform unveiled concomitancy with minor RAS mut. allele fractions in certain tumors)

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ROLE OF BRAF V600E IN LOCALIZED CRC

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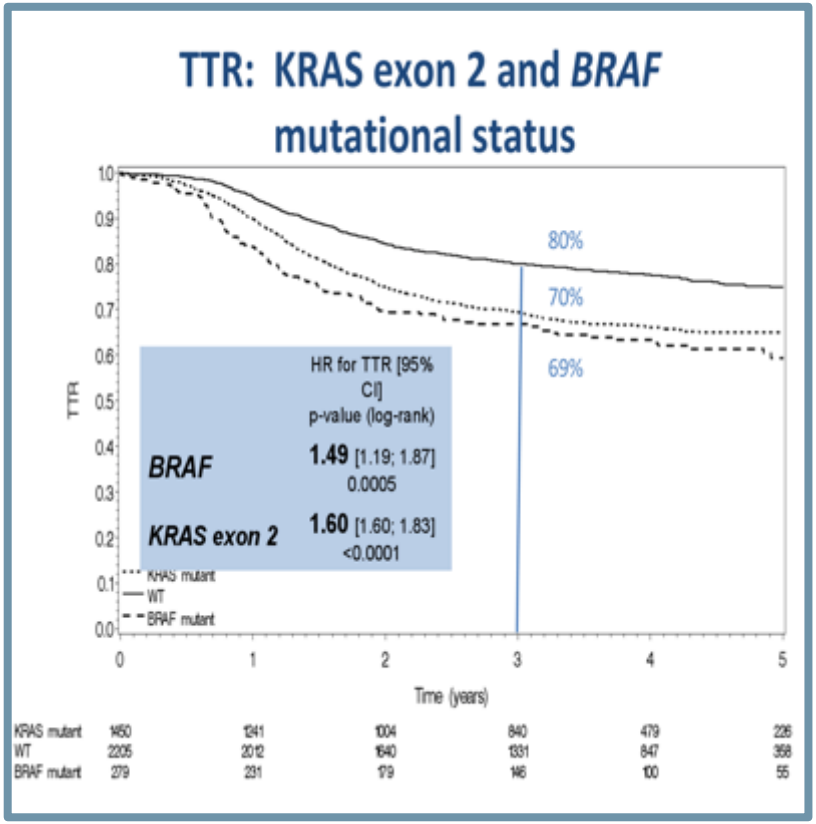

MOSAIC

Table 3. Univariable and Multivariable Analysis of Prognostic Factors for OS

Variable	No. Patients	No. Events	Univariable Analysis* HR	95% CI	P	Multivariable Analysis* HR	95% CI	P	Internal Validation Bootstrap BCa 95% CI
Age, years									
< 70	1,931	508	1			1			
≥ 70	315	123	1.57	1.29 to 1.91	< .001	1.81	1.36 to 2.42	< .001	1.30 to 2.45
BMI, kg/m^2									
< 25	1,206	338	1						
≥ 25	1,036	293	1.00	0.86 to 1.17	.989				
CEA, ng/dL									
< 5	2,047	566	1			1			
≥ 5	112	44	1.73	1.27 to 2.35	< .001	1.93	1.19 to 3.15	.008	1.10 to 3.04
Differentiation grade									
Well	108	31	1			1			
Moderate	1,414	389	1.21	0.98 to 1.51		1.13	0.82 to 1.54		0.82 to 1.58
Poor	290	105	1.81	1.38 to 2.38	< .001	2.18	1.41 to 3.35	< .001	1.36 to 3.40
Obstruction									
No	1,828	473	1			1			
Yes	418	158	1.60	1.33 to 1.91	< .001	1.32	0.97 to 1.79	.077	0.92 to 1.86
Perforation									
0	2,090	566	1			1			
ECOG PS									
> 1	1,952	525	1			1			
< 1	294	106	1.46	1.18 to 1.80	< .001	1.40	1.01 to 1.94	.042	0.95 to 1.96

BRAF testing on limited disease not recommended, prognostic and predictive data are pending to be clarified (disparate results in different trials)

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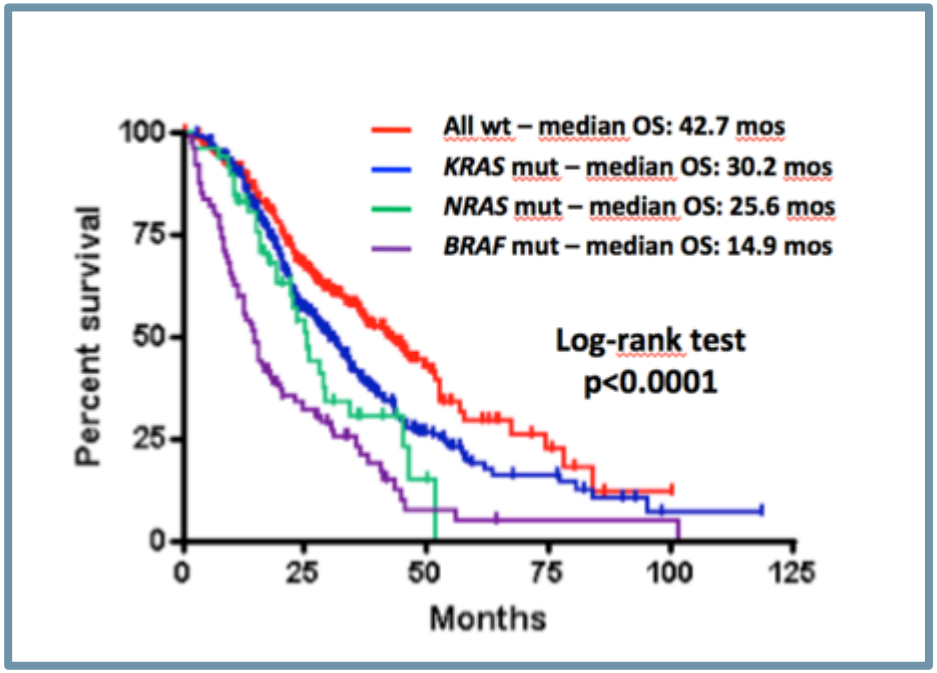
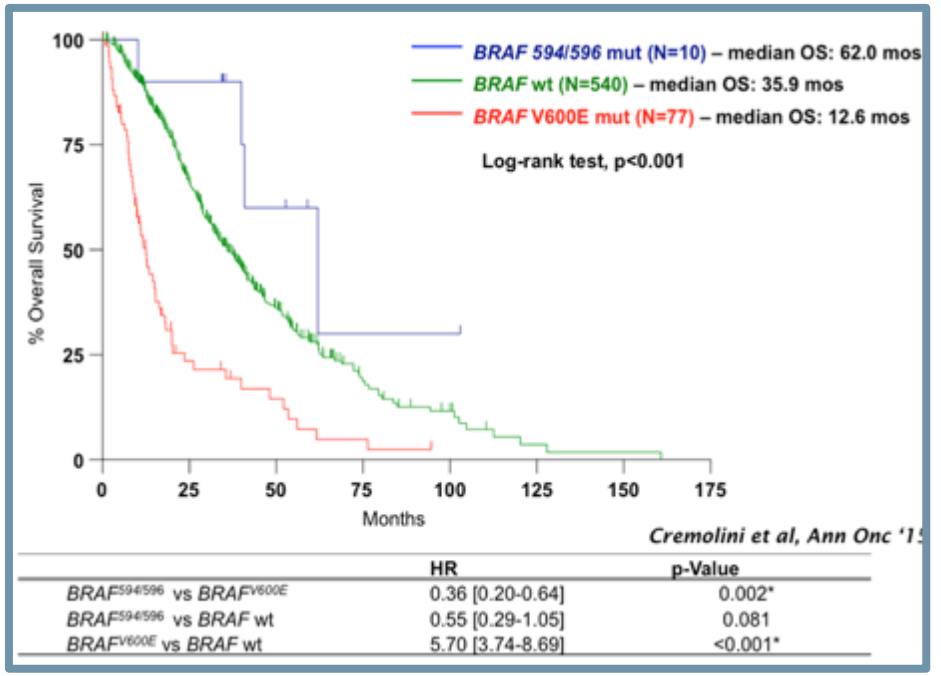
PROGNOSTIC ROLE OF BRAF MUTATION IN MCRC

BRAF mutations confer bad prognosis



Different biology of *BRAF* mutations



	HR	p-Value
$BRAF^{594/596}$ vs $BRAF^{V600E}$	0.36 [0.20-0.64]	0.002*
$BRAF^{594/596}$ vs *BRAF* wt	0.55 [0.29-1.05]	0.081
$BRAF^{V600E}$ vs *BRAF* wt	5.70 [3.74-8.69]	<0.001*

BRAF V600E mutation confers bad prognosis in the metastatic setting

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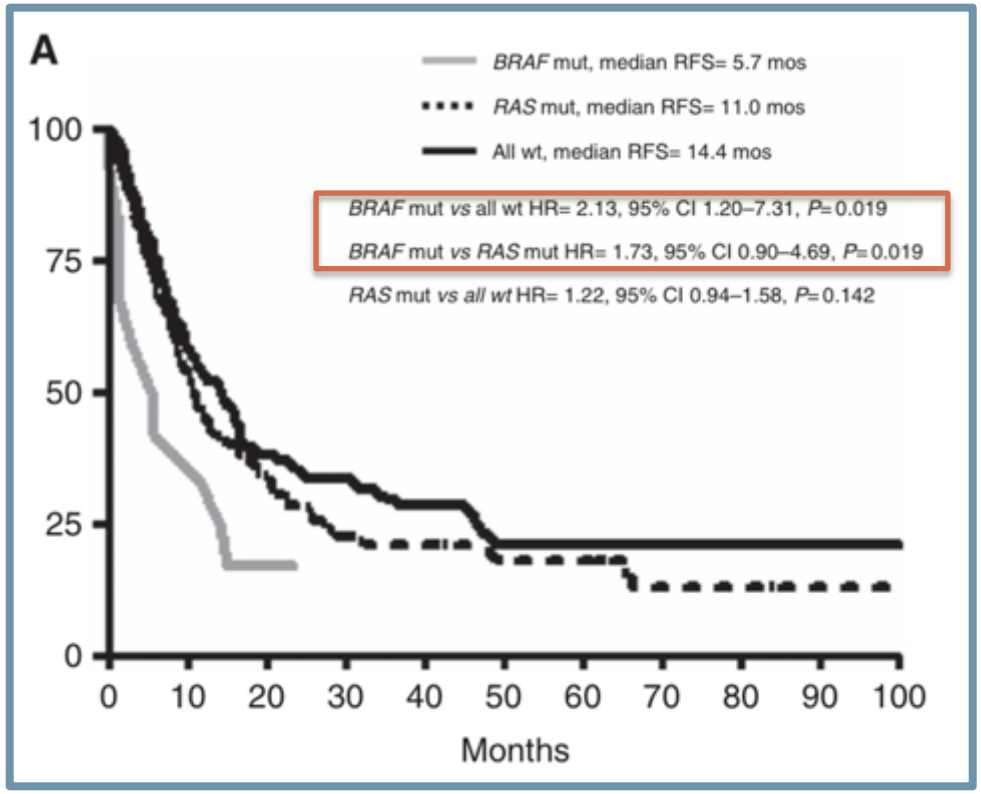
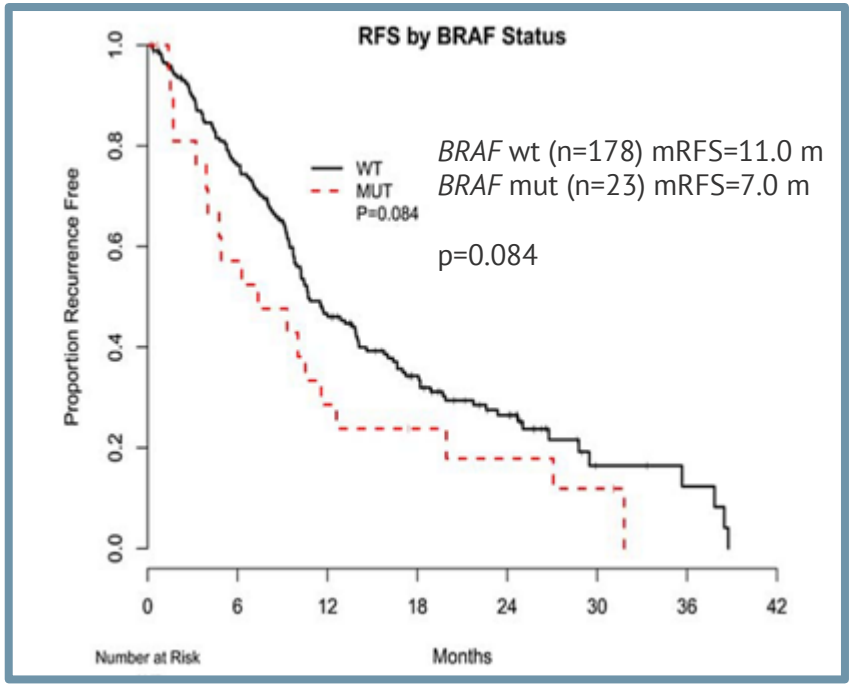
Schirripa et al, Int J Canc 2005, Cremolini et al, Ann Onc 2015

PROGNOSTIC ROLE OF BRAF MUT. IN mCRC (2)



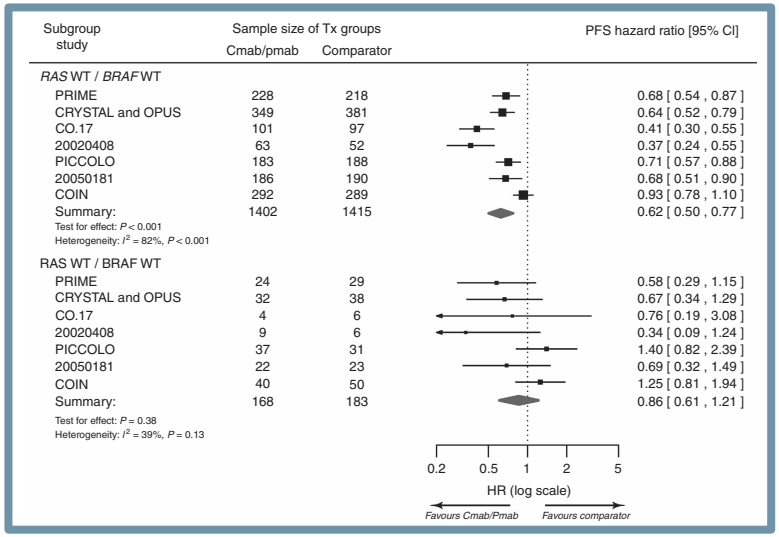


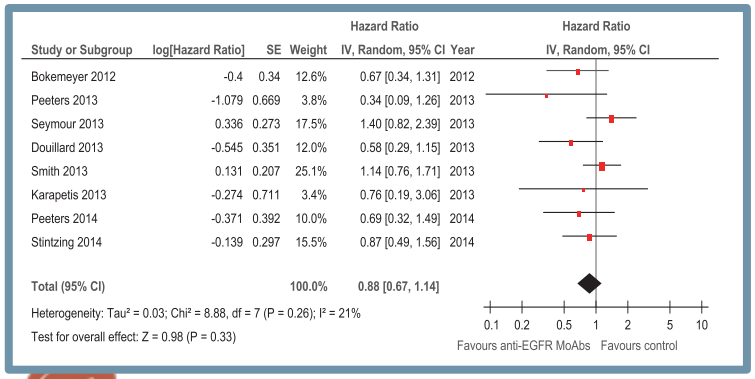
The trend towards bad prognosis still persist in BRAF V600E metastatic patients undergoing resection

Schirripa et al, BJC 2015 Yaeger et al, Cancer 2014

PREDICTIVE ROLE OF BRAF MUTATION IN MCRC

Role of BRAF Mut anti-EGFR predictive factor





TRIBE: subgroup analysis according to RAS/BRAF status

	N	FOLFIRI + bev Median OS	FOLFOXIRI + bev Median OS	HR [95% CI]	p
ITT population	508	25.8	29.8	0.80 [0.65-0.98]	0.030
RAS and *BRAF* evaluable	357	24.9	28.6	0.84 [0.66-1.07]	0.159
RAS and *BRAF* wt	93	33.5	41.7	0.77 [0.46-1.27]	
RAS mutated	236	23.9	27.3	0.88 [0.65-1.18]	0.522*
BRAF mutated	28	10.7	19.0	0.54 [0.24-1.20]	

* P for interaction

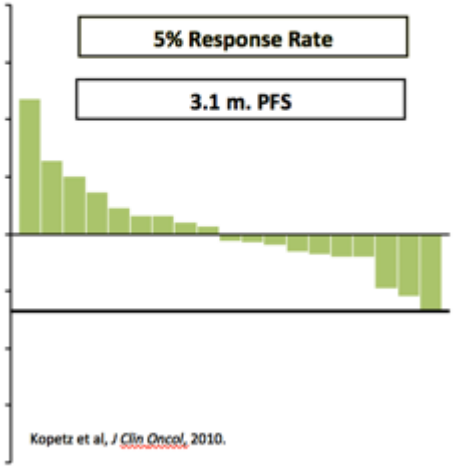
BRAF V600E diminish benefit derived from anti-EGFR MoAbs

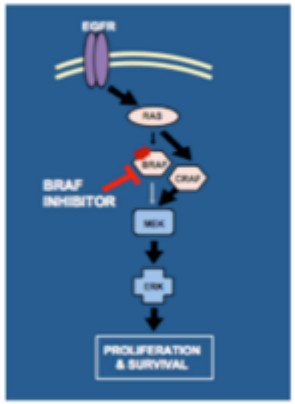
However intensive strategies using extended cytostatic combinations seem to improve patient outcomes

Rowland et al, Br J Canc 2015, Pietrantonio et al, Eur J Canc 2015, Cremolini et al, Lancet Oncol 2015

FIRST GENERATION OF BRAF THERAPEUTICS

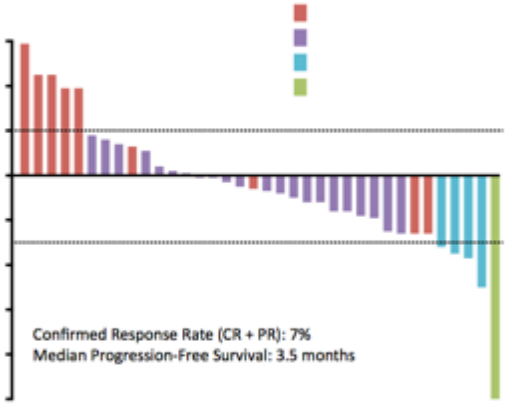
Vemurafenib Monotherapy: Not Effective in BRAFm CRC



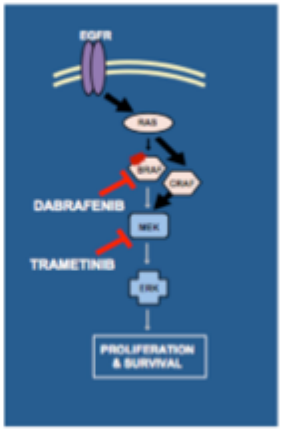


Kopetz et al, *J Clin Oncol*, 2010.

Dabrafenib (D) + Trametinib (T): Limited Activity in BRAFm CRC



Confirmed Response Rate (CR + PR): 7%
Median Progression-Free Survival: 3.5 months



BRF113220 — Corcoran et al, *J Clin Oncol*, 2014

Contrary to melanoma, initial trials with BRAF V600E inhibitors s/a failed to demonstrate clinical activity in mCRC

BRAF INHIBITORS + EGFR INHIBITORS HAVE *IN VIVO* ACTIVITY IN BRAF ^{V600E} MUTATED CRC XENOGRAFTS

LETTER

doi:10.1038/nature10868

Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR

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Inhibition of the BRAF(V600E) oncoprotein by the small-molecule drug PLX4032 (vemurafenib) is highly effective in the treatment of melanoma[1]. However, colon cancer patients harbouring the same *BRAF(V600E)* oncogenic lesion have poor prognosis and show only a very limited response to this drug[2–4]. To investigate the cause of the limited therapeutic effect of PLX4032 in *BRAF(V600E)* mutant colon tumours, here we performed an RNA-interference-based genetic screen in human cells to search for kinases whose knockdown synergizes with BRAF(V600E) inhibition. We report that blockade of the epidermal growth factor receptor (EGFR) shows strong synergy with BRAF(V600E) inhibition. We find in multiple *BRAF(V600E)* mutant colon cancers that inhibition of EGFR by the antibody drug cetuximab or the small-molecule drugs gefitinib or erlotinib is strongly synergistic with BRAF(V600E) inhibition, both *in vitro* and *in vivo*. Mechanistically, we find that BRAF(V600E) inhibition causes a rapid feedback activation of EGFR, which supports continued proliferation in the presence of BRAF(V600E) inhibition. Melanoma cells express low levels of EGFR and are therefore not subject to this feedback activation. Consistent with this, we find that ectopic expression of *EGFR* in melanoma cells is sufficient to cause resistance to PLX4032. Our data suggest that *BRAF(V600E)* mutant colon cancers (approximately 8–10% of all colon cancers[5,6,7]), for which there are currently no targeted treatment options available, might benefit from combination therapy consisting of BRAF and EGFR inhibitors.

Activating mutations in the *BRAF* oncogene (*BRAF(V600E)*) are seen in some 70% of primary melanomas[8], some 10% of colorectal cancers[9] and some 30–70% of papillary thyroid carcinoma[8–10]. However, clinical responses to the highly selective small-molecule inhibitor of the BRAF(V600E) oncoprotein, PLX4032, differ widely, ranging from a response rate of approximately 80% in melanoma to

determined by next generation sequencing of the barcode identifiers present in each shRNA vector (Fig. 1c, see Methods). We arbitrarily considered only shRNA vectors that had been sequenced at least 300 times and which were depleted at least fivefold by the drug treatment. Figure 1d shows that only very few of the 3,388 shRNA vectors in the library met this stringent selection criterion, among which were three independent shRNA vectors targeting the EGFR (see Supplementary Table 2 for all selected shRNAs). This suggested that suppression of EGFR synergizes with BRAF inhibition in these CRC cells. To validate this finding, we infected WiDr cells with each of these three EGFR shRNA vectors (all of which reduced EGFR levels; Fig. 1f) and cultured these cells with or without PLX4032 for 2 weeks. Figure 1e shows that inhibition of EGFR does not significantly affect proliferation of EGFR in WiDr cells, consistent with the clinical observations that *KRAS* or *BRAF* mutant CRC cells do not respond to EGFR-targeted monoclonal antibodies[7,13,14]. In contrast, suppression of EGFR in combination with PLX4032 caused a marked inhibition of proliferation in WiDr cells (Fig. 1e). This suggested that *BRAF(V600E)* mutant CRC cells are responsive to treatment with a combination of BRAF inhibitor plus an EGFR inhibitor.

At present, two classes of anti-EGFR drugs are clinically available; these include the monoclonal antibodies cetuximab and panitumumab, and the small-molecule kinase inhibitors gefitinib and erlotinib. We found that three BRAF mutant CRC cell lines (WiDr, VACO432 and KM20) all lack a significant response to monotherapy with PLX4032, cetuximab or gefitinib. However, strong synergy was seen when PLX4032 was combined with either cetuximab or gefitinib (Fig. 2a and Supplementary Fig. 1A, C) or erlotinib (data not shown), consistent with the notion derived from the shRNA screen that EGFR inhibition is required to elicit a response to BRAF inhibition in CRC cells.

To address the molecular mechanism underlying the synergy

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EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of *BRAF* mutant colorectal cancers to RAF inhibition with vemurafenib

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Abstract

BRAF mutations occur in 10–15% of colorectal cancers (CRCs) and confer adverse outcome. While RAF inhibitors such as vemurafenib (PLX4032) have proven effective in *BRAF* mutant melanoma, they are surprisingly ineffective in *BRAF* mutant CRCs, and the reason for this disparity remains unclear. Compared to *BRAF* mutant melanoma cells, *BRAF* mutant CRC cells were less sensitive to vemurafenib, and P-ERK suppression was not sustained in response to treatment. Although transient inhibition of phospho-ERK by vemurafenib was observed in CRC, rapid ERK re-activation occurred through EGFR-mediated activation of RAS and CRAF. *BRAF* mutant CRCs expressed higher levels of phospho-EGFR than *BRAF* mutant melanomas,

The genetic context is different in CRC. EGFR constitutive expression lead to a feedback crosstalk with BRAF downstream effectors that functionally rescue BRAF inhibition

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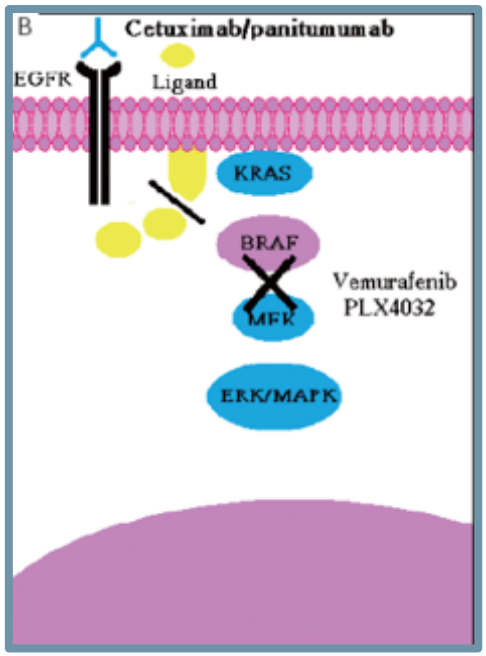
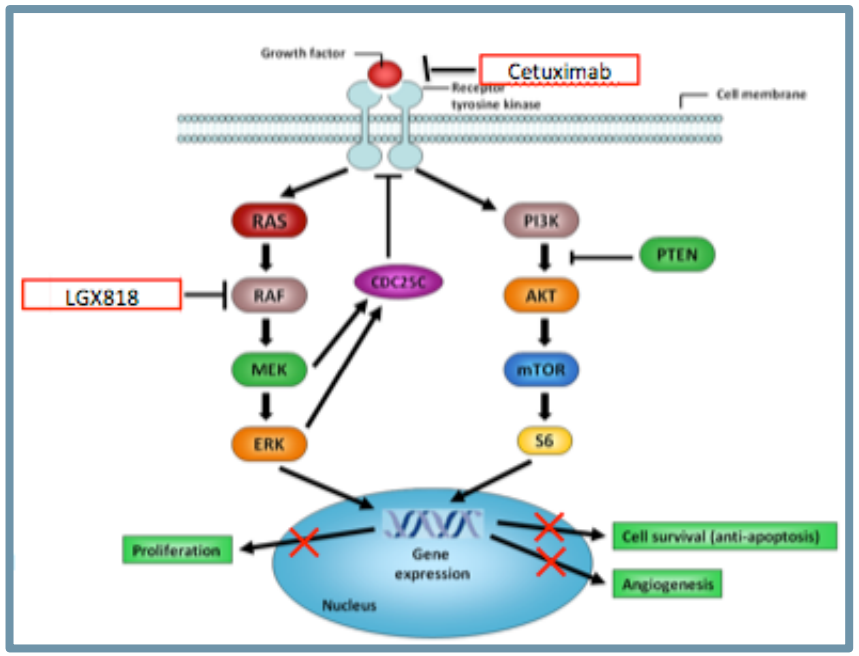
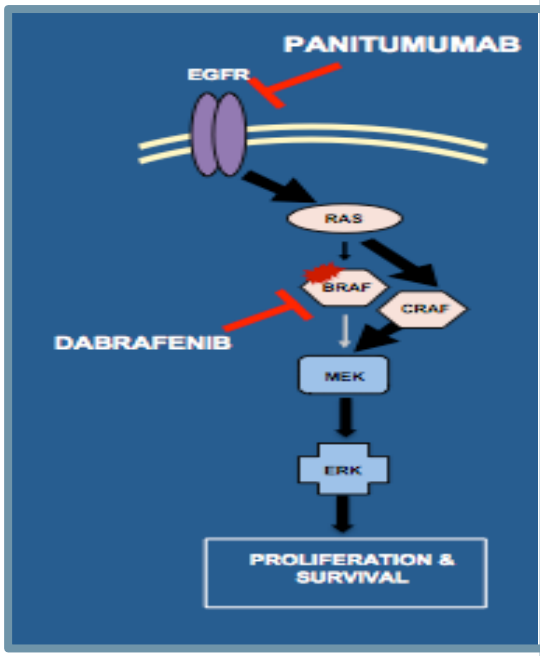
Corcoran RB et al, Cancer Discov 2012; 2:227-35, Prahallad A et al, Nature 2012; 483:100-3

SECOND GENERATION BRAF THERAPEUTICS

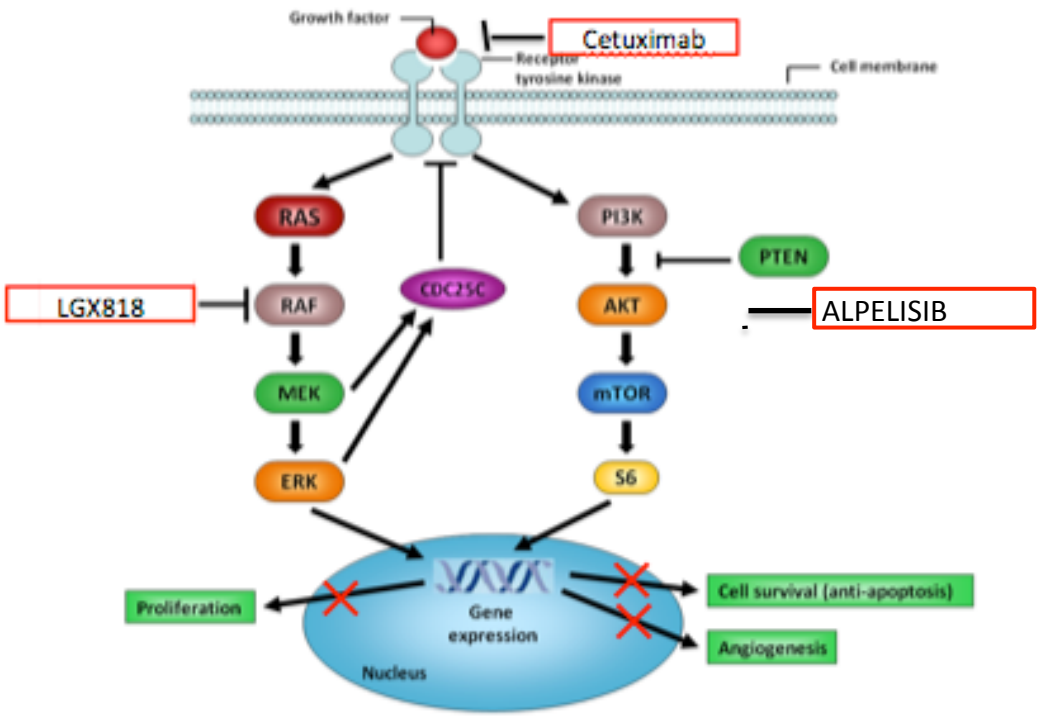


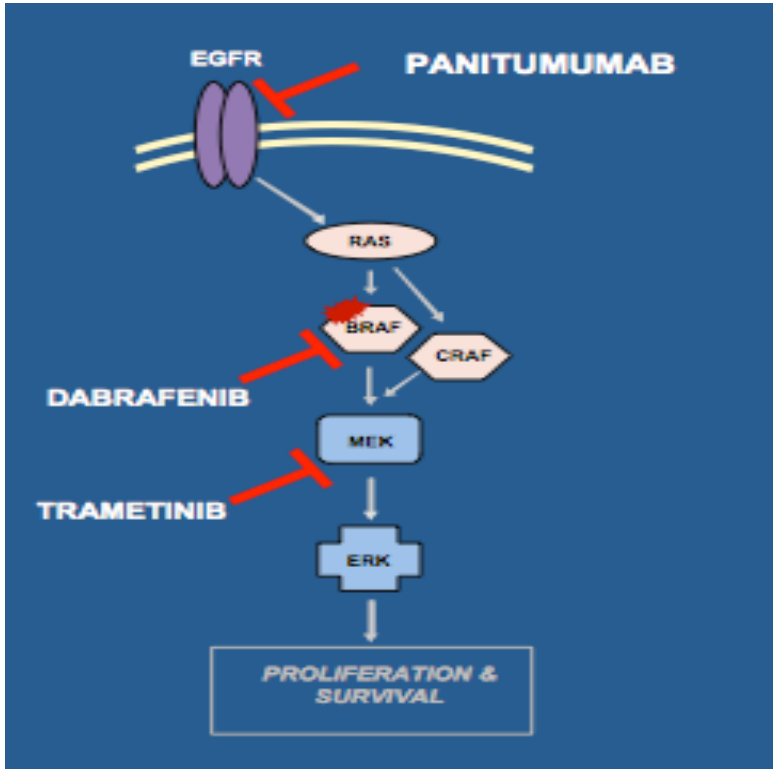




Regimen	N	PR/CR (%)	SD (%)	mPFS (m)
Dabrafenib + Panitumumab	20	10%	80	3.4 Van Cutsem WGIC 2015
Encorafenib + Cetuximab (ph II)	26	11%	54 (53)	3.7 Elez WGIC 2015
Vemurafenib + Cetuximab	26	4%	16(40)	3.7 Hyman NEJM 2015

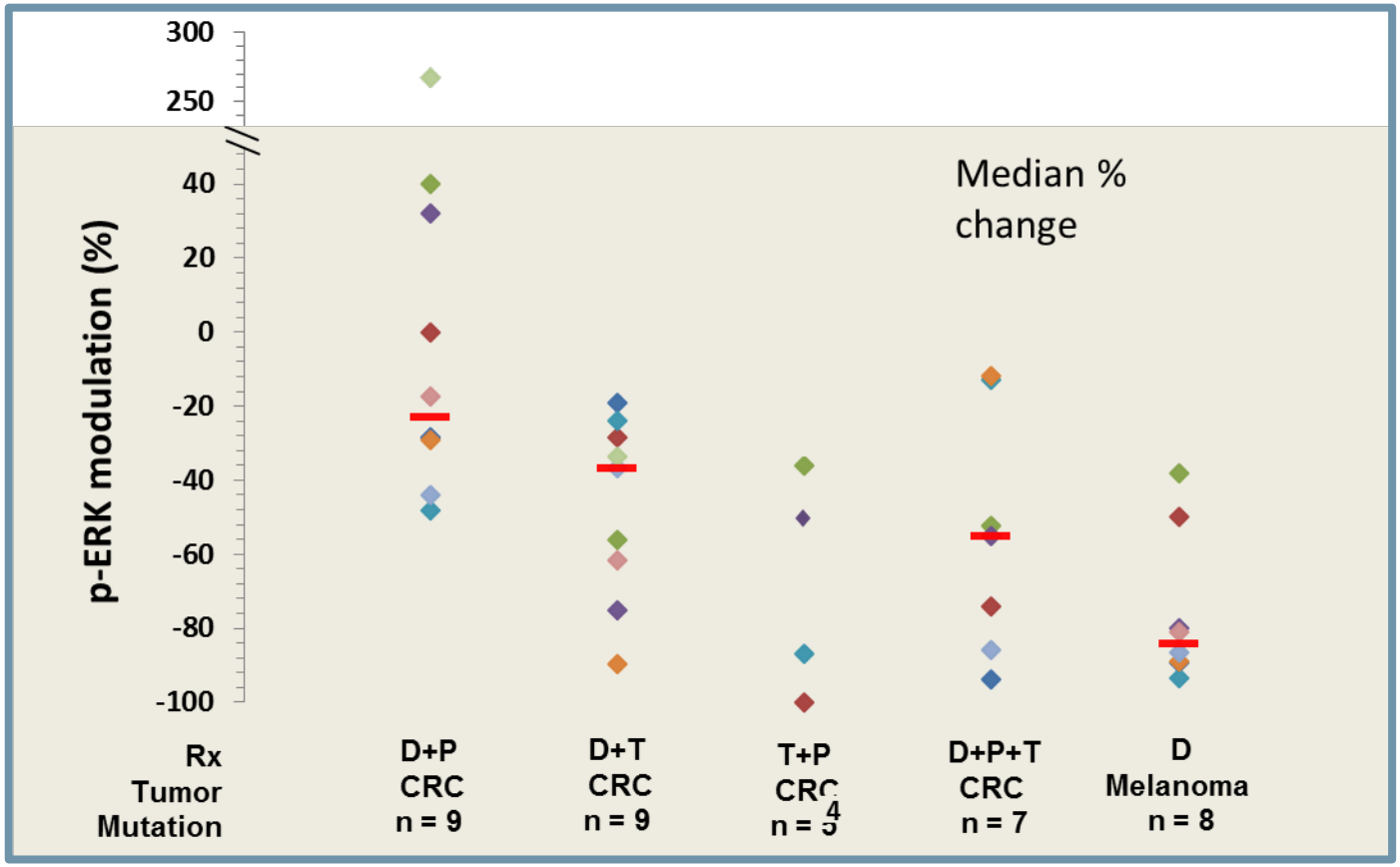
THIRD GENERATION BRAF INHIBITORS COMBOS





Regimen	N	PR/CR (%)	SD (%)	mPFS (m)
Dabrafenib + Trabetinib + Panitumumab	35	26%	50	4.1 Van Cutsem WGIC 2015
Encorafenib + Cetuximab + Alpelisib (ph II)	28 (49)	32%	44	4.3 Elez WGIC 2015
Vemurafenib + Cetuximab + CPT 11	ONGOING			

DIFFERENTIAL DEGREE OF MODULATION OF PERK BY VARIOUS TREATMENTS IN BRAF V600MUT CRC AND MELANOMA



Even so... numbers are still far distant fom those seen in melanoma. We have a long and fascinating way to walk

CONCLUSIONS

- BRAF testing can not be recommended in localized setting
- BRAF V600E testing should be perform at the debut of metastatic disease, based on:
  - Bad prognostic implications
  - Need from intensive chemotherapy combos to overcome bad outcome (FOLFOXIRI-bevacizumab)
  - Less benefit from anti-EGFR monoclonal antibodies
  - Refer patients to trials including BRAF inhibitor combos with anti-EGFR monoclonal antibodies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