

How to select patients for treatment with EGFR inhibitors: KRAS and beyond

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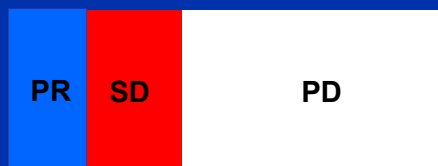
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Approved anti-EGFR targeted therapies in metastatic colorectal cancer

- Cetuximab was registered in 2004 in combination with irinotecan after irinotecan failure in both USA and EU, but also as monotherapy after irinotecan failure or irinotecan intolerance in USA.
- On July 2008: EMEA approval for EGFR-positive, KRAS wild-type tumors in combination with chemotherapy (any line of treatment) or as monotherapy after oxaliplatin and irinotecan failure or irinotecan intolerance.
- Panitumumab is registered as monotherapy in the third line treatment (after failure of fluoropyrimidine, oxaliplatin and irinotecan based therapies) in USA. In Europe, the approval is limited to KRAS wild-type tumors.

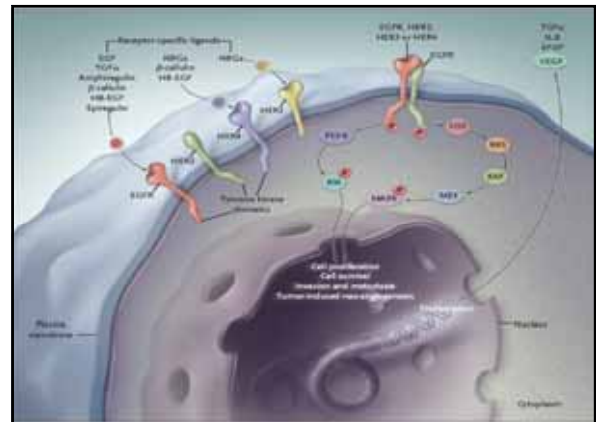
Efficacy of EGFR inhibitors monotherapy in chemorefractory unselected CRC patients



10% 30% 60%

Apoptosis Growth arrest No effect on tumor growth

EGFR-dependent Non-EGFR-dependent



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

EGFR inhibitors: Potential positive predictive factors

Predictive of efficacy:

- Markers of EGFR activation
 - Immunohistochemistry (IHC)
 - Fluorescence in situ hybridization (FISH)
 - Gene mutations
 - Gene expression levels
 - Gene polymorphisms
- Markers of EGFR ligand (amphiregulin, epiregulin) activation
 - Immunohistochemistry (IHC)
 - Gene expression levels

Potential predictors of clinical activity of EGFR inhibitors in metastatic CRC: Can we select patients for treatment?

- Skin reactions ("rash") correlate with survival. The strongest evidence of correlation with efficacy from all studies in CRC.
- No role of EGFR-IHC in CRC.
- No role of somatic EGFR gene mutations in CRC.

Potential predictors of clinical activity of EGFR inhibitors in metastatic CRC: Can we select patients for treatment?

- Increased EGFR gene copy numbers assessed by FISH correlate with higher RR and PFS.
 - Retrospective evidence from different studies, mainly in chemotherapy refractory patients treated with panitumumab or cetuximab (+/- irinotecan).
 - Major methodology issues for clinical practice.

EGFR inhibitors: Potential negative predictive factors

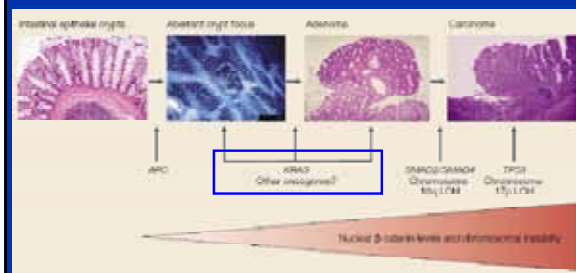
Predictive of lack of efficacy:

- Markers of activation of EGFR-independent signalling pathways in cancer cells
 - Intrinsic resistance to EGFR inhibitors.
 - Acquired resistance to EGFR inhibitors.

Possible Mechanisms of Intrinsic and Acquired Resistance to EGFR Inhibitors

- Activation of downstream signaling pathways through EGFR-independent mechanisms:
 - Activation through different cell membrane growth factor receptors (IGF1-R; ErbB3; MET).
 - **PTEN-PI3K-AKT-mTOR** pathway.
 - **Ras-Raf-MEK-ERK** pathway.
 - Pro-angiogenic growth factors (VEGF) production.
 - Expression of VEGFRs in cancer cells.
 - Bcl-2/Bcl-xL pathway.

Vogelstein paradigm for multistep colorectal carcinogenesis



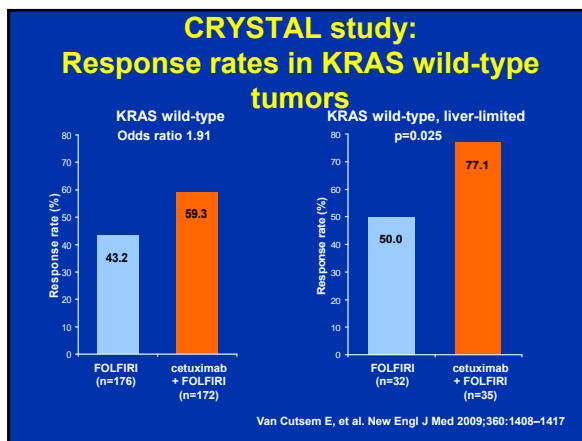
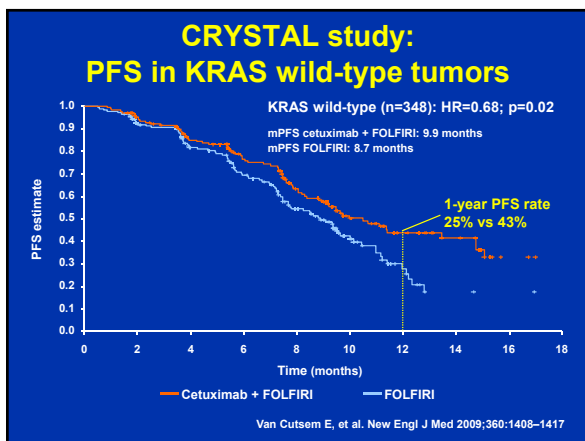
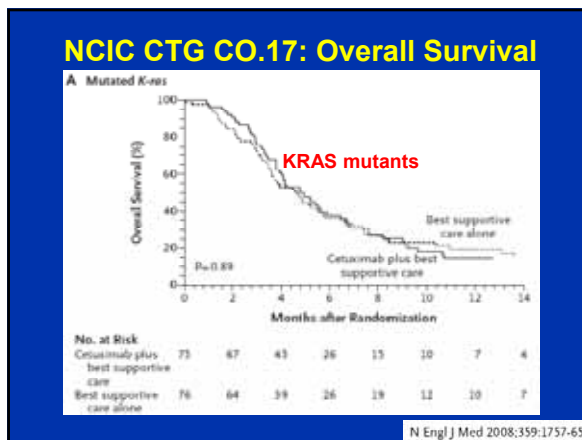
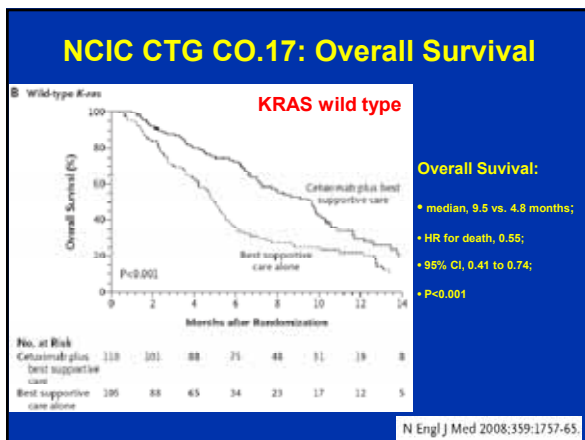
Fodde R, et al. Nature Rev Cancer 2001;1:55-67

KRAS is involved in the EGFR pathway in CRC

- Activating KRAS gene mutations are early events in the multi-step CRC carcinogenesis process:
 - Detected as early as in aberrant crypt foci
 - Detected in approximately 40% of patients with CRC
- Hot spot point mutations mainly within codons 12 or 13 of the KRAS gene result in the translation of a constitutively active KRAS protein
- A constitutively active KRAS protein is able to promote cancer cell growth and survival through the RAF-MEK-ERK and PI3K-AKT pathways independently from EGFR signaling

Potential predictors of clinical activity of EGFR inhibitors in metastatic CRC: Can we select patients for treatment?

- Strong evidence that KRAS wild type gene status predicts efficacy:
 - Concordance in different studies in chemotherapy-refractory patients treated with panitumumab or cetuximab (+/- irinotecan) and in the first-line treatment with cetuximab + chemotherapy (045, CRYSTAL and OPUS trials)



Influence of KRAS status on OS in patients treated with cetuximab + FOLFIRI vs FOLFIRI alone

Parameter	ITT		KRAS wild-type		KRAS mutant	
	FOLFIRI (n=599)	Cetuximab + FOLFIRI (n=599)	FOLFIRI (n=176)	Cetuximab + FOLFIRI (n=172)	FOLFIRI (n=87)	Cetuximab + FOLFIRI (n=105)
Median OS [95% CI]	18.6 [16.6-19.8]	19.9 [18.5-21.3]	21.0 [19.2-25.7]	24.9 [22.2-27.8]	17.7 [14.4-20.6]	17.5 [15.6-20.2]
Hazard ratio [95% CI]	0.93 [0.81-1.07]		0.84 [0.64-1.11]		1.03 [0.74-1.44]	
P-value	0.30		0.22		0.85	

Median follow up time: 30 months

Van Cutsem E, et al. New Engl J Med 2009;360:1408-1417

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

Federica Di Nicolantonio, Miriam Martini, Francesca Molinari, Andrea Sartore-Bianchi, Sabrina Arena, Piercarlo Saletti, Sara De Dosso, Luca Mazzuchelli, Milo Frattini, Salvatore Siena, and Alberto Bardelli

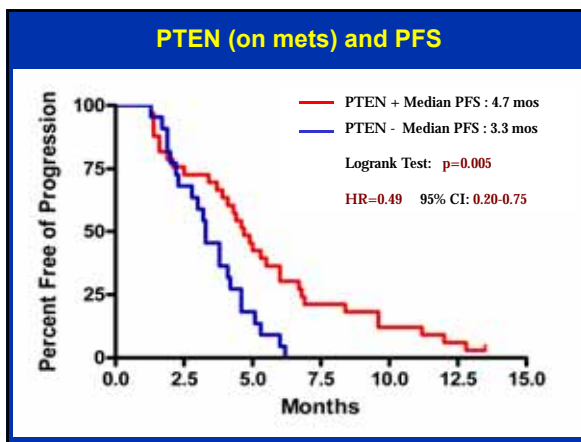
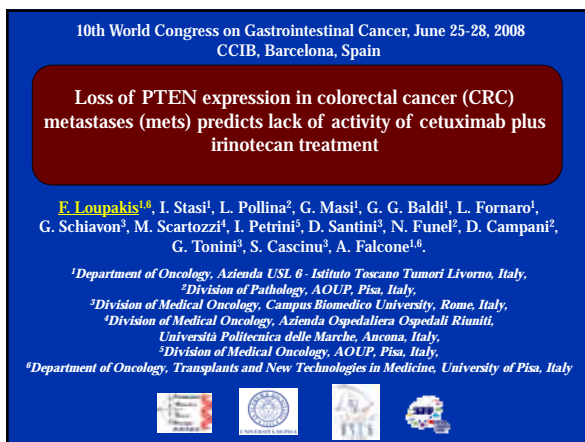
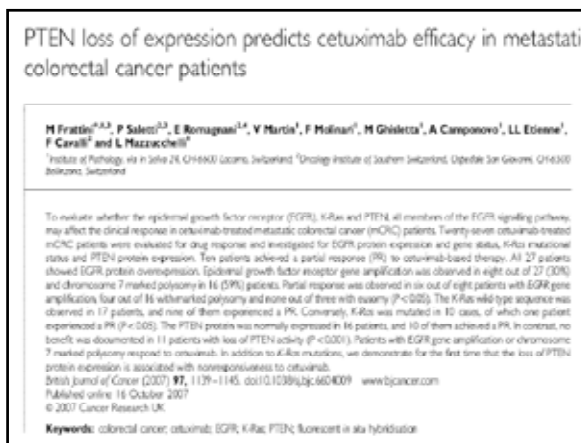
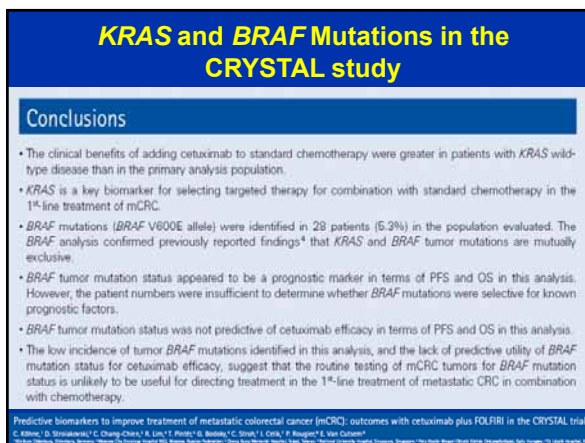
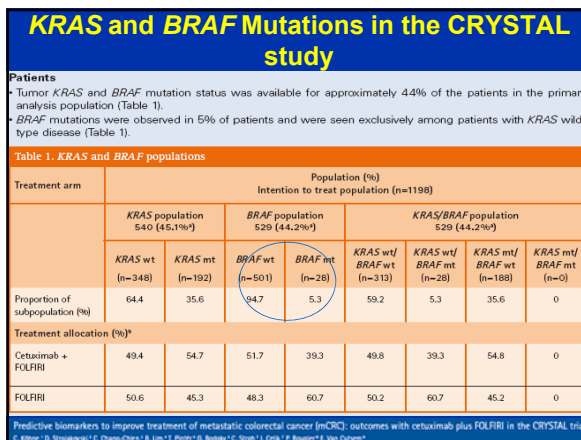
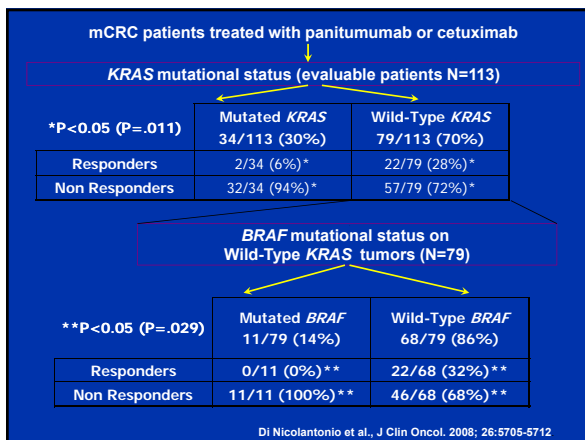
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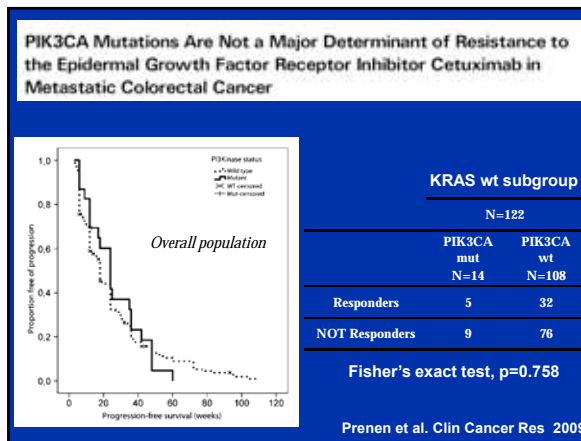
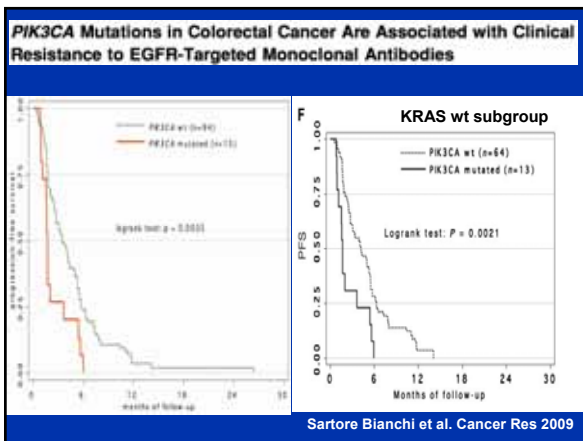
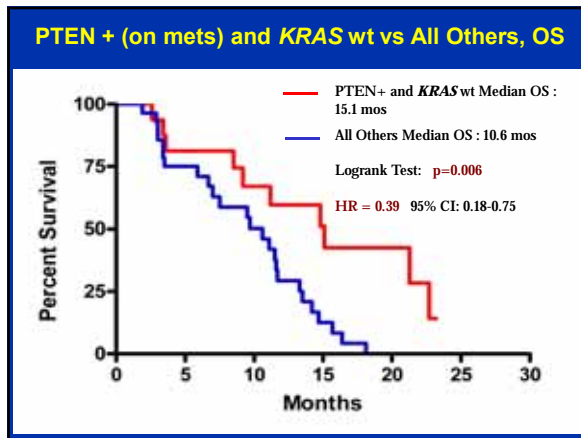
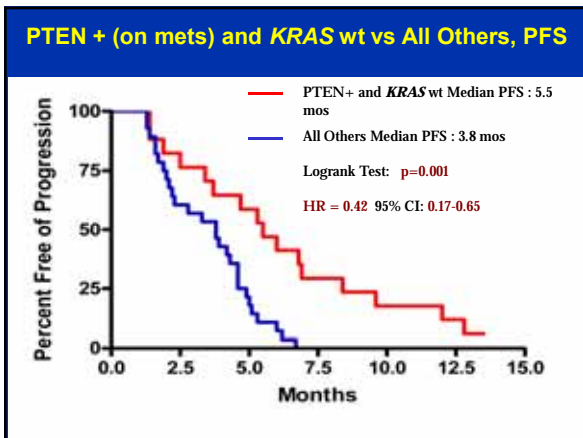
Purpose
Cetuximab or panitumumab are effective in 10% to 20% unselected metastatic colorectal cancer (CRC) patients. KRAS mutations account for approximately 30% to 40% of patients who are not responsive. The serine-threonine kinase BRAF is the principal effector of KRAS. We hypothesized that, in KRAS wild-type patients, BRAF mutations could have a predictive/prognostic value.

Patients and Methods
We retrospectively analyzed objective tumor responses, time to progression, overall survival (OS), and the mutational status of KRAS and BRAF in 113 tumors from cetuximab- or panitumumab-treated metastatic CRC patients. The effect of the BRAF V600E mutation on cetuximab or panitumumab response was also assessed using cellular models of CRC.

Results
KRAS mutations were present in 30% of the patients and were associated with resistance to cetuximab or panitumumab (P = .011). The BRAF V600E mutation was detected in 11 of 79 patients who had wild-type KRAS. None of the BRAF-mutated patients responded to treatment, whereas none of the responders carried BRAF mutations (P = .029). BRAF-mutated patients had significantly shorter progression-free survival (P = .011) and OS (P < .0001) than wild-type patients. In CRC cells, the introduction of BRAF V600E allele impaired the therapeutic effect of cetuximab or panitumumab. Treatment with the BRAF inhibitor sorafenib restored sensitivity to panitumumab or cetuximab of CRC cells carrying the V600E allele.

Conclusion
BRAF wild-type is required for response to panitumumab or cetuximab and could be used to select patients who are eligible for the treatment. Double-hit therapies aimed at simultaneous inhibition of epidermal growth factor receptor and BRAF warrant exploration in CRC patients carrying the V600E oncogenic mutation.





K-Ras, B-Raf, N-Ras and PIK3CA mutations and cetuximab efficacy: a multicenter European consortium study: Lambrechts et al., P ASCO 2009

Endpoint	Evaluation of 4 tumor based tests: <i>K-Ras</i> , <i>B-Raf</i> , <i>N-Ras</i> and <i>PIK3CA</i> mutation status
Utility	Predictive biomarker
Specimen	Tumor specimens (paraffin-embedded)
Patients	Refractory mCRC treated with Irinotecan + Cetuximab
Sample size	580 tumors (European consortium)
Assay	Sequenom MALDI TOF MassArray system

K-Ras, B-Raf, N-Ras and PIK3CA mutations and cetuximab efficacy: a multicenter European consortium study: Lambrechts et al., P ASCO 2009

	Mutations included	% coverage of potential mutations (Cosmic)	Mutation rate detected
KRAS	G12S, G12R, G12C, G12D, G12A, G12V, G13D, A146T, G13A, G13V, A59T, Q61K, Q61E, Q61P, Q61R, Q61L, Q61H	99.2%	36.5% (622 samples)
BRAF	V600E, K601E, D594G, V600M	97%	5% (589 samples)
NRAS	Q61P, Q61L, Q61H, Q61K, Q61Q, Q61E, G13S, G13C, G13R, Q61K, Q61R, G12D, G12S, G12C	97%	6% (261 samples)
PI3K	H1047R, H1047L, K179T, P539R, Q546K, Q546E, E81K, R88Q, G106V, N345K, R39W, S158L, H160N, R38H, E542K, E542Q, E545K, E545Q, G118D, G12D, K567R, H1047Y, P134S, R108H, C420R, H701P, K184E, C901F, M1004I, G1049R, G1007R, G1049S	86%	13% (578 samples)

K-Ras, B-Raf, N-Ras and PIK3CA mutations and cetuximab efficacy: a multicenter European consortium study: Lambrechts et al., P ASCO 2009

- K-Ras, B-Raf and N-Ras are mutually exclusive
- 17.7% K-Ras mt and 10.4% K-Ras wt had a PIK3CA mutation (p= 0.009 Pearson Chi square)
- 6% B-Raf mutants and 13% B-Raf wt had a PIK3CA mutation (p= 0.412 Fisher's Exact test)

Univariate analysis

KRAS	CR + PR	SD + PD	Total	P
WT	130 (36%)	226 (64%)	356	p<.001
Mut	11 (8%)	192 (95%)	203	

BRAF	CR + PR	SD + PD	total	p
WT	141 (26%)	399 (74%)	540	p=.035
Mut	2 (8%)	24 (92%)	26	

NRAS	CR + PR	SD + PD	total	p
WT	50 (21%)	179 (79%)	239	p=.317
Mut	1 (6%)	14 (94%)	15	

PI3K	CR + PR	SD + PD	total	p
WT	128 (27%)	357 (73%)	485	p=.028
Mut	10 (14%)	60 (86%)	70	

PI3K In KRAS wt	CR + PR	SD + PD	Total	P
WT	117 (38%)	195 (62%)	312	p=0.107
Mut	8 (24%)	26 (76%)	34	

Multivariate analysis

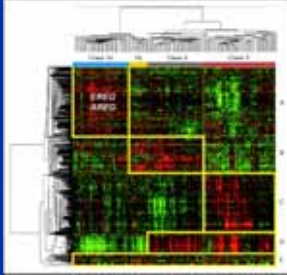
OR Logistic regression	OR	95%CI	P value
KRAS	0.093	0.048 – 0.177	p<.001
BRAF	0.140	0.032 – 0.604	p=.008
PI3K	Not retained		p=.136

PFS Cox regression	HR	95%CI	P value
KRAS	0.523	0.434 – 0.631	p<.001
BRAF	0.328	0.217 – 0.497	p<.001
PI3K	0.798	0.620 – 1.027	p=.079

OS Cox regression	HR	95%CI	P value
KRAS	0.549	0.452 – 0.667	p<.001
BRAF	0.378	0.250 – 0.572	p<.001
PI3K	Not retained		p=.187

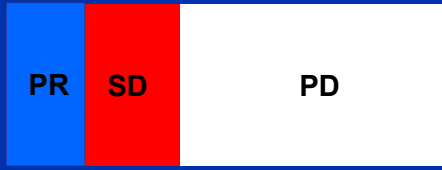
Amphiregulin and Epiregulin

- EGFR ligands:
 - 1 in C. Elegans
 - 4 in Drosophila
 - 7 in mammals: EGF, TGF- α , HB-EGF, amphiregulin (AREG), betacellulin, epiregulin (EREG) and epigen¹
 - EREG and AREG bind more weakly to EGFR than EGF but much more potently and prolonged
 - EREG preferentially activates heterodimers²
- High gene expression levels of EREG and AREG predict response to cetuximab³



¹Singh, AB et al. Cell Signal; 17:1183-1193,2005
²Shelly, M et al. J Biol Chem; 273:10496-10505,1998
³Khambata-Ford, S. et al. J Clin Oncol; 25:3230-3237, 2007

Efficacy of EGFR inhibitors monotherapy in chemorefractory unselected CRC patients



10% PR 30% SD 60% PD

Apoptosis Growth arrest No effect on tumor growth

EGFR-dependent Non-EGFR-dependent

Which biomarkers should be used?

- Accepted for clinical practice:
 - K-Ras gene status
- Far advanced in clinical development:
 - B-Raf gene status
- To be defined (more translational research studies needed):
 - N-Ras, PIK3CA gene status
 - Loss of PTEN gene and/or protein expression
 - Ligands: AREG, EREG
 - Genetic polymorphisms: EGFR, EGF, Fc receptors (ADCC)
 -

Modified from an original concept by Josep Tabernero