

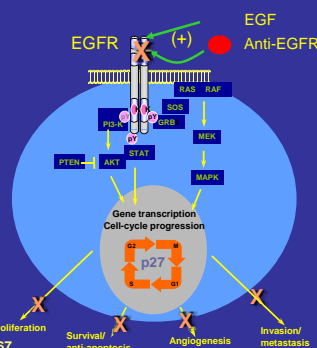
## Targeting EGFR: Implications in Colorectal Cancer Management

Al B. Benson III, MD, FACP  
 Professor of Medicine  
 Feinberg School of Medicine  
 Associate Director for Clinical Investigations  
 Robert H. Lurie Comprehensive Cancer Center  
 of Northwestern University

Thanks to the investigators for providing their slides.

### EGF-induced Signal Transduction and Tumorigenesis

- Epidermal growth factor receptor (EGFR)
  - A large tyrosine kinase growth factor receptor
- Natural ligands
  - TGF- $\alpha$ , EGF
- Potential to block multiple steps in the signal transduction process
  - Extracellular surface
  - Intracellular targets



Perez-Soler R. *Oncologist*. 2004;9:58-67.

### Potential Biomarkers: Methods of Testing

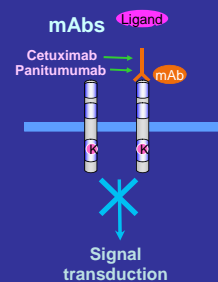
- EGFR protein expression
- *EGFR* gene copy number
- *K-ras* gene mutations
- EGFR ligands and phosphorylation

### EGFR Pathway and *K-ras* Gene Mutations

- EGFR pathway is the entry way to multiple intracellular signaling cascades
- When *K-ras* gene is mutated, *K-ras* protein is active regardless of EGFR activation
- *K-ras* gene mutations are an early event and are found in 40% to 45% of CRC patients
- Significantly less response in case of *K-ras* mutation and better survival in wild-type *K-ras*
- Mutation status may predict disease control in patients with CRC
  - Patients with *K-ras* tumor mutations have significantly shorter progression-free survival (PFS) than those who do not have mutations ( $P < 0.000005$ )
  - Shorter time to progression (TTP) with mutated *K-ras* vs wild-type ( $P = 0.0443$ )

Mendelsohn J, et al. *Oncogene*. 2000;19:6550-6565; Khambata-Ford S, et al. *J Clin Oncol*. 2007;25:3230-3237; Lievre A, et al. *Cancer Res*. 2006; Benvenuti S, et al. *Cancer Res*. 2007;67:2643-2648.

### Anti-EGFR Monoclonal Antibodies



Adapted from Raymond E, et al. *Drugs*. 2000;60(suppl 1):15-23.

Fig 1. CONSORT diagram

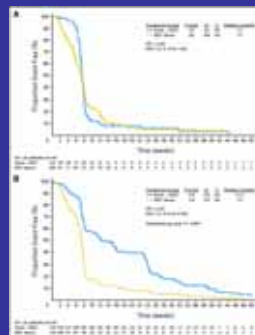


Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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Fig 2. Progression-free survival by treatment within KRAS groups

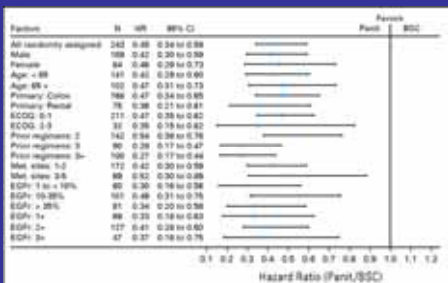


Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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Fig 3. Subset analyses of progression-free survival in the KRAS wild-type group

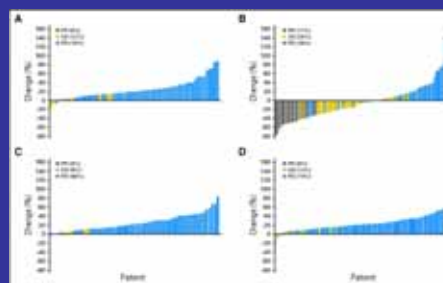


Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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Fig 4. Waterfall plots showing maximum percent decrease in target lesions (blinded central radiology)

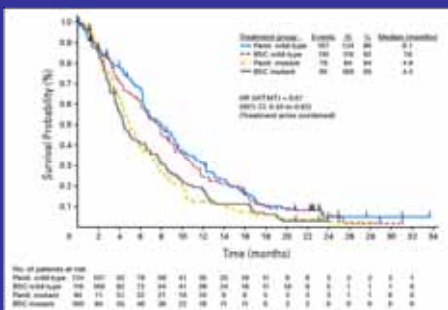


Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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Fig 5. Kaplan-Meier curves for overall survival by treatment and KRAS status

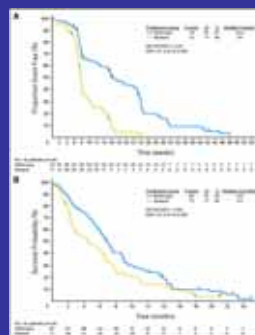


Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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Fig A1. (A) Progression-free survival and (B) overall survival by KRAS status among patients receiving panitumumab after progression on best supportive care alone




Amado, R. G. et al. J Clin Oncol; 26:1626-1634 2008

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### KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer treated with FOLFIRI with or without cetuximab: The CRYSTAL experience



**Eric Van Cutsem\***  
 I Lang, G D'Haens, V Moiseyenko, J Zaluski, G Folprecht, S Tejpar, O Kisker, C Stroh, P Rougier  
 \*University Hospital Gasthuisberg, Leuven, Belgium

### CRYSTAL trial in first-line mCRC

EGFR-expressing metastatic CRC → R

**Cetuximab + FOLFIRI**

Cetuximab IV 400 mg/m<sup>2</sup> on day 1, then 250 mg/m<sup>2</sup> weekly  
 + Irinotecan (180 mg/m<sup>2</sup>)  
 + 5-FU (400 mg/m<sup>2</sup> bolus + 2400 mg/m<sup>2</sup> as 46-hr continuous infusion)  
 + FA every 2 weeks

**FOLFIRI**

Irinotecan (180 mg/m<sup>2</sup>)  
 + 5-FU (400 mg/m<sup>2</sup> bolus + 2400 mg/m<sup>2</sup> as 46-hr continuous infusion)  
 + FA every 2 weeks

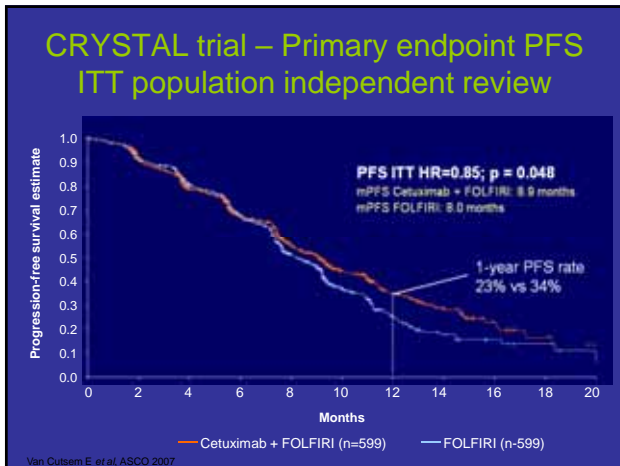
Stratification factors

- Regions
- ECOG PS

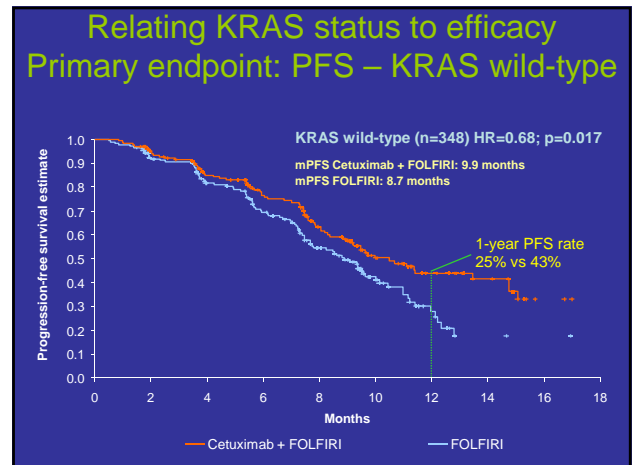
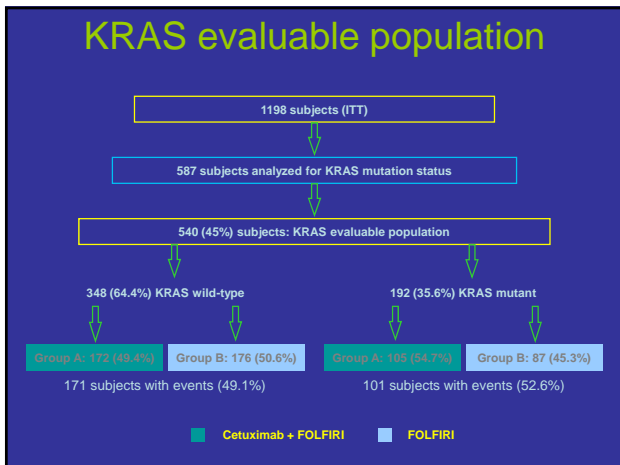
Populations

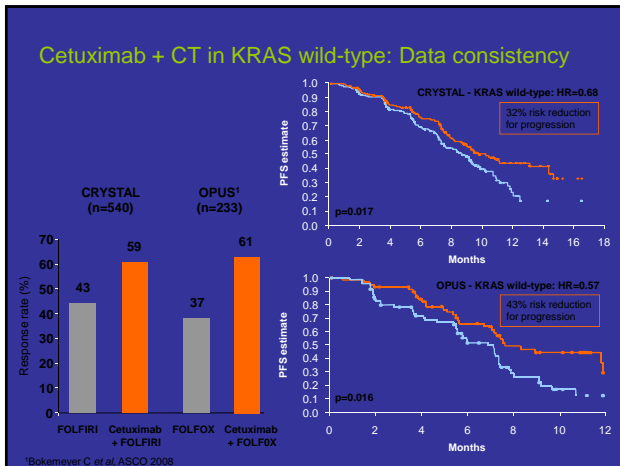
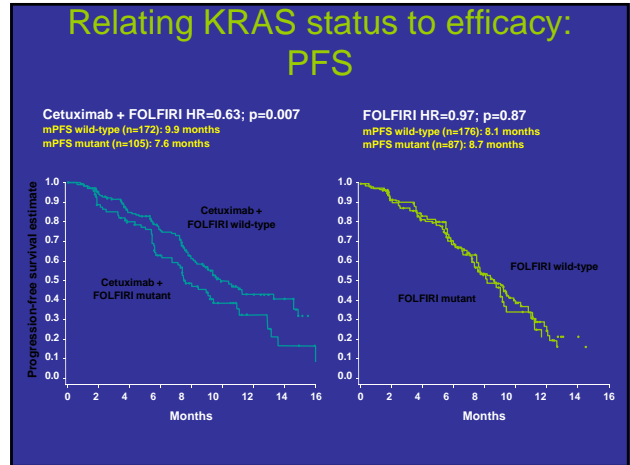
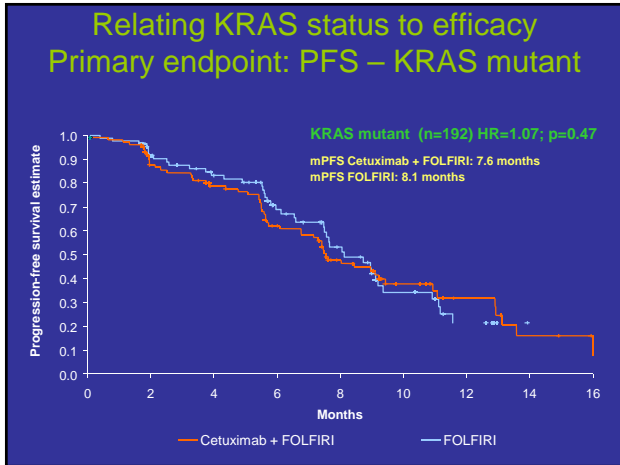
- Randomized patients n=1217
- Safety population n=1202
- ITT population n=1198

Van Cutsem E et al, ASCO 2007



- ### Relating KRAS status to efficacy
- Efficacy analyses repeated on KRAS evaluable population
  - Genomic DNA isolated from archived tumor material
  - Paraffin-embedded, formalin-fixed tissue
  - KRAS mutation status of codons 12/13 determined using quantitative PCR-based assay



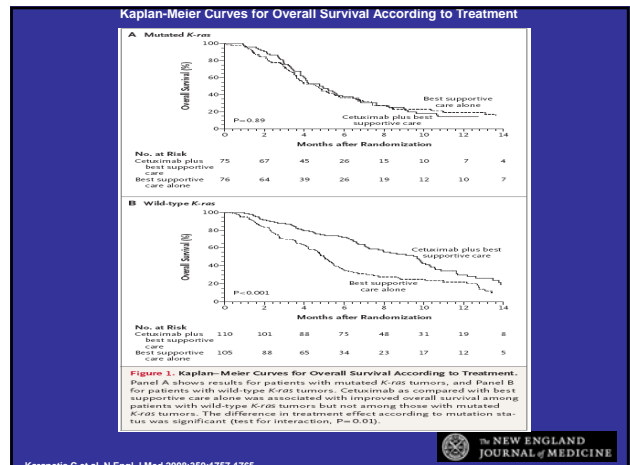


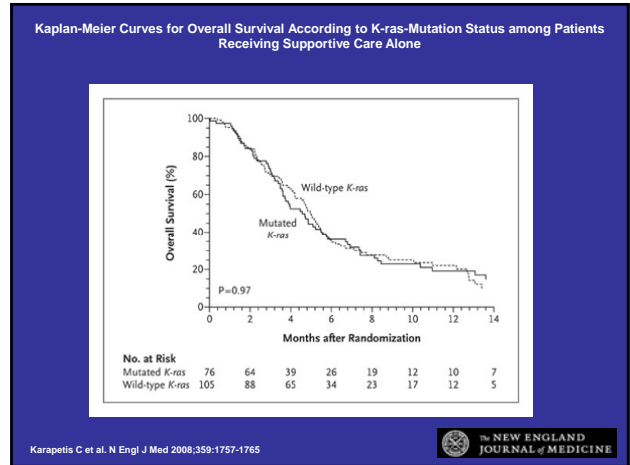
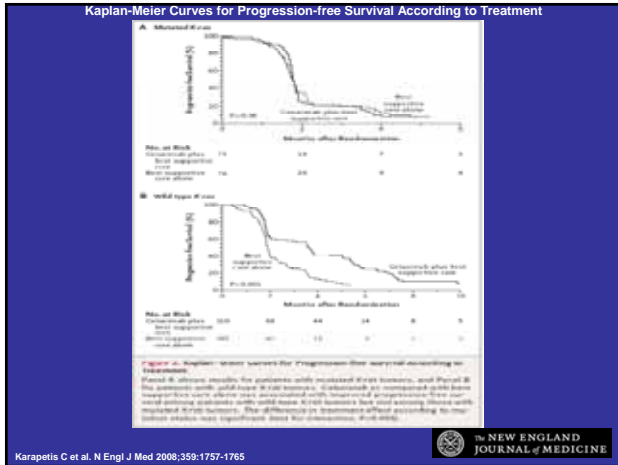
- ### CRYSTAL Subset Results: Considerations
- Retrospective study conducted on subset of patients assessable for KRAS status
    - However, subsets were similar to the ITT population in terms of demographics and disease characteristics
  - Assay methodology
    - Sensitive mutation-specific PCR assay was used which included the most frequent mutations in codons 12 and 13
  - Analysis largely conducted on primary tumor site
    - Available data (although scant) indicates that primary and metastatic colorectal tumors exhibit the same KRAS mutation status
  - Prognostic effect of KRAS
    - The lack of an untreated arm makes it more difficult to eliminate prognostic effects

### Distribution of K-ras Mutations According to Treatment Group

Mutation <sup>a</sup>	All Patients (N=184)	Patients Receiving Cetuximab plus Best Supportive Care (N=81)	Patients Receiving Best Supportive Care Alone (N=83)
G12A	11 (6.4)	7 (8.3)	4 (4.6)
G12C	9 (5.1)	6 (7.1)	3 (3.4)
G12D	61 (31.7)	39 (49.5)	33 (37.8)
G12E	2 (1.2)	1 (1.2)	1 (1.1)
G12V	17 (9.6)	7 (8.3)	10 (11.3)
G13V	48 (28.2)	29 (33.3)	30 (33.8)
G13A	1 (0.6)	0	1 (1.1)
G13C	1 (0.6)	0	1 (1.1)
G13D	20 (11.7)	7 (8.3)	17 (19.3)
G13V	1 (0.6)	0	1 (1.1)

<sup>a</sup> Seven patients had more than one mutation type.





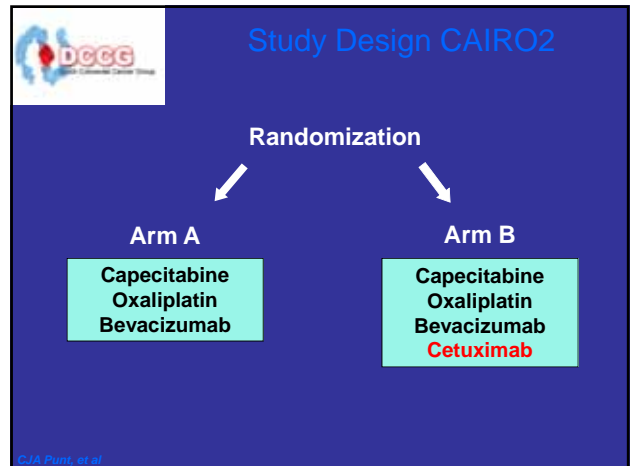
**DCCG**

Randomized phase III study of capecitabine, oxaliplatin and bevacizumab with or without cetuximab in advanced colorectal cancer

**CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG)**

J Tol, M Koopman...CJA Punt, et al.

NEJM Feb 5, 2009; 360 (6): 563-72



Association of the Mutation Status of the KRAS Gene with Progression-free Survival, Overall Survival, and Response Rate

**Table 1. Association of the Mutation Status of the KRAS Gene with Progression-free Survival, Overall Survival, and Response Rate.\***

Variable	Wild-type KRAS	Mutated KRAS	P Value
No. of patients	104	108	
CR group	104	108	
CRC group	104	108	
Median progression-free survival (mo)	10.6	11.1	0.81
CR group	10.6	11.1	0.81
CRC group	10.6	11.1	0.81
P value	0.81	0.81	0.81
Median overall survival (mo)	21.9	21.9	0.96
CR group	21.9	21.9	0.96
CRC group	21.9	21.9	0.96
P value	0.96	0.96	0.96
Response rate (%)	59.0	59.2	0.91
CR group	59.0	59.2	0.91
CRC group	59.0	59.2	0.91
P value	0.91	0.91	0.91

\* CR denotes capecitabine, oxaliplatin, and bevacizumab; and CRC denotes capecitabine, oxaliplatin, bevacizumab, and cetuximab.

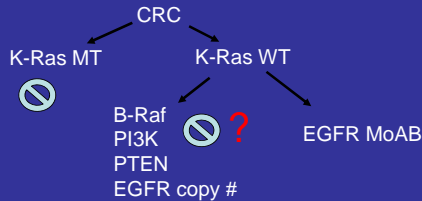
Tol J et al. N Engl J Med 2009;360:563-72

THE NEW ENGLAND JOURNAL OF MEDICINE

- DCCG**
- Conclusions - I**
- The addition of cetuximab to capecitabine, oxaliplatin and bevacizumab results in a significantly decreased progression-free survival, without affecting overall survival
  - The addition of cetuximab to chemotherapy and bevacizumab results in a significant increase of skin toxicity and diarrhea, however the toxicity is acceptable in both treatment arms
  - The grade of cetuximab-related skin toxicity significantly correlates with PFS



Now that Mutated K-Ras is an Established Marker of Resistance, the *Next Advance* will be in the identification of resistance markers in K-Ras Wild-type tumors



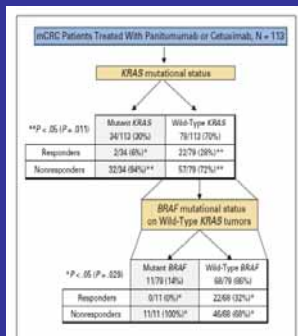
### B-Raf and Resistance to EGFR MoABs

- Mutated in 3-15% CRC
- Ras and Raf mutations exclusive of each other
- Gain-of-function mutations
  - Inability to convert the active form to inactive conformation



Schubbert et al. Nat Rev Ca, 2007

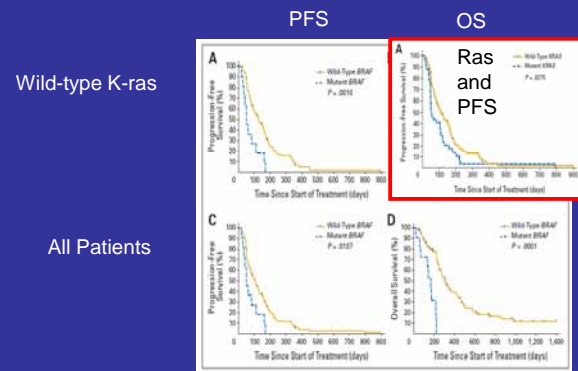
*"We hypothesized that in K-ras wild-type patients, B-raf mutations could have prognostic/predictive value."*



- All pts had progressed on at least one line of therapy
  - ~50% received monotherapy with EGFR MoAB
  - ~50 MoAB with chemotherapy
- In patients with tumors with Mut B-raf, there were NO objective responses

Nicolantonio et al. JCO 2008  
Wild-Type BRAF is Required for Response to EGFR MoABs

### B-Raf Predicts for Benefit of Anti-EGFR Therapy in Patients with WT Ras and the Entire Cohort



### BRAF Mutation in Metastatic Colorectal Cancer

- CAIRO 2
- Retrospective assessment of BRAF V600E
- 516 tumors (755 randomized PTs)
- 314 tumors wild type KRAS
- 45 tumors
- BRAF and KRAS mutations mutually exclusive
- KRAS restricted to outcome of cetuximab tx
- BRAF not restricted to outcome of cetuximab tx

Tol et al, NEJM 361: 98-99, 2009

### Association of the Mutation Status of the BRAF Oncogene with Progression-free Survival, Overall Survival, and Response Rate

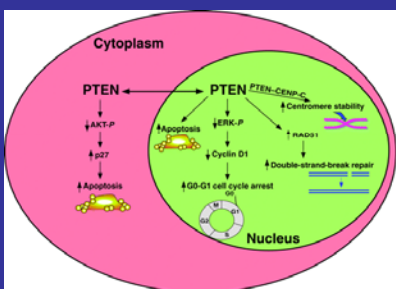
Variable	Wild-Type BRAF	Mutated BRAF	P Value
No. of patients			
CR group	243	27	
CRC group	333	39	
Median progression-free survival (mo)			
CR group	12.2	5.9	<0.001
CRC group	10.4	6.6	<0.010
Median overall survival (mo)			
CR group	24.6	13.0	<0.002
CRC group	22.5	11.2	<0.001
Response rate (%)			
CR group	30	33	0.53
CRC group	48	39	0.43

\* BRAF denotes B-type Raf kinase; CR, capecitabine, oxaliplatin, and bevacizumab; and CRC, capecitabine, oxaliplatin, and bevacizumab plus cetuximab.

Tol J et al. N Engl J Med 2009;361:98-99



### PTEN Pathway – Functions in the Cytoplasm and Nucleus



Nuclear PTEN plays a role in chromosome stability, DNA repair, cell cycle arrest and cellular stability.

Planchon et al., J Cell Sci, 2008

44th ASCO Annual Meeting May 30-June 3, 2008  
McCormick Place, Chicago, Illinois

### Evaluation of PTEN expression in colorectal cancer (CRC) metastases (mets) and in primary tumors as predictors of activity of cetuximab plus irinotecan treatment

E. Loupakis<sup>1,6</sup>, L. Pollina<sup>2</sup>, I. Stasi<sup>1</sup>, G. Masi<sup>1</sup>, N. Funel<sup>2</sup>, M. Scartozzi<sup>3</sup>, I. Petrini<sup>4</sup>, D. Santini<sup>5</sup>, S. Cascinu<sup>3</sup>, A. Falcone<sup>1,6</sup>.

<sup>1</sup>Department of Oncology, Azienda USL 6 - Istituto Toscano Tumori Livorno, Italy,

<sup>2</sup>Division of Pathology, AOUP, Pisa, Italy,

<sup>3</sup>Division of Medical Oncology, Azienda Ospedaliera Ospedali Riuniti,

Università Politecnica delle Marche, Ancona, Italy,

<sup>4</sup>Division of Medical Oncology, AOUP, Pisa, Italy,

<sup>5</sup>Division of Medical Oncology, Campus Biomedico University, Rome, Italy,

<sup>6</sup>Department of Oncology, Transplants and New Technologies in Medicine, University of Pisa, Italy



### STUDY DESIGN and TREATMENT

#### DESIGN

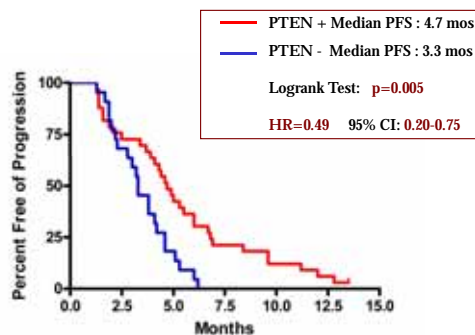
Retrospective evaluation of 102 EGFR-positive metastatic CRC patients treated with Cetuximab plus Irinotecan and progressed after previous Irinotecan-containing therapies

Centers: 4 Medical Oncology Divisions from Central Italy

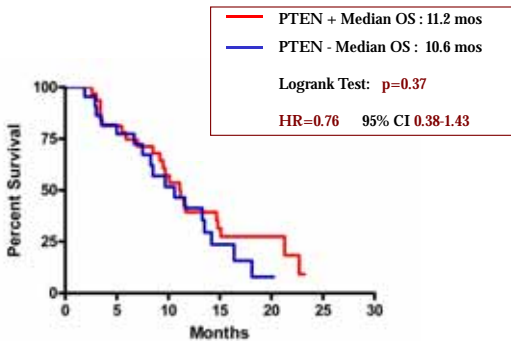
#### TREATMENT

- **Cetuximab** initial dose of 400 mg/sqm i.v. d1 followed by 250 mg/sqm i.v. weekly  
*or*  
500 mg/sqm i.v. d1 every 2 weeks.
- **Irinotecan** 130-180 mg/sqm i.v. d1 every 2 weeks.

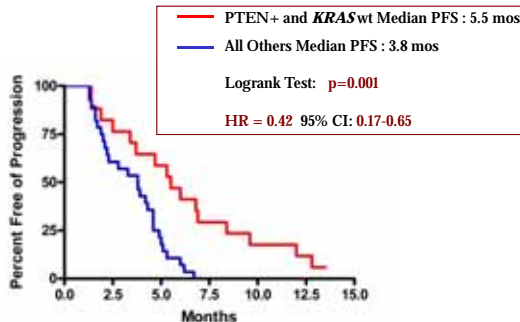
### PTEN (on mets) and PFS

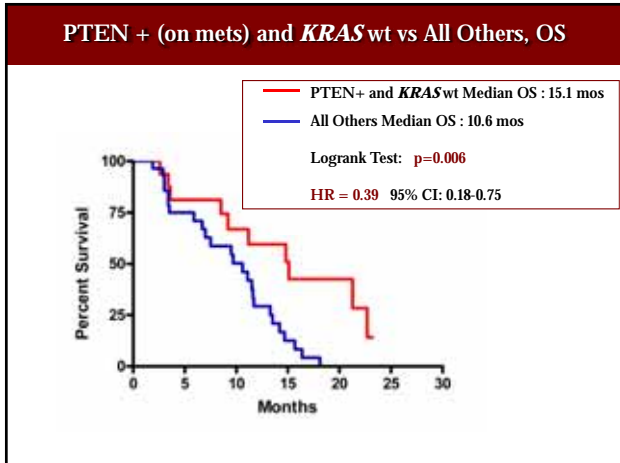


### PTEN (on mets) and OS

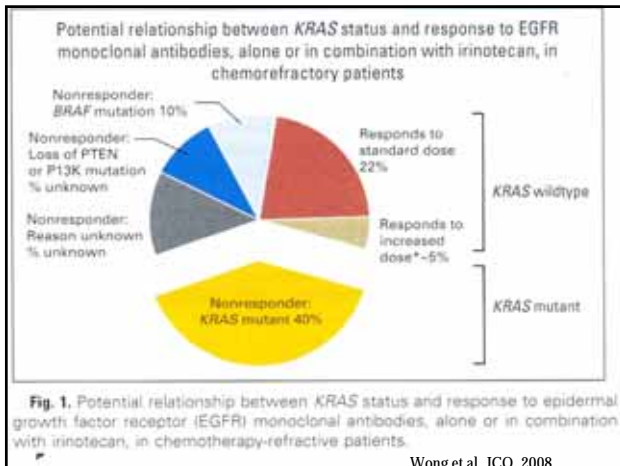


### PTEN + (on mets) and KRAS wt vs All Others, PFS





- ### CONCLUSIONS
- ✓ Primaries and related mets from CRC differed in terms of PTEN immunoreactivity in 40% of cases.
  - ✓ KRAS mutations found on primaries are almost always (95% of cases) confirmed on mets. Such analysis may be ruled out on any available tumor sample.
  - ✓ Loss of PTEN tested on mets predicted lack of activity of cetuximab plus irinotecan combination treatment in metastatic CRC pts.
  - ✓ KRAS is confirmed to be a predictor of resistance to cetuximab plus irinotecan combination treatment in metastatic CRC pts.
  - ✓ The combination of PTEN IHC performed on mets and KRAS mutational analysis identified a subgroup of patients with higher chances of benefiting from cetuximab plus irinotecan treatment.



### Detection of KRAS Oncogene in Peripheral Blood as a Predictor of the Response to Cetuximab Plus Chemotherapy in Patients with Metastatic Colorectal Cancer

Li-Chen Yen, Yung-Sung Yeh, Chao-Wen Chen, Hwei-Ming Wang, Hsiang-Lin Tsai, Chien-Yu Lu, Yu-Tang Chang, Koung-Shing Chu, Shiu-Ru Lin and Jaw-Yuan Wang

Yen et al, CCR, 15 (13), 4508-4513, July 1, 2009.

#### KRAS mutation detection in tumor tissues by direct sequencing and in peripheral blood by membrane-array method

	No. (%)
Tumor tissues	76
KRAS mutations	
Mutations	33 (43.4)
Wild type	43 (56.6)
KRAS mutation sites ( n = 33 )	
Codon 12	11 (33.3)
Codon 13	9 (27.3)
Codon 15	7 (21.2)
Codon 18	1 (3.0)
Codon 20	2 (6.1)
Codon 30	2 (6.1)
Codon 31	1 (3.0)
Activating KRAS mutant ( n = 33 )	
Positive (codons 12, 13, 15, 18)	28 (84.8)
Negative (codons 20, 30, 31)	5 (15.2)
Peripheral blood	
KRAS mutations	
Activating mutations (codons 12, 13, 15, 18)	30 (39.5)
Wild type	46 (60.5)

Yen et al, CCR, 15 (13), 4508-4513, July 1, 2009.

#### Correlation between clinical response to cetuximab plus chemotherapy and clinicopathologic features of 76 metastatic colorectal cancer patients

	Responders (n=45), no. (%)	Nonresponders (n=31), no. (%)	P
Chemotherapy regimen			
Cetuximab + FOLFOX	34 (75.6)	20 (64.5)	0.297
FOLFIRI	11 (24.4)	11 (35.5)	
Activating KRAS mutant in tumor tissues			
Mutant	4 (8.9)	24 (77.4)	<0.0001
Wild type	41 (91.1)	7 (22.6)	
KRAS mutant in peripheral blood			
Mutant	5 (11.1)	25 (80.6)	<0.0001
Wild type	40 (88.9)	6 (19.4)	

Yen et al, CCR, 15 (13), 4508-4513, July 1, 2009.

Correlation between progression-free and overall survival in metastatic colorectal cancer patients using multivariate Cox proportional hazard regression analysis according to KRAS mutations in tumor or peripheral blood

Variables	Progression-free survival		Overall survival	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Activating KRAS mutations in tumor				
No	0.253 (0.149-0.478)	<0.0001	0.151 (0.077-0.365)	<0.0001
Yes	1.000 (reference)		1.000 (reference)	
KRAS mutations in peripheral blood				
No	0.189 (0.113-0.305)	<0.0001	0.112 (0.053-0.274)	<0.0001
Yes	1.000 (reference)		1.000 (reference)	

Yen *et al*, *CCR*, 15 (13), 4508-4513, July 1, 2009.