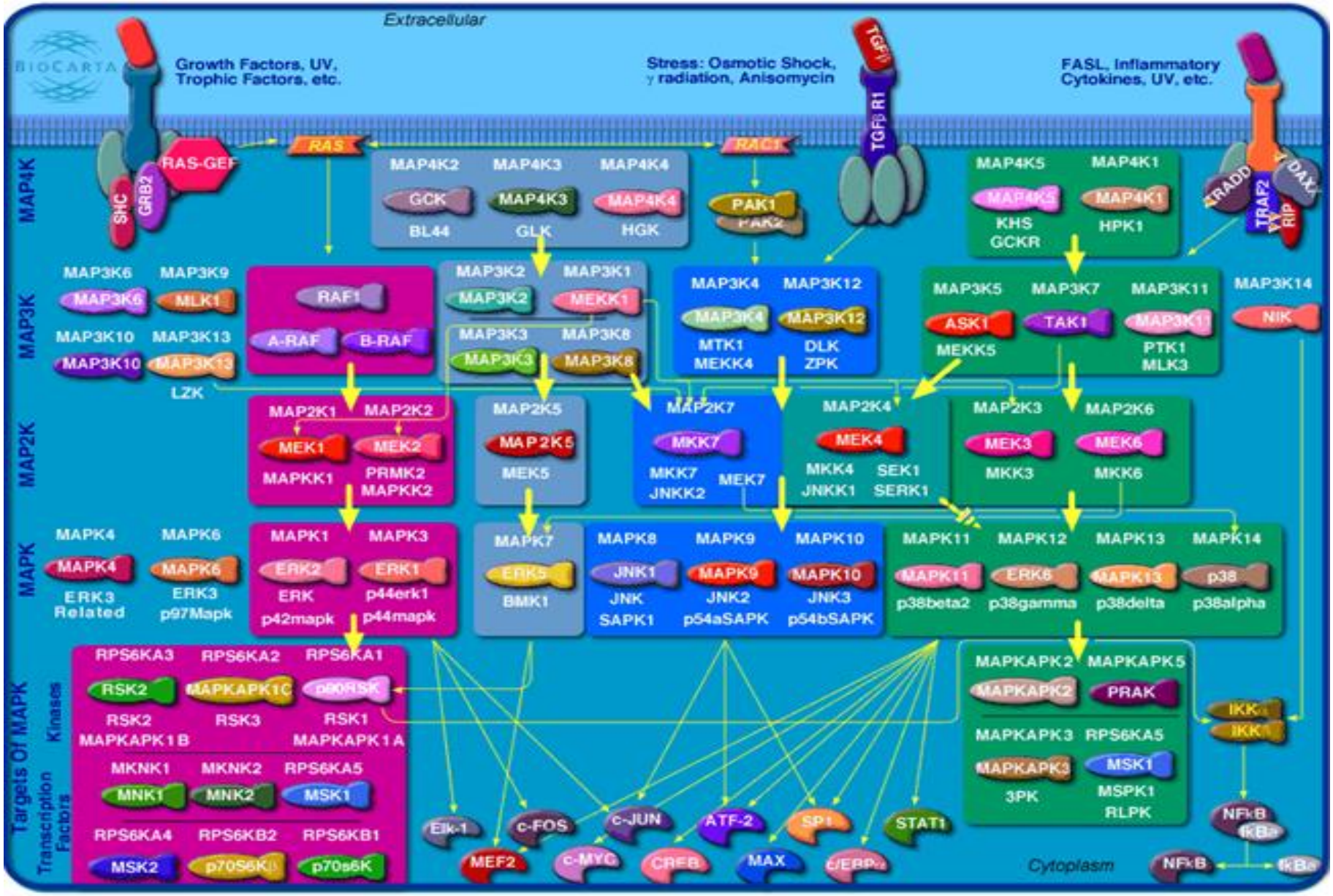
# Clinical therapeutic application in cancer Colorectal cancer (RAS, BRAF)

JY DOUILLARD MD PhD
Professor of Medical Oncology
Integrated Centres of Oncology R. Gauducheau
University of Nantes France

UNIVERSITÉ DE NANTES

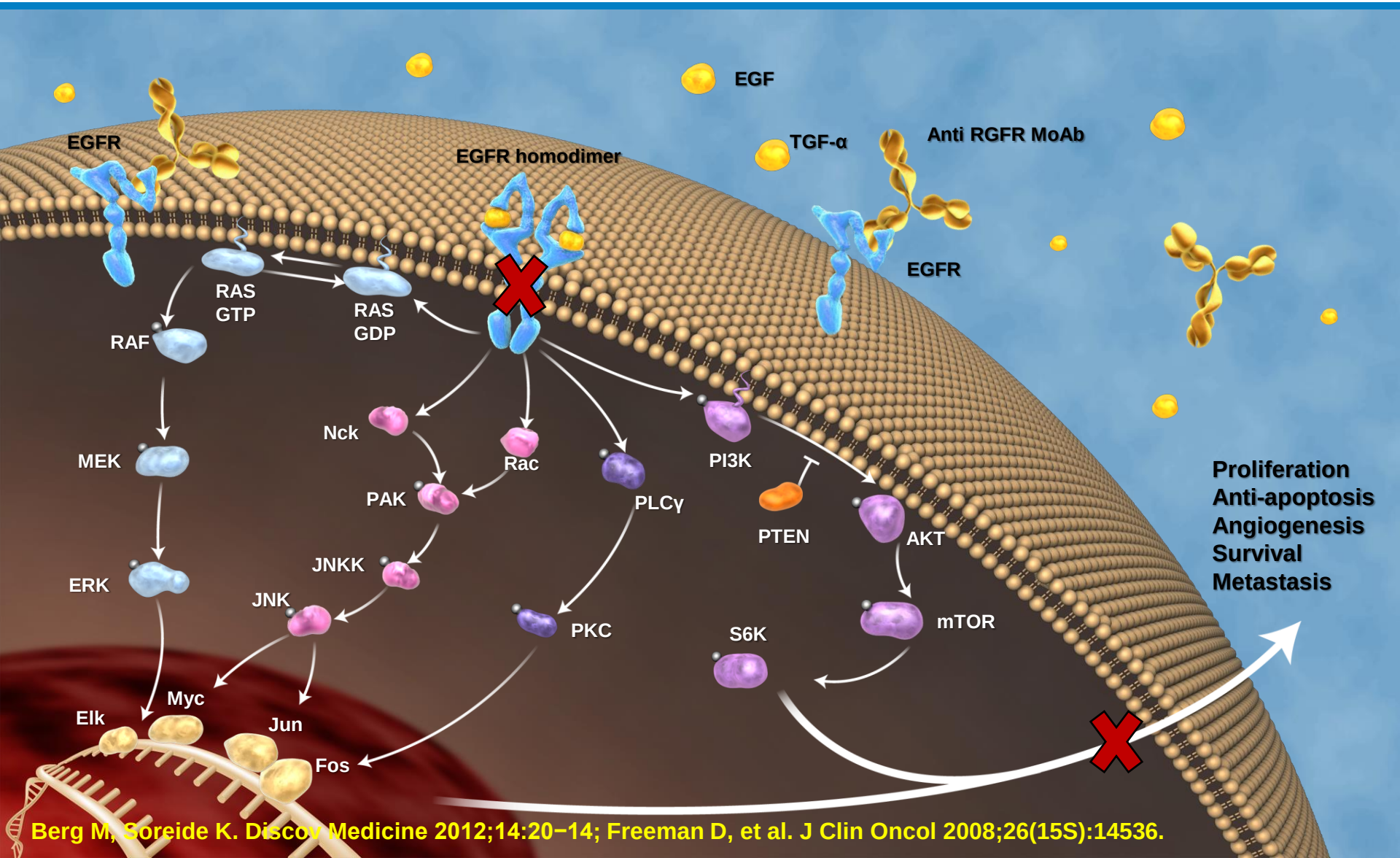
Institut de Cancérologie de l'Ouest

# The MAP Kinase map




Glenn Croston, PhD, Roger Anderson, PhD www.biocarta.com/pathfiles/h_mapkpathway.asp

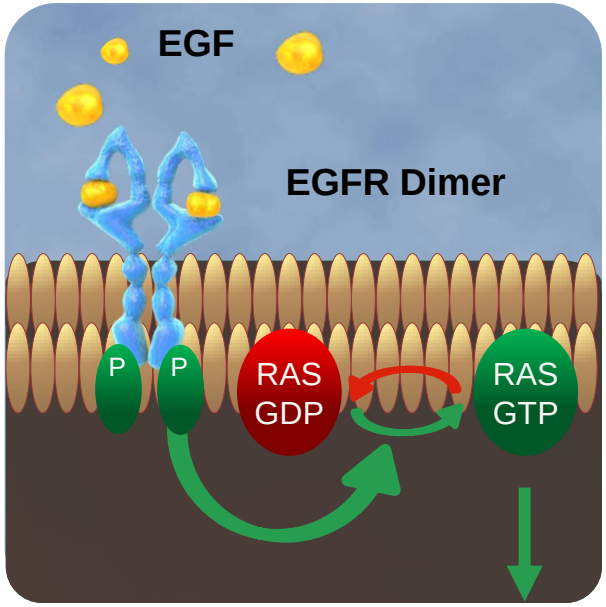
# RAS-RAF-MEK-ERK as downstream pathway of the EGFR activation




Berg M, Soreide K. Discov Medicine 2012;14:20−14; Freeman D, et al. J Clin Oncol 2008;26(15S):14536.

# EGFR Signaling and RAS mutation

## EGFR signalling via RAS



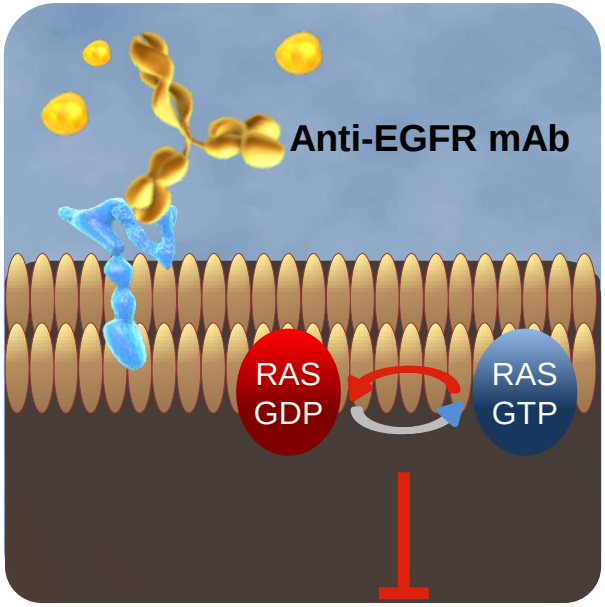

RAS effector pathways

↓

e.g. Tumour proliferation

## Anti-EGFR inhibition of EGFR signalling

### *RAS* WT

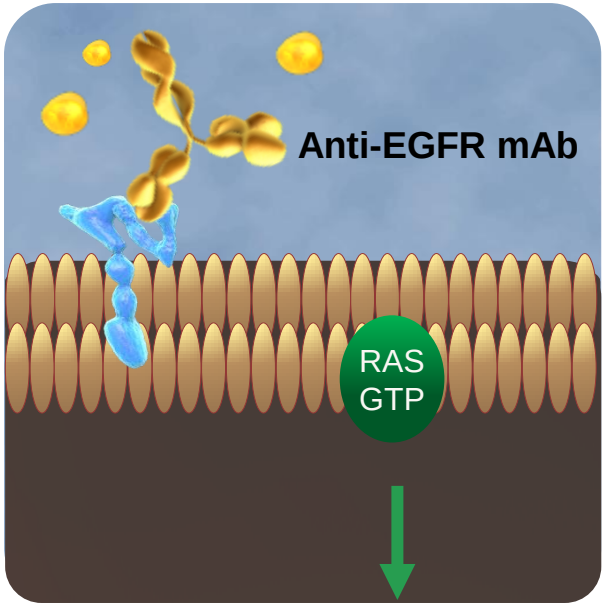



Attenuation of signalling

↓

Inhibition of tumour proliferation

### *RAS* MT




Constitutive signalling

↓

Tumour proliferation

Schubbert S, et al. Nat Rev Cancer 2007;7:295–308; Foon KA, et al. Int J Radiat Oncol Biol Phys 2004;58:984–90;
Berg M, Soreide K. Discov Med 2012;14:207–14; Custodio A, Feliu J. Crit Rev Oncol Hematol 2013;85:45–81.

Example signalling pathways, simplified for illustrative purposes
WT, wild type; MT, mutant.

# Panitumumab single agent
# Response rates in patients with *KRAS* WT (exons 2/3) mCRC

| Genotype | | Combined panitumumab + BSC[#] (n = 138) | |
|---|---|---|---|
| | | n | Response rate, % (95% CI) |
| *NRAS* | WT | 126 | 17 (11–25) |
| | MT | 9 | 0 (0–34) |
| *EGFR* | WT | 134 | 16 (11–24) |
| | MT | 0 | NA |
| *BRAF* | WT | 107 | 17 (10–25) |
| | MT | 13 | 0 (0–25) |
| *PTEN* | WT | 122 | 16 (10–24) |
| | MT | 9 | 11 (0–48) |
| *PIK3CA* | WT | 117 | 15 (9–22) |
| | MT | 10 | 20 (3–56) |
| *AKT1* | WT | 121 | 17 (10–24) |
| | MT | 1 | 0 (0–98) |
| *TP53* | WT | 50 | 14 (6–27) |
| | MT | 84 | 17 (9–26) |
| *CTNNB1* | WT | 118 | 15 (9–23) |
| | MT | 2 | 50 (1–99) |

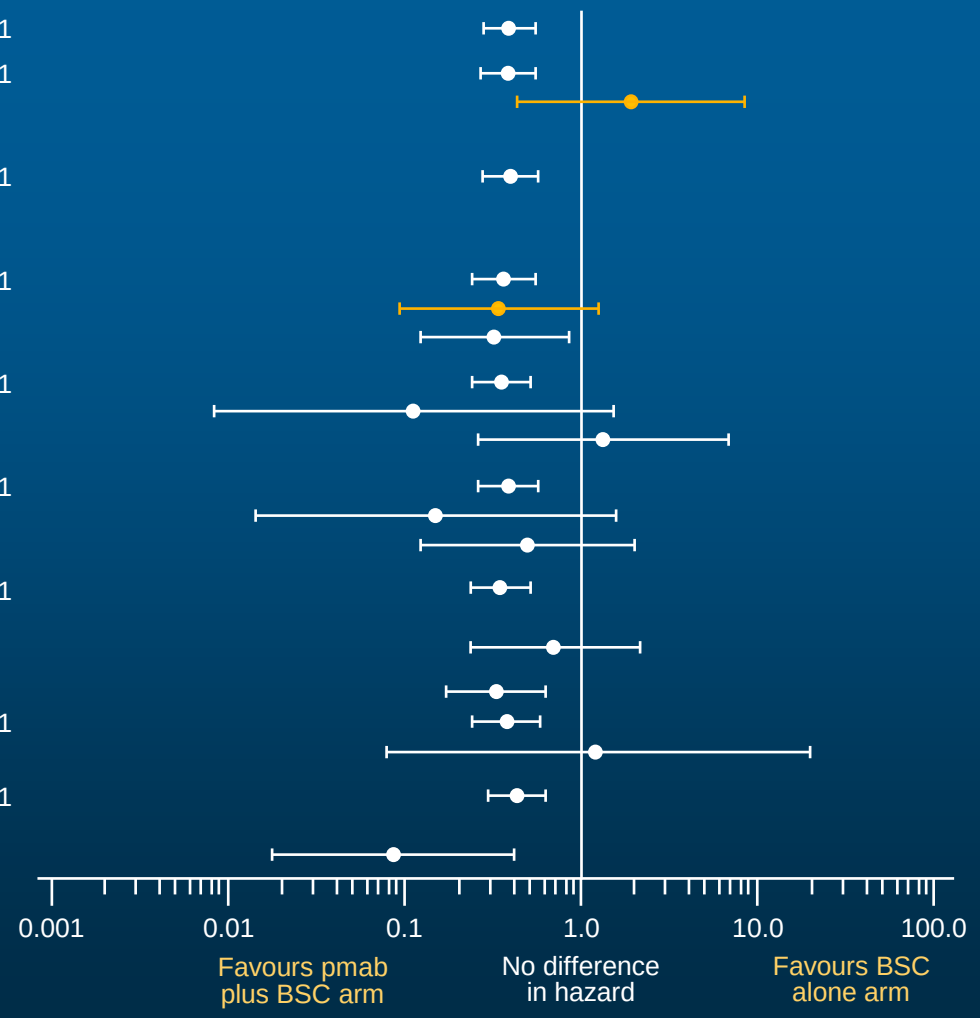
[#]Per local review.
BSC, best supportive care.

Peeters M, et al. Clin Cancer Res 2013;19:1902−12.

# Panitumumab single agent trial *RAS* (Exon 2/3)

## PFS—Differential Effect of Pmab in MT *NRAS* vs MT *BRAF*

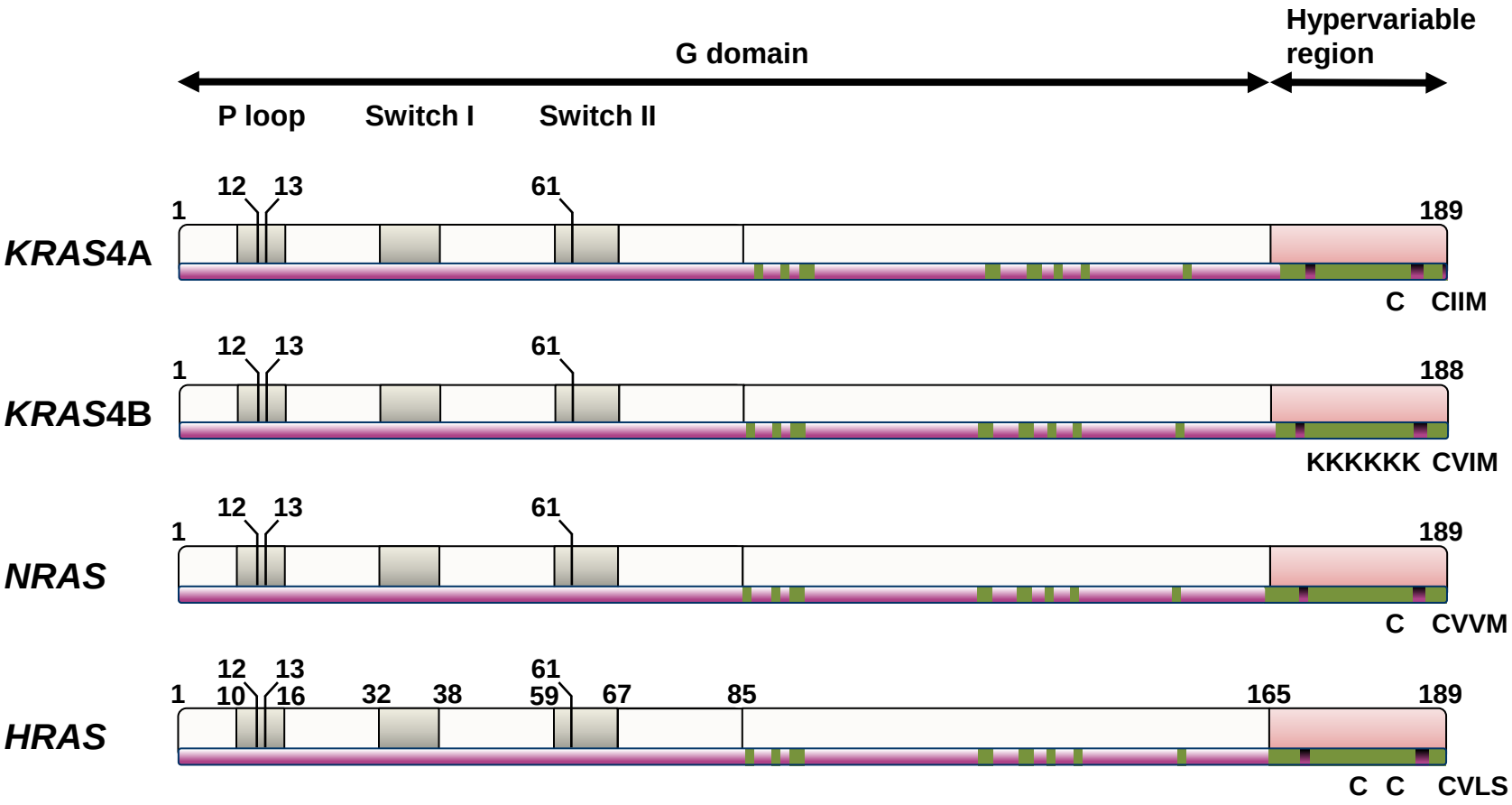
| Gene | Genotype | n | HR (95% CI) | *p*-value |
|---|---|---|---|---|
| ***KRAS* WT** | Total | 153 | 0.39 (0.28–0.56 | <0.0001 |
| ***NRAS*** | WT | 138 | 0.39 (0.27–0.56) | <0.0001 |
| | **MT** | 11 | **1.94 (0.44–8.44**) | 0.3789 |
| | ND | 4 | 0.00 (0.00–NE) | 1.0000 |
| *EGFR* | WT | 149 | 0.40 (0.28–0.58) | <0.0001 |
| | MT | 0 | NE | NE |
| | ND | 4 | NE | NE |
| ***BRAF*** | WT | 115 | 0.37 (0.24–0.55) | <0.0001 |
| | **MT** | 15 | **0.34 (0.09–1.24)** | 0.1035 |
| | ND | 23 | 0.32 (0.12–0.85) | 0.0223 |
| *PTEN* | WT | 135 | 0.36 (0.25–0.52) | <0.0001 |
| | MT | 9 | 0.11 (0.01–1.52) | 0.1001 |
| | ND | 9 | 1.34 (0.26–6.85) | 0.7290 |
| *PIK3CA* | WT | 128 | 0.39 (0.26–0.57) | <0.0001 |
| | MT | 10 | 0.15 (0.01–1.57) | 0.1139 |
| | ND | 15 | 0.50 (0.12–2.00) | 0.3254 |
| *AKT1* | WT | 133 | 0.35 (0.24–0.51) | <0.0001 |
| | MT | 1 | NE | NE |
| | ND | 19 | 0.70 (0.23–2.13) | 0.5346 |
| *TP53* | WT | 56 | 0.33 (0.18–0.63) | 0.0006 |
| | MT | 93 | 0.38 (0.24–0.60) | <0.0001 |
| | ND | 4 | 1.21 (0.07–19.71) | 0.8957 |
| *CTNNB1* | WT | 132 | 0.44 (0.30–0.64) | <0.0001 |
| | MT | 3 | 0.00 (0.00–NE) | 0.9990 |
| | ND | 18 | 0.09 (0.02–0.43) | 0.0026 |




BSC, best supportive care; MT, mutant; ND, not determined; NE, not evaluable; Pmab, panitumumab; WT, wild-type

Peeters M, et al. Clin Cancer Res 2013; 19:1902-12.

# Three cellular *RAS* genes encode four highly homologous 21 kD proteins




Adapted from Schubbert S, et al. Nat Rev Cancer 2007; 7:295–308.

# Anti-EGFR monoclonal antibodies in mCRC
# Cetuximab (Erbitux®) Panitumumab (Vectibix®)

- Anti-EGFR MoAbs are approved in the treatment of mCRC:
  - 1st line with chemotherapy Folfox or Folfiri
  - 2nd line irinotecan Cetuximab
  - 2nd line Folfiri Panitumamab
  - 3rd line single agents

- The target population are patients with a wt RAS phenotype
  - Kras and Nras exons 2, 3 and 4

- Oral fluoropyrimidines and bolus 5FU should not be used

- A proven detrimental effect on PFS and OS in patients with any type of RAS mutation trated with Folfox and anti EGFR

# Anti-EGFR monoclonal antibodies in mCRC

- 2 mAbs are available:

- CETUXIMAB[1]
  - IgG1 Human:mouse mAb
  - Extensively evaluated in CRC
  - Mediates ADCC in vitro
  - Administered weekly

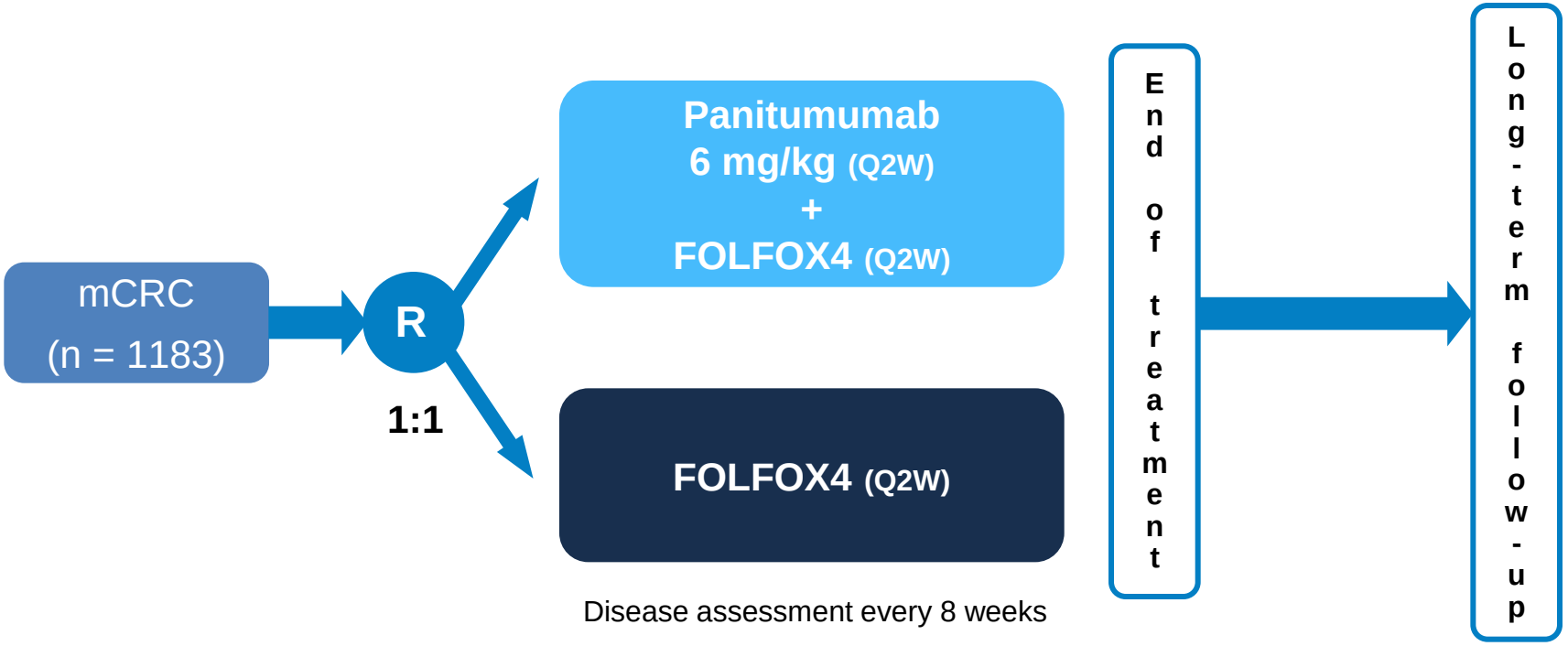
- PANITUMUMAB[2]
  - IgG2 fully human mAb
  - Extensively evaluated in CRC
  - May activate C2 complement
  - Administered Q 2 weeks

# Impact of extended RAS analysis vs.Kras Exon 2 on anti-EGFR MoAb efficacy in mCRC

Data from the PRIME trial

# PRIME trial

## FOLFOX4 ± panitumumab in first-line treatment of mCRC

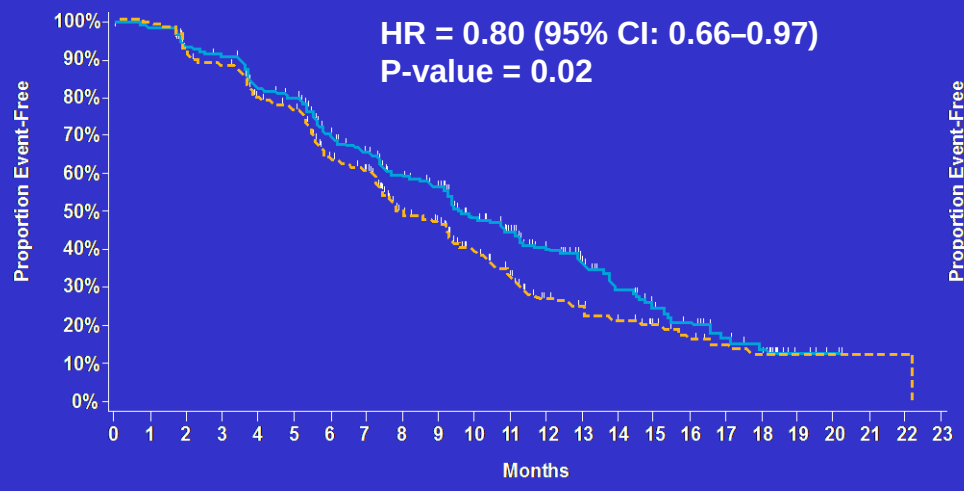



- Study endpoints: PFS (1º), OS, ORR, safety
- Design amended to focus on prospective hypothesis testing in the *KRAS* WT# stratum

Douillard J-Y, et al. J Clin Oncol 2010;28:4697–705;

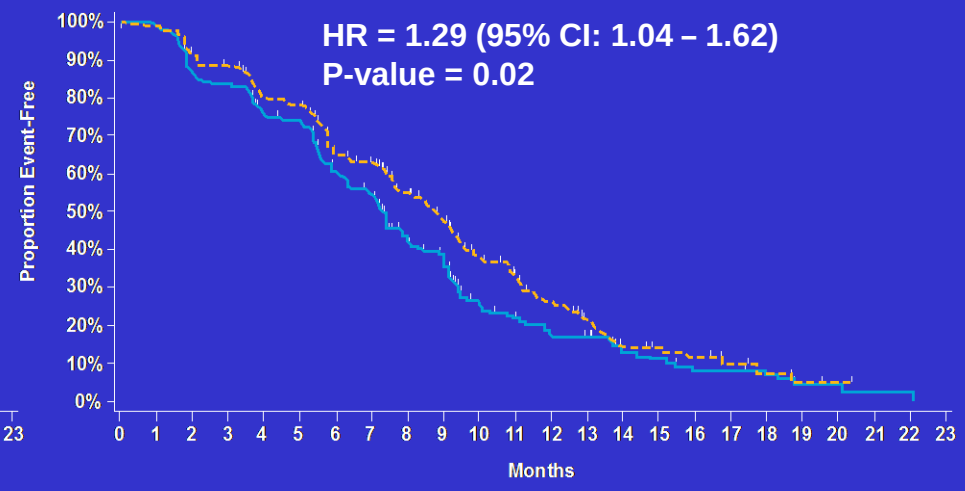
# PRIME: PFS by *KRAS* Mutation Status

## WT *KRAS*




| | Events n (%) | Median (95% CI) months |
|---|---|---|
| Panitumumab + FOLFOX | 199 (61) | 9.6 (9.2–11.1) |
| FOLFOX | 215 (65) | 8.0 (7.5–9.3) |

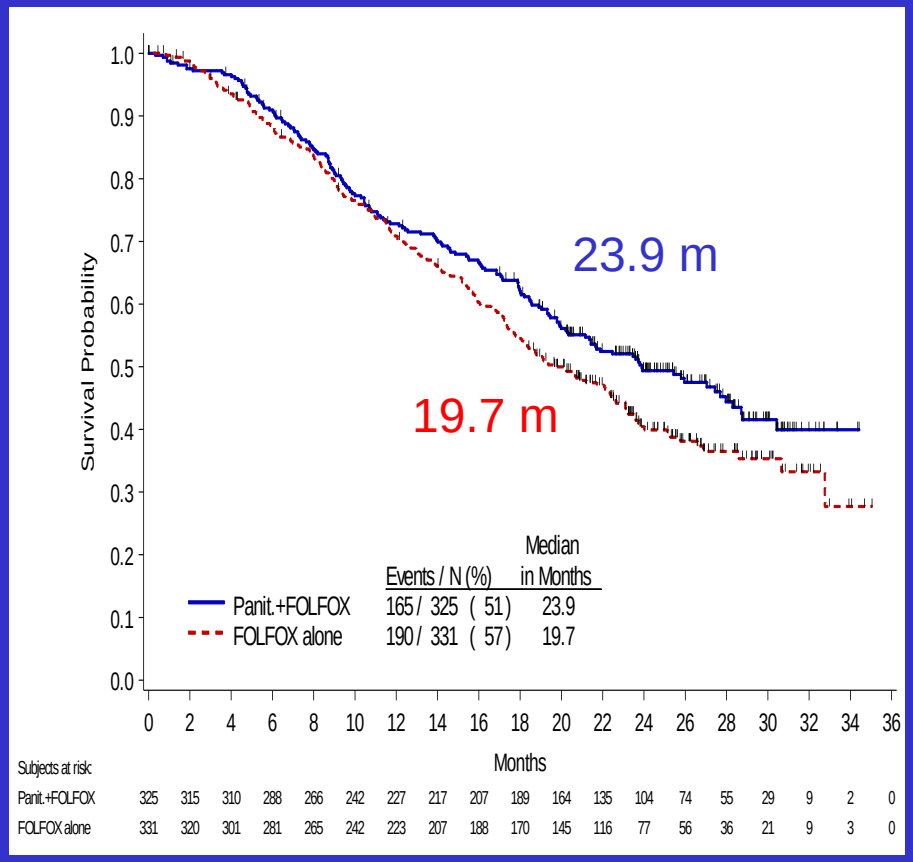
## MT *KRAS*




| | Events n (%) | Median (95% CI) months |
|---|---|---|
| Panitumumab + FOLFOX | 167 (76) | 7.3 (6.3 – 8.0) |
| FOLFOX | 157 (72) | 8.8 (7.7 – 9.4) |

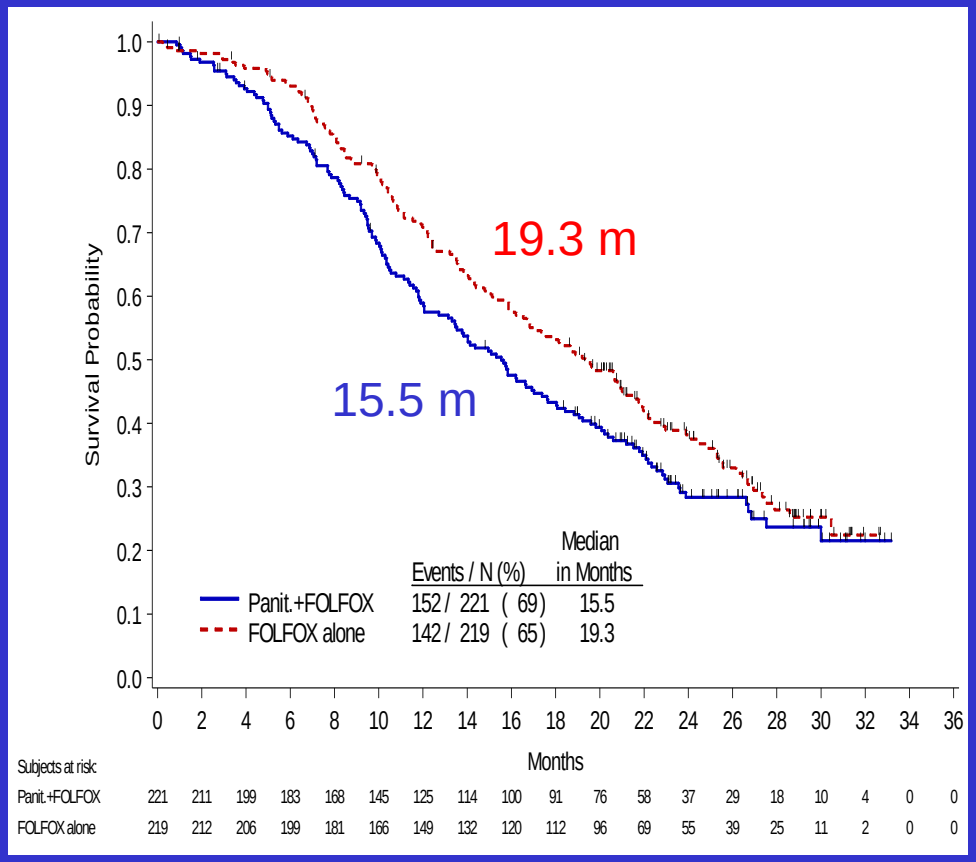
# PRIME: OS by *KRAS* Status

## WT *KRAS*




HR 0.83 (0.67-1.02) p=0.072

## MT *KRAS*



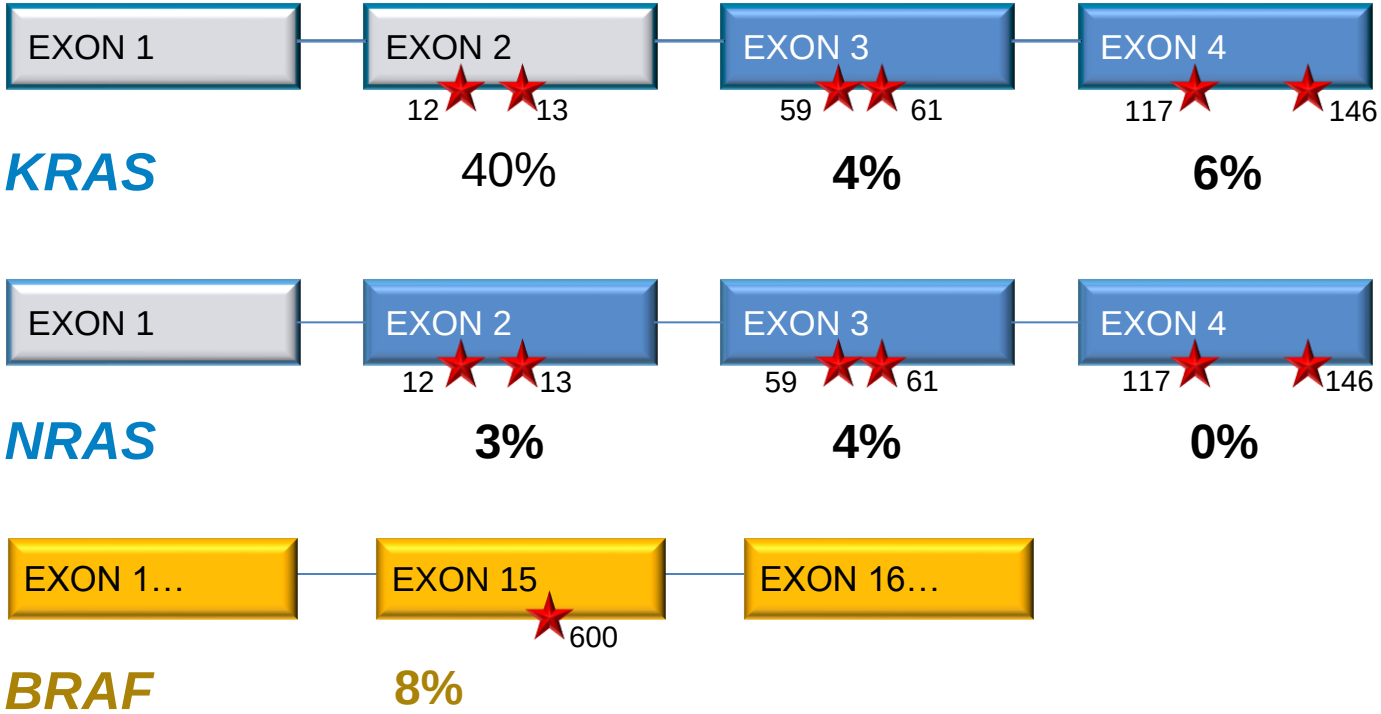

HR 1.24 (0.98-1.57) p=0.068

# Benefit of anti EGFR MoAbs in Kras exon2 mCRC1 st line

| wt Kras | Folfiri [1] | Folfiri cetux | Folfox [2] | Folfox Cetux | Folfox [3] | Folfox Pani |
|---|---|---|---|---|---|---|
| RR % | 43 | 57 p=0.0001 | 34 | 57 p=0.002 | 48 | 57 p=0.02 |
| PFS m | 8.4 | 9.9<br>HR 0.70* | 7.2 | 8.3<br>HR 0.56* | 8 | 9.6<br>HR 0.80* |
| OS m | 20 | 23.5<br>HR 0.80* | 18.5 | 22.8<br>HR 0.85** | 19.4 | 23.8<br>HR 0.83* |
| | | | | | | |
| mt Kras | | | | | | |
| RR% | 36 | 31 | 52 | 34 0.02 | 40 | 41 |
| PFS m | 7.7 | 7.4 HR 1.17** | 8.6 | 5.5 HR 1.72* | 8.8 | 7.3 HR 1.29* |
| OS m | 16.7 | 16.2 HR 1.03** | 17.5 | 13.4 HR 1.29** | 19.2 | 15.5 HR 1.16** |

1. Van Cutsem E, et al. *N Engl J Med* 2009; 360:1408-17, 2. Bokemeyer C, et al. *Ann Onc* 2011; doi:10.1093/annonc/mdq632, 3. Douillard JY, et al. *J Clin Onc* 2010;*27:* 4697-4705 (updated Jan 2013)

# *KRAS*, *NRAS* and *BRAF* mutation hotspots in the PRIME trial




Overall *RAS* ascertainment rate: 90%

Among WT *KRAS* exon 2 patients, an additional 17% of tumours with *RAS* mutations were found

**Douillard J-Y, et al. N Engl J Med 2013;369:1023–34**.

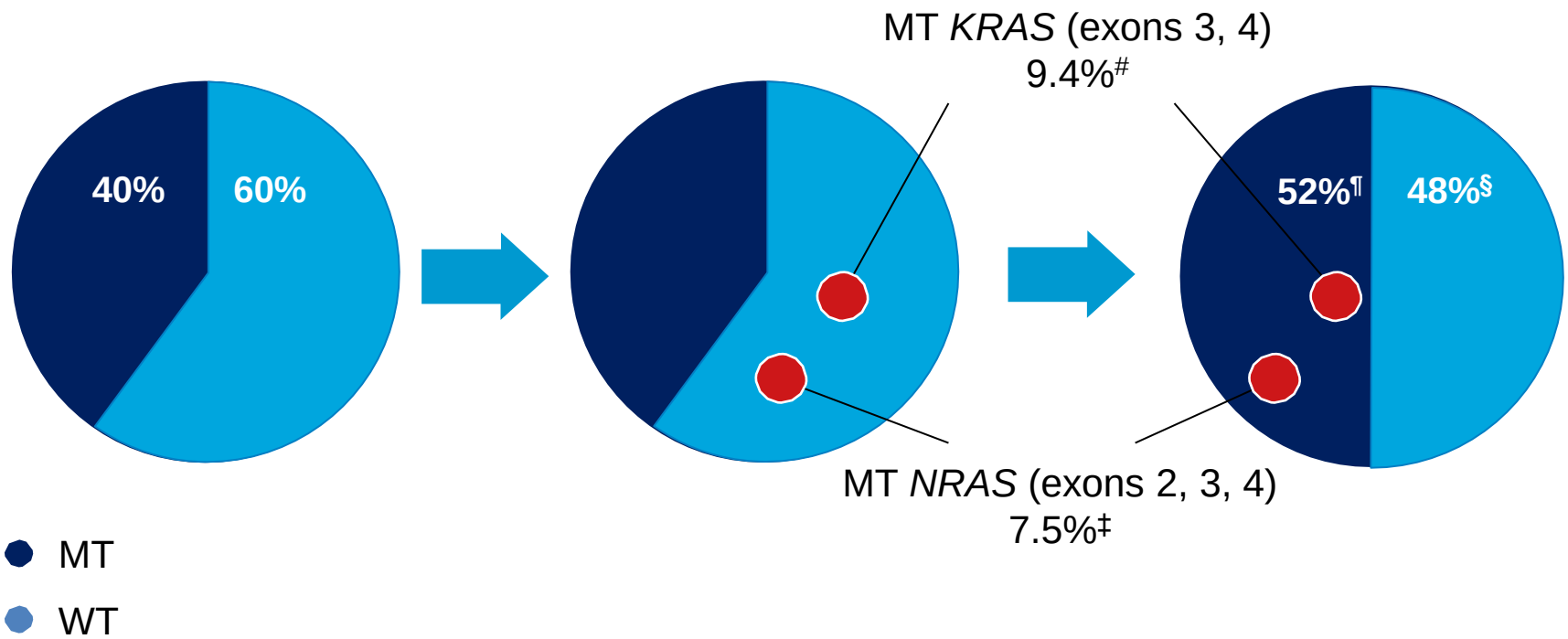
Percentages have been rounded. 7 patients harboured either *KRAS* or *NRAS* codon 59 mutations. *RAS* and *BRAF* ascertainment rate: 89%.

# PRIME trial *RAS* primary analysis

## *RAS* status – prevalence of *RAS* mutations among 1,060 evaluable patients

**PRIME (*KRAS* exon 2)[1]**

**PRIME *RAS* analysis[2]**
**(refinement of patient population by *RAS* mutation status)**





1. Douillard J-Y, et al. J Clin Oncol 2013;31(Suppl):abstract 3620 (and poster);
2. Douillard J-Y, et al. N Engl J Med 2013;369:1023–34.

# PRIME trial *RAS* analysis

## Refinement of patient population by WT *RAS* status (primary analysis)

| | WT *KRAS* exon 2[1] | | WT *RAS*[2] | |
|---|---|---|---|---|
| | Panitumumab + FOLFOX4 (n = 325) | FOLFOX4 (n = 331) | Panitumumab + FOLFOX4 (n = 259) | FOLFOX4 (n = 253) |
| Median PFS, months | **9.6** | 8.0 | **10.1** | 7.9 |
| Hazard ratio (P-value) | **0.80 (P = 0.02)** | | **0.72 (P = 0.004)** | |
| Median OS, months | **23.9** | 19.7 | **26.0** | 20.2 |
| Hazard ratio (P-value) | **0.83 (P = 0.072)** | | **0.78 (P = 0.043)** | |
| ORR*, % (95% CI) | 55 (50–61) (n = 175) | 48 (42–53) (n = 154) | **59** (52–65) (n = 149) | 46 (40–53) (n = 114) |
| Odds ratio (P-value) | 1.35† (P = 0.068) | | 1.63‡ (P = 0.009) | |

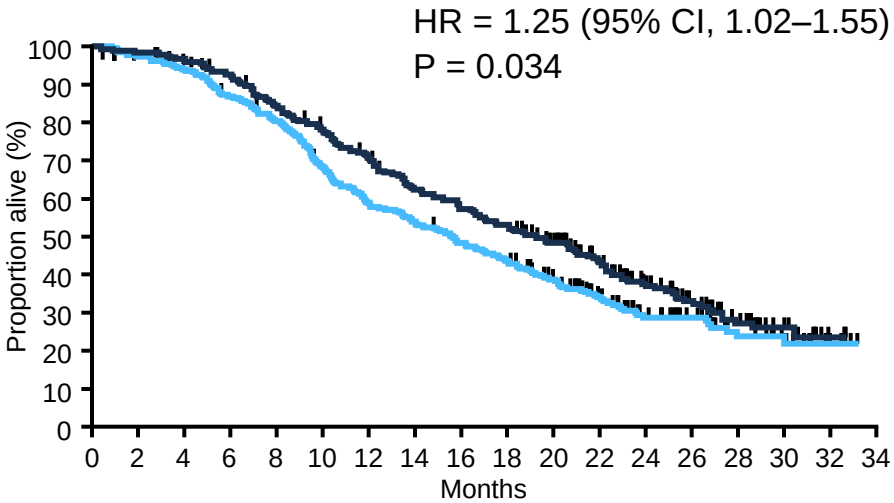

**1. Douillard J-Y, et al. J Clin Oncol 2010;28:4697–705;**
**2. Douillard J-Y, et al. N Engl J Med 2013;369:1023–34;**


*By central radiological assessment; †Stratified odds ratio; ‡Adjusted odds ratio; WT *RAS*, WT *KRAS* and *NRAS* exons 2, 3, 4. Includes 7 patients harbouring *KRAS/NRAS* codon 59 mutations.

# PRIME trial *RAS* primary analysis

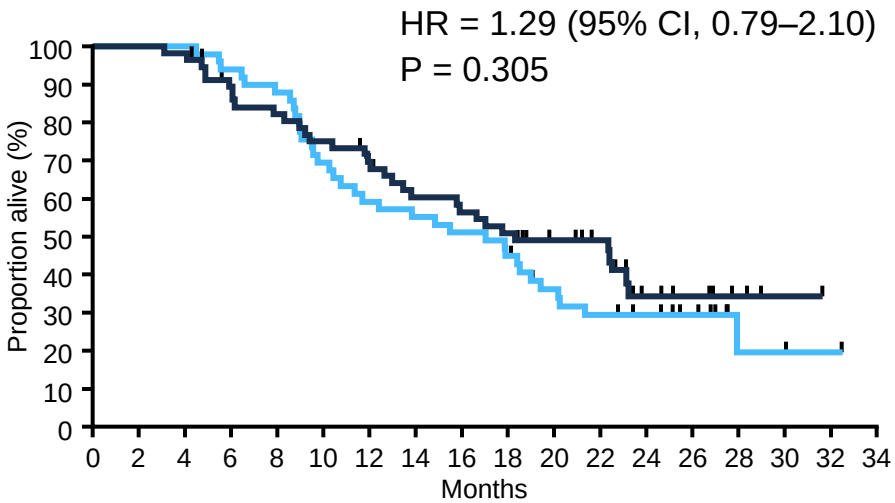
## *RAS* mutations predicted negative OS outcome with panitumumab + FOLFOX

### MT *RAS*




| | Events n (%) | Median, months (95% CI) |
|---|---|---|
| Panitumumab + FOLFOX4 (n = 272) | 187 (69) | 15.6 (13.4–17.9) |
| FOLFOX4 (n = 276) | 175 (63) | 19.2 (16.7–21.8) |

### WT *KRAS* exon 2, <u>other MT *RAS*</u>




| | Events n (%) | Median, months (95% CI) |
|---|---|---|
| Panitumumab + FOLFOX4 (n = 51) | 35 (69) | 17.1 (10.8–19.4) |
| FOLFOX4 (n = 57) | 33 (58) | 18.3 (13.0–23.2) |

**Douillard J-Y, et al. N Engl J Med 2013;369:1023–34,**

Predefined retrospective analysis.
Excludes 7 patients harbouring *KRAS/NRAS* codon 59 mutations.

# Improved outcome with better patient selection

| | | Kras exon 2 | | All Ras |
|---|---|---|---|---|
| CRYSTAL | PFS | 0.70 | → | 0.56 |
| | OS | 0.80 | → | 0.69 |
| OPUS | PFS | 0.57 | → | 0.43 |
| | OS | 0.86 | → | 0.83 |
| PRIME | PFS | 0.80 | → | 0.72 |
| | OS | 0.83 | → | 0.78 |

# Importance of Targeting the Right Population

Annals of Oncology Advance Access published September 22, 2014

review

*Annals of Oncology* 00: 1–9, 2014
doi:10.1093/annonc/mdu378

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

M. J. Sorich[1*], M. D. Wiese[2], A. Rowland[1], G. Kichenadasse[3], R. A. McKinnon[3] & C. S. Karapetis[3]

Sorich MJ, et al. *Ann Oncol.* 2015;26:13-21.

# Prevalence of New *RAS* Mutations Across Studies

| | New *RAS* Total | *KRAS* Exon 3 (59, 61) | *KRAS* Exon 4 (117, 146) | *NRAS* Exon 2 (12, 13) | *NRAS* Exon 3 (59, 61) | *NRAS* Exon 4 (117, 146) |
|---|---|---|---|---|---|---|
| OPUS | **26.3%** | 5.9% | 9.3% | 6.8% | 5.1% | 0.8% |
| PICCOLO | **9.8%** | NR | 3.7% | 6.8% | NR | NE |
| 20020408 | **17.6%** | 4.8% | 5.0% | 4.2% | 3.0% | 1.1% |
| 20050181 | **20.5%** | 4.6% | 7.9% | 2.3% | 5.8% | 0.0% |
| PRIME | **17.4%** | 3.7% | 5.6% | 3.4% | 4.1% | 0.0% |
| FIRE-3 | **16.0%** | 4.3% | 4.9% | 3.8% | 2.0% | 0.0% |
| PEAK | **20.1%** | 4.1% | 7.7% | 5.4% | 5.9% | 0.0% |
| COIN | **8.4%** | 2.1% | NE | 0.9% | 3.0% | NE |
| CRYSTAL | **14.7%** | 3.3% | 5.6% | 3.5% | 2.8% | 0.9% |
| SUMMARY | **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%) |

**Sorich MJ, et al. *Ann Oncol.* 2015;26:13-21.**

# Braf V600E mutation in mCRC

- Braf mutations occur **in 8-12%** of patients with mCRC.
  - They are in vast majority V600E mutations
  - They are **mutually exclusive with RAS** mutation and **will be found in RAS wt type patients**
- Braf mutations are a **poor prognostic factor** in mCRC
- Single agent Braf inhibitors have failed to demonstrate significant activity in Braf mutant mCRC

# PRIME trial *RAS* primary analysis

## BRAF mutations appeared to be prognostic

| | Panitumumab + FOLFOX4 | FOLFOX4 | HR | P-value |
|---|---|---|---|---|
| WT *RAS*/MT *BRAF*, n | 24 | 29 | | |
| Median PFS, months (95% CI) | **6.1** (3.7–10.7) | **5.4** (3.3–6.2) | **0.58 (0.29–1.15)** | **0.12** |
| Median OS, months (95% CI) | **10.5** (6.4–18.9) | **9.2** (8.0–15.7) | **0.90 (0.46–1.76)** | **0.76** |
| WT *RAS*/WT *BRAF*, n | 228 | 218 | | |
| Median PFS, months (95% CI) | 10.8 (9.4–12.4) | 9.2 (7.4–9.6) | **0.68 (0.54–0.87)** | **0.002** |
| Median OS, months (95% CI) | 28.3 (23.7–NE) | 20.9 (18.4–23.8) | **0.74 (0.57–0.96)** | **0.02** |

**Douillard J-Y, et al. N Engl J Med 2013;369:1023–34.**

NE, not estimable.
Predefined retrospective analysis.
Includes 7 patients harbouring *KRAS/NRAS* codon 59 mutations.

# TRIBE Folfiri-Bev. vs. Folfoxiri-Bev
# Impact of Ras and Braf mutation

| PFS (m) | Folfiri+Bev | HR | Folfoxiri+Bev |
|---|---|---|---|
| All wt (n 129) | 11.3 | 0.75 (ns) | 13.5 |
| RAS mt (n 218) | 9.5 | 0.82 (ns) | 12 |
| Braf mt (n 28) | 5.5 | 0.55 (ns) | 7.5 |
| OS (m) | | | |
| All wt | 34.4 | 0.85 (ns) | 41.7 |
| RAS mt | 23.1 | 0.88 (ns) | 30.8 |
| Braf mt | 10.8 | 0.55 (ns) | 19.8 |

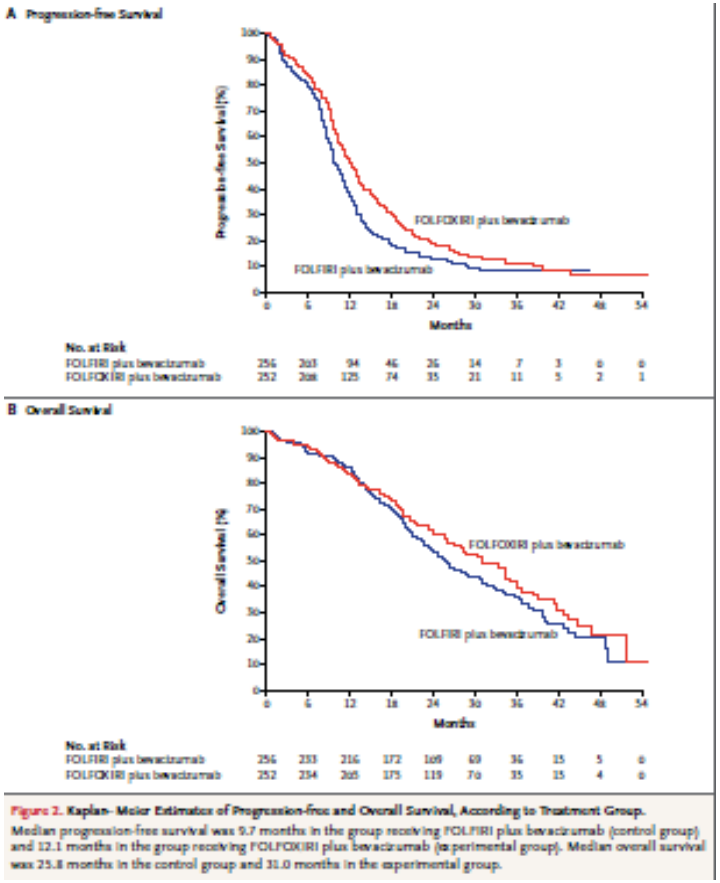



Figure 2. Kaplan-Meier Estimates of Progression-free and Overall Survival, According to Treatment Group.
Median progression-free survival was 9.7 months in the group receiving FOLFIRI plus bevacizumab (control group) and 12.1 months in the group receiving FOLFOXIRI plus bevacizumab (experimental group). Median overall survival was 25.8 months in the control group and 31.0 months in the experimental group.

**Loupakis F. et al N Engl J Med 371;17 October 23, 2014**

# Mechanisms of Braf inhibition resistance

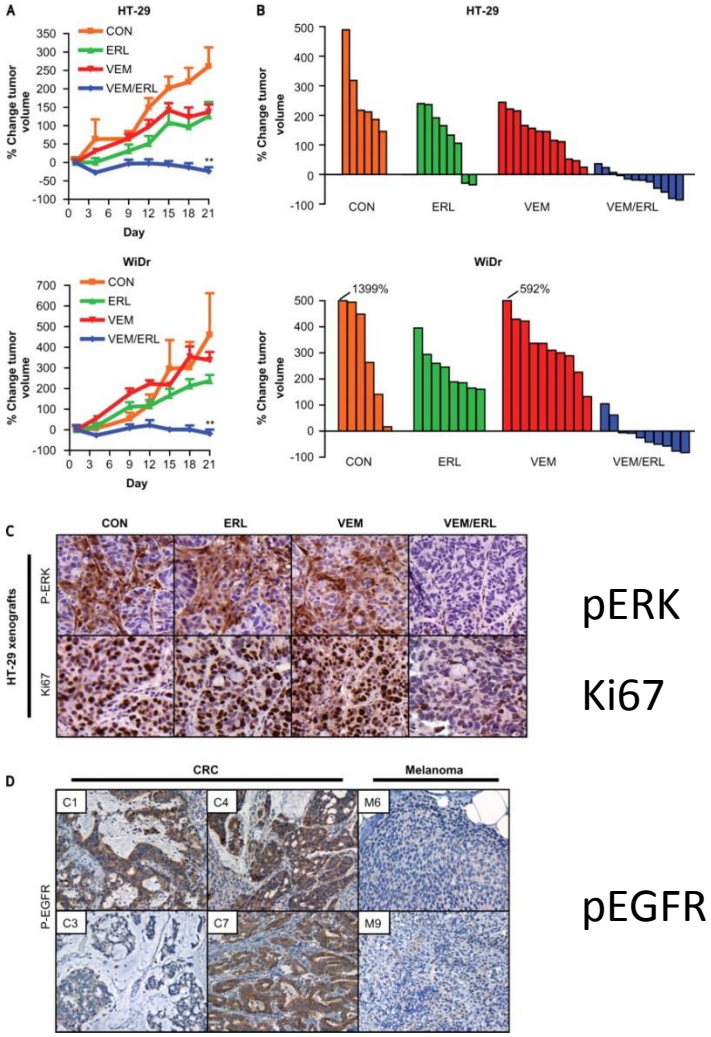
- Blockade of Braf-mutated mCRC induces:
  - Rapid activation of EGFR and RAS phosphorylation
  - Reactivation of the MAPKinase pathway
  - Resulting in activation of MEK1 and MEK2
  - As a consequence ERK Kinases phosphorylate a variety of cellular substrates involved in cell proliferation

- In additionBraf resistance may trigger other pathways
  - PI3K/AKT pathway

# Mechanisms of Braf inhibition resistance

- Pre-clinical data suggest that RAF inhibitors should be used in combination with
  - EGFR inhibitors
  - MEK inhibitors
  - To suppress the feed-back activation

# Combined RAF and EGFR inhibition leads to improved in vivo efficacy in BRAF-mutant colorectal cancer.




Ryan B. Corcoran et al. Cancer Discovery 2012;2:227-235

©2012 by American Association for Cancer Research

AACR American Association for Cancer Research
CANCER DISCOVERY

# Resistance to Braf inhibitors

Early clinical evaluation of combined treatments

# Vemurafenib and Panitumumab treatment in Braf mutant patients

- 15 patients with a Braf mutation
  - Previously treated with chemotherapy
  - 12 evaluable patients
    - 2 OR: (100% and 64% shrinkage)
    - 8 patients with stable disease

- Tolerance:
  - Rash (G1 and 2) 53%
  - Fatigue 53%
  - Arthralgia 40%

**Yaeger R et al ASCO GI 2015 Abst 611 J Clin Oncol 2015 suppl 3**

# CALGB 1606 trial on going

- Randomized phase II trial
  - Irinotecan+ cetuximab+/-Vemurafenib
  - V600E Braf mutant, wt RAS
  - One or two prior chemo regimen
    - (Irinotecan permitted)
    - Anti EGFR excluded
  - Cross over allowed at progression in the control arm

- Primary end-point: PFS
- 78 patients planned
- Ancillary study: resistance mechanisms in patient-derived xenograft

# Clinical therapeutic application in cancer
# Colorectal cancer (RAS, BRAF): conclusion

- The EGFR/RAS/RAF/MAPK pathway plays an important role in CRC treatment
- Patients selection on K and Nras phenotype allows using anti EGFR MoAbs with improved benefit risk

- Braf mutation is rather rare but associated with poor prognosis in RAS wt patients
  - Chemo-intensification has already improved PFS and OS
  - Present on going trials of combination therapy may improve outcome

- In patients with RAS mutation, all targeted agents have failed
  - Search for RAS mutant should be a priority