# Adjuvant FOLFOX +/- cetuximab in full *RAS* and *BRAF* wildtype stage III colon cancer patients

J. Taieb[1], R. Balogoun[2] K. Le Malicot[3], J. Tabernero[4], E. Mini[5], G. Folprecht[6], J-L. Van Laethem[7], J-F. Emile[8], C. Mulot[9], S. Fratté[10], C-B. Levaché[11], L. Saban-Roche [12] J. Thaler[13], L N. Petersen[14], J. Bridgewater[15], G. Perkins[1,2], C. Lepage[16] , E. Van Cutsem[17], A. Zaanan[1,2] and P. Laurent-Puig[2] for PETACC8 investigators*

1- Department of Gastroenterology and GI oncology, Sorbonne Paris Cité, Université Paris Descartes, Hopital Européen Georges Pompidou, Paris, France

2- Department of Biology, Université Paris Descartes, Sorbonne Paris Cité, France; Assistance Publique Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France; INSERM UMR-S1147,

3 - Fédération francophone de cancérologie digestive (FFCD), Dijon, France

4- Medical Oncology Department, Vall d'Hebron University Hospital and Institute of Oncology (VHIO), Barcelona, Universitat Autònoma de Barcelona, Spanish Gastrointestinal Tumours TTD Group, Spain.

5- Department of Experimental and Clinical Medicine, Section of Internal Medicine, University of Florence, Florence, Italy

6- Medical Department I, University Hospital Carl Gustav Carus, Dresden, Germany

7- Department of Gastroenterology, Hôpital Universitaire Erasme, Brussels, Belgium

8- Pathology Department, Ambroise Paré Hospital, Boulogne, France

9- Université Paris Descartes, Sorbonne Paris Cité, CRB EPIGENETEC, INSERM UMR-S1147

10- Department of Gastroenterology, Centre Hospitalier de Belfort-Montbeliard, France

11 - Department of Radiotherapy and medical oncology , Polyclinique Francheville, Périgueux, France

12 - Department of medical Oncology, Institut de Cancerologie de la Loire, Saint-Priest-En-Jarez, France

13- Department of Internal Medicine IV, Klinikum Wels-Grieskirchen, Wels, Austria;

14 - Department of Oncology, Rigshospitalet, København, Denmark;

15- UCL Cancer Institute, University College London, London, United Kingdom;

16 - Hepato-Gastroenterology Department, Dijon University Hospital and INSERM U 866, Dijon, France;

17- Digestive Oncology, University Hospitals Leuven and KU Leuven, Leuven, Belgium

*Please see the Supplementary Appendix for a list of the Investigators/Collaborators.

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Correspondence:

Pr Julien Taieb, Georges Pompidou European Hospital, Department of hepatogastroenterology and GI oncology, Sorbonne Paris Cité / Université Paris Descartes, 20 rue Leblanc 75015 Paris, France Phone: 33 1 56093551; Fax: 33 1 56093441 julien.taieb@egp.aphp.fr / jtaieb75@gmail.com

**Background**: RAS mutations have been shown to confer resistance to anti-EGFR treatment. We analyzed the results of the PETACC8 trial (cetuximab + FOLFOX vs FOLFOX) in full RAS and BRAF wildtype (WT) patients (pts) with resected stage III colon cancer.

**Methods**: Exons 2, 3 and 4 of KRAS and NRAS, and BRAF exons 11 and 15, were sequenced using the Ampliseq colon-lung cancer panel version 2, in PETACC8 trial pts who consented to translational research. The impact of cetuximab on time to recurrence (TTR), disease-free survival (DFS) and overall survival (OS) was investigated in pts with tumors harboring RAS & BRAF WT and RAS mutations. The prognostic value of each individual mutation was also tested.

**Results**: Among the 2559 pts analyzed, 745 pts (29%) were known to have KRAS exon 2 mutations and 163 pts (6.4%) the BRAF V600E mutation. Of the remaining 1651 pts, 1054 were assessed by NGS, showing that a further 227 pts (21%) had KRAS exon 2,3,4 or NRAS exon 2,3,4 mutations, and that 46 pts (4.4%) had a newly diagnosed BRAF mutation. Cetuximab added to FOLFOX did not significantly improve TTR, DFS or OS in pts with RAS WT or RAS & BRAF WT tumors (HR 0.77 to 1.03, all $P$>0.05). Cetuximab addition was not either significantly deleterious in RAS mutant pts or in pts with rare RAS or BRAF mutations. In the overall trial population, NRAS and KRAS codon 61 mutations were the only rare mutations with the same pejorative prognostic value as KRAS exon 2 or BRAF V600E mutations.

**Conclusion**

Though not significant, the clinically relevant 0.76 adjusted HR observed for DFS in favor of adding cetuximab to FOLFOX, in full RAS and BRAF WT stage III colon

cancer pts, may justify a new randomized controlled trial testing EGFR inhibitors in this setting.

Clinical trial number: This is an ancillary study of the PETACC8 trial: EUDRACT 2005-003463-23.

**Keywords**: stage III colon cancer; *RAS* mutations; *BRAF* mutations; cetuximab; FOLFOX; phase III; adjuvant; prognosis

**Key message:** In 1900 stage III colon cancer patients fully characterized by NGS for *RAS* and *BRAF*, adding cetuximab to the standard FOLFOX results in a trend to a better outcome in *RAS* & *BRAF* wildtype patients. Though not significant, the clinically relevant 0.76 adjusted/HR observed for DFS in favor of adding cetuximab may justify a new randomized trial with anti-EGFRs in this setting.

## Introduction

The Pan-European Trials in Alimentary traCt Cancer 8 (PETACC-8) study tested FOLFOX4, with or without cetuximab, after curative resection of stage III colon cancer [1]. Promising phase II and III studies of cetuximab adjunction to FOLFOX4 in metastatic colorectal cancer showed impressive response and disease-control rates, suggesting possible synergy of this new combination [2, 3]. The PETACC-8 protocol was amended on 17 June 2008, restricting enrolment to patients with *KRAS* exon 2 WT tumours and increasing the sample size. The first analysis of the trial results was negative, with no improvement in disease-free survival (DFS) or overall survival (OS) when cetuximab was added to FOLFOX [1].

*KRAS* exon 2 mutations are predictive of resistance to anti–epidermal growth factor receptor (EGFR) therapy in patients with metastatic colorectal cancer [3-6], as are activating mutations in *KRAS* exon 3 or 4 and in *NRAS* exon 2, 3 or 4 [7, 8].

*BRAF* mutations are typically exclusive of *RAS* mutations, and clinical data suggest that the *BRAF* V600E mutation is predictive of poorer survival but not of anti-EGFR efficacy in patients with metastatic colorectal cancer [9, 10], however, the low prevalence of these mutations makes it difficult to evaluate their possible biomarker status.

Patient selection based on tumour mutational status might thus improve the harm-benefit profile of anti-EGFR therapy. This has been largely demonstrated in metastatic colorectal cancer [7, 8] but not yet in the adjuvant setting. We and others recently found that *BRAF* V600E and *KRAS* exon 2 mutations were prognostic in stage III colon cancer, being associated with shorter time to recurrence (TTR), OS, and survival after relapse [11-14]. However, anti-EGFR efficacy has not yet been evaluated in selected patients with *RAS* WT and *BRAF* WT resected stage III colon

cancer.

We used the Ampliseq colon-lung cancer panel version 2 to sequence exons 2, 3 and 4 of *KRAS* and *NRAS*, as well as *BRAF* exons 11 and 15, amongst those PETACC8 trial participants who consented to translational research. TTR, DFS and OS were analyzed in full *RAS* WT patients and full *RAS* and *BRAF* WT patients. The prognostic impact of individual rare *RAS* and *BRAF* mutations was also investigated.

## Materials and methods

### Patients

PETACC8 trial participants underwent complete resection of histologically proven stage III colon adenocarcinoma, and were then randomly assigned to receive 6 months of either FOLFOX or FOLFOX+cetuximab, with regular monitoring, as described elsewhere [1]. The trial started in December 2005. The protocol was amended in June 2008 to enroll only patients with *KRAS* exon 2 WT tumours, and the sample size was increased to maintain power of statistical analyses. The study ended on 9 November 2009. Specific written informed consent was required from each patient included in the planned translational program of the trial.

### DNA Extraction and Mutation Analysis

Tumour samples were prospectively banked. Tumour DNAs were extracted from FFPE tissues containing more than 50% of tumour cells by using the QIAamp® DNA Mini Kit (Qiagen®). Molecular analysis, centralised at Georges Pompidou European Hospital, was performed retrospectively for the 2096 patients included before the trial amendment and prospectively for the other 463 patients. *KRAS* hotspot mutations

(c.34G>A/p.G12S, c.34G>C/p.G12R, c.34G>T/p.G12C, c.35G>A/p.G12D, c.35G>C/p.G12A, c.35G>T/p.G12V and c.38G>A p.G13D) and the *BRAF* V600E mutation (c.1799T>A/p.V600E) were detected by real-time PCR with TaqMan® probes (Applied Biosystems). The assays are alteration-specific and robustly detect 10% of mutated alleles for all the mutations tested.

Exons 2, 3 and 4 of *KRAS* and *NRAS*, as well as *BRAF* exons 11 and 15, were sequenced with the Ampliseq colon-lung cancer panel version 2 in the PETACC8 trial participants who consented to translational research.

Statistical Analyses

TTR, DFS and OS were analysed in patients with any *RAS* or *BRAF* mutations, *RAS* & *BRAF* WT status, and rare *RAS* mutations. The individual prognostic value of each mutation was also analysed.

TTR was defined as the time between randomization and local or metastatic recurrence or death related to disease recurrence, whichever occurred first. DFS was defined as the time between randomization and local or metastatic recurrence or diagnosis of a second colorectal cancer, or death from any cause, whichever occurred first. OS was defined as the time between randomization and death from any cause.

For baseline comparisons, categorical factors were compared with $\chi 2$ tests and continuous factors with standard parametric or non-parametric tests, depending on their normality. Continuous variables are reported as mean (SD) and median (interquartile range, IQR) values.

TTR, DFS and OS curves were estimated using the Kaplan–Meier method. Differences between groups of patients were analysed with log-rank tests. Cox models, Kaplan-Meier curves and forest plots were used for all analyses. Factors included in multivariate analyses were the treatment group and baseline prognostic factors that were clinically relevant or significant in univariate analysis, namely tumour grade, pT stage, pN stage, venous embolism, lymphatic invasion (VELI), bowel obstruction/perforation, and tumour location.

A two-sided significance level of 5% was applied for all analyses. Results were not adjusted for multiple comparisons. All statistical analyses were done by FFCD statisticians using SAS statistical software (version 9.4). The database was locked in July 2015.

## Results

*Study population*

Among the 2559 patients included in the PETACC8 phase III study, 741 were *KRAS* exon 2 mutated and 167 were *BRAF* V600E mutated. Of the remaining 1651 patients, 1054 gave their written consent for translational research and had sufficient tumour material for NGS analyses. NGS failed in 62 cases. The remaining 992 patients were fully analysed. A total of 1900 patients (including RAS mutated patients) met all the criteria for full molecular analysis (informed consent, sufficient material, and technical success) (Supplementary Figure 1). The patients' baseline and tumour characteristics are summarized in Table 1. The demographic and clinical characteristics of the patients included in the molecular study (*N*=1900) were not

significantly different from those of the entire randomized population (*N*=2559) (Supplementary Table 1).

*RAS and BRAF mutational status*

Amongst the 1900 patients included in the molecular study, 719 (38%) were double wildtype, 968 (51%) were *RAS* mutated and 213 (11%) were *BRAF* mutated (Figure 1). *KRAS*, *NRAS* and *BRAF* mutation frequencies are summarized in Figure 1.

The most frequently mutated *KRAS* exon was exon 2 (80.9%), followed by exons 4 (8.3%) and 3 (4.6%); two tumours (0.2%) were mutated on two different exons (Table 2). As expected, codon 12 was the most frequently mutated codon (75.9%), followed by codon 13 (18.4%). *NRAS* exons 2, 3 (codon 61) and 4 were mutated in respectively 30, 31 and 2 cases.

*BRAF* was mutated in 213 tumours, including 192 tumours (90%) harbouring the V600E mutation. The second most frequent mutation affected codon 469, in 8 cases (3.8%). The mutations were grouped for analysis into V600E and non V600E.

*KRAS* and *BRAF* mutations were both present in 8 tumours (4 V600E and 4 non V600E). *KRAS* and *NRAS* mutations were both present in two tumours (*KRAS* pA146T associated with *NRAS* p.G12D and with *NRAS* p.A146V in one case each). *NRAS* and *BRAF* mutations were both present in 3 tumours, all with non V600E *BRAF* mutations.

*Clinical outcomes according to RAS and BRAF mutational status*

As previously reported, adding cetuximab to FOLFOX did not improve TTR in the whole trial population [1] (Figure 2-A). In the *RAS* WT and *BRAF* WT population, a trend towards better outcomes was seen in the cetuximab group but the difference did not reach statistical significance for TTR (HR:0.77 (0.55-1.08); *P*=0.12) (Figure 2-B), DFS (HR:0.85 (0.63-1.14); *P*=0.27) or OS (HR:1.03 (0.70-1.50); *P*=0.89). In multivariate analyses, the results were better but still not significant: TTR (HR=0.70 (95%CI: 0.48 - 1.03); *P*=0.07), DFS (HR=0.76 (95%CI: 0.54 - 1.06); *P*=0.11), and OS (HR=0.90 (95%CI: 0.59 - 1.36); *P*=0.60).

In patients with *RAS*-mutated tumours, the addition of cetuximab to FOLFOX was associated with a trend towards poorer TTR (HR:1.14 (0.91-1.44); *P*=0.25) (Figure 2-C), DFS (HR:1.13 (0.91-1.40); *P*=0.27) and OS (HR:1.29 (0.99-1.69); *P*=0.061). These trends were less pronounced in multivariate analyses: TTR (HR=1.09 (95%CI: 0.84 - 1.41); *P*=0.51), DFS (HR=1.06 (95%CI: 0.83 - 1.35); *P*=0.64), OS (HR= 1.17 (95%CI: 0.87 - 1.57); *P*=0.30).

As DFS is the usual endpoint for adjuvant trials, DFS Kaplan Meier curves are shown in supplementary figure 2-A, 2-B and 2-C.

Rare *RAS* and *BRAF* mutations (i.e. KRAS exon 3,4 ; NRAS exon 2,3,4 and BRAF non V600E) tended to be associated with a deleterious effect of cetuximab, with HRs of 1.6 for TTR (*P*=0.09) and 1.61 for OS (*P*=0.13).

*Prognostic value of RAS and BRAF mutations*

In the overall study population, *KRAS* exon 2 and *BRAF* V600E mutations were associated with worse outcomes when compared to *RAS* and *BRAF* WT status, as

previously described [12]. This was also the case of *KRAS* and *NRAS* (exon 3) codon 61 rare mutants with respect to TTR and OS, contrary to other rare *RAS* or *BRAF* mutants (Figure 3).

The number of rare mutations was too small for meaningful multivariable analysis.

## Discussion

*KRAS* and *NRAS* are closely related to *RAS* oncogene family members, and mutations at codon 12, 13, 61, 117 or 146 of either gene result in increased levels of guanosine triphosphate–bound *RAS* proteins [15, 16]. *KRAS* and *NRAS* mutations at these codons tend to be mutually exclusive in colorectal tumours, suggesting functional redundancy [17]. Mutations in H*RAS*, the third member of the *RAS* family, are infrequent in colorectal cancer [17, 18]. Clinical data suggest that *RAS* genes mutations are also associated with worse outcomes in the adjuvant setting [11-14].
Previous trials of anti-EGFR therapies combined with irinotecan or oxaliplatin-containing regimens showed no benefit in patients with *KRAS* exon 2 mutations [2, 6]. Randomized phase 3 trials of panitumumab, given alone [19] or in combination with FOLFOX or FOLFIRI [3, 5, 7], showed no response to this anti-EGFR therapy in patients with metastatic colorectal tumours harboring a mutation in *KRAS* or *NRAS*. This was also the case in recent analyses of randomized trials with cetuximab [8, 20]. All these studies involved patients with metastatic colorectal cancer. By contrast, we assessed here the effect of adjuvant cetuximab plus FOLFOX in patients with fully resected primary stage III colon tumours and full *KRAS*, *NRAS* and *BRAF* characterization.

Removing patients with rare RAS and BRAF mutations, with a poor outcome, from the target efficacy population reveals a trend to a positive effect of the addition of cetuximab to standard FOLFOX in patients with RAS & BRAF wildtype tumours. Although the impact of cetuximab was not statistically significant, it might be clinically relevant. In the MOSAIC pivotal trial, adding oxaliplatin to 5-FU improved DFS, with an HR of 0.8. Here, multivariate analysis adjusted for pT, pN, histological grade, VELI and tumour location yielded an HR of 0.76 (95%CI:0.54-1.06). This suggests that a new randomized trial powered to demonstrate such a difference in wildtype colon cancer patients may be relevant, especially after a 12-year period with no advances in adjuvant treatment of stage III colon cancer. If such trial is not forthcoming, our results would have to be confirmed using at least internally (other sequencing approaches for example) and externally (on other datasets) before discussing any practice change. New markers of colon cancer sensitivity to anti-EGFRs are emerging and could in future also be assessed in samples from PETACC8 and other adjuvant trials of anti-EGFRs, such as the NCCTG N0147 study, in order to generate hypotheses for future trials [21, 22].

Although adding cetuximab to FOLFOX tended to be beneficial in terms of TTR and DFS, this was not the case for OS (HR of 0.9 in adjusted analyses). This discordance between OS and TTR/DFS suggests that survival after relapse may differ between patients who do and do not receive adjuvant cetuximab, possibly because of lower cetuximab prescription rates in the metastatic setting when patients have received adjuvant cetuximab. Further analyses of survival after recurrence, and of treatments received at recurrence, are needed to clarify this point.

A deleterious effect of cetuximab and panitumumab has been reported in some patients with *RAS*-mutated tumours treated with FOLFOX in the metastatic setting [7,

20]. This was not the case of patients with *RAS*-mutated metastatic colorectal cancer receiving irinotecan-based backbone chemotherapy [8]. In our study of stage III colon cancer, there was only a non-significant trend towards worse outcomes with cetuximab in *RAS*-mutant patients.

This trend towards a deleterious effect of cetuximab was even stronger in patients with rare *RAS* mutations, but again it did not reach statistical significance, possibly owing to the small number of patients with rare *RAS* mutations (n=185).

We and others have shown that *KRAS* exon 2 and *BRAF* V600E mutations are associated with a poor prognosis in stage III colon cancer and especially in the 90% of patients with MSS tumours [14]. However, the prognostic value of rare *KRAS*, *NRAS* and *BRAF* mutations has rarely been studied in this setting. Gavin et al reported in 2299 stage II and III colon tumours a similar frequency of *NRAS* mutations (2.9%) that were associated with a worse TTR (HR=1.53; 95%CI, 1.01-2.31; *P*=0.04), but this difference disappeared in multivariate analysis and was not significant for OS [23]. A recent retrospective study of rare *KRAS* mutations at codons 12, 13 and 61 in stage II-III colon cancer patients showed no significant impact on DFS or OS [24]. However, the impact of individual *KRAS* mutations was not studied, the sample was quite small, and the study was retrospective. Modest et al. very recently studied the prognostic impact of *RAS* mutations in metastatic patients and found that only G13D and G12C had prognostic value and not rare mutations [25]. We found no recent data on the prognostic value of rare *BRAF* mutations in the adjuvant setting. In the metastatic setting, *BRAF* non V600E-mutated tumours seem to carry a better prognosis [26]. In the present work, we found that only *KRAS* and *NRAS* codon 61 mutations had significant negative prognostic

value, while other rare *RAS* or *BRAF* non V600E mutations did not seem to affect patient outcome. However, these results need to be confirmed in larger series with full *RAS* and *BRAF* mutational analyses.

In conclusion, adding cetuximab to standard FOLFOX adjuvant therapy in stage III colon cancer results in a non significant trend towards better outcomes in *RAS* & *BRAF* WT patients. No significant detrimental effect was observed in *RAS* mutant patients. Though not significant, the clinically relevant 0.76 adjusted HR observed for DFS in favor of adding cetuximab to FOLFOX in full RAS and BRAF WT stage III colon cancer pts, may justify a new randomized controlled trial testing EGFR inhibitors in this setting.

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**Conflict of Interest Disclosures:**

Dr Taieb has participated in consulting or/and advisory boards for Merck, Sanofi, Roche Genentech, Pfizer and Amgen; Dr Zaanan for Roche, Merck Serono, Amgen, Sanofi and Lilly; Dr Tabernero from Amgen, ImClone Systems, Lilly, Millennium, Novartis, Roche/Genentech, Sanofi, Celgene, Chugai Pharma, Taiho Pharmaceutical, Boehringer Ingelheim and Merck Serono; Dr Folprecht from Roche, Merck KGaA, Lilly and Bristol; Dr Laurent-Puig from Sanofi, Merck Serono, Amgen, Roche, Genomic Health, Myriad Genetics and Pfizer.

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**Figure legends**

Figure 1:

Distribution of mutations.

Figure 2:

Kaplan Meier curves for time to relapse according to study treatment (A) in the *KRAS* exon 2 WT intention-to-treat population, (B) In patients with *RAS* WT and *BRAF* WT tumours, and (C) In patients with *RAS*-mutated tumours. TTR=time to recurrence. HR=hazard ratio.

Figure 3:

Prognostic impact of individual *RAS* and *BRAF* mutations on recurrence (A) and survival (B).

Supplementary Figure 1:

Flow chart of PETACC8 trial molecular study evaluating the impact of. full *RAS* and *BRAF* wildtype mutations

Supplementary Figure 2:

Kaplan Meier curves for disease-free survival according to study treatment (A) in the *KRAS* exon 2 WT intention-to-treat population, (B) In patients with *RAS* WT and *BRAF* WT tumours, and (C) In patients with *RAS*-mutated tumours. DFS=Disease-Free Survival. HR=hazard ratio.

Table 1- Baseline patient and tumour characteristics in the *RAS* mutant, *BRAF* mutant and double wildtype subpopulations

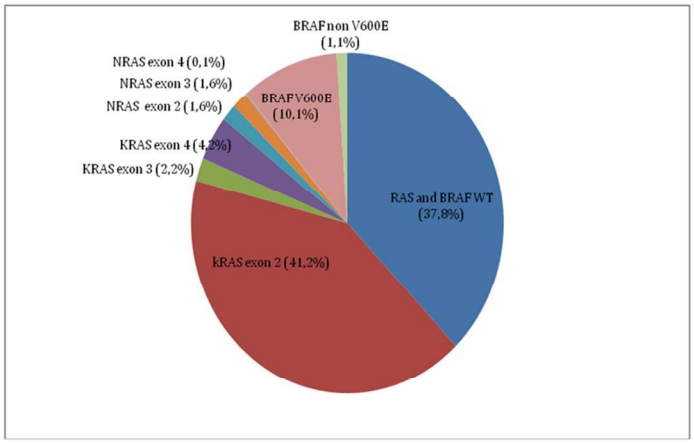
| | | **Double WT** | | ***RAS* Mutant** | | ***BRAF* Mutant** | |
|---|---|---|---|---|---|---|---|
| | | Folfox | Folfox+Cetux | Folfox | Folfox+Cetux | Folfox | Folfox+Cetux |
| Gender | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | Male | 224 (61.0%) | 225 (63.9%) | 255 (52.7%) | 270 (55.8%) | 49 (49.5%) | 55 (48.2%) |
| | Female | 143 (39.0%) | 127 (36.1%) | 229 (47.3%) | 214 (44.2%) | 50 (50.5%) | 59 (51.8%) |
| Age | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | Mean (SD) | 58.83 (9.21) | 58.33 (10.12) | 60.03 (9.47) | 59.68 (9.37) | 60.85 (8.86) | 59.78 (9.26) |
| | Median | 60.00 | 60.00 | 61.00 | 61.00 | 62.00 | 60.00 |
| | Q1; Q3 | 53.00; 66.00 | 52.00; 66.00 | 54.00; 68.00 | 54.00; 67.00 | 54.00; 68.00 | 53.00; 67.00 |
| | Range | 25.00; 75.00 | 19.00; 75.00 | 25.00; 75.00 | 23.00; 74.00 | 28.00; 73.00 | 27.00; 74.00 |
| Age | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | Age <= 70 years | 336 (91.6%) | 318 (90.3%) | 425 (87.8%) | 429 (88.6%) | 89 (89.9%) | 99 (86.8%) |
| | Age > 70 years | 31 (8.4%) | 34 (9.7%) | 59 (12.2%) | 55 (11.4%) | 10 (10.1%) | 15 (13.2%) |
| | Missing | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| WHO Performance Status | *n* | 355 | 344 | 463 | 465 | 96 | 108 |
| | 0 | 293 (82.5%) | 284 (82.6%) | 387 (83.6%) | 380 (81.7%) | 73 (76.0%) | 83 (76.9%) |
| | 1 | 60 (16.9%) | 60 (17.4%) | 75 (16.2%) | 83 (17.8%) | 23 (24.0%) | 25 (23.1%) |
| | 2 | 2 (0.6%) | 0 (0.0) | 1 (0.2%) | 2 (0.4%) | . | . |
| Tumour Location | *n* | 366 | 352 | 479 | 483 | 99 | 114 |
| | Left | 276 (75.4%) | 260 (73.9%) | 261 (54.5%) | 254 (52.6%) | 31 (31.3%) | 32 (28.1%) |
| | Right | 88 (24.0%) | 91 (25.9%) | 211 (44.1%) | 216 (44.7%) | 67 (67.7%) | 82 (71.9%) |
| | Both sides | 2 (0.5%) | 1 (0.3%) | 7 (1.5%) | 13 (2.7%) | 1 (1.0%) | 0 (0.0) |
| Tumour grade | *n* | 367 | 352 | 483 | 484 | 99 | 114 |
| | Missing | 5 (1.4%) | 3 (0.9%) | 7(1.4%) | 6 (1.2%) | 1 (1.0%) | 2 (1.8%) |
| | Well differentiated | 79 (21.5%) | 80 (22.7%) | 95 (19.7%) | 102 (21.1%) | 14 (14.1%) | 16 (14.0%) |
| | Moderately differentiated | 227 (61.9%) | 207 (58.8%) | 295 (61.1%) | 298 (61.6%) | 50 (50.5%) | 54 (47.4%) |
| | Poorly differentiated | 54 (14.7%) | 60 (17.0%) | 84 (17.4%) | 76 (15.7%) | 34 (34.3%) | 40 (35.1%) |
| | Undifferentiated | 2 (0.5%) | 2 (0.6%) | 2 (0.4%) | 2 (0.4%) | 0 (0.0) | 2 (1.8%) |
| pN stage | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | pN1 | 240 (65.4%) | 224 (63.6%) | 304 (62.8%) | 305 (63.0%) | 60 (60.6%) | 58 (50.9%) |
| | pN2 | 127 (34.6%) | 128 (36.4%) | 180 (37.2%) | 179 (37.0%) | 39 (39.4%) | 56 (49.1%) |

| | | **Double WT** | | ***RAS* Mutant** | | ***BRAF* Mutant** | |
|---|---|---|---|---|---|---|---|
| | | Folfox | Folfox+Cetux | Folfox | Folfox+Cetux | Folfox | Folfox+Cetux |
| pT stage | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | pT1 | 11 (3.0%) | 10 (2.8%) | 14 (2.9%) | 10 (2.1%) | 1 (1.0%) | 2 (1.8%) |
| | pT2 | 32 (8.7%) | 30 (8.5%) | 29 (6.0%) | 30 (6.2%) | 5 (5.1%) | 3 (2.6%) |
| | pT3 | 259 (70.6%) | 229 (65.1%) | 332 (68.6%) | 346 (71.5%) | 72 (72.7%) | 84 (73.7%) |
| | pT4 | 65 (17.7%) | 83 (23.6%) | 109 (22.5%) | 98 (20.2%) | 21 (21.2%) | 24 (21.1%) |
| | pTis | . | . | . | . | 0 (0.0) | 1 (0.9%) |
| Bowel obstruction and perforation | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | Bowel obstruction and/or perforation | 64 (17.4%) | 65 (18.5%) | 99 (20.5%) | 97 (20.0%) | 17 (17.2%) | 18 (15.8%) |
| | No bowel obstruction and no perforation | 303 (82.6%) | 287 (81.5%) | 385 (79.5%) | 387 (80.0%) | 82 (82.8%) | 96 (84.2%) |
| VELI | *n* | 367 | 352 | 484 | 484 | 99 | 114 |
| | Vascular Invasion or Lymphatic infiltration | 210 (57.2%) | 207 (58.8%) | 262 (54.1%) | 248 (51.2%) | 67 (67.7%) | 62 (54.4%) |
| | No Vascular Invasion and no Lymphatic infiltration | 104 (28.3%) | 95 (27.0%) | 144 (29.8%) | 154 (31.8%) | 24 (24.2%) | 29 (25.4%) |
| MMR Status | N | 340 | 324 | 406 | 390 | 89 | 105 |
| | pMMR | 309 (90.9%) | 301 (92.9%) | 377 (92.9%) | 372 (95.4%) | 56 (62.9%) | 71 (67.6%) |
| | dMMR | 31 (9.1%) | 23 (7.1%) | 29 (7.1%) | 18 (4.6%) | 33 (37.1%) | 34 (32.4%) |

Abbreviations: MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR

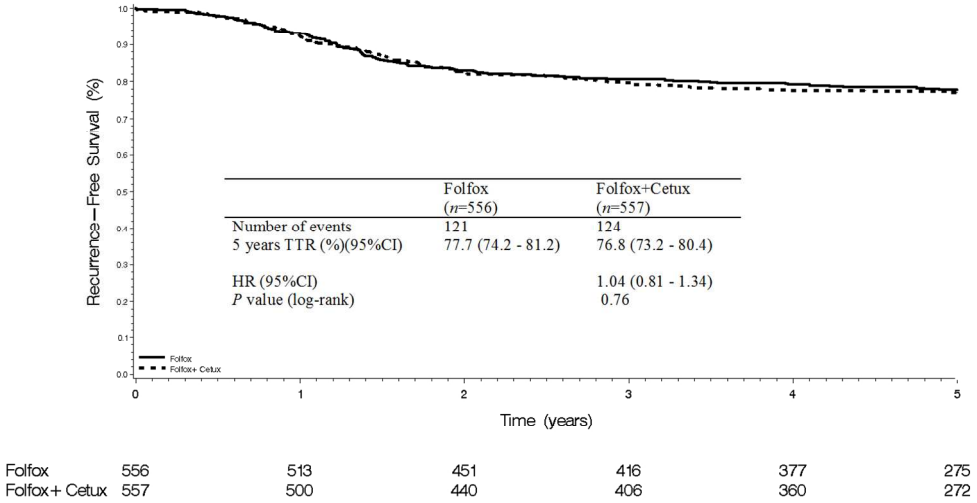
Table 2: *RAS* and *BRAF* mutations

| | *RAS* Mutant (*n*=968) | *BRAF* Mutant (*n*=213) |
|---|---|---|
| ***KRAS* mutations** | | |
| **Exon 2** | **783 (80.9%)** | **-** |
| **Codon 12** | **594 (75.9%)** | **-** |
| **Codon 13** | **178 (18.4%)** | **-** |
| **Other** | **11 (1.4%)** | **-** |
| **Exon 3** | **42 (4.3%)** | **-** |
| **Codon 59** | **8 (19.0%)** | **-** |
| **Codon 61** | **34 (81.0%)** | **-** |
| **Exon 4** | **80 (8.3%)** | **-** |
| **Codon 146** | **68 (85.0%)** | **-** |
| **Codon 117** | **11 (13.8%)** | **-** |
| **Other** | **1 (1.2%)** | **-** |
| ***NRAS* mutations** | | |
| **Exon 2** | **30 (3.1%)** | **-** |
| **Codon 12** | **26 (86.7%)** | **-** |
| **Codon 13** | **4 (13.3%)** | **-** |
| **Exon 3** | **31 (3.2%)** | **-** |
| **Exon 4** | **2 (0.2%)** | **-** |
| ***BRAF* mutations** | **-** | |
| **V600E** | **-** | **192 (90.1%)** |
| **Other mutations** | **-** | **21 (9.9%)** |




Distribution of mutations.

304x171mm (96 x 96 DPI)

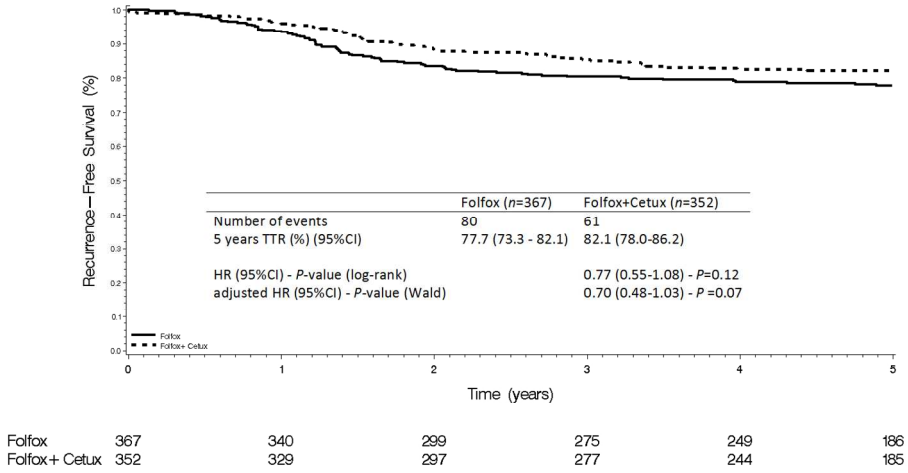



| | Folfox (*n*=556) | Folfox+Cetux (*n*=557) |
|---|---|---|
| Number of events | 121 | 124 |
| 5 years TTR (%)(95%CI) | 77.7 (74.2 - 81.2) | 76.8 (73.2 - 80.4) |
| HR (95%CI) | | 1.04 (0.81 - 1.34) |
| *P* value (log-rank) | | 0.76 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 556 | 513 | 451 | 416 | 377 | 275 |
| Folfox+ Cetux | 557 | 500 | 440 | 406 | 360 | 272 |

Kaplan Meier curves for time to relapse according to study treatment (A) in the KRAS exon 2 WT intention-to-treat population

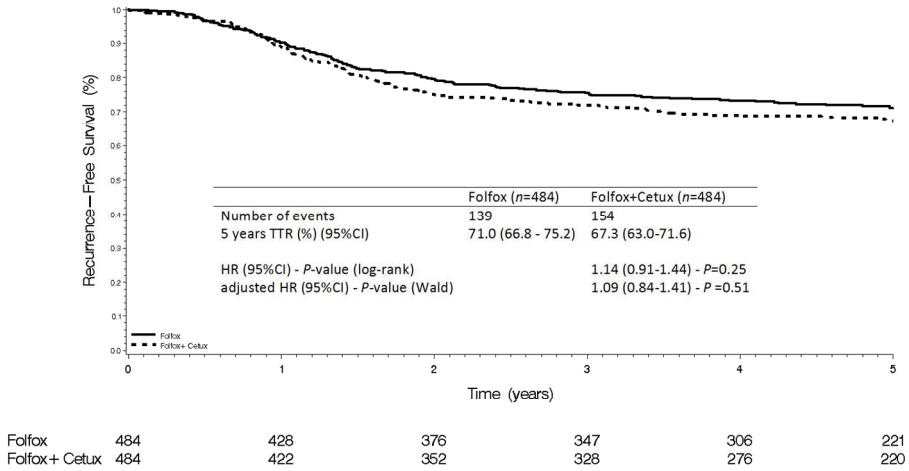
272x160mm (144 x 144 DPI)




| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 367 | 340 | 299 | 275 | 249 | 186 |
| Folfox+ Cetux | 352 | 329 | 297 | 277 | 244 | 185 |

Kaplan Meier curves for time to relapse according to study treatment (B) In patients with RAS WT and BRAF WT tumours,

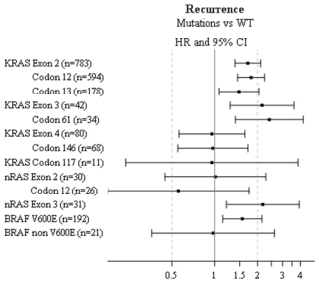
292x162mm (144 x 144 DPI)




| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 484 | 428 | 376 | 347 | 306 | 221 |
| Folfox+ Cetux | 484 | 422 | 352 | 328 | 276 | 220 |

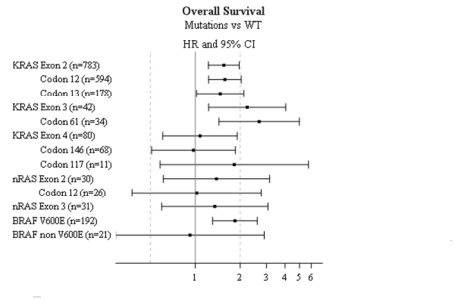
Kaplan Meier curves for time to relapse according to study treatment (C) In patients with RAS-mutated tumours

292x160mm (144 x 144 DPI)



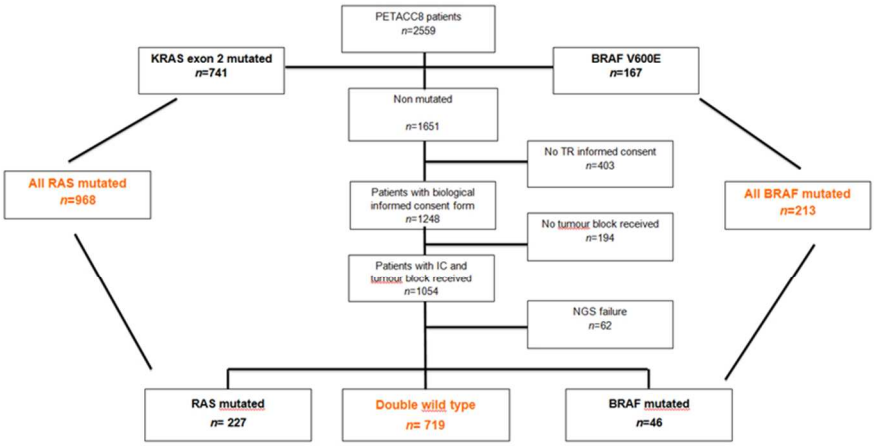

Prognostic impact of individual RAS and BRAF mutations on recurrence (A)

349x209mm (96 x 96 DPI)

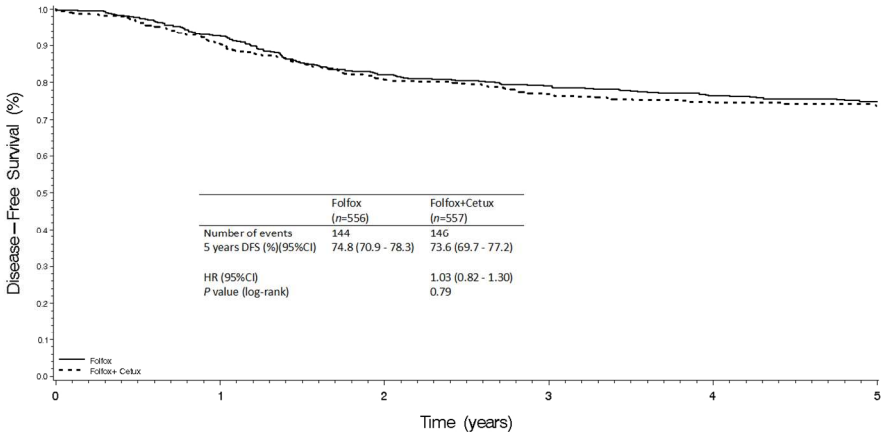



Prognostic impact of individual RAS and BRAF mutations on overall Survival (B)

349x209mm (96 x 96 DPI)

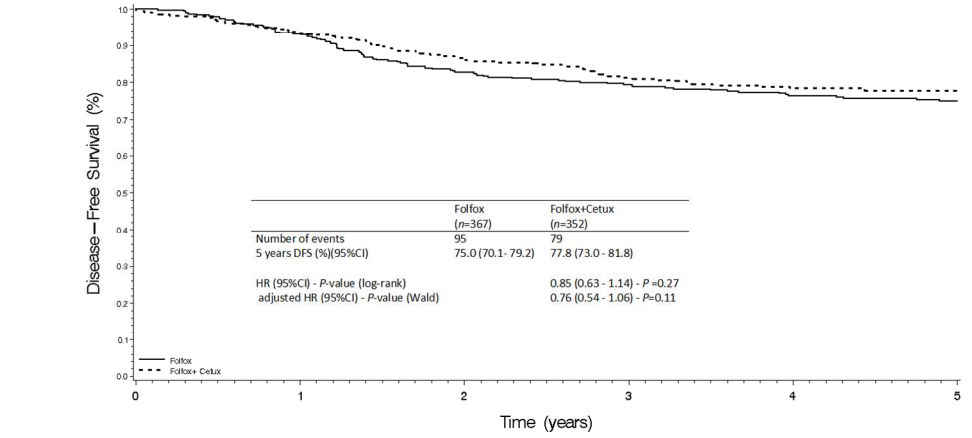



169x127mm (144 x 144 DPI)




| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 556 | 512 | 449 | 413 | 374 | 273 |
| Folfox+ Cetux | 557 | 497 | 438 | 402 | 356 | 270 |

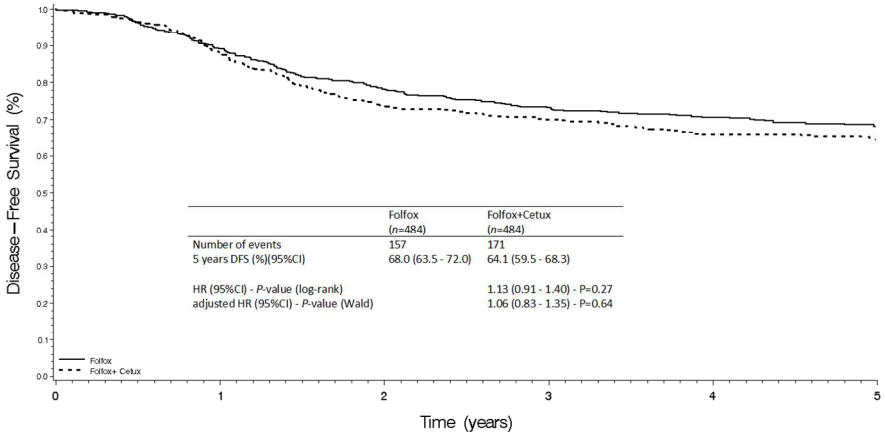
408x244mm (96 x 96 DPI)




| | Folfox (*n*=367) | Folfox+Cetux (*n*=352) |
|---|---|---|
| Number of events | 95 | 79 |
| 5 years DFS (%)(95%CI) | 75.0 (70.1- 79.2) | 77.8 (73.0 - 81.8) |
| HR (95%CI) - *P*-value (log-rank) | | 0.85 (0.63 - 1.14) - *P* =0.27 |
| adjusted HR (95%CI) - *P*-value (Wald) | | 0.76 (0.54 - 1.06) - *P*=0.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 367 | 340 | 299 | 275 | 248 | 186 |
| Folfox+ Cetux | 352 | 327 | 296 | 273 | 240 | 183 |

408x244mm (96 x 96 DPI)




| | | | | | | |
|---|---|---|---|---|---|---|
| Folfox | 484 | 427 | 375 | 344 | 303 | 219 |
| Folfox+ Cetux | 484 | 421 | 349 | 325 | 273 | 218 |

408x244mm (96 x 96 DPI)

Supplementary Table 1. Study population compared to all patients enrolled in the PETACC8 trial.

| | | Full *RAS* patients (N=1900) | PETACC8 patients (N=2559) | *P*-value |
|---|---|---|---|---|
| Gender | *n* | 1900 | 2559 | $X^2$: 0.7923 |
| | Male | 1078 (56.7%) | 1462 (57.1%) | . |
| | Female | 822 (43.3%) | 1097 (42.9%) | . |
| Treatment Group | *n* | 1900 | 2559 | $X^2$: 0.9897 |
| | Folfox | 950 (50.0%) | 1279 (50.0%) | . |
| | Folfox+Cetux | 950 (50.0%) | 1280 (50.0%) | . |
| Age | *n* | 1900 | 2559 | *W*: 0.4962 |
| | Mean (SD) | 59.42 (9.49) | 59.19 (9.67) | . |
| | Median | 61.00 | 60.00 | . |
| | Q1; Q3 | 54.00; 67.00 | 53.00; 67.00 | . |
| | Range | 19.00; 75.00 | 19.00; 75.00 | . |
| Age | *n* | 1900 | 2559 | $X^2$: 0.8422 |
| | Age <= 70 years | 1696 (89.3%) | 2289 (89.4%) | . |
| | Age > 70 years | 204 (10.7%) | 270 (10.6%) | . |
| | Missing | 0 (0.0) | 0 (0.0) | . |
| WHO performance status | *n* | 1831 | 2441 | $X^2$: 0.8073 |
| | 0 | 1500 (81.9%) | 2004 (82.1%) | . |
| | 1 | 326 (17.8%) | 431 (17.7%) | . |
| | 2 | 5 (0.3%) | 5 (0.2%) | . |
| | 3 | 0 (0.0) | 1 (0.0%) | . |
| Tumour Location | *n* | 1893 | 2546 | $X^2$: 0.3069 |
| | Left | 1114 (58.8%) | 1552 (61.0%) | . |
| | Right | 755 (39.9%) | 968 (38.0%) | . |
| | Both sides | 24 (1.3%) | 26 (1.0%) | . |

| | | Full *RAS* patients | PETACC8 patients | *P*-value |
|---|---|---|---|---|
| | | (N=1900) | (N=2559) | |
| Tumour grade | *n* | 1899 | 2557 | $X^2$: 0.9978 |
| | .A | 1 (0.1%) | 1 (0.0%) | . |
| | .D | 3 (0.2%) | 5 (0.2%) | . |
| | .K | 20 (1.1%) | 25 (1.0%) | . |
| | Well differentiated | 386 (20.3%) | 527 (20.6%) | . |
| | Moderately differentiated | 1131 (59.6%) | 1527 (59.7%) | . |
| | Poorly differentiated | 348 (18.3%) | 461 (18.0%) | . |
| | Undifferentiated | 10 (0.5%) | 11 (0.4%) | . |
| pN grade | *n* | 1900 | 2559 | $X^2$: 0.8501 |
| | pN1 | 1191 (62.7%) | 1597 (62.4%) | . |
| | pN2 | 709 (37.3%) | 962 (37.6%) | . |
| pT grade | *n* | 1900 | 2559 | $X^2$: 0.5889 |
| | pT1 | 48 (2.5%) | 71 (2.8%) | . |
| | pT2 | 129 (6.8%) | 194 (7.6%) | . |
| | pT3 | 1322 (69.6%) | 1768 (69.1%) | . |
| | pT4 | 400 (21.1%) | 522 (20.4%) | . |
| | pTis | 1 (0.1%) | 1 (0.0%) | . |
| | pTx | 0 (0.0) | 3 (0.1%) | . |
| Bowel obstruction and perforation | *n* | 1900 | 2559 | $X^2$: 0.7152 |
| | Bowel obstruction and/or perforation | 360 (18.9%) | 496 (19.4%) | . |
| | No bowel obstruction and no perforation | 1540 (81.1%) | 2063 (80.6%) | . |
| VELI | *n* | 1900 | 2559 | $X^2$: 0.8758 |
| | Vascular Invasion or Lymphatic infiltration | 1056 (55.6%) | 1442 (56.4%) | . |
| | No Vascular Invasion and no Lymphatic infiltration | 550 (28.9%) | 729 (28.5%) | . |

Abbreviations: MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR ; $X^2$: ; Chi-Square Test; W: Wilcoxon Test