Adjuvant FOLFOX4 with or without cetuximab in patients with resected stage III colon cancer: DFS and OS results and subgroup analyses of the PETACC8 intergroup phase III trial

J. Taieb on the behalf of the PETACC8 investigators

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The PETACC8 randomized phase III trial

- 1602 Stage III CRC patients with a KRAS wild type tumor were randomized to 6 months adjuvant treatment with FOLFOX4 or with FOLFOX4 plus cetuximab.
- Primary end-point: Disease Free Survival (DFS).

- No difference between the treatment arms:
  - 3 Year DFS: 75.1% (experimental arm) vs 78.0% (standard arm);
  - 3 Year OS: 88.3% (experimental arm) vs 90.5% (standard arm);
  - No difference in DFS and OS in “double wild type” (KRAS and BRAF) tumors.

- Subgroup analyses suggested that:
  - Patients with pT4N2 tumors may benefit from cetuximab treatment;
  - Patients >70 years, with right-sided colon cancer and females may have a worse outcome with cetuximab.
DFS: KRAS wt pT4N2 tumors

- **Number of patients at risk**
  - FOLFOX4 + C: 79, 64, 38, 28, 16, 0, 0
  - FOLFOX4: 67, 48, 24, 15, 7, 0, 0

- **HR for DFS [95% CI]**: 0.555 [0.348, 0.885]
- **p-value (log-rank)**: 0.0122
Why was the PETACC8 trial negative?

- In unselected fully resected Stage III CRC patients treated for 6 months with a combination of a fluoropyrimidine and of oxaliplatin (MOSAIC; NSABP-C0-7; XELOXA) a plateau of efficacy has been reached (75% 3 year DFS and 90% 3 year OS).

- The biology of micrometastases in fully resected Stage III CRC patients is different from the biology of metastases in Stage IV CRC patients and, therefore, anti-angiogenic drugs (bevacizumab: AVANT; NSABP-C0-8) or anti-EGFR drugs (cetuximab: NCCTG-N0147) are not effective in the adjuvant setting.

- The chemotherapy backbone (FOLFOX) is not the most appropriate combination for cetuximab.
Arms B and E from NCCTG-N0147 trial: is FOLFIRI a better chemotherapy partner for cetuximab?

- Arm B: FOLFIRI
- Arm E: FOLFIRI + Cetuximab

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=156)</th>
<th>KrAS wt (n=99)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Arm B</td>
<td>Arm E</td>
</tr>
<tr>
<td>N</td>
<td>111</td>
<td>45</td>
</tr>
<tr>
<td>3-yr DFS</td>
<td>65%¹</td>
<td>80%</td>
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<tr>
<td>3-yr OS</td>
<td>83%²</td>
<td>90%</td>
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| FOLFOX: ¹76%, ²88% |
CRYSタル and OPUS clinical trials

Adding cetuximab to first-line CT improves efficacy in *KRAS* wt mCRC

<table>
<thead>
<tr>
<th></th>
<th>CRYSTAL study¹</th>
<th>OPUS study²</th>
<th>Pooled analysis³</th>
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<tbody>
<tr>
<td></td>
<td>FOLFIRI + cetuximab</td>
<td>FOLFOX4 + cetuximab</td>
<td>CT + cetuximab</td>
</tr>
<tr>
<td><strong>PFS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio*</td>
<td>0.696</td>
<td>0.567</td>
<td>0.66</td>
</tr>
<tr>
<td>p-value†</td>
<td>0.0012</td>
<td>0.0064</td>
<td>&lt;0.0062</td>
</tr>
<tr>
<td><strong>OS</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hazard ratio*</td>
<td>0.796</td>
<td>0.855</td>
<td>0.81</td>
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<tr>
<td>p-value†</td>
<td>0.0093</td>
<td>0.39</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Tumor response</strong></td>
<td>2.069</td>
<td>2.551</td>
<td>2.16</td>
</tr>
<tr>
<td>Odds ratio*</td>
<td>&lt;0.001</td>
<td>0.0027</td>
<td>&lt;0.0001</td>
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<tr>
<td>p-value‡</td>
<td></td>
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¹Hazard and odds ratios are for CT + cetuximab vs CT groups; †log-rank test; ‡Cochran-Mantel-Haenszel test

CT, chemotherapy; wt, wild-type

1st-line treatment: PFS (KRAS wt)

Anti-EGFR drugs as monotherapy in unselected chemorefractory metastatic CRC: clinical results

EGFR-dependent Growth  Non-EGFR-dependent Growth

PR  SD  Non-Responders

10%  30%  60%
Possible Mechanisms of Resistance to EGFR Inhibitors

- Target changes in cancer cells (selection of cancer cell clones with somatic EGFR gene mutations which confer resistance, i.e. the T790M mutation in lung adenocarcinoma, the S492R mutation in colon adenocarcinoma).

- Activation of downstream signaling pathways through EGFR-independent mechanisms:
  - Other cell membrane growth factor receptors (IGF1-R; ErbB2; ErbB3; MET);
  - PTEN-PI3K-AKT pathway;
  - RAS-RAF-MEK-ERK pathway;
  - Pro-angiogenic growth factors (VEGF) production;
  - Expression of VEGFRs in cancer cells.

- Epithelial to mesenchimal cancer cell transition (loss of E-Cadherin expression; acquisition of Vimentin expression).
Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy in chemotheray-refractory metastatic colorectal cancer: a retrospective consortium analysis

Wendy De Roock, Bart Claes, David Bernasconi, Jef De Schutter, Bart Biesmans, George Fountzilas, Konstantine T Kalogeras, Vassiliki Kotoula, Demetris Papamichael, Pierre Laurent-Puig, Frédérique Penault-Llorca, Philippe Rougier, Bruno Vincenzi, Daniele Santini, Giuseppe Tonini, Federico Cappuzzo, Milo Frattini, Francesca Molinari, Piercarlo Saletti, Sara De Dosso, Miriam Martini, Alberto Bardelli, Salvatore Siena, Andrea Sartore-Bianchi, Josep Tabernero, Teresa Macarulla, Frédéric Di Fiore, Alice Oden Gangloff, Fortunato Ciardiello, Per Pfeiffer, Camilla Qvortrup, Tine Plato Hansen, Eric Van Cutsem, Hubert Pissesvaux, Diether Lambrechts, Mauro Delorenzi, Sabine Tejpar

**Summary**

**Background** Following the discovery that mutant *KRAS* is associated with resistance to anti-epidermal growth factor receptor (EGFR) antibodies, the tumours of patients with metastatic colorectal cancer are now profiled for seven *KRAS* mutations before receiving cetuximab or panitumumab. However, most patients with *KRAS* wild-type tumours still do not respond. We studied the effect of other downstream mutations on the efficacy of cetuximab in, to our knowledge, the largest cohort to date of patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab plus chemotherapy in the pre-*KRAS* selection era.

**Methods** 1022 tumour DNA samples (73 from fresh-frozen and 949 from formalin-fixed, paraffin-embedded tissue) from patients treated with cetuximab between 2001 and 2008 were gathered from 11 centres in seven European countries. 773 primary tumour samples had sufficient quality DNA and were included in mutation frequency analyses; mass spectrometry genotyping of tumour samples for *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* was done centrally. We analysed objective response, progression-free survival (PFS), and overall survival in molecularly defined subgroups of the 649 chemotherapy-refractory patients treated with cetuximab plus chemotherapy.

**Findings** 40.0% (299/747) of the tumours harboured a *KRAS* mutation, 14.5% (108/743) harboured a *PIK3CA* mutation (of which 68.5% [74/108] were located in exon 9 and 20.4% [22/108] in exon 20), 4.7% (36/761) harboured a *BRAF* mutation, and 2.6% (17/644) harboured an *NRAS* mutation. *KRAS* mutants did not derive benefit compared with wild types, with a response rate of 6.7% (17/253) versus 35.8% (126/352; odds ratio [OR] 0.13, 95% CI 0.07–0.22; p<0.0001), a median PFS of 12 weeks versus 24 weeks (hazard ratio [HR] 1.98, 1.66–2.36; p<0.0001), and a median overall survival of 32 weeks versus 50 weeks (1.75, 1.47–2.09; p<0.0001). In *KRAS* wild types, carriers of *BRAF* and *NRAS* mutations had a significantly lower response rate than did *BRAF* and *NRAS* wild types, with a response rate of 8.3% (2/24) in carriers of *BRAF* mutations versus 38.0% in *BRAF* wild types (124/326; OR 0.15, 95% CI 0.02–0.51; p=0.0012); and 7.7% (1/13) in carriers of *NRAS* mutations versus 38.1% in *NRAS* wild types (110/289; OR 0.14, 0.007–0.70; p=0.013). *PIK3CA* exon 9 mutations had no effect, whereas exon 20 mutations were associated with a worse outcome compared with wild types, with a response rate of 0.0% (0/9) versus 36.8% (121/329; OR 0.00, 0.00–0.89; p=0.029), a median PFS of 11.5 weeks versus 24 weeks (HR 2.52, 1.33–4.78; p=0.013), and a median overall survival of 34 weeks versus 51 weeks (3.29, 1.60–6.74; p=0.0057). Multivariate analysis and conditional inference trees confirmed that, if *KRAS* is not mutated, assessing *BRAF*, *NRAS*, and *PIK3CA* exon 20 mutations (in that order) gives additional information about outcome. Objective response rates in our series were 24.4% in the unselected population, 36.3% in the *KRAS* wild-type selected population, and 41.2% in the *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* exon 20 wild-type population.

**Interpretation** While confirming the negative effect of *KRAS* mutations on outcome after cetuximab, we show that *BRAF*, *NRAS*, and *PIK3CA* exon 20 mutations are significantly associated with a low response rate. Objective response rates could be improved by additional genotyping of *BRAF*, *NRAS*, and *PIK3CA* exon 20 mutations in a *KRAS* wild-type population.
Figure 1: Associations between mutations
Absolute numbers of KRAS wild type, KRAS mutant, BRAF mutant, NRAS mutant, PIK3CA exon 9 mutant samples (A), and PIK3CA exon 20 mutant (B) samples are shown.
Gene expression profiles of 80 mCRC showed that patients with tumors that express high levels of epiregulin and amphiregulin are more likely to have disease control with cetuximab (\(EREG, P=0.000015\); \(AREG, P=0.000025\)) and significantly longer PFS than patients with low expression (\(EREG: P=0.0002, \text{median PFS, } 103.5 \text{ v 57 days}; \ AREG: P=0.0001, \text{median PFS, } 115.5 \text{ v 57 days}\).
Patients with KRAS wt tumors: Epiregulin as a biomarker of efficacy

Gene expression measurements of EREG and AREG and KRAS mutation analysis were performed on archival FFPE primary tumors of 220 cmCRC patients

In KRAS wild type patients, there was a significant association between ligand expression and response for EREG and for AREG; ligand expression was significantly associated with PFS and OS

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<th>Low epiregulin</th>
<th>High epiregulin</th>
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<tr>
<td>Median OS, weeks [95% CI]</td>
<td>31 [23–40]</td>
<td>65 [50–82]</td>
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<tr>
<td>Log rank test: p&lt;0.001 HR: 0.418 [95%CI: 0.278–0.628]</td>
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Jacobs, B et al. J Clin Oncol 2009
Overall survival by treatment: Subset of patients “Combimarker” positive (K-ras wild-type and high EREG):

OS was better for cetuximab than BSC among patients with high EREG based on both approaches (HR 0.43 and 0.46 respectively; p<0.0001)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mOS</th>
<th>1yOS</th>
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<tbody>
<tr>
<td>CET/BSC</td>
<td>9.8m</td>
<td>33.8%</td>
</tr>
<tr>
<td>BSC</td>
<td>5.1m</td>
<td>18.5%</td>
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Overall survival by treatment: Subset of patients with *K-ras* wild type but low EREG who don’t benefit from cetuximab?

- In the *K-ras* wild-type subset:
  - OS was better for cetuximab than BSC among patients with high EREG but not for low EREG patients
  - The p-value of interaction was 0.13 for pre-specified threshold
Is EREG gene expression prognostic?

- In K-ras wild-type patients on BSC, high EREG expression did not correlate with OS using:
  - pre-specified threshold: adjusted HR 0.82 [0.58-1.15], p=0.24
  - minimum p threshold: adjusted HR 0.85 [0.59-1.22], p=0.38
Pharmacogenomic and Pharmacoproteomic Studies of Cetuximab in Metastatic Colorectal Cancer: Biomarker Analysis of a Phase I Dose-Escalation Study


See accompanying article on page 1254

ABSTRACT

Purpose
This study assessed biomarkers for cetuximab efficacy in tissue samples collected during a phase I dose-escalation study exploring every second week administration of cetuximab as first-line therapy in patients with metastatic colorectal cancer (mCRC).

Patients and Methods
Sixty-two patients received cetuximab monotherapy for 6 weeks, followed by cetuximab plus infusional fluorouracil, leucovorin, and irinotecan until disease progression. Patients in the control arm received cetuximab as a 400 mg/m² initial dose then 250 mg/m² per week; patients in the dose-escalation arms received 400 to 700 mg/m² every second week. Tumor and skin biopsies were taken for immunohistochemical and microarray expression analyses (tumor only) at baseline and week 4. Plasma was collected for proteomic analysis at baseline and week 4. KRAS tumor mutation status was assessed.

Results
In subsets of paired skin samples from 35 patients, cetuximab treatment was associated with substantial downregulation of phospho(p)-EGFR, p-MAPK and proliferation and substantial upregulation of p27Kip1 and p-STAT3 levels. No marked difference in these effects was noted for different schedules of administration and dose levels. In the cetuximab monotherapy phase, responses were seen only in patients whose tumors were wild-type for KRAS (eight of 29 versus zero of 19 for KRAS mutant tumors; P = .015). Progression-free survival was longer for patients with KRAS wild-type compared with KRAS mutant tumors (log-rank P = .048). Genomics/proteomics analyses (42 and 45 patients, respectively) identified candidate biomarkers associated with response.

Conclusion
Biomarker analysis supported the functional equivalence of weekly and every second week administration of cetuximab and provided further confirmation that patients with KRAS wild-type mCRC were those most likely to benefit from cetuximab treatment.

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How do we select an EGFR-dependent CRC?

- KRAS wild type CRC is an heterogeneous disease.
- Only a subset of KRAS wild type CRC (approximately 50%) is dependent on the EGFR pathway.

Potential markers of high dependence on the EGFR pathway:
- Quadruple negative mutations (KRAS, NRAS, BRAF, PI3KCA exon 20 wild type genes);
- High amphiregulin and/or high epiregulin.
Why was the PETACC8 trial negative?

- The KRAS wild type (and also double KRAS/BRAF wild type) tumor group is an heterogeneous biologic and pathologic group.

- Therefore, a better definition of an EGFR-dependent CRC is necessary to identify those patients that could benefit from treatment also in an adjuvant setting in combination with a chemotherapy regimen which is on the average effective in obtaining a 3 year DFS of 70-75% in Stage III CRC patients.

- The authors are strongly encouraged to conduct further translational research in the PETACC8 KRAS wt patient population.