Clinical Translational Research

Oncology

Oncology 2012;83:241–247
DOI: 10.1159/000339534

Received: February 3, 2012
Accepted after revision: May 2, 2012
Published online: August 29, 2012

Impact of the Specific Mutation in *KRAS* Codon 12 Mutated Tumors on Treatment Efficacy in Patients with Metastatic Colorectal Cancer Receiving Cetuximab-Based First-Line Therapy: A Pooled Analysis of Three Trials

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Key Words
Metastatic colorectal cancer · *KRAS* mutation · Codon 12 · Cetuximab · Anti-epidermal growth factor receptor

Abstract
Purpose: This study investigated the impact of specific mutations in codon 12 of the Kirsten-ras *(KRAS)* gene on treatment efficacy in patients with metastatic colorectal cancer (mCRC). ***Patients:*** Overall, 119 patients bearing a *KRAS* mutation in codon 12 were evaluated. All patients received cetuximab-based first-line chemotherapy within the Central European Cooperative Oncology Group (CECOG), AIO KRK-0104 or AIO KRK-0306 trials. ***Results:*** Patients with *KRAS* codon 12 mutant mCRC showed a broad range of outcome when treated with cetuximab-based first-line regimens. Patients with tumors bearing a *KRAS* p.G12D mutation showed a strong trend to a more favorable outcome compared to other mutations (overall survival 23.3 vs. 14–18 months; hazard ratio 0.66, range 0.43–1.03). An interaction model illustrated that *KRAS* p.G12C was associated with unfavorable outcome when treated with oxaliplatin plus cetuximab. ***Conclusion:*** The present analysis suggests that *KRAS* codon 12 mutation may not represent a homogeneous entity in mCRC when treated with cetuximab-based first-line therapy.

Introduction

Kirsten-ras *(KRAS)* is a proto-oncogene encoding a small G protein. The KRAS protein is located at the inner cell membrane and has guanosine triphosphatase (GTPase) activity. Extracellular binding of ligands to transmembrane receptors like the epidermal growth fac-

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tor receptor (EGFR) causes activation of the downstream signal transduction cascade to the nucleus. In the first step, the intracellular tyrosine kinase domain of the EGFR is phosphorylated which in turn induces a transient activation of the RAS protein. While in its inactive state, RAS is bound to guanosine diphosphate (GDP), activation occurs by the conversion of GDP to GTP [1, 2].

Mutations of the *KRAS* gene are described to occur in approximately 40% of metastatic colorectal cancers. The mutations of *KRAS* are point mutations and are mostly (>90%) detected in codon 12 (approx. 80%) and codon 13 (approx. 20%) and are far less frequent in other codons like codon 61 [2–6]. In some studies, different types of *KRAS* mutations, mostly in codon 13, were reported to be associated with a more aggressive biology concerning the risk of relapse after initial surgery was performed for colorectal cancer [7, 8]. For metastatic colorectal cancer (mCRC), mutations in the *KRAS* proto-oncogene have been identified as determinants of poor response to anti-EGFR antibodies such as cetuximab and panitumumab leading to an exclusion of affected patients from anti-EGFR therapy [9–13]. However, several recent studies did not identify wild-type *KRAS* alone to be a strong predictor of response to anti-EGFR antibodies [14–16].

In patients with *KRAS* mutated tumors, the combination of FOLFOX plus anti-EGFR-directed antibodies showed trends to be associated with shorter overall survival (OS) when compared to FOLFOX alone in the PRIME study (15.5 vs. 19.3 months; p = 0.068) and in the smaller OPUS study (13.4 vs. 17.5 months; p = 0.2) [11, 17].

Disappointing outcomes in patients undergoing treatment with anti-EGFR antibodies and chemotherapy were mostly observed in studies using an oxaliplatin-based chemotherapy backbone, while this was not the case in studies using irinotecan-based chemotherapy backbones [10, 18]. Therefore, it has been hypothesized that an unknown mechanism of inhibitory interaction may exist for the combined use of anti-EGFR agents and oxaliplatin in patients with *KRAS* mutant mCRC.

The concept of *KRAS* mutant tumors as a uniform entity generally resistant to anti-EGFR antibodies was first questioned by de Roock and coworkers [19]. In a recent report based on preclinical and clinical investigations, this group indicated that *KRAS* p.G13D mutant mCRC compared to other *KRAS* mutant tumors is associated with unfavorable outcome receiving best supportive care, but with a longer OS and progression-free survival (PFS) when treated with cetuximab.

These findings are further supported by studies investigating the role of *KRAS* mutations in the risk of relapse and death after primary resection of colorectal cancer. A decade ago, *KRAS* mutation in codon 13 was identified as a risk factor of poor outcome [7, 8, 20]. Other authors described codon 12 mutation (p.G12V) as a marker of a highly aggressive disease [8, 21].

In the present analysis, we investigate patients with mCRC exclusively characterized by a mutation in *KRAS* codon 12. The study aims to analyze the question whether subtypes of *KRAS* codon 12 mutation have an impact on treatment outcome.

Methods

KRAS Mutation Detection

All tumor samples were evaluated using validated methods of high sensitivity for KRAS diagnostics [22, 23]. In terms of the AIO KRK-0104 and the AIO KRK-0306 trial, *KRAS* testing was performed in a German reference laboratory for *KRAS* analysis (Department of Pathology, University of Munich, Munich, Germany). Detection of mutations in codons 12 and 13 of the *KRAS* proto-oncogene was performed by pyrosequencing using Qiagen's PyroMark Gold kits together with a Q24 pyrosequencer device [5]. This procedure resulted in a specificity of 0.98 and a sensitivity of 0.99 for the detection of mutations in the *KRAS* proto-oncogene [5, 15, 18, 24]. The tumor samples belonging to the Central European Cooperative Oncology Group (CECOG) trial were retrospectively tested for *KRAS* by an allele-specific real-time polymerase chain reaction assay which demonstrated comparable precision in *KRAS* testing (DxS Ltd., Manchester, UK) [22, 23, 25].

Treatment Schedules

The AIO KRK-0104 study was designed as an open-label, randomized phase II study as previously reported by Moosmann et al. [15] and Modest et al. [18]. It was conducted at 35 centers in Germany. In both study arms, cetuximab was given at an initial dose of 400 mg/m^2 as a 120-min infusion, followed by weekly infusions of 250 mg/m^2 over 60 min. Patients in arm A received chemotherapy with CAPIRI (i.e. oral capecitabine 800 mg/m^2 twice daily on days 1–14, followed by a 1-week rest period plus irinotecan 200 mg/m^2 as a 30-min intravenous infusion on day 1). In patients >65 years, doses were further reduced by 20%. Patients in arm B received chemotherapy with CAPOX (i.e. capecitabine 1,000 mg/m^2 twice daily on days 1–14, followed by a 1-week rest period plus oxaliplatin 130 mg/m^2 as a 120-min intravenous infusion on day 1). Treatment cycles were repeated every 3 weeks until disease progression or unacceptable toxicity [15].

The ongoing AIO KRK-0306 study was designed as a randomized study and is presently conducted at 177 centers in Germany and Austria. This trial compares FOLFIRI plus cetuximab to FOLFIRI plus bevacizumab. Following an amendment, the study was closed for patients with *KRAS* mutant mCRC in 2009. Therefore, only patients with *KRAS* mutated mCRC could be analyzed in the present analysis. As described before, only patients included in the cetuximab arm were evaluated. FOLFIRI was applied as previously reported by Tournigand and coworkers [26]. Irinotecan (180 mg/m^2) was given as a 60-min infusion followed by fo-

linic acid (400 mg/m^2), as a 120-min infusion and by an intravenous bolus of 5-fluorouracil (5-FU; 400 mg/m^2) and 5-FU (2,400 mg/m^2) applied as a 46-hour continuous infusion. The regimen was repeated every 2 weeks. Cetuximab was given at an initial dose of 400 mg/m^2 as a 120-min infusion, followed by weekly infusions of 250 mg/m^2 over 60 min. Treatment was performed until disease progression or unacceptable toxicity [24].

The CECOG study was a two-arm randomized multicenter, open-label, parallel-group phase II study involving 28 participating centers across 13 countries (CECOG/CORE1.2.001). Eligible patients were centrally randomized 1:1, using a minimization technique, stratifying patients according to study site, the number of organs involved and prior neoadjuvant/adjuvant therapy. Patients received cetuximab (400 mg/m^2 initial infusion on day 1, then 250 mg/m^2 weekly) and were then placed either in arm A or arm B: arm A, oxaliplatin (100 mg/m^2 on day 1) with FA [400 mg/m^2 (racemic) or 200 mg/m^2 (L-form)] plus 5-FU (400 mg/m^2 bolus plus 2,400 mg/m^2 as a 46-hour continuous infusion) every 2 weeks (FOLFOX6); arm B, irinotecan (180 mg/m^2) with the 5-FU/FA regimen described (FOLFIRI) [25].

Patients

The present analysis investigated 119 mCRC patients with a confirmed *KRAS* mutation in codon 12 that received cetuximab-based first-line therapy for mCRC. All patients were treated within the AIO KRK-0104 (NCT00254137; 41 patients), the AIO KRK-0306 (NCT00433927; 39 patients) or the CECOG trial (39 patients). A total of 62 patients received FOLFIRI, while 16 patients were treated with FOLFOX. Further 27 patients received CAPIRI and 14 patients CAPOX. The protocols of the clinical trials were approved by an independent ethics committee and governmental authorities. The trials were conducted in accordance with the Declaration of Helsinki (1996). All patients provided written and oral informed consent to be treated within the clinical trials.

Endpoints

The present investigation was performed as an exploratory analysis. Radiological tumor response (complete remission, partial remission, stable disease, progressive disease) was assessed according to RECIST criteria. Response evaluation was performed at 6- to 8-week intervals. PFS was defined as the interval between randomization and first documentation of progression or death; OS was calculated as the time between randomization and death due to any reason. Patients alive were censored at the last time point of patient contact.

Statistical Analysis

Data were summarized by adequate measures of location and variation for continuous variables and by proportions for discrete variables. Adequate tests for discrete data (χ2-test) were used. Survival data were described by using the Kaplan-Meier estimator. Differences in survival between the groups were tested by Cox regressions. Differences in OS and PFS were modelled using Cox proportional hazards regression where a treatment-covariate interaction (irinotecan vs. oxaliplatin and codon 12 mutation subtype) was included. The resulting hazard ratios were tested by the Wald test. All statistical tests were performed two-sided, and a p value <0.05 was considered statistically significant. All statistical analyses were performed using R (version 2.13.2) and IBM SPSS (version 19).

Results

Study Population

This exploratory analysis includes 119 patients who received first-line treatment for mCRC. Characteristics of the patients analyzed in this study do not indicate significant differences regarding age, sex, ECOG status or primary disease sites when compared between the different subgroups. The frequencies of *KRAS* codon 12 mutations within our study population were 44 p.G12D (37%), 36 p.G12V (30%), 14 p.G12A (12%), 13 p.G12C (11%), 8 p.G12S (7%) and 4 p.G12R (3%; table 1).

Impact of Codon 12 Subgroups on Parameters of Treatment Efficacy

Patients with *KRAS* codon 12 mutated tumors showed significantly different response rates depending on the single mutation. Mutations in position 35 (p.G12D, p.G12A, p.G12V) showed response rates of at least 40%, while mutations in position 34 (p.G12C, p.G12S, p.G12R) were associated with lower response rates of $<$20%. Correspondingly, PFS reached durations from 4.9 (p.G12C) to 9.8 months (p.G12A; table 2).

Median OS observed in this pooled analysis revealed marked differences between the single mutations. While patients with *KRAS* p.G12D mutant mCRC showed an OS of 23.3 months, survival observed in patients with tumors bearing other *KRAS* codon 12 mutations was generally shorter, reaching 14–18 months (table 2; figure 1).

Interaction Tests: PFS and OS

PFS was illustrated by an interaction model including irinotecan versus oxaliplatin and the *KRAS* mutation as possible parameters. If all *KRAS* mutations were taken into account and p.G12D was defined as the 'reference mutation', a significant negative interaction ($p = 0.03$) was found in patients with *KRAS* p.G12C mutant tumors who were treated with cetuximab- and oxaliplatin-containing regimens. This interaction test modeling OS instead of PFS again illustrated the negative interaction in patients with *KRAS* p.G12C mutant tumors undergoing cetuximab plus oxaliplatin-based first-line therapy ($p = 0.002$; table 3).

Discussion

Mutations of the *KRAS* gene are observed in approximately 40% of sporadic colorectal cancers [3, 6]. Generally, metastatic colorectal tumors are tested only for mu-

Table 1. Patient characteristics according to *KRAS* mutation

Characteristics	p.G12D	p.G12V	p.G12A	p.G12C	p.G12S	p.G12R	p value
Patients	44	36	14	13	8	4	
Age, years							
Median	63	63.5	62	67	66	62.5	
Range	39–78	40–76	38–71	49–81	47–72	58–70	
Sex, %							0.71
Female	36	28	36	38	12	50	
Male	64	72	64	62	88	50	
ECOG status, %							0.30
0	45	56	43	31	38	75	
1	55	44	57	62	63	25	
2	0	0	0	7	0	0	
Primary tumor site, %							0.43
Colon	68	56	50	62	75	25	
Rectum	32	44	50	38	25	75	
Disease site, %							
Liver	79	89	71	85	88	25	0.06
Lung	36	28	50	46	38	100	0.05
Peritoneum	14	11	8	0	0	0	0.55
Other	40	28	29	31	25	25	0.94
Subsets							0.20
FOLFIRI	26	16	7	5	5	3	
FOLFOX	3	9	1	0	2	1	
CAPIRI	10	8	5	4	0	0	
CAPOX	5	3	1	4	1	0	

Percentages are based on non-missing data. The χ^2 test was used for p values.

tations in codons 12 and 13 of the *KRAS* gene since the other possible mutations are less frequent. Codons 12 and 13 of the *KRAS* gene code for two adjacent glycine residues located in the proximity of the catalytic site of the RAS protein. The *KRAS* mutations in codon 12 occur as point mutations in position 34 [c.34G→A (p.G12S), c.34G→C (p.G12R) and c.34G→T (p.G12C)] or in position 35 [c.35G→A (p.G12D), c.35G→C (p.G12A) and c.35G→T (p.G12V)]. These mutations lead to a base exchange which in turn codes for different amino acids at the catalytic site of the resulting *KRAS* protein. Although, the intrinsic level of KRAS-GTPase activity may be variably affected, the described clinical impact of the specific mutation is not consistent between different studies and tumor stages [19, 27, 28].

Several studies performed in chemotherapy-refractory mCRC patients have indicated that anti-EGFR agents are not effective when tumors bear *KRAS* mutations [29–33]. Subsequently, also in first-line studies, no benefit of anti-EGFR treatment was observed in patients with *KRAS* mutant tumors [10, 11, 17]. Therefore, *KRAS* mutant mCRCs were generally regarded as resistant to anti-EGFR treatment. This assumption was maintained until recently, when a study performed in pretreated patients demonstrated that also tumors with a c.38G→A (p.G13D) mutation in codon 13 responded to treatment with cetuximab [19].

Our pooled analysis focused on *KRAS* codon 12 mutations and was designed to perform a comparative subgroup analysis of the observed mutations.

In our study, outcome parameters of patients with tumors bearing a p.G12D mutation were generally more favorable compared to other mutations. A median OS of 23.3 months observed for this subgroup compares well to the survival of patients with *KRAS* wild-type tumors, as shown in various other trials [10, 11, 17]. It might be important to note that the majority of patients with *KRAS* p.G12D mutant mCRC in our pooled analysis were treated with cetuximab plus irinotecan-based chemotherapy. By contrast, no prognostic impact of p.G12D mutation compared to other *KRAS* mutations in patients with mCRC receiving first-line treatment with FOLFOX plus

Table 2. Treatment efficacy by *KRAS* codon 12 mutation in patients with mCRC undergoing cetuximab-based first-line treatment

Mutation position	Mutation	n	ORR %	PFS, months	OS, months
Codon 12–35	p.G12D	44	46	7.5 (6.8–8.2)	23.3 (17.8–28.8)
	p.G12V	36	44	6.2 (3.2–9.2)	18.4 (13.9–22.9)
	p.G12A	14	42	9.8 (5.3–14.3)	17.9 (12.6–23.1)
Codon 12–34	p.G12C	13	17	4.9 (3.7–6.2)	14.3 (6.7–21.9)
	p.G12S	8	13	8.3 (1.4–15.2)	15.2 (6.4–24.0)
	p.G12R	4	0	5.3 (3.1–7.6)	15.5 (11.0–20.0)
p value			0.12		

Percentages are based on non-missing data. Figures in parentheses are 95% confidence intervals. ORR = Overall response rate.

Table 3. Interaction model with p.G12D as reference mutation

Mutation/treatment	Hazard ratio	95% CI	p value
PFS			
Oxaliplatin	0.9	0.33–2.19	0.8
p.G12C	0.92	0.42–2.00	0.83
p.G12A	0.89	0.43–1.87	0.76
p.G12V	0.86	0.49–1.49	0.58
Oxaliplatin/p.G12C	5.35	1.21–23.70	*0.03*
Oxaliplatin/p.G12A	1.62	0.28–9.30	0.59
Oxaliplatin/p.G12V	1.81	0.59–5.52	0.30
OS			
Oxaliplatin	0.81	0.30–2.17	0.68
p.G12C	1.41	0.60–3.30	0.43
p.G12A	1.71	0.81–3.58	0.16
p.G12V	1.23	0.67–2.26	0.50
Oxaliplatin/p.G12C	13.37	2.63–68.1	*0.002*
Oxaliplatin/p.G12A	1.75	0.18–17.7	0.63
Oxaliplatin/p.G12V	1.65	0.48–5.68	0.43

Cox proportional hazards regression/treatment covariate interaction. p.G12S and p.G12R were not included due to limited sample size. p values by the Wald test.

panitumumab/placebo was described by a recent report [34]. Taken together, these observations may point to a heterogeneous sensitivity of *KRAS* codon 12 mutant tumors to the combined use of chemotherapy plus anti-EGFR antibody or to a prognostic impact of the mutation position. Our pooled analysis might be biased by the fact that the majority of patients received irinotecan-based regimens.

The PRIME trial, comparing FOLFOX to FOLFOX plus panitumumab, demonstrated that patients with *KRAS* mutant tumors have a better outcome when treated with FOLFOX alone compared to FOLFOX plus panitumumab. This difference was nearly significant [17]. A similar effect was observed in the OPUS trial (FOLFOX vs. FOLFOX plus cetuximab), although no statistical significance was reached either [11]. However, the CRYSTAL trial, using irinotecan-based chemotherapy, did not describe any differences in terms of OS in patients with *KRAS* mutant tumors when cetuximab was added to FOLFIRI [10]. Therefore, interactions between some *KRAS* mutations and oxaliplatin might be suspected to cause negative effects in patients with mCRC. In our dataset, an interaction model in which p.G12D was used as the 'reference mutation' demonstrated that p.G12C mutation correlates with poor outcome when treated with oxaliplatin-based first-line treatment. This finding might be supported by the translational analysis of the PRIME trial, which describes *KRAS* p.G12C mutations to correlate with a poor prognostic impact on PFS and OS (OS not significant) when treated with FOLFOX alone compared to other *KRAS* mutations [34].

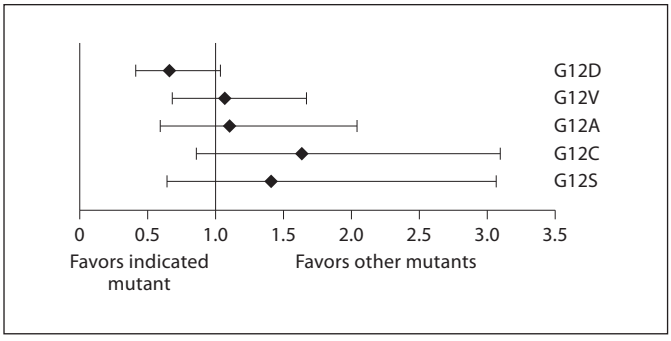

Fig. 1. Prognostic impact of *KRAS* subgroups on OS in patients receiving cetuximab-based first-line therapy. G12R was not assessed due to limited sample size. Hazard ratios by Cox regressions.

Due to the limited number of patients involved in the oxaliplatin-treated subgroup, conclusions must be drawn very carefully. However, our observations suggest that the combination of anti-EGFR-directed agents with specific chemotherapeutics may be an important determinant of outcome with different effects in the respective mutation subgroup. Based on the present data, it could be hypothesized that the poor outcome of patients with

KRAS codon 12–34 mutated tumors, especially those with p.G12C mutation, may be an important factor determining the disappointing survival of *KRAS* mutant patients exposed to oxaliplatin plus anti-EGFR antibodies [11, 17]. Although our retrospective evaluation only allows the formation of hypotheses, future studies evaluating drugs targeting the MAP kinase pathway in patients with *KRAS* mutant tumors may expect differences within the *KRAS* codon 12 mutant group.

Finally, the influence of the three single studies on our results cannot be evaluated properly due to the small numbers of patients in our subgroups observed in each trial.

In conclusion, this analysis suggests that *KRAS* codon 12 mutations are associated with variable outcomes in patients with mCRC. This survival variety might be influenced by the chemotherapy combined with cetuximab as first-line regimen. Prospective trials will have to clarify the sensitivity of *KRAS* codon 12 subgroups to chemotherapy.

Acknowledgments

We thank all of the patients and their families, the participating centers of the AIO KRK-0104,AIO KRK-0306 and CECOG-CORE trials and the entire medical staff who contributed to patient care. We also thank Matthias Wolff for his expert secretarial help and organization. All trials included in this analysis were supported by Merck.

References

1 Heinemann V, Stintzing S, Kirchner T, Boeck S, Jung A: Clinical relevance of EGFR- and *KRAS*-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. Cancer Treat Rev 2009;35:262–271

2 Khosravi-Far R, Der CJ: The Ras signal transduction pathway. Cancer Metastasis Rev 1994;13:67–89.

3 Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb AJ, Vogelstein B: Prevalence of ras gene mutations in human colorectal cancers. Nature 1987;327: 293–297.

4 Boughdady IS, Kinsella AR, Haboubi NY, Schofield PF: K-ras gene mutations in adenomas and carcinomas of the colon. Surg Oncol 1992;1:275–282.

5 Neumann J, Zeindl-Eberhart E, Kirchner T, Jung A: Frequency and type of *KRAS* mutations in routine diagnostic analysis of metastatic colorectal cancer. Pathol Res Pract 2009;205:858–862.

6 Finkelstein SD, Sayegh R, Christensen S, Swalsky PA: Genotypic classification of colorectal adenocarcinoma. Biologic behavior correlates with K-ras-2 mutation type. Cancer 1993;71:3827–3838.

7 Bazan V, Migliavacca M, Zanna I, Tubiolo C, Grassi N, Latteri MA, La Farina M, Albanese I, Dardanoni G, Salerno S, Tomasino RM, Labianca R, Gebbia N, Russo A: Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. Ann Oncol 2002;13:1438–1446.

8 Cerottini JP, Caplin S, Saraga E, Givel JC, Benhattar J: The type of K-ras mutation determines prognosis in colorectal cancer. Am J Surg 1998;175:198–202.

9 Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL: American Society of Clinical Oncology provisional clinical opinion: testing for *KRAS* gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27: 2091–2096.

10 Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360: 1408–1417.

11 Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M, Koralewski P: Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol 2011;22:1535–1546.

12 Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C, Moore MJ: Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357:2040–2048.

13 Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR: K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–1765.

14 Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP, MRC COIN Trial Investigators: Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet 2011;377:2103–2114.

15 Moosmann N, von Weikersthal LF, Vehling-Kaiser U, Stauch M, Hass HG, Dietzfelbinger H, Oruzio D, Klein S, Zellmann K, Decker T, Schulze M, Abenhardt W, Puchtler G, Kappauf H, Mittermüller J, Haberl C, Schalhorn A, Jung A, Stintzing S, Heinemann V: Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104 – a randomized trial of the German AIO CRC Study Group. J Clin Oncol 2011;29:1050–1058.

16 Tveit K, Guren T, Glimelius B, et al: Randomized phase III study of 5-fluorouracil/folinate/oxaliplatin given continuously or intermittently with or without cetuximab, as first-line treatment of metastatic colorectal cancer: the NORDIC VII Study (NCT00145314) (abstract LBA20). Ann Oncol 2010;21(suppl 8):9.

17 Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010;28:4697–4705.

18 Modest DP, Jung A, Moosmann N, Laubender RP, Giessen C, Schulz C, Haas M, Neumann J, Boeck S, Kirchner T, Heinemann V, Stintzing S: The influence of KRAS and BRAF mutations on the efficacy of cetuximab-based first-line therapy of metastatic colorectal cancer: an analysis of the AIO KRK-0104-trial. Int J Cancer 2011, Epub ahead of print.
19 De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, Lamba S, Arena S, Frattini M, Piessevaux H, Van Cutsem E, O'Callaghan CJ, Khambata-Ford S, Zalcberg JR, Simes J, Karapetis CS, Bardelli A, Tejpar S: Association of *KRAS* p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 2010; 304:1812–1820.
20 Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML: Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000;9:1193–1197.
21 Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N, Beranek M, Jandik P, Benamouzig R, Jullian E, Laurent-Puig P, Olschwang S, Muller O, Hoffmann I, Rabes HM, Zietz C, Troungos C, Valavanis C, Yuen ST, Ho JW, Croke CT, O'Donoghue DP, Giaretti W, Rapallo A, Russo A, Bazan V, Tanaka M, Omura K, Azuma T, Ohkusa T, Fujimori T, Ono Y, Pauly M, Faber C, Glaesener R, de Goeij AF, Arends JW, Andersen SN, Lövig T, Breivik J, Gaudernack G, Clausen OP, De Angelis PD, Meling GI, Rognum TO, Smith R, Goh HS, Font A, Rosell R, Sun XF, Zhang H, Benhattar J, Losi L, Lee JQ, Wang ST, Clarke PA, Bell S, Quirke P, Bubb VJ, Piris J, Cruickshank NR, Morton D, Fox JC, Al-Mulla F, Lees N, Hall CN, Snary D, Wilkinson K, Dillon D, Costa J, Pricolo VE, Finkelstein SD, Thebo JS, Senagore AJ, Halter SA, Wadler S, Malik S, Krtolica K, Urosevic N: Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 2001;85:692–696.
22 Lamy PJ, Montels F, Servanton AC, Ychou M, Crapez E: Diagnostic of KRAS gene mutations in colorectal cancer: evaluation of direct sequencing, pyrosequencing and allele specific amplification (abstract 1322). ECCO 15 and 34th ESMO Multidisc Congr, Berlin, 2009.
23 Juan T, Suggs S, Wolf M, Sarosi I, Freeman D, Oliner K, Bakkar A, Patterson SD: A comparatibility study of 4 commercial KRAS tests (abstract 1811). Am Assoc Cancer Res Annu Meet, San Diego, 2008.
24 Stintzing S, Fischer von Weikersthal L, Decker T, Vehling-Kaiser U, Jäger E, Heintges T, Stoll C, Giessen C, Modest DP, Neumann J, Jung A, Kirchner T, Scheithauer W, Heinemann V, et al: FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer-subgroup analysis of patients with KRAS: mutated tumours in the randomised German AIO study KRK-0306. Ann Oncol 2012, Epub ahead of print.
25 Ocvirk J, Brodowicz T, Wrba F, Ciuleanu TE, Kurteva G, Beslija S, Koza I, Pápai Z, Messinger D, Yilmaz U, Faluhelyi Z, Yalcin S, Papamichael D, Wenczl M, Mrsic-Krmpotic Z, Shacham-Shmueli E, Vrbanec D, Esser R, Scheithauer W, Zielinski CC: Cetuximab plus FOLFOX6 or FOLFIRI in metastatic colorectal cancer: CECOG trial. World J Gastroenterol 2010;16:3133–3143.
26 Tournigand C, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A: FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 2004;22:229–237.
27 Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F: Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010;28:466–474.
28 Scheffzek K, Ahmadian MR, Wittinghofer A: GTPase-activating proteins: helping hands to complement an active site. Trends Biochem Sci 1998;23:257–262.
29 Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD: Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26: 1626–1634.
30 Cappuzzo F, Varella-Garcia M, Finocchiaro G, Skokan M, Gajapathy S, Carnaghi C, Rimassa L, Rossi E, Ligorio C, Di Tommaso L, Holmes AJ, Toschi L, Tallini G, Destro A, Roncalli M, Santoro A, Jänne PA: Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br J Cancer 2008;99:83–99.
31 Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, Camponovo A, Etienne LL, Cavalli F, Mazzucchelli L: PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007;97:1139–1145.
32 Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, Wong TW, Huang X, Takimoto CH, Godwin AK, Tan BR, Krishnamurthi SS, Burris HA 3rd, Poplin EA, Hidalgo M, Baselga J, Clark EA, Mauro DJ: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–3237.
33 Lièvre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Côté JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P: *KRAS* mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66: 3992–3995.
34 Peeters M, Douillard YI, van Cutsem E, Siena S, Zhang K, Williams R, Wiezorek J: Evaluation of individual codon 12 and 13 mutant *KRAS* alleles as prognostic and predictive biomarkers of response to panitumumabin patients with metastatic colorectal cancer (mCRC). EJC 2011;47(suppl 2):33LBA.