

Panitumumab in Metastatic Colorectal Cancer: The Importance of Tumour *RAS* Status

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Abstract Tumour biomarker status is being used more and more frequently to guide treatment decisions in patients with metastatic colorectal cancer (mCRC). Continued cycles of hypothesis generation and biomarker testing in retrospective, prospective–retrospective and prospective analyses from studies of the epidermal growth factor (EGFR)-targeted monoclonal antibodies (mAbs), panitumumab and cetuximab, have resulted in improved patient selection in mCRC. Initial data suggested EGFR-targeted mAb treatment should be limited to patients with *KRAS* exon 2 wild-type (WT) tumours, but the availability of tumour samples from large phase III studies permitted evaluation of additional potential biomarkers of activity for these agents. Subsequent analyses further refined the target population to those patients whose tumours were WT for *KRAS* and *NRAS* exons 2, 3 and 4 (i.e., those with *RAS* WT status). Here, we review key clinical data for panitumumab in mCRC across the lines of treatment, assessing in detail the impact of more comprehensive *RAS* selection on patient outcomes. Panitumumab data across first- to third-line

therapy consistently demonstrate that by testing tumour *RAS* status, it is possible to select patients more likely to benefit from treatment.

Key Points

RAS mutations predict a lack of response to panitumumab.

Detecting *RAS* mutations improves patient selection in mCRC.

Panitumumab has an optimal risk/benefit profile in tumours without *RAS* mutations.

1 Introduction

A predictive biomarker is a characteristic that can be objectively measured and evaluated as an indicator of treatment response (positively predictive) or lack of response (negatively predictive) [1]. Biomarker-guided treatment has the potential to improve clinical outcomes by allowing physicians to tailor therapy to those patients most likely to benefit, thereby sparing potential side effects in patients who are unlikely to respond to treatment. Avoiding treating patients who are unlikely to benefit improves the overall risk/benefit profile of targeted agents; it also has the advantage of being potentially cost-saving, in terms of reducing the use of ineffective drugs, the strain on hospital resources and the need for side-effect management.

Tumour biomarker status is increasingly used to guide treatment decisions in patients with cancer and has been a rapidly developing area of research in metastatic colorectal

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cancer (mCRC). Improved patient selection through the use of biomarkers is likely to be particularly beneficial in mCRC because of the heterogeneity of response amongst these patients and the costs and toxicities associated with the available targeted therapies [2]. Approximately 27–43 % of mCRC tumours harbour *KRAS* exon 2 mutations that lead to constitutive activation of downstream signalling pathways [3]. Results of several uncontrolled [4, 5] and phase II [6] studies led to the hypothesis that the presence of *KRAS* exon 2 mutations might be associated with a lack of response to the epidermal growth factor receptor (EGFR)-targeted monoclonal antibodies (mAbs) panitumumab and cetuximab (Fig. 1) [6–15]. Initial analyses from the 408 study comparing panitumumab + best supportive care (BSC) with BSC alone in patients with mCRC receiving predominantly third-line treatment, supported the use of *KRAS* as a biomarker [13].

As the availability of tumour samples for biomarker analysis was a requirement for entry into several phase III panitumumab trials [10, 11, 13], prospective–retrospective analyses were possible to further test the hypothesis that tumour *KRAS* status may predict response to anti-EGFR therapy in mCRC. In this context, a prospective–retrospective analysis is defined as the prospective analysis of a new biomarker hypothesis that was not prespecified at study start; these analyses are conducted on banked tumour samples from a clinical trial [16]. Wang et al. [17] defined such analyses as “a completed or post-interim analysis from a trial where biomarker samples were collected prior to treatment initiation and where the mechanistic hypothesis would be ‘prospectively specified’ prior to an approved diagnostic assay testing”. Hence, the data analysis is considered a prospective analysis of the hypothesis.

As more data were generated around the sensitivity of tumours to EGFR inhibitors, there was an ongoing cycle of

hypothesis generation and testing. As a result, study protocols were developed to enable prospective analysis of new biomarkers, including prospective analyses of *KRAS* in the phase III PRIME [10] and 181 [11] panitumumab studies (Fig. 1; Table 1). The subsequent confirmation of *KRAS* exon 2 mutations as being negatively predictive of EGFR-targeted mAb efficacy led to their use being restricted to patients with *KRAS* exon 2 wild-type (WT) tumours [18] and meant that for the first time patients with mCRC could be specifically selected for treatment based on the molecular profile of their tumours. This was among the most important developments in personalised mCRC management in recent years.

Continued investigation of the molecular mechanisms underpinning tumour development and growth in mCRC led to the hypothesis that, even with a *KRAS* exon 2 WT population, other mutations might confer resistance to EGFR inhibitors. Based on the similarity between *NRAS* and *KRAS* oncogenes, and the fact that mutations in either gene at codons 12, 13, 61, 117 and 146 had similar biochemical effects [19, 20], these additional *RAS* mutations were hypothesised as biomarkers of resistance to EGFR-targeted mAb activity. Furthermore, in the clinic, colorectal tumours harbour mutations at these same codons of *NRAS* and *KRAS*, and these mutations tend to be mutually exclusive within tumour cells, suggesting functional redundancy [21]. As tumour samples were available for testing, this preclinical molecular hypothesis led to prospective–retrospective analyses of the impact of tumour *RAS* status in the PRIME and 181 trials (Fig. 1). The resulting data showed for the first time that mutations in *KRAS* beyond those in exon 2, as well as mutations in the related *NRAS* gene, also have a significant impact on the risk/benefit profile of panitumumab in mCRC [12, 14]. Patients whose tumours harbour *RAS* mutations do not

Fig. 1 The cycle of hypothesis generation/testing to refine the target metastatic colorectal cancer population for panitumumab treatment. *ASCO* American Society of Clinical Oncology, *NCCN* National Comprehensive Cancer Network, *NGS* next-generation sequencing, *PCR* polymerase chain reaction, *PRA* prospective–retrospective analysis

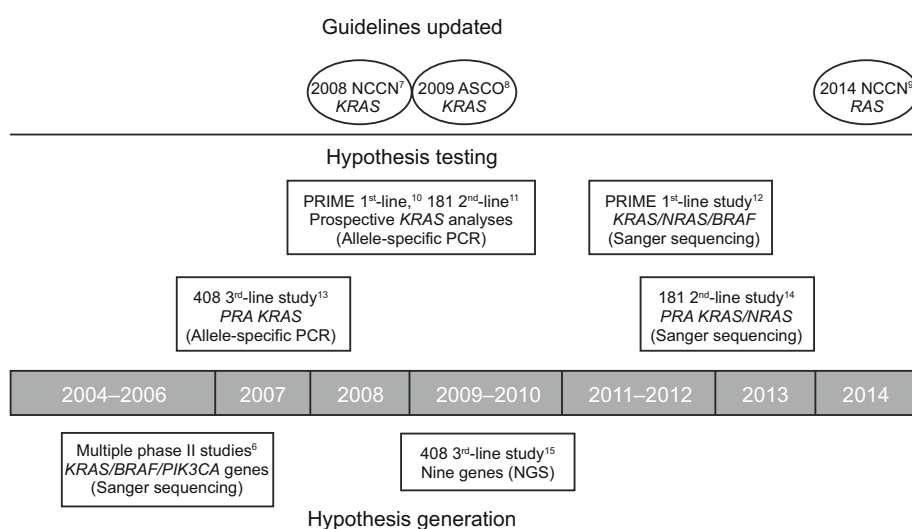


Table 1 Full trial names and abbreviations

Trial acronym	Full trial name
181	A randomised, multicentre phase III study to compare the efficacy of panitumumab in combination with FOLFIRI to the efficacy of FOLFIRI alone in patients with previously treated metastatic colorectal cancer
314	A single arm multicentre phase II study of panitumumab in combination with irinotecan/5-fluorouracil/leucovorin in patients with metastatic colorectal cancer
408	An open-label, randomised, phase III clinical trial of panitumumab plus best supportive care versus best supportive care in subjects with metastatic colorectal cancer
ASPECCT	A randomised, multicentre, open-label, phase III study to compare the efficacy and safety of panitumumab and cetuximab in subjects with previously treated, wild-type <i>KRAS</i> , metastatic colorectal cancer
CRYSTAL	Cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer
FIRE-3	FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer
OPUS	Oxaliplatin and cetuximab in first-line treatment of metastatic colorectal cancer
PEAK	Panitumumab efficacy in combination with mFOLFOX6 against bevacizumab plus mFOLFOX6 in mCRC subjects with wild-type <i>KRAS</i> tumours
PLANET	Safety and efficacy study of FOLFOX4 + panitumumab vs FOLFIRI + panitumumab in subjects WT <i>KRAS</i> colorectal cancer and liver-only metastases
PRIME	The panitumumab randomised trial in combination with FOLFOX4 for metastatic colorectal cancer to determine efficacy
SPIRITT	Second-line panitumumab, irinotecan treatment trial

benefit from treatment with an EGFR inhibitor, whereas those with *RAS* WT status appear to gain from an improved risk/benefit profile compared with those having *KRAS* exon 2 WT tumours. Similar results have since been seen in retrospective analyses of older cetuximab trials [22, 23].

Patients can, therefore, now be selected for panitumumab or cetuximab therapy based on their *RAS* status, meaning that patients whose tumours are WT for *KRAS* and *NRAS* (exons 2, 3 and 4) are eligible, but patients whose tumours harbour mutations in these exons should not receive EGFR inhibitor treatment [24, 25]. An ongoing study (NCT01412957) of panitumumab monotherapy in the third-line mCRC setting will be the first trial to prospectively analyse outcomes for patients based on their tumour *RAS* status. This is a rapidly moving field, with new data supporting the shift from *KRAS* to *RAS* testing being published regularly over the last 18 months and regulatory bodies in Europe responding quickly to these advances, updating the labels of both panitumumab and cetuximab to reflect the need for *RAS* testing in mCRC [24, 25]. Here we review the key clinical data for panitumumab in mCRC across the lines of treatment, and assess in detail the impact of more comprehensive *RAS* selection on patient outcomes.

2 First-Line Setting

There have been four key first-line panitumumab trials in patients with mCRC, including a total of 1699 patients. Prospective and prospective-retrospective analyses of

tumour samples from these studies have allowed *RAS* status to be determined for 1500 (88 %) of the included patients. PRIME, the largest of these trials, was conducted in an unselected patient population, as was the 314 study, whereas PEAK and PLANET only included patients with known *KRAS* exon 2 WT status.

2.1 Phase III Data—The PRIME trial

PRIME (NCT00364013) was a randomised (1:1) phase III study comparing the efficacy and safety of first-line panitumumab 6.0 mg/kg once every 2 weeks (Q2W) + FOLFOX4 versus FOLFOX4 alone in patients with previously untreated mCRC [10]. PRIME was the first randomised, first-line study in which results were prospectively analysed by tumour *KRAS* exon 2 status. A key inclusion criterion was that paraffin-embedded tumour tissue from the primary tumour or metastasis had to be available for central biomarker analyses. Importantly, sufficient tumour tissue was collected to enable additional hypothesis-driven biomarker analyses after the primary efficacy analysis had been conducted.

In PRIME, a prespecified mutational analysis was conducted on banked tumour samples that were previously characterised as *KRAS* exon 2 WT to test for mutations in *NRAS* exon 2 (codons 12 and 13), and *KRAS* and *NRAS* exons 3 (codon 61) and 4 (codons 117 and 146). Assessment of tumour *BRAF* status was also prespecified (exon 15 codon 600). Gene alterations in *KRAS* and *NRAS* exon 3 (codon 59) were investigated as an exploratory endpoint. The *RAS* WT population, therefore, included

patients whose tumours harboured no mutations in any of the following: *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 [exploratory analysis set only] and 61), and 4 (codons 117 and 146).

2.1.1 Impact of *KRAS* Exon 2 Status

Overall, 1183 patients were randomised in PRIME. In total, 1096 patients (93 %) had tumour *KRAS* results, of whom 656 (60 %) were found to have *KRAS* exon 2 WT tumours. The primary analysis from this study was prespecified to be performed when >50 % of patients with *KRAS* exon 2 WT mCRC had died from any cause. In the primary analysis (54 % of patients had died at this point), panitumumab + FOLFOX4 significantly improved progression-free survival (PFS) compared with FOLFOX4 alone in patients with *KRAS* exon 2 WT tumours (Table 2) [10]. Overall survival (OS) and objective response rate (ORR) were numerically higher in the panitumumab + FOLFOX4 versus FOLFOX4 group, but these between-treatment differences were not statistically significant. In *KRAS* exon 2 WT patients, surgical resection was attempted in 10.5 % of those receiving panitumumab + FOLFOX4 and 9.4 % of those receiving FOLFOX4 alone; complete resections were performed in 8.3 versus 7.0 % of patients, respectively [10]. In contrast, in patients with *KRAS* exon 2 mutant (MT) tumours, PFS was significantly reduced in the panitumumab + FOLFOX4 group versus the FOLFOX4 group, and median OS was numerically lower (Table 2).

Significant PFS improvements (median 10.0 vs 8.6 months; hazard ratio (HR) 0.80 [95 % confidence intervals {CI} 0.67–0.95]; $p = 0.01$) were maintained in the *KRAS* exon 2 WT panitumumab + FOLFOX4 group in a prespecified ‘final’ analysis scheduled to occur ~30 months after the last patient enrolled (68 % of patients had died at this point) [26]. Median OS (23.9 vs 19.7 months; HR 0.88 [95 % CI 0.73–1.06]; $p = 0.17$) was also numerically higher for the panitumumab + FOLFOX4 group versus FOLFOX4 in the *KRAS* exon 2 WT population in this analysis. An exploratory analysis of updated survival (>80 % OS events, providing the most up-to-date estimation of OS) was also carried out, which demonstrated a significant OS improvement for *KRAS* exon 2 WT patients receiving panitumumab + FOLFOX4 versus FOLFOX4 (HR 0.83 [95 % CI 0.70–0.98]; $p = 0.03$) [26].

2.1.2 Impact of *RAS* Mutations on Efficacy

RAS ascertainment rates in PRIME were high, with tumour *RAS* status determined for 1060 of the 1183 (90 %) patients randomised in this study; among those 1060 patients, 512 (48 %) were found to have *RAS* WT mCRC [12]. Of the 620 patients with *KRAS* exon 2 WT tumours who could be

evaluated for tumour *RAS* status, 108 (17 %) had mutations elsewhere in either the *KRAS* or *NRAS* genes. Sites of the *RAS* mutations found in the PRIME study are shown in Fig. 2a. A further seven patients had mutations in codon 59 of *KRAS* or *NRAS* exon 3, which were not originally prespecified for analysis—these patients were excluded from the exploratory analysis population ($n = 505$). Tumour *RAS/BRAF* ascertainment rate was also high, with evaluable samples available for 89 % (1047/1183) of patients. Of the 619 patients with *KRAS* exon 2 WT mCRC who were evaluated for tumour *BRAF* status, 53 (9 %) were found to have *BRAF* V600E mutations.

In the primary *RAS* analysis, PFS, OS and ORR were significantly improved in patients with *RAS* WT tumours receiving panitumumab + FOLFOX4 versus FOLFOX4 alone (Table 2) [12]. A clinically significant 5.8-month improvement in median OS was observed in *RAS* WT patients treated with panitumumab + FOLFOX4 compared with those receiving FOLFOX4 alone.

In patients with *RAS* WT tumours, the PFS and OS benefits observed in favour of panitumumab + FOLFOX4 versus FOLFOX4 were observed across all subpopulations predefined according to baseline covariates, with the exception of the Eastern Cooperative Oncology Group (ECOG) performance status 2 subgroup (Fig. 3). Consistent OS benefits were seen in the panitumumab + FOLFOX4 group versus the FOLFOX4 group in an exploratory, updated OS analysis performed when >80 % of all patients in PRIME had died from any cause. In this analysis, median OS was 25.8 versus 20.2 months, respectively (HR 0.77 [95 % CI 0.64–0.94]; $p = 0.009$), supporting the robustness of the primary analysis. In an additional exploratory analysis excluding the seven patients with *KRAS* or *NRAS* exon 3 codon 59 mutations, PFS (median 10.4 vs 7.9 months; HR 0.71 [95 % CI 0.57–0.89]; $p = 0.002$) and OS (median 26.0 vs 20.2 months; HR 0.77 [95 % CI 0.60–0.98]; $p = 0.032$) outcomes were further improved in the panitumumab + FOLFOX4 versus FOLFOX4 group.

In the 108 patients without *KRAS* exon 2 mutations but with mutations elsewhere in *KRAS* or *NRAS*, PFS (median 7.3 vs 8.0 months; HR 1.28 [95 % CI 0.79–2.07]; $p = 0.33$) and OS (median 17.1 vs 18.3 months; HR 1.29 [95 % CI 0.79–2.10]; $p = 0.31$) were numerically shorter in the panitumumab + FOLFOX4 versus FOLFOX4 group. In the *RAS* MT population overall, median PFS and OS were shorter for patients receiving panitumumab + FOLFOX4 versus FOLFOX4 (Table 2), highlighting the importance of limiting the use of panitumumab + FOLFOX4 to patients with *RAS* WT tumours.

In an additional analysis, *BRAF* MT status was found to be a negative prognostic factor. In the 446 patients with neither *RAS* nor *BRAF* mutations, panitumumab + FOLFOX4 was associated with a 1.6-month improvement in PFS (HR 0.68

Table 2 Impact of *KRAS* exon 2 [10] and *RAS* [12, 24] status on outcomes in the PRIME trial (primary analysis data)

	<i>KRAS</i> exon 2 WT [10]		<i>RAS</i> WT ^a [12, 24]	
	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
HR [95 % CI]; p value	0.80 [0.66–0.97]; 0.02		0.72 [0.58–0.90]; 0.004	
Median OS, months	23.9	19.7	26.0	20.2
HR [95 % CI]; p value	0.83 [0.67–1.02]; 0.072		0.78 [0.62–0.99]; 0.043	
ORR, ^a % [95 % CI]	n = 317 55 [50–61]	n = 323 48 [42–53]	n = 149 59 [52–65]	n = 114 46 [40–53]
OR [95 % CI]; p value	1.35 [NA]; 0.068		1.63 [1.13–2.38]; 0.009	
	<i>KRAS</i> exon 2 MT [10]		<i>RAS</i> MT ^b [12, 24]	
	Panitumumab + FOLFOX4 (n = 221)	FOLFOX4 (n = 219)	Panitumumab + FOLFOX4 (n = 272)	FOLFOX4 (n = 276)
Median PFS, months	7.3	8.8	7.3	8.7
HR [95 % CI]; p value	1.29 [1.04–1.62]; 0.02		1.31 [1.07–1.60]; 0.008	
Median OS, months	15.5	19.3	15.6	19.2
HR [95 % CI]; p value	1.24 [0.98–1.57]; 0.068		1.25 [1.02–1.55]; 0.034	
ORR, ^c % [95 % CI]	n = 215 40 [33–46]	n = 211 40 [34–47]	NA	NA
OR [95 % CI]; p value	NA		NA	

CI confidence interval, HR hazard ratio, MT mutant, NA not available, OR odds ratio, ORR objective response rate, OS overall survival, PFS progression-free survival, WT wild type

^a Wild type at *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146)

^b Mutations at any of *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146). Of 620 patients with *KRAS* exon 2 WT tumours and *RAS* data, 108 (17 %) had other *RAS* mutations (*RAS* status determined using bidirectional Sanger sequencing and WAVE-based Surveyor[®] Scan Kits (Transgenomic))

^c Included only patients with baseline measurable disease per central review

[95 % CI 0.54–0.87]; $p = 0.002$) and a 7.4-month improvement in OS (HR 0.74 [95 % CI 0.57–0.96]; $p = 0.02$), versus FOLFOX4 alone. In patients with no *RAS* mutations but with *BRAF* mutations ($n = 53$), small absolute PFS (median 6.1 vs 5.4 months; HR 0.58 [95 % CI 0.29–1.15]; $p = 0.12$) and OS (median 10.5 vs 9.2 months; HR 0.90 [95 % CI 0.46–1.76]; $p = 0.76$) differences observed in favour of the panitumumab + FOLFOX4 versus FOLFOX4 group were not statistically significant, likely due to the small sample size.

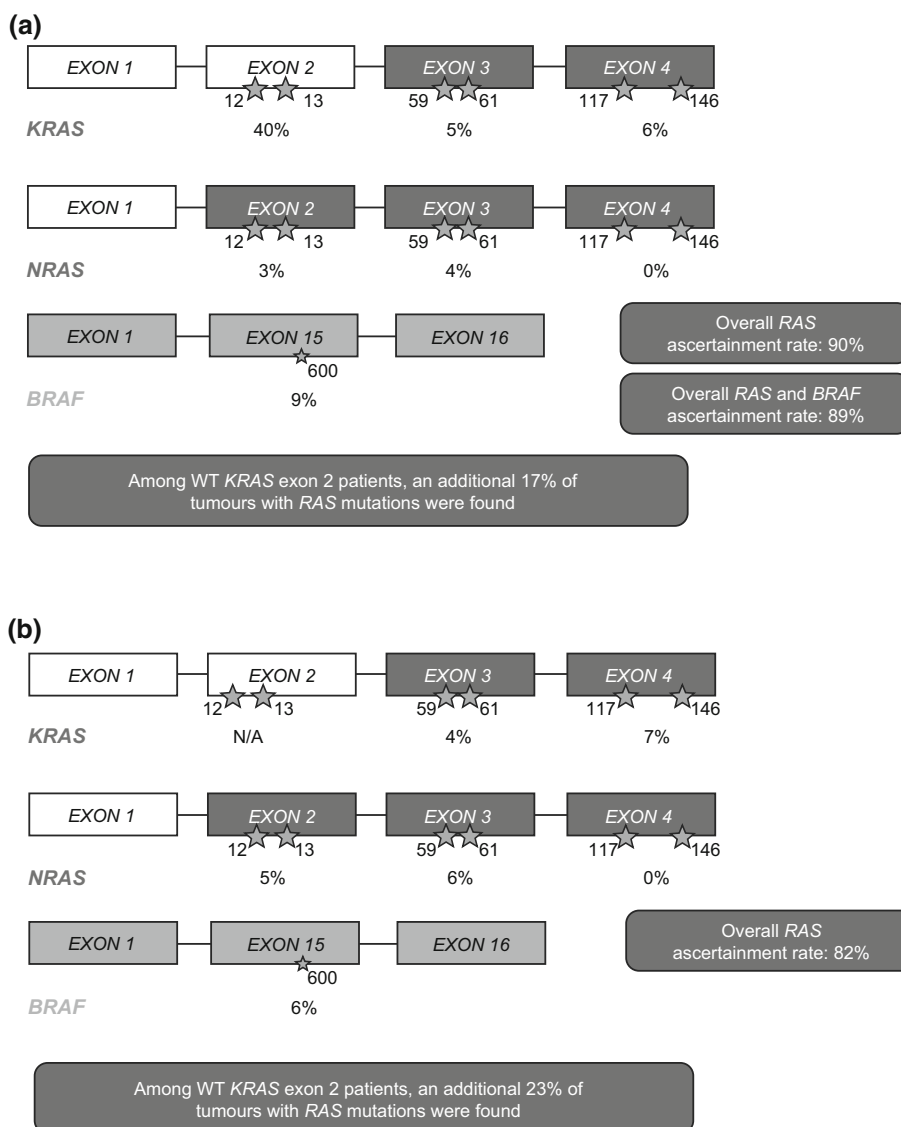
Tumour shrinkage is an important treatment aim for patients with mCRC as it increases the possibility of potentially curative surgical resection [27] and would also be expected to be accompanied with symptom relief. The presence of early tumour shrinkage of ≥ 30 % at week 8 has also been associated with improved PFS and OS outcomes versus shrinkage < 30 % at this time point [28–30]. By week 8, significantly more *RAS* WT patients in the panitumumab + FOLFOX4 versus FOLFOX4 group had achieved ≥ 30 % tumour shrinkage (59 vs 38 %; $p < 0.001$) [28]. Surgical resections were performed in 14

versus 12 % of *RAS* WT patients (exploratory analysis population; $n = 505$) and complete resections were performed in 10 versus 8 % of panitumumab + FOLFOX4 versus FOLFOX4-treated patients, respectively [28].

2.2 Phase II Data—The PEAK Trial

PEAK (NCT00819780) was a 1:1 randomised, open-label, phase II study comparing modified (m)FOLFOX6 + either panitumumab (6 mg/kg Q2W) or bevacizumab (5 mg/kg Q2W) as first-line treatments in patients with *KRAS* exon 2 WT mCRC and unresectable metastatic disease [31]. PEAK was the first randomised trial to compare the treatment effect of an EGFR-targeted mAb with that of an anti-vascular endothelial growth factor (VEGF) therapy in combination with oxaliplatin-containing chemotherapy in the first-line treatment of patients with *KRAS* exon 2 WT mCRC. In this study, *KRAS* exon 2 WT status (codons 12 and 13 were assessed) of paraffin-embedded tumour tissue had to be determined using a validated test. A prespecified extended *RAS* analysis was also performed, with mutations assessed in

Fig. 2 RAS mutation hotspots in the first-line **a** PRIME [12] and **b** PEAK [31, 32] studies. Stars denote codon position; percentages denote the proportion of patients with available data who had a mutation within the specified gene exon. N/A not applicable as *KRAS* exon 2 WT status was defined in the trial eligibility criteria, WT wild type



NRAS exon 2 (codons 12 and 13), and *KRAS* and *NRAS* exons 3 (codons 59 and 61) and 4 (codons 117 and 146). *BRAF* exon 15 (codon 600) mutations were also assessed [32].

Two clinical snapshots were reported in PEAK—an event-driven prespecified primary analysis (performed when ~168 PFS events had occurred) and an exploratory analysis performed ~1 year after the last patient enrolled.

2.2.1 Efficacy in the *KRAS* Exon 2 WT Population

Overall, 285 patients with *KRAS* exon 2 WT mCRC were randomised and comprised the primary analysis population [31]. PFS was similar between the panitumumab + mFOLFOX6 and bevacizumab + mFOLFOX6 arms in the primary analysis (Fig. 4a), despite the relative dose intensity being 8 % lower in the panitumumab arm. OS outcomes were immature at the time of this analysis, with 87

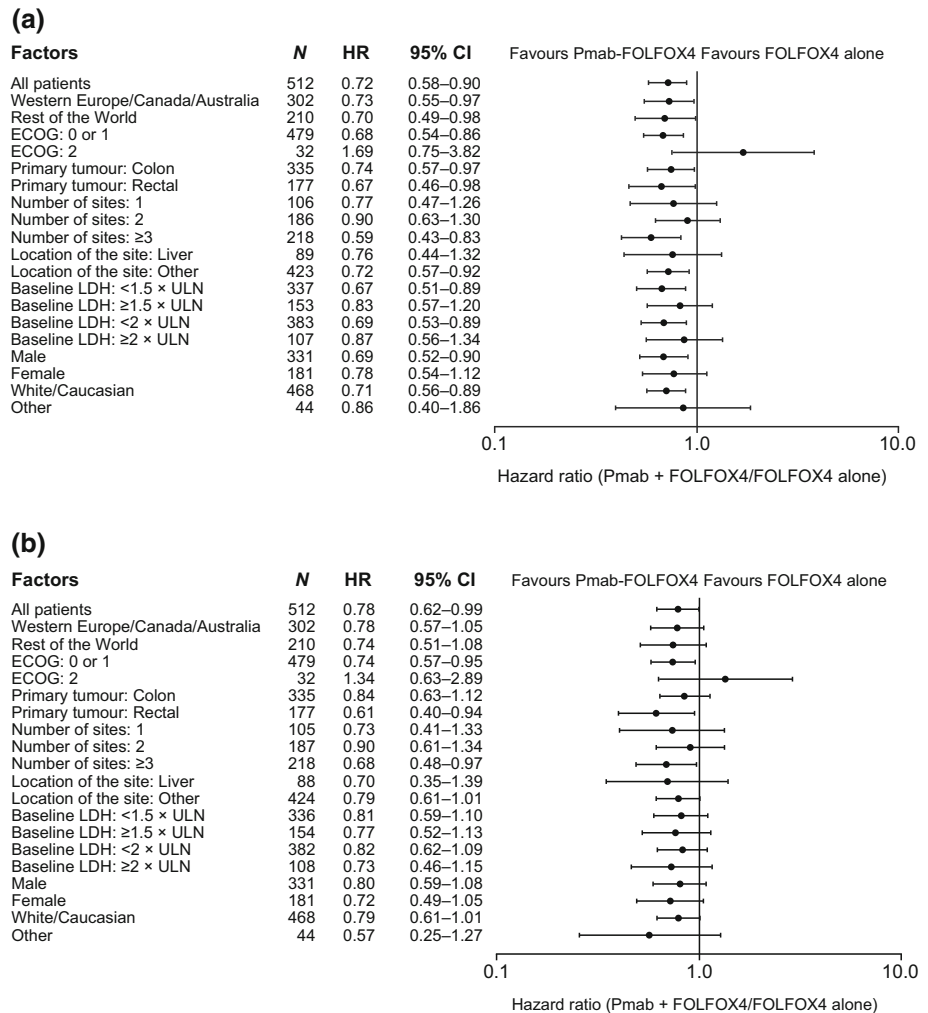
deaths (31 %) reported in the *KRAS* exon 2 WT population overall. At the time of the exploratory analysis, 130 deaths (46 %) had been reported. In this analysis, a significant OS benefit was observed for panitumumab + mFOLFOX6 versus bevacizumab + mFOLFOX6 (Fig. 5a). Post-study treatment included an EGFR-targeted mAb in 21 and 38 % of patients and included anti-VEGF therapy in 40 and 24 % of patients in the panitumumab and bevacizumab arms, respectively.

In PEAK, ORRs were similar between treatments (58 % [95 % CI 49–66] vs 54 % [95 % CI 45–62]) for the panitumumab vs bevacizumab arms, respectively).

2.2.2 Efficacy in the RAS WT Population

In PEAK, tumour samples from 250/285 (88 %) patients underwent a prespecified extended RAS analysis and 233

Fig. 3 PRIME: Hazard ratios (95 % confidence intervals) for **a** progression-free survival; **b** overall survival (*RAS* wild-type primary analysis population) [12]. From Douillard et al. [12]. Copyright© 2013 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society. *CI* confidence intervals, *ECOG* Eastern Cooperative Oncology Group, *HR* hazard ratio, *LDH* lactate dehydrogenase, *Pmab* panitumumab, *ULN* upper limit of normal



(82 %) results were obtained; 170/221 (77 %) patients with *KRAS* exon 2 WT mCRC had *RAS* WT tumours and 51/221 (23 %) had other *RAS* mutations [31]. The sites of mutations found in the PEAK study are shown in Fig. 2b.

PFS was significantly improved in patients with *RAS* WT tumours receiving panitumumab + mFOLFOX6 versus bevacizumab + mFOLFOX6 (Fig. 4b). The OS benefits seen in *KRAS* exon 2 WT patients receiving panitumumab + mFOLFOX6 versus bevacizumab + mFOLFOX6 were greater in the *RAS* WT population (Fig. 5b). Median OS in the *KRAS* exon 2 WT population was 34.2 versus 24.3 months in the panitumumab versus bevacizumab arms, respectively (HR 0.62 [95 % CI 0.44–0.89]; $p = 0.009$) in comparison to 41.3 versus 28.9 months (HR 0.63 [95 % CI 0.39–1.02]; $p = 0.058$) for these agents, respectively, in the *RAS* WT population. Post-study treatment in the *RAS* WT population included EGFR-targeted mAbs in 22 and 37 % of patients and anti-VEGF therapy in 40 versus 33 % of patients in the panitumumab and bevacizumab arms, respectively,

suggesting that second-line treatment was unlikely to be driving differences in outcome.

In PEAK, ORRs in the *RAS* WT group were 64 % [95 % CI 53–74] versus 60 % [95 % CI 49–71] for panitumumab + mFOLFOX6 versus bevacizumab + mFOLFOX6, respectively. ORR results for patients with *KRAS* exon 2 WT/other *RAS* MT tumours were consistent with those reported for the *KRAS* exon 2 WT population.

2.3 Phase II Data—The PLANET Trial

PLANET (NCT00885885) was a phase II, open-label, randomised, parallel-group study including patients with *KRAS* exon 2 WT mCRC and unresectable liver-limited metastases, who had recurrence after prior adjuvant and/or surgical treatment [33]. Patients were randomised to receive preoperative panitumumab (6 mg/kg Q2W) with either FOLFOX4 or FOLFIRI for 4–8 cycles. Patients with stable disease or who remained unresectable received additional cycles until disease progression (PD) or

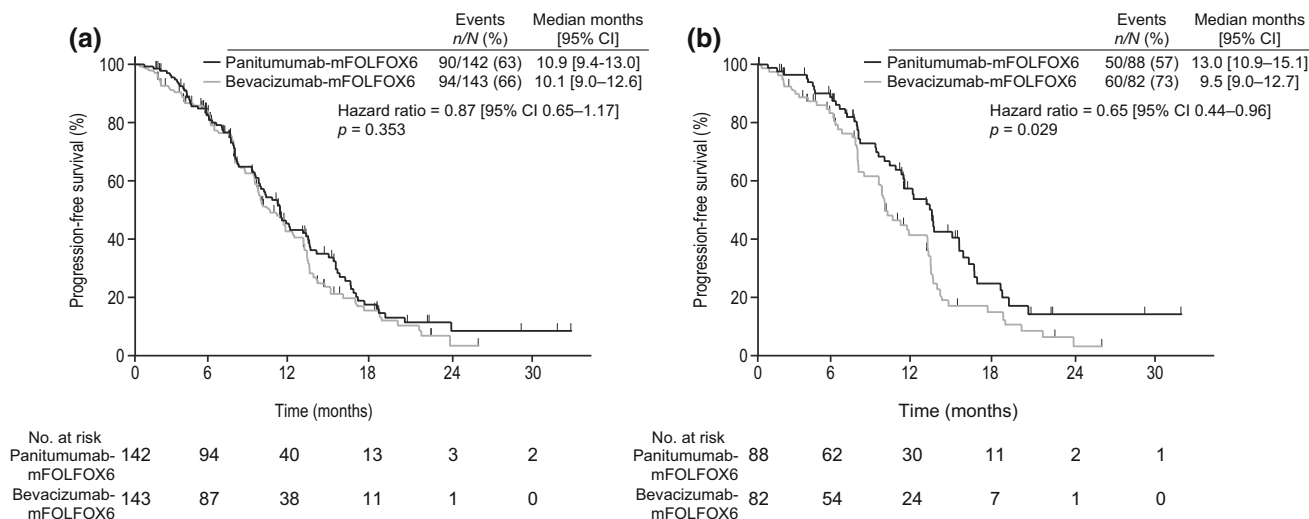


Fig. 4 PEAK: progression-free survival in the **a** Wild-type *KRAS* exon 2 population (primary analysis, data cut-off 30 May 2012); **b** Wild-type *RAS* population (prespecified analysis, data cut-off Jan 3 2013) [31]. From Schwartzberg et al. [31]. Reprinted with permission

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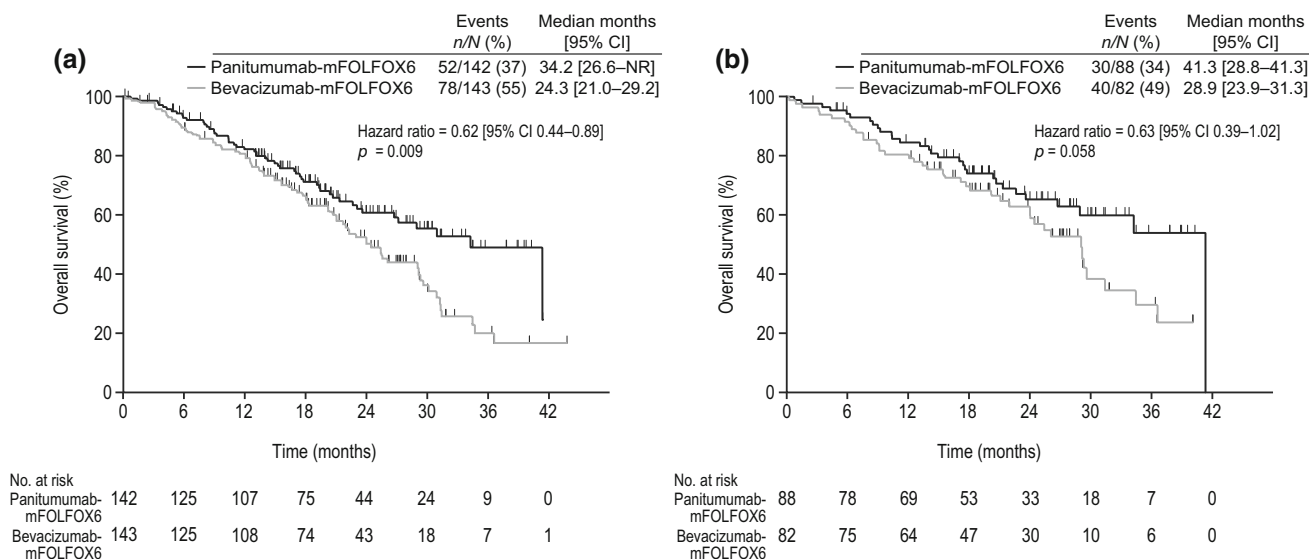


Fig. 5 PEAK: overall survival in the **a** Wild-type *KRAS* exon 2 population; **b** Wild-type *RAS* population (primary analysis, data cut-off 3 January 2013) [31]. From Schwartzberg et al. [31]. Reprinted

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unacceptable toxicity. Six cycles of adjuvant treatment were also administered after surgery. The impact of *RAS* mutations in exons 2, 3 and 4 of *KRAS* and *NRAS* on treatment efficacy was also determined in an exploratory analysis of this study.

2.3.1 Efficacy in the *KRAS* Exon 2 WT Population

Overall, 77 patients with *KRAS* exon 2 WT mCRC were randomised and comprised the primary analysis set in

PLANET [33]. Efficacy results were generally similar between treatments. Overall, the ORR was 70 % and was 74 % for panitumumab + FOLFOX4 versus 67 % for panitumumab + FOLFIRI. Liver resection was reported in 52 % of patients (45 % for panitumumab + FOLFOX4 vs 59 % for panitumumab + FOLFIRI) after preoperative treatment. Median PFS was 12.6 months for both treatments ($p = 0.943$) and median OS was 32.5 versus 42.4 months for patients receiving panitumumab + FOLFOX4 versus panitumumab + FOLFIRI, respectively ($p = 0.848$).

2.3.2 Impact of RAS Mutations Beyond KRAS Exon 2

RAS status was determined for 64 patients (83 %) in PLANET, of whom 53 (83 %) were found to have RAS WT and 11 (17 %) were found to have RAS MT mCRC [33]. Overall, in RAS WT patients, the ORR increased to 76 % and was 78 % for panitumumab + FOLFOX4 versus 73 % for panitumumab + FOLFIRI. In RAS MT patients, the ORR was 55 % overall and was 50 % versus 57 % for panitumumab + FOLFOX4 versus panitumumab + FOLFIRI. In the RAS WT population, median PFS was 12.8 versus 14.8 months ($p = 0.621$) and median OS was 39.0 versus 45.8 months ($p = 0.935$) for panitumumab + FOLFOX4 versus panitumumab + FOLFIRI groups, respectively.

2.4 Phase II Data—The 314 Trial

The 314 trial (NCT00508404) was a phase II, single-arm study in which panitumumab (6 mg/kg Q2W) + FOLFIRI was administered to patients with previously untreated mCRC [34]. After the importance of KRAS as a biomarker was demonstrated in patients receiving anti-EGFR therapies [5, 13], the protocol of this study was amended to enable prospective evaluation of outcomes by tumour KRAS exon 2 status. Subsequently, an exploratory analysis of panitumumab + FOLFIRI activity by tumour RAS/BRAF status was performed using tumour samples from participating patients with known KRAS exon 2 WT status. In this analysis, mutations were assessed in NRAS exon 2 (codons 12 and 13), KRAS and NRAS exons 3 (codons 59 and 61) and 4 (codons 117 and 146) and BRAF exon 15 (codon 600) using bidirectional Sanger sequencing.

2.4.1 Impact of KRAS Exon 2 Status

Overall, 145/154 (94 %) patients included in the study had KRAS evaluable samples, of whom 86 (59 %) had KRAS exon 2 WT tumours and 59 (41 %) had KRAS exon 2 MT tumours [34]. A higher proportion of patients in the KRAS exon 2 WT group (56 % [95 % CI 45–67]) than the MT group (38 % [95 % CI 26–52]) had an objective response (difference 18 % [95 % CI 1–35]; odds ratio [OR] 2.1 [95 % CI 1.0–4.4]). Median duration of response (DoR; 13.0 vs 7.4 months; HR 0.3 [95 % CI 0.1–0.6]), PFS (8.9 vs 7.2 months; HR 0.5 [95 % CI 0.3–0.7]) and time to progression (TTP; 11.2 vs 7.3 months; HR 0.4 [95 % CI 0.3–0.6]) also favoured the KRAS exon 2 WT versus MT group. Seven patients (8 %) in the KRAS exon 2 WT group had a complete resection, compared with three (5 %) in the KRAS MT group. Most patients undergoing a complete resection had a complete resection of liver metastases (KRAS exon 2 WT: $n = 6$ vs MT: $n = 1$).

2.4.2 Impact of RAS/BRAF Mutations on Activity

RAS/BRAF status was retrospectively determined in 143/154 (93 %) patients from the 314 study. Of these, 69 patients (48 %) had RAS WT and 74 (52 %) had RAS MT tumours; 60 patients (42 %) had RAS WT/BRAF WT tumours, and 83 (58 %) had RAS MT or BRAF MT tumours [35].

The ORR was higher in patients with RAS WT versus RAS MT mCRC (59 vs 41 %; OR 2.0 [95 % CI 1.0–4.2]). Longer median DoR (13.0 vs 5.8 months; HR 0.16 [95 % CI 0.07–0.37]) and PFS (11.2 vs 7.3 months; HR 0.37 [95 % CI 0.24–0.58]) and numerically longer TTP (13.2 vs 7.3 months) were also observed in the RAS WT versus MT groups. Patients with RAS WT/BRAF WT versus RAS MT or BRAF MT status also had improved ORR (68 vs 37 %; OR 3.6 [95 % CI 1.7–7.9]), median DoR (13.0 vs 5.8 months; HR 0.16 [95 % CI 0.07–0.37]) and PFS (13.2 vs 6.9 months; HR 0.25 [95 % CI 0.15–0.41]) and numerically longer TTP (13.3 vs 7.2 months). Resection rates were 13 versus 9 % in RAS WT versus RAS MT populations and 15 versus 8 % for RAS WT/BRAF WT versus RAS MT or BRAF MT populations, respectively.

3 Second-Line Setting

There has been one large second-line panitumumab trial, conducted in an unselected patient population ($n = 1186$); RAS data were available for 1014 patients (85 %). There has also been a smaller ($n = 182$) second-line trial conducted in patients with KRAS exon 2 WT mCRC; however, RAS analyses are not available from this study.

3.1 Phase III Data—The 181 Trial

20050181 (NCT00339183) was an open-label, phase III, randomised (1:1) study comparing panitumumab 6 mg/kg Q2W + FOLFIRI versus FOLFIRI alone as second-line treatments for patients who had progressed on one prior fluoropyrimidine-based mCRC therapy [11]. As the impact of KRAS mutations on EGFR inhibitor therapy became apparent, the study protocol was amended to permit data to be prospectively analysed by tumour KRAS exon 2 status.

Two clinical snapshots were reported for the KRAS exon 2 WT population in this study. The primary analyses of PFS and OS were conducted after 381 PFS events had occurred and after 407 deaths, respectively [11]. A further prespecified final analysis of PFS and OS was conducted ~30 months after the last patient enrolled [36].

Mutations were subsequently assessed in NRAS exon 2 (codons 12 and 13), KRAS and NRAS exons 3 (codons 59 and 61) and 4 (codons 117 and 146) and BRAF exon 15

Table 3 Impact of *KRAS* exon 2 [36] and *RAS* [14, 30] status on outcomes in the 181 trial (primary analysis data)

	<i>KRAS</i> exon 2 WT [11]		<i>RAS</i> WT ^a [14, 30]	
	Panitumumab + FOLFIRI (n = 303)	FOLFIRI (n = 294)	Panitumumab + FOLFIRI (n = 208)	FOLFIRI (n = 213)
Median PFS, months	5.9	3.9	6.4	4.6
HR [95 % CI]; p value	0.73 [0.59–0.90]; 0.004		0.70 [0.54–0.91]; 0.007	
Median OS, months	14.5	12.5	16.2	13.9
HR [95 % CI]; p value	0.85 [0.70–1.04]; 0.12		0.81 [0.63–1.03]; 0.08	
ORR, ^c % [95 % CI]	35 [30–41]	10 [7–14]	41 [34–48]	10 [6–15]
OR [95 % CI]; p value	NA [NA]; 0.001		NA	
	<i>KRAS</i> exon 2 MT [11]		<i>RAS</i> MT ^b [14]	
	Panitumumab + FOLFOX4 (n = 238)	FOLFOX4 (n = 248)	Panitumumab + FOLFOX4 (n = 299)	FOLFOX4 (n = 294)
Median PFS, months	5.0	4.9	4.8	4.0
HR [95 % CI]; p value	0.85 [0.68–1.06]; 0.14		0.86 [0.71–1.05]; 0.14	
Median OS, months	11.8	11.1	11.8	11.1
HR [95 % CI]; p value	0.94 [0.76–1.15]; ND		0.91 [0.76–1.10]; 0.34	
ORR, ^c % [95 % CI]	13 [9–18]	14 [10–19]	NA	NA
OR [95 % CI]; p value	NA [NA]; 1.0		NA	

CI confidence interval, HR hazard ratio, MT mutant, NA not available, ND not done, OR odds ratio, ORR objective response rate, OS overall survival, PFS progression-free survival, WT wild type

^a Wild type at *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146)

^b Mutations at any of *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146). Of 597 patients with *KRAS* exon 2 WT tumours and *RAS* data, 107 (18 %) had other *RAS* mutations (*RAS* status determined using bidirectional Sanger sequencing and WAVE-based Surveyor[®] Scan Kits (Transgenomic))

^c Included only patients with baseline measurable disease per central review

(codon 600). Results of this prospective–retrospective analysis have recently been reported [14].

3.1.1 Efficacy in the *KRAS* Exon 2 WT Population

Overall, 1083/1186 patients (91 %) in the 181 study had tumour *KRAS* data available, of whom 597 patients (55 %) had *KRAS* WT tumours [11]. In the primary analysis, a statistically significant PFS benefit was observed in the panitumumab + FOLFIRI versus FOLFIRI group (Table 3). There was no significant difference in OS between panitumumab + FOLFIRI versus FOLFIRI groups, however, significantly more patients receiving panitumumab + FOLFIRI versus FOLFIRI alone had an objective response (Table 3).

Results of the final analysis were consistent with the primary analysis. The addition of panitumumab to FOLFIRI significantly improved PFS (median 6.7 vs 4.9 months; HR 0.82 [95 % CI 0.69–0.97]; $p = 0.023$) and ORR (35 vs 10 %; OR 5.5 [95 % CI 3.32–8.87]; $p < 0.0001$) but not OS (median 14.5 vs 12.5 months; HR 0.92 [0.78–1.10]; $p = 0.37$) in patients with *KRAS* exon 2 WT mCRC [36].

3.1.2 Efficacy in the *RAS* WT Population

Tumour *RAS/BRAF* status was determined for 1014/1186 patients (85 %) in the 181 study; 107/597 *KRAS* exon 2 WT patients (18 %) were found to harbour additional *RAS* mutations. *BRAF* mutations were found in 45/541 patients (8 %) [14].

In the *RAS* WT population ($n = 421$), patients receiving panitumumab + FOLFIRI versus FOLFIRI had significantly improved PFS and a trend towards improved OS (Table 3). Analysis of prespecified subgroups showed that the treatment benefit of panitumumab was generally consistent across subgroups, although the HR did not favour panitumumab in patients who had longer time (>6 months) to PD. *RAS* WT patients receiving panitumumab + FOLFIRI had significantly longer PFS if they were aged <65 years (HR 0.64 [95 % CI 0.45–0.89]; $p = 0.008$), had an ECOG performance status of 0 or 1 (HR 0.68 [95 % CI 0.52–0.88]; $p = 0.0042$) or had *BRAF* WT mCRC (HR 0.68 [95 % CI 0.51–0.90]; $p = 0.0063$) [37]. Those who had received prior oxaliplatin (HR 0.64 [95 % CI 0.47–0.86]; $p = 0.0035$) or no prior bevacizumab (HR 0.72 [95 % CI 0.54–0.95]; $p = 0.0204$) also showed

significant PFS benefits on addition of panitumumab to FOLFIRI. Overall, patients with *BRAF* mutations had worse prognosis, irrespective of treatment arm. Finally, more *RAS* WT patients in the panitumumab + FOLFIRI versus FOLFIRI group achieved tumour shrinkage $\geq 30\%$ at week 8 (37 vs 7 %; difference: 30 % [95 % CI 22–38]) and the ORR was also higher in this group (41 % [95 % CI 34–48] vs 10 % [95 % CI 6–15]; difference 31 % [95 % CI 23–38]) [30].

Patients with *RAS* MT tumours had similar PFS and OS in the panitumumab + FOLFIRI versus FOLFIRI groups (Table 3). *BRAF* mutations were associated with numerically worse PFS (median 2.5 vs 1.8 months; HR 0.69 [95 % CI 0.32–1.49]; $p = 0.34$) and OS (median 4.7 vs 5.7 months; HR 0.64 [95 % CI 0.32–1.28]; $p = 0.20$) in patients with *RAS* WT tumours, irrespective of treatment received [14].

3.2 Phase II Data—The SPIRITT trial

SPIRITT (NCT00418938) was a randomised, open-label, phase II study of panitumumab 6 mg/kg Q2W + FOLFIRI versus bevacizumab 5 or 10 mg/kg Q2W + FOLFIRI as second-line treatments for patients with *KRAS* exon 2 WT mCRC previously treated with first-line bevacizumab + oxaliplatin-based therapy. No prior irinotecan or anti-EGFR therapy was permitted [38].

3.2.1 Efficacy in the *KRAS* Exon 2 WT Population

One hundred and eighty-two patients with *KRAS* exon 2 WT mCRC were included in the initial analysis ($n = 91$ in each treatment arm). Median PFS (primary endpoint: 7.7 vs 9.2 months; HR 1.01 [95 % CI 0.68–1.50]) and OS (18.0 vs 21.4 months; HR 1.06 [95 % CI 0.75–1.49]) did not differ significantly between panitumumab + FOLFIRI and bevacizumab + FOLFIRI groups [38]. Objective responses were reported in 32 % [95 % CI 23–43] versus 19 % [95 % CI 11–29] of patients in the panitumumab versus bevacizumab groups, respectively. Currently, no *RAS* data have been reported from this study.

4 Third-Line and Beyond

RAS data have been reported from one large panitumumab trial conducted in an unselected mCRC population ($n = 463$) who had PD following at least two chemotherapy regimens. *RAS* data were available for 288 patients (62 %). A second large trial (ASPECCT) compared panitumumab and cetuximab specifically in patients ($n = 999$) with *KRAS* exon 2 WT mCRC. *RAS* analyses are not yet available from this study.

4.1 Phase III Data—The 408 Trial

The 20020408 trial (NCT00113763) was a randomised, open-label, phase III study of panitumumab 6 mg/kg Q2W + BSC versus BSC alone as treatment for patients with EGFR-expressing mCRC after failure of fluoropyrimidines and oxaliplatin- and irinotecan-containing regimens [39]. To ensure adequate exposure to prior chemotherapy, average dose intensity of irinotecan (≥ 65 mg/m² per week) and of oxaliplatin (≥ 30 mg/m² per week) were required. All but one patient in this study had received at least two prior chemotherapy lines; 37 % had received three prior lines. This study also incorporated an optional open-label extension study in which BSC patients experiencing PD in the pivotal phase III study could cross over to receive panitumumab monotherapy [40].

A prospective–retrospective analysis from the 408 study [13] first tested the clinical hypothesis that a larger treatment effect of EGFR-targeted mAbs would be observed in patients with *KRAS* exon 2 WT tumours compared with patients with *KRAS* exon 2 MT tumours. An exploratory analysis was also subsequently performed in patients with *KRAS* exon 2 WT tumours to assess the impact of mutations in *NRAS* exon 2 (codons 12 and 13), and *KRAS* and *NRAS* exon 3 (codon 61), using massively parallel sequencing [15].

4.1.1 Impact of *KRAS* Exon 2 Status

Of the 231 patients included in the panitumumab + BSC arm, 208 (90 %) had biomarker data; of these, 124 (60 %) had *KRAS* exon 2 WT mCRC [13]. Of the 232 patients included in the BSC arm, 219 (94 %) had biomarker data; of these, 119 (54 %) had *KRAS* exon 2 WT mCRC. Overall, 176 BSC patients subsequently received panitumumab in the crossover study. Biomarker data were available for 167 (95 %) of the patients who underwent crossover; 90 (54 %) had *KRAS* WT mCRC.

In the pivotal phase III study, the treatment effect on PFS was significantly greater in patients with *KRAS* exon 2 WT tumours than in patients with *KRAS* exon 2 MT tumours [13]. Median PFS was significantly longer for panitumumab + BSC versus BSC alone (median 12.3 weeks vs 7.3 weeks; HR 0.45 [95 % CI 0.34–0.59]; $p < 0.0001$) and ORRs were 17 % [95 % CI 11–25 %] versus 0 %, respectively. Median PFS was similar between treatments for patients with *KRAS* exon 2 MT tumours (median 7.4 vs 7.3 weeks; HR 0.99 [95 % CI 0.73–1.36]). PFS was also significantly longer in patients with *KRAS* exon 2 WT versus MT tumours receiving panitumumab treatment in the crossover study (median 16.4 vs 7.9 weeks; HR 0.32 [95 % CI 0.22–0.45]).

OS was similar between treatments among patients with *KRAS* exon 2 WT (HR 0.99 [95 % CI 0.75–1.29]) or *KRAS* exon 2 MT tumours (HR 1.02 [95 % CI 0.75–1.39]). However, OS analysis was confounded by the high proportion of patients who crossed over from the BSC arm to receive panitumumab therapy following PD.

4.1.2 Impact of RAS Mutations on Efficacy

Overall, 320 archival tumour samples were available from the 463 patients originally included in the 408 study, 288 of whom (90 %) provided RAS data [15]. In an exploratory analysis, panitumumab was associated with longer PFS versus BSC in patients with *KRAS* WT mCRC (codons 12, 13 and 61 assessed; HR 0.39 [95 % CI 0.28–0.56]) [15]. Among *KRAS* WT ($n = 153$) patients, a treatment effect for PFS favouring panitumumab occurred in patients with *NRAS* WT mCRC ($n = 138$; HR 0.39 [95 % CI 0.27–0.56]) and *BRAF* WT mCRC ($n = 115$; HR 0.37 [95 % CI 0.24–0.55]), but not in patients with *NRAS* mutations ($n = 11$; HR 1.94 [95 % CI 0.44–8.44]).

4.2 Phase III Data—The ASPECCT Trial

ASPECCT (NCT01001377) was a randomised, open-label, phase III non-inferiority study of panitumumab (6.0 mg/kg Q2W) versus cetuximab (initial dose of 400 mg/m², followed by 250 mg/m² once a week [QW]) in patients with *KRAS* exon 2 WT, chemorefractory mCRC [41]. Patients must have failed prior regimens containing both irinotecan and oxaliplatin for metastatic disease and have previously received a thymidylate synthase inhibitor (fluorouracil, capecitabine, raltitrexed, or fluorouracil-uracil). Patients in the cetuximab group received premedication with an H1 antagonist before infusion; premedication for infusion reactions was not required in the panitumumab group.

4.2.1 Efficacy in the *KRAS* Exon 2 WT Population

At the time of primary analysis ($n = 999$), 77 % (383/499) versus 78 % (392/500) of patients in the panitumumab and cetuximab arms had died and 477 patients in each group had progressed or died [41]. Median PFS was similar between panitumumab and cetuximab arms (4.1 vs 4.4 months, respectively; HR 1.00 [95 % CI 0.88–1.14]). For the primary endpoint of OS, panitumumab was found to be non-inferior to cetuximab (Z-score: -3.19 ; $p = 0.0007$; median OS 10.4 vs 10.0 months, respectively; HR 0.97 [95 % CI 0.84–1.11]). Similar proportions of patients also experienced an objective response (22 % [95 % CI 18–26] vs 20 % [95 % CI 16–24] for panitumumab and cetuximab groups, respectively; OR 1.15

[95 % CI 0.83–1.58]). No RAS data have been reported from this study.

5 Safety and Tolerability

Across the *KRAS* exon 2 WT patients included in these studies, treatment-related grade 3/4 adverse events (AEs) occurred in 82 % of patients receiving panitumumab + FOLFOX4 [10], 68–78 % of patients receiving panitumumab + FOLFIRI [11, 38] and 25 % of those receiving panitumumab + BSC [13]. The most common grade 3/4 AEs of interest (AEs known to be associated with EGFR-targeted mAbs and/or relevant chemotherapy regimen) were integument toxicity (36 % in patients receiving panitumumab + FOLFIRI [34], 25 % in patients receiving panitumumab + BSC [13]), skin toxicity (32–36 % in patients receiving panitumumab + oxaliplatin-based regimens [10, 31], 31–37 % in patients receiving panitumumab + FOLFIRI [11, 34]) and diarrhoea (18 % in patients receiving panitumumab + FOLFOX4 [10], 14–24 % in patients receiving panitumumab + FOLFIRI [11, 34], 2 % in patients receiving panitumumab + BSC [13]). Discontinuations due to AEs occurred in 24 % of patients receiving panitumumab + mFOLFOX6 [31], 29 % of patients receiving panitumumab + FOLFIRI [38] and 7 % of those receiving panitumumab + BSC [13]. In ASPECCT, AE profiles were generally similar for the two EGFR-targeted mAbs. However, despite the use of premedication in the cetuximab group, there was a numeric difference in the incidence of grade 3/4 infusion reactions for the panitumumab versus cetuximab group (<0.5 vs 2 %, respectively). In contrast, grade 3/4 hypomagnesaemia was numerically more frequent in the panitumumab versus cetuximab arm (7 vs 3 %) [41].

In general, the type, incidence and severity of AEs and overall safety profile of panitumumab were similar in patients with RAS WT tumours [12, 31, 35, 42] to those previously reported for those with *KRAS* exon 2 WT tumours receiving panitumumab treatment [10, 13, 31, 34]. No new safety signals were identified in these extended RAS analyses. Safety data specifically in the RAS WT population have not yet been reported in the PLANET and 181 studies. Selection of patients according to tumour RAS status does not, therefore, appear to be associated with an increase in EGFR-related toxicities.

6 Discussion

Preclinical and clinical studies of panitumumab support the concept that hypothesis-generating molecular analyses can be used to develop retrospective studies that inform

prospective trials on the use of tumour biomarkers, to predict response to targeted anti-cancer treatment. The 'RAS story' represents a breakthrough in personalised medicine for patients with mCRC, and illustrates well how molecular selection can be developed to define patient populations for targeted oncology treatment. To date, six key panitumumab trials including a total of 3348 patients have reported extended RAS analyses, with RAS data currently available from 2802 patients (84 %), of whom 1378 (49 %) had RAS WT tumours. In the trials in which collection of tumour samples was mandatory, RAS ascertainment rates were ~90 % [12, 14, 31].

In the first- [10, 12, 31, 33], second- [11, 14, 36] and third-line [13, 15, 42] settings overall, the efficacy benefits of panitumumab treatment were generally more pronounced in RAS WT patients than in the corresponding KRAS exon 2 WT populations, demonstrating the benefit of further refining the target patient population for this agent. Notably, median OS for RAS WT patients in the panitumumab + mFOLFOX6 group from PEAK was 41.3 months [31]; however, patient numbers and the number of deaths at last analysis were relatively low in this phase II study.

The impact of RAS mutations in KRAS exon 3 and 4 and NRAS exons 2, 3 and 4 appears to be similar to that of KRAS exon 2 mutations, with all mutations being predictive of a lack of response to panitumumab therapy. The impact of tumour BRAF mutations was also assessed in retrospective and prospective-retrospective analyses from some of these panitumumab studies, with data generally suggesting that these mutations are indicative of poor prognosis, irrespective of treatment received [12, 35]. No new safety signals were noted for the RAS WT populations [12, 31, 35, 42], with the overall safety profile being similar to that observed in patients with KRAS WT tumours [10, 13, 31, 34]. Safety profiles in all of these studies were as expected based on the mechanisms of action and previously reported data for the study treatments. In general, these studies have consistently reported negative outcomes for patients with RAS MT tumours receiving an EGFR-targeted mAb combined with oxaliplatin-containing chemotherapy [10, 12, 31, 43]. Retrospective data from clinical trials of cetuximab generally appear to support these observations [22, 23].

Efficacy benefits have been noted on addition of cetuximab to FOLFOX4 in RAS WT patients from the first-line OPUS trial (RAS ascertainment rate: 66 % [n = 118/179]) [22] and on addition of cetuximab to FOLFIRI in the corresponding patient population in the first-line CRYSTAL trial (RAS ascertainment rate: 65 % [n = 430/666]) [23]. These data confirm the impact of tumour RAS status on efficacy of all EGFR-targeted mAbs in patients with mCRC. The efficacy and safety of these

two EGFR-targeted mAbs has recently been compared in ASPECCT, the first head-to-head monotherapy study of Q2W panitumumab versus QW cetuximab in patients with KRAS exon 2 WT, chemorefractory mCRC [41]. In the primary analysis, panitumumab was non-inferior to cetuximab for OS in the KRAS exon 2 WT population; no RAS analyses have been performed to date. Safety was similar for these two agents with the exception of grade 3/4 infusion reactions (less common with panitumumab: <0.5 vs 2 %, respectively) and grade 3/4 hypomagnesaemia (less common with cetuximab 7 vs 3 %) [41]. Because of the key role of EGFR signalling in skin, skin toxicity is a common side effect of all EGFR-targeted therapies [44]. Proactive treatment with skin moisturizers, sunscreen, topical steroid and/or doxycycline is recommended to help limit the impact of these AEs on quality of life. In a recent panitumumab study comparing pre-emptive or reactive treatment (after skin toxicity developed) of skin toxicity, pre-emptive treatment reduced the incidence of grade 2 + skin toxicities by more than 50 % and patients in this group experienced less quality-of-life impairment compared with those receiving reactive treatment [45].

There are currently no studies investigating optimal treatment sequence of biologics in mCRC; nonetheless, head-to-head trials support the use of EGFR-targeted agents as first-line therapy, as they suggest an OS benefit for these agents relative to anti-VEGF therapy in patients with KRAS exon 2 WT mCRC, with patients with RAS WT tumours even more likely to benefit from anti-EGFR therapy [31, 46, 47]. For example, in the phase III FIRE-3 trial, improved OS was seen with cetuximab + FOLFIRI versus bevacizumab + FOLFIRI in both KRAS exon 2 WT (n = 592; 28.7 vs 25.0 months, HR 0.77 [95 % CI 0.62–0.96]; p = 0.017) and RAS WT patients (n = 342; 33.1 vs 25.6 months, HR 0.70 [95 % CI 0.53–0.92]; p = 0.011) [46]. However, ORR was the primary endpoint in this study and no differences were found between treatments for this measure in either the KRAS exon 2 WT (62 % [95 % CI 56–68] vs 58 % [95 % CI 52–64]; OR 1.18 [95 % CI 0.85–1.64]; p = 0.18) or RAS WT (66 % [95 % CI 58–73] vs 60 % [95 % CI 52–67]; OR 1.28 [95 % CI 0.83–1.99]; p = 0.32) intent-to-treat populations. However, in a predefined per-protocol analysis (n = 526), ORR was higher in the cetuximab versus bevacizumab arms (72 vs 63 %, respectively; p = 0.017) [46]. Furthermore, in a subsequent analysis in which response data were independently, centrally reviewed, significant benefits in favour of the cetuximab + FOLFIRI versus bevacizumab + FOLFIRI group were noted. In this analysis, ORRs were 67 % [95 % CI 60–73] versus 54 % [95 % CI 48–61] in the KRAS exon 2 WT population, respectively (OR 1.68; p = 0.0076) and 71 % [95 % CI 63–79] versus 56 % [95 % CI 48–65] in the RAS WT

population (OR 1.93; $p = 0.015$), respectively [47]. The *RAS* analyses from FIRE-3 included 407 of the 592 patients (69 %) with *KRAS* exon 2 WT tumours, who had tumour tissue available [46].

Preliminary results from the randomised, phase III Cancer and Leukaemia Group B (CALGB) 80405 trial comparing first-line bevacizumab or cetuximab combined with chemotherapy in 1137 patients with mCRC have also recently been reported [48]. In this study, 73 % of patients overall received mFOLFOX6 and 27 % received FOLFIRI as their chemotherapy backbone. Although this trial enrolled patients over a 10-year period, only 24-month follow-up data for the 11th interim analysis have so far been presented. After 24 months' follow up, PFS (10.8 vs 10.4 months; HR 1.04 [95 % CI 0.91–1.17]; $p = 0.55$) and OS (29.0 vs 29.9 months; HR 0.92 [95 % CI 0.78–1.09]; $p = 0.34$) were similar in the bevacizumab and cetuximab arms, respectively. Median OS was numerically higher specifically in cetuximab patients receiving the mFOLFOX6 backbone; median OS was 26.9 versus 30.1 months in the bevacizumab versus cetuximab groups, respectively (HR 0.9 [95 % CI 0.7–1.0]; $p = 0.09$). Overall, 27 % of those receiving bevacizumab versus 32 % of those receiving cetuximab discontinued treatment due to PD. The proportions withdrawing from treatment due to an AE were also similar (56 vs 55 %, respectively). This study included patients with *KRAS* exon 2 WT tumours, and data from a retrospective analysis of outcomes according to *RAS* status have recently been reported. In this analysis, median OS was 31.2 versus 32.0 months for *RAS* WT patients receiving bevacizumab or cetuximab combined with chemotherapy, respectively (HR 0.9 [95 % CI 0.7–1.1]; $p = 0.40$) [49].

Retrospective analyses assessing the impact of post-PD therapy on efficacy can also give insights into optimal treatment sequencing. In an analysis from the PRIME trial, *RAS* WT patients receiving first-line panitumumab + FOLFOX4 followed by anti-VEGF therapy had the longest median OS. This sequence was associated with a median OS of 40 months versus 36 months for FOLFOX4 alone followed by anti-VEGF treatment (HR 0.64 [95 % CI 0.41–1.00]; $p = 0.0494$) and an OS of 26 months for panitumumab + FOLFOX4 versus 21 months for FOLFOX4 followed by treatment not containing an anti-VEGF agent (HR 0.69 [95 % CI 0.53–0.92]; $p = 0.0096$) [50]. Similarly, in PEAK, median OS was numerically longer for *RAS* WT patients receiving first-line therapy with panitumumab + mFOLFOX6 (41.3 months, 40 % received subsequent anti-VEGF therapy) than in those who received bevacizumab + mFOLFOX6 (28.9 months, 38 % subsequently received an EGFR-targeted mAb; HR 0.63 [95 % CI 0.39–1.02]; $p = 0.058$) [31].

Given the importance of tumour *RAS* testing, particularly for individuals in whom oxaliplatin-based chemotherapy is being planned, it is essential that *RAS* testing is of a sufficiently high quality to give robust results. The summary of product characteristics for panitumumab acknowledge this point and state that “Mutational status should be determined by an experienced laboratory using validated test methods for detection of *KRAS* (exons 2, 3 and 4) and *NRAS* (exons 2, 3 and 4) mutations”. External quality assurance (EQA) programmes have a critical role in ensuring that *RAS* testing is carried out to a high standard [51]. For example, the European Society of Pathology EQA programme has provided recommendations and an overview of validated laboratory methods, standardised operating procedures, and accreditation criteria relevant for *RAS* mutation testing [52, 53]. The cost of incorporating tumour *RAS* testing into clinical practice is important to consider. However, these upfront costs are likely to be offset by subsequent savings in patient care (e.g., by avoiding unnecessary treatment and hospital stays in patients unlikely to respond), as was seen with the introduction of *KRAS* [54–58] and also *BRAF* [59] testing.

Although *RAS* WT status is clearly important for activity of EGFR-targeted mAbs, a proportion of patients with *RAS* WT tumours still do not respond to these agents, suggesting that other mutations/alterations may also impact on their activity. The PI3K pathway is partly modulated by *KRAS* activation during EGFR signalling and alterations in this pathway, such as *PI3KCA* mutations and loss of PTEN expression/activity, may also lead to lack of response to EGFR-targeted mAbs [1, 60–64]. Furthermore, other studies have suggested that overexpression of EGFR ligands such as amphiregulin and epiregulin may predict response to cetuximab [65–67]. However, these potential biomarkers need to be evaluated/validated in properly designed studies to assess their potential usefulness in clinical practice.

To enable biomarker analysis to continue to optimise the treatment of patients with mCRC, collection of tumour samples should be recommended for all future mCRC trials. If biomarker analyses are contemplated, then banked tumour samples may be used to perform prospective analyses prior to the primary trial analysis, potentially providing more robust data. Ideally, sufficient sample should be taken to allow biomarker analyses to continue for several years after initial trial results are published, as new biomarkers are continually being proposed that require validation in a clinical trial setting.

Currently, biopsy in solid tumours has several disadvantages, including its invasive nature and the limitation that it provides a single ‘snapshot’ of the tumour before treatment with biologics. In the future, liquid

biopsies of circulating plasma DNA may allow for a less invasive approach to assessing biomarkers in mCRC patients: mutations detected in circulating plasma DNA show good concordance with tumour mutations, particularly when samples are paired [68–70]. When combined with sensitive molecular testing, this technique has the potential to improve patient selection and aid the early detection of PD and the emergence of resistance. For example, *KRAS* mutations have been detected in the sera of previously *KRAS* WT patients during treatment with panitumumab [71] or cetuximab [70], in some cases up to 10 months ahead of radiographic progression [70]. Data suggest that EGFR-targeted mAb treatment causes amplification of rare, pre-existing cells harbouring these mutations in ostensibly *KRAS* WT tumours [71]. Recently, liquid biopsies from patients in the ASPECCT study detected EGFR S492R mutations, which appear to confer resistance to cetuximab but not panitumumab, emerging in 16 % versus 1 % of patients during cetuximab versus panitumumab treatment, respectively [72], supporting the idea that this technique may be particularly useful for assessing biomarkers of resistance and progression. Nonetheless, further clinical studies are required to progress and refine this approach to molecular monitoring.

Most of the current evidence regarding the use of *RAS* as a biomarker comes from retrospective or prospective-retrospective analyses, using banked samples from completed phase III trials. Debate continues around how much evidence is sufficient when assessing a potential new biomarker using retrospective analyses. Nonetheless, with consistent observations noted in all of these analyses, the weight of evidence from the studies reviewed here suggests that *RAS* mutations are a robust biomarker for a lack of EGFR-targeted mAb activity. In future studies, there may be sufficient supporting evidence to prospectively test biomarker hypotheses by randomising patients based on their biomarker status to further define the optimum patient population for EGFR-targeted mAbs. Future studies will likely prospectively evaluate the use of these agents specifically in *RAS* WT patients, while others will exploit the improved molecular understanding of processes underpinning tumour growth and development to assess new targeted combination treatments. Biomarker research in mCRC continues to rapidly evolve and promises to further optimise outcomes via better patient selection and improved tailoring of anti-cancer treatment to the molecular profile of individual patients' tumours.

In conclusion, data across first- to third-line therapy consistently demonstrate that by testing tumour *RAS* status, it is possible to select patients more likely to benefit from panitumumab therapy. Consistent effects have also been noted in trials that have reported *RAS* data for cetuximab in patients with mCRC. EGFR-targeted mAbs have not

shown a positive risk/benefit ratio in patients whose tumours harbour mutations in any of *KRAS* and *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146); accordingly, their licensed indications in Europe now state that treatment should be confined to patients with *RAS* WT tumours [24, 25]. The risk/benefit profile of panitumumab is thereby improved by restricting treatment to patients whose tumours do not harbour *RAS* mutations. Notably, EGFR-targeted mAbs may even cause harm when combined with oxaliplatin-containing regimens in patients with *RAS* MT tumours, highlighting the importance of *RAS* testing in mCRC. Biomarker studies are ongoing in mCRC and will help to further define the optimum patient population for EGFR-targeted mAbs.

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Ethical Standards All panitumumab studies mentioned in this review article were approved by the appropriate ethics committee and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients included in these studies gave their informed consent prior to their inclusion.

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