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Exploratory pooled analysis evaluating the effect of sequence of biological therapies on overall survival in patients with *RAS* wild-type metastatic colorectal carcinoma

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ABSTRACT

Background The aim of this study was to evaluate the optimal sequence of targeted therapies (epidermal growth factor receptor inhibitors (EGFRi) and vascular endothelial growth factor inhibitors (VEGFi)), combined with chemotherapy, in patients with *RAS* wild-type (WT) metastatic colorectal carcinoma (mCRC). Exploratory analyses of overall survival (OS) for patients treated with either first-line panitumumab (EGFRi) and second-line VEGFi therapy, or first-line bevacizumab (VEGFi) and second-line EGFRi, were conducted.

Methods Patients from PEAK (NCT00819780), PRIME (NCT00364013) and Study 181 (NCT00339183), with *RAS* WT or *RAS* WT/*BRAF* WT tumours, were included in the analyses. OS data were pooled for patients receiving first-line panitumumab (PEAK and PRIME) or first-line bevacizumab (PEAK and 181), followed by second-line VEGFi or EGFRi, respectively.

Results Overall, 104 *RAS* WT patients were included (n=66 panitumumab→VEGFi, n=38 bevacizumab→EGFRi). At the time of final data analysis, 63.6% versus 92.1% of patients in the panitumumab→VEGFi versus bevacizumab→EGFRi arms had died; median OS was 36.8 versus 27.8 months, respectively (HR 0.65; 95% CI 0.42 to 1.03). The OS HR for patients with *RAS* WT/*BRAF* WT mCRC overall was 0.58 (95% CI 0.36 to 0.95) and was 0.56 (95% CI 0.30 to 1.04) in those with left-sided tumours.

Conclusion Although numbers are small, these exploratory analyses suggest a trend towards improved OS for first-line panitumumab plus chemotherapy followed by second-line VEGFi, compared with first-line bevacizumab followed by second-line EGFRi in patients with *RAS* WT and *RAS* WT/*BRAF* WT mCRC. Large prospective randomised trials are needed to further evaluate the optimum sequence of EGFRi/VEGFi in mCRC.

Key questions

What is already known about this subject?

- Two classes of targeted therapy are available for upfront treatment of patients with *RAS* wild-type (WT) metastatic colorectal carcinoma (mCRC): epidermal growth factor receptor inhibitors (EGFRi) and vascular endothelial growth factor inhibitors (VEGFi).
- Results from the available head-to-head studies and meta-analyses generally favour first-line treatment with an EGFRi in patients with *RAS* WT mCRC, with some authors suggesting that the sequence of biological therapies may be an important factor.
- Preclinical studies have suggested that pretreatment with an EGFRi may sensitise tumours to VEGFi therapy, while resistance to a VEGFi may result in simultaneous resistance to EGFRis.

What does this study add?

- Results of the current exploratory pooled analysis suggest a potential survival benefit for patients with *RAS* WT mCRC who receive first-line EGFRi treatment followed by second-line VEGFi therapy, compared with the reverse sequence.
- The observed benefits appear greatest in patients with *RAS* WT/*BRAF* WT mCRC and those with left-sided tumours.

How might this impact on clinical practice?

- These exploratory data provide further evidence supporting upfront EGFRi treatment followed by second-line treatment with a VEGFi in patients with *RAS* WT mCRC.
- The results of prospective trials evaluating optimal treatment sequencing in patients with *RAS* WT mCRC are awaited.

INTRODUCTION

Recent advances in the treatment landscape for metastatic colorectal carcinoma (mCRC) have led to significant improvements in clinical outcomes. In particular, the addition of targeted biological therapies to chemotherapy-based regimens in the first-line setting has increased median overall survival (OS) to 25–30 months for patients with mCRC.[1–5] Current first-line treatment options for these patients include 5-fluorouracil, leucovorin

and oxaliplatin (FOLFOX) or folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) chemotherapy schedules combined with either epidermal growth factor receptor inhibitors (EGFRi: panitumumab and cetuximab) or the vascular endothelial growth factor inhibitor (VEGFi) bevacizumab. The cytotoxic triplet FOLFOXIRI can also be used with VEGFi in selected fit patients.[1 6] While there are no validated predictive molecular biomarkers for bevacizumab, *RAS* mutations (exons 2–4 of *KRAS* and *NRAS*) represent a negative predictive marker for EGFRi therapy efficacy. Patients whose tumours harbour *RAS* mutations are thus unlikely to benefit from EGFRi treatment and, consequently, current guidelines recommend using EGFRi only in patients with *RAS* wild-type (WT) mCRC.[1] In addition, *BRAF* mutations are associated with poor prognosis in mCRC; however, it remains unclear if these mutations also predict response to EGFRi.[1]

With two classes of targeted therapies available for upfront treatment of patients with *RAS* WT mCRC, physicians are challenged with an important decision with respect to assigning the optimal biological agent for first-line therapy. Three prospective, randomised trials, FIRE-3,[3] PEAK (Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours)[4] and CALGB/SWOG 80405,[5] have directly compared EGFRi and VEGFi in combination with chemotherapy (FOLFIRI or FOLFOX), for first-line treatment of patients with *RAS* WT mCRC. The results of these studies, along with those from two meta-analyses,[7 8] generally favour upfront treatment with an EGFRi. It has been proposed that the observed OS improvement with first-line EGFRi therapy may be due to the impact of subsequent non-study therapy; however, current data suggest that this is not the case,[3–5] leading to the hypothesis that the sequence of targeted therapies may be a key factor.[2 9 10]

To further explore whether an optimal treatment sequence of targeted agents in mCRC can be identified, we conducted exploratory pooled analyses comparing OS for patients who received either first-line panitumumab followed by second-line VEGFi therapy, or first-line bevacizumab followed by second-line EGFRis, using data from three prospective randomised panitumumab trials.

METHODS

Data from patients enrolled in the PEAK (NCT00819780),[4] PRIME (Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy: NCT00364013)[11] and 181 (NCT00339183)[12] studies, whose tumours were *RAS* WT (WT for *KRAS* and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61) and exon 4 (codons 117 and 146)) or *RAS* WT/*BRAF* WT (WT at *BRAF* exon 15 (codon 600)), were included in these exploratory analyses. The study designs of these trials have been published previously.[4 11 12] Briefly, PEAK was a phase II study of first-line panitumumab (6 mg/kg every 2 weeks) plus modified FOLFOX (mFOLFOX6) versus bevacizumab (5 mg/kg every 2 weeks) plus mFOLFOX6 in patients who had not received prior treatment for mCRC. The phase III PRIME study compared first-line panitumumab (6 mg/kg every 2 weeks) plus FOLFOX4 versus FOLFOX4 alone in treatment-naïve patients with mCRC. The phase III 181 study compared second-line panitumumab (6 mg/kg every 2 weeks) plus FOLFIRI with FOLFIRI alone in patients with previously treated mCRC. In all three studies, data for non-study therapies (subsequent/second-line (PRIME and PEAK) or prior/first-line (181)) were not prospectively collected but were extracted from study case report forms, where consent was available.

Patient-level OS data were pooled for all patients with *RAS* WT mCRC and all those with *RAS* WT/*BRAF* WT tumours who received first-line panitumumab (PEAK and PRIME studies) followed by second-line VEGFi treatment, as well as first-line bevacizumab (PEAK and 181 studies), followed by second-line treatment with an EGFRi. Patients receiving any other sequence of agents, including those who had received both biological agents (ie, a VEGFi and an EGFRi) but not specifically as first-line and second-line therapies, were excluded. OS was also analysed for patients with left-sided *RAS* WT and *RAS* WT/*BRAF* WT tumours receiving first-line panitumumab followed by second-line VEGFi or first-line bevacizumab followed by second-line EGFRi treatment. Primary tumours located in the caecum to transverse colon were coded as right sided. Tumours located from the splenic flexure to rectum were categorised as left sided.

The Kaplan-Meier method was used to analyse OS (from time of treatment with first-line biologic until death) for each treatment sequence. HRs for OS, and associated 95% CIs, were calculated using a Cox proportional hazards model. All analyses are descriptive.

All patients provided signed informed consent before any study-related procedures were performed. No formal consent was required for these retrospective analyses.

RESULTS

Patients

Overall, 104 patients with *RAS* WT mCRC from these three trials received the specified first-line and second-line treatment sequences of interest and so were included in these exploratory analyses (n=35 from PRIME, n=40 from PEAK, n=29 from 181). Of these patients, 66 were treated with first-line panitumumab followed by second-line VEGFi, while 38 received first-line bevacizumab followed by second-line EGFRi (figure 1). A total of 93 patients with *RAS* WT/*BRAF* WT disease were included, of whom 58 received first-line panitumumab followed by second-line VEGFi, and 35 received first-line bevacizumab followed by second-line EGFRi. In patients with *RAS* WT disease, baseline demographics and disease characteristics were well balanced between patients receiving the two treatment sequences (table 1).

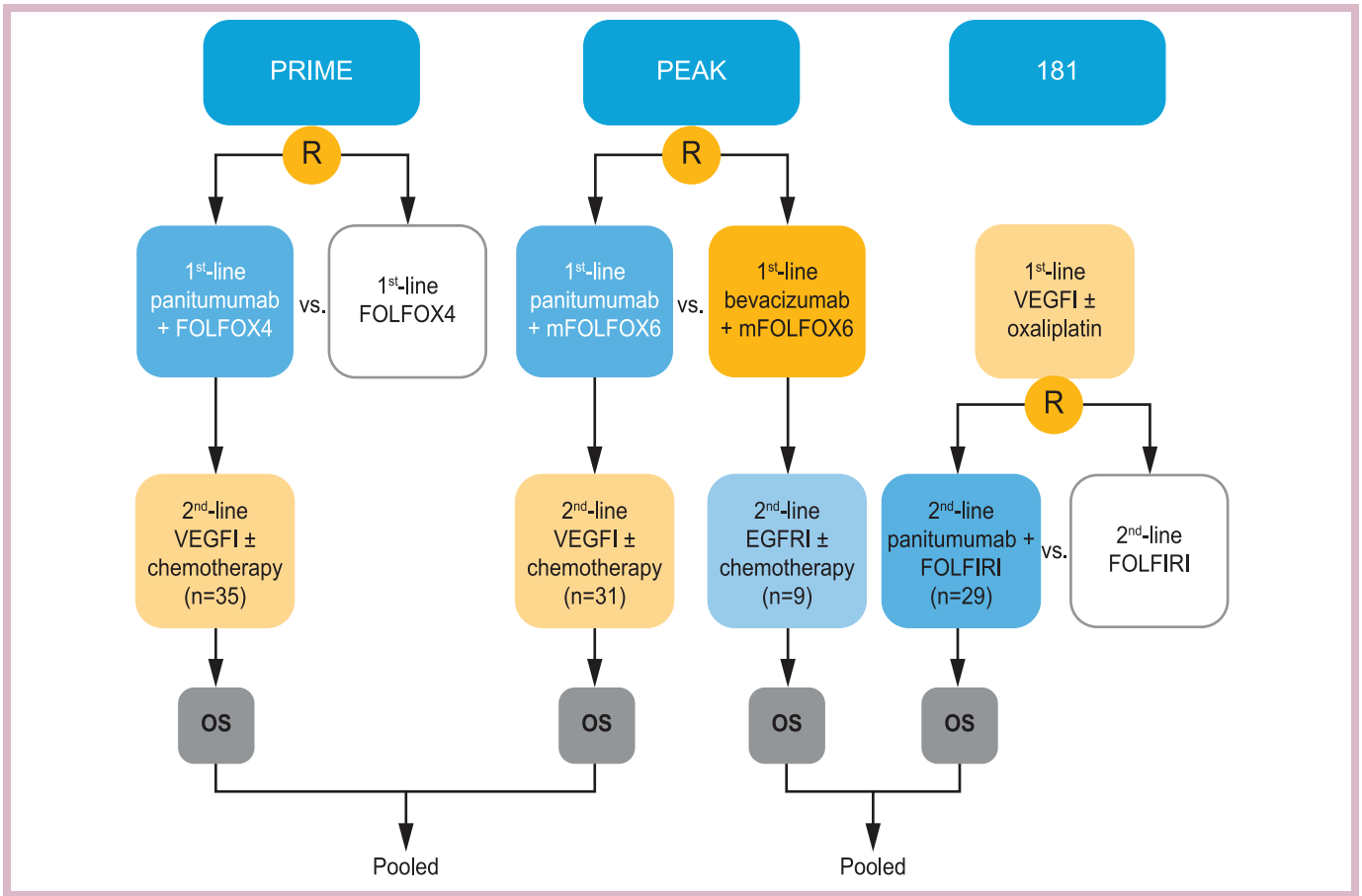

Figure 1 Schema of the exploratory analyses (*RAS* wild-type population). EGFRi, epidermal growth factor receptor inhibitor; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; OS, overall survival; PEAK, Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours; PRIME, Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy; R, randomisation; VEGFi, vascular endothelial growth factor inhibitor.

Overall, most patients had an Eastern Cooperative Oncology Group performance status of 0 (46 patients (70%) in the panitumumab→VEGFi arm; 29 (76%) in the bevacizumab→EGFRi arm). The majority of patients had left-sided tumours (40 patients (61%) receiving panitumumab→VEGFi; 25 patients (66%) receiving bevacizumab→EGFRi). A slight imbalance between treatment arms was observed with respect to the sites of metastatic disease: more patients receiving the panitumumab→VEGFi sequence had colon cancer (53 (80%)) compared with those treated with bevacizumab→EGFRi (22 (58%)) (table 1).

The use of non-study therapies was also generally well balanced between treatment sequences, with most patients receiving EGFRi/VEGFi in combination with chemotherapy (second line for PEAK and PRIME studies, and first line for Study 181) (table 2).

Most patients in Study 181 received first-line bevacizumab combined with oxaliplatin-based chemotherapy (22 (76%)), while in PEAK and PRIME, most received second-line EGFRi (PEAK: 8 (89%)) or VEGFi (PEAK: 24 (77%); PRIME: 27 (77%)) with irinotecan-based chemotherapy (table 2). Six patients (9%) from the panitumumab→VEGFi group received the anti-angiogenesis multikinase inhibitor regorafenib in third or later lines of (non-study) treatment, compared with no patients in the bevacizumab→EGFRi arm (data not shown).

Efficacy

At the time of the data analysis, 63.6% of patients with *RAS* WT mCRC receiving first-line panitumumab followed by second-line VEGFi, and 92.1% of patients receiving the first-line bevacizumab followed by second-line EGFRi sequence had died (table 3).

Similar results were observed in patients with *RAS* WT/*BRAF* WT mCRC: 58.6% and 91.4% of patients had died, respectively (table 3).

In the *RAS* WT pooled analysis, median OS was 36.8 versus 27.8 months in patients treated with first-line panitumumab followed by second-line VEGFi, compared with first-line bevacizumab followed by second-line EGFRi (HR 0.65; 95% CI 0.42 to 1.03) (figure 2 and table 3). The OS benefit associated with the panitumumab→VEGFi treatment sequence was more pronounced in patients with *RAS* WT/*BRAF* WT mCRC (median OS: 41.3 vs 28.9 months in the panitumumab→VEGFi vs bevacizumab→EGFRi

Table 1 Baseline demographics and disease characteristics* (*RAS* wild-type population)

	PEAK+PRIME	PEAK+181
	Panitumumab→VEGFi (n=66)	Bevacizumab→EGFRi (n=38)
Male sex, n (%)	43 (65)	26 (68)
White ethnicity, n (%)	57 (86)	34 (89)
Age, years—median (range)	59 (38 to 77)	61 (28 to 75)
ECOG performance status, n (%)		
0	46 (70)	29 (76)
1	18 (27)	8 (21)
2	2 (3)	1 (3)
Primary tumour diagnosis, n (%)		
Colon†	53 (80)	22 (58)
Rectum	13 (20)	16 (42)
Tumour side, n (%)		
Left	40 (61)	25 (66)
Right	13 (20)	6 (16)
Unknown	13 (20)‡	7 (18)
Time since mCRC diagnosis, months—median (range)§	1.5 (0.2 to 15.2)	1.1 (–0.6 to 15.3)
CEA>normal range, n (%)	55 (87)¶	30 (83)¶
Number of sites of metastatic disease, n (%)		
1	18 (27)	9 (24)
2	21 (32)	15 (39)
≥3	27 (41)	14 (37)
Sites of metastatic disease, n (%)		
Liver only	17 (26)	8 (21)
Liver+other	40 (61)	24 (63)
Other only	9 (14)	6 (16)

*Data were collected at randomisation (first-line treatment for PEAK and PRIME studies and second-line treatment for Study 181).
†Includes patients with either left-sided or right-sided disease.
‡Percentages do not add up to 100% due to rounding.
§Date of first-line treatment minus date of metastatic disease.
¶Denominators for the CEA data are n=63 and n=36 for the panitumumab→VEGFi and bevacizumab→EGFRi groups, respectively.
CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; EGFRi, epidermal growth factor receptor inhibitor; mCRC, metastatic colorectal carcinoma; PEAK, Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours; PRIME, Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy; VEGFi, vascular endothelial growth factor inhibitor.

groups, respectively; HR 0.58; 95% CI 0.36 to 0.95) (figure 2 and table 3). Median OS was most prolonged in patients with left-sided mCRC receiving the panitumumab→VEGFi treatment sequence (43.4 vs 32.4 months in the panitumumab→VEGFi vs bevacizumab→EGFRi groups, respectively; HR 0.61; 95% CI 0.33 to 1.11 and HR 0.56; 95% CI 0.30 to 1.04 in the *RAS* WT and *RAS* WT/*BRAF* WT groups, respectively) (table 3). OS data from the individual studies for patients with *RAS* WT and *RAS* WT/*BRAF* WT tumours treated with these sequences were consistent with the results from the pooled analysis (online supplementary table 1).

Among patients with *RAS* WT mCRC, complete resection was performed in six patients from the panitumumab→VEGFi group (9.1%, 95% CI 3.4 to 18.7) and two patients receiving the bevacizumab→EGFRi treatment sequence (5.3%, 95% CI 0.6 to 17.8).

DISCUSSION AND CONCLUSIONS

We report here the results from exploratory pooled analyses of OS for patients with *RAS* WT and *RAS* WT/*BRAF* WT mCRC, treated with either first-line panitumumab followed by second-line VEGFi, or first-line bevacizumab followed by second-line EGFRi, in three randomised trials: PEAK,[4] PRIME[11] and Study 181.[12] Although the patient numbers were relatively small and the CIs wide, the results suggest a trend towards improved OS for first-line panitumumab plus chemotherapy followed by second-line VEGFi treatment, compared with first-line

Table 2 Details of non-study therapy use in PEAK, PRIME and 181 (*RAS* wild-type population)

	PEAK		PRIME	181
	Panitumumab →VEGFi (n=31)	Bevacizumab →EGFRi (n=9)	Panitumumab →VEGFi (n=35)	VEGFi →Panitumumab (n=29)
	Second-line (VEGFi)	Second-line (EGFRi)	Second-line (VEGFi)	First-line (VEGFi)
EGFRi/VEGFi therapy, n (%)				
EGFRi/VEGFi monotherapy	4 (13)	1 (11)	2 (6)	0 (0)
EGFRi/VEGFi+oxaliplatin-containing chemotherapy	2 (6)	0 (0)	4 (11)	22 (76)
EGFRi/VEGFi+irinotecan-containing chemotherapy	24 (77)	8 (89)	27 (77)	0 (0)
EGFRi/VEGFi+other chemotherapy*	1 (3)	0 (0)	2 (6)	7 (24)

*Other chemotherapy=fluoropyrimidine-based chemotherapy, not known or other chemotherapy.
EGFRi, epidermal growth factor receptor inhibitor; PEAK, Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours; PRIME, Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy; VEGFi, vascular endothelial growth factor inhibitor.

bevacizumab plus chemotherapy followed by second-line EGFRi. Although an OS benefit was observed in both patient populations, it was most evident in patients with *RAS* WT/*BRAF* WT mCRC and in those with left-sided tumours, consistent with previous reports.[1 11 13]

Overall, the results from these exploratory analyses are also in line with data from previous reports suggesting a potential clinical benefit for first-line EGFRi treatment compared with first-line VEGFi therapy in *RAS* WT mCRC.[3 4 7–9 14–17] These include meta-analyses of head-to-head first-line randomised trials of EGFRis versus bevacizumab (FIRE-3, PEAK and CALGB/SWOG 80405),[7 8] as well as small retrospective analyses suggesting that prior bevacizumab treatment may decrease efficacy of EGFRi therapy.[14–17] Similar results were also seen in a retrospective analysis of subsequent therapy use and outcomes in the FIRE-3[10] trial and in analyses from the SPIRITT[18] and Prodige 18[19] studies. In FIRE-3, an OS benefit was observed for patients receiving first-line EGFRi followed by second-line VEGFi therapy, compared with the reverse sequence.[10] Conversely, after progression on first-line bevacizumab-based therapy in the SPIRITT[18] and Prodige 18[19] studies, numeric differences in median OS were seen in favour of second-line regimens including bevacizumab

Table 3 Pooled analysis of overall survival in patients receiving panitumumab→VEGFi (PEAK and PRIME) versus bevacizumab→EGFRi (PEAK and 181) (*RAS* wild-type and *RAS* wild-type/*BRAF* wild-type populations)

	All *RAS* WT		Left-sided *RAS* WT	
	PEAK+PRIME	PEAK+181	PEAK+PRIME	PEAK+181
	Panitumumab →VEGFi (n=66)	Bevacizumab →EGFRi (n=38)	Panitumumab →VEGFi (n=40)	Bevacizumab →EGFRi (n=25)
OS events, n (%)	42 (63.6)	35 (92.1)	21 (52.5)	22 (88.0)
Median OS, months (95% CI)	36.8 (30.3 to 43.8)	27.8 (24.2 to 35.6)	43.4 (31.6 to 49.4)	32.4 (23.9 to 41.3)
P value*	0.06		0.10	
HR (95% CI)	0.65 (0.42 to 1.03)		0.61 (0.33 to 1.11)	
	All *RAS* WT/*BRAF* WT		Left-sided *RAS* WT/*BRAF* WT	
	PEAK+PRIME	PEAK+181	PEAK+PRIME	PEAK+181
	Panitumumab →VEGFi (n=58)	Bevacizumab →EGFRi (n=35)	Panitumumab →VEGFi (n=38)	Bevacizumab →EGFRi (n=25)
OS events, n (%)	34 (58.6)	32 (91.4)	19 (50.0)	22 (88.0)
Median OS, months (95% CI)	41.3 (31.6 to 46.1)	28.9 (24.2 to 39.2)	43.4 (36.8 to 55.4)	32.4 (23.9 to 41.3)
P value*	0.03		0.06	
HR (95% CI)	0.58 (0.36 to 0.95)		0.56 (0.30 to 1.04)	

*Log-rank test.
EGFRi, epidermal growth factor receptor inhibitor; OS, overall survival; PEAK, Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours; PRIME, Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy; VEGFi, vascular endothelial growth factor inhibitor; WT, wild type.

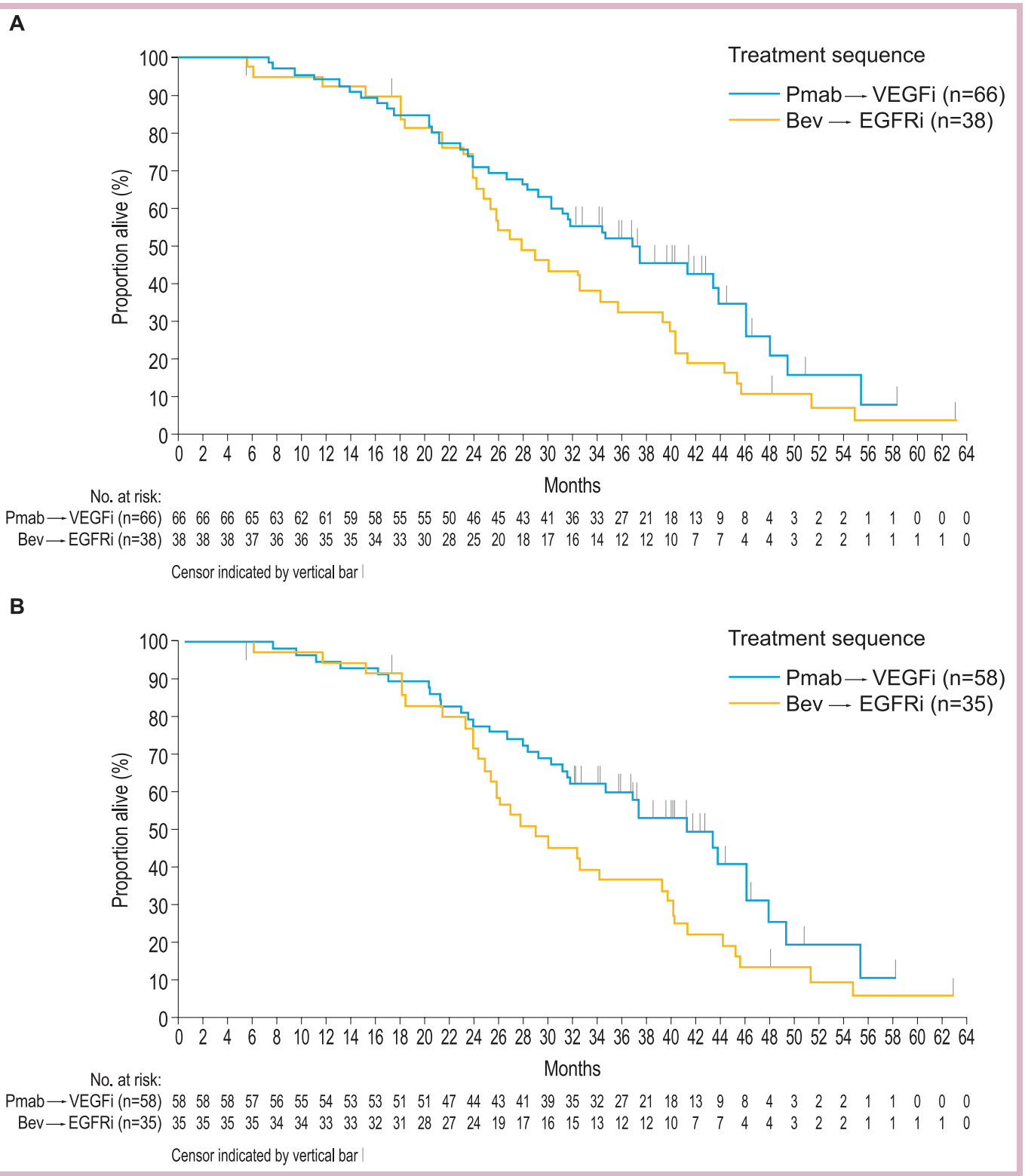

Figure 2 Kaplan-Meier analysis of overall survival in patients receiving panitumumab→VEGFi (PEAK and PRIME) versus bevacizumab→EGFRi (PEAK and 181) in the (A) *RAS* wild-type and (B) *RAS* wild-type/*BRAF* wild-type populations. Bev, bevacizumab; EGFRi, epidermal growth factor receptor inhibitor; P E A K , Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with *KRAS* WT tumours; PRIME, Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy; Pmab, panitumumab; VEGFi, vascular endothelial growth factor inhibitor.

rather than panitumumab or cetuximab (although no significant differences were observed between arms).

Although not directly related to the discussion of optimum sequencing of biologics, our data are also consistent with the results of several other trials. For example, OS benefits have been demonstrated for second-line bevacizumab,[20] aflibercept[21] and ramucirumab[22] versus chemotherapy alone, although after progression on prior bevacizumab (rather than EGFRi) therapy. In contrast, an OS benefit for second-line EGFRi therapy after non-specified first-line mCRC therapy[23–25] has so far not been observed except in a subgroup of patients from the PICCOLO (Panitumumab, Irinotecan and Ciclosporin in COLOrectal cancer) trial whose tumours had high HER3 expression.[26]

In our analyses, the OS improvement observed in patients with *RAS* WT mCRC receiving first-line panitumumab followed by second-line VEGFi does not seem to be influenced by the type of non-study therapies used, as these were well balanced between treatment sequences. Across all three studies examined, most patients received EGFRi/VEGFi in combination with chemotherapy. The proportions of patients receiving first-line oxaliplatin-based chemotherapy followed by a second-line irinotecan-containing regimen were also comparable between treatment sequences. This is supportive of the view that the different sequence of biologics is the major factor impacting the OS results in our analyses.

Treatment with the anti-angiogenic receptor tyrosine kinase inhibitor regorafenib has been shown to provide survival benefits in patients with mCRC whose disease has progressed after standard therapies.[27] In our exploratory analyses, a very small imbalance in the use of subsequent regorafenib as third or later line therapy was observed between treatment arms (9% in the panitumumab→VEGFi arm vs 0% in the bevacizumab→EGFRi group). However, these results should be interpreted with caution due to the small numbers of patients. Furthermore, data on regorafenib use were not prospectively collected, but were rather extracted from patient case report forms where it was recorded as 'other' non-study therapy, and may have therefore been under-reported in the bevacizumab→EGFRi arm.

One of the potential biological mechanisms underlying the OS benefit observed with first-line EGFRi treatment followed by second-line VEGFi therapy is that the emergence of EGFRi resistance does not seem to lead to resistance to VEGFis. Preclinical studies have shown that, at the molecular level, resistance to EGFRis can lead to increased VEGF expression levels[28 29] and the promotion of angiogenesis, thereby sensitising the tumour to subsequent treatment with a VEGFi.[2 14] In contrast, resistance to a VEGFi may result in simultaneous resistance to EGFRis.[2 14] This may be due to the hypothesis that VEGFi therapy causes evasion from hypoxia-dependent apoptosis, which normally operates via EGFR/RAS-mediated activation of Akt, and can therefore potentially lead to reduced EGFRi efficacy.[2 9] The lower OS benefit observed with the first-line VEGFi→second-line EGFRi treatment sequence may also be due to reduced targeting of EGFRi to the tumour, as a consequence of the VEGFi-mediated decrease in tumour vascularisation.[30]

Intriguingly, recent studies have indicated that left-sided mCRC tumours are more responsive to EGFRi therapy than right-sided tumours.[13] Consistent with this, in the present analyses, median OS was most prolonged for patients with left-sided mCRC receiving first-line panitumumab followed by second-line VEGFi treatment, rather than first-line bevacizumab followed by second-line EGFRi treatment. The number of patients with right-sided tumours was too small in the present study to permit analysis of OS by treatment sequence in this population, but as the proportions of patients with right-sided tumours were balanced between sequences, this is unlikely to be a confounding factor.

A key strength of these analyses is that patient-level data were used and the OS data from the PEAK, PRIME and 181 studies are mature. On the other hand, the small sample size, the retrospective nature of the analyses and the use of pooled data from independent trials, which were heterogeneous in their study design, represent limitations of the analyses.

In summary, these exploratory analyses of patient-level OS data from the PEAK, PRIME and 181 studies, suggest that patients with *RAS* WT mCRC derive greater clinical benefit from first-line EGFRi treatment followed by second-line VEGFi therapy than from the reverse sequence. Nonetheless, prospective phase III studies are required to further evaluate the optimal sequence for administering biological therapies in these patients.

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Contributors MP, FF, MK, MVA, AZ, GD and FR were involved in the acquisition and/or interpretation of data, drafting or critical revision of the manuscript for important intellectual content, and they all approved the final version for submission. XG analysed and interpreted the data, critically reviewed the manuscript for important intellectual content and approved the final version for submission.

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received research funding from Amgen, Merck Serono, Roche and Sanofi. GD is an employee of Amgen (Europe) and owns restricted shares in Amgen. XG is an employee of Amgen and owns restricted shares in Amgen. FR has acted on advisory boards and/or received research funding from Amgen, Bayer, Celgene, Lilly, Merck Serono, Merck Sharp & Dohme, Roche, Sanofi, and Servier. AZ has no conflicts of interest to declare.

Patient consent Not required.

Ethics approval The original PRIME, PEAK and 181 studies were conducted in accordance with the ethical standards of the relevant institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Provenance and peer review Not commissioned; externally peer reviewed.

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