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ORIGINAL ARTICLE

Neo-adjuvant FOLFOX with and without panitumumab for patients with *KRAS*-wt locally advanced colon cancer: results following an extended biomarker panel on the FOxTROT trial embedded phase II population☆

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Available online 11 January 2025

Background: The FOxTROT trial has reported advantages of neoadjuvant chemotherapy (NAC) in locally advanced colon cancer (LACC). In this article, we present results of the embedded randomised phase II trial testing the addition of panitumumab to neoadjuvant FOLFOX compared with FOLFOX alone in *RAS* and *BRAF*-wild-type (wt) patients and with biomarker hyperselection.

Patients and methods: Patients had operable, computed tomography-predicted stage T3-4, N0-2, M0 colon adenocarcinoma. *KRAS*-wt patients allocated to NAC could optionally be sub-randomised 1 : 1 to FOLFOX ± panitumumab during the preoperative phase. *RAS*/*BRAF* were tested by next-generation sequencing; and epiregulin (*EREG*)/amphiregulin (*AREG*) by RNAseq. The primary endpoint was time to recurrence (TTR) in *RAS*/*BRAF*-wt patients; secondary endpoints included safety, histological down-staging, disease-free survival (DFS), colon cancer-specific survival (CCSS), overall survival (OS) and impact of primary tumour location and *EREG*/*AREG*.

Results: In total 269 *KRAS*-wt patients were enrolled into the embedded phase II trial. Extended *RAS*/*BRAF* data were available for 232 (83%) patients; 22/232 (9.5%) were *RAS*-mutant; 41/210 (20%) were *BRAF*-mutant. Median follow-up was 42 months. In 169 *RAS*/*BRAF*-wt patients, there was a trend towards reduced recurrences with FOLFOX plus panitumumab compared with FOLFOX (12% versus 21%, hazard ratio = 0.51, $P = 0.09$); significant improvements were seen for DFS, CCSS and OS. Within the hyperselected *EREG*/*AREG*-high group, there was significant reduction in recurrences with panitumumab. Panitumumab was not associated with increased pathological regression of the primary tumour (tumour regression grade 1-3 16% versus 22%, $P = 0.27$). FOLFOX plus panitumumab was associated with higher rates of grade 3 diarrhoea (8% versus 3%) and rash (22% versus 2%).

Conclusion: This exploratory analysis from a randomised phase II study shows a non-significant improvement in TTR from the addition of neoadjuvant panitumumab to perioperative FOLFOX in *RAS/BRAF*-wt LACC. Hyperselection with *EREG*/*AREG* status was associated with increased efficacy. A dedicated prospective trial within a biomarker-selected population is under development.

Key words: colon, neoadjuvant, panitumumab, hyperselection, epiregulin, amphiregulin

INTRODUCTION

Personalisation of therapy is a standard strategy in the treatment of metastatic colorectal cancer (mCRC), and biomarker testing is considered mandatory ahead of starting therapy.[1] Biomarker stratification directly influences treatment decisions for patients with microsatellite instability-high (MSI-H) or *RAS* and *BRAF*-wild-type (*RAS*/*BRAF*-wt) tumours,[1] and targeted treatment options are

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☆Note: This research was previously presented in the Proffered Paper Lower Gastrointestinal Session of the ESMO Congress 2023.

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available for patients with *BRAF*-mutant,[2] *KRAS*^{G12C}-mutant[3] and human epidermal growth factor receptor 2-amplified tumours.[4] For *RAS*/*BRAF*-wt disease, the combination of chemotherapy with an anti-epidermal growth factor receptor (EGFR) agent is a licensed treatment; however, guidelines currently only endorse treatment in patients with left-sided primary tumour location (PTL) due to a lack of survival benefit in patients with right PTL compared with chemotherapy alone.[5] In an attempt to improve patient selection, novel predictive biomarkers for anti-EGFR agents have been evaluated: high expression of the EGFR ligands epiregulin (*EREG*) and amphiregulin (*AREG*),[6,7] or negative hyperselection through detection of genetic alterations associated with anti-EGFR resistance.[8]

Standard treatment for locally advanced colon cancer (LACC) is usually a combination of surgery and chemotherapy with oxaliplatin and a fluoropyrimidine.[9] The Fluoropyrimidine, Oxaliplatin and Targeted-Receptor pre-Operative Therapy for patients with high-risk, operable colon cancer (FOxTROT) trial was the first international phase III randomised study to test neoadjuvant treatment using oxaliplatin and 5-fluorouracil in LACC. It reported safety, fewer recurrences at 2 years and improved 3-year disease-free survival (DFS) with delivering 6 weeks of planned chemotherapy ahead of surgery, compared with the standard sequence of upfront surgery followed by adjuvant chemotherapy. It also indicated differential benefit by mismatch repair (MMR) status, suggesting that personalisation of care is relevant in LACC as well as mCRC.[10]

Currently the only biomarker testing routinely recommended for LACC is MMR or MSI status to establish the likelihood of Lynch syndrome, and to guide adjuvant chemotherapy (AC) decisions only in stage 2 cancers.[9] In the neoadjuvant setting, MMR/MSI status predicts non-response to chemotherapy and more recently has been shown to predict dramatic pathological responses to immune checkpoint inhibitors.[11] *RAS* and *BRAF* mutation status are prognostic factors for stage 2 and 3 cancers that are microsatellite stable (MSS), providing additional information to TNM (tumour—node—metastasis) staging.[12]

Given the benefits in response rate and overall survival (OS) with anti-EGFR agents in mCRC, the PETACC 8 study tested whether the addition of cetuximab to FOLFOX resulted in improved DFS in patients with stage 2/3 *KRAS*-mutant colon cancer.[13] While the trial was negative for its primary endpoint, subsequent analyses in biomarker-selected patients demonstrated a non-significant trend towards DFS benefit for adding cetuximab to adjuvant FOLFOX; the analysis was hindered by a lack of power by reduced patient numbers with samples available for translational analyses.[14] Therefore this is not a recommended strategy.

The FOxTROT trial included an embedded randomised phase II study which tested the addition of panitumumab to neoadjuvant FOLFOX, versus neoadjuvant FOLFOX alone in patients with $KRAS_{c.12\text{-}13,61}$-wt disease. Initial results were reported with the main FOxTROT trial publication: in *KRAS*-wt patients there was no significant improvement in pathological tumour down-staging or 2-year recurrence-free survival with panitumumab. While a trend towards benefit with panitumumab was observed across all efficacy endpoints, this was not significant compared with FOLFOX alone.[10]

In this article, we report an exploratory translational substudy testing the addition of panitumumab to neoadjuvant FOLFOX firstly in a contemporary biomarker-selected population ($KRAS_{c.12,13,59\text{-}61,117,146}$, $NRAS_{c.12,13,59\text{-}61}$ and $BRAF^{V600E}$ wild-type), then in a biomarker-hyperselected population by tumour *EREG*/*AREG* expression.

PATIENTS AND METHODS

Trial procedures

The trial procedures of the overall FOxTROT trials (ISRCTN87163246) have been previously described[1,2]; this article focuses on patients treated in panitumumab sub-randomisation. This sub-randomisation was restricted to patients already randomised to receive neoadjuvant chemotherapy (NAC). The eligibility criteria were: biopsy-confirmed colon adenocarcinoma that was *KRAS*-wt (codons 12, 13 and 61), computed tomography-predicted T3-4 with extramural extension >5 mm (modified to >1 mm after pilot phase), M0, and being fit for both surgery and chemotherapy. Patients with bowel obstruction were eligible if first diverted with a stoma. Treatment allocations were previously described; patients were randomised 1 : 1 to receive FOLFOX $\pm$ panitumumab.

National and institutional approvals were obtained, and patients provided written informed consent for trial treatment and for translational research.

Treatment

The FOxTROT trial treatment was a planned 24-week perioperative chemotherapy, with all patients having three cycles delivered ahead of surgery. An optional total perioperative duration of 12 weeks was permitted. Oxaliplatin and fluorouracil were given using a modified FOLFOX schedule. Panitumumab was infused at 6 mg/kg over 30-90 min before the first three cycles of FOLFOX. The use of capecitabine with oxaliplatin was not permitted within panitumumab sub-randomisation.

The initial trial protocol randomised *KRAS*-wt patients to receive NAC to 24 weeks of FOLFOX $\pm$ panitumumab, but the protocol was amended following publication of PETACC 8 showing no advantage with panitumumab in the adjuvant setting in *KRAS*-wt patients.[13] The amended protocol limited treatment with panitumumab to the neoadjuvant setting (6 weeks of total duration; no adjuvant panitumumab). Figure 1 outlines the number of patients allocated to each trial treatment in the original protocol. Due to small numbers, no further analyses on patients receiving adjuvant panitumumab will be presented.

Dose reductions, treatment delays and early cessation for toxicity were permitted as in routine practice. Surgery was scheduled 4-6 weeks after completing neoadjuvant

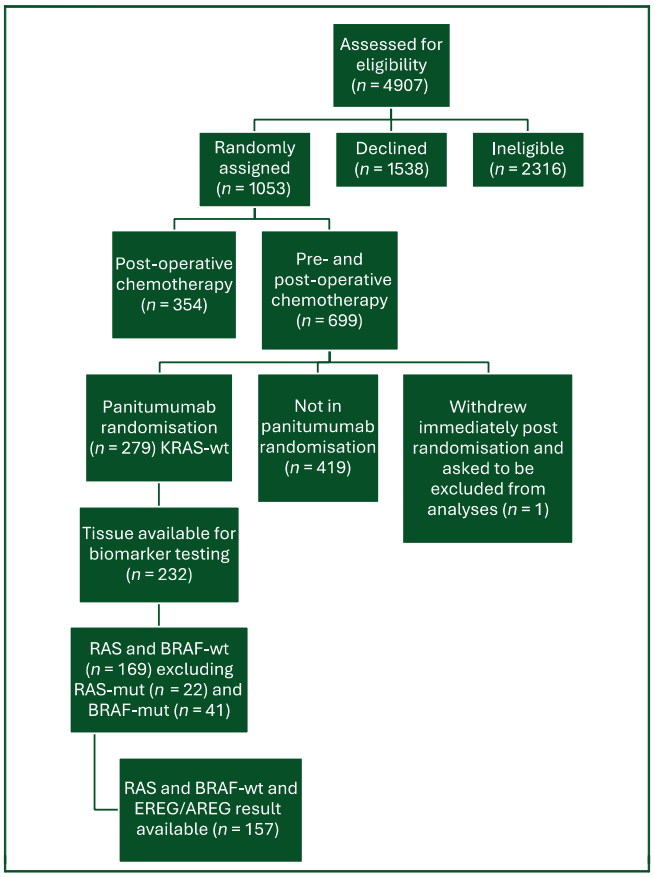

Figure 1. CONSORT diagram.
AREG, amphiregulin; *EREG*, epiregulin.

treatment. AC was scheduled to start 6-10 weeks after surgery. Clinical follow-up was according to routine practice and previously described.[1]

Extended biomarker analysis

Where sufficient tumour material was available 10 µM formalin-fixed, paraffin-embedded (FFPE) sections were macrodissected and extracted using a Qiagen (Netherlands MiSeq, San Diego, CA) AllPrep FFPE DNA/RNA kit as previously described. Extended *RAS* and *BRAF* status was assessed in both pre- and post-treatment samples using previously described methods.[7] PCR products using two sets of primers (A and B) covering each codon of interest (*KRAS* codons 12/13/59/61/117/146, NRAS codons 12/13/59/61 and *BRAF* codon 600) were pooled and sequenced on an Illumina MiSeq before mutation calling. Mutations were deemed present if they were called at >5% variant allele frequency in both A and B PCR products.

A minimum of 5 ng extracted RNA underwent library preparation using the Lexogen QuantSeq 3′ sequencing kit. Samples were pooled and sequenced to a minimum of 5 million reads on an Illumina NextSeq 500 instrument. Data analysis was carried out using nf-core/RNAseq and STAR/Salmon-quantified CPMs (count per million) as the output.

EREG and *AREG* mRNA values were assessed by taking the CPM value for each gene; values were then assigned high and low using thresholds established in the PICCOLO trial (*EREG* $\geq$ 10 CPM; *AREG* $\geq$ 16 CPM).[6] Data from both were combined to give a clinically usable single dichotomous classifier by taking the upper/middle tertile cut point for each ligand and dividing the population into 'high expressors' (either *EREG* or *AREG* in the top tertile) or 'low expressors' (neither *EREG* nor *AREG* in the top tertile).

Outcome measures

The primary outcome measure for this study was freedom from recurrence [time to recurrence (TTR)] in the *RAS*/*BRAF*-wt population, where recurrences are events and deaths are censored. Secondary endpoints were DFS, colon cancer-specific survival (CCSS), OS and short-term efficacy [assessed by rate of complete resection (R0 versus R1-2) involved lymph nodes, extramural venous invasion and Dworak tumour regression grade].

Statistical analyses

Frequency tables of categorical variables and descriptive statistics of quantitative variables were produced by treatment allocation for baseline characteristics in the extended *RAS*/*BRAF*-wt population. *P* values were determined from Pearson chi-square tests, Fisher's exact tests and two-tailed *t*-tests. Both toxicity reported during the first 6 weeks of chemotherapy and intraoperative and post-operative complications were assessed by treatment allocation in the 279 *KRAS*-wt intention-to—treat (ITT) population. Unadjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using the Cox proportional hazards model for the effect of FOLFOX plus panitumumab versus FOLFOX on each of the four endpoints and Kaplan—Meier curves produced. All endpoints were assessed in the *RAS*/*BRAF*-wt subgroup and in the ITT population ($n = 279$). These analyses were repeated in right PTL, left PTL, low *EREG*/*AREG* expression (both *AREG* and *EREG* in the lowest two-thirds of expression as previously defined[6]) and high *EREG*/*AREG* expression (either *AREG* or *EREG* in the highest third of expression) to explore differential effects. To determine short-term efficacy in the *RAS*/*BRAF*-wt subgroup, two-way frequency tables of the pathology variables versus treatment allocation were produced and *P* values were generated from Pearson's chi-square tests and Fisher's exact tests. Tumour regression grade, grouped as complete/marked/moderate versus mild/none, was analysed further by estimation of odds ratios and 95% CIs for treatment effect from logistic regression models. These analyses were repeated in low and high *EREG*/*AREG* expression separately to explore differential effects. Throughout, for analyses of differential effects, *P* values for interaction were generated from likelihood ratio tests. All statistical analyses were carried out using STATA StataCorp (2019).

RESULTS

A total of 279 *KRAS*-wt patients were randomised to the neoadjuvant FOLFOX ± panitumumab sub-randomisation (ITT population); results based upon *KRAS* testing and ITT have been reported as part of the main FOxTROT analysis.[10]

Table 1. Estimated crude hazard ratios (HRs) for the effect of treatment (FOLFOX + Pan versus FOLFOX) on time to recurrence, disease-free survival, death from colon cancer and overall survival in different mutation subgroups

Mutation subgroup	Treatment	Events/total (%)	Unadjusted HR (95% CI)	*P* value
Overall TTR				
Ext *RAS*/*BRAF*-wt	FOLFOX	16/77 (20.8)	1.0	
	FOLFOX + Pan	11/92 (12.0)	0.51 (0.24-1.10)	0.09
KRAS-wt	FOLFOX	31/136 (22.8)	1.0	
	FOLFOX + Pan	25/143 (17.5)	0.71 (0.42-1.20)	0.20
Overall DFS				
Ext *RAS*/*BRAF*-wt	FOLFOX	22/77 (28.6)	1.0	
	FOLFOX + Pan	17/92 (18.5)	0.51 (0.27-0.97)	0.04
KRAS-wt	FOLFOX	38/136 (27.9)	1.0	
	FOLFOX + Pan	31/143 (21.7)	0.68 (0.42-1.09)	0.11
Death from colon cancer				
Ext *RAS*/*BRAF*-wt	FOLFOX	10/77 (13.0)	1.0	
	FOLFOX + Pan	3/92 (3.3)	0.23 (0.06-0.83)	0.03
KRAS-wt	FOLFOX	22/136 (16.2)	1.0	
	FOLFOX + Pan	13/143 (9.1)	0.53 (0.27-1.05)	0.07
Overall survival				
Ext *RAS*/*BRAF*-wt	FOLFOX	16/77 (20.8)	1.0	
	FOLFOX + Pan	9/92 (9.8)	0.36 (0.16-0.85)	0.02
KRAS-wt	FOLFOX	29/136 (21.3)	1.0	
	FOLFOX + Pan	20/143 (14.0)	0.61 (0.34-1.08)	0.09

CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; Pan, panitumumab; TTR, time to recurrence; wt, wild-type.

The primary analysis population for this extended biomarker study is the extended *RAS*/*BRAF*-wt population, as per contemporary practice for anti-EGFR agents in mCRC. In total, 232/279 (83.2%) patients had extended *RAS* and *BRAF* data available. In the other 47 cases, 15 cases failed sequencing and the remainder either had insufficient tumour or no block submitted for central testing. After testing, there were 169/232 extended *RAS*/*BRAF*-wt patients having excluded 22/232 *RAS*-mutant patients and 41/232 *BRAF*-mutant patients. A total of 157/169 patients (93%) also had *EREG*/*AREG* results available (Figure 1).

Baseline characteristics are described in Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2024.12.013. Mean age was 60 years; baseline computed tomography assessment described T4 disease in 32/169 (19%). Regarding tumour location, 102 (60%) had left PTL and 67 (40%) had right PTL. There were no major imbalances between treatment arms (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2024.12.013).

Safety and perioperative outcomes

The tolerability of FOLFOX ± panitumumab is shown in Supplementary Tables S2 and S3 in the 279 *KRAS*-wt population. Treatment with FOLFOX + panitumumab was associated with numerically increased toxicity compared with FOLFOX alone (Supplementary Table 2). The only significant difference in grade 3/4 toxicity however was panitumumab related skin toxicity. In terms of peri-operative outcomes, there were numerical increases in anastomotic leaks and abscess formation rates, wound infections and bronchopneumonia in the panitumumab treated patients (Supplementary Table 3). There were no differences between the rate of complications prolonging hospital stay, need for re-operation or post-operative death.

Efficacy

In the primary analysis population, the median follow-up was 42 months. A total of 11/92 (12%) *RAS*/*BRAF*-wt patients experienced a recurrence event in the FOLFOX plus panitumumab group, versus 16/77 (21%) in the FOLFOX group (HR 0.51, 95% CIs 0.24-1.10, $P = 0.09$) (Table 1 and Figure 2). Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2024.12.013, shows the effect of panitumumab on TTR in relevant subgroups in the *RAS*/*BRAF*-wt population. While there were trends towards increased panitumumab benefit in female patients, no significant differential effect was observed in those with left PTL and T4 tumours.

When considering other outcome measures, the addition of panitumumab to neoadjuvant FOLFOX in *RAS*/*BRAF*-wt patients was associated with a significant improvement (DFS HR 0.51, 95% CIs 0.27-0.97, $P = 0.04$; death from colon cancer HR 0.23, 0.06-0.83, $P = 0.03$; OS HR 0.36, 95% CIs 0.16-0.85, $P = 0.02$) (Table 1 and Supplementary Figure S2A-C, available at https://doi.org/10.1016/j.annonc.2024.12.013). Deaths related to causes other than colon cancer were similar between the two arms (7% versus 7%).

Among MMR-proficient (pMMR) patients, there was no difference in recurrence rate between treatment groups (13.4% in FOLFOX + Pan versus 17.5% in FOLFOX, $P = 0.50$). Among the 10 MMR-deficient (dMMR) FOLFOX + Pan patients, there were no recurrences and among the 14 dMMR FOLFOX patients, there were 5 recurrences (35.7%), $P = 0.05$.

Impact of PTL on effectiveness of panitumumab

Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2024.12.013, shows survival analyses by PTL. Numerically fewer patients with left PTL experienced

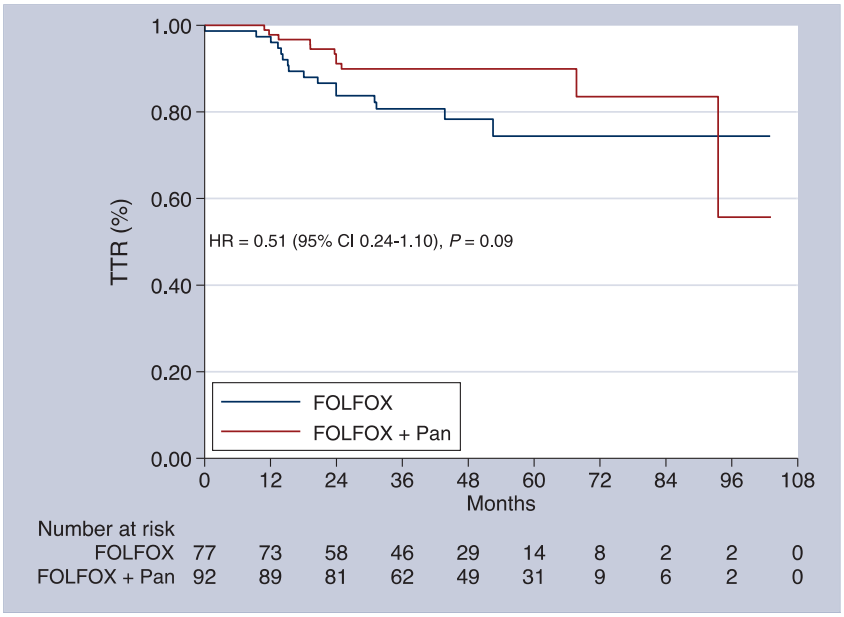

Figure 2. Overall TTR Kaplan–Meier curves for *RAS/BRAF* wild-type patients for FOLFOX ± panitumumab.
CI, confidence interval; HR, hazard ratio; Pan, panitumumab; TTR, time to recurrence.

a recurrence event when treated with FOLFOX +panitumumab than FOLFOX alone (10.9% versus 21.3%; HR 0.45, 95% CIs 0.16-1.23, $P = 0.12$) compared with those with right PTL (13.5% versus 20.0%; HR 0.67, 95% CIs 0.20-2.21, $P = 0.51$); interaction testing by PTL was not significant, $P = 0.63$ (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2024.12.013). Similar data were seen for DFS, CCSC and OS but analyses were limited by low patient numbers.

Results by EREG *and* AREG *status*

Using a prospectively defined model for dichotomisation,[6] 74 patients (47.1%) had high *EREG* and/or *AREG* expression, and 83 (52.9%) had low *EREG* and/or *AREG* expression. Patients with high *EREG/AREG* expression were more likely to be left PTL (68.9% versus 51.8% with low *EREG/AREG*, $P = 0.03$); patients with low *EREG/AREG* expression were more likely to have lymph node involvement (35.8% versus 13.7% with high *EREG/AREG*, $P = 0.002$) (data not shown).

Patients with high *EREG/AREG* expression were less likely to experience a recurrence when treated with FOLFOX + Pan versus FOLFOX alone (11.9% versus 25.0%; HR 0.29, 95% CIs 0.09-0.99, $P = 0.047$, Table 2 and Figure 3A); for patients with low *EREG/AREG* there were no signs of improvement with the addition of panitumumab to FOLFOX (14.0% versus 17.5%; HR 0.75, 95% CIs 0.25-2.22, $P = 0.60$, Table 2 and Figure 3B), although interaction testing by *EREG/AREG* status was not statistically significant ($P = 0.33$). Consistent results were seen for DFS. For CCSS and OS, the effects of panitumumab were similar when comparing low and high *EREG/AREG* expression, whereas CCSS analysis was limited by low event rate.

Impact of panitumumab on histopathological endpoints in the resected tumour

Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2024.12.013, shows histopathological measures of treatment efficacy in the resected tumour by treatment allocation. Unlike for longer-term efficacy endpoints, there were no differences observed with the addition of panitumumab to neoadjuvant FOLFOX. In patients treated with FOLFOX plus panitumumab, the rate of major histological response overall as assessed by tumour regression grade (complete/marked/moderate) was 19.3%, compared with 23.4% in FOLFOX alone, $P = 0.53$. Tumour regression was further analysed stratified by EREG/AREG expression (Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2024.12.013) and no differences were observed even in this hyperselected population.

DISCUSSION

This study suggests that precision molecular testing can define a group of patients with LACC more likely to benefit from the addition of panitumumab to a spine of FOLFOX NAC. It also showed that unlike for FOLFOX alone, down-staging at the time of surgery does not predict long-term benefit.

The understanding of biological sub-types and response to therapy in mCRC has greatly improved over the past decades, particularly for anti-EGFR agents. It is therefore necessary to revisit conclusions of studies where drugs were tested in sub-optimal populations—based upon best evidence at the point of trial conception. In this study, we tested the addition of panitumumab to neoadjuvant FOLFOX compared with neoadjuvant FOLFOX alone in LACC and have demonstrated clinically relevant activity limited to a rationally selected and predefined biomarker group. Within

Table 2. **Estimated crude hazard ratios (HRs) for the effect of treatment (FOLFOX + Pan versus FOLFOX) on time to recurrence, disease-free survival, death from colon cancer and overall survival separately in patients with low *EREG/AREG* expression and high *EREG/AREG* expression including likelihood ratio tests for *EREG/AREG* treatment interaction**

Mutation subgroup	Treatment	All patients with *EREG/AREG* data available			Low *EREG/AREG* expression			High *EREG/AREG* expression			*P* Value for interaction
		Events/total (%)	Unadjusted HR (95% CI)	*P* value	Events/total (%)	Unadjusted HR (95% CI)	*P* value	Events/total (%)	Unadjusted HR (95% CI)	*P* value	
Overall TTR											
RAS/BRAF-wt	FOLFOX	15/72 (20.8)	1.0		7/40 (17.5)	1.0		8/32 (25.0)	1.0		
	FOLFOX + Pan	11/85 (12.9)	0.56 (0.26-1.21)	0.14	6/43 (14.0)	0.75 (0.25-2.22)	0.60	5/42 (11.9)	0.29 (0.09-0.99)	0.047	0.33
Overall DFS											
RAS/BRAF-wt	FOLFOX	20/72 (27.8)	1.0		9/40 (22.5)	1.0		11/32 (34.4)	1.0		
	FOLFOX + Pan	16/85 (18.8)	0.55 (0.28-1.07)	0.08	7/43 (16.3)	0.67 (0.25-1.81)	0.43	9/42 (21.4)	0.27 (0.10-0.74)	0.01	0.33
Death from colon cancer											
RAS/BRAF-wt	FOLFOX	10/72 (13.9)	1.0		5/40 (12.5)	1.0		5/32 (15.6)	1.0		
	FOLFOX + Pan	3/85 (3.5)	0.24 (0.07-0.87)	0.03	2/43 (4.7)	0.37 (0.07-1.90)	0.23	1/42 (2.4)	[a]	—	—
Overall survival											
RAS/BRAF-wt	FOLFOX	15/72 (20.8)	1.0		7/40 (17.5)	1.0		8/32 (25.0)	1.0		
	FOLFOX + Pan	8/85 (9.4)	0.36 (0.15-0.88)	0.03	3/43 (7.0)	0.39 (0.10-1.51)	0.17	5/42 (11.9)	0.15 (0.03-0.69)	0.02	0.49

AREG, amphiregulin; CI, confidence interval; DFS, disease-free survival; *EREG*, epiregulin; Pan, panitumumab; TTR, time to recurrence.
[a]Numbers are too small to estimate H.

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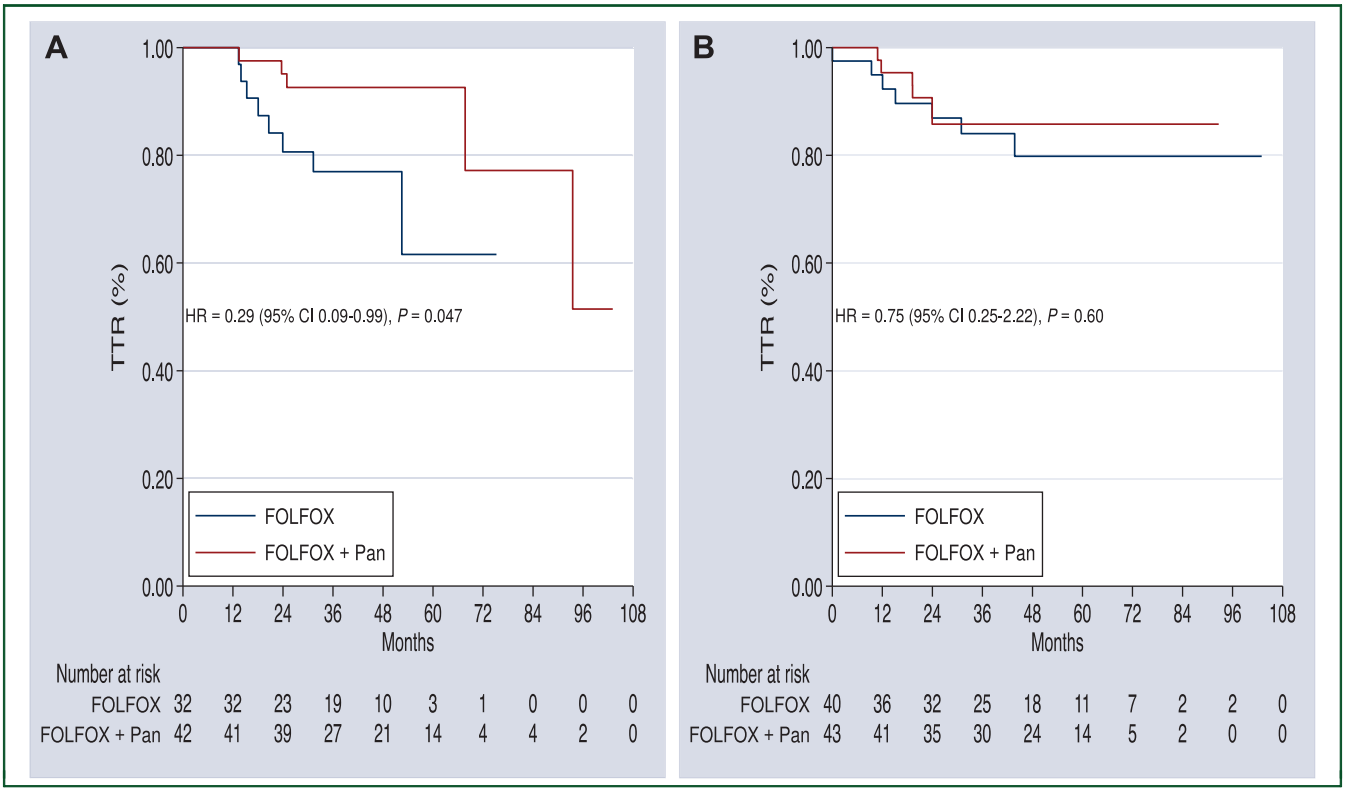

Figure 3. Overall TTR Kaplan-Meier curves for *RAS/BRAF* wild-type patients by Pan randomisation. (A) *EREG/AREG*-high patients and (B) *EREG/AREG*-low patients. *AREG*, amphiregulin; CI, confidence interval; *EREG*, epiregulin; HR, hazard ratio; Pan, panitumumab; TTR, time to recurrence.

the *RAS/BRAF*-wt primary analysis population of this exploratory study, the addition of neoadjuvant panitumumab to perioperative FOLFOX was associated with a trend towards improvement in the primary outcome measure of freedom from recurrence, and a statistically significant improvement in DFS, CCSS and OS, compared with perioperative FOLFOX alone. Importantly, within a hyperselected population with additional high tumour expression of *EREG/AREG* expression, a statistically significant improvement in all longer-term cancer outcomes was observed.

While personalisation of care has rapidly progressed in mCRC with several novel target/drug combinations, similar advances have not been made in LACC. For stage 3, adjuvant treatment is only guided by TNM staging,[9] and there have not been any new drug approvals since the introduction of oxaliplatin in 2004.[15] However, the relevance of biomarker selection is apparent in LACC: MSI-H or dMMR status predicts benefit from neoadjuvant immunotherapy[11]; tumour *KRAS* and *BRAF* status are highly prognostic in stage 3 MSS colon cancer.[12] FOxTROT demonstrated that over a third of pMMR patients had no pathological response to 6 weeks of NAC when assessed using conventional regression grading systems.[10] This challenges the concept that LACC should be treated as a single entity with standard chemotherapy; one alternative would be to test novel strategies within rational biomarker groups.

Here, improvements in panitumumab effect mirrored what would be expected in mCRC: step-wise improvement by biological selection, limited codons in *KRAS*, to extended *RAS*, to *RAS* and *BRAF* wild-type, in left PTL compared with right PTL (not statistically significant), and in *EREG/AREG*-high versus -low tumours. In mCRC, high *EREG/AREG* expression has shown consistent correlation with outcomes following anti-EGFR agents, both when assessed by RT—PCR[6,16] and by immunohistochemistry.[7,17] In this study *EREG/AREG* was a more discriminate biomarker than PTL for panitumumab benefit; similar results have been noted for *EREG/AREG* and hyperselection by circulating tumour DNA in mCRC.[8,18]

Thus our biological understanding of mCRC has been shown to be relevant in LACC and this study provides a proof of principle that studies in LACC should be extended into other molecular alteration/treatment combinations which have been successfully targeted in mCRC.

This was an exploratory translational study from a randomised phase II trial, and the number of patients within the study was determined by the availability of tissue for extended molecular testing on previously collected diagnostic biopsies. While the primary endpoint of the study was freedom from recurrence, the study lacked power to provide definitive results to change practice and fully explore subgroups of interest. However, these results for the first time demonstrate activity on longer-term cancer control of a targeted agent in LACC. Reassuringly, the results are consistent with the trends observed with adjuvant cetuximab in PETACC 8 when the analysis was limited to *RAS/BRAF*-wt patients. This analysis was limited by its

retrospective design with reduced power for similar reasons.

Surprisingly, panitumumab did not improve histological down-staging in the primary tumour compared with FOLFOX alone even in patients with *EREG*/*AREG*-high tumours; conversely, pathological response to neoadjuvant OxFp and longer-term cancer outcomes were strongly correlated in FOxTROT. Similarly, EXPERT-C (which tested the addition of cetuximab to oxaliplatin and capecitabine before chemoradiotherapy in *KRAS*-wt locally advanced rectal cancer) did not meet its primary endpoint of an improvement in complete response rate, but showed improved OS (HR 0.27, $P = 0.034$).[19]

Pathological response was assessed using tumour regression grading which assesses tumour fragmentation, but not tumour shrinkage. This may be more applicable to testing the actions of cytotoxic agents rather than cancer treatments with other mechanisms of action; however, no typical measures of short-term efficacy were observed with panitumumab. The mechanism of action may instead suppress proliferation and reduce metastatic potential. Preclinical work suggests that metastatic recurrence in colon cancer is due to survival of a unique population of 'high-relapse cells', with EGFR/MAPK inhibition pushing cells towards a WNT-high stem or Paneth cell-like state that may be acutely sensitive to chemotherapy and reduce the likelihood of survival of the 'high-relapse cells'.[20] Such hypotheses will be explored in future translational work, including antibody-dependent cellular cytotoxicity, natural killer cells and pattern of immune infiltrates.

Perioperative safety is critically important as the field develops. Here there was an increase in perioperative complications with the addition of panitumumab to FOLFOX; however, numerically these were no different from complication rates in patients who had upfront surgery in the FOxTROT trial. This will need to be monitored in future studies.

The neoadjuvant treatment of LACC is a rapidly progressing area. This study adds to the growing evidence by demonstrating that biomarker testing is possible and relevant, and that treatment of rational biomarker—drug combinations could lead to longer-term survival gains in LACC, and conceivably with locally advanced rectal cancer in the era of total neoadjuvant treatment. Several ongoing studies are exploring the role of neoadjuvant-targeted agents in LACC, including FOxTROT 4 (ISRCTN83842641) and UNICORN (NCT05845450).

To progress these results, a prospective phase III trial is urgently required to establish the role of anti-EGFR agents in the curative setting for a biomarker-defined population of colon cancer patients.

ACKNOWLEDGEMENTS

We wish to thank the patients who participated in FOxTROT and all staff who have supported the trial. The patient representative for the trial was Alf Oliver. He contributed for the entire duration of the trial and was an active member of the trial management group. Susan Richman and Gemma Hemmings carried out the prospective testing in Leeds, and Philippe Taniere did the prospective testing in Birmingham.

FUNDING

The FOxTROT trial was funded by Cancer Research UK. (C551/A8283). Amgen supported both the study and provided panitumumab and funded extended biomarker testing. Yorkshire Cancer Research (L386) supported the pathology work.

DISCLOSURE

JFS: advisory/steering committee: AstraZeneca, Boehringer-Ellison, BMS, GSK, Johnson & Johnson, Merck Serono, Sanofi and Servier Takeda; speaker fees: GSK, Merck Serono, Pierre Fabre Medicament, Servier and Takeda; and research funding: Pierre Fabre Medicament, Merck Serono, GSK. CJMW: speaker fees: Servier. PQ: advisory and research funding Roche Diagnostics. NPW: Amgen, Astellas, Bristol Myers Squibb, GSK and Pfizer; and research funding to institution from Adlai Nortyem GSK and Pierre Fabre. All other authors have declared no conflicts of interest.

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