

Keywords: biliary tract cancer; cholangiocarcinoma; epidermal growth factor receptor EGFR; KRAS; panitumumab

Phase II study of gemcitabine, oxaliplatin in combination with panitumumab in KRAS wild-type unresectable or metastatic biliary tract and gallbladder cancer

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Background: Current data suggest that platinum-based combination therapy is the standard first-line treatment for biliary tract cancer. EGFR inhibition has proven beneficial across a number of gastrointestinal malignancies; and has shown specific advantages among KRAS wild-type genetic subtypes of colon cancer. We report the combination of panitumumab with gemcitabine (GEM) and oxaliplatin (OX) as first-line therapy for KRAS wild-type biliary tract cancer.

Methods: Patients with histologically confirmed, previously untreated, unresectable or metastatic KRAS wild-type biliary tract or gallbladder adenocarcinoma with ECOG performance status 0–2 were treated with panitumumab 6 mg kg⁻¹, GEM 1000 mg m⁻² (10 mg m⁻² min⁻¹) and OX 85 mg m⁻² on days 1 and 15 of each 28-day cycle. The primary objective was to determine the objective response rate by RECIST criteria v.1.1. Secondary objectives were to evaluate toxicity, progression-free survival (PFS), and overall survival.

Results: Thirty-one patients received at least one cycle of treatment across three institutions, 28 had measurable disease. Response rate was 45% and disease control rate was 90%. Median PFS was 10.6 months (95% CI 5–24 months) and median overall survival 20.3 months (95% CI 9–25 months). The most common grade 3/4 adverse events were anaemia 26%, leukopenia 23%, fatigue 23%, neuropathy 16% and rash 10%.

Conclusions: The combination of gemcitabine, oxaliplatin and panitumumab in KRAS wild type metastatic biliary tract cancer showed encouraging efficacy, additional efforts of genetic stratification and targeted therapy is warranted in biliary tract cancer.

Biliary tract cancer (BTC) encompasses both intra- and extra-hepatic cholangiocarcinomas and gallbladder carcinoma. The annual incidence is >12 000 cases (Siegel *et al*, 2013) in the

United States with a rising number of intrahepatic cholangiocarcinomas (Patel, 2001). Curative therapy for BTC is surgical resection, however, over 80% of patients present with metastatic disease, for

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whom treatment options are palliative. Based on the ABC-02 trial, the standard of care for advanced BTC is cisplatin and gemcitabine, which demonstrated significant improvement in median overall survival (OS) of 11.7 months compared to 8.3 months with gemcitabine alone (Valle *et al*, 2010). Additional trials have evaluated gemcitabine in combination with oxaliplatin with response rates in excess of 30% and favourable toxicity profile, providing another option for first-line therapy (Andre *et al*, 2004, Pracht *et al*, 2012).

The epidermal growth factor receptor (EGFR) is broadly expressed in BTC and *in vitro* studies have suggested the potential efficacy of inhibitors of this pathway (Harder *et al*, 2009). It has been further suggested that tumour genetics may influence the impact of EGFR inhibition. Preclinical data has demonstrated KRAS wild-type (KRAS WT) human cholangiocarcinoma cell lines show increased sensitivity to cetuximab when compared to KRAS mutant cell lines (Xu *et al*, 2010). In another study vandetanib, a partial EGFR inhibitor, suppressed growth of TKKK (KRAS WT) BTC cell line, while HuCCT (KRAS mutant) required 10 times the amount of drug for similar effect (Yoshikawa *et al*, 2009). KRAS mutations are found in 3–50% of tumours depending on tumour location and methodology used to evaluate for mutations in different studies (Suto *et al*, 2000; Rashid *et al*, 2002; Ong *et al*, 2012). The most recent report suggests that KRAS mutations are most common in extrahepatic tumours and that rates for intrahepatic cancers are on the lower end of this spectrum at 9–22% (Voss *et al*, 2013). Early phase trials evaluating EGFR inhibition in BTC demonstrated clinical activity with observed responses (Philip *et al*, 2006; Lubner *et al*, 2010). These early trials, however, were not guided by tumour genetics and the pattern of responses was variable with no clear anatomic or pathologic corollaries identified. Given this, and the paradigm of sensitivity to EGFR blocking antibodies as established in colon cancer (Douillard *et al*, 2010), we undertook a molecularly driven approach evaluating panitumumab, a fully human EGFR blocking monoclonal, in combination with gemcitabine and oxaliplatin (GEMOX) in KRAS WT BTC.

MATERIALS AND METHODS

This phase II clinical trial was reviewed and approved by the institutional review boards at the Wilmot Cancer Center, University of Rochester (Rochester, NY, USA) and the Dana-Farber/Harvard Cancer Center (Boston, MA, USA).

All patients provided written informed consent before participation in accordance with institutional and federal guidelines.

At the University of Rochester tumour-derived DNA (isolated from FFPE biopsy material) was assessed for KRAS mutations in codons 12 and 13 via PCR using Roche Light Cycler. At Massachusetts General Hospital Cancer Center, total nucleic acids were extracted from FFPE diagnostic tumour tissue and mutational profiling was performed using a custom modified ABI PRISM SNaPshot Multiplex System on an ABI PRISM 3730 DNA-Analyzer (Life Technologies/Applied Biosystems, Grand Island, NY, USA), as previously described (Dias-Santagata *et al*, 2010).

Eligibility criteria. Eligibility included histological confirmation metastatic or unresectable KRAS WT biliary tract adenocarcinoma (bile ducts, hepatic duct, cystic duct, common bile duct, ampulla of Vater or gallbladder adenocarcinoma) with measurable disease defined as at least one lesion >2 cm that can be accurately measured with standard imaging techniques. Participants were 18 years of age or older with no history of chemotherapy or anti-EGFR therapy for biliary tract or gallbladder cancer. Eligible patients required an ECOG performance status of 0 or 1, adequate hepatic, renal and haematologic function (serum bilirubin

<2.5 mg dl⁻¹, aspartate aminotransferase and alanine aminotransferase <2.5 × institutional upper limit of normal, serum creatinine below upper institutional limits or creatinine clearance >60 ml min⁻¹ per 1.73 m⁻², absolute neutrophil count (ANC) >1500 mcl⁻¹, platelet count >100 000 mcl⁻¹ and a life expectancy >3 months. Patients with concurrent malignancies were excluded unless they were treated with curative intent with no known active disease for 3 years prior to enrolment and these include adequately treated non-melanomatous skin cancer or lentigo maligna, treated cervical carcinoma *in situ* without evidence of disease, prostatic intraepithelial neoplasia without evidence of disease or DCIS without evidence of invasive breast cancer. Patients with known brain metastasis were also excluded. Patients with pre-existing grade 2 or higher peripheral neuropathy were excluded. Prior chemoembolization or radiation to the liver was allowed as long as there was measurable disease outside the radiation area and at least 4 weeks had lapsed since therapy. Women of childbearing potential and men were required to agree to the use adequate contraception and pregnant women were excluded.

Study design and treatment. The trial was designed as an open-label, single-arm phase II study. Eligible patients were treated first with panitumumab at 6 mg kg⁻¹ over 1 h, followed by gemcitabine at 1000 mg m⁻² as dose rate infusion at 10 mg m⁻² min⁻¹, and then with oxaliplatin at 85 mg m⁻² over 2 h, on days 1 and 15 of every 28-day cycle. Patients were screened with computed tomography (CT) scans of the chest, abdomen and pelvis, physical examination, blood chemistries and KRAS evaluation. During treatment patients were assessed prior to therapy on days 1 and 15 of each cycle; CT scans and CA19-9 levels were performed every 8 weeks.

Toxicity was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Treatment was discontinued in the event of disease progression, performance status of ≥3, or participant withdrawal. Panitumumab was held for symptomatic skin or nail-related toxicity or any clinically related ≥ grade 3 toxicity. When panitumumab was withheld due to skin or nail toxicity the administration of GEMOX was left to the clinical discretion of the treating physician. Gemcitabine and oxaliplatin were held for ANC <1000 mcl⁻¹ or platelet count <75 000 mcl⁻¹, or other ≥ grade 3 non-haematologic toxicities. Treatment could be delayed for up to 3 weeks to allow for recovery from toxicity, if the patient did not meet re-treatment criteria after a 3-week delay, then the patient will be removed from the study. Antibiotics and steroids were permitted for panitumumab-related rash at the discretion of the treating physician.

The trial was registered at Clinical Trials.gov with the identifier NCT01308840.

Statistical analysis. The primary endpoint was the radiographic response rate by RECIST criteria to GEMOX + panitumumab and secondary endpoints included progression-free survival (PFS), OS and toxicity. A sample size of 30 patients was selected to achieve a power of 80% to detect an absolute difference in response rate of 20% (50% vs 30%) using a one-sided binomial test with a type 1 error set at 0.10. Adverse events and response data were presented as frequencies and percentages. Associated two-sided 95% confidence intervals (CIs) were constructed. Progression-free survival was defined as the time from study enrolment to date of cancer progression or death, whichever occurred first, and OS was defined as the time of enrolment in the study until the date of death from any cause. The distributions of PFS and OS were estimated using the method of Kaplan–Meier. The analysis was performed using intent-to-treat (ITT) principles. All analyses were conducted using SAS software (version 9.3; SAS Institute, Cary, NC, USA).

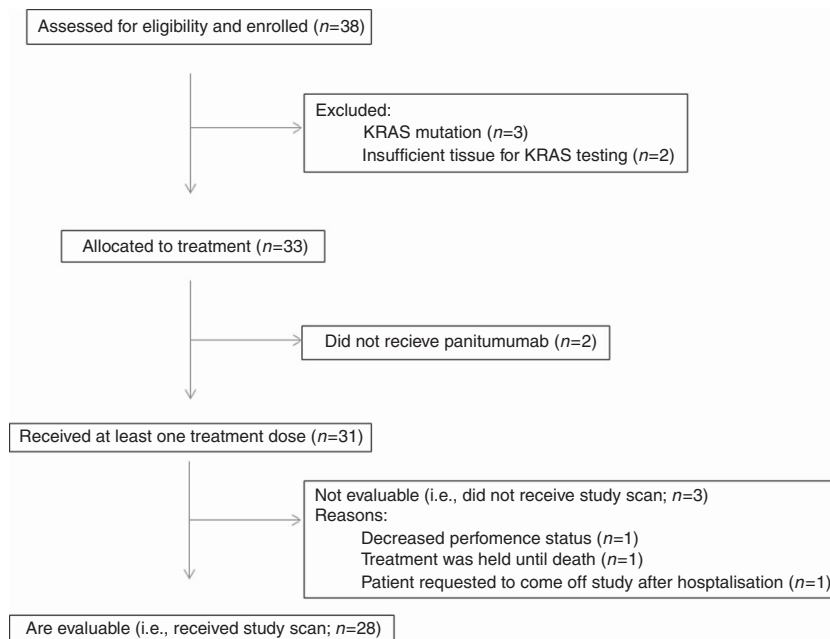


Figure 1. Study flow diagram depicts all patients assessed for eligibility and enrolled in the study.



Figure 2. Waterfall plot of percentage of tumour change from baseline in 28 evaluable patients.

RESULTS

Patient characteristics. Between 2010 and 2012, 38 patients were enrolled in the trial; of these, 28 were evaluable for efficacy and 31 for toxicity (Figures 1 and 2). Baseline characteristics are shown in Table 1. Three patients were excluded due to having a KRAS mutation and two due to having insufficient tissue for KRAS testing. Of note, a number of patients were selected based on known KRAS status established by clinical broad-based institutional tumour genotyping efforts independent of this trial. Thirty-three patients were allocated to receive treatment but 2 patients did not receive treatment as they were removed from the study due to rapidly progressing symptoms while awaiting KRAS testing. Of the 31 patients who received at least one dose of study drug, 28 patients were evaluable for response because 3 patients did not undergo initial restaging CT evaluation. Reasons for not receiving a second CT evaluation included one patient whose performance status declined rapidly, one patient who was hospitalised during the first cycle followed by an extended treatment delay and then requested to withdraw from the study, and a third patient who developed grade 3

Table 1. Baseline characteristics		
Characteristics	No. of patients 31	%
Sex		
Male	17	54.8
Female	14	45.2
Median age, years (range)	61 (36–74)	
ECOG performance status		
0	10	32.3
1	21	67.7
Extent of disease		
Locally advanced	2	6.5
Metastatic	29	93.5
Primary tumour site		
Intrahepatic cholangiocarcinoma	25	80.6
Gallbladder	3	9.7
Extrahepatic cholangiocarcinoma	3	9.7
Metastases		
Lymph nodes	24	77.4
Lung	8	25.8
Bone	4	12.9
Omentum/peritoneal	4	12.9
Adrenal	3	9.7
Pelvic soft tissue	1	3.2

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

anaemia, hyponatremia, and hyperbilirubinemia and was not able to tolerate additional chemotherapy. Of the included 31 patients, 17 were male and 14 female, the median age was 61 (36–74), 29 patients with metastatic disease and, 2 with locally advanced disease. Baseline characteristics are shown in (Table 1).

Table 2. Efficacy estimates

	Number (%; 95% CI)
	(n = 31)
Best response	
Complete response	0 (0)
Partial response	14 (45; 27.3–64.0)
Stable disease	14 (45; 27.3–64.0)
Progressive disease	3 (9.7; 2.0–25.8)
Overall response rate	14 (45; 27.3–64.0)
Disease control rate	28 (90; 79.4–100)
Median PFS	10.6 Months (4.8–24.2)
Median OS	20.3 Months (9.1–25.1)

Abbreviations: CI = confidence interval; OS = overall survival; PFS = progression-free survival.

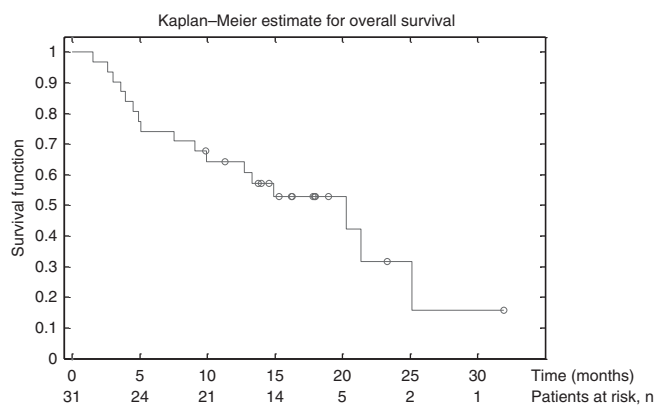


Figure 3. Kaplan-Meier curve of overall survival (OS).

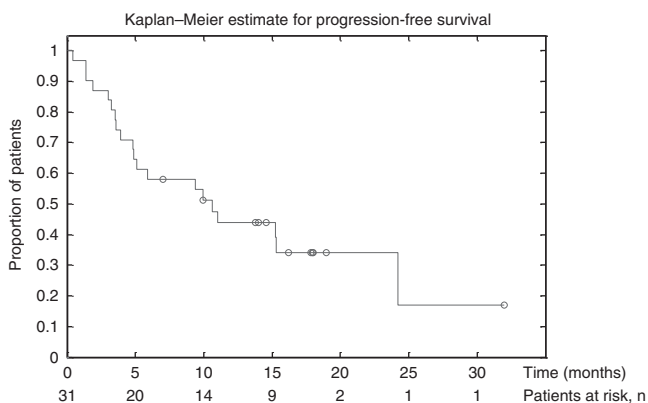


Figure 4. Kaplan-Meier curve of progression-free survival (PFS).

Dose intensity. The median number of completed treatment cycles was 6 (range 2–24). The most common indication for dose reductions of gemcitabine was thrombocytopenia and occurred in 32% of patients. Oxaliplatin was dose reduced in 42% of patients secondary to neuropathy. In the 11 patients who completed 10 or more cycles of chemotherapy, 7 patients had to permanently discontinue oxaliplatin for the remainder of their treatment. Patients with preserved performance status of ≤ 2 were allowed to remain on gemcitabine and panitumumab after discontinuation of oxaliplatin as long as they maintained clinical benefit (stable

Table 3. Adverse events

	Grade 1, n (%)	Grade 2, n (%)	Grade 3, n (%)	Grade 4, n (%)
Anaemia	10 (32)	8 (26)	7 (23)	1 (3)
Neutropenia	2 (6)	3 (10)	2 (6)	0
Leukopenia	4 (13)	8 (26)	4 (13)	3 (10)
Thrombocytopenia	10 (32)	12 (39)	2 (6)	0
Fatigue	13 (42)	5 (16)	7 (23)	0
Nausea	15 (48)	4 (13)	4 (13)	0
Abdominal pain	5 (16)	1 (3)	3 (10)	0
Diarrhoea	7 (23)	5 (16)	2 (6)	0
Infection	0	0	4 (13)	0
Neuropathy	10 (32)	5 (16)	6 (19)	0
EGFR-related rash	12 (39)	6 (19)	2 (6)	0
Elevated alkaline phosphatase	12 (39)	6 (19)	2 (6)	0
Hypomagnesaemia	10 (32)	9 (29)	2 (6)	0

Abbreviation: EGFR = epidermal growth factor receptor.

disease or objective response). Dose adjustments for panitumumab were infrequent, it was held for one patient who had a grade 3 finger fissures and dose reduced in another patient due to rash.

Efficacy. Of the 31 patients who received treatment, 14 patients had a confirmed partial response resulting in an objective response rate of 45% (95% CI 27–64) in the ITT population (Table 2). Of the 28 patients who had a post treatment follow-up scan, the response rate was 50%. Median OS was 20.3 months (95% CI 9–25 months; Figure 3) and median PFS was 10.6 months (95% CI 5–24 months; Figure 4) in the ITT population.

Toxicity. Thirty-one patients received at least one treatment cycle and were eligible for toxicity assessment. In general, the combination of GEMOX and panitumumab was tolerated reasonably well. Table 3 highlights grade 3 and 4 toxicities. Grade 3 fatigue, anaemia, neuropathy, and electrolyte changes were fairly common; there were no grade 3 or 4 cases of motor toxicity. Patients were not prophylactically treated for EGFR-related rash.

Correlative analysis. EGFR-related rash was seen in 20 patients and hypomagnesaemia in 21 patients, there was no statistical correlation between the presence of rash/hypomagnesaemia and response.

DISCUSSION

This open-label phase II trial was designed to evaluate the efficacy of the addition of the EGFR inhibitor panitumumab to standard systemic therapy among selected patients with a KRAS WT allele. The chemotherapeutic backbone of GEMOX was chosen given the favourable side-effect profile, convenient schedule (once every 2 weeks) and similar efficacy when compared to gemcitabine and cisplatin (Harder *et al*, 2006; Verderame *et al*, 2006; Andre *et al*, 2008). We found a response rate of 50%, in evaluable patients and 45% in patients who received at least one dose of study drug. The median PFS was 10.6 months and median OS 20.3 months. Furthermore, the combination of gemcitabine, oxaliplatin and panitumumab was well tolerated with manageable grade 3 and 4 toxicities.

Table 4. Completed EGFR inhibitor trials in BTC/GEMOX

Treatment	Phase	No. of subjects	ORR	mPFS (m)	mOS (m)	Reference
GEMOX GEMOX + erlotinib	III	268	16% 30%	4.2 5.8	9.5 9.5	Lee <i>et al</i> , 2012
GEMOX GEMOX + cetuximab	II	150	29% 23%	5.5 6.1	12.4 11	Malka <i>et al</i> , 2012
GEMOX GEMOX + cetuximab	II	122	17% 27%	4.1 6.7	9.8 10.6	Chen <i>et al</i> , 2013
GEMOX Panitumumab	II	31	45%	10.6	20.3	Hezel <i>et al</i> , 2010
GEMOX Cetuximab	II	30	63%	8.8	15.2	Gruenberger <i>et al</i> , 2010
Gemcitabine Capecitabine Cetuximab	II	34	18%	7.8	14.5	Rubovszky <i>et al</i> , 2013
GEMOX Capecitabine Panitumumab	II	46	33%	8.3	10	Jensen <i>et al</i> , 2012
Gemcitabine Irinotecan Panitumumab	II	31	31%	9.7	12.7	Sohal <i>et al</i> , 2013
Erlotinib (2nd line)	II	42	8%	2.6	7.5	Philip <i>et al</i> , 2006

Abbreviations: BTC = biliary tract cancer; EGFR = epidermal growth factor receptor; GEMOX = in combination with gemcitabine and oxaliplatin; mOS = median overall survival; mPFS = median progression free survival; ORR = objective response rate.

There are three randomised trials evaluating targeted EGFR inhibition in BTC and these have offered limited understanding on the impact of KRAS in response to EGFR therapy. BTC is a heterogeneous group of tumours with varying genetic drivers based on geographic region, which may contribute to variable responses to systemic therapy. Such disease heterogeneity makes comparison of trials between geographic regions and with differing composition of diseases (gallbladder, intra- and extra-hepatic tumours) challenging. A phase III trial evaluating anti-EGFR therapy among unselected populations assessed the combination of GEMOX with either erlotinib or placebo, the response rate with erlotinib was 30% compared to 16% in the placebo arm, but failed to show a significant increase in PFS or OS (Lee *et al*, 2012). Only 6 out of 60 patients with evaluable tissue were found to harbour a KRAS mutation limiting any conclusions about the impact of mutations on clinical efficacy. The largest randomised II trial with an EGFR monoclonal antibody (BINGO) enrolled 150 patients to cetuximab and GEMOX vs GEMOX alone, the addition of cetuximab failed to show a statistically significant increase in PFS (Malka *et al*, 2012). The response rate in the cetuximab-GEMOX arm was 23%, compared to 29% in the GEMOX arm with a trend towards increased OS (12.4 vs 11 months) in the GEMOX arm. Although not statistically significant, there was a trend towards increased PFS in the cetuximab arm, 6 vs 5.3 months. Tumour samples were available for genetic analysis in 50% of patients, KRAS mutations were found in 19% of patients tested, and there was no prognostic or predictive impact based on genetic characterisation identified among this cohort. A third more recent randomised phase II experience with GEMOX and cetuximab found a trend towards increased progression-free and OS in KRAS mutant patients who received cetuximab compared to those who received GEMOX (Chen *et al*, 2013). This is a surprising finding given the understanding of the molecular biology of mutant RAS signalling as well as paradigms in other tumour types where RAS mutations are predictive of EGFR insensitivity.

In the other phase II marker-driven trial of panitumumab in BTC the response rate was 33% and median PFS 8.3 months (Jensen *et al*, 2012). In comparison to that trial rates of toxicity with GEMOX and panitumumab were similar; however, we found a lower incidence of rash possibly due to the fact that we did not include capecitabine. The most recent trial evaluating panitumumab in BTC used the chemotherapeutic backbone of gemcitabine and irinotecan resulting in a response rate of 31% with median PFS 9.7 months and OS 12.9 months (Sohal *et al*, 2013). This study did not exclude patients who were KRAS mutant, and among 17 patients with adequate samples, 7 harboured KRAS mutations.

A summary of published EGFR trials in BTC is outlined in Table 4 (Gruenberger *et al*, 2010; Rubovszky *et al*, 2013).

The PFS of 10.6 months and mOS of 20.3 months were the longest among anti-EGFR trials reported to date. The response rate in the intrahepatic cholangiocarcinoma subset (25 pts) was 48%, among the non-intrahepatic cholangiocarcinoma patients there were two responses, one patient with extrahepatic cholangiocarcinoma, and one patient with gallbladder carcinoma. A notable difference in our trial compared to other GEMOX anti-EGFR combinations is that we allowed patients to remain on study receiving gemcitabine and panitumumab even after discontinuation of oxaliplatin secondary to neurotoxicity. Oxaliplatin was discontinued in 25% of patients, who then continued to receive gemcitabine and panitumumab, and it is possible that such an approach, utilising the two agents with little accumulated toxicity as maintenance therapy enabled a relatively long PFS. Given the single-arm design, it is unknown whether the favourable OS outcomes were contributed by including patients with only the KRAS WT signature.

The recent uncovering of the genetic landscape of BTC demonstrates this to be a heterogeneous disease influenced by both anatomic location within the biliary system and on geographic region (Hezel *et al*, 2010). This will not only make clinical study increasingly challenging, but also much more likely successful, as we should aim to parse out subgroups of patients

from each other to test the impact of the new and promising therapeutic targets that have emerged in BTC.

Predicting response to treatment stratified by genetic subtype may spare patients unlikely to respond to targeted therapy unnecessary toxicity, and it may result in cost saving for the healthcare system. At this time the role of anti-EGFR therapy in BTC is modest, with variable results and negative, though relatively small, unselected randomised trials. The prognostic or therapeutic significance of KRAS testing in BTC is unknown highlighting the importance of continued analysis with subsequent trial design. We realise that limitations of our study include single-arm phase II design, which may lead to selection bias and presently survival data is not yet mature. The single group nature of our phase II design precludes definitive conclusions on the added efficacy of panitumumab when added to GEMOX. Nonetheless, here we report the combination of GEMOX and panitumumab with a response rate of 45% in evaluable patients was well tolerated and led to a relatively favourable mPFS and OS. Although further randomised trials excluding KRAS mutant patients may demonstrate a benefit, this would need to be balanced against using efforts and resources in evaluating other new promising targeted agents in BTC and ongoing efforts at genetic stratification to better guide therapeutic approaches.

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CONFLICT OF INTEREST

AFH has served as a consultant to Bayer and Novartis and has received speaking honoraria from Celgene, Bayer, and Novartis. MSN has served as a consultant for Bayer. DRB is a consultant for Bio-Reference Laboratories. CF has served as a consultant for Amgen, Takeda, Metamark, Genetics, Genentech, Acceleron and Momenta Pharmaceuticals. AXZ has served as a consultant for Acceleron Pharmaceuticals, Sanofi-aventis, Exelixis, Daiichi Sankyo, Eisai, Celgene and Jennerex. The remaining authors declare no conflict of interest.

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