

A *KRAS* mutation status-stratified randomized phase II trial of gemcitabine and oxaliplatin alone or in combination with cetuximab in advanced biliary tract cancer

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Key Message: "C-GEMOX was well tolerated but failed to demonstrate significantly therapeutic superiority than GEMOX alone for ABTC in current randomized phase II trial. The trend of improvement did not correlate with KRAS mutation status. Further exploration of predictive and prognostic molecular biomarkers for selecting patients who will benefit most from EGFR targeted therapy is warranted."

ABSTRACT

Background

Previous clinical trials have not proved that adding epidermal growth factor receptor inhibitors to chemotherapy confers a survival benefit for patients with advanced biliary tract cancer (ABTC).

Whether the *KRAS* mutation status of tumor cells confounded the results of past studies is unknown.

Patients and methods

ABTC patients stratified by *KRAS* status, Eastern Cooperative Oncology Group performance status, and primary tumor location were randomized 1:1 to receive GEMOX (800 mg/m² gemcitabine and 85 mg/m² oxaliplatin) or C-GEMOX (500 mg/m² cetuximab plus GEMOX) every 2 weeks. The primary endpoint was objective response rate (ORR).

Results

The study enrolled 122 patients between December 2010 and May 2012 (62 treated with C-GEMOX and 60 with GEMOX). Compared to GEMOX alone, C-GEMOX was associated with trend to better ORR (27% vs. 15%; $p = 0.12$) and progression-free survival (PFS, 6.7 vs. 4.1 months; $p = 0.05$), but not overall survival (OS, 10.6 vs. 9.8 months; $p = 0.91$). *KRAS* mutations, which were detected in 36% of tumor samples, did not affect the trends of difference in ORR and PFS between C-GEMOX and GEMOX. The two treatment arms had similar adverse events, except that more patients had skin rashes, allergic reactions, and neutropenia in the C-GEMOX arm. Of patients with C-GEMOX, the presence of a grade 2 or 3 skin rash was associated with significantly better ORR,

PFS, and OS.

Conclusions

Addition of cetuximab did not significantly improve the ORR of GEMOX chemotherapy in ABTC, although a trend of PFS improvement was observed. The trend of improvement did not correlate with *KRAS* mutation status.

Keywords:

KRAS mutation, cetuximab, chemotherapy, biliary tract cancer

This study is registered at ClinicalTrials.gov (NCT01267344). All patients gave written informed consent.

INTRODUCTION

Biliary tract cancer (BTC), which includes intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC), and gallbladder cancer (GBC), exhibits significant variations in incidence ethnically and geographically [1]. The current standard of care for patients with advanced BTC (ABTC) is gemcitabine plus platinum combination chemotherapy. However, the achievable median overall survival (OS) with such regimens is generally less than 12 months [2]. Therefore, new combination regimens to improve treatment efficacy are warranted.

Inhibitors targeting the epidermal growth factor receptor (EGFR) signaling pathway have been evaluated for the treatment of various epithelial cell malignancies, including ABTC [3, 4]. Compared to chemotherapy alone, combining cetuximab with chemotherapy delivers substantial improvement in clinical outcomes of patients with metastatic colorectal cancer (mCRC), non-small cell lung cancer (NSCLC), and head and neck cancer [5-7]. Recent studies demonstrated *KRAS* mutations as a negative predictor for the therapeutic efficacy of anti-EGFR antibodies in mCRC patients [6]. However, *KRAS* mutations did not preclude the potential benefits of adding cetuximab in NSCLC patients with chemotherapy [8].

Two randomized trials have tested the combination of an EGFR antagonist and chemotherapy in ABTC patients. Such combination regimens achieved an objective response rate (ORR) of 24% to 30% and a median progression-free survival (PFS) of 5.8 to 6.1 months, compared to an ORR of 16% to 23% and a median PFS of 4.2 to 5.5 months with chemotherapy alone [9, 10]. Jensen et al.

showed that panitumumab plus GEMOX followed by capecitabine yielded a 33% response rate and a median PFS of 8.3 months in patients with *KRAS* wild-type (WT) ABTC [11]. However, no, randomized, large-scale ABTC trials have prospectively evaluated the influence of *KRAS* mutations on the therapeutic effect of anti-EGFR agents.

Herein we reported the results of a *KRAS* mutation status-stratified randomized phase II trial in which the impact of candidate predictive factors, including skin rash, *KRAS* mutation, and EGFR expression level on therapeutic efficacy of cetuximab in combination with GEMOX in ABTC were evaluated. *NRAS* and *BRAF* mutations, newly identified negative predictors for EGFR-targeted antibody therapy in mCRC [12], were also included in the *post-hoc* analysis.

PATIENTS AND METHODS

Eligibility criteria

Key eligibility criteria included: histologically proven advanced or metastatic BTC; at least 20 years old; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; systemic therapy-naïve; at least 1 measurable lesion; adequate organ function (supplement). Key exclusion criteria included: major surgery or radiotherapy within 4 weeks before study entry; brain metastases; active infections; pre-existing peripheral neuropathy $\geq$ grade 2; and other clinically significant comorbidities. The ethics committees of each participating center approved this study. This study is registered at ClinicalTrials.gov (NCT01267344). All patients gave written informed consent.

Study design and randomization

This was an open-label randomized phase II trial conducted at 12 Taiwan Cooperative Oncology Group (TCOG)-affiliated medical centers. Eligible patients were stratified by *KRAS* mutation status (wild-type vs. mutated) of tumors, ECOG PS (0 vs. 1), and primary tumor location (IHCC vs. EHCC/GBC) then randomly assigned in a 1:1 ratio to receive either GEMOX or C-GEMOX. Randomization was performed centrally at the Statistical Center of TCOG, Taiwan, with a permuted block randomization.

The primary endpoint of this study was ORR according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Secondary endpoints included disease control rate (DCR, objective response plus stable disease [SD] ≥ 16 weeks), PFS, OS, safety profile, and treatment efficacy across *KRAS* mutation status subgroups. Tumor responses were determined by radiologists at each participating center who were blinded to treatment assignments.

Procedures

GEMOX treatment was consisted of a fixed-dose rate (FDR, 10 mg/m²/min) intravenous infusion of 800 mg/m² gemcitabine followed by a 2-hr infusion of 85 mg/m² oxaliplatin every 2 weeks. Patients in the C-GEMOX arm received an intravenous infusion of 500 mg/m² cetuximab on day 1 before each GEMOX cycle. Treatment courses were continued until disease progression, unacceptable toxicity, or withdrawal of consent. Adverse reactions were assessed according to the Common Terminology Criteria for Adverse Events, version 4.0. Dose modifications were made

based on the most serious adverse events encountered during the preceding cycle (see supplement for dose modification plan details). A new treatment cycle would be given when absolute neutrophil and platelet counts were no less than 1,500/ μ L and 75×10^3 / μ L, respectively, and any treatment-related non-hematologic toxicities had resolved to grade 1 or less.

At study entry and before each cycle of treatment, patients underwent a complete medical history review, physical examination, routine hematological and biochemical analyses. Computed tomography (CT) or magnetic resonance imaging (MRI) to define the tumor extent and tumor response were performed at study entry and every 8 weeks during treatment. Patients with first radiographical objective tumor response had confirmatory imaging examination 4 weeks later, according to RECIST 1.1. Archived tumor tissue sections and peripheral blood mononuclear cells were prospectively collected from all patients for biomarkers and pharmacogenomics studies at study entry.

Biomarker analysis

Genomic DNA was extracted from tumor tissues using TaKaRa DEXPAT™ (TaKaRa Bio Inc, Japan). *KRAS* mutation status was then determined using direct sequencing of the coding sequences of exons 2 and 3, as well as the TaqMan® assay for *KRAS* codon 12/13 mutations using the LightMix® Kit (TIB MOLBIOL GmbH, Germany). Tumors were considered to be *KRAS* mutation if any test was positive. The methodology of *BRAF*/*NRAS* mutation and EGFR protein expression is described in the supplement.

Statistical analysis

We assumed uninterested and interested ORRs of 20% and 30%, respectively, for the C-GEMOX arm; the GEMOX arm served as a control. Given 60 assessable patients per treatment arm, the probability of correctly ranking the two arms according to the observed ORR would be 90% [13]. Chi-squared or Fisher's exact tests were applied to compare discrete variables. PFS and OS were estimated using the Kaplan-Meier method and compared by two-sided log-rank tests. Exploratory univariate and multivariate logistic regression for ORR and Cox regression for survival were used. All clinical data were gathered and analyzed at the Statistical Center, TCOG using SAS software, version 9.1. Two-sided *p* value less than 0.05 were considered statistically significant.

RESULTS

Patient enrollment

Between December 2010 and May 2012, 122 of 266 pre-screened patients were enrolled (Figure 1). Baseline demographics were well-balanced between the two treatment arms (Table 1). As of April 30, 2013, 4 patients (1 in C-GEMOX arm and 3 in GEMOX arm) remained on the study treatment and the median follow-up time of the intent-to-treat (ITT) population was 10.1 months (range, 0.9–24.4 months).

KRAS mutations and EGFR positivity (+ to +++) were detected in 36% and 63% of tumors, respectively. Of *KRAS* mutations, Gly12Asp was the most common mutation (64%), followed by Gly13Asp (14%). The incidence of *KRAS* mutations and EGFR positivity was similar between

IHCC, EHCC, and GBC.

Treatment efficacy

Compared to GEMOX alone, C-GEMOX-treated patients had a significantly longer treatment duration (median: 4.9 vs. 3.0 months; $p = 0.01$) and received more treatment cycles (median: 10 vs. 6.5 cycles; $P = 0.03$). Tumor response was assessed in 118 patients (Figure 1). In ITT analysis, C-GEMOX was associated with a trend of improvement in ORR (27% [95% CI, 17%–40%] vs. 15% [95% CI, 7%–27%]; $p = 0.12$) and median PFS (6.7 months [95% CI, 5.0–8.1 months] vs. 4.1 months [95% CI, 2.3–6.1 months]; $p = 0.05$), and significant better long-term DCR (58% vs. 37%; $p = 0.02$) compared with GEMOX (Table S1). However, the median OS was similar (10.6 months [95% CI, 8.8–13.1 months] vs. 9.8 months [95% CI, 6.7–12.8 months]; $p = 0.91$) (Figure 2). The ORR and DCR in IHCC, EHCC and GBC, respectively, were shown in table S2.

After withdrawal from study treatment, 48% of C-GEMOX-treated patients and 63% of GEMOX-treated patients received second-line chemotherapy ($p = 0.10$). The most commonly used second-line chemotherapy agents were cisplatin plus intravenous fluorouracil (>90%) followed by oral capecitabine or S-1 monotherapy.

Safety and dose intensity

Incidence of significant adverse events were similar between two study arms, except significantly more grade 3-4 neutropenia and grade 2-3 skin rash in C-GEMOX arm (Table 2). No patients discontinued cetuximab treatment because of skin toxicity, and only 1 patient required a

cetuximab dose reduction. Chemotherapy dose reduction due to adverse events was required in 60% and 48% of patients in the C-GEMOX and GEMOX arms, respectively. Treatment-related mortality occurred in one GEMOX-treated patient. The dose-intensities of gemcitabine and oxaliplatin were similar between two arms (Table S3).

Factors correlated with treatment efficacy

Patients with wild-type *KRAS* tumors had a significantly better long-term DCR and trends toward longer median PFS and OS than patients with *KRAS* mutated tumors. However, EGFR positivity did not affect clinical outcomes (Table S4).

A consistent trend favoring the C-GEMOX arm in terms of ORR and PFS was observed for all analyzed subgroups (Table S5 and Figure S1), the hazard ratio (HR) for PFS in patients with *KRAS* wild-type and mutated tumors was 0.65 (95% CI=0.41-1.03) and 0.73 (95% CI=0.39-1.35), respectively. Multivariate analyses of predictive or prognostic factors for ORR and PFS were shown in tables S6–7, in which C-GEMOX treatment was a borderline predictive factor for ORR (odds ratio=2.60, 95% CI=0.96-7.06, $p=0.06$) and a significant prognostic factor for PFS (HR=0.67, 95% CI=0.45-0.98; $p = 0.04$).

Of patients with C-GEMOX, those with grade 2 or higher skin rashes had significantly better ORR, DCR, PFS and OS than patients with less severe or no skin rashes (Table S8 and Figure S2).

Post-hoc biomarker analyses

In *post-hoc* analysis, there were 119 patients with enough tumor samples for *NRAS* and *BRAF*

mutation analysis, *NRAS* mutations were detected in 9 tumors, in which best tumor response was 1 PR and 3 SD ≥ 16 weeks. Three tumors exhibited both *KRAS* and *NRAS* mutations. No *BRAF* mutations (V600E) were detected. Therapeutic efficacy based on *RAS* mutation (*KRAS* plus *NRAS*) was summarized in Table S5. Overall, the results were similar to the *KRAS* analysis in 122 patients.

DISCUSSION

This study is the first randomized trial to evaluate the efficacy of cetuximab plus chemotherapy stratified by tumor *KRAS* mutation status and the one in Asian ABTC patients, as shown table 3. Addition of cetuximab only marginally, not significantly, improve the ORR and PFS of GEMOX chemotherapy in ABTC in this phase II trial. The results are largely consistent with that of two previous randomized trials evaluating adding either erlotinib or cetuximab to GEMOX in ABTC [9, 10].

The 36% of *KRAS* mutation rate in current study by the TaqMan[®] assay was similar to that of 41% by pyrosequencing in the report of Sohal et al. [14] and higher than the 10%–26% mutation rate in previous reports using direct sequencing [9, 15–17] However, our analysis showed patients with *KRAS* mutated ABTC had marginally lower responses and shorter survival than those with *KRAS* wild-type tumor, regardless of treatment group (Table S4). Whether *KRAS* mutation to be a negative prognostic factor for ABTC, as it did for IHCC after hepatectomy [16], requires further validation in larger study cohort. In addition, although patients with C-GEMOX did not achieve significantly better ORR and PFS than those with GEMOX alone, the trend of improvement in

ORR and PFS with C-GEMOX was consistently observed in both *KRAS* wild-type and mutated subpopulations (Figure S1). A similar trend was also observed in the *post-hoc* analysis of BINGO study in which, although not statistically significant, patients with C-GEMOX had numerical higher 4-months PFS rate as compared to patients with GEMOX alone regardless of *KRAS* mutation status [10]. Our *post-hoc* analysis further demonstrated a similar trend in *KRAS/NRAS* wild-type and mutated subgroups (table S5), indicating *RAS* mutation didn't preclude the efficacy of cetuximab in ABTC.

We chose GEMOX as the backbone chemotherapy because modified GEMOX was the first regimen to demonstrate the survival benefit of systemic chemotherapy over best supportive care in ABTC patients and the experience of better compliance of GEMOX than GC in clinical practice [18]. However, we adopted the modified GEMOX by replacing the administration schedule of gemcitabine from 900 mg/m² bolus injection with 800 mg/m² FDR infusion, based on previously reported pharmacokinetic advantage of FDR [19]. With such a relatively low-dose of gemcitabine and oxaliplatin regimen, our patients had comparable clinical outcomes with lower incidence of all grade sensory neuropathy and grade 3-4 neutropenia as compared to those with 100 mg/m² oxaliplatin and 1,000 mg/m² gemcitabine in the Korean and BINGO trials [9, 10]. It is also interested to note that of patients with either GC or GEMOX in randomization trials for ABTC, the ORR and median PFS in trials from western countries (ABC02 and BINGO trials) were generally better than those from Asian countries (BT-22, Korean and current studies), with ORR of 23-26%

versus 15%-20% and PFS of 5.5-8.0 months *versus* 4.1-5.8 months [9, 10, 20]. Whether the therapeutic effects of gemcitabine and platinum doublets in ABTC would be affected by ethnicity or the choice of platinum agent can only be answered by well-designed, prospective, large-scale randomization study.

In current study, the trend toward better therapeutic efficacy of C-GEMOX was not affected by the status of EGFR expression and *KRAS* mutation in ABTC. Recent studies linked the molecular abnormalities in cancer cells and the activation of EGFR signaling of intratumor mesenchymal stem cells with angiogenic and/or inflammatory responses in the tumor microenvironment, which can play crucial roles in cancer progression [21, 22]. Therefore, targeting EGFR-expressing stromal cells and the induction of antibody-dependent cell-mediated cytotoxicity are potential mechanisms to explain the effectiveness of cetuximab in EGFR-negative and/or *KRAS*-mutated tumors. A more comprehensive illustration of how molecular abnormalities regulate the tumor microenvironment will facilitate identification of optimal BTC patient subgroups for EGFR-targeted therapy.

The close correlation between the severity of skin rashes and cetuximab efficacy in current study was consistent with the findings in two previous studies of anti-EGFR therapy in ABTC, a Belgian study with cetuximab and a Korean study with erlotinib [9, 15]. These findings concur with the predictive role of skin rashes for EGFR antagonists in patients with mCRC and NSCLC [23, 24]. On the other hand, three trials combining chemotherapy with panitumumab in ABTC and the BINGO study did not describe such an association [10, 11, 14, 25].

There are several limitations in our study. Firstly, randomized phase II trials can help balance patient heterogeneity and avoid selection bias, however, insufficient patient number frequently undermines the statistical power of such studies, especially when the effect of improvement is moderate or lower, as in the current study. Secondly, emerging data suggests that some BTC tumors could be driven by oncogenic alterations such as *MET* amplification, *FGFR2* translocation, and *ROS1* fusion, which might lead to anti-EGFR therapy resistance [26-28]. However, these biomarkers were not validated in ABTC studies, and not included as stratification or enrichment factors in current study.

In conclusion, C-GEMOX was well tolerated but failed to demonstrate significantly therapeutic superiority than GEMOX alone for ABTC in current randomized phase II trial. Further exploration of predictive and prognostic molecular biomarkers for selecting patients who will benefit most from EGFR targeted therapy is warranted.

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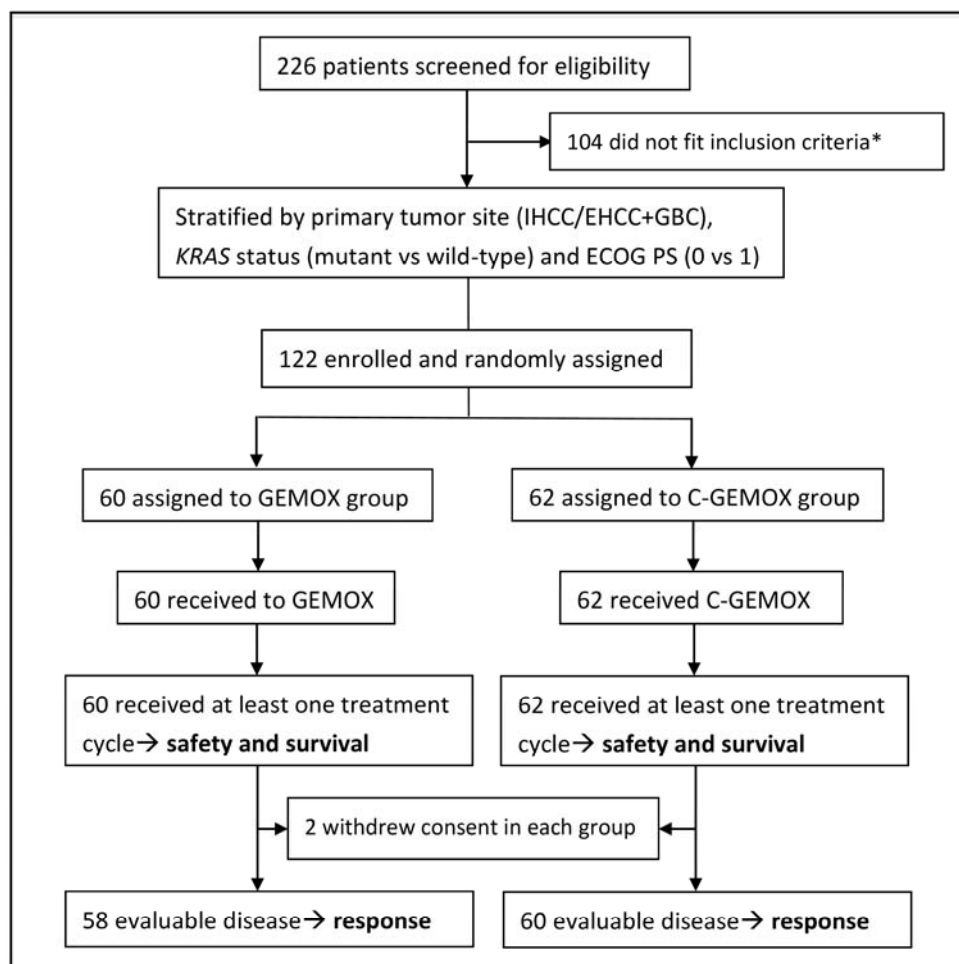
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FIGURE LEGENDS

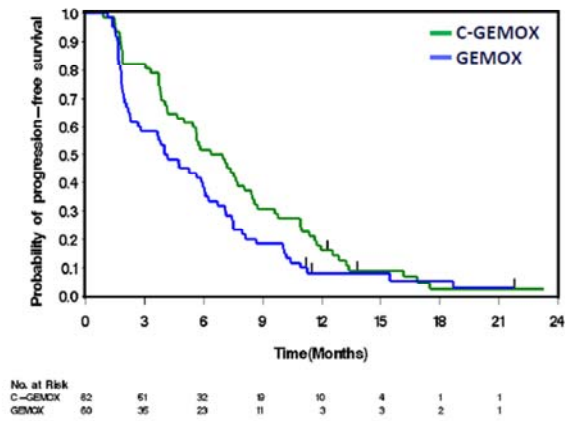
Figure 1. Study flow diagram. IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GB, gallbladder; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

* The cause of ineligibility (number of patients): previous chemotherapy (5), patient refusal (18), physician judgment (co-morbid conditions/compliance) (10), other concomitant cancer (4), no measurable lesions (5), age/performance status (9), central nervous system metastasis (1), no adequate tissue/*K-RAS* failure (10), liver enzyme/bilirubin (30), renal function (3), hemoglobin/platelet (9).

Figure 2. (A) Progression-free survival (PFS) and (B) overall survival (OS) in all patients.



A



B

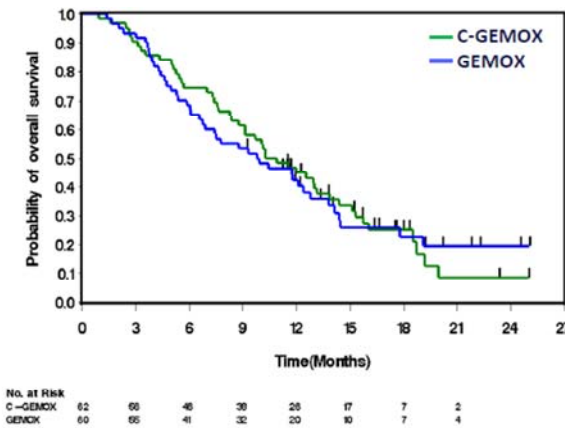


Table 1. Demographic and clinical characteristics of randomized patients

	C-GEMOX <i>n</i> = 62	GEMOX <i>n</i> = 60	<i>p</i> ^{**}
Age, years			
Median (range)	61 (32–78)	59 (32–80)	
Gender			0.72
Male	28 (45%)	30 (50%)	
Female	34 (55%)	30 (50%)	
ECOG PS			1.00
0	18 (29%)	17 (28%)	
1	44 (71%)	43 (72%)	
Primary site			0.61
Intrahepatic	44 (71%)	45 (75%)	
Extrahepatic	9 (15%)	10 (17%)	
Gallbladder	9 (15%)	5 (8%)	
T stage			1.00
≤ T ₃	49 (79%)	47 (78%)	
T ₄	13 (21%)	13 (22%)	
N stage			0.54
N ₀	15 (24%)	18 (30%)	
N ₁	47 (76%)	42 (70%)	
Disease status at entry			0.34
Locally advanced	23 (37%)	17 (28%)	
Distant metastasis	39 (63%)	43 (72%)	
Previous surgery			0.72
No	35 (57%)	36 (60%)	
Yes	27 (44%)	24 (40%)	
KRAS status			0.85
Negative	39 (63%)	39 (65%)	
Positive	23 (37%)	21 (35%)	
EGFR expression [*]			0.06
Negative	17 (28%)	28 (47%)	
Positive	43 (72%)	32 (53%)	

^{*}Only 120 tumors were available for EGFR expression assessment (60 in each arm).

^{**}*p* was obtained by Fisher's exact test, 2-sided.

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin;

ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor

Table 2. Treatment-related adverse events

	All grades			Grades 3–4		
	C-GEMOX	GEMOX	<i>p</i> ^{**}	C-GEMOX	GEMOX	<i>p</i> ^{**}
	<i>n</i> = 62	<i>n</i> = 60		<i>n</i> = 62	<i>n</i> = 60	
Neutropenia	23 (37%)	11 (18%)	0.03	11 (18%)	2 (3%)	0.02
Thrombocytopenia	43 (69%)	40 (67%)	0.85	8 (13%)	2 (3%)	0.10
Oral mucositis	27 (44%)	15 (25%)	0.04	2 (3%)	1 (2%)	1.00
Diarrhea	17 (27%)	13 (22%)	0.53	2 (3%)	2 (3%)	1.00
Nausea	32 (52%)	32 (53%)	0.86	0 (0%)	2 (3%)	0.24
Vomiting	17 (27%)	21 (35%)	0.44	0 (0%)	0 (0%)	-
Fatigue	38 (61%)	45 (75%)	0.12	2 (3%)	2 (3%)	1.00
ALT increased	27 (44%)	15 (25%)	0.04	2 (3%)	1 (2%)	1.00
Anorexia	43 (69%)	44 (73%)	0.69	2 (3%)	5 (8%)	0.27
Neuropathy	33 (53%)	34 (57%)	0.72	5 (8%)	5 (8%)	1.00
Allergic reaction	15 (24%)	4 (7%)	0.01	1 (2%)	0 (0%)	1.00
Skin rash	51 (82%)	2 (3%)	<0.0001	27 (44%)	0 (0%)	<0.0001 [*]

Data are number of patients with one or more episodes of an adverse event (% of patients).

^{*}Grade 2–3 skin rash in the C-GEMOX versus GEMOX arms

^{**}*p* was obtained using Fisher's exact test, 2-sided.

ALT, alanine aminotransferase; C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin

Table 3. Selected trials of EGFR antibody in advanced biliary tract cancer

Regimen, (n)	KRAS wild-type n/N (%)	ORR (%)	Median PFS (months)	Median OS (months)
Rubovszky et al [29] Gemcitabine and capecitabine + cetuximab (34)	24/24 (100%)	18%	8.6	15.7
Borbath et al [15] Gemcitabine + cetuximab (44)	20/27 (74%) [§]	20%	5.8	13.5
Gruenberger et al [17] GEMOX + cetuximab (30)	27/30 (90%)	63%	8.8	15.2
BINGO [10] GEMOX+ cetuximab (76)	61/75 (81%) [§]	24%	6.1	11.0
GEMOX (74)		23%	5.5	12.4
TCOG T1210 (present study) GEMOX+ cetuximab (62)	78/122(64%)*	27%	6.7	10.6
GEMOX (60)		15%	4.1	9.8
Jensen et al [11] GEMOX + panitumumab and capecitabine (46)	46/46 (100%)	33%	8.3	10.0
Sohal et al [14] Gemcitabine and irinotecan + Panitumumab (35)	10/17 (59%) [§]	32%	9.7	12.9
Hezel et al [26] GEMOX + panitumumab (31)	31/31 (100%)	45%	10.6	20.3

Online only.

GEMOX, gemcitabine and oxaliplatin; ORR, objective response rate; PFS, progression-free survival; OS, overall survival

* Prospective analysis showed *KRAS* mutation didn't preclude the efficacy of cetuximab in advanced biliary tract cancer

[§] Post-hoc analysis showed no significant difference of treatment efficacies between the *KRAS* wild-type and mutated subpopulations

Supplement

Adequate organ function

neutrophil count $\geq 1,500/\mu\text{L}$, hemoglobin ≥ 10 g/dL, platelet count $\geq 100 \times 10^3/\mu\text{L}$, creatinine ≤ 1.5 times the upper limit of normal (ULN), calculated glomerular filtration rate ≥ 40 mL/min, serum bilirubin ≤ 1.5 times the ULN, alanine aminotransferase ≤ 2.5 times the ULN.

Dose modification

Dose levels were as follows: level 1 (600 mg/m² gemcitabine and 85 mg/m² oxaliplatin), level 2 (600 mg/m² gemcitabine and 65 mg/m² oxaliplatin), and level 3 (400 mg/m² gemcitabine and 65 mg/m² oxaliplatin). If patients had grade 2, 3 and 4 adverse events of neutropenia and thrombocytopenia, the dose of chemotherapeutic drug was adjusted to level 1, level 2, and level 3, respectively. Absolute neutrophil count (ANC) ≥ 1500 cells/ μL and platelet count $\geq 75 \times 10^3$ cells/ μL were required before each treatment cycle. If ANC < 1500 cells / μL , platelet count $< 75 \times 10^3$ cells/ μL or chemotherapy-related $\geq$ grade 2 non-hematologic toxicities persist more than 4 weeks, GEMOX were discontinued afterwards. If patients had gastrointestinal toxicities, such as stomatitis and diarrhea which were grade 3 or higher, the dose of chemotherapeutic drugs were adjusted to level 2 for all subsequent doses. Additionally, for grade 2 more than 7 days or higher than grade 2 of neurotoxicity,

oxaliplatin was adjusted to 65 mg/m²; for the second time of severe neurotoxicity despite a dose reduction, oxaliplatin was withdrawn but therapy with cetuximab and gemcitabine was continued at the same dose and schedule. In the event of neurotoxicity, only oxaliplatin was interrupted, reduced, or discontinued. GEMOX was not withheld if the cetuximab infusion was suspended due to skin toxicity. The first time a patient experienced grade 3 skin toxicity, the subsequent cetuximab dose was delayed for up to 14 days without changing the dose level. If grade 3 skin toxicity occurred a second or third time, cetuximab treatment could be delayed again for up to 14 days, and the dose was reduced to 400 mg/m² or 300 mg/m², respectively.

Biomarker analysis

For *post-hoc* analysis, the LightMix *BRAF* V600E Kit (TIB MOLBIOL GmbH, Berlin, Germany), and the *NRAS* Pyro Kit (Qiagen, Venlo, Netherlands) with the PyroMark Q24 system (Qiagen, Venlo, Netherlands) were used to detect *BRAF* and *NRAS* mutations, respectively. All procedures were performed according to the manufacturers' instructions. EGFR protein expression was determined by immunohistochemical (IHC) staining (primary antibody: DAKO, H11 Cytomation Denmark A/S, Glostrup, Denmark), followed by staining with the ultraView Universal DAB detection kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). Tumor EGFR protein expression was considered weak (1+), moderate (2+), or strong

(3+) if 1% to 10%, 10% to 50%, or >50% of tumor cells were positive, respectively.

Table S1. Treatment efficacy stratified by treatment arm

	C-GEMOX <i>n</i> = 62	GEMOX <i>n</i> = 60	<i>p</i> **
Objective response rate	17 (27%; 17–40)	9 (15%; 7–27)	0.12
Disease control rate*	36 (58%; 45–71)	22 (37%; 25–50)	0.02
Partial response [§]	15 (24%; 14–37)	9 (15%; 7–27)	0.26
Stable disease	34 (55%; 42–68)	27 (45%; 32–58)	0.37
Progressive disease	9 (15%; 7–26)	22 (37%; 25–50)	0.01
Not evaluable	2 (3%; 0.4–11)	2 (3%; 0.4–12)	1.00
PFS (months)	6.7 (5.0–8.1)	4.1 (2.3–6.1)	0.05
OS (months)	10.6 (8.8–13.1)	9.8 (6.7–12.8)	0.91

Online only.

Data are number (%; 95% CI) for response and time (95% CI) for survival

*Disease control rate = complete response + partial response + stable disease ≥ 16 weeks.

**Objective response rates and disease control rates were compared using Fisher's exact test, 2-tailed; PFS and OS were compared using log-rank tests.

[§]Three patients underwent secondary curative resections after achieving partial response with C-GEMOX

Abbreviations: C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin; OS, overall survival; PFS, progression-free survival.

Table S2. ORR and DCR in IHCC, EHCC and GBC

C-GEMOX	IHCC <i>n</i> = 44	EHCC <i>n</i> = 9	GBC <i>n</i> = 9
Objective response rate	32%	11%	22%
Disease control rate*	84%	78%	82%

GEMOX	<i>n</i> = 45	<i>n</i> = 10	<i>n</i> = 5
Objective response rate	18%	0%	20%
Disease control rate*	60%	70%	40%

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*Disease control rate = complete response + partial response + stable disease $\geq$ 16 weeks

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin.

IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer

Table S3. Drug administration and dose-intensity

	C-GEMOX <i>n</i> = 62	GEMOX <i>n</i> = 60
Gemcitabine		
Duration (weeks)	20.9 (2–76.6)	14.6 (2–104.3)
Total dose (% of theoretical dose)	88 (46–100)	86 (40–100)
Dose-intensity*	343 (161–400)	358 (137–420)
Patients with dose reduction, <i>n</i> (%)	32 (52%)	26 (43%)
Patients with delayed administration, <i>n</i> (%)	49 (79%)	37 (62%)
Oxaliplatin		
Duration (weeks)	17.5 (2–56.1)	14.6 (2–104.3)
Total dose (% of theoretical dose)	93 (47–105)	90 (34–105)
Dose-intensity ¹	37 (20–43)	40 (20–44)
Patients with dose reduction, <i>n</i> (%)	31 (50%)	21 (35%)
Patients with delayed administration, <i>n</i> (%)	50 (81%)	37 (62%)
Cetuximab		
Duration (weeks)	20.0 (2–76.6)	
Dose-intensity ¹	232 (186–252)	
Patients with dose reduction, <i>n</i> (%)	3 (5%)	
Patients with delayed administration, <i>n</i> (%)	46 (74%)	

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Data are percentage or medians (range)

*Defined as the total dose received (mg/m²) divided by total treatment duration (weeks) (i.e., the time elapsed between the first and the last injection of the considered drug, plus two weeks)

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin.

Table S4.**Associations between treatment efficacy and *KRAS* mutation status and EGFR expression**

	<i>KRAS</i> wild-type <i>n</i> = 78	<i>KRAS</i> mutant <i>n</i> = 44	<i>p</i> ^{**}
Objective response rate	23%	18%	0.65
Disease control rate [*]	56%	32%	0.01
Median PFS (95% CI, months)	6.0 (5.0–7.4)	3.8 (2.0–6.7)	0.42
Median OS (95% CI, months)	11.5 (9.3–13.0)	8.0 (6.0–13.1)	0.74
	EGFR-negative <i>n</i> = 45	EGFR-positive <i>n</i> = 75	
Objective response rate	22%	20%	0.82
Disease control rate [*]	40%	52%	0.26
Median PFS (95% CI, months)	4.7 (3.8–6.4)	5.8 (4.1–7.1)	0.89
Median OS (95% CI, months)	10.1 (6.9–14.4)	10.9 (8.8–13.0)	0.84

Online only.

^{*}Disease control rate = complete response + partial response + stable disease ≥ 16 weeks.

^{**}ORR and DCR compared using Fisher's exact tests, 2-sided; PFS and OS compared using log-rank tests.

EGFR, epidermal growth factor receptor; CI, confidence interval; PFS, progression-free survival; OS, overall survival.

Table S5.

Treatment efficacy (ORR, DCR, PFS) stratified by *KRAS* mutation, *RAS* mutation, and EGFR expression

	Case number		ORR (% , 95% CI)			DCR (% , 95% CI)			PFS (months, 95% CI)		
	C-GEMOX	GEMOX	C-GEMOX	GEMOX	<i>p</i>	C-GEMOX	GEMOX	<i>p</i>	C-GEMOX	GEMOX	<i>p</i>
<i>KRAS</i> (n = 122)											
Wild-type	39	39	31 (17–48)	15 (6–31)	0.18	64 (47–79)	49 (32–65)	0.25	7.1 (5.5–8.5)	5.6 (3.8–7.1)	0.06
Mutant	23	21	22 (8–44)	14 (3–36)	0.70	48 (27–69)	14 (3–36)	0.02	5.6 (3.8–8.7)	2.0 (1.8–4.7)	0.31
<i>RAS</i> (n = 119)											
Wild-type	37	32	32 (18–50)	16 (5–33)	0.16	68 (50–82)	44 (26–62)	0.06	7.2 (5.6–8.6)	5.0 (2.7–7.4)	0.03
Mutant	24	26	21 (7–42)	15 (4–35)	0.72	46 (26–67)	27 (12–48)	0.24	5.6 (3.8–8.7)	2.9 (1.9–5.9)	0.31
EGFR expression (n = 120)											
Negative	17	28	35 (14–62)	14 (4–33)	0.14	59 (33–82)	29 (13–49)	0.06	7.2 (3.8–9.8)	4.1 (1.9–5.6)	0.29
Positive	43	32	23 (12–39)	16 (5–33)	0.56	58 (42–73)	44 (26–62)	0.25	5.8 (5.0–8.5)	4.8 (2.3–7.1)	0.05

Online only

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin; CI, confidence interval; DCR, disease control rate; EGFR, epidermal growth factor receptor; GEMOX, gemcitabine plus oxaliplatin; ORR, objective response rate; PFS, progression-free survival.

Table S6. Multivariate logistic regression analysis for ORR

Variables		Univariate			Multivariate*		
Variable		OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
Age							
<60 years		1.00			1.00		
≥60 years		0.43	0.17–1.06	0.07	0.30	0.11–0.84	0.02
Assigned Treatment							
GEMOX		1.00			1.00		
C-GEMOX		2.14	0.87–5.28	0.10	2.60	0.96–7.06	0.06
ECOG PS							
0		1.00			-	-	-
1		1.12	0.42–5.28	0.82	-	-	-
Gender							
Male		1.00			1.00		
Female		1.97	0.80–4.85	0.14	3.05	1.09–8.56	0.03
Primary site							
Intrahepatic		1.00			1.00		
Extrahepatic or GB		0.42	0.13–1.33	0.14	0.37	0.11–1.27	0.11
Disease Status at Diagnosis							
Locally advanced		1.00			-	-	-
Metastatic disease		0.73	0.30–1.79	0.49	-	-	-
Prior surgery							
No		1.00			-	-	-
Yes		1.53	0.64–3.65	0.34	-	-	-
KRAS							
Negative		1.00			1.00		
Positive		0.74	0.29–1.88	0.53	0.57	0.20–1.63	0.30
EGFR expression							
Negative		1.00			1.00		
Positive		0.87	0.35–2.16	0.77	0.61	0.22–1.67	0.34

Online only.

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin; OR, odds ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GB, gallbladder; EGFR, epidermal growth factor receptor.

*All predictors with $p < 0.2$ in the univariate analysis, and *KRAS* mutation and EGFR expression status were included.

Table S7. Multivariate Cox regression analysis for PFS

Variables		Univariate			Multivariate*		
Variable		HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age							
<60 years		1.00			1.00		
≥60 years		1.38	0.95–2.00	0.09	1.44	0.99–2.12	0.06
Assigned Treatment							
GEMOX		1.00			1.00		
C-GEMOX		0.70	0.48–1.01	0.06	0.67	0.45–0.98	0.04
ECOG PS							
0		1.00			-	-	-
1		1.07	0.71–1.59	0.75	-	-	-
Gender							
Male		1.00			1.00		
Female		0.68	0.47–0.98	0.04	0.63	0.43–0.92	0.02
Primary site							
Intrahepatic		1.00			-	-	-
Extrahepatic or GB		1.29	0.85–1.94	0.23	-	-	-
Disease Status at Diagnosis							
Locally advanced		1.00			1.00		
Metastatic disease		1.31	0.89–1.94	0.18	1.20	0.80–1.80	0.39
Prior surgery							
No		1.00			-	-	-
Yes		0.80	0.55–1.17	0.25	-	-	-
KRAS							
Negative		1.00			1.00		
Positive		1.17	0.80–1.71	0.42	1.25	0.84–1.87	0.27
EGFR expression							
Negative		1.00			1.00		
Positive		1.03	0.70–1.51	0.89	1.15	0.77–1.72	0.50

Online only.

C-GEMOX, cetuximab plus gemcitabine and oxaliplatin; GEMOX, gemcitabine plus oxaliplatin; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GB, gallbladder; EGFR, epidermal growth factor receptor.

*All predictors with $p < 0.2$ in the univariate analysis, and *KRAS* mutation and EGFR expression status were included.

Table S8.**Rash development and response to treatment in patients treated with cetuximab plus chemotherapy**

	Acne rash, grade 2–3 <i>n</i> = 27	Acne rash, grade 0–1 <i>n</i> = 35	<i>p</i> ^{**}
Objective response rate	44%	14%	0.011
Disease control rate [*]	74%	46%	0.038
Median PFS (months)	7.6 (5.6–11.0)	5.0 (3.7–7.7)	0.011
Median OS (months)	13.1 (10.3–18.7)	8.3 (5.6–12.0)	0.013

Online only.

^{*}Disease control rate = complete response + partial response + stable disease ≥ 16 weeks.

^{**}Objective response rate and disease control rate compared using Fisher's exact tests, 2-sided; PFS and OS compared using log-rank test

OS, overall survival; PFS, progression-free survival.

Supplementary figure legends

Supplementary figure 1.

(A) Objective response rate (ORR) and (B) progression-free survival (PFS) in subgroup analysis

ECOG=Eastern Cooperative Oncology Group;

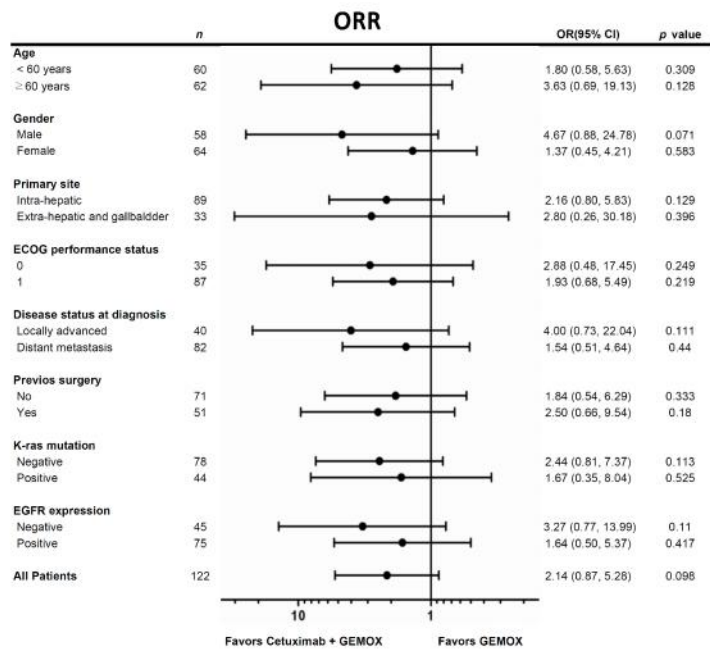
p value: logistic regression analysis (for ORR); Cox proportional hazards analysis (for PFS)

Supplementary figure 2.

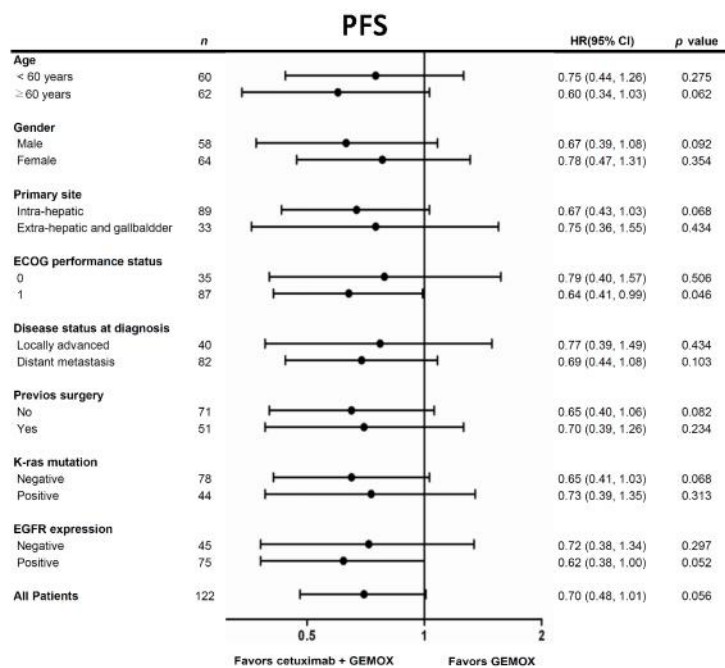
(A) Progression-free survival (PFS) and (B) overall survival (OS) in patients receiving C-GEMOX

(cetuximab plus gemcitabine and oxaliplatin) who experienced grade 0-1 and grade 2-4 acne rash.

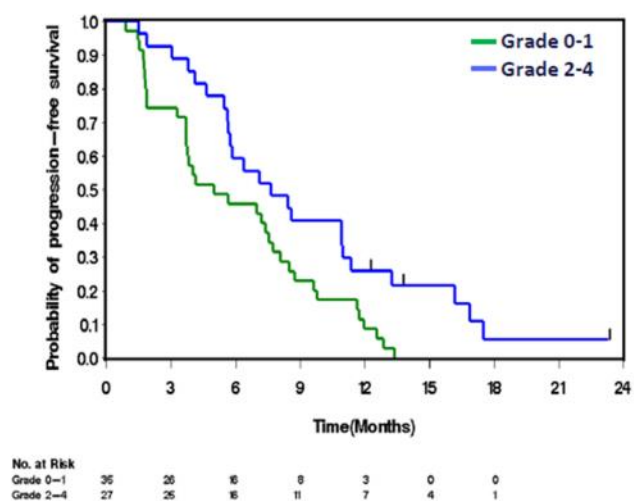
A



B



A



B

