Trastuzumab plus FOLFOX for HER2-positive biliary tract cancer refractory to gemcitabine and cisplatin: a multi-institutional phase 2 trial of the Korean Cancer Study Group (KCSG-HB19–14)

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Summary

Background HER2 overexpression or amplification, which is present in 15% of all cases of biliary tract cancer, has been identified as a druggable molecular target by genomic profiling. In the phase 3 ABC-06 trial, the folinic acid, fluorouracil, and oxaliplatin (FOLFOX) regimen showed a survival benefit compared with active symptom control as second-line therapy for biliary tract cancer. We aimed to evaluate the clinical activity of FOLFOX plus anti-HER2 antibody trastuzumab as a second-line or third-line treatment for HER2-positive biliary tract cancer.

Methods This study was an investigator-initiated, open-label, non-randomised, single-arm, multi institutional, phase 2 trial in participants aged 19 years or older with HER2-positive (defined as immunohistochemistry 3+ or immunohistochemistry 2+ and in-situ hybridisation positive or *ERBB2* gene copy number ≥6·0 by next-generation sequencing) biliary tract cancer (intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer) who progressed on chemotherapy containing gemcitabine and cisplatin (with one or two previous chemotherapy lines permitted). In cycle one, patients received intravenous trastuzumab-pkrb at 6 mg/kg on day 1, and FOLFOX (consisting of intravenous oxaliplatin [85 mg/m²], intravenous leucovorin [200 mg/m²], and fluorouracil [400 mg/m² bolus] all on day 1, and fluorouracil [2400 mg/m² infusion] on days 1–2. In cycle two onwards, participants were administered intravenous trastuzumab-pkrb at 4 mg/kg and FOLFOX, every 2 weeks, until unacceptable toxic effects or disease progression. The primary endpoint of the study was objective response rate based on RECIST version 1.1, assessed in the participants who completed at least one study cycle. The response rate threshold for a positive objective response rate was 25%. This trial is registered with ClinicalTrials.gov (NCT04722133) and is ongoing.

Findings 34 participants were enrolled between June 26, 2020, and Sept 1, 2021. At the time of data cutoff on May 1, 2022, median follow-up was 13·0 months (IQR 11·0–16·9), with three participants remaining on treatment. Ten patients had a partial response and 17 had stable disease; the overall response rate was 29·4% (95% CI 16·7–46·3) and the disease control rate was 79·4% (95% CI 62·9–89·9). Median progression-free survival was 5·1 months (95% CI 3·6–6·7); median overall survival was 10·7 (95%CI 7·9–not reached). The most common treatment-related grade 3 or 4 adverse events were neutropenia (ten [29%] participants with grade 3 and nine [26%] with grade 4), grade 3 anaemia (five [15%] participants), and grade 3 peripheral sensory neuropathy (four [12%] participants). There were no treatment-related cardiac toxic effects or deaths. The overall health assessment (EuroQoL-VAS) score did not change significantly throughout the treatment. Sensory and motor neuropathy symptoms as assessed by the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy twenty-item scale questionnaire did not change significantly over time.

Interpretation For HER2-positive biliary tract cancer, second-line or third-line trastuzumab biosimilar plus FOLFOX exhibited promising activity with acceptable toxicity, warranting further investigation.

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Introduction

Biliary tract cancers are rare malignancies that include intrahepatic and extrahepatic cholangiocarcinomas and gallbladder cancer.[1] Most patients present with locally advanced or metastatic biliary tract cancer, and their therapeutic options are few. Gemcitabine and cisplatin has become the standard first-line regimen after the phase 3 ABC-02 trial,[2] in which the median overall survival was 11·7 months, which was superior to 8·1 months with gemcitabine alone. For second-line

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Research in context

Evidence before this study

We searched PubMed for HER2-targeted clinical studies in English on HER2-positive (amplified or overexpressed) individuals with biliary tract cancer, using the search terms "HER2-positive" and "biliary tract cancer". Articles published before Dec 31, 2021, were included. Before 2021, no study reported prospective trial results using HER2-targeted agents for individuals with HER2-positive biliary tract cancer, except for pilot studies, case series, or pooled analyses. In 2021, Javle and colleagues reported a phase 2 study of second-line or later pertuzumab and trastuzumab treatment for HER2-positive biliary tract cancer, which was part of a non-randomised, multicentre, multiple basket study (NCT02091141). The trial showed a promising response rate of 23%, supporting the need for HER2-targeting trials against HER2-positive biliary tract cancer.

In 2021, a phase 3 trial (the ABC-06 trial; NCT01926236) showed an overall survival benefit with second-line folinic acid, fluorouracil, and oxaliplatin (FOLFOX) chemotherapy over active symptom control, with FOLFOX becoming the standard-of-care chemotherapy as second-line treatment for advanced biliary tract cancer.

Added value of this study

To our knowledge, this is the first study to evaluate the combined synergistic effect of an anti-HER2 agent with FOLFOX in HER2-positive advanced biliary tract cancer refractory to gemcitabine and cisplatin. Our study met its primary endpoint with an objective response rate of 29%. Thxdition of trastuzumab to the current second-line standard-of-care chemotherapy regimen for biliary tract cancer, FOLFOX, had encouraging survival and response outcomes with an acceptable safety profile.

Implications of all the available evidence

The results of this multi-institutional, open-label, phase 2 study suggest that adding a HER2-targeting agent to the chemotherapeutic treatment of individuals with advanced HER2-positive biliary tract cancer is effective and safe. Because retrospective studies suggest that individuals with biliary tract cancer with HER2 overexpression or amplification have a worse prognosis compared with HER2-negative biliary tract cancer, our data support further efforts to identify HER2-positive populations among individuals with advanced biliary tract cancer and to initiate larger randomised clinical trials to test HER2-targeted agents with standard-of-care chemotherapy.

treatment, the folinic acid, fluorouracil, and oxaliplatin (FOLFOX) regimen has shown improved survival[3] compared with active symptom control, with a median overall survival of 6·2 months (compared with 5·3 months), a median progression-free survival of 4·0 months, and a 5% objective response rate. The FOLFOX regimen has become the standard-of-care chemotherapy as a second-line treatment for advanced biliary tract cancer and the reference regimen for further clinical trials. However, the low median overall survival benefit compared with active symptom control implies an unmet need for novel and effective therapeutic options for biliary tract cancer refractory to gemcitabine and cisplatin.

Molecular profiling studies published since 2017 on biliary tract cancer have identified actionable genetic alterations,[4] including *FGFR2* fusion or rearrangement and *IDH1* mutation. Targeted agents have shown clinically meaningful efficacy outcomes when used as monotherapy in phase 2 or 3 trials for individuals previously treated for biliary tract cancer with *FGFR2* fusions or rearrangements and *IDH1* mutations.[5–7] HER2 (also known as *ERBB2*) is a transmembrane tyrosine kinase receptor and is overexpressed or amplified in approximately 14·8–24·0% of all gallbladder cancers, 7·8–12·9% of extrahepatic cholangiocarcinomas, and 3·1–4·5% of intrahepatic cholangiocarcinomas.[8–12] The anti-HER2 humanised monoclonal antibody trastuzumab is one of the standard treatments, combined with cytotoxic chemotherapy, for HER2-positive gastric cancer[13] and breast cancer;[14–16] it has not been actively studied prospectively in HER2-positive biliary tract cancer. The addition of trastuzumab to cytotoxic chemotherapy has shown enhanced efficacy with no overlapping toxicity among HER2-positive tumours.

Here, we report the results of a single-arm, multi-institutional, phase 2 trial evaluating the addition of trastuzumab biosimilar to FOLFOX chemotherapy as a palliative second-line or third-line therapy for HER2-overexpressed or amplified advanced biliary tract cancer (KCSG-HB19–14).

Methods

Study design and participants

The trial was phase 2 and open-label, performed at eight academic cancer centres in South Korea, to evaluate the efficacy and safety of adding trastuzumab biosimilar to FOLFOX chemotherapy as a second-line or third-line therapy for HER2-positive advanced biliary tract cancer. The trial was conducted according to the Declaration of Helsinki and Guidelines for Good Clinical Practice. The institutional review boards of Severance Hospital, CHA Bundang Medical Centre, Ulsan University Hospital, Seoul St Mary's Hospital, Chung-Ang University Hospital, Seoul National University Bundang Hospital, St Vincent's Hospital, and Inje University Haeundae Paik Hospital approved the trial's protocol, including ethical approval. All participants provided written informed consent before enrolment.

Eligible participants were men and women aged 19 years or older with HER2-positive histologically

confirmed advanced (ie recurrent, metastatic or unresectable) biliary tract cancer (intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, or ampulla of Vater cancer). HER2 positivity was defined as an immunohistochemistry test result of 3+ or 2+ combined with in-situ hybridisation positivity or an *ERBB2* gene copy number of 6·0 or more, assessed by next-generation sequencing (NGS) tested in Clinical Laboratory Improvement Amendments-certified pathology laboratories at each cancer centre. Other major inclusion criteria were: documented radiological disease progression graded by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 when on previous treatment (one or two previous palliative lines of therapies permitted, including one regimen containing gemcitabine and cisplatin, with washout periods of 2 weeks); an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; adequate haematological, cardiac, renal, and liver function; negative urine or serum pregnancy test results and willingness to use birth control during the study in women who were menstruating (no tests were performed on women with menopause). Immunotherapy monotherapy was not considered as a line of therapy. The main exclusion criteria were: previous oxaliplatin or HER2-targeted treatment; receipt of systemic anti-cancer therapy or having participated in an investigational study within 2 weeks of the first treatment dose; a known history of active tuberculosis; hypersensitivity to fluorouracil or oxaliplatin or trastuzumab; non-recovery (ie, grade 1 adverse events or worse or at baseline) from previously administered agent-induced adverse events; known additional progressive malignancy, or that which requires active treatment within 3 years, except basal or squamous cell carcinoma of the skin; in-situ cervical cancer; thyroid cancer subjected to curative therapy, active or untreated CNS metastases or carcinomatous meningitis, or a combination; active infection requiring systemic therapy; known psychiatric or substance abuse disorders that would interfere with cooperation during the trial; a pregnant or breastfeeding state; a known history of HIV (HIV 1 and 2 antibodies), hepatitis B (HBsAg reactive and hepatitis B virus DNA more than 100 copies per mL) or hepatitis C (anti-hepatitis C virus reactive and hepatitis C virus RNA [qualitative] detected); and being a live vaccine recipient within 30 days of commencing the trial. The full inclusion and exclusion criteria are available in the protocol (appendix pp 15–18).

Procedures

Blood samples were collected before and after treatment to confirm HER2 status and elucidate the molecular mechanisms underlying the response or resistance. Before the study, tumour tissues were obtained from either primary or metastatic tumours (archival or fresh). For establishing HER2 status, an anti-HER2/neu antibody (clone 4B5; Ventana Medical Systems, West Sussex, UK) was used for immunohistochemistry; the HER2 expression scoring system was applied using the American Society of Clinical Oncology and College of American Pathologists guidelines for HER2-positive gastric cancer.[17] Silver in-situ hybridisation was performed using INFORM HER2 DNA and chromosome 17 (CEP17) probes (Ventana Medical Systems). HER2 amplification was defined as a HER2:CEP17 ratio of 2·0 or more, or an average HER2 gene copy number of 6 or more signals per cell. Local testing for HER2 status was permitted.

In cycle one, participants were administered intravenous trastuzumab-pkrb (CT-P6 or Herzuma; Celltrion, Incheon, South Korea)[18] at 6 mg/kg on day 1 and FOLFOX (consisting of intravenous oxaliplatin [85 mg/m²], intravenous leucovorin [200 mg/m²], and fluorouracil [400 mg/m² bolus] all on day 1; and fluorouracil [2400 mg/m² infusion] on days 1–2). In cycle two onwards, participants were administered intravenous trastuzumab-pkrb at 4 mg/kg and FOLFOX, every 2 weeks. All participants were treated until disease progression, death, or unacceptable toxicity was documented. CT or MRI was done within the 28 days before the first dose of treatment, as the baseline measurement; tumour responses were evaluated every 8 weeks according to the RECIST version 1.1 criteria by the investigators at each site (CL, HJC, JC, MAL, H-SI, J-SJ, MHK, BK, JWK, HSP, MJK, and HJC). During the clinic visits, which were once every 2 weeks, a physical examination, assessment of ECOG performance status, symptom monitoring, a review of concomitant medication, and an assessment of complete blood count, serum chemistry, and electrolytes were done. Carcinoembryonic antigen and carbohydrate antigen 19–9 measurements were taken every four cycles until disease progression. After treatment discontinuation, participants were monitored for survival every 3 months until death, loss to follow-up, consent withdrawal, or study termination. Quality of life was assessed before treatment and on day 1 of every four cycles until the end of treatment, using the EuroQoL-5D and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy twenty-item scale (EORTC QLQ-CIPN20) participant-reported outcomes. The EuroQoL-5D defines health in terms of the five dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression.[19] The EuroQoL-5D questionnaire's second part is the EuroQoL-Visual Analog Scale, incorporating a visual analog scale of the overall health assessment from 0 (worst health imaginable) to 100 (best health imaginable). Since our study regimen contained oxaliplatin, which can worsen chemotherapy-induced peripheral neuropathy from previous cisplatin use, the EORTC QLQ-CIPN20 was also assessed to provide information on chemotherapy-induced peripheral neuropathy-related symptoms and the functional limitations of participants exposed to potentially

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neurotoxic chemotherapeutic and neuroprotective agents.[20]

Safety and tolerability were assessed from the study treatment's first dose at each clinic visit every 2 weeks until the treatment ended. Toxic effects were graded on the basis of the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0). The left ventricular ejection fraction was measured using a multi-gated acquisition scan or echocardiography every 12 weeks. Doses were reduced for the FOLFOX regimen according to the type and severity of the adverse events, but not for trastuzumab biosimilar. A maximum of two dose reductions of fluorouracil and oxaliplatin were allowed for grade 3–4 toxicities (predefined in the study protocol; appendix p 54). Trastuzumab biosimilar dosing could be delayed until resolution or the medical stabilisation of grade 3 or 4 treatment-related toxicity associated with clinical symptoms, or of congestive heart failure or other cardiac dysfunction.

Protocol-based liquid biopsies were prospectively performed. Circulating tumour DNA analyses were performed using DNA extracted from plasma collected at baseline and at disease progression, the results of which will be reported seperately.

Outcomes

The primary endpoint was the objective response rate according to RECIST version 1.1. Secondary endpoints were progression-free survival, overall survival, disease control rate, safety, and quality of life. Circulating tumour DNA and tissue HER2 status were exploratory endpoints. The objective response rate was calculated as the percentage of participants who had a confirmed complete response or partial response. Disease control rate was the proportion of participants with a confirmed complete response, partial response, and stable disease for at least 4 weeks. Confirmatory tumour assessments were not mandatory. Progression-free survival was the time from treatment initiation to the date of disease progression or death from any cause. For participants with no disease progression or who did not die, the censoring date was the date of their last response evaluation. Overall survival was the time from treatment initiation to the date of death from any cause. The overall survival censoring date was the last date the participant was known to be alive as the data cutoff date for analysis. The response time was the time from treatment initiation to the date of the first response (complete response or partial response). Safety endpoints included the incidences and severities of adverse events. Biomarker exploratory endpoints included progression-free survival and overall survival stratified by tissue HER2 status.

Statistical analysis

According to Simon's[21] two-stage minimax design, a minimum sample size of 31 participants was needed to accept the alternative hypothesis that the true response rate was 25% with 80% power and to reject the null hypothesis that the response rate was 10% or less,[3] with a type I error of 0·1. In the first stage, the study was stopped if there were fewer than two responses among the initial 16 participants. The null hypothesis would be rejected if six or more responses were observed in 31 participants. Considering a 10% dropout rate, 34 participants were enrolled. Patients who completed at least one study cycle were considered evaluable for the primary outcome.

Survival analyses and the assessment of objective response rate included participants who had received at least one trial dose. Safety was assessed in all participants who received at least one trial dose. The duration of response was analysed using a post-hoc test for participants who showed a complete response or partial response and was defined as the time of best response (complete response or partial response, whichever came first) until the date of progression. Objective response and disease control rates with 95% CIs were estimated using the Agresti-Coull method.

All statistical analyses and graphing were performed using SAS software version 9.4, R software version 4.1.0, or GraphPad Prism version 8. The Kaplan–Meier method was used to estimate survival and the log-rank test was applied to identify the differences in survival between two groups, stratified by pretreatment HER2 overexpression by immunohistochemistry or *ERBB2* amplification by NGS. Median follow-up duration was estimated by reverse Kaplan-Meier method and the corresponding 95% CI was constructed on the basis of the Brookmeyer and Crowley method using log-log transformation. A Cox proportional hazards regression model was used for univariate analyses to assess the significant prognostic factors associated with survival, with the hazard ratio (HR) and 95% CIs. Statistical differences in quality-of-life assessments were compared using a Kruskal-Wallis test and post-hoc Dunn's test for multiple comparison. Two-sided p values of less than 0·05 were considered statistically significant. This study is registered with ClinicalTrials.gov (NCT04722133).

Role of the funding source

The funders supplied the study drug trastuzumab biosimilar (Celltrion) and oxaliplatin (Boryung Pharmaceutical). The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

34 participants were enrolled in the study between June 26, 2020, and Sept 1, 2021, and four were excluded on the basis of eligibility criteria (appendix p 1). Baseline participant characteristics are shown in table 1. The participants' median age was 66 years (IQR 60–70); most had an ECOG performance status of 1 (28 [82%]). The majority of participants had gallbladder cancer

(n=18 [53%]). 18 (53%) participants underwent curative surgery before recurrence. Two (6%) patients had previous anti-PD-1 treatment as second-line therapy (appendix p 2). The most common sites of metastasis were the liver (24 [71%]) and lymph nodes (18 [53%]). All participants were HER2-positive, as detected using immunohistochemistry 3+ (n=23 [68%]) or immunohistochemistry 2+ with in-situ hybridisation + (11 [32%]). One participant was enrolled in the study with pretreatment *ERBB2* amplification results (copy number 10·27) on local NGS. Most participants (n=32 [94%]) were confirmed as HER2-positive with archival tissues acquired before or during gemcitabine and cisplatin treatment. The median carbohydrate antigen 19–9 value was 292·0 units per mL (range 2·0–25 811·6) at baseline.

At the time of data cutoff on May 1, 2022, median follow-up was 13·0 months (95% CI 11·0–16·9); three (9%) participants remained on the treatment. 25 (81%) of 31 participants discontinued treatment due to radiological disease progression, and six (19%) of 31 participants discontinued due to investigator's decision (appendix p 1). The confirmed objective response rate was 29·4% (95% CI 16·7–46·3), with ten participants having a partial response. 17 (50%) participants had stable disease, with a disease control rate of 79·4% (95% CI 62·9–89·9; figure 1A). 14 (82%) of 17 participants had stable disease at 4·0 months or after. Non-target lesions (escape lesions) represented the only site of progressive disease in five (16%) of 31 participants at the time of the treatment discontinuation (figure 1B).

Median progression-free survival was 5·1 months (95% CI 3·6–6·7), with a 6-month progression-free survival rate of 36·7% (20·7–52·8) and 1-year progression-free survival rate of 11·4% (3·0–26·0; figure 2A). Median overall survival was 10·7 months (7·9–not reached [NR]), with a 6-month overall survival rate of 76·3% (58·0–87·4) and a 1-year overall survival rate of 49·2% (30·8–65·3; figure 2B). Among the participants achieving a partial response as the best response, the median time to response was 1·7 months (range 1·4–1·8), and the median duration of response was 4·9 months (2·1–10·4; figure 2C).

Post-hoc exploratory analyses of efficacy outcomes according to tumour location are shown in the appendix (p 3). Patients with gallbladder cancers showed the highest objective response rate of 38·9% (95% CI 17·3–64·3), whereas there were no responders among participants with intrahepatic cholangiocarcinoma. Among all participants, participants with extrahepatic cholangiocarcinoma had the longest survival (median progression-free survival, 9·70 months [95% CI 1·3–11·8]; median overall survival, 15·7 months [95% CI 2·2–NR]). There were no differences in progression-free survival or overall survival when stratified by baseline clinical characteristics (appendix p 4).

The median number of treatment cycles was 8·5 (IQR 6·3–16·0). Treatment-related adverse events occurred in all participants (table 2). The commonly reported grade 3 adverse events were mostly haematological, including neutropenia (29%), anaemia (15%), and thrombocytopenia (9%). Grade 4 adverse events occurred in nine participants (all neutropenia, 26%). Non-haematological adverse events were mostly graded as 1 or 2, and four participants (12%)

	Participants (N=34)
Age, year	66 (60–70)
Sex	
Male	17 (50%)
Female	17 (50%)
Eastern Cooperative Oncology Group performance status	
0	6 (18%)
1	28 (82%)
Primary tumour type	
Intrahepatic cholangiocarcinoma	6 (18%)
Extrahepatic cholangiocarcinoma	10 (29%)
Gallbladder cancer	18 (53%)
Pathological differentiation	
Poorly differentiated	3 (9%)
Moderately differentiated	21 (62%)
Well differentiated	1 (3%)
Others*	9 (26%)
Number of previous systemic treatments as palliative aim	
1	27 (79%)
2	7 (21%)
History of surgery	
Yes	18 (53%)
No	16 (47%)
HER2 status	
Immunohistochemistry 3+	23 (68%)
Immunohistochemistry 2+ and in-situ hybridisation +	11 (32%)
HER2-examined tissue origin	
Primary tumour	14 (41%)
Metastatic tumour	20 (59%)
Site of metastatic lesion	
Liver	24 (71%)
Lymph node	18 (53%)
Lung	14 (41%)
Peritoneum	10 (29%)
Baseline carbohydrate antigen 19–9†	
Median, units per mL	292·0 (27·6–1779·8)
<34 units per mL	8 (24%)
≥34 units per mL	26 (77%)

Data shown as n (%) or median (IQR). Immunohistochemistry 3+ or 2+ was defined using the HER2 expression scoring system from American Society of Clinical Oncology and College of American Pathologists guidelines for HER2-positive gastric cancer. In-situ hybridisation + was defined as HER2:CEP17 ratio of 2·0 or more, or an average *ERBB2* gene copy number of 6 or more signals per cell. *Others include unknown, n=8, and intracystic papillary neoplasm with associated invasive carcinoma, n=1. †Carbohydrate antigen 19–9 units are grouped by the upper limit of the normal range.

Table 1: **Baseline participants' characteristics**

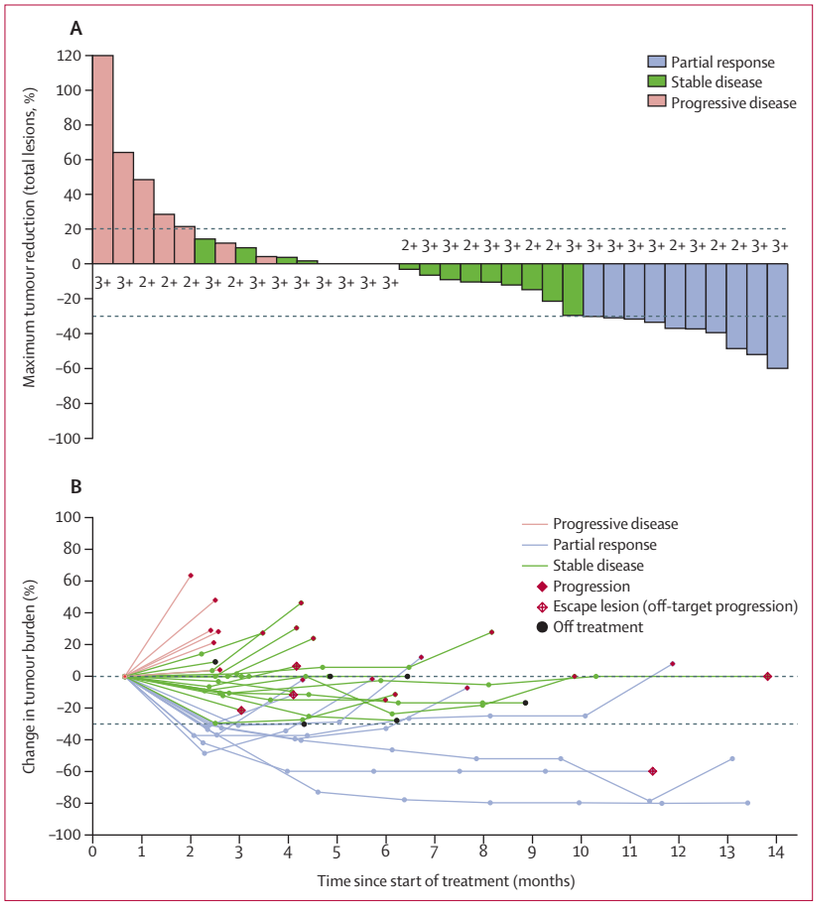

Figure 1: **Changes in tumour burden in participants administered trastuzumab combined with FOLFOX**
(A) Maximum percentage change from baseline in the size of target lesions with corresponding confirmed best responses based on RECIST version 1.1 and HER2 immunohistochemistry values from pretreatment tissues in all patients. The lower dotted line represents the cutoff value for a partial response, and the upper dotted line represents the cutoff value for progression. (B) Percentage change from baseline in the total number of target lesions over time. The dotted lines represent a 0% and 30% tumour reduction from baseline. FOLFOX=folinic acid, fluorouracil, and oxaliplatin.

had grade 3 peripheral sensory neuropathy because of oxaliplatin. 30 (88%) participants required one or more dose reductions of fluorouracil or oxaliplatin chemotherapy owing to adverse events. The most common reason for neutropenia-induced dose-reduction was fluorouracil (18 [60%] of 30) and oxaliplatin (17 [57%] of 30). The median number of cycles to the first dose reduction for fluorouracil and oxaliplatin was four (IQR 3–6). None of the treatment-related adverse events led to trastuzumab discontinuation. There were no treatment-related cardiac toxic effects or deaths.

The mean overall health assessment (EuroQoL-Visual Analog Scale) score at baseline was significantly (p=0·0080) lower for the participants with the best response of progressive disease than for those who had a partial response or stable disease. EuroQoL VAS score did not change significantly throughout the treatment. Sensory and motor neuropathy symptoms assessed by the EORTC QLQ-CIPN20 questionnaire score increased throughout the treatment, although not significantly (appendix p 5).

We performed a post-hoc analysis of the effect of HER2 overexpression or *ERBB2* amplification. When we compared the pretreatment HER2 overexpression and survival rates, participants with immunohistochemistry 3+ (n=23) did not show a difference in progression-free survival or overall survival compared with participants with immunohistochemistry 2+ with in-situ hybridisation positivity (appendix p 6). Of the 34 enrolled participants, 32 had available pretreatment-targeted local NGS results from either primary or metastatic tissues. *ERBB2* amplification by NGS (copy number ≥6) was detected in ten (31%) participants of 32; nine of them were HER2 immunohistochemistry 3+ (appendix p 7). Only one participant with HER2 2+ in-situ hybridisation positivity showed *ERBB2* amplification on NGS (10%). There was no difference regarding progression-free survival and overall survival between participants with pretreatment *ERBB2* amplification by NGS and *ERBB2* non-amplified participants (appendix p 8).

Discussion

In this multi-institutional, single-arm, phase 2 study, second-line or third-line trastuzumab biosimilar and FOLFOX showed promising activity for participants with HER2-positive biliary tract cancer refractory to gemcitabine and cisplatin. To our knowledge, this is the first prospective study reporting the results of HER2-targeting agents combined with chemotherapy in the biliary tract cancer population. The study reached its primary endpoint, with an objective response rate of 29·4% (95% CI 15·1–47·5; planned target, 25%). Considering the poor survival after gemcitabine and cisplatin failure in participants with biliary tract cancer and that approximately 20% of participants were treated as third-line, absolute survival results (median progression-free survival 5·1 months and median overall survival 10·7 months) were encouraging. Response rates in our trial were higher than current second-line cytotoxic chemotherapy options (appendix p 9),[3,22] which might be attributable to trastuzumab's additive effect, although differences between the study populations mean that outcomes should be interpreted cautiously.

The poor prognosis of HER2-positive biliary tract cancer has not been prospectively confirmed. However, retrospective HER2 overexpression studies among participants with biliary tract cancer have suggested that these populations have a worse survival compared with HER2-negative biliary tract cancer,[23,24] which was also true for breast cancer and gastric cancer before the HER2-targeted treatment era.[25,26] *IDH1*-targeted and *FGFR*-targeted therapies are effective and approved for use in *IDH1* R132 mutations and *FGFR2* fusions, which occur in intrahepatic cholangiocarcinoma. Gallbladder cancer and extrahepatic cholangiocarcinomas do not have these genetic aberrations and represent an area of

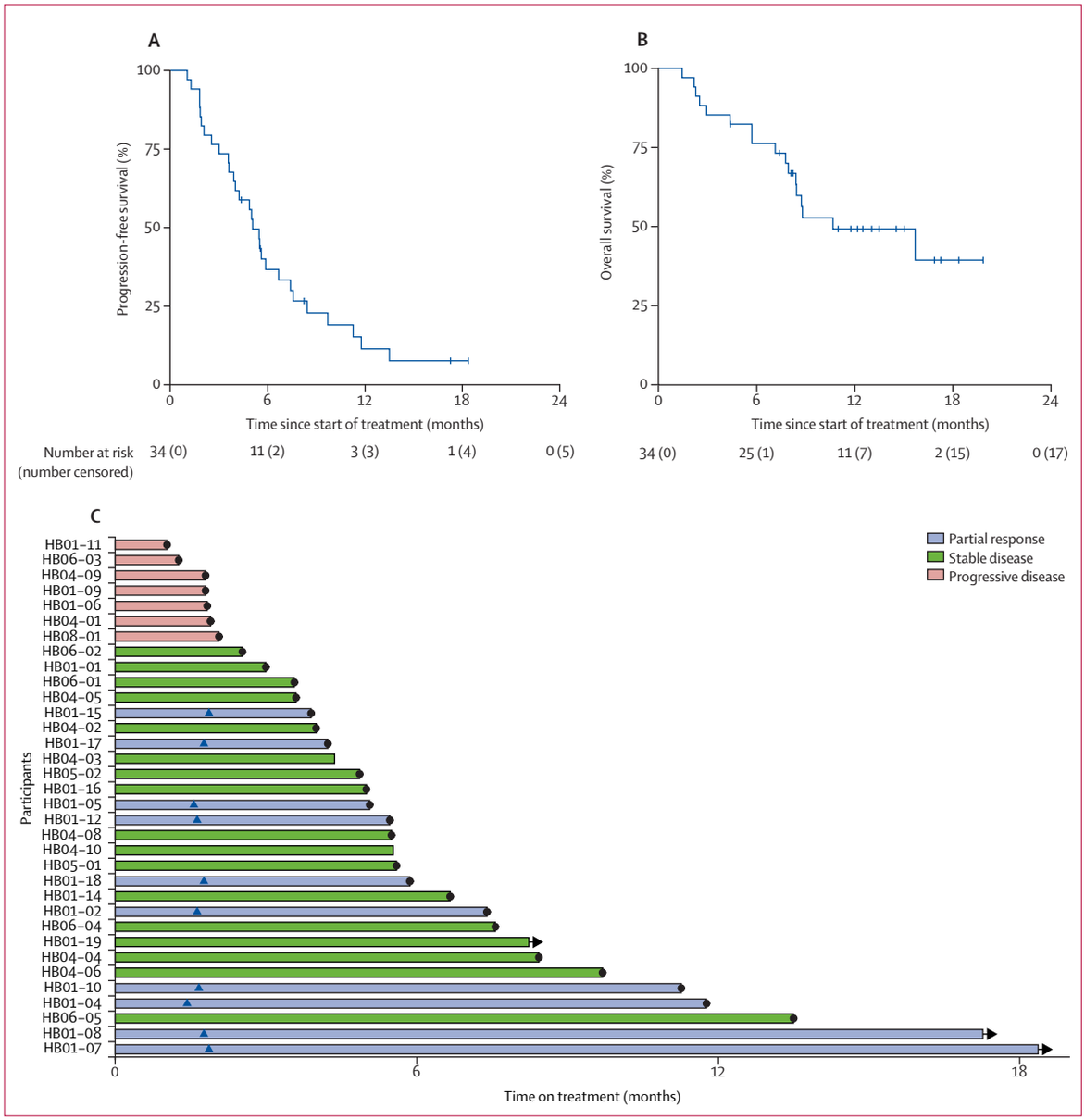

Figure 2: **Progression-free survival and overall survival in the study participants**

(A) Progression-free survival in all patients over time. (B) overall survival in all patients over time. (C) A swimmer plot showing outcomes in all patients from the start of treatment to either disease progression or the date of last follow-up. Triangles indicate partial reponse start, and arrows indicate ongoing treatment.

unmet need. HER2 targeting is an attractive option for some of these participants.

Few single-arm trials have shown the efficacy of HER2-targeting agents against HER2-positive biliary tract cancer (appendix p 9). The results of a single-arm, open-label, phase 2a, multiple basket study (MyPathway) with a cohort with biliary tract cancer were reported in 2021.[27] Among 39 participants who were HER2-positive, participants with metastatic biliary tract cancer with a median of two previous treatment lines were administered pertuzumab and trastuzumab, a chemotherapy-free regimen. Pertuzumab plus trastuzumab resulted in an objective response rate of 23%, a median progression-free survival of 4·0 months, a median overall survival of 10·9 months, and median duration of response of 10 months, with low incidences of grade 3 or higher treatment-related adverse events (46%). Notably, similar to in our trial, there were no objective responses for intrahepatic cholangiocarcinoma in the MyPathway study, which warrants further investigation. The phase 2 HERB trial,[28] presented in 2022, tested the efficacy of the HER2 antibody-drug conjugate trastuzumab deruxtecan for

	Any grade	Grade 3	Grade 4
Haematological			
Neutrophil count decreased	21 (62%)	10 (29%)	9 (26%)
Anaemia	8 (24%)	5 (15%)	0
Platelet count decreased	5 (15%)	3 (9%)	0
Non-haematological			
Peripheral sensory neuropathy	17 (50%)	4 (12%)	0
General weakness	6 (18%)	3 (9%)	0
Diarrhoea	7 (21%)	2 (6%)	0
Abdominal pain	4 (12%)	1 (3%)	0
Fever	3 (9%)	1 (3%)	0
Biliary tract infection	1 (3%)	1 (3%)	0
Oral mucositis	7 (21%)	0	0
Anorexia	5 (15%)	0	0
Nausea	5 (15%)	0	0
Infusion-related reaction	4 (12%)	0	0
Dyspepsia	4 (12%)	0	0
Total	34	23 (68%)	9 (26%)

Data shown as n (%) of all participants (N=34). Any grade treatment-related adverse events with an incidence of more than 10% or any treatment-related adverse events that were grade 3 or worse are shown.

Table 2: **Treatment-related adverse events**

previously treated individuals with biliary tract cancer, resulting in an objective response rate of 36·4% for 22 HER2-positive participants, with a median progression-free survival of 4·4 months and median overall survival of 7·1 months. The trial also included eight HER2-low expressing participants, for whom the objective response rate was 12·5% with a median progression-free survival of 3·5 months and median overall survival of 8·9 months. Treatment-related adverse events leading to drug discontinuation occurred in eight participants, and two participants had grade 5 interstitial lung disease. The bispecific anti-HER2 antibody, zanidatamab, also showed promising efficacy (eight partial responses of 20 individuals with HER2-positive biliary tract cancer) in a phase 1 trial.[29] Including ours, these trial results support the need for HER2-targeted agents for HER2-positive biliary tract cancer. Trastuzumab plus FOLFOX requires toxicity management for cytotoxic chemotherapy, but might be more cost-effective than other novel anti-HER2 agents, which are expensive and unafforable in many countries.

Although HER2-overexpressing participants were mainly enrolled, not every participant had *ERBB2* amplification through NGS. Reports regarding concordance between *ERBB2* amplification through NGS and HER2 expression through immunohistochemistry or in-situ hybridisation are controversial.[30] In this study, participants with biliary tract cancer with HER2 overexpression or *ERBB2* amplification had similar survival rates to those without. One explanation for this is tumour heterogeneity; in addition, the biopsied sample might not reflect the HER2 status of the entire tumour. Another explanation is the tissue acquisition timing. It would have been more accurate to investigate HER2 positivity in pre-trastuzumab treatment tissues acquired after the previous line failure. New trials to test the efficacy of anti-HER2 agents for HER2-positive gastric or breast cancers as second-line or later treatments are mostly designed to confirm HER2 positivity after previous-line failure since acquired resistance mechanisms to anti-HER2 treatment include the loss of HER2. However, HER2-positive gastric and breast cancers should be treated with first-line anti-HER2 agent combination regimen. Since none of the participants were treated with anti-HER2 agents before enrolment in this study, it is likely that HER2-positive subclones of the tumours still exist.

Since HER2 testing is not a standard procedure for biliary tract cancer, we defined pathological HER2 positivity according to the American Society of Clinical Oncology and College of American Pathologists guidelines for HER2-positive gastric cancer,[17] similar to other HER2-targeting trials for biliary tract cancer, which define HER2 positivity based on 10% or more of tumour cells. Because it is not validated whether the definition of HER2 immunohistochemistry status or in-situ hybridisation amplification for gastric cancer can also serve as a predictive biomarker for HER2-targeted agents in treating HER2-positive biliary tract cancer, we need a large validation study concurrently with a phase 3 trial to establish the pathological definition of HER2 positivity for biliary tract cancer. Another method to assess HER2 status is to investigate the *ERBB2* copy number variation in circulating tumour DNA. We prospectively collected blood samples for plasma-based NGS analyses at baseline and post-progression for all the participants in this study.

This study had some limitations, including the single-arm design, the small number of participants tested in a single Asian country, the absence of tissue samples pre-trastuzumab treatment (after gemcitabine and cisplatin failure) for HER2 investigation, built-in selection biases in the hazard ratio,[31] and there were no predefined assumptions for statistical power for post-hoc analyses. Quality of life might have been assessed better by a generic scale such as the Quality of Life Questionnaire-C30 and a specific scale such as the Quality of Life Questionnaire-BIL21. Despite these limitations, this study is strengthened by multi-institutional enrolments from South Korea, where the incidence of biliary tract cancer is one of the highest globally. We believe that the response rate and survival observed in this study support the direct comparison of trastuzumab and chemotherapy combination to the existing standard of care second-line chemotherapy regimens for HER2-positive biliary tract cancer, requiring further validation in randomised clinical trials. We believe that our data supports the consideration

of trastuzumab plus FOLFOX as a treatment option in the second-line or third-line therapy and encourage randomised clinical trials to further explore the efficacy of HER2-targeted regimens in biliary tract cancer.

Contributors

C-kL, MHK, and HJChoi designed the study and developed the protocol. C-kL and HJChoi secured funding. C-kL, HJChon, JC, MAL, H-SI, J-SJ, MHK, BK, JWK, HSP, MJK, and HJChoi participated in the recruitment of participants and collection of data. C-kL, SP, MH, YNP, and HJChoi analysed the data. All authors interpreted the data. C-kL wrote the first draft of the manuscript. All authors contributed to the review and revision of the manuscript for important intellectual content and approved the final version for submission. C-kL, SP, and HJChoi had access to the raw data. C-kL and HJChoi accessed and verified the data. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

C-kL reports research grant from GC Biopharma; consulting fees from Roche; honoraria from Boryung Pharmaceutical, Dong-A ST, Norvatis, and Servier; and an advisory role at AstraZeneca. HJChon reports research grants from Boryung Pharmaceutical, Dong-A ST, and Roche; honoraria from Bristol Myers Squibb, Dong-A ST, Roche, Sanofi, and Servier; and a consulting or advisory role at AstraZeneca, Bayer, BMS, Celgene, Eisai, GreenCross Cell, Menarini, Merck Sharpe and Dohme, ONO pharmaceuticals, Roche, Sanofi, Servier, and Sillajen. JC reports research grants from Bayer; and honoraria from Eisai and Roche. MHK reports honoraria and consulting fees from Boryung Pharmaceutical and Celltrion. JWK reports grants from inno.N and Jeil Pharm; consulting fees from AstraZeneca, BeiGene, Beyond Bio, Bristol Myers Squibb and Celgene, Eisai, GC Cell, MSD, ONO, Sanofi-Aventis, Servier, and TCUBEit. HJChoi reports an advisory role at AstraZeneca and Roche. All other authors declare no competing interests.

Data sharing

The data collected for this study will not be made available to the public. Investigators interested in the deidentified participant data should contact the corresponding author at choihj@yuhs.ac after publication of this article for data sharing and collaboration. The protocol can be found in the appendix (pp 11–76).

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