

# Systemic treatment of advanced or recurrent biliary tract cancer

Wei Zhang<sup>1,2,\*</sup>, Hongyuan Zhou<sup>1,2</sup>, Yingying Wang<sup>1,2</sup>, Zewu Zhang<sup>1,2</sup>, Guangtai Cao<sup>3</sup>, Tianqiang Song<sup>1,2</sup>, Ti Zhang<sup>1,2</sup>, Qiang Li<sup>1,2</sup>

<sup>1</sup>Department of Hepatobiliary Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China;

<sup>2</sup>National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin. Tianjin's Clinical Research Center for Cancer, Tianjin, China;

<sup>3</sup>Cangzhou Hospital of Integrated TCM-WM Cangzhou, Hebei, China.

**SUMMARY** Biliary tract cancer (BTC) is a disease entity comprising diverse epithelial tumors with features of cholangiocyte differentiation, and it includes cholangiocarcinoma (CCA) and gallbladder cancer (GBC). Depending on its anatomical location, cholangiocarcinoma is categorized as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA). Nearly two-thirds of patients with biliary tract cancer present with advanced disease at diagnosis and in 68-86% of resections the cancer eventually recurs either loco-regionally or at a distance. Chemotherapy is the first-line therapy for advanced or recurrent BTC. With the development of next-generation sequencing (NGS)-guided molecular targeted therapy, more options are available for treatment of advanced BTC. Chemotherapy, and especially a triplet regimen based on gemcitabine/cisplatin/nab-paclitaxel, has had the most significant effect, and fluorouracil, leucovorin, irinotecan plus oxaliplatin (FOLFIRINOX) combined with bevacizumab is promising. Molecular targeted therapy should be based on genome sequencing and appears essential to precision medicine. Fibroblast growth factor receptor (FGFR) inhibitors and isocitrate dehydrogenase (IDH) inhibitors are promising emerging targeted therapies mainly for iCCA. Other targeted therapies such as anti-human epidermal growth factor receptor-2 (HER2) therapies, MEK inhibitors, BRAF inhibitors, and poly ADP ribose polymerase (PARP) inhibitors had tentatively displayed efficacy. Further evaluations of combination strategies in particular are needed. An immune checkpoint inhibitor (ICI) alone is less efficacious, but an ICI in addition to chemotherapy or radiotherapy has resulted in a response according to many case series. However, ICIs are still being evaluated in several ongoing studies. Combination therapies have garnered attention because of interactions between signaling pathways of carcinogenesis in BTC.

**Keywords** biliary tract cancer, chemotherapy, targeted therapy, immune checkpoint inhibitor, next-generation sequencing

## 1. Introduction

Biliary tract cancer (BTC) is a disease entity comprising diverse epithelial tumors with features of cholangiocyte differentiation, and it includes cholangiocarcinoma (CCA) and gallbladder cancer (GBC). Depending on its anatomical location, cholangiocarcinoma is categorized as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA) (1). The overall incidence of BTC has increased progressively worldwide over the past four decades (2-6). Unfortunately, the prognosis remains poor, with a 5-year survival rate of around 5-15% (7). Surgical resection remains the mainstay of potentially curative treatment for all three disease subtypes, whereas liver transplantation after neoadjuvant chemoradiation is restricted to a subset of patients with early-stage pCCA (1,8). However, nearly

two-thirds of patients with CCA present with advanced disease at diagnosis and in 68-86% of resections the cancer eventually recurs either loco-regionally or at a distance (9-11). Chemotherapy is the first-line therapy for advanced or recurrent BTC. With the development of next-generation sequencing (NGS)-guided molecular targeted therapy, more options are available for treatment of advanced BTC, and a growing number of studies have reported achieving a partial response or even a complete response (CR) after molecular targeted therapy or immune checkpoint inhibitors (ICIs). Systemic treatment of advanced or recurrent BTC is summarized here.

## 2. Chemotherapy and beyond

2.1. Chemotherapy: The first-line and the second line

Chemotherapy is the standard systemic therapy for BTC. Since 2010, the landmark UK ABC-02 trial established the doublet cisplatin and gemcitabine (GEMCIS) as the first-line standard of care for advanced CCA (12). In this randomized phase III study, 410 patients with BTC were randomly allocated to receive gemcitabine alone or gemcitabine combined with cisplatin. The doublet regimen conferred a statistically significant overall survival (OS) advantage over gemcitabine alone (11.7 vs. 8.1 months; HR, 0.64; 95% CI, 0.52-0.80;  $P < 0.001$ ). In addition, cisplatin plus gemcitabine was well tolerated, and adverse events were similar between the treatment arms (Table 1).

After the ABC-02 trial, many gemcitabine-based regimens have been developed, including the gemcitabine plus oxaliplatin (GEMOX) regimen, the gemcitabine plus S-1 regimen (GS), and the gemcitabine plus nab-paclitaxel regimen (Table 1). The GEMOX regimen, which substitutes oxaliplatin for cisplatin, represents a valuable alternative as the first-line option in patients ineligible or unwilling to receive cisplatin based on promising results from a non-randomized phase II study (13), with fewer adverse reactions compared to GEMCIS. According to the Japanese experience, Morizane *et al.* (14) conducted a phase III clinical trial and found that GS is comparable to the GEMCIS regimen. The median progression-free survival (PFS) was 5.8 months with GC and 6.8 months with GS (HR: 0.86, 95% CI: 0.70-1.07). The median OS was 13.4 months with GEMCIS and 15.1 months with GS (HR: 0.945, 95% CI: 0.777-1.149,  $p$  for non-inferiority = 0.0459 < 0.05). In a phase II clinical trial where nab-paclitaxel and gemcitabine were administered as first-line treatment of advanced or metastatic cholangiocarcinoma, patients received intravenous nab-paclitaxel followed by gemcitabine on days 1, 8, and 15 of each 28-day treatment cycle until disease progression or unacceptable toxicities. Median OS was 12.4 months (95% CI, 9.2-15.9), and median time to progression was 7.7 months (95% CI, 6.1-13.1). The confirmed best overall response rate was 30% and the disease control rate was 66% (15,16). Although the trial did not meet its primary efficacy end point, its results indicated that a nab-paclitaxel plus gemcitabine regimen was well tolerated and may be an alternative to the current therapeutic approaches for advanced BTC.

Compared to the duplet gemcitabine, the triplet regimen based on GEMCIS resulted in a more objective response (Table 1). Shroff *et al.* (17) investigated the addition of nab-paclitaxel to standard doublet therapy (known as the GAP regimen: gemcitabine, nab-paclitaxel [Abraxane], and cisplatin [Platinol]). In this open-label, single-arm, phase II clinical trial, 60 patients with advanced BTC were treated with gemcitabine, cisplatin, and nab-paclitaxel. A point worth noting is that the standard starting doses of gemcitabine and nab-paclitaxel were reduced from 1000 mg/m<sup>2</sup> and 125 mg/m<sup>2</sup> to 800 mg/m<sup>2</sup> and 100 mg/m<sup>2</sup>, respectively. The majority of

**Table 1. Summary of first-line chemotherapy for advanced BTC**

Study, Year	Study design	N	Chemotherapy regimen	Objective response (PR or CR)	PFS (months)	OS (months)
Valls (12), 2010	Advanced BTC Phase III (ABC-02)	410	GEMCIS vs. GEM; GEM: 1000 mg/m <sup>2</sup> ; CIS: 5 mg/m <sup>2</sup>	PR: 26.1% vs. 15% DCR: 81.4% vs. 71.8%	8 vs. 5 4m-PFS 70% in CIS/GEM	11.7 vs. 8.1
Andre' T (13), 2008	Advanced BTC Phase II	67	GEMOX gemcitabine 1000 mg/m <sup>2</sup> (day 1) and oxaliplatin 100 mg/m <sup>2</sup> (day 2), every 2 weeks	ORR: 20.5% in non-GBC 4.3% in GBC	3.4	8.8
Morizane (14), 2019	Unresectable locally advanced BTC, Phase III, FUGA-BT (JCOG1113)	354	GS (141 cases) vs. GEMCIS (148 cases); S-1 60/80/100 mg/day	ORR: 29.8% (42/141) vs. 32.4% (48/148)	6.8 vs. 5.8	15.1 vs. 13.4
Sahai V (15), 2019	Metastatic CCA Phase II	74	Gemcitabine, nab-paclitaxel on days 1, 8, and 15 of each 28-day treatment cycle	ORR 30%; DCR 66%	7.7 (6-m PFS: 61%)	12.4
Shroff (17), 2019	Phase II 63% ICCA 78% metastatic disease	60	GAP regimen (gemcitabine, nab-paclitaxel, cisplatin): Gemcitabine and nab-paclitaxel were reduced from 1000 mg/m <sup>2</sup> and 125 mg/m <sup>2</sup> to 800 mg/m <sup>2</sup> and 100 mg/m <sup>2</sup> .	ORR: 48.3%; PRR: 45%; DCR 84%. CR was achieved in 2 patients. Conversion to resectable: 20%(12/60)	11.8	19.2
Belkouz (18), 2020	Advanced BTC (salvage)	40	FOLFIRINOX-single arm Oxaliplatin (85 mg/m <sup>2</sup> ), LV (400 mg/m <sup>2</sup> ), irinotecan (180 mg/m <sup>2</sup> ), and FU bolus (400 mg/m <sup>2</sup> ) then FU (2400 mg/m <sup>2</sup> ) over 46 h, 12 cycles	ORR: 10%	6.2	10.7

BTC, biliary tract cancer. CCA, cholangiocarcinoma. ICCA, intrahepatic cholangiocarcinoma. GBC, gallbladder cancer. LV, leucovorin. FU, fluorouracil. GEM, gemcitabine. CIS, cisplatin. Oxa, Oxaliplatin. GS, gemcitabine plus S-1. PFS, progression-free survival. OS, overall survival. PR, partial response. CR, complete response. ORR, objective response rate. DCR, disease control rate. na, not available.

patients (63%) had intrahepatic cholangiocarcinoma, and 78% of the entire cohort had metastatic disease. PFS was 11.8 months (vs. 8.0 months for ABC-02) and median OS was 19.2 months (vs. 11.7 months for ABC-02). Moreover, the triplet regimen allowed conversion to resectable disease in 12 patients, and a pathologic complete remission was achieved in 2 of those patients. SWOG 1815 is a phase III trial currently underway comparing the gemcitabine/cisplatin/nab-paclitaxel regimen to the GEMCIS regimen (16) and if it yields positive results, it has the potential to establish a new standard of care.

Another triplet regimen is fluorouracil, leucovorin, irinotecan plus oxaliplatin (FOLFIRINOX), which is the standard therapy for pancreatic duct adenocarcinoma (PDAC). However, a single arm of FOLFIRINOX (18) to treat advanced BTC resulted in an objective response rate (ORR) of only 10% and a PFS of 6.2 months and an OS of 10.7 months, indicating that it was less efficacious than GEMCIS. A trial of modified FOLFIRINOX versus GEMCIS as first-line chemotherapy for locally advanced non resectable or metastatic BTC (AMEBICA)-PRODIGE 38 (NCT02591030) is now underway (19). This is a randomized controlled multicenter phase II/III study aiming to clarify the efficacy of FOLFIRINOX over GEMCIS.

Ten years ago when ABC-02 was published, there were fewer than 50 trials listed for this disease site on ClinicalTrials.gov. Currently, there are over 400 hundred BTC trials listed all over the world. More phase III clinical trials of different regimens are expected to help eradicate this aggressive disease.

## 2.2. Chemotherapy combined with antiangiogenic therapies

Antiangiogenic inhibitors, such as cabozantinib (20) or sunitinib (21), did not have better efficacy when used alone (not in combination with chemotherapy), and adverse reactions to cabozantinib and sunitinib precluded their combination with chemotherapy. Gemcitabine plus sorafenib provided comparable disease control and survival to GEMCIS (22). The best result came from a phase II study, which revealed that FOLFIRI plus bevacizumab (23) resulted in a PFS of 8 months and an OS of 20 months. In the future, the combination of chemotherapy with bevacizumab may offer hope.

## 2.3. Chemotherapy combined with anti-EGFR therapy

Combining GEMCIS with an epidermal growth factor receptor (EGFR) antibody, such as panitumumab (24-26), cetuximab (27), or erlotinib (28), did not provide a survival benefit compared to GEMCIS alone. Although the addition of erlotinib to gemcitabine and oxaliplatin had antitumor activity in advanced BTC as indicated by a higher ORR (30% vs. 16%) and a prolonged PFS

(5.9 vs. 3.0 months,  $p = 0.049$ ) (28), no significant difference in OS was noted between erlotinib/GEMCIS and GEMCIS groups. One possible reason for the lack of aq benefit could be because these trials were conducted in unselected populations. Further development of anti-EGFR therapy for cholangiocarcinoma should include a biomarker-driven approach.

## 3. Molecular targeted therapy

There is no standard second-line treatment for advanced cholangiocarcinoma. With the development of NGS, more driven genes are being identified, helping to explain the underlying mechanism of the pathogenesis of BTC and to develop new therapies (29). BTCs are clinically and genetically heterogeneous. Different forms of NGS have been reported to yield different results.

Wardell *et al.* (30) examined 412 BTC samples from Japanese and Italian populations including 136 of iCCA, 101 of dCCA, 109 of pCCA, and 66 of GBC. They identified 32 significantly mutated genes, some of which negatively affected prognosis. TP53 (26%), KRAS (17%), SMAD4 (8%), NF1 (6%), ARID1A (6%), PBRM1 (6%), ATR (6%), PIK3CA (5%), and ERBB3 (5%) are among the 32 significantly and commonly mutated genes. Nakamura *et al.* (31) performed comprehensive whole-exome and transcriptome sequencing in a large cohort of 260 patients with BTC, including 145 with iCCA, 86 with pCCA/dCCA, and 29 with GBC. The repertoire of genetic alterations varied across the different cholangiocarcinoma subtypes. For example, recurrent mutations in IDH1, IDH2, FGFR1, FGFR2, FGFR3, EPHA2, and BAP1 were noted predominantly in iCCA, whereas ARID1B, ELF3, PBRM1, PRKACA, and PRKACB mutations occurred preferentially in iCCA/pCCA/dCCA (31).

Lowery *et al.* (32) reported that the most commonly altered genes in iCCA were IDH1 (30%), followed by ARID1A (23%), BAP1 (20%), TP53 (20%), and FGFR2 gene fusions (14%).

In a cohort of 80 Chinese patients with eCCA, Xue *et al.* (33) reported that the most frequently altered genes were TP53 (68%), followed by KRAS (46%), SMAD4 (22%), ARID1A (20%), and CDKN2A (19%). The top three actionable alterations included CDKN2A ( $n = 11$ ), BRAF ( $n = 5$ ), and ERBB2 ( $n = 4$ ). Montal *et al.* (34) identified KRAS (36.7%), TP53 (34.7%), ARID1A (14.0%), and SMAD4 (10.7%) as the prevalent mutations in 189 patients with BTC (76% had pCCA and 24% had dCCA) in the US and Europe, while recurrent chromosomal amplifications were observed in YEATS4 (6.0%), MDM2 (4.7%), CCNE1 (2.7%), CDK4 (1.3%), and ERBB2 (1.3%).

Paraffin-embedded tumors from a cohort of 108 Chinese and 107 American patients with GBC were subjected to comprehensive genomic profiling (CGP) with an NGS panel (35). The most frequent alterations

were TP53 (69.4%), followed by CDKN2A/B (26%), ERBB2 (18.5%), PIK3CA (17%), and CCNE1 (13%) in the Chinese cohort, and TP53 (57.9%), CDKN2A/B (25%), SMAD4 (17%), ARID1A (14%), PIK3CA (14%), and ERBB2 (13.1%) in American patients.

In patients with BTC, the disease is highly targetable, thus allowing precision medicine. In a study by Lowery *et al.* (36) with a total of 195 patients of iCCA/pCCA/dCCA, genetic alterations with potential therapeutic implications were identified in 47% of the patients, leading to biomarker-directed therapy or clinical trial enrollment in 16%. Nakamura *et al.* (31) also found potentially targetable genetic driver alterations in ~40% of the patients. With the development of NGS-guided molecular targeted therapy, many inhibitors of molecular targets are reported to achieve a PR or even a CR (36).

### 3.1. Targeting FGFR

Several studies have consistently identified fibroblast growth factor receptor (FGFR) fusions in patients with BTC, and especially patients with iCCA (29). FGFR2 fusion events have been identified in 5.5% (31) to 28% (37) of patients with iCCA. Clinically, FGFR2 fusion-positive status was associated with a shorter OS. A few therapies targeting FGFR-fusions have yielded promising results, including BGJ398 (infigratinib; QED Therapeutics), INCB54828 (pemigatinib; Incyte), ARQ-087 (derazantinib; Arqule), and TAS-120 (Table 2).

BGJ398 (infigratinib) is an orally bioavailable, selective, ATP-competitive pan-FGFR kinase inhibitor with activity in tumor models harboring FGFR alterations. A phase II study of BGJ398 (infigratinib; QED Therapeutics) (38) involved patients with pFGFR-altered advanced cholangiocarcinoma, and it found that the overall response rate was 14.8% (18.8% FGFR2 fusions only), the disease control rate was 75.4% (83.3% FGFR2 fusions only), and the estimated median PFS was 5.8 months (95% CI, 4.3 to 7.6 months). Adverse events included hypophosphatemia (72.1% all grade), fatigue (36.1%), stomatitis (29.5%), and alopecia (26.2%). A phase III clinical trial is ongoing (39).

Derazantinib (ARQ087) is an orally bioavailable, multikinase inhibitor with potent pan-FGFR activity. In a multicenter, open-label, phase I-II trial, Mazzaferro *et al.* (40) enrolled 29 patients with unresectable intrahepatic cholangiocarcinoma with FGFR2 fusion. The overall response rate was 20.7% and the disease control rate was 82.8%.

Pemigatinib (INCB54828; Incyte) is a selective, potent, oral inhibitor of FGFR1-3. A multicenter, open-label, phase II study (41) obtained an objective response (a CR in 3, a PR in 35, and a disease control rate of 82%) in 38 (35.5%) of 107 patients with FGFR2 fusions or rearrangements. Despite the low level of resistance caused by pemigatinib, tumor heterogeneity associated with acquired drug resistance remains a major barrier

**Table 2. Small molecule inhibitor targeting FGFR**

Study, Year	Study design	N	Chemotherapy regimen	PR or CR	PFS	OS (months)
Javle M (39), 2018	FGFR-altered advanced CCA	61	BGJ398 (infigratinib, a pan FGFR inhibitor) 125 mg once daily for 21 days, then 7 days off (28-day cycles) FGFR2 fusion ( $n = 48$ ), mutation ( $n = 8$ ), or amplification ( $n = 3$ )	ORR: 18.8% (FGFR2 fusions) DCR: 83.3% (FGFR2 fusions)	5.8	na
Mazzaferro (40), 2019	Unresectable iCCA with FGFR2 fusion	29	Derazantinib (ARQ 087), a potent pan-FGFR inhibitor	ORR: 20.7% DCR: 82.8%	5.7	na
Ghassan About-Ali (41), 2020	Phase II, previously treated, locally advanced or metastatic CCA	107	Pemigatinib (INCB054828, highly selective FGFR-1, 2, and 3 TKI) (the FIGHT-202 trial) 13.5 mg once daily (21-day cycle; 2 weeks on, 1 week off) in 38/107 (35.5%) of patients with FGFR2 fusions or rearrangements	ORR: 35.5% (38/107, CR achieved in 3 patients, PR in 35)	6.9	21.1
Meric-Bernstam F (44), 2018	CCA previously treated with chemotherapy and another FGFR inhibitor	45	TAS120 (a FGFR 1-4 inhibitor)	PR 25%, SD 53%, CR 0, DCR 79%	na	na

CCA, cholangiocarcinoma. iCCA, intrahepatic cholangiocarcinoma. PFS, progression-free survival. OS, overall survival. PR, partial response. CR, complete response. ORR, objective response rate. DCR, disease control rate. na, not available. FGFR, fibroblast growth factor receptor.

to the long-term use of targeted therapy. Recent studies have noted the emergence of recurrent secondary single-nucleotide variants in FGFR following the inhibition of FGFR; these variants desensitize tumor cells to such therapies (42). Therefore, the mutations that develop in response to FGFR inhibition need to be comprehensively identified in order to investigate novel inhibitors (43).

TAS-120 is an irreversible FGFR inhibitor. A phase I study evaluated the efficacy of TAS-120 (44) in patients with cholangiocarcinoma and FGFR pathway alterations who previously received chemotherapy and other FGFR inhibitors. Forty-five patients with CCA (intra-hepatic  $n = 41$ ) harboring FGF/FGFR aberrations were treated with 16 mg ( $n = 24$ ), 20 mg ( $n = 14$ ), and 24 mg ( $n = 7$ ) QD. The tumor shrank in 20 (71%) of 28 patients with FGFR2 gene fusion, and a PR was achieved in 7. The ORR was 25%. Of the 7 responders, 6 remain on treatment, including 1 patient with an ongoing PR of > 1 year. SD was achieved in 15 (54%) of the 28 patients, and this was their best response. Seven patients are still on treatment. The overall disease control rate was 79%.

In conclusion, FGFR2 inhibitors resulted in the highest ORR and DCR among different targeted therapies, and those inhibitors offer promise for the future development of targeted therapies. In addition, combining FGFR inhibitors with chemotherapy or immunotherapy could increase survival benefits in patients with advanced or metastatic cholangiocarcinoma; this approach requires further investigation.

### 3.2. Targeting IDH1/2

Isocitrate dehydrogenase (IDH) is part of the Krebs cycle; this enzyme converts isocitrate to alpha-ketoglutarate (AKG). Various enzymes such as DNA and histone modifiers require AKG as a cofactor. Mutations in the IDH1 and IDH2 genes occur in about 15-20% of iCCA, with R132 and R172 being the most frequently mutated codons, respectively. An IDH mutation is found exclusively in iCCA, and the prognostic significance of an IDH mutation in advanced iCCA is a subject of debate. Goyal *et al.* (45) reported that the median OS did not differ significantly between patients with an IDH mutant and wild-type IDH (15.0 vs. 20.1 months, respectively;  $p = 0.17$ ), but that patients with iCCA and an IDH mutant had a lower median serum CA19-9. Jiao *et al.* (46) reported that the status of IDH gene mutations was significantly associated with a worse prognosis: subjects with an IDH mutation had a 3-year survival of 33% compared to a 3-year survival of 81% for subjects with wild-type IDH genes ( $P = 0.0034$ ). However, Wang *et al.* (47) found that mutations in IDH1 or IDH2 were associated with a longer OS ( $p = 0.028$ ) and were independently associated with a longer time to tumor recurrence after intrahepatic cholangiocarcinoma resection according to multivariate analysis ( $p = 0.021$ ).

Molecular targeted therapy for mutant IDH1 or

IDH2 in cholangiocarcinoma is limited. Ivosidenib (AG-120) (Tibsovo; Agios) is an oral, targeted mutant IDH1 inhibitor that was approved for the treatment of IDH1 mutant acute myeloid leukemia by the FDA on July 20, 2018 (48). Lowery *et al.* (49) conducted a phase I study on IDH1-mutant iCCA. Seventy-three patients with IDH1-mutant cholangiocarcinoma were enrolled and received ivosidenib. A PR was achieved in 4 patients (5%). Median PFS was 3.8 months, 6-month PFS was 40.1%, and 12-month PFS was 21.8%. Median OS was 13.8 months, though data were censored for 48 patients (66%).

The ClarIDHy phase III clinical trial (NCT02989857) (50) evaluated the role of ivosidenib in patients with IDH1 mutant (R132C/L/G/H/S mutation variants) cholangiocarcinoma following progression during prior chemotherapy. PFS was significantly improved by ivosidenib in comparison to a placebo (median 2.7 months vs. 1.4 months; HR: 0.37; 95% CI: 0.25-0.54; one-sided  $p < 0.0001$ ). However, data on survival time have not been available up to this point.

Other IDH inhibitors are also undergoing clinical trials. A phase I-II, multicenter, open-label, dose-escalation study of enasidenib (AG-221/CC-90007), a selective inhibitor of mutant-IDH2 enzymes, is underway in patients with advanced solid tumors including intrahepatic cholangiocarcinoma (NCT02273739). Patients with advanced malignancies that harbor IDH1R132 mutations are now being recruited for a study of IDH305 (targeted inhibitor of IDH1).

### 3.3. Targeting MEK1

A mutation in the MAP kinase signaling cascade, *i.e.* the RAS/RAF/MEK/ERK signaling pathway, is commonly found in BTC and occurs by multiple mechanisms including ERBB2 overexpression and KRAS, BRAF, and NRAS mutations. A few therapies that target MEK-1 have yielded preliminary results, including selumetinib, trametinib, and binimetinib. A combination of MEK-1 inhibitor and chemotherapy seems better, but the efficacy of MEK1 inhibitors still needs to be improved (Table 3).

Furuse *et al.* (51) reported the results of a phase II study of selumetinib in patients with metastatic biliary cancer. Selumetinib is an inhibitor of MEK1/2 targeting the RAS/RAF/MEK/extracellular signal-related kinase pathway. A PR was achieved in 3 of 28 patients, representing a response rate of 12%. The median PFS was 3.7 months and the median OS was 9.8 months. All toxicities were manageable and reversible. Bridgewater *et al.* (52) conducted a phase Ib study of selumetinib combined with cisplatin/gemcitabine. Objective response (Response Evaluation Criteria in Solid Tumors, RECIST v1.1) was evaluable in 8 patients: PR was achieved in 3 and SD was achieved in 5, with an ORR 25%. The median PFS was 6.4 months. Toxicities related to selumetinib were mostly edema and a rash of grade 1-2

Table 3. MEK1 inhibitors

Study, Year	Study design	N	Chemotherapy regimen	PR or CR	PFS	OS (months)
Furuse J (51), 2011	Phase II	28	Selumetinib	PR was achieved in 3 patients (ORR: 12%)	3.7	9.8
Bridgewater J (52), 2016	Phase Ib	12	The ABC-04 study: Selumetinib (75 mg bid) + cisplatin/gemcitabine (GEMCIS)	PR was achieved in 3 patients (ORR 25%); SD was achieved in 5 (objective response was evaluable in 8 patients) (RECIST v1.1)	6.4	Two patients remained on treatment at 14 and 19 months post registration.
Kim RD (53), 2020	Phase II trial (SWOG S1310)	44 (24 vs. 20)	Trametinib (arm 1) versus 5-fluorouracil or capecitabine (arm 2)	ORR: 8% vs. 10%	1.4 vs. 3.3	4.3 vs. 6.6
Shroff RT (54), 2017	advanced cholangiocarcinoma	25	Pazopanib and trametinib	ORR: 5%; DCR 75%	3.6	6.4
Finn RS (55), 2018	Phase Ib	25	Binimetinib monotherapy	SD was achieved in 12 patients (43%); CR in 1; PR 1; ORR: 2/25 (8%); DCR: 14/25(56%)	na	na
Kim JW (56), 2019	Phase Ib	34	Binimetinib + capecitabine	ORR: 20.6%; DCR:76.5%	4.1	7.8
Lowery MA (32), 2019	Phase II	35	Binimetinib + GEMCIS The efficacy signals observed were modest and not superior to GEMCIS alone	ORR: 36% (12/35) CR was achieved in 3 patients; PR in 9; SD in 14; PD in 7. DCR: 74.2%	6; PFS 6 = 54%	13.3

MEK1, mitogen-activated protein kinase 1. GEM, gemcitabine. CIS, cisplatin. na, not available. RECIST, Response Evaluation Criteria in Solid Tumors. PFS, progression-free survival. OS, overall survival. PR, partial response. CR, complete response. ORR, objective response rate. DCR, disease control rate. na, not available.

and manageable.

Another MER-1 inhibitor, trametinib, was less efficacious than selumetinib. Kim *et al.* (53) studied a total of 44 eligible patients with cholangiocarcinoma (68%) and GBC (32%) who were randomly assigned to treatment arms (24 patients in arm 1 and 20 in arm 2). The response rate was 8% in arm 1 versus 10% in arm 2 ( $p > 0.99$ ). Median OS was 4.3 months for arm 1 and 6.6 months for arm 2. The median PFS was 1.4 months for arm 1 and 3.3 months for arm 2. Shroff RT (54) reported that a combination of trametinib and pazopanib, a VEGF receptor inhibitor, improved DCR but not ORR in advanced cholangiocarcinoma.

Binimetinib monotherapy resulted in an ORR of 8% and a DCR of 56% (55), and a combination of binimetinib and chemotherapy resulted in an ORR of 20.6% and a DCR of 36% (32,56). Using an MSK-IMPACT 410-gene panel, Lowery *et al.* (32) found aberrations in the RAS-RAF-MEK-ERK pathway and mutations in PIK3CA, AKT2, PIK3CG, BRAF, and MAP3K1 in responders. Binimetinib with gemcitabine and cisplatin did not improve the 6-month PFS or ORR. However, the recruiting criteria were not based on molecular signatures in those clinical trials. Molecular profiling may help to select patients who may benefit from MEK-1 targeted therapy.

### 3.4. Targeting BRAF-V600E

Several other solid tumors with a BRAF mutation have benefited from a combination of BRAF and MEK inhibitors. Planchard *et al.* (57) conducted an open-label phase II trial examining the efficacy of dabrafenib plus trametinib in patients with BRAFV600E-mutant metastatic non-small-cell lung cancers that were previously untreated. Thirty-six 36 patients were enrolled. Twenty-three patients had an overall response (64%, 95% CI 46-79); a CR was achieved in 2 (6%) and a PR in 21 (58%). Robert *et al.* (58) reported the first-line treatment with dabrafenib plus trametinib led to a long-term benefit in approximately one-third of patients who had unresectable or metastatic melanoma with a BRAF V600E or V600K mutation. A CR, which was associated with an improved long-term outcome, was achieved in 109 patients (19%). The overall survival rate at 5 years was 71% (95% CI, 62 to 79).

Only case reports have evaluated dabrafenib plus trametinib in advanced BTC (Table 4). Bunyatov *et al.* (59) described a rare case with poorly differentiated cholangiocarcinoma with an atypical genetic mutation in the BRAF V600E gene; the cancer was stage T4N1M0, and a successful outcome was obtained. A 38-year-old female patient underwent surgery at the National Surgery Institute for iCCA of the left lobe of the liver with invasion of the anterior abdominal wall, the diaphragm, and the pericardium. Liver resection, lymph node dissection, and pericardial resection were performed.

**Table 4. Targeting BRAF V600E**

Study, Year	Study design	N	Chemotherapy regimen	PR or CR	PFS	OS (months)
Kocsis J (62), 2017	Case report, a 59-year-old female with chemotherapy-refractory metastatic eCCA	eCCA (n = 1)	Dabrafenib + Trametinib Next-generation sequencing (NGS) revealed a BRAF V600E mutation	1 CR	3	At 12 weeks, PET CT confirmed further tumor regression with complete regression of multiple cerebral metastases.
Bunyatov T (59), 2019	Case report, iCCA of the left lobe of the liver with invasion of the anterior abdominal wall, the diaphragm, and the pericardium	iCCA (n = 1)	Dabrafenib + Trametinib Mutation in the BRAF V600E gene	1 CR	na	The patient has been tumor-free for 2 years with no signs of recurrence.
Lavingia V (60), 2016	Case report	iCCA (n = 2)	Dabrafenib and trametinib Mutation in the BRAF V600E gene	1 CR 1 PR	6 5	CR was achieved at 6 months in 1 patient who died at 9 months. PR was achieved at 2 months in 1 patient who had a PFS for 5 months.
Loaiza-Bonilla A (61), 2014	Case report	iCCA (n = 1)	Dabrafenib + Trametinib Mutation in the BRAF V600E gene	1 PR	na	Symptomatic and sustained near-complete radiological improvement.

iCCA, intrahepatic cholangiocarcinoma. eCCA, extra-hepatic cholangiocarcinoma. PFS, progression-free survival. OS, overall survival. PR, partial response. CR, complete response. na, not available.

Adjuvant chemotherapy (GEMOX) did not yield any results. Treatment with pembrolizumab did not result in any improvement, either. NGS and molecular profiling of the tumor revealed the mutation in BRAF V600E gene. Target therapy with dabrafenib and trametinib was initiated and resulted in a full response. The patient has been tumor-free for 2 years with no signs of recurrence.

Lavingia *et al.* (60) reported on 2 cases of BRAF V600E refractory iCCA treated with dual BRAF and MEK inhibitors (dabrafenib and trametinib) with an excellent clinical and radiological response to therapy and a protracted duration of disease control. A CR was achieved in 1 patient after 6 months of treatment, and disease progression ultimately occurred at 9 months. PR was achieved in the second patient 2 months after treatment, and that patient has been progression-free 5 months after treatment.

Loaiza-Bonilla *et al.* (61) reported on a 47-year-old woman diagnosed with chemotherapy and radiation-refractory BRAF V600E mutant, poorly differentiated iCCA. The patient was stage IV and had multiple metastatic lesions in the liver, lungs, pleura, and bone. NGS genomic information suggested that the patient was a suitable candidate for dual BRAF and MEK inhibition therapy. After dual therapy with dabrafenib and trametinib, the patient's tumor almost disappeared completely, as confirmed by computed tomography, but the patient is still symptomatic.

The outcome of the dual targeting therapy appears superior to that of BRAF inhibition alone and cytotoxic chemotherapy. Given the poor outlook and refractoriness of BRAF mutant iCCA, future studies should focus on early integration of BRAF/MEK inhibition.

### 3.5. Targeting HER-2

HER family receptors (EGFR/HER1, HER2neu, HER3, and HER4) trigger multiple signaling cascades, including the mitogen-activated protein kinase (MAPK) cascade phosphatidylinositol 3-kinase (PI3K)/AKT pathway and signal transducer and activator of transcription (STAT) transcription factor, leading to various phenomena, including cell proliferation, cell differentiation, angiogenesis, metastasis, and inhibition of apoptosis, that are involved in the development of several carcinomas. HER2 alterations, including overexpression, amplifications, and other mutations, are found in a variety of solid tumors (63). In BTC, HER2 overexpression is observed in ~ 5% of intrahepatic CCA, ~20% of extrahepatic CCA, and ~19% of GBC.

HER-2 inhibitors include trastuzumab, pertuzumab, lapatinib, neratinib, and afatinib. Trastuzumab plus chemotherapy is the first-line therapy for patients with HER2-positive gastric cancer, although trials involving pertuzumab, lapatinib, and T-DM1 have failed to improve outcomes.

Lapatinib monotherapy (64) or afatinib plus GEMCIS

(65) has failed to yield any survival benefit in advanced BTC. However, these studies were not treating patients with specific molecular biomarkers. Moreover, a PR was achieved in 2 patients with metastatic GBC who received HER-2 inhibitors with amplification of the ERBB2 gene (66,67). Furthermore, treatment of advanced GBC and CCA with HER2/neu genetic aberrations or protein overexpression with monotherapy or a combination of two HER-2 inhibitors resulted in an ORR ranging from 22-55% (64-70) (Table 5). In the future, both novel antibody-drug conjugates and bispecific antibodies targeting HER2 and HER2-targeted therapies in combination with immune-checkpoint inhibition will be tested in clinical trials (67).

### 3.6. PARP inhibitors targeting BRAC1/2, BAP1, and ATM

Poly [ADP-ribose] polymerase (PARP) inhibitors are involved in cell repair. Somatic mutations of the tumor-suppressor genes BRCA1 and BRCA2 have been reported in cholangiocarcinomas (31). BRCA-mutated tumors are often sensitive to PARP inhibitors. Accordingly, a retrospective clinical analysis of patients with BRCA-mutated cholangiocarcinoma ( $n = 18$ ) found that a sustained disease response was achieved in 1 of 4 patients who received PARP inhibitors, with a PFS of 42.6 months; the OS for patients with stage III/IV cancer was 25 months (71). Although PARP inhibitors and inhibitors of ataxia-telangiectasia mutated (ATM), another DNA repair protein, are currently being evaluated in multiple clinical trials on BRCA-mutated breast cancer, they need to be prospectively evaluated in patients with cholangiocarcinoma. Zhang *et al.* (72) reported on the efficacy of olaparib in a patient with gallbladder cancer with an ATM-inactivating mutation. SD was achieved, and the patient survived for 16 months on olaparib. A phase II trial of the PARP inhibitor niraparib is planned in patients with advanced-stage malignancies, including cholangiocarcinoma, and with known mutations in BAP1 and other DNA double-strand break repair pathway genes – excluding BRCA1/2 mutations (NCT03207347).

### 3.7. Immune checkpoints inhibitors (ICI) for BTC

Immune checkpoints inhibitors (ICI) targeting programmed cell death protein 1 (PD-1) or its ligand PD-L1 or cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) checkpoints have demonstrated the potential to target tumor-specific immune suppression. According to data from the literature, inhibition of immune checkpoints has yielded promising results in several malignancies such as melanoma (73,74), non-small cell lung cancer (75), urothelial carcinoma (76), renal cell carcinoma (77), head and neck cancer (78) and hepatocellular carcinoma (79). Thus far, the clinical data

**Table 5. Targeting HER2/ERBB2**

Study, Year	Study design	N	Chemotherapy regimen	PR or CR	PFS	OS (months)
Moehler M (64), 2019	advanced BTC	9	Afatinib + GEMCIS	failed to have a survival benefit in combination with GEMCIS	6.0	7.7
Ramanathan RK (65), 2009	Advanced BTC and HCC, Phase II	BTC 17, HCC 40	Lapatinib	The response was 0% in BTC and 5% in HCC	1.8 vs. 2.3	5.2 vs. 6.2
Czink E (66), 2016	metastatic GBC with ERBB2 gene amplification	1	Pertuzumab and trastuzumab in combination with nab-paclitaxel	1 case report of PR	> 12	> 12
Ye MF (67), 2019	HER2 amplification in GBC	1	Trastuzumab and lapatinib	1 case report of PR	na	na
Hyman DM (70), 2018	SUMMIT; phase II; Second-line or later. ERBB2 and/or ERBB3 alterations identified using NGS	11	Neratinib (a pan-HER-kinase inhibitor)	ORR 22%	na	na
Hainsworth JD (69), 2018	MyPathway Second-line or later. HER2-IHC 3+ and/or ISH-positive; Phase IIa (basket study)	11	Trastuzumab + pertuzumab	PR in 2/7 patients (ORR: 29%), SD in 3/7 >120 days. PR in 4/11, SD in 3/11, ORR: 36%	na	na
Javle M (68), 2015	advanced GBC and CCA with HER2/neu genetic aberrations or protein overexpression	14	HER2/neu-directed therapy (trastuzumab, lapatinib, or pertuzumab)	9 cases of GBC: CR was achieved in 1, PR in 4, SD in 3, MR (mixed response) in 1, ORR: 55%. 5 cases of CCA: PD was achieved in 5 patients	na	8-178 weeks 7-29weeks

BTC, biliary tract cancer. GBC, gall bladder cancer. CCA, cholangiocarcinoma. HCC, hepatocellular carcinoma. GEM, gemcitabine. CIS, cisplatin. PFS, progression-free survival. OS, overall survival. PR, partial response. CR, complete response. SD, stable disease. na, not available. HER2, human epidermal growth factor receptor-2. ERBB2, erythroblastic leukemia viral oncogene homolog 2.



on immunotherapy for CCA and other BTCs are limited, and several trials are underway; they are exploring, for instance, the role of the monoclonal antibodies ipilimumab or tremelimumab (anti-CTLA4) or antibodies targeting PD-L1 or PD-1, such as pembrolizumab or nivolumab (80).

Gou *et al.* (81) reported on 30 patients with metastatic BTC who voluntarily received nivolumab. CR was achieved in 1 patient, a PR in 5, SD in 12, and PD in 12. ORR was 20%, DCR was 60%, and PFS was 3.1 months. Fifty-four patients with BTC included 59% with iCCA, 11% with eCCA and 30% with GBC who received nivolumab monotherapy; ORR was 22%, median PFS was 3.8 months, and median OS was 10.3 months (82). Durvalumab monotherapy has also displayed limited efficacy. In a phase I study of 42 patients, ORR was only 4.8%, median PFS was 1.6 months, and median OS was 8.1 months (83).

The efficacy of pembrolizumab monotherapy is also limited. The PD-L1 inhibitor pembrolizumab was administered to 104 patients with advanced BTC. Pembrolizumab achieved a PR in 6 patients, resulting in an ORR of 5.8%. Median PFS was 2.0 months, and median OS was 9.1 months (84). Kang *et al.* (85) conducted a prospective cohort study in 40 patients with PDL1-positive BTC that progressed despite first-line gemcitabine plus cisplatin. Pembrolizumab 200 mg was administered intravenously every 3 weeks. The ORR was 10% according to RECIST v1.1 and 12.5% according to the immune-modified RECIST (imRECIST). The median PFS was 1.5 months, and OS was 4.3 months. This checkpoint inhibitor is currently being tested in combination with cisplatin and gemcitabine in the phase II ABC-09 trial (NCT03260712).

Combining two ICIs does not look promising. Arkenau *et al.* (86) reported that ramucirumab plus pembrolizumab in patients with advanced or metastatic BTC had limited efficacy even in the patients with biomarker-unselected progressive BTC, with an ORR of 4%, a median PFS of 1.6 months, and an OS of 6.4 months.

A combination of an ICI and chemotherapy resulted in a better ORR and DCR compared to an ICI alone. Nivolumab combined with chemotherapy resulted in a better tumor response and patient survival than nivolumab monotherapy. Ueno *et al.* (87) conducted a multicenter, open-label, phase I trial at four cancer centers in Japan. Thirty patients were enrolled in each cohort. In the monotherapy cohort, median OS was 5.2 months, median PFS was 1.4 months, and a PR was achieved in 1 of the 30 patients. In the combined therapy cohort, median OS was 15.4 months, median PFS was 4.2 months, and a PR was achieved in 11 of the 30 patients. Phase II studies are ongoing: patients with BTC are receiving either nivolumab alone (NCT02829918), or in combination with chemotherapy (gemcitabine/cisplatin) or with another immunotherapy (ipilimumab;

NCT03101566).

Numerous case series have involved patients receiving immunotherapy with PD-1/PD-L1 inhibitors combined with radiotherapy or chemotherapy that achieved a CR or PR (Table 6). Clinical trials studying immunotherapy combinations designed to augment the immune antitumor response are also underway. Hyperactivated PD1/PD-L1 signals in tumor tissues are a negative prognostic marker for iCCA after resection (88). In addition, PD-L1 expression in both cancer and stroma cells of patients with CCA was an independent predictor of poor OS (89). However, evidence of PD-L1 expression was not always related to a longer PFS in contrast to a lack of PD-L1 expression (81). PD-L1 protein expression is determined using the tumor proportion score (TPS), which is the percentage of viable tumor cells with partial or complete membrane staining at any intensity. The TPS is an indicator of the degree of PD-L1 immunostaining. Some studies have reported that patients with a TPS  $\geq 50\%$  (85) had a higher rate of tumor response to ICI than patients with a TPS  $< 50\%$ . Immunotherapy could become an important part of treatment of iCCA in the future. Future studies of immunotherapies need to collect and report information on important clinical covariates, such as the anatomical site, along with blood and tumor samples. In addition, potential biomarkers including MSI, MMR, TMB, and PD-L1 and tumor somatic mutations (TMB) should be quantified in order to identify those patients who are most likely to benefit from immunotherapy (80,90).

#### 4. Conclusion and perspectives for the future

In conclusion, advanced BTC has a poor prognosis. Chemotherapy, and especially a triplet GAP regimen based on GEMCIS, has the most significant effect on that cancer, and FOLFIRINOX combined with bevacizumab is promising. Molecular targeted therapy based on genome sequencing appears essential to precision medicine. FGFR inhibitors and IDH inhibitors are promising emerging targeted therapies mainly for iCCA. Other targeted therapies such as anti-HER2 therapies or MEK-1/2 or BRAF inhibitors should be used in accordance with biomarkers. Further evaluation of combination strategies in particular is needed. Case series have reported that ICIs combined with chemotherapy or radiotherapy result in a good response, though this is still being evaluated in several studies. Combination therapies have garnered attention because of interactions between signaling pathways of carcinogenesis in BTC

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Table 6. Summary of cases of biliary tract cancer where PR/CR was achieved with immune checkpoint inhibitors.

NO.	Author, Year	Tumor type	Stage	TMB	PD-L1	MS status	Pre-treatment	Treatment	Response	PFS/DFS
1	Chen (91), 2019	iCCA	IV	18.46	< 1%	na	Liver tumor recurred 5 months after surgery	Nivolumab + lenvatinib	PR	> 21 m
2	Liu (92), 2019	iCCA	IVA	1.2	< 1%	MSS, pMMR	na	SBRT (Cyberknife) + Nivolumab* 15 cycles	PR	7 m
3	Liu (92), 2019	iCCA	IIIA	3.8	< 1%	MSS, pMMR	Lapatinib* 3 cycles	Cyberknife + Pembrolizumab* 5 cycles	PR	5 m
4	Alshari (93), 2019	iCCA	IIIB	0.98	< 1%	MSS, pMMR	11 months after surgery, liver tumor recurred with LNM	Cyberknife + Pembrolizumab* 16 cycles. Furflucil* 6 cycles, Endostatin* 4 cycles	CR	11 m
5	Sui (94), 2018	iCCA	IIIB	2.95	< 5%	MSS, pMMR	11 month after surgery, liver tumor recurred with LNM	Cyberknife + Tegafur* 3 cycles + Pembrolizumab* 15 cycles	CR	16 m
6	Sui (94), 2019	iCCA	IIIB	7.09	< 5%	MSS, pMMR	3 months after surgery, liver tumor recurred with LNM	Tegafur continuous + Pembrolizumab* 6 cycles	CR	13 m
7	Mou (95), 2018	iCCA	IIIB	19.3	80%	pMMR	na	Pembrolizumab + SOX* 8 cycles, Pembrolizumab monotherapy for 6 months	CR	10 m
8	Alshari (93), 2019	GBC	IVB	na	na	na	Liver tumor recurred with LNM	Pembrolizumab* 8 cycles	CR	24 m

iCCA, intrahepatic cholangiocarcinoma. GBC, gall bladder cancer. LNM, lymph node metastasis. SOX, S-1 plus oxaliplatin. SBRT, stereotactic body radiation therapy. PR, partial response. CR, complete response. PFS, progression-free survival. DFS, disease-free survival. na, not available. MS, microsatellite status. MSS, microsatellite stable. MMR, mismatch repair status. TMB, tumor mutational burden.

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*\*Address correspondence to:*

Wei Zhang, Department of Hepatobiliary Cancer, Tianjin Medical University Cancer Institute and Hospital; National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin; Tianjin's Clinical Research Center for Cancer. Huan Hu Xi Road, Tianjin, 300060, China.

E-mail: zhangweitjch@163.com

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