Review Article

Targeted therapies in advanced biliary tract cancers—a narrative review

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Background and Objective: Biliary tract cancers (BTCs), including cholangiocarcinoma and gallbladder cancer, are a relatively rare group of cancers with a poor prognosis. Over the past decade, the utilization of next-generation sequencing has led to the identification of multiple actionable somatic aberrations in BTCs. Subsequently, new therapies have been created to target these molecular alterations and have been incorporated into clinical practice. In this review, we outline therapies that have been previously studied, and those that are under investigation, to target genomic alterations with the goal of improving survival in patients with advanced disease.

Methods: A literature search was performed to identify phase I, II, and III trials of targeted therapies in patients with advanced BTCs published between January 1, 2010 and October 1, 2022. Medline (via PubMed) and ClinicalTrials.gov were searched for relevant studies and 415 trials were identified. The search strategy was performed using keywords including: biliary tract cancer, cholangiocarcinoma, gallbladder cancer, chemotherapy, targeted therapy, randomized trials, controlled trials, phase I, phase II, and phase III. Search results were imported into EndNote X 9.1.

Key Content and Findings: Overall, immune checkpoint inhibitors, fibroblast growth factor receptor (FGFR) inhibitors, isocitrate dehydrogenase (IDH) inhibitors, and human epidermal growth factor receptor 2 (HER2)-directed therapies have all shown promising results with regard to efficacy in patients with advanced BTCs studied in clinical trials. A number of other agents have also been studied in early-phase trials.

Conclusions: Targeted agents can improve survival in patients with advanced BTCs and have substantially increased the number of potential therapeutic options in patients with refractory disease. The therapeutic landscape of targeted therapies for patients with advanced BTCs continues to evolve based on improvements in detection of genomic alterations.

Keywords: Biliary tract cancer (BTC); cholangiocarcinoma; gallbladder cancer; targeted therapies

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Introduction

Intrahepatic cholangiocarcinoma (IC), distal extrahepatic cholangiocarcinoma (EC), hilar (Klatskin) cholangiocarcinoma, and gallbladder cancer, collectively known as biliary tract cancers (BTCs) arise from the epithelium of the biliary tree and are relatively rare cancers. The global incidence of BTCs is estimated to be between 0.3 to 6 per 100,000 people, with the highest incidence in southeast Asia (age-standardized rate as high as 3.00 in South Korea) and lowest in Western countries (age-standardized rate as low as 0.66 in the United Kingdom) (1,2). In the United States, the incidence of cholangiocarcinoma, particularly IC, is rising (3,4). Risk factors for BTCs include liver fluke infection, primary sclerosing cholangitis, hepatolithiasis, choledochal cysts, alcohol consumption, tobacco use, hepatitis B and hepatitis C infection, and metabolic conditions such as obesity, diabetes, and nonalcoholic fatty liver disease (5-9). Although some progress has been made with respect to improving the survival of patients with BTCs over the past decade, historically, these malignancies are associated with a poor prognosis, with 5-year overall survival (OS) rates of less than 15% (10-12).

Early detection of BTCs greatly impacts prognosis. Currently, no screening guidelines exist or are recommended for BTCs. When BTC is suspected, contrast-enhanced magnetic resonance cholangiopancreatography (MRCP) or computed tomography (CT) are the imaging modalities of choice to evaluate for vascular anomalies, satellite lesions, lymph node involvement, and metastatic disease (13,14). These characteristics are crucial in determining whether a biliary tract tumor is surgically resectable and thereby potentially curable. A tissue diagnosis is often obtained via endoscopic retrograde cholangiopancreatography (ERCP) and via biliary tract brushings. Resectable disease is defined by the absence of multifocal liver disease, lymph node metastasis beyond the porta hepatis, and distant metastasis. Resectability status is ultimately determined by a multidisciplinary team of experienced radiologists and surgeons. In patient's with hilar cholangiocarcinoma, biopsy should not be performed until resectability status and transplant candidacy have been determined because transperitoneal biopsy may preclude transplantation depending on institutions' protocols. In patients with unresectable disease, biopsies should be performed for tissue analysis and molecular profiling, including next-generation sequencing, to identify actionable mutations. In addition, positron emission tomography (PET) and diagnostic laparoscopy can help detect regional lymph node involvement and distant metastasis, although PET scans have a higher propensity for falsely detecting sites of disease that are not actually sites of metastasis (15-17).

While chemotherapy has historically been the mainstay of treatment for patients with advanced cholangiocarcinoma, targeted molecular therapies are increasingly utilized in clinical practice due to newly identified alterations and the desire to reduce adverse effects associated with cytotoxic therapy (*Figure 1*). One analysis postulated that approximately 68% of patients with BTCs have an actionable mutation (18). In this review, we examine the evidence supporting the utilization of targeted therapies for patients with advanced BTCs. We present the following article in accordance with the Narrative Review reporting checklist (available at https://cco.amegroups.com/article/view/10.21037/cco-22-93/rc).

Methods

A literature search was performed to identify phase I, II, and III trials of targeted therapies in patients with advanced BTCs published between January 1, 2010 and October 1, 2022 (*Table 1*). MEDLINE (via PubMed) and ClinicalTrials.gov were searched for relevant studies and 415 trials were identified. The search strategy was performed using keywords including: biliary tract cancer, cholangiocarcinoma, gallbladder cancer, chemotherapy, targeted therapy, randomized trials, controlled trials, phase I, phase II, and phase III. Search results were imported into EndNote X 9.1.

Chemotherapy

For patients with unresectable (hereto referred to as advanced) cholangiocarcinoma who are treatment naive, therapeutic options include clinical trial enrollment, gemcitabine-based chemotherapy, and chemotherapy combined with immunotherapy. Other options are also acceptable depending on a patient's comorbidities and drug toxicity profiles, including fluoropyrimidine-based chemotherapy, chemoradiation, or radiation alone. The survival benefit conferred by gemcitabine or fluoropyrimidine-based chemotherapy for patients with advanced, BTCs has been demonstrated in several trials (*Table 2*). Based on the ABC-02 study, combination gemcitabine and cisplatin for patients with advanced,

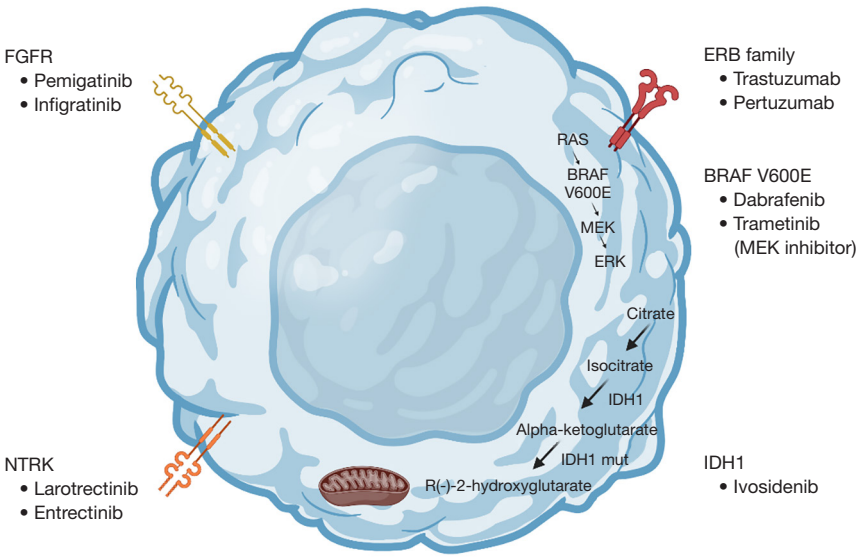

Figure 1 Key targets in biliary tract cancers. Created with BioRender.com. FGFR, fibroblast growth factor receptor; NTRK, neurotrophic tyrosine receptor kinase; RAS, rat sarcoma; BRAF, v-raf murine sarcoma viral oncogene homolog B1; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; IDH1, isocitrate dehydrogenase 1; ERB, epidermal growth factor.

Table 1 Search strategy

Items	Specification
Date of search	06/01/2022
Databases and other sources searched	PubMed, ClinicalTrials.gov
Search terms used	Biliary tract cancer, cholangiocarcinoma, gallbladder cancer, chemotherapy, targeted therapy, randomized trials, controlled trials, phase I, phase II, phase III
Timeframe	January 1, 2010 and October 1, 2022
Inclusion and exclusion criteria	All clinical trials (phase I, phase II, phase III) for the treatment of biliary tract cancers were included, based on search criteria above. Trials were excluded if they were not completed, closed early, or did not report their outcome in the form of an abstract or manuscript
Selection process	An independent search was conducted by the authors

untreated BTC was the standard of care regimen for over a decade (19). Alternative first-line chemotherapeutic options that are suitable depending on a patient's comorbidities include: gemcitabine plus oxaliplatin, gemcitabine plus capecitabine, gemcitabine plus albumin-bound paclitaxel, capecitabine plus oxaliplatin, 5-fluorouracil plus oxaliplatin, single-agent fluorouracil, single-agent capecitabine, or single-agent gemcitabine.

No consensus exists on which chemotherapy regimens should be utilized in the second-line (or further) setting, however the ABC-06 study demonstrated in patients with advanced, refractory BTCs, that 5-fluorouracil with leucovorin and oxaliplatin (FOLFOX) plus active symptom control improved median OS compared to active symptom control alone [6.2 *vs.* 5.3 months, hazard radio (HR) 0.69; 95% confidence interval (CI): 0.50–0.97; P=0.031] (20). Another option for patients with advanced BTCs who have progressed on gemcitabine and cisplatin is liposomal irinotecan in combination with fluorouracil, which was shown to be superior with regard to median OS compared to fluorouracil alone in the phase II NIFTY trial (8.6 *vs.* 5.3 months, HR 0.68; 95% CI: 0.48–0.95; P=0.024) (21).

Table 2 Completed landmark trials utilizing chemotherapy

Investigational arm	Comparison arm	N	Line	Phase	mPFS	mOS
Gemcitabine plus cisplatin, ABC-02 Study (19)	Gemcitabine	410	1L	3	8.0 *vs.* 5.0 months (P<0.001)	11.7 *vs.* 8.1 months (HR 0.64; 95% CI: 0.52, 0.80; P<0.001)
FOLFOX plus active symptom control, ABC-06 Study (20)	Active symptom control	162	2L	3	4.0 months (95% CI: 3.2–5.0) *vs.* not reported	6.2 *vs.* 5.3 months (HR 0.69; 95% CI: 0.50, 0.97; P=0.031)
Liposomal Irinotecan (nal-IRI) plus 5FU/LV, NIFTY Study (21)	5FU/LV	174	2L	2B	7.1 *vs.* 1.4 months (P=0.0019)	8.6 *vs.* 5.3 months (HR 0.68; 95% CI: 0.48, 0.95; P=0.024)

mPFS, median progression-free survival; mOS, median overall survival; CI, confidence interval; HR, hazard ratio; nal-IRI, nanoliposomal irinotecan; 5FU, fluorouracil; LV, leucovorin.

Table 3 Completed landmark trials utilizing first-line immunotherapy

Investigational arm	Comparison arm	N	Line	Phase	mPFS	mOS
Durvalumab plus gemcitabine plus cisplatin, TOPAZ-1 Study (27)	Gemcitabine plus cisplatin	685	1L	3	7.2 *vs.* 5.8 months (HR 0.75; 95% CI: 0.64–0.89; P=0.001)	12.8 *vs.* 11.5 months (HR 0.80; 95% CI: 0.66–0.97; P=0.021)

mPFS, median progression-free survival; mOS, median overall survival; CI, confidence interval; HR, hazard ratio.

Immunotherapy

Broadly, immunotherapy refers to the class of agents that block the ability of tumor cells to evade recognition and destruction by immune cells. Several prognostic biomarkers of response to immunotherapy for specific cancer types have been identified. However, their significance in predicting response to immunotherapy in BTCs is under investigation (22). A tumor's mutational burden (TMB), or the quantity of a tumor's genetic mutations, is associated with susceptibility to immunotherapy across many cancer types (23). High tumoral expression of PD-L1 is another marker that may be associated with susceptibility to immunotherapy (24). TMB status and PD-L1 expression do not typically correlate with one another, as each provides unique information about a tumor's genome (25). A third prognostic biomarker for immunotherapy sensitivity involves mutations in genes that encode proteins responsible for the repair of single base pair insertions and deletions (mismatch repair, or MMR) at repetitive sequences of deoxyribonucleic acid (DNA), known as microsatellites. Tumoral genomes are classified as having either proficient (pMMR) or deficient MMR (dMMR) as well as microsatellite stability (MSS) or instability (MSI). In one analysis of mutational load by cancer type, 7% of all cholangiocarcinomas (n=1,327) had a TMB of greater than ten mutations per megabase, whereas only 1% of cholangiocarcinomas were classified as having microsatellite instability (26).

Recently, in the TOPAZ-1 study, the addition of durvalumab, an antibody against programmed death-ligand 1 (PD-L1), in combination with gemcitabine and cisplatin was shown to improve OS and progression-free survival (PFS) without an increase in grade 3 or 4 treatment-related adverse events in patients with advanced, BTCs (27). A median OS of 12.8 months was observed in patients who received durvalumab with gemcitabine and cisplatin compared to 11.5 months in patients who received gemcitabine and cisplatin (HR 0.80; 95% CI: 0.66–0.97; P=0.021) (*Table 3*).

Nivolumab, an antibody against programmed cell death 1 (PD-1) receptor, has been studied in patients with advanced, refractory BTCs. In a phase II trial of 54 patients (46 evaluable from the United States) who received nivolumab, an objective response rate (ORR) of 22% was observed by investigator review (11% by blind independent review) and a disease control rate (DCR) of 59% was observed by investigator review (50% by blind independent review). All patients who responded had tumors with proficient mismatch repair, and nine of the ten patients who responded had PD-L1 expressed by 1% or more of the tumor cells analyzed (28). In another phase II trial, patients (n=39) who received nivolumab in combination with ipilimumab, an antibody against cytotoxic lymphocyte-associated protein 4 (CTLA-4), resulted in an ORR of 23% and DCR of 44%. Of note, only patients with IC or gallbladder cancer responded (29). As a result of these studies, nivolumab can be considered as a subsequent-line treatment option for

patients with unresectable or metastatic progressive BTC who have not previously been treated with a checkpoint inhibitor.

Pembrolizumab, an antibody against PD-1, has also been studied in patients with advanced, refractory BTCs. In a phase II trial of 233 patients (with 27 cancer types, including 22 patients with cholangiocarcinoma) with MSI-H/dMMR tumors who received pembrolizumab, an ORR of 34.3%, median PFS of 4.1 months, and median OS of 23.5 months were observed. In addition, 29% of patients with a high TMB responded to pembrolizumab, whereas only 6% of patients without a high TMB had a response (30,31). In another analysis of 86 patients (with 12 cancer types, including four patients with cholangiocarcinoma) with dMMR tumors who received pembrolizumab, all patients with cholangiocarcinoma demonstrated disease control, and one had a complete response (32). As a result of these studies, pembrolizumab is another option as a first or subsequent-line (when a checkpoint inhibitor was not used in the first-line) treatment option for patients with advanced MSI-H/dMMR and TMB-H BTCs. Pembrolizumab was also studied in combination with lenvatinib, a multikinase inhibitor, in a phase II trial of 31 patients with advanced, refractory BTC. An ORR of 10%, DCR of 68%, and median PFS of 6.1 months were observed. All patients who responded had tumors with PD-L1 expressed by 1% or more of the tumor cells analyzed (33).

Dostarlimab, another antibody against PD-1, was studied in a phase I study of 209 patients with MSI-H/dMMR or polymerase epsilon catalytic subunit (POLE) hypermutated tumors (34). One patient in this study had gallbladder cancer, and another had cholangiocarcinoma. Both had a complete response (35). As a result of this study, dostarlimab can be considered as a subsequent-line treatment option for patients with MSI-H/dMMR recurrent or advanced BTCs as a subsequent-line therapy who have no satisfactory alternative treatment option and have not been treated with a checkpoint inhibitor.

Lastly, besides the aforementioned benefit from adding durvalumab to gemcitabine and cisplatin in the first-line setting from the TOPAZ-1 study, durvalumab has also been studied as a single agent and in combination with tremelimumab, an antibody against CTLA-4, in a phase I study of 107 patients with advanced, refractory BTCs. In the 42 patients who received durvalumab alone, a DCR of 16.7% and median OS of 8.1 months were observed. In the 65 patients who received durvalumab and tremelimumab, a DCR of 32.2% and median OS of 10.2 months were observed (36). Several trials studying the efficacy and safety of immunotherapy in patients with BTCs are ongoing (*Table 4*).

Targeted therapies

Fibroblast growth factor receptor (FGFR) inhibitors

FGFRs are transmembrane receptors that, when bound by FGF ligands, activate downstream signaling pathways that promote cell survival, proliferation, and differentiation, including the extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathway (37). *FGFR2* gene mutations, fusions, and rearrangements are identified in 13–14% of ICs and are associated with a favorable prognosis (38-41).

The FGFR inhibitor futibatinib, in the phase II FOENIX-CCA2 trial, was recently shown to show activity in 103 patients with advanced, refractory IC harboring FGFR2 fusion/rearrangements. The median duration of response was 9.5 months, PFS was 8.9 months, and OS was 20.0 months (42). Pemigatinib, another FGFR inhibitor, was studied in the phase II FIGHT-202 trial of 146 patients with advanced, refractory cholangiocarcinoma containing *FGFR* fusions, rearrangements, or alterations. 35.5% of patients achieved an objective response (95% CI: 26.5–45.4%) (43). Pemigatinib was also studied in another phase II trial of 87 patients with advanced, refractory cholangiocarcinoma (47 with *FGFR2* translocations, 22 with other *FGFR* alterations, and 18 without *FGFR* alterations). Responses were only observed in eight patients with *FGFR2* translocations. A median PFS of 6.8 months (95% CI: 3.6–9.2 months) in patients with *FGFR2* translocations was observed compared to 1.4 months in patients with other *FGFR* alterations and 1.5 months in patients without FGFR alterations (44). Infigratinib, a different FGFR inhibitor, was studied in a phase II trial of 108 patients with advanced, refractory cholangiocarcinoma containing *FGFR* fusions or rearrangements. In this study, a median PFS of 7.3 months (95% CI: 5.6–7.6 months) and ORR of 23.1% (95% CI: 15.6–32.2%) were demonstrated, with a median duration of response of 5.0 months (45). As a result of these studies, pemigatinib and infigratinib are considered to be subsequent-line treatment options for patients with unresectable or metastatic cholangiocarcinoma with FGFR2 fusions or rearrangements.

Other FGFR inhibitors, such as derazantinib and RLY-4008, have been evaluated in early phase trials with modest

Table 4 Ongoing trials utilizing immunotherapy in advanced disease

Investigational arm	Comparison arm	n	Line	Phase	NCT number
Pembrolizumab plus gemcitabine plus cisplatin (KEYNOTE-966)	Gemcitabine plus cisplatin	1,048	1L	3	NCT04003636
Durvalumab plus SNDX-6532 after intra-arterial chemoembolization or radioembolization	None	30	Unspecified	2	NCT04301778
Sitravatinib plus tislelizumab	None	43	2L and beyond	2	NCT04727996
Durvalumab plus tremelimumab plus radiation	None	70	2L and beyond	2	NCT03482102
Nivolumab plus rucaparib	None	35	Maintenance following platinum therapy	2	NCT03639935
Nivolumab plus DKN-01	None	30	2L and beyond	2	NCT04057365
Durvalumab plus tremelimumab with or without paclitaxel	None	106	2L	2	NCT03704480
Nivolumab plus nanoliposomal-irinotecan plus 5-FU	None	34	2L	1/2	NCT03785873
XmAb22841	XmAb22841 with pembrolizumab	242*	2L and beyond	1	NCT03849469
Toripalimab plus lenvatinib	None	44	2L	2	NCT04211168
Toripalimab plus S1 plus albumin paclitaxel	None	30	1L	2	NCT04027764
Toripalimab plus gemcitabine plus oxaliplatin	none	20	1L	2	NCT04191343
Avelumab plus regorafenib	None	482*	2L and beyond	1/2	NCT03475953
Camrelizumab plus apatinib	Camrelizumab plus either GEMOX or FOLFOX	157*	Arm A: 2L or beyond; arm B: 1L	2	NCT03092895
Durvalumab plus guadecitabine	None	55*	2L	1	NCT03257761
Envafolimab plus GEMOX	GEMOX	480	1L	3	NCT03478488

*, includes several cancer types. NCT, National Clinical Trial; FOLFOX, leucovorin (folinic acid), fluorouracil, oxaliplatin; GEMOX, gemcitabine, oxaliplatin.

to comparable results. Derazantinib was studied in a phase I/II trial of 29 patients with advanced IC with *FGFR* fusions, of which 27 patients had refractory disease. Patients treated with derazantinib had an ORR of 20.7%, DCR of 82.8%, and median PFS of 5.7 months (95% CI: 4.0–9.2 months) (46). Derazantinib was also studied in a phase II trial of 28 patients with IC containing *FGFR2* mutations or amplifications. Of the 23 patients who received derazantinib, the DCR was 73.9% (95% CI: 51.6–89.8%) (47). RLY-4008 was studied in a phase I trial of 45 patients with advanced, refractory tumors (35 had cholangiocarcinoma) containing *FGFR2* alterations (26 with *FGFR* fusions, 13 with *FGFR* mutations, and 5 with *FGFR* amplifications). Radiographic tumor reduction of 10% or more was observed in 59% of patients treated with RLY-4008 (48). RLY-4008 was also studied in a phase II trial of 38 patients advanced solid tumors with FGFR fusions or rearrangements and showed an ORR of 88% (at the "recommended phase 2 dose") (49). Several trials studying the efficacy and safety of FGFR inhibitors in patients with advanced BTCs are ongoing (*Table 5*).

Isocitrate dehydrogenase (IDH) inhibitors

IDH1 and IDH2 are enzymes involved in cellular aerobic respiration, catalyzing the conversion of isocitrate to alpha-ketoglutarate. Mutations in genes encoding these proteins result in the abnormal production of R(-)-2-hydroxyglutarate and downstream, uncontrolled cellular differentiation (50). *IDH* mutations exist in 10–23% of ICs and less than 1% of patients with ECs (38,51-53). In patients with EC, *IDH1* mutations are associated with a

Table 5 Ongoing trials in patients with *FGFR* alterations

Investigational arm	Comparison arm	N	Line	Phase	NCT number
Infigratinib	Gemcitabine plus cisplatin	300	1L	3	NCT03773302
Infigratinib	None	143	2L and beyond	2	NCT02150967
Pemigatinib	Gemcitabine plus cisplatin	434	1L	3	NCT03656536
Futibatinib	Gemcitabine plus cisplatin	216	1L	3	NCT04093362
Erdafitinib	None	35*	2L and beyond	2	NCT02699606
Gunagratinib	None	56*	2L and beyond	1/2	NCT03758664
Pazopanib plus trametinib	None	89*	2L and beyond	1	NCT01438554
Derazantinib	None	148	2L and beyond	2	NCT03230318
E7090	None	60	2L and beyond	2	NCT04238715

*, includes several cancer types. NCT, National Clinical Trial; FGFR, fibroblast growth factor receptor.

Table 6 Ongoing trials in patients with *IDH* mutations

Investigational arm	Comparison arm	N	Line	Phase	NCT number
Olaparib	None	145*	2L and beyond	2	NCT03212274
Olaparib plus durvalumab	None	78*	2L or 3L	2	NCT03991832
Olaparib plus ceralasertib	None	50*	2L and beyond	2	NCT03878095
LY3410738	LY3410738 with gemcitabine plus cisplatin or with durvalumab	200*	Dependent on cohort (e.g., dose expansion cohort limited to 1L)	2	NCT04521686
HMPL-306	None	90*	2L and beyond	1	NCT04762602
Dasatinib	None	8	2L and beyond	2	NCT02428855
FT-2102 plus gemcitabine plus cisplatin	None	93*	2L	1/2	NCT03684811
Ivosidenib plus nivolumab	None	35*	2L and beyond	2	NCT04056910
Ivosidenib or pemigatinib plus gemcitabine plus cisplatin	None	40	Maintenance	1	NCT04088188

*, includes several cancer types. NCT, National Clinical Trial; IDH, isocitrate dehydrogenase.

poor prognosis (54).

Ivosidenib, an IDH1 inhibitor, was studied and compared to placebo in the phase III ClarIDHy trial of 185 patients with advanced, refractory *IDH1*-mutant cholangiocarcinoma. In the 124 patients treated with ivosidenib, a median PFS of 2.7 months was observed compared to 1.4 months in the placebo arm (HR 0.37; 95% CI: 0.25–0.52; P<0.0001) (55). In the final survival analysis, a median OS of 10.3 months in the ivosidenib arm was observed compared to 7.5 months in the placebo arm (HR 0.79; 95% CI: 0.34–0.70; P<0.0001) (56). As a result of this study, ivosidenib is recommended as a subsequent-line treatment option for patients with unresectable or metastatic *IDH1*-mutated cholangiocarcinoma. Several early-phase trials studying the efficacy and safety of therapies in patients with *IDH*-mutated BTCs are ongoing (*Table 6*).

Human epidermal growth factor receptor 2 (HER2)-directed therapy

HER2 is a receptor encoded by *ERBB2* and belongs to the epidermal growth factor receptor (EGFR) family. Overexpression of HER2 leads to abnormal cell survival and proliferation (57). Typically, HER2 status is graded by

Table 7 Ongoing trials in patients with *HER2* alterations

Investigational arm	Comparison arm	N	Line	Phase	NCT number
Trastuzumab plus tucatinib	None	12	2L and beyond	2	NCT04579380
Multiple targeted therapies	None	6,452*	2L and beyond	2	NCT02465060
Multiple targeted therapies	None	3,581*	2L and beyond	2	NCT02693535
BDTX-189	None	91*	2L and beyond	1/2	NCT04209465

*, includes several cancer types. NCT, National Clinical Trial; HER2, human epidermal growth factor receptor 2.

immunohistochemical (IHC) staining from 0 (negative, or less than 10% of cells displaying membranous reactivity) to 3+ (positive). In situ hybridization (ISH) is used for IHC equivocal (2+) HER2 status to determine the ratio of copies of the *HER2* gene to chromosome 17 centromeres (CEP17) within the nucleus of tumor cells. *HER2* gene amplification is identified in as high as 18% of EC and is associated with a poor prognosis (58).

One of the first indicators of efficacy of HER2-directed therapy in patients with BTC was a retrospective study of eight patients with advanced gallbladder cancer containing *HER2/neu* gene amplification who received HER2-directed treatment. Three patients experienced disease stability, four experienced a partial response, and one had a complete response (59).

Neratinib, an oral pan-HER inhibitor, was studied in the phase II basket trial SUMMIT and showed modest response in patients with advanced, refractory *HER2*-mutated BTC. Of 25 patients in the updated analysis (11 cholangiocarcinoma, 10 gallbladder, 4 ampullary cancers), the ORR was 16% (95% CI: 4.5–36.1%), median PFS was 2.8 months (95% CI: 1.1–3.7 months), and median OS was 5.4 months (95% CI: 3.7–11.7 months) (60,61).

Varlitinib, another oral pan-HER inhibitor, was studied in a phase II trial of 127 patients with advanced, refractory BTCs. Sixty-four patients received varlitinib plus capecitabine and 63 received placebo plus capecitabine. No differences among the two arms were detected with regard to median PFS (2.8 *vs.* 2.8 months; P=0.63) and median OS (7.8 *vs.* 7.5 months; P=0.66) (62).

Regarding HER2-directed antibodies, the combination of pertuzumab and trastuzumab, both monoclonal antibodies against HER2, was studied in a phase II trial of 39 patients with *HER2*-amplified or *HER2*-overexpressed (or both) advanced, refractory BTC. This study revealed that dual HER2-directed therapy resulted in an ORR of 23% (95% CI: 11–39%). Notably, patients with gallbladder cancer had an ORR of 31% (95% CI: 11–59%) and DCR of 63% (95% CI: 35–85%), which was higher than the ORR in patients with intrahepatic and EC (63).

Moreover, trastuzumab-deruxtecan, an antibody-drug conjugate, was studied in a phase II trial of 32 patients with advanced, refractory BTCs; 24 patients were HER2-expressing and 8 patients were classified as having HER2-low disease. HER2-expressing was defined as IHC/ISH status of 3+ or 2+/+ while HER2-low was defined as IHC/ISH status of 0/+, 1+/–, 1+/+, or 2+/–. In HER2-expressing patients, an ORR of 36.4% (90% CI: 19.6–56.1%), DCR of 81.8% (95% CI: 59.7–94.8%), median PFS of 4.4 months (95% CI: 2.8–8.3 months), and median OS of 7.1 months (95% CI: 4.7–14.6 months) were observed compared to an ORR of 12.5% (95% CI: 0.3–52.7%), DCR of 75.0% (95% CI: 34.9–96.8%), median PFS of 4.2 months (95% CI: 1.3–6.2 months), and median OS of 8.9 months (95% CI: 3.0–12.8 months) in HER2-low patients (64).

Lastly, zanidatamab, a bispecific HER2-targeted antibody, was studied in a phase I trial of 20 patients with advanced, refractory BTCs with HER2 overexpression. Of 17 evaluable patients, an ORR of 47% (95% CI: 23–72%), DCR of 65% (95% CI: 38–86%), and median duration of response of 6.6 months (95% CI: 3.2–not estimable) were observed (65). Several trials studying the efficacy and safety of HER2-directed therapies in patients with advanced BTCs are ongoing (*Table* 7).

Neurotrophic tyrosine receptor kinase (NTRK) gene fusion inhibitors

NTRK genes encode transmembrane tyrosine kinase receptors that, when bound by neurotrophins, activate downstream signaling pathways promoting cell growth, differentiation, and survival such as MAPK, phosphatidylinositol 3-kinase (PI3K), and protein kinase C (PKC). Molecular aberrations that commonly affect *NTRK*, including gene fusions, mutations, and amplifications, result in constitutive activation of these signaling pathways (66).

NTRK fusions exist in less than 1% of BTCs (67).

Both entrectinib and larotrectinib have shown activity in the very rare setting of BTCs with NTRK fusions. Entrectinib, a multikinase inhibitor that targets oncogenic rearrangements in genes such as *NTRK*, c-ros oncogene 1 (*ROS1*), and anaplastic lymphoma kinase (*ALK*), was studied in three early-phase trials of patients with advanced or metastatic *NTRK* fusion-positive tumors. In a pooled efficacy analysis of 54 patients enrolled in these trials, 57% had an objective response; however, only one patient in this analysis had cholangiocarcinoma (68). Larotrectinib, a tropomyosin receptor kinase (TRK) inhibitor, was studied in an early-phase trial of 55 patients with *TRK* fusion-positive tumors, in which two patients had cholangiocarcinoma. 75% achieved an objective response (95% CI: 61–85%). At one year, 71% of the responses were ongoing, and 55% of patients did not have disease progression (69). Entrectinib and larotrectinib are therefore viable first or subsequent-line treatment options for patients with unresectable or metastatic cholangiocarcinoma with *NTRK* gene fusions.

B-Raf proto-oncogene (BRAF) inhibitors

BRAF kinase is a protein that regulates signaling networks, primarily the MAPK/ERK cascade, responsible for regulating cell growth, development, and division (70). *BRAF V600E* mutations are identified in less than 1% of BTCs and are more common in IC (71).

Dabrafenib, a BRAF inhibitor, combined with trametinib, a mitogen-activated extracellular signal regulated kinase (MEK) 1/2 inhibitor, was studied in a phase II trial of 43 patients with *BRAF V600E*-mutated advanced, refractory BTC. 51% of patients treated with dabrafenib and trametinib achieved a response (95% CI: 36–37%) (72). In an open-label, single-arm study of 35 patients with advanced, refractory *BRAF V600E*-mutated tumors, four of which had cholangiocarcinoma, the ORR was 38% (90% CI: 22.9–54.9%) in patients treated with dabrafenib and trametinib. Three of the four patients with cholangiocarcinoma demonstrated a partial response, with individual PFS of 29.4, 12.8, and 9.1 months (73). Accordingly, combination therapy with dabrafenib and trametinib is a subsequent-line treatment option in unresectable or metastatic cholangiocarcinoma with *BRAF V600E* mutations.

EGFR inhibitors

EGFRs are transmembrane receptors that transduce signals through the MAPK, PI3K-Protein kinase B (PBK, or Akt), and phospholipase C (PLC)/protein kinase C (PKC) signaling pathways upon ligand binding that stimulate cell proliferation, differentiation, growth, and migration (74). Overexpression of EGFR is thought to be present in 8–27% of BTCs and is more common in IC (75,76); however, randomized studies have shown no benefit when adding EGFR inhibitors to a chemotherapy backbone.

Cetuximab, an EGFR inhibitor, was studied in combination with gemcitabine plus oxaliplatin in a phase II trial of 30 patients with advanced, untreated BTC. An objective response occurred in 63% of patients (95% CI: 56.2–69.8%) (77). This study led to further evaluation of cetuximab in the randomized phase II BINGO trial of 150 patients with advanced BTC, in which 76 patients received cetuximab in combination with gemcitabine plus oxaliplatin and 74 received gemcitabine and oxaliplatin alone. Overall, this study demonstrated no significant survival differences between study arms (78).

Panitumumab, another EGFR inhibitor, was studied in a phase II trial of 28 patients with advanced, untreated cholangiocarcinoma. This combination of therapies led to a DCR of 74%, median PFS of 9.7 months (95% CI: 5.1–12.9 months), and median OS of 12.9 months (95% CI: 9.5–27.8 months) (79). The addition of panitumumab to chemotherapy was subsequently evaluated in the randomized, phase II Vecti-BIL trial of 89 patients with untreated, *KRAS* wild-type, advanced BTCs. Forty-five patients received panitumumab in combination with gemcitabine and oxaliplatin, and 44 received gemcitabine and oxaliplatin alone. Similar to the results from the BINGO study, no survival benefit was observed with the addition of panitumumab to chemotherapy. A subgroup analysis by disease site demonstrated that panitumumab with chemotherapy may have resulted in a marginal OS benefit compared to chemotherapy alone in patients with IC, although there was a higher incidence of EGFR-related toxicity in the combination arm (80).

Finally, erlotinib, an oral EGFR inhibitor, was studied in a phase II trial of 42 patients with advanced, refractory BTC. 81% of patients included for analysis had *EGFR* overexpression. Seventeen percent of patients treated with erlotinib monotherapy were progression-free at 6 months (95% CI: 7–31%) and 8% of patients achieved a response (95% CI: 2–20%) (81). Erlotinib was later studied in a phase III trial of 268 patients with advanced, untreated BTC in which 135 patients received erlotinib in combination with gemcitabine and oxaliplatin and 133 received gemcitabine and

oxaliplatin alone. Again, there were no significant PFS or OS differences when adding erlotinib to chemotherapy, although a subgroup analysis by disease site demonstrated a possible PFS benefit in patients with cholangiocarcinoma (82).

Other novel therapies

A number of other therapies, including praseltinib, regorafenib, bintrafusp alfa, INCB001158, sotorasib, and chimeric antigen receptor (CAR) T cells have been studied, largely in the context of early phase trials, in patients with advanced BTCs.

Rearranged during transfection (RET) fusions and mutations occur in approximately 5.7% of cholangiocarcinoma (83). Pralsetinib, a RET inhibitor, was studied in an early-phase trial of 27 patients with advanced, refractory *RET*-altered solid tumors. Of the two patients with cholangiocarcinoma, both had an objective response (84). Regorafenib, a multikinase inhibitor, was studied in three phase II trials in patients with advanced, refractory BTCs. In these studies, median PFS was found to be 3.6 months (90% CI: 3.0–5.7 months), 3.7 months (95% CI: 1.8–5.4 months), and, 1.5 months (95% CI: 1.2–2.0 months) while median OS was found to be 3.6 months (90% CI: 3.1–17.1 months), 5.4 months (95% CI: 3.4–12.8 months), and 5.3 months (95% CI: 2.7–10.5 months), respectively (85). A bifunctional fusion protein against transforming growth factor-beta and PD-L1, called bintrafusp alfa, was studied in a phase I trial of 30 patients with advanced, refractory BTC. Treatment with bintrafusp alfa in this cohort resulted in an ORR of 20% (95% CI: 8.0–39.0%), median PFS of 2.5 months (95% CI: 1.3–5.6 months), and median OS of 12.7 months (95% CI: 6.1–15.7 months) (86). The arginase inhibitor INCB001158, in combination with gemcitabine plus cisplatin, was administered to 33 patients with advanced, untreated BTC in an early phase trial. Patients experienced an ORR of 24% (95% CI: 11.1–42.3%), median duration of response of 5.8 months (4.1 months–not yet reached), and median PFS of 8.5 months (5.7–10.1 months) (87). Sotorasib, an inhibitor of Kirsten rat sarcoma viral oncogene homolog (KRAS), was studied in a phase I trial of 25 patients with advanced, refractory solid tumors harboring *KRAS p.G12C* mutations, which included one patient who had BTC and who achieved disease control (88).

Finally, one patient achieved a complete response and ten patients experiences stable disease in a phase I study of 17 patients with advanced, refractory EGFR-positive BTCs treated with anti-EGFR CAR T cells. In this study, a median PFS of 4 months (range, 2.2–22 months) was observed (89). In another phase I trial of 11 patients with advanced, refractory HER2-positive BTC and pancreatic cancer treated with anti-HER2 CAR T cells, one patient achieved a partial response, and five patients experienced stable disease. Combinations of previously studied therapies and a number of new agents are being studied in patients with advanced BTCs (*Table 8*).

Future directions

Molecular profiles of biliary tract tumors vary by anatomic location, with IC displaying a higher rate of actionable mutations than other BTCs (90-92). For example, FGFR2 fusions and mutations in genes such as breast cancer gene-associated protein 1 (*BAP1*), *IDH1*, and polybromo 1 (*PBRM1*) are more common in IC while *HER2* and tumor protein p53 (*TP53*) mutations are more common in EC and gallbladder cancer (91,92). An emerging understanding of the importance of biliary tract tumor transcriptomics and proteomics has helped identify "clusters" of biliary tract tumors based on genetic aberrations and immunophenotypes, supporting the hypothesis that molecular alterations may be used in conjunction with RNA and protein expression when searching for new targets and potentially choosing a therapeutic regimen. In an analysis of genomic and transcriptomic records of over 400 patients in North America with matched DNA, ribonucleic acid (RNA), and clinical data, four distinct genomic clusters (grouped based on driver mutations in similar genes) were identified (*Table 9*) (93). This analysis showed actionable biomarkers (defined by the authors as TMB > ten mutations per megabase, MSI-H, dMMR, *NTRK* gene fusions, *FGFR2* fusions or rearrangements, *IDH1* mutations, *HER2* alterations, or *BRAF V600E* mutations) were present in 30.5% of all BTCs—39.1% of IC, 29.6% of EC, and 15% of gallbladder cancer. Another analysis, using a combination of genomic and proteomic data from 110 patients with IC, identified three molecular subtypes (chromatin remodeling, metabolism, and chronic inflammation) and showed that clinical outcomes varied by subtype. Others have also identified similar clusters and subtypes of BTCs based on shared tumor microenvironmental profiles (94-98). These data support the use of tumor molecular profiling and potentially incorporating multi-omic information to determine first and subsequent-line therapies in all patients with BTCs.

To date, most targeted therapies developed for BTCs

Table 8 Ongoing trials of other targeted and novel therapies

Investigational arm (mechanism)	Comparison arm	n	Line	Phase	NCT number
Olaparib (PARP inhibitor)	None	36	Maintenance following platinum therapy	2	NCT04042831
Ramucirumab (monoclonal antibody against VEGFR2	None	61	2L and beyond	2	NCT02520141
Niraparib (PARP inhibitor)	None	35*	2L and beyond	2	NCT03207347
Anlotinib (multikinase inhibitor) and levamisole (T cell activation and proliferation)	Anlotinib	152	2L and beyond	3	NCT03940378
Bortezomib (proteasome inhibitor)	None	50	2L and beyond	3	NCT03345303
CB-103 (diaryl ether against Notch receptors)	None	200*	2L and beyond	1/2	NCT03422679
Opaganib (SK2 inhibitor) with or without hydroxychloroquine	Opaganib	65	1L or 2L	2	NCT03377179
Apatinib (VEGFR2 inhibitor)	None	55	2L and beyond	2	NCT03427242
Apatinib (VEGFR2 inhibitor)	None	30	2L and beyond	2	NCT03521219
Surufatinib (pan-VEGFR and CSF-1R inhibitor)	Capecitabine	298	2L	2/3	NCT03873532
Copanlisib (PI3K inhibitor) plus gemcitabine plus cisplatin	None	24	1L (although patients who received adjuvant therapy more than 6 months prior are also eligible)	2	NCT02631590
RXC004 (porcupine inhibitor) plus denosumab	None	15	2L	2	NCT04907851
Crizotinib (multikinase inhibitor)	None	246*	2L and beyond	2	NCT02034981
Natural killer cells plus pembrolizumab	None	40	2L and beyond		NCT03937895
Tumor infiltrating lymphocytes plus aldesleukin (recombinant interleukin-2)	None	59	2L and beyond	2	NCT03801083
Memory T cells plus chemotherapy	Chemotherapy	20	Resection without recurrence	2	NCT03820310
MUC-1 CAR-T cells plus fludarabine plus cyclophosphamide	None	9	1L	1/2	NCT03633773
Anti-HER2 CAR-macrophages	None	18*	2L and beyond	1	NCT04660929
Gavocabtagene autoleucel (cell therapy) plus fludarabine plus cyclophosphamide with or without nivolumab and ipilimumab	None	175*	2L and beyond (but not more than 5 lines)	1/2	NCT03907852
Oncolytic adenovirus encoding TMZ-CD40L and 4-1BBL plus gemcitabine plus cisplatin	None	50*	1L and beyond	1/2	NCT03225989

*, includes several cancer types. NCT, National Clinical Trial; VEGFR, vascular endothelial growth factor receptor; CSF-1, colony stimulating factor 1; PI3K, phosphoinositide 3-kinase; CAR, chimeric antigen receptor; HER2, human epidermal growth factor receptor 2; PARP, poly adenosine diphosphate-ribose polymerase.

Table 9 Genomic clusters in biliary tract cancers

Cluster 1	Cluster 2	Cluster 3	Cluster 4
TP53, *KRAS*, *HER2*, and *ATM* mutations	*CDKN2A/B* mutations	*IDH1* and chromatin remodeling gene (*ARID1A* and *PBRM1*) mutations	*FGFR2* fusions and *BAP1* mutations

BAP1, BRCA1 associated protein-1; FGFR, fibroblast growth factor receptor; IDH1, isocitrate dehydrogenase 1; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; HER2, human epidermal growth factor receptor 2; ARID1A, AT-rich interactive domain-containing protein 1A; PBRM1, polybromo 1; ATM, ataxia telangiectasia mutated.

take advantage of DNA mutations identified in tumor tissue. Efforts to detect targets in blood, bile, and cytologic specimens (i.e., from brushings obtained on endoscopic procedures or small tissue samples obtained on fine-needle aspiration) are underway. Circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs), collectively referred to as cell-free DNA (cfDNA), are released into peripheral blood via necrosis and apoptosis of tumor cells (99). cfDNA is sometimes utilized to identify targetable mutations when tissue cannot be obtained for technical or logistical reasons and is being studied as a mechanism to detect early disease, acquired resistance to therapy, and response to treatment (100). In BTCs, mutational concordance between tissue and cfDNA is between 50–100% and is higher for IC (compared to other types of BTCs) and tissue obtained from metastatic sites of disease (compared to the primary tumor) (101-104). In a large analysis of 2,068 samples of cfDNA from 1,671 patients with advanced BTCs, targetable mutations were detected in 44% of patients. In this analysis, concordance between cfDNA was highest for *IDH1* mutations (87%) and *BRAF V600E* mutations (87%) but low for *FGFR2* fusions (18%) (105). Biliary tract tumors also shed ctDNA into bile. The mutational concordance between bile ctDNA and tissue DNA is as high as 88% (106). Alterations in non-DNA markers, particularly non-coding RNA (including micro RNA) shed from tumors into blood and bile, have also been studied to detect early disease, prognosticate, and monitor response to therapy for patients with BTCs (107-110).

Conclusions

BTCs are a heterogeneous group of malignancies, both in terms of anatomic location and mutational profile, with a poor prognosis. The development and refinement of next-generation sequencing has led to the identification of numerous actionable targets over the past decade. The integration of multi-omic information will likely lead to the development of new therapies targeting genomic alterations. Targeted agents can improve survival in patients with advanced disease and have substantially increased the number of potential therapeutic options in patients with refractory or progressive disease. Moreover, with ongoing and rapid drug development, these drugs may be studied in the first-line setting for patients with advanced BTCs who have actionable mutations. Data should also be gathered on patients who progress on targeted therapies to better understand the development of acquired resistance and define optimal treatment sequencing. In addition, non-invasive techniques to detect early disease and monitor response to therapy, which represent a promising alternative to tissue-based target identification, will likely be studied in trials with potential to change clinical practice. In conclusion, the therapeutic landscape of BTCs continues to evolve, based on improvements in detection of genomic alterations and effective utilization of molecularly-driven therapies. Discovering novel agents for genomic alterations in BTCs will be key in the coming years in order to improve patient outcomes.

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Footnote

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