Review Article

Targets for therapy in biliary tract cancers: the new horizon of personalized medicine

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Abstract: Biliary tract cancers (BTCs) are a set of molecularly distinct and heterogeneous diseases. While cytotoxic chemotherapy remains the current standard of care for treatment-naïve and treatment-refractory unresectable disease, recently identified mutations driving oncologic development offer opportunities for targeted therapy. Currently, alterations in the fibroblast growth factor receptor (FGFR), isocitrate dehydrogenase (IDH), v-Raf murine sarcoma viral oncogene homolog B (BRAF), DNA damage repair, and HER2 pathways have demonstrated promising new therapeutic avenues, among others, and various studies have demonstrated clinical activity with targeted tyrosine kinase inhibitors and/or antibodies. In this review, we will discuss the currently identified targets for therapy in BTCs and review currently available data regarding clinical development of treatment options in these molecularly distinct subsets.

Keywords: Biliary tract cancer (BTC); targeted therapy; cholangiocarcinoma

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Biliary tract cancers (BTCs) have traditionally been classified according to their location. Intrahepatic cholangiocarcinoma (IH-CCA) arises from the peripheral bile ducts within the liver beyond the secondary biliary radicals while extrahepatic cholangiocarcinoma (EH-CCA) arises from bile ducts outside the liver and can be subclassified into either hilar cholangiocarcinoma or distal cholangiocarcinoma. Gallbladder carcinoma (GBC) arise from the gallbladder. While these anatomic distinctions continue to be highly relevant for the diagnosis, prognosis, and management of BTC, an increasing awareness of their molecular heterogeneity has paved the way for new therapeutic approaches in these diseases.

In China, BTC is the 24th most common cancer and the 17th leading cause of cancer death (1). The incidence of BTC appears to be increasing globally, due in large part to a rising incidence of IH-CCA (2,3). In fact, BTCs were the most rapidly rising malignancy in Shanghai, China between 1972 and 1994, with an increase of 119% in men and 124% in women and an incidence rate of ~5.5 per 100,000 people (4,5). The mortality rate of BTC is 1.8/100,000 in China versus 1.0/100,000 in the United States (6). Epidemiologic variance between Eastern and Western populations is thought to be due in part to a difference in risk factors. For example, obesity and chronic inflammation from primary sclerosing cholangitis (PSC) are more prevalent in Western countries (7), whereas the prevalence of the liver parasite *Opisthorchis viverrini* is significantly higher in Thailand, Laos, and Cambodia (8,9). The liver fluke *Clonorchis sinensis* has also been associated with the development of cholangiocarcinoma and is endemic to rural China, especially in the northeastern province of Heilongjiang and the southern provinces of Guangdong and Guangxi (10,11). Other risk factors for CCA include diabetes, chronic viral

hepatitis B or C, anatomical abnormalities in the biliary tract such as choledochal cysts, and Lynch syndrome. Risk factors for GBC include polyps, *Salmonella Typhi*, and chronic cholecystitis (12,13).

Unresectable or advanced BTC is associated with a 5-year survival rate of 5–10% (14). The current standard of care for unresectable BTCs is systemic chemotherapy. Gemcitabine was established as palliative therapy in 1996 (15), and remained the standard of care until the phase III ABC-02 trial demonstrated a survival advantage for the combination of cisplatin and gemcitabine over gemcitabine in the front-line setting for advanced disease [median overall survival (OS) 11.7 *vs.* 8.1 months, respectively, hazard ratio 0.64, P<0.001] (16). The survival advantage of the combination over gemcitabine alone was confirmed in a Japanese population (17). Recently, the ABC-06 trial demonstrated a modest survival benefit with oxaliplatin/5-fluoruracil (mFOLFOX) and active symptom control (ASC) after progression on cisplatin and gemcitabine (compared to ASC alone), with a median OS of 6.2 *vs.* 5.3 months, respectively (adjusted hazard ratio 0.69 (P=0.03) (18).

While cytotoxic regimens continue to be evaluated in clinical trials, several genotyping efforts have identified specific potentially targetable molecular aberrations in BTC and the investigational paradigm for unresectable BTC now includes targeted therapy in the front-line and treatment-refractory settings. An understanding of these evolving treatment paradigms requires an understanding of the molecular and genomic underpinnings of the disease. In this review we identify the molecular aberrations identified in BTC and the emerging therapies which target these molecular mutations (*Table 1*).

Molecular and genomic characteristics

The complex molecular pathogenesis of BTC involves alterations in multiple pathways including protein kinases (FGFR2, BRAF, HER2), epigenetic modification (IDH1/IDH2), and DNA damage repair (BRCA2, TP53) (30-33). The advent of genomic tumor profiling via next-generation sequencing (NGS) has allowed for the characterization of specific somatic aberrations. In addition, improved detection of circulating plasma cell-free DNA may aid in genomic profiling when inadequate tissue is obtained at biopsy (34). The anatomic location of the tumor—intrahepatic, extrahepatic, or gallbladder—greatly influences the mutational profile likely to be found within the tumor. In one series, potentially targetable genetic alterations were identified in 39% of 239 analyzed BTC cases (35). FGFR2 fusions and IDH1/2 mutations were observed most commonly in IH-CCA, PRKACA/PRKACB fusions were most common in EH-CCA, and ERBB2/3, TSC1, and PTEN alterations were seen in gallbladder cancer.

Exposure to specific risk factors may be associated with differing BTC molecular profiles. A study of 209 patients with CCA in Asia and Europe reported higher rates of TP53 mutations in *O. viverrini*-related CCA compared to non-*O. viverrini*-related CCA (40% *vs.* 9%), along with higher rates of SMAD4 (19.4% *vs.* 5.8%) and GNAS (5.6% *vs.* 0%) mutations (36). Conversely, there were lower rates of BAP1 in *O. viverrini*-related CCA (2.8% *vs.* 10.5%) along with lower rates of IDH1/2 (2.8% *vs.* 9%) mutations. In another study, TP53 mutations in IH-CCA were more likely to be associated with Hepatitis B surface antigen (HBsAg) seropositivity and KRAS mutations associated with HBsAg-seronegative patients (31). Hepatitis B or C positivity may also be associated with an increased likelihood of FGFR alteration (37). Finally, genomic sequencing of over 500 CCA tumors demonstrated four distinct molecular clusters (33). The clusters with higher rates of fluke infections had significantly more somatic mutations, including higher rates of aberrations in ERBB2, TP53, and BRCA 1/2 and lower rates of FGFR and IDH 1/2 mutations, and were associated with a poorer survival compared to the clusters with lower rates of fluke-associated tumors (P<0.001). Further research is needed to further characterize the link between risk factors, pathogenesis and mutational drivers of the various BTCs.

Multiple studies have found prognostic implications for specific genetic alterations in BTC (38-41). Mutations in TP53, KRAS, and the MAP/ERK pathway mutation have been associated with a poor prognosis, while FGFR pathway alterations, and specifically FGFR fusions, have been associated with a favorable prognosis and improved survival compared to those without fusions (41-43). Data on the prognostic implication of aberrations in IDH1/IDH2, HER2/neu, c-MET, and the PI3K/AKT/mTOR pathway are conflicting (32,44-48). While the data are still maturing regarding the possible prognostic implications of specific genomic mutations, emerging data suggest that certain mutations drive cancer growth and progression, thus making them prime targets for therapeutic intervention.

FGFR2

The complex fibroblast growth factor (FGF) pathway

Table 1 Prospective trials of promising targeted therapies in BTC

Targetable pathway	Study	Drug	Total N (N with FGFR fusions/alterations)	Results* (overall population, FGFR fusion population, FGFR alteration** population)
FGFR	Javle *et al.* 2018 (19)	Infigratinib (BGJ398)	61 (48 FGFR2 fusions, 11 FGFR2 alterations**)	Overall population: • ORR 14.8% • DCR 75.4% • PFS 5.8 months *FGFR2* fusion cohort: • ORR 18.8% • DCR 83.3% *FGFR* altered** cohort: • ORR 0%
	Mazzaferro *et al.* 2019 (20), Droz Dit Bussett, *et al.* 2019 (21)	Derazantinib (ARQ 087)	44 (29 FGFR2 fusions, 6 FGFR alterations, 9 no alteration)	*FGFR* fusion cohort: • ORR 20.7% • DCR 82.8% • PFS 5.7 *FGFR* altered cohort: • ORR 0% • DCR 67% • PFS 6.7 mo No *FGFR* alteration** cohort: • ORR 0% • DCR 22% • PFS 1.5 mo
	Park *et al.* 2019 (22)	Erdafitinib (JNJ42756493)	17 (11 FGFR 2/3 fusions, 6 FGFR alterations)	Overall population: • ORR 47% • DCR 80% • PFS 5.6 months *FGFR2/3* fusion cohort: • ORR 67% • DCR 100% • PFS 12.65 months *FGFR* altered** cohort: • ORR 16.67%

Table 1 (*continued*)

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Targetable pathway	Study	Drug	Total N (N with FGFR fusions/alterations)	Results* (overall population, FGFR fusion population, FGFR alteration** population)
	Vogel *et al.* 2019 (23)	Pemigatinib (INCB054828)	146 (107 FGFR2 fusions, 20 FGFR alterations, 18 no alteration)	*FGFR2* fusion cohort: • ORR 35.5% • DCR 82% • PFS 6.9 months • OS 21 months *FGFR* altered** cohort: • ORR 0% • PFS 2.1 months No *FGF*/*FGFR* alteration cohort: • ORR 0% • DCR 22% • PFS 1.7 mo
	Tran *et al.* 2018 (24)	TAS-120	45 (28 FGFR2 fusions, 17 FGF/FGFR alterations**)	*FGFR2* fusion cohort: • ORR 25% • DCR 78.6% • PFS 7.4 months *FGFR* altered** cohort: • ORR 17.6% • DCR 76.4% • PFS 6.8 months
IDH 1/2	Lowery *et al.* 2019 (25)	Ivosidenib (AG-120)	73	• ORR 5% • DCR 61% • PFS 3.8 months • OS 13.8 months
	Abou-Alfa *et al.* 2019 (26)	Ivosidenib (AG-120)	185	• ORR 2.4% • DCR 53% • PFS 2.7 months • OS 10.8 months
$BRAF^{V600E}$	Wainberg *et al.* 2019 (27)	Dabrafenib/ Trametinib	33	• ORR 41% • PFS 7.2 months • OS 11.3 months

Table 1 (*continued*)

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Targetable pathway	Study	Drug	Total N (N with FGFR fusions/alterations)	Results* (overall population, FGFR fusion population, FGFR alteration** population)
PI3K/AKT/mTOR	Lau *et al.* 2018 (28)	Everolimus	27	• ORR 12% • DCR 48% • PFS 5.5 months • OS 9.5 months
VEGF	Sun *et al.* 2019 (29)	Regorafenib	43	• ORR 11% • DCR 56% • PFS 15.6 weeks • OS 31.8 weeks

*, as reported per study population and publication; **, other alterations include mutations and amplifications in FGFR. N, number of enrolled subjects; ORR, overall response rate; DCR, disease control rate; PFS, median progression-free survival; OS, median overall survival; FGFR, fibroblast growth factor receptor; IDH, isocitrate dehydrogenase; BRAF, v-Raf murine sarcoma viral oncogene homolog B; MEK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor.

is involved in a wide array of biological processes from embryonal development to angiogenesis and wound repair (49). It consists of four transmembrane tyrosine kinase receptors (FGFR 1-4) and 22 FGFs, with downstream activation of RAS/RAF/MEK, JAK/STAT, PI3K/AKT pathways (40). Dysregulated FGF signaling has been associated with tumor proliferation, migration, and angiogenesis in a variety of malignancies (42,50). In BTC, the most common FGFR pathway aberrations are gene fusions involving FGFR2, which are seen at a frequency of 10–16% in IH-CCA, although point mutations and gene amplifications are also observed (46,51,52). FGFR2-BICC1 was the first fusion reported in cholangiocarcinoma in 2013 (53), and several subsequent studies have uncovered more than 50 additional fusion partners. FGFR pathway abnormalities are rarely seen in EH-CCA or GBC (46,51). In addition, studies in North American cohorts have shown a significantly higher proportion of FGFR2 aberrations among women (39,40), a trend not seen in Asian cohorts (54).

FGFR inhibitors have demonstrated efficacy in patients with FGFR-altered advanced refractory cholangiocarcinoma in multiple phase I and II studies. Infigratinib (BGJ398), an oral selective pan-FGFR kinase inhibitor, showed an overall response rate (ORR) of 14.8%, disease control rate (DCR) of 75.4%, and a median PFS of 5.8 months in a phase II single arm trial (19). This was driven by patients with FGFR2 fusions where the ORR was 18.8% and the DCR was 83.3%. No responses were seen in patients with FGFR amplifications or mutations. Adverse events included hyperphosphatemia (72.1%), fatigue (36.1%), stomatitis (29.5%), and alopecia (26.2%). Derazantinib demonstrated an ORR of 21% and DCR of 83%, with a median progression-free survival of 5.7 months, in patients with FGFR fusions (20,21). While no responses were seen in the FGFR mutated or amplified group, 67% achieved disease control with a median progression-free survival of 6.7 months and OS has not been reached in either arm (20).

Preliminary data on pemigatinib, an FGFR-selective inhibitor of FGFR 1-3, demonstrated an ORR of 35.5%, including a partial response rate of 32.7% in patients with FGFR2 fusions, in the recently reported FIGHT-202 study (23). DCR in the fusion population was 82%, with a duration of response of 7.5 months, and a median progression-free survival and preliminary OS of 6.9 and 21.1 months, respectively. Similar to infigratinib, no objective responses were seen with pemigatinib in patients with FGFR amplified or mutated patients (DCR was 40%, consisting of stable disease only), and median progression-free survival was 2.1 months. The adverse event profiles of pemigatinib and derazantinib were similar to that of infigratinib (20,23). Additional studies of futibatinib (TAS120) (55), Debio 1347 (56), and Erdafitinib (57) are ongoing. Phase III studies comparing single agent FGFR inhibitor to standard cisplatin/gemcitabine in the

first line metastatic setting are underway with infigratinib (NCT03773302) and pemigatinib (NCT03656536).

A limitation of the ATP-competitive FGFR inhibitors is the development of resistance after exposure. *In-vitro*, resistance develops via FGFR2 kinase domain mutations (58). Goyal *et al.* reported that three patients with IH-CCA harboring an *FGFR2* translocation who initially responded to treatment with infigratinib subsequently acquired resistance via polyclonal recurrent mutations in the FGFR2 kinase domain (50). Of note, *FGFR2* V564F mutation, a gatekeeper mutation that leads to steric clash between the FGFR2 binding domain and infigratinib, emerged in all three patients. The irreversible pan-FGFR inhibitor, TAS120, covalently binds to the P-loop cysteine residue of FGFR and has shown potency in preclinical models against acquired FGFR2 kinase domain mutations (59). Goyal *et al.* recently showed proof of concept of TAS120 overcoming acquired resistance to the ATP-competitive inhibitors infigratinib and Debio1347 in four patients with *FGFR2*-fusion positive IH-CCA (60). Functional assessment and modeling of the acquired FGFR2 kinase domain mutations showed that each of the three inhibitors has different spectrums of activity, with strategically sequencing of FGFR inhibitors potentially prolonging duration of benefit from an FGFR inhibition strategy in these patients.

IDH 1/2

Isocitrate dehydrogenase 1 and 2 (IDH1/2) are enzymes which regulate metabolism, DNA repair, and epigenetic modulation (61). IDH1/2 normally catalyze the conversion of isocitrate to α-ketoglutarate. However, mutations result in neomorphic activity of IDH, leading to increased production of 2-hydroxyglutarate (2HG), a suspected oncometabolite that can lead to aberrant DNA and histone methylation and epigenetic changes (51,62,63). 2-hydroxygluatarate can be measured in serum and may have a role as a possible biomarker as significantly higher levels of 2HG have been found in patients with IDH1/2 mutations than patients with wildtype *IDH1/2* (64,65). Initially discovered in hematologic malignancies, mutations in *IDH1/2* occur in approximately 20–25% of IH-CCA without significant frequency in EH-CCA or gallbladder cancer. Unlike *FGFR2* fusions, there appears to be no consistent report of difference in age, sex, or OS between patients with *IDH* mutant and *IDH* wild-type tumors (48).

Ivosidenib, an *IDH1* inhibitor, was studied in a phase I trial of multiple solid tumors including cholangiocarcinoma, chondrosarcoma, and glioma (66). In the cholangiocarcinoma cohort (25), 6% of patients had a confirmed PR while 56% experienced stable disease; the PFS at 6 months was 40% with no dose-limiting toxicities. Adverse events included fatigue (21%), nausea (18%), vomiting (12%), diarrhea (10%). The subsequent ClarIDHy study is a randomized phase III trial of 185 patients with IDH-1 mutated, treatment-refractory cholangiocarcinoma who received either ivosidenib or placebo with crossover allowed for the placebo arm at disease progression. Preliminary data show a DCR of 53% *vs.* 28% for placebo and a median PFS of 2.7 *vs.* 1.4 months (P<0.001); no patients on the placebo arm were progression-free at 6 or 12 months. Median OS showed a trend towards benefit with ivosidenib (10.8 *vs.* 9.7 months, P=0.06) and all subgroups favored ivosidenib (26).

Other inhibitors of *IDH1/2* are currently being evaluated in trials of *IDH1* or *IDH2* mutant advanced solid tumors including cholangiocarcinoma. The *IDH1* inhibitor FT2012 is being studied alone and in combination with other drugs in a phase I/II trial in relapsed/refractory patients with *IDH1* mutations across multiple tumor types (NCT03684811). The *IDH1* inhibitor BAY1436032 has shown efficacy in AML (67) and is currently undergoing a phase I trial in advanced solid tumors (NCT02746081). Additionally, the PARP inhibitor olaparib is being studied in a phase II trial of relapsed/refractory advanced solid tumors with IDH1/2 mutations NCT03212274).

Work to identify resistance mechanisms to ivosidenib and other IDH1 inhibitors is ongoing. In the phase I study of ivosidenib in cholangiocarcinoma, 37 (59%) of patients had paired pre-treatment and post-treatment sequencing of tumor (68). Six patients, including four with stable disease and one with a partial response, developed new oncogenic mutations. These included mutations in *IDH1* and *IDH2* (*IDH2-R172V*, *IDH1-R132F*) and mutations in *TP53*, *ARID1A*. *POLE*, *PIK3R1*, and *TBX3*. Further work to define potential resistance mechanisms will be necessary to better understand optimal treatment strategies and additional targets in this patient population.

BRAF/MEK

The mitogen-activated protein kinase (*MAPK*)/extracellular signal-regulated kinase (*ERK*), or *MEK* pathway, is involved in cell proliferation and survival and is frequently mutated in tumorigenesis (69). One of the strongest activators of the *MEK* pathway is a mutation in v-Raf murine sarcoma viral

oncogene homolog B (*BRAF*); the most common mutation in BRAF is an activating mutation resulting from glutamic acid substituting for valine at amino acid 600 (V600E) (69,70). Although common in melanoma and papillary thyroid cancer, the incidence of this mutation is BTC is low at about 1–6% with a preponderance of cases seen in IH-CCA (52,71).

The ROAR trial, a phase II basket trial of 178 patients with BRAF V600E mutations, evaluated the combination of the *BRAF* inhibitor dabrafenib and the *MEK* inhibitor trametinib across multiple diseases including 33 patients with advanced refractory BTC (27). With a median follow-up of 8 months, the ORR was 41%, with a median PFS of 7.2 months and a median OS of 11.3 months, suggesting that this may be an active regimen in this small population. A phase I/IIa basket trial in patients with relapsed/refractory solid tumors including BTC using PLX8394, an oral inhibitor of mutant and wild-type *BRAF*, is ongoing (NCT02428712).

MEK inhibitors have also been evaluated in patients with cholangiocarcinoma and have shown modest activity. A phase II study of the *MEK* inhibitor selumetinib in 28 patients with advanced BTC, including 39% of whom had received prior therapy, showed a median PFS of 3.7 months and median OS of 9.8 months with a favorable safety profile (72). The phase 1b ABC-04 study of gemcitabine/cisplatin/selumetinib showed a median PFS of 6.4 months and manageable toxicities at the established dose but the regimen had insufficient efficacy to be developed further (73).

DNA damage repair proteins

Alterations in DNA damage repair pathways have also been associated with BTC, providing a potential target for therapy. *BRCA2* mutation carriers have an increased risk of BTC, with an incidence of ~4% (52). Olaparib is a PARP inhibitor approved for germline *BRCA1/2* mutation carriers with breast or ovarian cancers and activity in BRCA-associated unresectable or metastatic pancreatic cancer after front-line platinum-based chemotherapy. Olaparib has demonstrated clinical activity in case reports of 2 patients with advanced BTC and a BRCA-1 and BRCA-2 mutation, respectively, although further work is needed to define any potential benefit (74,75). Mismatch repair deficiency has also been noted in BTCs, potentially identifying a subset of patients susceptible to immune checkpoint inhibition (76). Studies of PARP inhibitors alone or in combination with immune checkpoint inhibitors in platinum-sensitive BTC patients are ongoing (NCT04042831, NCT03639935).

HER2/EGFR pathway

The HER pathway consists of four receptors (HER1/EGFR, HER2, HER3, HER4) that homo- or hetero-dimerize, resulting in downstream activation of multiple pathways including the MAPK and PI3K/AKT pathways (77). Approximately 5–15% of BTC tumors are HER2-positive by IHC and/or FISH, and are more commonly expressed in gallbladder cancers and EH-CCA with no differences in expression by geographic region (78,79). HER2 expression has not been shown to have clear prognostic significance in advanced BTC (78,80).

The importance of targeting the HER2 pathway in BTC is emerging, with some retrospective data suggesting efficacy of HER2-targeted therapy in HER2 amplified tumors (81). Preliminary activity of pertuzumab + trastuzumab in HER2-positive metastatic BTC has been reported in a study of 11 patients, with 4 PRs and 3 SDs (82). The pan-HER TKI neratinib was studied in a solid tumor basket trial involving 9 patients with HER-2 mutant BTC and showed a response in 2 of these patients at 8 weeks and a median PFS of 2.8 months (83). Surprisingly, other pan-HER TKIs including lapatinib and apatinib have not shown significant efficacy in advanced BTC (84,85). In a pooled analysis of three phase I studies of patients with refractory BTC, varlitinib, a pan-HER TKI, demonstrated a PR of 27%, SD of 43%, and a DCR of 70% across 37 evaluable patients (86). Trials of varlitinib, trastuzumab plus pertuzumab, and trastuzumab emtansine in are ongoing in patients with HER2-altered BTC or in basket trials of HER2 altered solid tumors (NCT03093870, NCT03613168, NCT02693535, NCT02992340).

While epidermal growth factor receptor (EGFR) is overexpressed in 11–27% of IH-CCA, 5–20% of EH-CCA, and 1–5% of GBC (79-81,87), studies are mixed regarding EGFR expression and prognosis (79,80). However, the clinical utility of targeting this pathway has been disappointing (88). A phase III study of gemcitabine and oxaliplatin, with or without the EGFR-targeted TKI erlotinib, showed an improved response rate but no difference in PFS or OS (89). A recent meta-analysis of trials evaluating gemcitabine and oxaliplatin plus EGFR-targeted therapy showed an overall improvement in PFS with both TKIs and antibodies [hazard ratio (HR) of 0.8 (P=0.03)], although there were higher rates of adverse events and no difference in OS (90). While combining EGFR inhibitors

with cytotoxic therapy should not be considered standard of care, these analyses suggest that further work to define the optimal EGFR-targeted combination or BTC subset may be warranted.

Vascular endothelial growth factor (VEGF)

VEGF signaling leads to tumorigenesis via neovascularization and cell proliferation. VEGF overexpression is common in CCA and associated with intrahepatic metastases in IH-CCA (79). Single arm studies of the humanized anti-VEGF monoclonal IgG1 antibody bevacizumab combined with chemotherapy have demonstrated a median PFS of 6–7 months (91,92) with variable OS, while a single arm phase II study combining bevacizumab with erlotinib in patients with no prior treatment for advanced disease demonstrated a high rate of disease control (12% PR, 51% SD) and a median OS of 9.9 months (93).

Small molecule TKIs directed against the VEGF pathway have also demonstrated mixed results. The randomized phase II ABC-03 study evaluated gemcitabine/cisplatin with or without cediranib in advanced BTC; no survival benefit was shown with the addition of cediranib and the rates of grade 3 or 4 toxicity were higher (94). Studies of cabozantinib, sunitinib, vandetanib, and sorafenib (with or without chemotherapy) have not demonstrated significant activity (95-100). However, a recent single-arm phase II trial of regorafenib in advanced refractory BTC showed modest activity with an objective response of 11% but a DCR of 56% and a median OS of 32 weeks (29). In addition, the combination of VEGF inhibitors and immune checkpoint inhibitors are currently being tested. A retrospective, real-world Chinese study of lenvatinib and nivolumab or pembrolizumab in 60 previously treated patients with BTC demonstrated an objective response rate of 29.4% and DCR of 86.3%, signaling synergy in targeting these two pathways simultaneously, with promising median progression-free survival of 5.0 months and median OS of 13.0 months (101).

Further work is needed to understand the optimal strategy for targeting angiogenesis in BTC. A meta-analysis of 964 patients across 7 trials showed an improvement in ORR when anti-VEGF therapy was added to chemotherapy, but did not detect a difference in PFS or OS (102). Newer agents are now being explored, including an ongoing phase III study of a combination of ramucirumab, a monoclonal antibody against VEGFR2 (NCT02520141), and apatinib, a VEGFR2 TKI, in the second line setting for IH-CCA (NCT03521219). Ramucirumab has also been evaluated with cisplatin and gemcitabine in a randomized global phase II study compared to cisplatin and gemcitabine alone, and results are awaited (NCT02711553). The combination of VEGF inhibition and immune checkpoint inhibition is also being prospectively evaluated in BTC, including lenvatinib/pembrolizumab (NCT03895970). Identifying susceptible subgroups or optimal patient populations who will benefit from antiangiogenic-based therapy will be necessary before this approach can be incorporated into routine standards of care.

PI3K/AKT/mTOR pathway

The *PI3K/AKT/mTOR* pathway regulates cell cycle progression, proliferation, and angiogenesis and interacts with the *RAS/RAF/MEK* pathway through mTOR signaling (103). PIK3CA mutations have been found in 0–6% of EH-CCA and 0–8% of IH-CCA, and in 5–15% of GBC (31,52,104), although mutations in the *PI3K/AKT/mTOR* pathway occur up to 40% in EH-CCA and 25% in IH-CCA (42,105). Although dysregulation of the *PI3K/AKT/mTOR* pathway is thought to contribute to tumor progression, no clear prognostic value of these mutations has been demonstrated in BTC (52,106-108). Preclinical studies of *PI3K* pathway inhibitors in BTC have led to clinical studies of both mTOR inhibitors and *PI3K* inhibitors in patients with BTC.

A small phase II study of everolimus monotherapy in previously treated BTC demonstrated a modest PFS of 3.2 months and OS of 7.7 months (109), while a phase II study of the same agent in the first-line setting demonstrated a PFS of 5.5 months and OS of 9.5 months, with disease control lowest for GBC (28). A phase I study of copanlisib, an *PI3K* inhibitor, in combination with gemcitabine and cisplatin in advanced malignancies including BTC reported a response rate of 17% in patients with BTC, although numbers were small (110). A phase II study of copanlisib (BAY 80-6946) in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma is ongoing (NCT02631590).

c-MET

c-MET and its ligand hepatocyte growth factor (HGF), involved with cell proliferation, migration, and invasion (111). *c-MET* is overexpressed in 11–58% in IH-CCA and 0–16% in EH-CCA (80,112,113). The prognostic value of *c-MET*

overexpression is unclear, with some studies associating c-Met overexpression with a worse overall 5-year OS (112) while another study found no association with survival (113). Studies of small molecule multikinase inhibitors that target MET have been evaluated in early phase studies in advanced BTC. A phase II study of cabozantinib, a multi-kinase inhibitor of *MET*, *AXL*, and VEGFR, demonstrated a median PFS of 1.8 months and median OS of 5.2 months in patients with previously treated advanced CCA but had high rates of grade 3/4 toxicity (114). Tivantinib, another oral *c-MET* inhibitor, has shown activity in combination with gemcitabine; out of 56 evaluable patients 11 (19%) patients had a PR and 26 (46%) had SD, and 10 of these patients with PR or SD had prior gemcitabine exposure (115). A phase I study of multiple tumor types treated with merestinib, a small molecule inhibitor of MET and several other kinases, showed one CR and three PRs in patients with cholangiocarcinoma (116). A randomized global phase II trial of gemcitabine and cisplatin with or without merestinib in the first line setting has completed accrual and results are awaited (NCT02711553).

Conclusions

Once considered to be three diseases under one umbrella, BTC has been shown to have heterogeneity in presentation, prognosis, and response to treatment beyond anatomic subgroups. The advent of next generation sequencing has allowed for the classification of BTC into molecular subsets with distinct genomic profiles that have influenced the evolving treatment paradigms. Many of the mutations have proven to be ripe therapeutic targets, demonstrating efficacy in the refractory setting. Thus, early tumor molecular profiling is critical for patients with newly-diagnosed advanced BTC. Studies are currently underway to evaluate the targeting of driver mutations to the front-line setting and also as maintenance after initial clinical benefit on cytotoxic therapy (*Table 2*); the role of these targeted therapies in the adjuvant setting remains to be determined.

Table 2 Currently enrolling trials of targeted therapies for biliary tract cancers, as available on clinicaltrials.gov (117)

Pathway targeted	Clinical trial identifier	Trial arms	BTC population	Phase	Line of treatment
FGFR	NCT03773302	Infigratinib *vs.* Cisplatin/Gemcitabine	Cholangiocarcinoma with FGFR gene fusions/translocations	III	1st
FGFR	NCT03656536	Pemigatinib *vs.* cisplatin/gemcitabine	Cholangiocarcinoma with FGFR2 gene rearrangement	III	1st
FGFR	NCT04093362	Futibatinib *vs.* cisplatin/gemcitabine	Cholangiocarcinoma with FGFR2 gene rearrangement	III	1st
FGFR	NCT03230318	Derazantinib	FGFR2 Gene Fusion-, Mutation- or Amplification- Positive Intrahepatic Cholangiocarcinoma	II	2nd
FGFR	NCT02699606	Erdafitinib	FGFR pathway altered cholangiocarcinoma	II	2nd
FGFR	NCT02052778	Futibatinib	FGF/FGFR aberrant cancers	II	Multiple lines
FGFR/VEGF	NCT03873532	Surufatinib *vs.* capecitabine	All Biliary Tract Cancers	II/III	2nd
IDH1	NCT03684811	FT2012	IDH1 mutated biliary tract cancers	I/II	Refractory
IDH1	NCT02746081	BAY1436032	IDH1 mutated solid tumors	I	Refractory
IDH 1/2	NCT03212274	Olaparib	IDH1 or IDH2 mutated cholangiocarcinoma		Refractory
BRAF	NCT02428712	PLX8394	BRAF mutated solid tumors	I/II	Refractory
VEGF	NCT02520141	Ramucirumab	All Biliary Tract Cancers	II	Refractory

Table 2 (*continued*)

Table 2 (*continued*)

Pathway targeted	Clinical trial identifier	Trial arms	BTC population	Phase	Line of treatment
VEGF	NCT03521219	Apatinib	Intrahepatic Cholangiocarcinoma	II	Refractory
c-MET or VEGF	NCT02711553	Merestinib/placebo + cisplatin/gemcitabine or ramucirumab/placebo + cisplatin/gemcitabine	All Biliary tract Cancers	II	1st
VEGF and immune checkpoint inhibition	NCT03895970	Lenvatinib + pembrolizumab	All Biliary Tract Cancers	II	2nd
DNA damage repair	NCT04042831	Olaparib	BTCs with somatic or germline mutations in *ATM, ATR, CHEK2, BRCA 1/2, RAD51, BRIP1, PALB2, PTEN, FANC, NBN, EMSY, MRE11, ARID1A* without progression on platinum-based therapy	II	1st
DNA damage repair and immune checkpoint inhibition	NCT03639935	Rucaparib/nivolumab	All Biliary Tract Cancers without progression on platinum-based therapy	II	1st
HER2	NCT03613168	Trastuzumab + cisplatin/gemcitabine	HER2+ Biliary Tract Cancers	II	1st
HER2	NCT02992340	Varlitinib + cisplatin/gemcitabine	HER2+ Biliary Tract Cancers	I/II	1st
HER2	NCT03093870	Varlitinib/capecitabine *vs.* Placebo/capecitabine	HER2+ Biliary Tract Cancers	II/III	2nd
mTOR	NCT02631590	Copanlisib + cisplatin/gemcitabine	HER2+ Biliary Tract Cancers	II	1st

If targeted therapies become the preferred approach for subsets of BTCs, cumulative chemotherapy-related toxicity could be delayed or spared for many patients.

Molecular sequencing at the time of progression is also identifying mechanisms of resistance and potential therapeutic targets, as third-generation inhibitors are being developed to overcome point mutations that arise with initial targeted therapy. As this field evolves, it may be necessary to sample a tumor's genome sequentially with either repeat tumor biopsy or liquid biopsy platforms or both. As technologies advance to more comprehensively and more economically interrogate the tumor genome and transcriptome, we anticipate the identification of additional potential therapeutic targets and mechanisms of resistance ripe for investigation. For now, we await the results of a bevy of exciting trials in the refractory and front-line metastatic setting that will hopefully redefine how we approach BTCs in the years to come.

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Footnote

Conflicts of Interest: Dr. CS Denlinger has received advisory board honoraria from Astellas, Bayer, Merck, Bristol Myer Squibb, Eli Lilly & Co, EMD Serono, Exelixis, and BeiGene. Dr. Denlinger has received institutional grant support from Array BioPharma, Amgen, Advaxis, Bristol Myer Squibb, Astra Zeneca, Sanofi, BeiGene, Merrimack Pharmaceuticals, Roche/Genentech, Macrogenics, Lycera, Zymeworks, and Agios Pharmaceuticals. Dr. L Goyal is

a consultant/advisory board member for Debiopharm, H3 Biomedicine, Agios Pharmaceuticals, Taiho Pharmaceuticals, QED, Klus Pharmaceuticals, and Pieris Pharmaceuticals. Dr. MH Chen has received advisory board honoraria from Bayer, Sanofi, Merck, Bristol Myer Squibb and Ono, Eli Lilly & Co, and IPSEN. Dr. P Iyer has no conflicts of interest to declare.

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