

Emerging treatment evolutions and integrated molecular characteristics of biliary tract cancers

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Funding information

This paper is funded by the National Major Scientific and Technological Special Project for Significant New Drugs Development during the Thirteenth Five-Year Plan Period 2020ZX09201-003.

Abstract

Biliary tract cancers (BTCs) consist of a group of highly heterogeneous malignancies that are characterized by genomic differences among tumors from different anatomic sites. The current treatment for BTC includes surgery, chemotherapy, target therapy, and immunotherapy. Although surgery remains the primary option for localized disease, representing the only potential curative treatment, a high risk of recurrence cannot be neglected. Chemotherapy has been considered the standard of care for both advanced and metastatic disease and in adjuvant settings. However, drug resistance is a major obstacle associated with chemotherapy. The development of genetic testing technologies, including next-generation sequencing, has opened the door for the identification of drug targets and candidate molecules. A series of preclinical studies has demonstrated the role of gene mutations, abnormal signaling pathways, and immunosuppression in the pathogenesis of BTC, laying the foundation for the application of targeted therapy and immunotherapy. A variety of molecularly targeted agents, including pemigatinib, have shown promising survival benefits in patients with advanced disease. The rapidly evolving role of multimodal therapy represents the subject of this review.

KEYWORDS

biliary tract cancers, clinical trials, immunotherapy, molecular characterization, targeted therapy

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1 | BACKGROUND

Biliary tract cancers (BTCs) consist of a group of highly heterogeneous malignancies originating from the biliary system, including gallbladder cancer (GBC) and cholangiocarcinoma (CCA). BTC is a rare but fatal disease whose incidence varies according to geographic location and ethnicity.¹ The diagnosis of BTC often relies on imaging and tumor tissue biopsy, with the latter involving surgical resection or mass puncture. Due to its latent onset, BTC is usually diagnosed in late stage, thereby deprived of the opportunity for surgical resection.² The purpose of this review is to discuss the characteristics of BTC subtypes, current multimodal treatments (including surgery and adjuvant therapies for patients with early disease and chemotherapies for those with advanced disease), and recent progress related to targeted therapies and immune therapies.

2 | ANATOMICAL AND HISTOPATHOLOGICAL CLASSIFICATION OF BTC

According to anatomic location, BTCs can be classified as GBC, intrahepatic cholangiocarcinoma (ICC), perihilar cholangiocarcinoma (pCCA), or distal cholangiocarcinoma (dCCA). The latter two are collectively referred to as extrahepatic cholangiocarcinoma (ECC). Most cases of GBC (60%) arise from the fundus of the gallbladder, while 30% and 10% arise from the body and neck, respectively. ICC is defined as a CCA located proximally to the secondary bile ducts, while pCCA appears in the right and left hepatic ducts and the insertion of the cystic duct into the common bile duct.³ dCCA is confined to the area between the start of the common bile duct and the ampulla of Vater.

ICC has three main subtypes: the mixed subtype (derived from cuboidal mucin-negative cholangiocytes in small bile ducts), the mucinous subtype (derived from cylindrical mucous cholangiocytes in larger bile ducts), and the cholangiolocellular subtype (derived from hepatic progenitor cells [HPCs] in the most peripheral biliary branches).⁴ Some studies suggest that hepatitis B virus and hepatitis C virus (HCV) infections play a role in the development of mixed-ICC. In Japan, the risk of ICC in patients with liver cirrhosis caused by HCV is approximately 1000 times higher than that in the general population.⁵ Mixed-ICCs and cholangiolocellular-ICCs (CLCs) display almost mass-forming growth patterns, while muc-ICCs exhibit mass-forming patterns or a combination of periductal infiltration and mass formation.⁶ In addition to conventional ICC, CLC is a rare variant of ICC originating from bipotential HPCs.⁷ Studies have found that mice with genetic alterations that affect the Hippo pathway within the liver exhibit HPC expansion and subsequently develop CLC.⁸ In this case, a single tumor consisting of both differentiations develops, rather than one containing a mixture of hepatocytes and cholangiocytes. Although it shares biliary features with ICC, studies to date have shown that its radiological, clinicopathological, and molecular characteristics are different from those of ICC and hepatocellular carcinoma (HCC). Combined HCC may be more accurate

terminology.⁸ In addition, recent research indicates that the combination of activated Notch signaling and AKT overexpression may lead to the rapid formation of ICCs from hepatocytes.⁹ The molecular characteristics still need to be further elucidated.

ECC is believed to derive from cholangiocytes or pluripotent stem cells, which originate from the peribiliary glands located at the branch points of the biliary tree (eg, portal and ampulla regions).⁵ Conventional ECCs are mucin-producing adenocarcinomas. They frequently appear as flat or poorly defined nodular sclerosing tumors with a periductal infiltrating growth pattern (>80%) and are less common as intraductal polypoid or papillary tumors.^{6,10}

3 | MOLECULAR CHARACTERISTICS OF BTC

Several studies have reported the molecular characteristics of BTC, highlighting the genomic differences among tumors from different anatomic sites. Their findings may contribute to a more precise classification of BTC and have potential therapeutic significance (Figure 1).

3.1 | Gallbladder cancer

Two key pathways for the onset of GBC have been identified. The most common is gallstones and chronic inflammation of the gallbladder, which tend to occur in female patients over 65 years of age. The second, less frequent pathway is related to congenital abnormalities of the pancreatic bile duct junction (APBJ), which are especially common in Japanese patients and are more likely to occur in young patients.¹¹

Cases of GBC that develop in the context of an APBJ are consistently associated with KRAS (50%-83%) and late-onset p53 mutations. On the other hand, cholecystitis-associated GBCs rarely exhibit KRAS mutations and are characterized by early p53 mutations.¹² TP53 is the most frequently altered gene in patients with GBC. In general, the estimated frequency of p53 mutations in patients with GBC ranges from 21% to 39%.^{13,14} Studies investigating the PI3K pathway have also identified activating mutations in PIK3CA in cases of GBC (12%-13%).¹ HER2/neu amplification or protein overexpression has been detected in 12%-16% of GBC cases, a pattern unique to GBC.¹⁵ High-level microsatellite instability (MSI) has been reported in 5% of GBC cases,¹⁶ occurring more frequently in patients with APBJ.¹ A previous study reported that the incidence of breast cancer susceptibility gene (BRCA) mutations is higher in patients with MSI-H/deficient mismatch repair (dMMR) than in those without (19.5% vs. 1.7%, $P < .0001$).¹⁷ However, there is no significant difference in the frequency of BRCA mutations among tumor sites. In GBC and ICC, BRCA2 mutations (4.0% and 2.7%) are more frequent than BRCA1 mutations (0.3% and 0.4%, $P < .05$), although the observed frequency of the two mutation types is similar for ECC (BRCA2: 2.6%; BRCA1: 2.1%).¹⁷

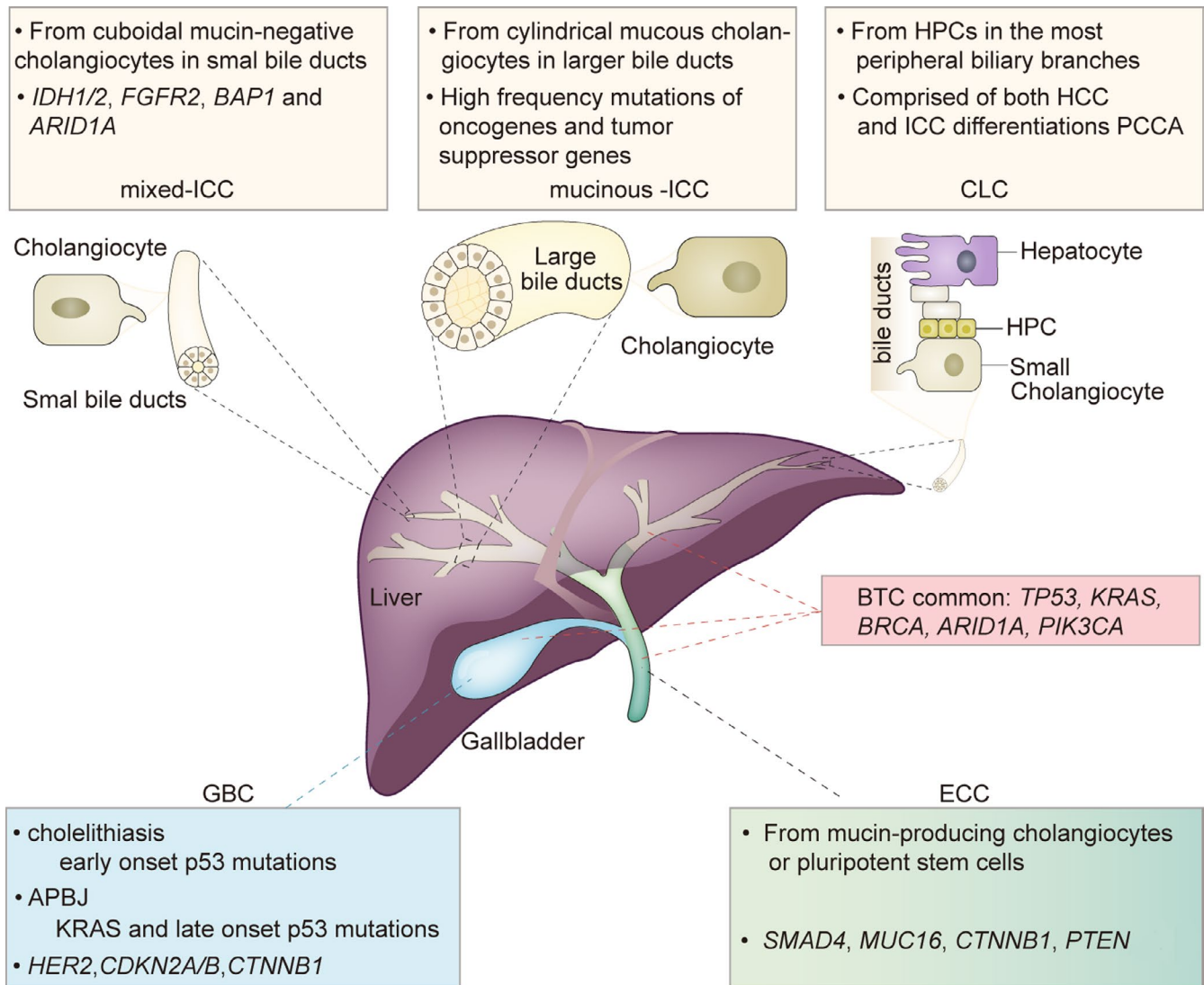


FIGURE 1 Molecular characterization of BTC (biliary tract cancer). APBJ, abnormalities of the pancreatic bile-duct junction; ARID1A, AT-rich interaction domain 1A; BAP1, BRCA1-associated protein 1; CDKN2A/B, cyclin-dependent kinase inhibitor 2A and B; CLC, cholangiolocellular-ICC; CTNNB1, catenin, cadherin-associated protein, beta 1; ECC, extrahepatic cholangiocarcinoma; FGFR2, fibroblast growth factor receptor 2; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; HPC, hepatic progenitor cell; ICC, intrahepatic cholangiocarcinoma; IDH1/2, isocitrate dehydrogenase 1 and 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; MUC16, mucoprotein 16; PCCA, perihilar cholangiocarcinoma; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, gene of phosphate and tension homology deleted on chromosome ten; ; SMAD4, SMAD family member 4; TP53, tumor protein 53

3.2 | ICC

Several independent studies have defined two distinctive molecular groups of ICCs: the proliferation molecular subclass with shorter survival and the inflammation subclass with better prognosis.¹² From the perspective of anatomic location and growth patterns, the former corresponds to the mucinous subtype to some extent. They both activate oncogenic signaling pathways, including KRAS (8.6%-25%), BRAF, SMAD4, and genes from the RAS-MAPK and MET signaling networks.^{3,10,14} Unfortunately, the latter do not have much in common with the mixed subtype, although they have both been observed in the setting of chronic liver disease.¹⁴ The

inflammation subclass is distinguished by overexpression of cytokines and IL-6-STAT3 signaling, while mixed-ICC displays a typically high rate of missense mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1/2*) genes (10%-23%),¹⁸ fibroblast growth factor receptor 2 (*FGFR2*) fusions (14%), and BRCA1-associated protein 1 (*BAP1*) (15%).^{10,12,13} Interestingly, KRAS and *IDH1* mutations are mutually exclusive. *FGFR2* aberrations appear to be mutually exclusive with other mutations such as *IDH1/2*, KRAS, and BRAF.^{19,20} MSI status is observed in 10% of patients with ICC and is more frequent than GBC (5%) and ECC (5%).¹⁶

IDH1/2 mutation is a molecular feature of intrahepatic bile duct origin and therefore occurs exclusively in ICC and rarely in

ECC and GBC.^{7,21} The correlation between IDH mutation status and prognosis remains controversial. In the Fudan cohort of 252 patients with ICC with follow-up data, the mutation status of IDH genes was associated with longer overall survival (OS) and a lower probability of recurrence,¹⁸ which is contrary to the findings of another study with a small sample size ($n = 34$).¹³ Data have also shown that IDH1/2 mutation predicts a good prognosis in mixed-ICC and a poor prognosis in muc-ICC.²² Notably, FGFR2 fusions that lead to ligand-independent activation of the receptor tyrosine kinase have been identified almost only in patients with ICC.^{21,23,24} FGFR2 aberrations in ICC have been associated with an apparent female predominance and an apparent tendency for a better prognosis.²⁴ Epidermal growth factor receptor (EGFR) expression is an independent prognostic factor in ICC, whereas vascular endothelial growth factor (VEGF) expression is associated with intrahepatic metastasis.²⁵

3.3 | ECC

ECC, ICC, and GBC exhibit partial overlap in terms of molecular expressions, albeit at different frequencies. These include TP53, KRAS, CDKN2A/B, ARID1A, and PTEN. KRAS mutations are consistently more prevalent in ECC than in ICC and GBC.⁶ HER2 is present in 9% of ECCs. However, unlike in cases of GBC, these alterations are mostly base substitution and insertion mutations rather than amplifications.²⁶

In recent years, Japanese scholars have reported a type of occupational CCA caused by long-term exposure to chlorinated organic solvents, including 1,2-dichloropropane (DCP) and dichloromethane (DCM).²⁷ Although the genetic mutations of occupational CCA is similar to that of conventional BTC, its tumor mutation burden (TMB) is about 30 times higher than that of the latter.²⁸

4 | STATE-OF-THE-ART TREATMENT FOR BTC

BTC treatment varies according to the stage defined by the Bismuth-Corlette and TNM classifications. Surgery with negative margins including lymphadenectomy remains the primary treatment option for localized disease, representing the only potential curative treatment. Chemotherapy has also been applied in adjuvant and metastatic/locally advanced settings (Figure 2).

4.1 | Neoadjuvant therapy

Due to atypical symptoms in the early stages of the disease, only about 20%-50% of patients with BTC have the opportunity to undergo surgery.^{2,29} Several retrospective studies indicated that neoadjuvant therapies, including preoperative radiotherapy and chemotherapy, could improve the resectability of locally advanced CCA and prolong the survival time.³⁰⁻³² Previous research provided

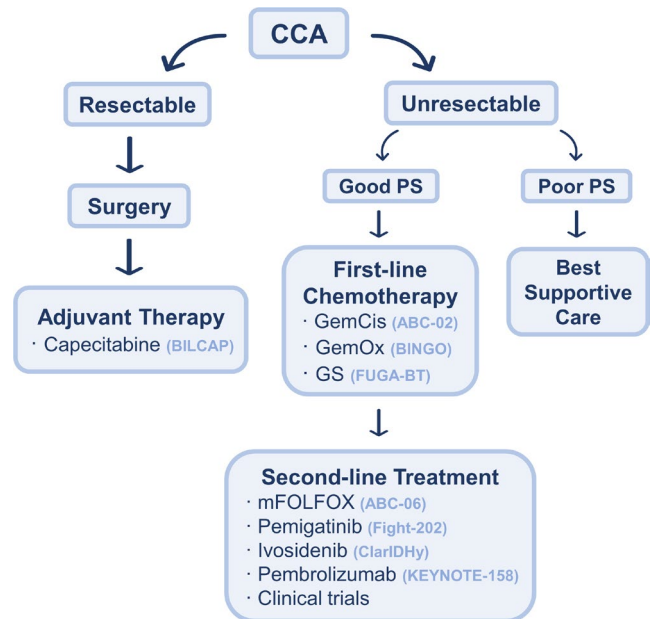


FIGURE 2 Therapeutic strategy in cholangiocarcinoma (CCA). GemCis, gemcitabine + cisplatin; GemOx, gemcitabine + oxaliplatin; GS, gemcitabine + S-1; mFOLFOX, modified 5-fluorouracil + oxaliplatin; PS, performance status

information that in patients with localized node-negative pCCA, compared with traditional resection, neoadjuvant chemoradiation combined with liver transplantation achieved a better 5-year survival rate (82% versus 21%, $P = .022$) and less recurrence rate.³³ A prospective case series showed that ICC with pretransplant stable disease (SD) in gemcitabine-based neoadjuvant chemotherapy may benefit from liver transplantation.³⁴ The application of neoadjuvant therapy in BTC requires further exploration in prospective trials.

4.2 | Adjuvant therapy

Even with R0 resection, the risk of 5-year recurrence is 64% for GBC and 53.5% for ICC.^{35,36} The reported 5-year survival rates for patients with CCA after surgery range from 22% to 44%.^{2,36} After surgery, patients are advised to undergo adjuvant therapy to reduce the likelihood of recurrence, especially those with positive lymph nodes or resection margins.³⁷ For occupational BTC, aggressive treatment including secondary surgical resection of the recurrent lesion appears to be effective.³⁸

Adjuvant chemotherapy is based on the results of the BILCAP study,³⁹ which was a randomized, controlled phase III study that compared capecitabine with observation in patients with resected BTC. The primary endpoint was not met in the intention-to-treat population, and the authors reported a median OS of 51.1 months in the capecitabine group and 36.4 months in the observation group (hazard ratio [HR]: 0.81, $P = .97$). In a protocol-specified analysis adjusted for minimization factor, nodal status, grade, and sex, the authors reported a statistically significant survival

advantage in the capecitabine group (HR: 0.75, $P = .028$, respectively). Nevertheless, in the same setting, the PRODIGE-12⁴⁰ and BCAT⁴¹ phase III studies reported no benefit from the combination of gemcitabine and oxaliplatin (GemOx) or from the single agent gemcitabine, respectively.

Survival data from a phase II randomized prospective study (KHBO 1208) in Japan indicate that S-1 adjuvant therapy may be better than gemcitabine adjuvant therapy after major hepatectomy in patients with BTC.⁴² A phase III prospective randomized trial (ACTICCA-1) is assessing the clinical performance of gemcitabine + cisplatin (GemCis) in patients after curative intent resection of BTC.⁴³

4.3 | First-line chemotherapy

Systemic chemotherapy is the standard of care for advanced or metastatic diseases. GemCis should be the preferred choice in patients with good Eastern Cooperative Oncology Group (ECOG) performance status (PS) and normal renal function based on the results of the ABC-02 trial.⁴⁴ This Phase III clinical trial demonstrated the superiority of GemCis compared with gemcitabine monotherapy, with an OS of 11.7 months vs 8.1 months, respectively (HR: 0.64 $P < .001$). GemOx is generally preferred if renal function is a concern.⁴⁵ In patients with ECOG PS 2, gemcitabine alone should be considered. In Asia, the combination of gemcitabine + S-1 (GS) is considered a new option for advanced BTC thanks to the phase III trial FUGA-BT, which demonstrated the noninferiority of GS to GemCis in terms of OS (GemCis: 11.2 months vs. GS: 13 months; HR: 0.94, $P = .046$).⁴⁶ Another phase III clinical trial revealed that capecitabine plus oxaliplatin (XELOX) exhibited similar efficacy to GemOx in terms of 6-month progression-free survival (PFS), highlighting its potential as an alternative first-line treatment.⁴⁷

It is worth noting that there are new therapeutic candidates with improved survival rates. A phase III clinical trial in Japan showed that the clinical efficacy of gemcitabine + cisplatin + S-1 (GCS) is significantly better than that of GemCis. The former was associated with a longer median PFS (mPFS; 7.4 months vs. 5.5 months, HR: 0.75; $P = .0015$), and there were no significant differences in adverse events (AEs), except for those related to S-1. Such therapy may thus become a new standard for the treatment of advanced BTC.⁴⁸ A phase II clinical trial has shown that adding nab-paclitaxel to first-line treatment with GemCis may prolong survival, with an mPFS of 11.8 months and an mOS of 19.2 months.⁴⁹

4.4 | Second-line treatment

Second-line chemotherapy has been widely utilized despite limited evidence to support a benefit. The results of the randomized phase III ABC 06 trial demonstrated an OS advantage for modified 5-fluorouracil + oxaliplatin (mFOLFOX) compared with best supportive care (6.2 vs. 5.3 months, HR: 0.69, $P = .031$), suggesting that

mFOLFOX is the treatment of choice in patients with ECOG PS 0-1 previously treated with GemCis first-line chemotherapy.⁵⁰ However, considering the limited survival benefit, studies have explored the possibility of other options in the second-line treatment of BTC. Retrospective data for 87 patients with patients receiving second-line therapy indicated that the capecitabine and irinotecan (XELIRI) regimen is safe and effective, with a disease control rate (DCR) of 41.3% and an OS of 8 months.⁵¹ A phase II trial conducted in 60 patients demonstrated that, compared with irinotecan monotherapy, the XELIRI regimen has significant advantages in terms of prolonging PFS and improving 9-month OS rates (3.7 vs. 2.4, $P = .036$, and 60.0% vs. 32.9%, $P = .045$, respectively).⁵²

For those who have used GemOx as first-line treatment, 5-fluorouracil-irinotecan (FOLFIRI)-based chemotherapy may be a good choice. And the sensitivity to this chemotherapy regimen is correlated with sensitivity to the first-line GemOx regimen ($P = .007$).⁵³ However, FOLFIRI is not superior to FOLFOX in patients with BTC after failure of first-line GemCis treatment.⁵⁴

Regorafenib is a potent inhibitor of angiogenic and oncogenic kinases and has been approved for the treatment of patients with metastatic colorectal cancer refractory to standard chemotherapy. An earlier study showed that, among 43 patients with chemotherapy-refractory advanced/metastatic BTC in whom the response to regorafenib treatment could be assessed, the mPFS was 15.6 weeks (90% confidence interval [CI]: 12.9-24.7 weeks).⁵⁵ The randomized phase II study REACHIN showed that, although regorafenib doubled the mPFS compared with placebo (3.0 months to 1.5 months; HR: 0.49; 95% CI: 0.29-0.81; $P = .004$), there was no significant difference in OS.⁵⁶

Based on the results of the Fight-202 trial, pemigatinib (a potent and selective oral inhibitor of FGFR 1-3 and VEGFR2) has been approved by the Food and Drug Administration (FDA) for the treatment of previously treated patients with CCA exhibiting FGFR2 rearrangement or fusions, with an overall response rate (ORR) of 35.5% and an overall median follow-up time of 17.8 months.⁵⁷ Based on the results of the phase III ClarIDHy study, ivosidenib has also been recommended as a second-line treatment for patients with IDH1-mutant CCA, which reduces the risk of death by 63% compared with placebo.⁵⁸ In addition, in 2018, the FDA included pembrolizumab as a treatment option for patients with unresectable or metastatic MSI-H/dMMR BTC.⁵⁹

5 | TARGETED THERAPY IN CCA

With the development of gene detection technologies, including next-generation sequencing, studies have elucidated the role of gene mutations and abnormal signaling pathways in the pathogenesis of BTC, leading to the discovery of new therapies for molecular targets. Liquid biopsy could overcome the limitations of tissue biopsy (limited tissue, tumor heterogeneity), aid in the analysis of biomarkers of drug resistance, and assist with longitudinal monitoring.⁶⁰ In the prospective clinical study MOSCATO-01, patients with BTC who

received targeted therapy based on identified molecules exhibited an encouraging improvement in mOS compared with those who received unselected therapy (17 months vs. 5 months; $P = .008$).⁶¹ The important targets involved in this pathway include FGFR, IDH, ERBB, c-MET, BRAF, NTRK, and anti-VEGF therapy (Figure 3).

5.1 | FGFR-targeted therapy

FGFR2 is one of the most promising therapeutic targets identified in CCA in recent years. Derazantinib (ARQ087), a potent orally bioavailable multikinase inhibitor with pan-FGFR activity, has shown encouraging antitumor activity and a manageable safety profile in patients with chemotherapy-refractory ICC harboring FGFR2 fusion.⁶² A pivotal phase II study of derazantinib is currently underway in patients with inoperable or advanced ICC (NCT03230318). A multicenter, open-label, phase II study demonstrated the acceptable toxicity and potential efficacy of infigratinib (BGJ398)—an orally bioavailable, selective pan-FGFR kinase inhibitor—in 61 patients with FGFR-altered advanced CCA.⁶³ The ORR was 14.8%, and the DCR was 75.4%, with an mPFS of 5.8 months (95% CI: 4.3 to 7.6 months). A phase III study compared the efficacy of infigratinib and GemCis as first-line treatment for patients with unresectable locally advanced or metastatic ICC with FGFR2 gene fusions/translocations (NCT03773302). Following the recent FDA approval of pemigatinib

and its appearance in the National Comprehensive Cancer Network (NCCN) Guidelines, the European Medicine Agency's Committee for Medicinal Products for Human Use (CHMP) also recommended it for patients with locally advanced and previously treated CCA and for those with metastatic CCA harboring an FGFR2 gene fusion or rearrangement. However, the rapid emergence of acquired resistance has often been observed. Recent studies have shown that the third-generation irreversible FGFR inhibitor futibatinib (TAS-120) provides clinical benefits for patients with ICC who have acquired resistance to infigratinib.⁶⁴ In a phase II study (FOENIX-CCA2), 67 patients with ICC harboring FGFR2 fusions/other rearrangements received futibatinib and had an ORR of 37.3%, a median duration of response (DOR) of 8.3 months, and a DCR of 82%.⁶⁵ Clinical trials of other selective FGFR inhibitors are underway (NCT04238715, NCT04526106, NCT04565275).

5.2 | IDH-targeted therapy

IDH gene mutations affect the proliferation and differentiation of HPCs by inhibiting HNF-4 α (a primary regulator of hepatocyte identity and quiescence), which plays an important role in the pathogenesis of CCA.⁶⁶ The phase III ClarIDHy study showed that ivosidenib improved PFS relative to placebo treatment: The mPFS was 2.7 months in the 126 patients treated with ivosidenib, while it was 1.4 months in the 61 patients receiving placebo (HR: 0.37, $P < .001$). The median OS for ivosidenib was 10.3 months, compared with 7.5 months for placebo (HR: 0.79; 95% CI: 0.56–1.12).⁵⁸ However, given the limited survival benefits and high costs, it is necessary to consider the patients' financial situation and ensure proper communication. A study of high-throughput drug screening in a large panel of cancer cell lines found that IDH-mutant ICC cells are highly sensitive to the multi-kinase inhibitor dasatinib.⁶⁷ Furthermore, a phase II clinical trial is exploring the safety and effectiveness of dasatinib in patients with IDH-mutant advanced ICC (NCT02428855).

5.3 | ERBB-targeted therapy

The receptor tyrosine protein kinase ERBB-2, also known as HER2, is a member of the EGFR family of receptor tyrosine kinases. In a retrospective analysis, eight patients with GBC with HER2/neu amplification or overexpression achieved a 100% DCR (1 CR, 4 PR, and 3 SD) after receiving HER2-directed therapy (trastuzumab, lapatinib, or pertuzumab).¹⁵ In the SUMMIT global basket study, treatment with neratinib (an irreversible inhibitor of HER1, HER2, and HER4) resulted in an ORR of 22% in nine patients with BTC exhibiting HER2 mutations.⁶⁸ Despite the limited number of patients involved, the results of these studies provide insights into the treatment of patients with HER2-positive BTC. However, two phase II trials demonstrated that lapatinib, a dual-target tyrosine kinase inhibitor (TKI) of HER1 and HER2, showed no benefits in patients with advanced BTC when

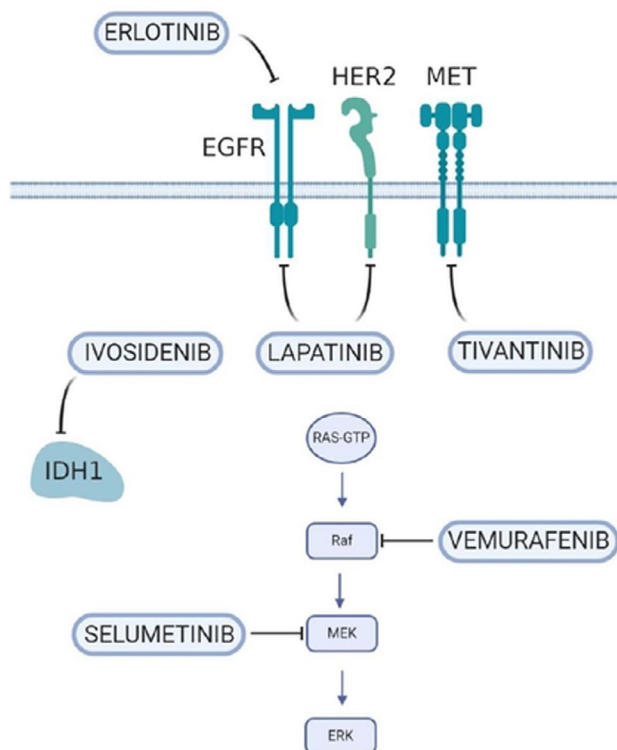


FIGURE 3 New therapeutic target in cholangiocarcinoma (CCA). EGFR, epidermal growth factor receptor; IDH1, isocitrate dehydrogenase 1; MEK, mitogen-activated protein kinase; MET, tyrosine protein kinase MET; RAF, serin-threonine protein

administered as monotherapy,^{69,70} although it improved the clinical outcome of patients with breast cancer with HER2 amplification or overexpression.

5.4 | KRAS-BRAF-MEK-ERK pathway

A phase II basket study of vemurafenib, a BRAF inhibitor, has reported a potential benefit in patients with CCA harboring BRAF V600 mutations.⁷¹ In another basket trial (ROAR) the strategy of simultaneously inhibiting BRAF (dabrafenib) and MEK (trametinib) was tested in solid tumors. In the subgroup of 32 evaluable patients with BTC, the ORR was 41% (13/32; 95% CI: 24-59%), mPFS was 7.2 months (95% CI: 4.6-10.1), and the mOS was 11.3 months (95% CI: 7.3-17.6).⁷² Based on these studies, routine screening for BRAF V600e mutations is recommended in patients with CCA. A phase II study of the MEK1/2 inhibitor selumetinib has reported an mPFS of 3.7 months and an mOS of 9.8 months in patients with advanced BTC.⁷³

5.5 | Neurotropic tyrosine receptor kinase (NTRK)-targeted therapy

The binding of NTRK to the tropomyosin-related kinase (TRK) receptor leads to the activation of the Ras/MAPK pathway, which leads to increased conduction and cell growth through ERK signaling, thus playing a carcinogenic role.⁷⁴ As a pioneer of broad-spectrum anti-cancer drugs, NTRK inhibitors may play a role in a small proportion of patients with BTC (0.67%).⁷⁵ In a previous study, 54 patients with advanced or metastatic NTRK fusion-positive solid tumors (one patient with CCA) treated with entrectinib achieved an ORR of 57%.⁷⁶ Larotrectinib (another highly selective NTRK inhibitor) also showed excellent antitumor activity in patients with NTRK fusion-positive tumors, with an ORR of 93% (14 of 15 patients).⁷⁷

5.6 | Anti-VEGF therapy

Two phase II clinical trials have revealed that regorafenib has promising efficacy in patients with BTC after failure of GemCis first-line treatment.^{55,56} However, treatment with vandetanib (an orally available, dual VEGFR2 and EGFR TKI) did not improve PFS in patients with advanced BTC, according to the VanGogh Phase II multicenter study.⁷² The ABC-03 study also showed that adding cediranib (an oral VEGFR inhibitor) to GemCis could not slow the growth of cancer.⁷⁸ The role of VEGF inhibition in patients with advanced BTC is still under investigation.

5.7 | c-MET targeted therapy

The activity of tivantinib, a c-MET kinase inhibitor, has been investigated both in cell lines and in a phase I study (ARQ 197), showing

antineoplastic activity.^{79,80} A phase II trial evaluated the activity of cabozantinib, an inhibitor of the tyrosine kinases c-Met and VEGFR2, in patients with advanced CCA after progression on first-line or second-line systemic therapy, which demonstrated limited clinical activity and significant toxicity⁸¹: In the 19 patients enrolled, the mPFS was 1.8 months, and the mOS was 5.2 months (95% CI: 2.7-10.5 months). A phase II study is now assessing the efficacy and safety of crizotinib in patients harboring alterations in ALK, MET, or ROS1 (NCT02034981).

5.8 | Poly (ADP-ribose) polymerase inhibitors (PARPi)

PARP1 is an abundant ribozyme that can be activated when DNA is notched or broken, playing an important role in maintaining genomic stability. Abnormal functioning of BRCA1 or BRCA2 makes cells significantly sensitive to the inhibition of PARP enzyme activity, leading to chromosomal instability, cell cycle arrest, and subsequent apoptosis.⁸² Ongoing trials are investigating the use of niraparib in patients with BAP1-mutant tumors (NCT03207347). In addition, *in vitro* and *in vivo* IDH mutant tumor models show sensitivity to PARPi,⁸³ and a phase II trial involving patients with CCA is underway to test this concept (NCT03212274).

5.9 | Anti-Dickkopf-1 (Dkk1) antibody

Dkk1 is an inhibitor of oncogenic beta-catenin-dependent Wnt signaling through negative feedback.⁸⁴ But DKK1 can also target β -catenin in myeloid-derived suppressor cells (MDSC), which may lead to immune escape and promote the proliferation of cancer cells.⁸⁵ DKN-01, an anti-DKK1 antibody, has proven safety in studies of various malignancies and showed potential efficacy in patients with Wnt activating mutations (such as CTNNB1 and APC mutations) or high expression of Dkk1.^{84,86} A phase II trial of the combination of DKN-01 and nivolumab is ongoing in previously treated patients with advanced BTC (NCT04057365).

6 | IMMUNOTHERAPY IN BTC

Many cancers are able to evade the immune system, mainly by overexpressing inhibitory ligands to suppress T cell attack. It is now evident that immune checkpoint modulation plays a relevant role in gastrointestinal cancers as well. Therefore, an increasing number of research groups aim to enhance T cell-mediated antitumor immune responses through checkpoint blockade and adoptive cell therapy (ACT).

6.1 | Immune checkpoint inhibition

The clinical benefits of immune checkpoint inhibitors (CPIs) have been demonstrated in tumors with MSI-H/dMMR and TMB-H

(TMB ≥ 10 mutations/megabase) status.⁵⁹ Higher PD-L1 expression in tumors is correlated with venous invasion and advanced tumor staging, leading to poor prognosis of BTC.^{87,88} According to a previous study of 652 patients with BTC, 8.6% were programmed cell death protein 1 ligand (PD-L1) positive with the following distribution: GBC 12.3%, ICC 7.3%, and ECC 5.2%.⁸⁹ It is worth stating that compared with conventional CCA, higher PD-L1 overexpression and TMB was observed in occupational CCA ($P < .01$).⁹⁰ The number of inhibitory ligand-positive tumor-infiltrating cells in the tumor microenvironment of occupational CCA was also significantly increased, which may be promising targets for mobilizing immune cells to attack the tumors.⁹¹ Taken together, these studies provide the basis for treating patients with BTC using CPIs.

Early data from KEYNOTE-028 investigating outcomes in PD-L1-positive patients with advanced BTC pretreated with blockers reported that four (17%) of the 24 patients had a PR, while another four achieved SD.⁹² A phase II study using another programmed cell death protein 1 (PD-1) antibody, nivolumab, was conducted in 54 patients with advanced BTC after progression despite standard-line therapy. The ORR was 22%, and the associated mPFS and mOS were 3.9 and 14.2 months, respectively.⁸⁹ It is also worth mentioning that, in KEYNOTE-158, pembrolizumab achieved an ORR of 40.9% and an mPFS of 4.2 months in patients with BTC presenting with MSI-H/dMMR status.⁵⁹

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and PD-1/PD-L1 suppress T cell-mediated immune responses at different stages and locations; thus, they may act synergistically to activate antitumor immune responses.⁹³ A phase II clinical trial evaluated the efficacy and safety of combining nivolumab and ipilimumab (an anti-CTLA-4 inhibitor) in patients with advanced BTC, with an ORR of 23% and DCR of 44%.⁹⁴ There are other ongoing clinical trials evaluating the effects of dual immune checkpoint suppression therapy in patients with BTC (Table 1b).

6.2 | ACT

ACT uses host cells that have been genetically engineered with anti-tumor T cell receptors (TCRs) or chimeric antigen receptors (CARs), as well as natural host cells such as natural killer (NK) cells, to induce antitumor reactivity.

Recently, a phase I trial of 19 patients with EGFR-positive advanced BTC reported that one patient achieved complete response (CR) and 10 patients achieved SD after CAR T-EGFR cell immunotherapy, demonstrating the safety and feasibility of this therapy.⁹⁵ Another phase I trial of 11 patients with HER2-positive (>50%) BTC reported an mPFS of 4.8 months (range: 1.5-8.3 months), suggesting encouraging clinical activity.⁹⁶

Other studies have indicated that ex vivo-expanded and activated NK cells exhibit effective antitumor activity both in vitro and in vivo. A phase I/IIa clinical trial is evaluating the safety and efficacy of

allogeneic NK cells ("SMT-NK") in combination with pembrolizumab for patients with gemcitabine-refractory BTC (NCT03937895).

The successful use of ACT lies in identifying appropriate targets that are selectively expressed in cancers, which could be challenging in solid tumors.

6.3 | Immunotherapy in combination with other therapies

The tumor microenvironment plays a vital role in accelerating the formation of new blood vessels, enhancing the proliferation and invasion of cancer cells, and preventing apoptosis of tumor cells.⁹⁷ Complementary combinations of immunotherapy are increasingly being explored as strategies to improve efficacy. Multiple clinical trials using CPIs plus targeted or cytotoxic drugs are underway (Table 1).

6.3.1 | Immunotherapy in combination with targeted therapy

Preclinical evidence indicates a close correlation between angiogenesis and suppression of the antitumor response. VEGF increases T cell exhaustion by enhancing the expression of inhibitory checkpoints on T cells, while simultaneous blocking of VEGFR and PD-1/PD-L1 can induce cumulative antitumor effects.⁹⁸ Lenvatinib is a multiple receptor TKI that mainly targets VEGFR and FGFR. Flow cytometric analysis has revealed that lenvatinib enhances the ability to induce CD8+ T cells via the interferon (IFN) signaling pathway when combined with PD-1 blockade.⁹⁹ The efficacy and safety of this strategy are being evaluated in patients with CCA (NCT03797326, NCT03895970). However, only one patient achieved an objective response in the trial of ramucirumab (an antagonist of VEGFR2) and pembrolizumab, in which 46.2% (11/26) of patients were PD-L1 positive.⁹⁸ PARPi can upregulate PD-L1 expression in breast cancer cells, which may lead to increased sensitivity to CPI therapy.¹⁰⁰ A phase II trial of this combination therapy is expected to start recruiting patients to assess the efficacy of IDH-mutated CCAs (NCT03991832).

6.3.2 | Immunotherapy in combination with chemotherapy

There is growing evidence that cytotoxic drugs can also strengthen the immune system by increasing the ratio of cytotoxic lymphocytes to regulatory T cells and the number of antigen-presenting cells. In vitro, CCA cells treated with gemcitabine can induce the mRNA expression of PD-L1.¹⁰¹ These encouraging experiments highlight the potential and feasibility of this treatment strategy, and larger clinical trials are thus underway. A phase II study demonstrated tolerability

TABLE 1 Selected ongoing clinical trials with immunotherapy in BTC

Therapeutic	Target	Phase	Status	Design	Trail NCT	Endpoints
(a) Checkpoint inhibitor monotherapy						
Pembrolizumab (MK-3475)	PD-1	Ib	Active, not recruiting	Single Group Assignment	NCT02054806	ORR/PFS
Pembrolizumab (MK-3475)	PD-1	II	Recruiting	Single Group Assignment	NCT03110328 NCT02628067	ORR/PFS; ORR
Nivolumab	PD-1	II	Active, not recruiting	Single Group Assignment	NCT02829918	ORR
STI-3031	PD-L1	II	Not yet recruiting	Single Group Assignment	NCT03999658	ORR/DOR/PFS
M7824	PD-L1	II	Recruiting	Single Group Assignment	NCT03833661	OR/DOR
Nivolumab or pembrolizumab	PD-1	-	Recruiting	Prospective Cohort Study	NCT03695952	ORR
(b) Dual checkpoint inhibition						
MEDI4736 Tremelimumab	PD-L1 monotherapy/ with CTLA-4	I	Active, not recruiting	Parallel Assignment	NCT01938612	Safety and tolerability
Nivolumab Ipilimumab	PD-1 CTLA-4	II	Recruiting	Parallel Assignment	NCT02834013	ORR
Gemcitabine + Cisplatin + Nivolumab; Nivolumab + Ipilimumab	PD-1 PD-1+ CTLA-4	II	Active, not recruiting	Parallel Assignment (Randomized)	NCT03101566	PFS
(c) Checkpoint inhibition plus myeloid cell immunosuppression						
Pembrolizumab Sargramostim	PD-1 GM-CSF	II	Active, not recruiting	Single Group Assignment	NCT02703714	ORR
Nivolumab Nivolumab + Cabrilizumab	PD-1 CSF-1R	-	Recruiting	Parallel Assignment (Randomized)	NCT03768531	Safety and tolerability
(d) Adoptive cell therapy						
IL-2 HER2Bi-Armed T Cells		I	Unknown	Single Group Assignment	NCT02662348	Safety and tolerability
CART-HER-2		I	Unknown	Single Group Assignment	NCT01935843	Safety and tolerability
CART-EGFR		I	Unknown	Single Group Assignment	NCT01869166	Safety and tolerability
Cytokine induced killer cells		I	Recruiting	Single Group Assignment	NCT01868490	Tumor size and CIK cell-homing
TC-210 T Cells TC-210 + fludarabine + cyclophosphamide TC-210+ fludarabine + cyclophosphamide + anti-PD1		I/II	Recruiting	Single Group Assignment	NCT03907852	Safety and tolerability
Tumor-Infiltrating Lymphocytes (TIL)		II	Recruiting	Single Group Assignment	NCT03801083	ORR/CRR/DOR
MUC-1 CART cell		-	Recruiting	Single Group Assignment	NCT03633773	DCR/ORR
(e) Adoptive cell therapy plus checkpoint inhibition						
CD8+ T Cell Aldesleukin Cyclophosphamide Pembrolizumab		I	Active, not recruiting	Single Group Assignment	NCT02757391	Safety and tolerability

(Continues)

TABLE 1 (Continued)

Therapeutic	Target	Phase	Status	Design	Trail NCT	Endpoints
'SMT-NK' Inj Pembrolizumab		I/IIa	Recruiting	Single Group Assignment	NCT03937895	Phase I: RP2D Phase IIa: ORR
(f) Checkpoint inhibition plus targeting						
Ramucirumab Pembrolizumab	VEGFR-2 PD-1	I	Active, not recruiting	Parallel Assignment	NCT02443324	Number of participants who experienced DLTs
Guadecitabine Durvalumab	DNMT PD-L1	Ib	Recruiting	Single Group Assignment	NCT03257761	Incidence of TEAEs, RP2D
Anlotinib TQB245	VEGFR, PDGFR, FGFR, c-Kit PD-L1	Ib	Recruiting	Single Group Assignment	NCT03996408	DLT/MTD/RP2D/ ORR
Regorafenib Avelumab	VEGFR1-3, PDGFR- β , Kit, RET, Raf-1 PD-L1	I/II	Recruiting	Sequential Assignment	NCT03475953	Phase I: RP2D Phase II: ORR
FT-2102 Nivolumab	IDH1 PD-1	Ib/II	Recruiting	Parallel Assignment	NCT03684811	Phase Ib: DLT/ RP2D Phase II: ORR
Lenvatinib Pembrolizumab	VEGFR1-3, FGFR1-4, PDGFR- β , RET, KIT PD-1	II	Recruiting	Single Group Assignment	NCT03895970	ORR/DCR/PFS
Lenvatinib Pembrolizumab	VEGFR1-3, FGFR1-4, PDGFR- β , RET, KIT PD-1	II	Active, not recruiting	Single Group Assignment	NCT03797326	ORR, Incidence of TEAEs
Rucaparib Nivolumab	PARP PD-1	II	Recruiting	Single Group Assignment	NCT03639935	PFS
Axitinib Toripalimab	VEGFR1-3, PDGFR- β , c-Kit PD-1	II	Not yet recruiting	Single Group Assignment	NCT04010071	ORR/PFS
Olaparib Durvalumab	PARP PD-L1	II	Not yet recruiting	Parallel Assignment	NCT03991832	ORR/DCR
DKN-01 Nivolumab	DKK1 PD-1	II	Recruiting	Parallel Assignment	NCT04057365	ORR
SHR-1210 + Apatinib SHR-1210 + FOLFOX4/ GEMOX regimen	PD-1, VEGFR-2 PD-1+ chemotherapy	II	Recruiting	Parallel Assignment	NCT03092895	Safety and tolerability
Entinostat Nivolumab	HDAC1, HDAC3 PD-1	II	Recruiting	Parallel Assignment	NCT03250273	ORR
Atezolizumab Atezolizumab + Cobimetinib	PD-L1 MEK	II	Active, not recruiting	Parallel Assignment (Randomized)	NCT03201458	PFS
(g) Checkpoint inhibition plus chemotherapy						
Nivolumab Gemcitabine Cisplatin	PD-1	I/II	Unknown	Single Group Assignment	NCT03311789	6-month PFS rate, mOS

(Continues)

TABLE 1 (Continued)

Therapeutic	Target	Phase	Status	Design	Trail NCT	Endpoints
INT230-6 Pembrolizumab or anti-CTLA-4 antibody	PD-L1 CTLA-4	I/II	Recruiting	Sequential Assignment	NCT03058289	Safety and tolerability
Manganese chloride Nab-paclitaxel Gemcitabine Anti-PD-1 antibody	PD-1	I/II	Recruiting	Single Group Assignment	NCT04004234	Incidence of TEAEs, PFS
Nivolumab Nanoliposomal-Irinotecan 5-Fluorouracil Leucovorin	PD-1	Ib/II	Recruiting	Single Group Assignment	NCT03785873	Phase Ib: Incidence of DLTs Phase II: mPFS
Nivolumab TS-1 Gemcitabine	PD-1	II	Recruiting	Single Group Assignment	NCT04172402	ORR
Toripalimab Gemcitabine 5- fluorine pyrimidine	PD-1	II	Recruiting	Single Group Assignment	NCT03982680	6-month PFS rate, mPFS, toxic side effects
Toripalimab S1 Albumin Paclitaxel	PD-1	II	Recruiting	Single Group Assignment	NCT04027764	ORR/PFS/DCR/ OS
SHR-1210 GEMOX	PD-1	II	Recruiting	Single Group Assignment	NCT03486678	6-month PFS rate, incidence of TEAEs
Pembrolizumab Oxaliplatin Capecitabine	PD-1	II	Recruiting	Single Group Assignment	NCT03111732	5-month PFS rate, safety, ORR, OS
Pembrolizumab Cisplatin Gemcitabine	PD-1	II	Recruiting	Single Group Assignment	NCT03260712	6-month PFS rate, ORR, toxicity
GS Toripalimab	PD-1	II	Recruiting	Single Group Assignment	NCT03796429	PFS/OS/ORR
Durvalumab Tremelimumab Gemcitabine/ Cisplatin	PD-L1 CTLA-4	II	Recruiting	Single Group Assignment	NCT03046862	ORR/DCR/PFS/ OS
Durvalumab + Tremelimumab + Gemcitabine Durvalumab + Tremelimumab + Gemcitabine + Cisplatin Gemcitabine + Cisplatin	PD-L1 CTLA-4	II	Recruiting	Parallel Assignment (Randomized)	NCT03473574	ORR, OS, incidence of TEAEs
M7824 + Gemcitabine + Cisplatin Placebo + Gemcitabine + Cisplatin	PD-L1	II/III	Recruiting	Parallel Assignment (Randomized)	NCT04066491	Number of participants who experienced DLTs, OS
Pembrolizumab + Gemcitabine + Cisplatin Placebo + Gemcitabine + Cisplatin	PD-1	III	Recruiting	Parallel Assignment (Randomized)	NCT04003636	PFS/OS/ORR

(Continues)

TABLE 1 (Continued)

Therapeutic	Target	Phase	Status	Design	Trail NCT	Endpoints
Durvalumab + Gemcitabine + Cisplatin Placebo + Gemcitabine + Cisplatin	PD-L1	III	Recruiting	Parallel Assignment (Randomized)	NCT03875235	OS/PFS/ORR
KN035 + Gemcitabine + oxaliplatin Gemcitabine + oxaliplatin	PD-L1	III	Recruiting	Parallel Assignment (Randomized)	NCT03478488	OS/PFS/ORR
(h) Checkpoint inhibition plus targeting and chemotherapy						
Oxaliplatin Gemcitabine Lenvatinib JS001	VEGFR1-3, FGFR1-4, PDGFR α , RET, KIT PD-1	II	Active, not recruiting	Single Group Assignment	NCT03951597	ORR/OS/PFS
(i) Checkpoint inhibition plus ablative local therapy						
Tremelimumab Durvalumab Radiation	CTLA-4 PD-L1	II	Recruiting	Single Group Assignment	NCT03482102	ORR, incidence of TEAEs, OS, DCR, PFS
Durvalumab + Tremelimumab Durvalumab + Tremelimumab + TACE Durvalumab + Tremelimumab + RFA Durvalumab + Tremelimumab + Cryoablation	PD-L1 CTLA-4	II	Recruiting	Parallel Assignment	NCT02821754	PFS, incidence of TEAEs
Radiotherapy + Camrelizumab Gemcitabine + Cisplatin	PD-1	II	Recruiting	Parallel Assignment (Randomized)	NCT03898895	PFS, OS, incidence of TEAEs
Nivolumab + RA Nivolumab + Ipilimumab + RA	PD-1 CTLA-4	II	Recruiting	Parallel Assignment (Randomized)	NCT02866383	CBR, incidence of TEAEs, ORR, PFS, OS

Abbreviations: CTLA-4, cytotoxic T lymphocyte-associated protein 4; DCR, disease control rate; DLT, dose-limiting toxicity; DOR, duration of response; GM-CSF, human granulocyte-macrophage colony-stimulating factor; HDAC, histone deacetylases; MEK, methyl ethyl ketone; mOS, median OS; mPFS, median PFS; ORR, objective response rate; OS, overall survival; PDGFR- β , platelet-derived growth factor receptor beta; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; PFS, progression-free survival; RP2D, recommended phase 2 dose; NCT, national clinical trial; MTD, maximum tolerated dose; CIK, cytokine-induced killer; TEAE, treatment emergent adverse event; CBR clinical benefit rate.

and good response durability for GemCis combined with durvalumab (D) \pm tremelimumab (T) in 121 first-line patients with advanced BTC, with an ORR of 70% and a DCR of 95%.¹⁰² The efficacy of GemCis combined with durvalumab is being further studied in the phase III TOPAZ-1 trial.

6.3.3 | Immunotherapy in combination with cancer vaccine

A phase I trial of 36 patients with ICC confirmed that adoptive T cell transfer combined with a dendritic cell vaccine may magnify tumor destruction. The mPFS and mOS were 18.3 and 31.9 months in patients receiving adjuvant immunotherapy and 7.7 and 17.4 months in patients undergoing surgery alone ($P = .005$ and $.022$, respectively).¹⁰³

7 | CONCLUSION

Given the heterogeneity of BTC, molecular characterization may represent the key to improving clinical outcomes through personalized therapy. Targeted therapies have shown remarkable results in patients with BTC harboring identified actionable mutations (such as FGFR2 fusions and IDH gene mutations) and have been successively approved as the standard treatment. Therefore, clinical research should focus on developing targeted therapies. Immunotherapy represents a promising approach for patients with BTC with MSI-H/dMMR disease. Nevertheless, identifying the characteristics of specific patients likely to benefit from each treatment remains a challenge that must be overcome to guide the precise treatment of BTC. Longitudinal liquid biopsies such as ctDNA analyses may aid in promoting the development of precision medicine and improve

therapeutic benefits in some patients. To date, chemotherapy remains the standard of care for advanced diseases. As we wait for new randomized clinical trials to shed light on the current dilemma, the possible options for patients with BTC should be discussed by a multidisciplinary team to ensure selection of the most appropriate treatment for each patient.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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How to cite this article: Zhang R, Puzzoni M, Mariani S, et al. Emerging treatment evolutions and integrated molecular characteristics of biliary tract cancers. *Cancer Sci*. 2021;112:4819-4833. doi:[10.1111/cas.15139](https://doi.org/10.1111/cas.15139)