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Review

The state of therapy modalities in clinic for biliary tract cancer

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Abstract

Biliary tract cancers (BTCs) include intrahepatic cholangiocarcinoma (iCCA), perihilar and distal cholangiocarcinoma (pCCA and dCCA), and gallbladder carcinoma based on the epithelial site of origin. BTCs are highly aggressive tumors associated with poor prognosis due to widespread metastasis and high recurrence. Surgery is the typical curative-intent treatment, yet the cornerstone of cure depends on the anatomical site of the primary tumor, and only a minority of patients (approximately 30%) has an indication necessitating surgery. Similarly, only a small subset of carefully selected patients with early iCCA who are not candidates for liver resection can opt for liver transplantation. Chemotherapy, target therapy, and immunotherapy are the main treatment options for patients who have advanced stage or unresectable disease. The genetic background of each cholangiocarcinoma subtype has been accurately described based on whole gene exome and transcriptome sequencing. Accordingly, precision medicine in targeted therapies has been identified to be aimed at distinct patient subgroups harboring unique molecular alterations. Immunotherapy such as immune checkpoint inhibitors (ICIs) was identified as antitumor responses in a minority of select patients. Current studies indicate that immunotherapy of adoptive cell therapy represents a promising approach in hematological and solid tumor malignancies, yet clinical trials are needed to validate its effectiveness in BTC. Herein, we review the progress of BTC treatment, stratified patients according to the anatomic subtypes of cholangiocarcinoma and the gene drivers of cholangiocarcinoma progression, and compare the efficacy and safety of chemotherapy, targeted therapy, and immunotherapy, which will be conducive to the design of individualized therapies.

Keywords: biliary tract cancer; surgery; chemotherapy; target therapy; immunotherapy

1. Introduction

Biliary tract cancers (BTCs) are highly invasive adenocarcinomas, including intrahepatic, perihilar, distal cholangiocarcinoma (based on anatomical location within the biliary tree), and gallbladder carcinoma. Intrahepatic cholangiocarcinoma (ICCA) is located proximal to the secondary bile duct in the liver parenchyma. Extrahepatic cholangiocarcinoma (eCCA) includes Perihilarcholangiocarcinoma (pCCA) and distalcholangiocarcinoma (dCCA). pCCA arising from the right and/or left hepatic duct and/or common hepatic duct is confined between the secondary bile duct and the cystic duct inserted into the common bile duct. dCCA is confined to the common bile duct, and the area between the origin of the cystic duct and the ampulla of Vater [1]. Approximately 50–60% of cholangiocarcinomas (CCAs) are pCCA, followed by dCCA (20–30%) and intrahepaticbile duct cancers (10–20%) [2]. CCA is more common among Hispanics and Asians (2.8 to 3.3 per 100,000) than among non-Hispanic whites and blacks (2.1 per 100,000). Males show a slight predominance in the incidence of BTCs over females (1.2 to 1.5 per 100,000 *vs*. 1 per 100,000), whereas the incidence rate of iCCA in Hispanic females is higher than that in Hispanic males (1.5 per 100,000 *vs*. 0.9 per 100,000) [3]. The iCCA age-standardized mortality rate increased from 2.15 per 100,000 persons in 2009 to 2.95 per 100,000 persons in 2018, with an annual growth rate of 3.5% (95% confidence interval (CI) 3.1–3.8%). Likewise, age-standardized mortality from eCCA increased from 0.28 per 100,000 persons in 2009 to 0.39 per 100,000 persons in 2018, with an annual increase of 3.2% (95% CI 1.7–4.8%). In contrast, gallbladder carcinoma related mortality declined over 10 years, from 1.0 per 100,000 in 2009 to 0.87 per 100,000 in 2018, with an annual growth rate of –1.6% (95% CI –2.1% to –1.1%). Compared with most malignant tumors, the survival rates of iCCA, eCCA, and gallbladder carcinoma were lower. The 1-year, 3-year and 5-year survival rates for gallbladder carcinoma were 44%, 24%, and 19%, respectively, followed by eCCA (40%, 15%, 10%) and iCCA (37%, 13%, 9%) [4]. Risk factors for BTCs include hepatolithiasis, cirrhosis, hepatitis B and C infection, primary sclerosing cholangitis, Caroli disease, liver fluke infection, and obesity-related liver diseases. Variations in geographic origin are partly related to risk factors for the incidence rate of BTC [5]. Meta-analysis showed that the regional distribution of hepatitis B and C was one of the strongest risk factors for BTCs, especially intrahepatic diseases. Many studies have shown that the incidence of BTCs is strongly related to hepatitis C in the USA and Europe, while hepatitis B as a risk factor is significantly related to the incidence of iCCA in

China and South Korea [6–8]. In some economically underdeveloped areas of South-East Asia, 113 per 100,000 people suffer from BTCs because of high rates of hepatobiliary flukes, *Opisthorchis viverrini* and *Clonorchissinensis* [9]. Hepatolithiasis is another risk factor for the high incidence of BTCs, especially iCCA, in Asian countries. Chronic biliary inflammation, often secondary to hepatolithiasis, would increase the risk of BTCs. Similar to hepatolithiasis, 70–90% of patients with gallbladder carcinoma have a history of chronic cholecystitis induced by gallbladder stones, which is one of the high-risk factors of gallbladder malignancy [10]. In addition, hepatobiliary fluke infection is more common in patients with hepatobiliary calculi [11]. Studies confirmed that the high incidence of primary sclerosing cholangitis in Western countries is strongly associated with BTCs, especially pCCA and gallbladder carcinoma [12,13]. Patients diagnosed with primary sclerosing cholangitis generally progress to BTCs within 24 months. Primary sclerosing cholangitis results in progenitor cell proliferation, and chronic inflammation with liver injury may be the pathogenic factor. Predisposing conditions causing biliary stasis and calculi are generally associated with choledochal cystic disorders, including Caroli's disease which usually develops to BTCs within a range of 7% to 14% [14].

It is difficult to diagnose early gallbladder tumor malignancy, which is found accidentally in the process of pathological examination after cholecystectomy. If gallbladder malignancy is suspected preoperatively, contrast-enhanced magnetic resonance imaging (MRI) is preferred to assess the mass in the gallbladder and characterize involvement of the bile duct. For the diagnosis of iCCA and eCCA, contrast enhanced multi temporal thin-layer MRI or computed tomography (CT) can be recommended to determine the location of bile duct tree, hepatic artery, and portal vein and their relationship with tumor [15,16]. In addition, contrast-enhanced MRI or MRCP can be employed to evaluate the degree of bile duct infiltration. In the case of bile duct dilatation, if non-mass-forming on CT or MRI, endoscopic retrograde cholangiopancreatography or endoscopic ultrasonography can be used for tissue biopsy and may provide an approach with which to relieve biliary obstruction. However, the high connective tissue proliferation of cholangiocarcinoma limits the accuracy of pathological and cytological methods, leading to high specificity but low sensitivity in the diagnosis of malignant bile duct stenosis. Cancer antigen 19-9 (CA19-9), also known as sialylated Lewis-A antigen, is a major serum biomarker for the diagnosis of CCA [17]. However, CA19-9 can be increased in other malignant tumors such as gastrointestinal malignant tumors, biliary obstruction, and benign diseases, but excluding Lewis antigen-negative patients, which arises due to a lack of specificity in the diagnosis of BTCs [18]. Therefore, there is an urgent need to develop new protocols to predict the diagnosis of BTC in the early and resectable stages and to obtain sufficient material for genomic analysis.

The peripheral blood of cancer patients may contain cellular components from primary or metastatic cancer, including circulating free DNA (cfDNA), exosomes containing nucleic acids, lipids, proteins, and even circulating tumor cells [19]. Kumari *et al*. [20] evaluated the role of cfDNA in the diagnosis of gallbladder carcinoma. cfDNA in gallbladder carcinoma groups was significantly higher than that in a cholecystitis group and among healthy subjects. Moreover, cfDNA was positively correlated with jaundice and TNM stage [20]. In addition, gene mutations of KRAS, NRAS, BRAF and PIK3CA in BTC tissue were consistent with those detected in plasma [21]. Mutations in FGFR2, KRAS, and TP53 have yet to be evaluated between tumor tissue and plasma in larger patient cohorts. Lastly, bile as another source of cfDNA, has received increasing attention. Studies suggest that the long cfDNA fragment contained in bile was highly consistent with the gene of tumor tissue [22]. On the basis of genetic concordance between plasma and tissue cfDNA, cfDNA analysis is helpful to detect tumor heterogeneity and find *de novo* point mutations in chemotherapy and targeted therapy resistance [23,24]. Furthermore, Lapitz *et al*. [25] isolated extracellular vesicles in blood and urine of patients with CCA, PSC, and ulcerative colitis and found significant differences in their RNA profiles. Interestingly, the RNA profiles of extracellular vesicles in blood and urine of CCA patients can reflect the transcription level of tumor tissue related genes, thus paving the way for potential targeted therapy.

After completing the disease-related examination, formulation of planned treatment should depend on the staging of the American Joint Committee on Cancer (AJCC) Cancer Staging Handbook, 8th Edition. Surgical resection is the cornerstone of curative therapy, whereas it is appropriate for early stage. Understanding of tumor stage (*e.g.*, oncogenic landscape, presence of distant metastases, vascular involvement), tumor biology, the identification of positive predictive biomarkers, and the molecular mechanisms of immunotherapy resistance, then formulating the optimal treatment plan may hold the best survival prospect. Herein, we review current state of treatment and summarize the recent clinical data on the efficacy of chemotherapy, targeted therapy, and immunotherapy for BTCs, and further propose the perspectives for future investigations.

2. Resection and transplantation

The objective of resection is to achieve radical clearance through negative margin (R0) resection when contraindications are precluded. Patients with early-stage disease are frequently asymptomatic, while it often progresses to the advanced stage after detection, and only 22% of patients with iCCA have surgical indications [26]. Resection is feasible in the absence of intrahepatic and distant metastasis, showing no major vessel invasion and suffi-

cient future liver remnant (FLR). Postresection liver failure that is strongly correlated with insufficient FLR is the most serious complication after hepatectomy. For patients without underlying disease (*e.g.*, cirrhosis, steatosis), 25% FLR volume is considered adequate, while it should increase to 40% or more with a compromised liver [27,28]. Since FLR volume often does not reflect FLR function, CT volume measurement should be supplemented with at least regional liver function examination. For correct interpretation of volumetry results, personalized adjustments should be made. Vauthey *et al*. [29] proposed an improved method for estimating total liver volume based on characteristics of Western populations: estimated total liver volume (eTLV; unit mL) = –794.41 + 1267.28 × body surface area. The threshold for safe hepatectomy using standard FLR volume was 20% in healthy liver parenchyma, 30% in chemotherapy-related liver injury, and 40% in chronic liver disease [30]. Notably, as advanced age is a strong independent risk factor for serious complications after hepatobiliary resection, FLR of patient age $\geq$69 should be greater than or equal to 45% [31]. Multi-center studies indicate that more than one-third of iCCA patients have lymph node metastasis, which is negatively correlated with patient prognosis (median survival: 24 *vs*. 30 months with or without lymph node metastasis) [32–34]. Therefore, the expert consensus in 2015 recommended routine regional lymphadenectomy for iCCA surgery due to the high incidence of lymph node metastasis, the potential possibility of locoregional recurrence, and potential benefits of lymph node dissection in predicting prognosis [35]. Patients with very-early iCCA (a single iCCA $\leq$20 mm across) or not suitable for liver resection (*e.g.*, due to cirrhosis) and without extrahepatic metastasis, lymph node spread, and vascular invasion are candidates for liver transplantation. The 5-year overall survival rate of liver transplantation was 65% and the 5-year recurrence rate was 18% [36]. In addition to the rarity of indications for very early iCCA, patients with locally advanced unresectable iCCA without positive lymph nodes, vascular infiltration, and distant metastasis should receive adjuvant therapy with capecitabine, gemcitabine, or both before liver transplantation. Surprisingly, the 5-year overall survival rate, recurrence rate, and median survival time were 83%, 50%, and 7.6 months, respectively, although a greater tumor burden was detected in the liver. These results exceed underwent liver resection or liver transplantation alone, in the absence of neoadjuvant treatments [37]. There is no evidence that immunotherapy can improve the prognosis as a bridging therapy before liver transplantation. However, a case reported an adolescent with advanced hepatocellular carcinoma (HCC) who was treated with pembrolizumab, a PD-1 inhibitor, and subsequently underwent successful liver transplantation and received no recurrence and allograft rejection 4 years post-liver-transplantation [38]. This case provides a basis for potential benefit from the application of immunotherapy as the bridge to liver transplant to improve outcomes in BTC patients.

The Bismuth-Corlette classification stratified the pCCA according to tumor invasion along the biliary tract as follows, Type I: tumor only involves the common hepatic duct below the confluence of the left and right hepatic ducts; Type II: tumor reaches the confluence, but there is no invasion of right and left hepatic ducts; Type IIIa and IIIb: tumor obstructs the right and the left hepatic ducts in addition to the common hepatic duct; Type IV: tumor involves the common and left and right hepatic ducts [39]. Whether major hepatectomy improves the outcome of patients with Type I and Type II pCCA is controversial. Several reports showed that major hepatectomy improved survival and bile duct excision alone associated with poor outcome [40,41]. However, another study showed no significant difference in survival between the two surgical methods [42]. Further prospective studies are needed to assess the effects of hepatectomy on Type I and Type II pCCA. The caudate lobe duct joins the confluence of the left and right hepatic ducts, but mainly drains to the left hepatic duct. Retrospective studies demonstrated an improvement in 5-year survival in patients who underwent concurrent caudate lobectomy [43,44], while another study indicated no improvement [45]. It is evident that caudate lobectomy reduces the possibility of margin-positive, thus decreasing local recurrence [46]. Similar to iCCA, lymph node dissection can only predict the prognosis of patients. The 5-year survival rates were 30%, 15%, and 12% for patients with no lymph node metastasis, regional lymph node positivity, and para-aortic lymph node positivity, respectively [47]. Liver transplantation is superior to hepatectomy in achieving R0 resection and avoiding post-operative liver failure, especially involving portal vein invasion and substantive diseases (*e.g.*, cirrhosis, steatosis) in patients with pCCA. Patient candidates for liver transplantation should meet following criteria: (1) locally advanced unresectable tumor, positive biopsy, or radiographically malignant stenosis with CA19-9 $\geq$100, and no extrahepatic metastasis, including regional lymph node involvement; (2) primary sclerosing cholangitis with resectable disease; and (3) no contraindications for liver transplantation. In addition, the selected patients should receive chemotherapy or chemotherapy and external radiation therapy up to transplantation according to the Mayo Clinic protocol [48]. Three-year and five-year survival were improved among patients who received liver transplantation compared with resection (72% *vs*. 33%), (64% *vs*. 18%). Similarly, in patients with early pCCA (tumors $<$30 mm across without lymph-node metastases) liver transplantation also showed better survival rate than resection (3-year: 54% *vs*. 44%; 5-year: 54% *vs*. 29%) [49].

dCCA can infiltrate the head of the pancreas and cause connective tissue hyperplasia, which is often indistinguishable from pancreatic head adenocarcinoma and often requires postoperative pathological diagnosis. The operation of dCCA involves pancreaticoduodenectomy and regional

lymphadenectomy: the key to R0 resection is detailed dissection of the superior mesenteric artery and perivenous tissue. Margin status, lymph node status, perineural invasion, lymphovascular invasion, pancreatic invasion, tumor invasion depth, tumor size (< or >20 mm), and degree of differentiation were important factors affecting prognosis. Five-year overall survival rates for patients with R0 or R1/R2 resection were 60% or 8%, respectively, and those with lymph node negative or positive were 46% or 18%, respectively [2,50–52].

Gallbladder carcinoma is often found incidentally after cholecystectomy. The AJCC 8th Edition classifies tumor stages based on depth of tumor invasion (T), lymph node spread (N), and metastasis (M). For incidentally discovered gallbladder carcinoma, T stage often decides whether to re-resection if distant metastasis is excluded. T1a stage is confined to the lamina propria; T1b stage penetrates the submucosa but does not invade the entire gallbladder wall; T2 invades the entire gallbladder wall, T2a invades on the peritoneal side of the gallbladder, and T2b involves the hepatic side of the gallbladder. T3 stage breaks through the serosa of gallbladder and enters the liver or nearby organs. Patients atT1a stage are treated with cholecystectomy without further treatment, while re-resection should be performed provided there are no contraindications at T1b, T2, and T3 stages. The scope of operation includes hepatic segments IVB and V, portal lymphadenectomy [53]. For patients at T2 stage, median overall survival (mOS) improved from 12.4 to 44.1 months after re-resection. Similarly, T3 stage extended from 9.7 to 23.0 months after re-resection [54]. However, there was no evidence that re-resection could significantly improve outcome in T1b stage, although this subgroup was small. Larger sample studies are needed to determine whether re-resection can improve the prognosis of T1b stage patients.

3. Chemotherapy and radiotherapy

The ABC-02 trial of 410 patients confirmed cisplatin plus gemcitabine as first-line chemotherapy for patients with locally advanced or metastatic cholangiocarcinoma and gallbladder carcinoma. Compared with gemcitabine alone, gemcitabine combined with cisplatin improved median progression free survival (mPFS) (8.0 months *vs*. 5.0 months; $p < 0.001$) and mOS (11.7 months *vs*. 8.1 months; HR 0.64, 95% CI 0.52–0.80; $p < 0.001$) without increasing AEs [55]. Subsequent phase II BT22 trial [56] and meta-analysis [57] reported similar conclusion to ABC-02 trial. In a Phase-2 trial, atriple chemotherapy regimen consisting of cisplatin, gemcitabine and nab-Paclitaxel showed a magnitude of benefit compared to cisplatin plus gemcitabine. The mPFS of triple chemotherapy regimen was 11.8 months (95% CI, 6.0–15.6) and mOS was 19.2 months (95% CI, 13.2 months to not estimable) [58]. Furthermore, The mOS of triple chemotherapy regimen with gemcitabine, cisplatin, and S-1 (GCS) was higher than that of gemcitabine and cisplatin (13.5 months *vs*. 12.6 months hazard ratio 0.79, 95% CI 0.60–1.04; $p = 0.046$) in a Japanese Phase-III trial, KHBO1401-Mitsuba. The mPFS was 7.4 months in the GCS group and 5.5 months in the gemcitabine and cisplatin groups, respectively (hazard ratio 0.75, 95% CI 0.58–0.97; $p = 0.0015$) [59]. Based on these results, standard chemotherapy for patients with advanced BTCs may be replaced by triple-agent therapies.

Adjuvant therapy after radical resection of BTCs includes chemotherapy, radiotherapy, and combinations of radiotherapy and chemotherapy. In BTCs, the study of adjuvant therapy first began after cholecystectomy of gallbladder carcinoma. This study implied that mitomycin and 5-fluorouracil could improve the OS time and PFS compared with placebo [60]. A meta-analysis involving 6712 patients verified the benefit of adjuvant chemotherapy in patients with R1 resection and lymph node positivity [61]. Compared with the observation group, an experimental group with patients subjected to eight cycles of capecitabine exhibited statistical significantly efficacy in the mOS, could be corrected for prognostic factors (51.1 months *vs*. 36.4 months) [62]. However, a randomized Phase-III trial (thePRODIGE12-ACCORD18-study) demonstrated no difference in prognosis between the GEMOX scheme (Gemcitabine/oxaliplatin) and observation scheme after R0 or R1 resection of BTCs [63]. Based on these data, the ACTICCA-1 study is ongoing with capecitabine as the control group [64]. Further Phase-III trials are needed to confirm capecitabine as the new standard for adjuvant chemotherapy after curative resection of BTCs. Other trials evaluating first-line chemotherapy agents in patients with advanced BTCs are summarized (Table 1, Ref. [55,56,65–73]).

With the exploration of animal models and the improvement of technology, radiotherapy has become a safe and efficacious treatment for advanced BTCs. The local control rate of conventionally fractionated radiotherapy, stereotactic body radiation therapy and intensity-modulated radiation therapy was found to be 45–100%, and the 1-year survival rate was 58–81% [74]. Cynomolgus monkeys administered with total parenteral nutrition containing 25% dextrose after high-dose liver directed radiotherapy ($\geq$36 Gy) developed liver failure, while dextrose $\leq$10% did not result in abnormal liver function [75]. On the basis of animal models, whole liver irradiation had been restricted to the standard dose range of 1.8 to 2.0 Gy per day with the total dose 30 to 35 Gy, because patients were at potential risk of fatal radiation liver disease when these dosages were exceeded. Surprisingly, with the development of individualized dosing strategies based on mean liver dose and the progress of modern radiotherapy technology, the tumor-free liver tissue might receive less radiation, thereby reducing the risk of liver function deterioration. In a retrospective dose response analysis, 79 iCCA patients received 3-d conformal intensity-modulated radiotherapy with passive scat-

Table 1. Phase-II or III clinical trials evaluate the first-line setting of chemotherapy in BTCs.

Authors (year of publication)	Phase	Number of patients	Experimentalarm	Controlarm	mOS (months)	mPFS (months)
Andréet *al*. (2004) [65]	II	26	Gemcitabine plus oxaliplatin	Observation	15.4 *vs*. 7.6	5.7 *vs*. 3.9
Sharma *et al*. (2010) [66]	IIR	88	Gemcitabine plus oxaliplatin	5-FU/supportive care	9.5 *vs*. 4.6/4.5	8.5 *vs*. 3.5/2.8
Okusakaet *al*. (2010) [56]	IIR	83	Gemcitabine plus cisplatin	Gemcitabine	11.2 *vs*. 7.7	5.8 *vs*. 3.7
Valle *et al*. (2010) [55]	III	410	Gemcitabine plus cisplatin	Gemcitabine	59.3 *vs*. 42.5	11.7 *vs*. 8.1
Phelipet *al*. (2014) [67]	IIR	34	RT plus 5-FU/cisplatin	Gemcitabine plus oxaliplatin	13.5 *vs*. 19.9	5.8 *vs*. 11.0
Zheng *et al*. (2018) [68]	II	60	Capecitabine plus irinotecan	Irinotecan	10.1 *vs*. 7.3	3.7 *vs*. 2.4
FUGA-BT/Ueno *et al*. (2018) [69]	III	354	gemcitabine plus cisplatin	Gemcitabine plus S-1	13.4 *vs*. 15.1	5.8 *vs*. 6.8
Sahai *et al*. (2018) [70]	II	74	Gemcitabine plus nab-paclitaxel	Observation	12.4	7.7
Sakai *et al*. (2018) [71]	III	246	Gemcitabine plus cisplatin and S-1	Gemcitabine plus cisplatin	13.5 *vs*. 12.6	7.4 *vs*. 5.5
Kim *et al*. (2019) [72]	III	222	Capecitabine plus oxaliplatin	Gemcitabine plus oxaliplatin	10.6 *vs*. 10.4	5.8 *vs*. 5.3
Shroff *et al*. (2019) [73]	II	60	Gemcitabine plus cisplatin and nab-paclitaxel	Observation	>20	11.4

ter proton beam techniques or 6-MV photon beams with a median dose of 58.08 Gy (35–100 Gy), and no radiation-induced liver disease occurred [76]. Further randomized trials are necessary to ascertain the positive effect of radiotherapy on localized, unresectable BTC.

4. Targeted therapies

In order to reveal substantial molecular heterogeneity across BTC subtypes, the genetic profiling studies delineated the genetic background of each anatomic subtypes of BTC [77,78]. Isocitrate dehydrogenase-1/2 (IDH1/2), fibroblast growth factor receptor (FGFR) 2, epoxide hydrolase (EPH)A2, and biofilm-associated surface protein (BAP)1 gene mutations have been reported almost exclusively in iCCA, while KRAS proto-oncogene (KRAS), receptor tyrosine-protein kinase erbB-2 (ERBB2), AT-rich interactive domain (ARID)1B, protein polybromo-1 (PBRM1), and E74-like factor (ELF)3 mutations occur frequently in eCCA and gallbladder carcinoma [79–81]. Compounds are tailored to target the key oncogenic drivers which result from translocation, substitution, deletion, or insertion mutations. Below we discuss the most common mutations with targeted therapeutic significance in BTCs.

A retrospective study of 5393 patients with cholangiocarcinoma showed that the functional mutation in the coding region of IDH occurred in about 13% of iCCA, and there were few reports of eCCA [82]. IDH2 mutations are less common than IDH1. IDH mutations lead to increases in IDH1/2 activity, resulting in the changes in cell metabolism and the accumulation of tumor metabolite 2-hydroxyglutaric acid (2-Hg) via NADPH-dependent reduction. The 2-HG blocks normal cell differentiation and promotes tumorigenesis by affecting chromatin structure and DNA methylation [83,84]. Inhibitors of IDH1 (ivosidenib) and IDH2 (enasidenib) are currently being evaluated in patients with iCCA. Ivosidenib is approved for the treatment of patients with acute myeloid leukemia with IDH1 mutations. A Phase-I clinical trial was performed for the first time in 73 patients with IDH1 mutant CCA. Median PFS was 3.8 months (95% CI 3.6–7.3), and mOS was 13.8 months (95% CI 11.1–29.3), and the partial response (PR) rate was 5%. The observed drug-related AEs included all degrees of loss of appetite, vomiting, abdominal pain, diarrhoea, nausea, and fatigue with incidences of 23%, 27%, 27%, 32%, 34%, and 42%, respectively [85]. 500 mg was selected as the recommended dose in this study, as the maximum tolerated dose was not reached and there was no dose-limiting toxicity. A subsequent Phase-3 randomized trial included 185 CCA patients with IDH1 mutations, whose disease progressed after one or two lines of systemic therapy. The ratio with ivosidenib was 2:1500 mg once daily or matched with placebo (NCT03173248) [86]. Patients who received placebo were allowed to cross-over to Ivosidenib after radiographic progression. Compared with placebo, Ivosidenib significantly increased mPFS (2.7 months *vs*. 1.4 months). 32% (95% CI 23–42) of patients received Ivosidenib had no progression at 6 months, 22% (95% CI 13–32) had no progression at 12 months, and none in the placebo group achieved non-progression at 6 months. However, there was no significant difference in mOS between

the ivosidenib and placebo groups (10.8 months *vs*. 9.7 months; HR 0.69, unilateral $p = 0.06$). More clinical trials are needed to confirm the efficacy of Ivosidenib in patients with IDH1 mutant cholangiocarcinoma. Enasidenib is highly selective against the mutant IDH2 [87]. A Phase-I/II, multi-center, dose-escalation clinical trial involves advanced IDH2-mutated solid tumors including iCCA is being assessed (NCT02273739).

The chromosomes fused by FGFR2 exons 1 to 17 encode complete extracellular and kinase domains that fuse within the framework to a 3' partner has a protein dimeric domain [88]. Genomic analysis showed that FGFR2 alterations are implicated in approximately 20% of iCCAs [89]. Several inhibitors of FGFR isoforms 1–3, including ATP-competitive, reversible inhibitors (infigratinib, derazantinib, pemigatinib, and erdaftinib) and a non-ATP competitive, covalent inhibitor futibatinib have shown activity in advanced cholangiocarcinoma harboring FGFR genetic aberrations. Infigratinib (BGJ398) is a pan-FGFR tyrosine kinase inhibitors (TKIs) preliminarily assessed in a Phase-I clinical trial involving three patients with cholangiocarcinoma with FGFR2 abnormalities (two FGFR2 fusions and one FGFR2 genetic mutation). All the three patients had stable disease with tumor burden reduction [90]. A subsequent Phase-II trial enrolled 61 patients with gemcitabine-resistant FGFR-fused, mutated, or amplified cholangiocarcinoma who received infigratinib. The overall response rate of FGFR2 fusion patients was 19% and the disease control rate (DCR) was 83%. The tumor burden of the patients with FGFR2 mutation and amplification was reduced by 23% and 27%, respectively. Common AEs include fatigue, hyperphosphatemia, alopecia, stomatitis, and palmar-plantar syndrome [91]. Another Phase-IItrial of infgratinib involving 71 patients with FGFR2 fusions showed the partial response rate and stable disease rate were 25% and 58%, with mPFS of 7 months and overall survival of 12 months [92].

Pemigatinib (INCB054828), is a highly selective FGFR-1, 2, and 3 TKI preliminarily assessed in basket trial that reported partial response in one cholangiocarcinoma patient with FGFR2-CCDC6 fusion but no other cholangiocarcinoma patients with FGFR genetic aberrations. A multi-center, open-label, single-arm, multi-cohort, Phase-II study (FIGHT-202) enrolled 146 previously treated metastatic or locally advanced cholangiocarcinoma patients with or without FGFR genetic aberrations. All enrolled patients received 13.5 mg pemigatinib orally once daily until tumor progression, unacceptable toxicity, physician decision, or patient consent withdrawal. Objective response rates (ORRs) were achieved in 35.5% of FGFR2 fusion or re-arrangement patients, with mPFS at 6.9 months and mOS at 21.1 months. In contrast, mPFS in patients with and without other FGFR2 alterations were 2.1 and 1.7 months, respectively (the mOS were 6.7 months and 4.0 months in the same groups) [93,94]. AEs patients suffered from included hypophosphataemia, arthralgia, stomatitis, hyponatraemia, abdominal pain, fatigue, abdominal pain, pyrexia, cholangitis, and pleural effusion. Subsequently, a global, randomized, active-controlled, multi-center Phase-III study named FIGHT-302 was designed to compare the safety and efficacy of pemigatinib with gemcitabine plus cisplatin in patients with advanced cholangiocarcinoma with FGFR2 gene rearrangements [95]. The mPFS, mOS, ORRs, and AEs are yet to be evaluated.

Derazantinib (ARQ087), another pan-FGFR inhibitor, was preliminarily assessed in a Phase-I/II open-label study (ARQ087-101) in patients with advanced cholangiocarcinoma with FGFR2 gene fusion [96]. The study enrolled 29 patients including two without therapy and 27 who experienced disease progression after at least one systemic treatment. Derazantinib showed promising anti-tumor activity with mPFS of 5.7 months. ORRs were achieved in 20.7% patients with a median duration of response (DR) of 4.6 months, and 82.8% patients took DCR with median DR of 5.8 months. 72.4% patients suffered from AEs of Grade $\leq$2, included asthenia or fatigue, hyperphosphatemia, and eye toxicity. These promising results subsequently led to a pivotal trial (NCT03230318) of derazantinib in iCCA patients with FGFR2 gene fusion.

In a Phase-I trial (NCT01703481), oral pan-FGFR TKI erdaftinib (JNJ-42756493) showed preliminary clinical activity in cholangiocarcinoma with FGFR mutation or fusion, indicating ORRs of 27.3% and median DR of 11.4 months [97]. The common AEs were hyperphosphatemia, followed by skin, nail, and eye changes. Most AEs were reversible after temporary doing interruption. A Phase-IIa trial (NCT02699606) of erdafitinib in Asian cholangiocarcinoma patients is ongoing.

Futibatinib (TAS-120) is a highly selective pan-FGFR inhibitor that inhibits FGFR mutants resistant to ATP competition inhibitors. The first Phase-I dose-escalation trial (NCT02052778) enrolled patients with advanced solid tumors harboring FGFR aberrations, including three iCCA patients. This trial observed partial responses in three-FGFR2 fusion iCCA patients. According to results of NCT02052778, a single-arm multi-center Phase-II trial (FoeniX-CCA2) enrolled iCCA patients with FGFR2 gene fusion or other re-arrangements who progressed after at least one line of systemic treatment. Among the 67 patients who received futibatinib, the complete response rate was 1.5% and the partial response rate was 35.8%. mPFS of 7.2 months was observed after a median follow-up of 11.4 months [98]. Similar to other FGFR inhibitors, toxicities frequently reported include hyperphosphatemia, dry mouth, diarrhoea, dry skin, and hair loss. Its promising efficacy and adequate safety resulted in a Phase-III study (the FOENIX-CCA3 trial, NCT04093362) being planned to compare the efficacy of futibatinib to cisplatin and gemcitabine as first-line treatment in patients with advanced or metastatic iCCA with FGFR2 gene re-arrangement.

The activation mutation of proto-oncogene KRAS is

Table 2. Phase-II trials evaluating molecularly targeted monotherapy or combination therapy in BTCs.

Authors (year of publication)	Targets	Number of patients	Treatment	ORR (%)	Median OS (months)	Median PFS (months)
Philip PA *et al.* (2006) [118]	EGFR	42	Erlotinib	8	7.5	2.6
Ramanathan RK *et al.* (2009) [119]	EGFR, HER2	17	Lapatinib	-	5.2	1.8
Bengala C *et al.* (2010) [120]	VEGFR, RAF	46	Sorafenib	2	4.4	2.3
Lubner SJ *et al.* (2010) [121]	EGFR, VEGF	53	Erlotinib plus bevacizumab	12	9.9	4.4
Bekaii-Saab T *et al.* (2011) [122]	MEK1/2	28	Selumetinib	12	9.8	3.7
El-Khoueiry AB *et al.* (2012) [123]	VEGFR, RAF	31	Sorafenib	-	9.0	3.0
El-Khoueiry AB *et al.* (2014) [124]	EGFR, VEGR, RAF	34	Erlotinib plus sorafenib	6	6.0	2.0
Denlinger CS *et al.* (2014) [125]	Proteasome	20	Bortezomib	-	9.0	5.8
Javle MM *et al.* (2016) [126]	FGFR	26	BGJ398	14	On-going	On-going
Subbiah IM *et al.* (2013) [127]	IDH1	25	AG-120	4	On-going	On-going

common in cholangiocarcinoma, and its incidence is 10%–60% [99]. KRAS activation up-regulates the RAS-MAPK pathway via downstream pathways, including the BRAF-MEK-ERK pathway [100]. BRAF mutations are reported in about 5–7% of cases of BTC [101]. Compared with wild-type patients, iCCA patients with BRAF-V600 mutations had higher TNM stage and poorer long-term overall survival [102]. Accordingly, BRAF or MEK inhibition may be amenable to KRAS mutant cholangiocarcinomas. In addition, activation of KRAS mutation-related signaling pathways was significantly associated with FGFR2 fusion, suggesting that it may play a synergistic role in driving iCCA pathogenesis [103]. A randomized, double-blind, Phase-II trial in metastatic or unresectable cholangiocarcinoma patients following failure of gemcitabine plus platinum-based treatment demonstrated that BRAF inhibitor, regorafenib, significantly improved PFS and tumor control rate. Confirmed stable disease rates were 74%, with mPFS of 3.0 months [104]. The common AEs were hypophosphatemia, hyperbilirubinemia, hypertension, and hand-foot skin reaction. In consideration of the modest antitumor activity of monotherapy with a BRAF inhibitor in BRAF-V600-mutated cholangiocarcinoma, the researchers conducted a continuing phase II, open-label, single-arm, multi-centerevaluation of combination therapy for another BRAF inhibitor (darafenib) and MEK inhibitor (trimeitinib) [105]. Data show that combination treatment of darafafenib + trametinib in patients with cholangiocarcinoma after disease progression on gemcitabine-based chemotherapy achieved 9.2 months of mPFS and 11.7 months of mOS with 36% of patients occurring partial responses. The common AEs included fever, rash, and nausea.

A comprehensive molecular analysis identified a class of proliferative iCCAs characterized by the activation of EGFR signaling [106]. EGFR signaling plays important role in tumorigenesis [107]. However, EGFR inhibitors (erlotinib, cetuximab, and panitumumab) showed no advantage in overall survival in comparison with gemcitabine and platinum based treatment in randomized controlled trials [108,109]. Alterations of the receptor tyrosine protein kinase ERBB2, a member of the EGFR family, play a tumorigenic role in cholangiocarcinoma and gallbladder carcinoma by promoting the proliferation and survival of cancer cells through downstream pathways such as MAPK-ERK or PI3k-AKT-mTOR [110,111]. Gallbladder carcinoma, eCCA, and iCCA with ERBB overexpression or gene amplification accounted for 19%, 17%, and 4.8% respectively [112,113]. A small gallbladder carcinoma cohort (n = 9) treated with trastuzumab, lapatinib, or pertuzumab resulted in clinical activity, with three showing disease stability, fourshowing a partial response, and one showing a complete response. Despite a high proportion of ERBB mutations in cholangiocarcinoma in this trial, no response could be seen [114]. Prospective studies in selected populations are needed in the future to evaluate the efficacy and safety of ERBB2-targeted therapy as a single agent or combination therapy for patients with ERBB2-activated BTC. BRCA mutations were detected in approximately 3.6% of the samples of patients with BTC (BRCA1: 0.6%, BRCA2: 3%), and there was no significant difference between different tumor sites [115]. BRCA1/2 mutations will accumulate DNA double strand breaks, leading to genomic instability and increased susceptibility to malignant transformation [116]. BRCA-mutated tumors confer sensitivity to poly [ADPribose] polymerase (PARP) inhibition. A multicenter retrospective analysis showed that four cholangiocarcinoma patients bearing BRCA-mutations treated with PARP inhibition resulted in the superior mOS, ranging from 11.01 to 64.78 months [117]. Phase-II trials in large populations are required to evaluate the efficacy and safety of PARP inhibitors against BRCA mutated BTC. Other Phase-II trials evaluating molecularly-targeted monotherapy or combination therapy in BTCs are demonstrated in Table 2 [118–127].

5. Immunotherapy

The immune system, regulated by a complex system of immune checkpoint proteins, has the capability of

identifying and destroying aberrant cells. In recent years, ICIs, including programmed cell death protein-1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), have been detected to inhibit antitumor immune responses in solid tumors with a low rate of immune-mediated AEs [128,129]. Although ICIs, combination targeted therapies, and novel adoptive cell therapies have shown efficacy in many cancers, the response rate, refined treatment selection, and safety of immunotherapy for BTC remain to be established. BTC, as a highly heterogeneous tumor caused by tumor gene aberration, may be related to the expression of neoantigen. The biochemical milieu of immunosuppression is generated by the tumor microenvironment.

Tumor antigenicity due to certain mutations leads to abnormal expression of tumor proteins through the major histocompatibility complex [130]. During normal DNA replication, the proficient DNA mismatch repair (pMMR) pathway is responsible for detecting and correcting small DNA mismatch mismatches. The quantitative or qualitative abnormalities of key proteins *MLH1*, *MSH2*, *MSH6*, and *PMS2* lead to the deletion of the DNA MMR pathway, the accelerated accumulation of genetic errors on microsatellites, and diffuse high-level microsatellite instability (MSI-H), resulting in increased tumor-associated antigens expression [131,132]. Previous studies indicated that 1 to 10% of CCAs had MMR deficiency [132–134]. In KEYNOTE-158 (Phase II) and KEYNOTE-028 (Phase Ib), a small amount of BTC patients who failed the standard treatment regimen were enrolled and received pembrolizumab (an ICI that inhibits PD-1). In KEYNOTE-158, without stratification analysis of MMR status, the mOS and mPFS of patients were 7.4 months and 2.0 months, respectively, with 5.8% of ORR. In KEYNOTE-028, ORR was 13.0%, while mOS and mPFS were similar to KEYNOTE-158 with 5.7 and 1.8 months [135]. In contrast, the outcomes for BTC patients with MSI-H/dMMR showed a significant improvement in the KEYNOTE-158 study. Patients with MSI-H/dMMR achieved a higher mOS and mPFS of 24.3 and 4.2 months, respectively, with 40.9% of ORRs [136]. However, in a Phase-II study of Nivolumab (an ICI that inhibits PD-1), all respondents were microsatellite stable (MSS) with mOS and mPFS of 14.24 and 3.68 months, respectively [137]. Tumor mutation burden (TMB) is another biomarker which is related to immunotherapeutic response [138]. High TMB was defined as more than 10 mutations per Mb ($\geq$10 Mut/Mb). The key to the immunotherapy activity of checkpoint inhibitors is the recognition by ICIs of the neoantigens produced by increased TMB, leading to lymphocyte infiltration in tumors [139,140]. Based on a genomic study with 1502 BTC patients, the proportion of TMB-H tumors was found to be different in distinct primary sites, with 3.5% (7/198), 2% (1/50), and 5.8% (6/104) of iCCA, eCCA, and GBC [141]. A recent study published by Hongsik Kim and colleagues revealed significant differences in ORR (60.0% *vs*. 11.1%) and mPFS (7.4 *vs*. 2.2 months) with ICIs between patients with and without TMB-H [142]. In addition, survival analysis indicated that TMB was significantly associated with poor prognosis of iCCA [143]. Prospective research with greater populations should be enrolled to validate the TMB in predicting the response to ICIs and prognosis in BTC patients. The expression of PD-L1 is associated with ICI responses in several solid tumors, including non-small cell lung cancer and gastric cancer [144,145]. PD-L1 positive expression was categorized according to the proportion of tumor cells expressing PD-L1, and a threshold of 1% was positive ($\geq$1%). According to previous reports, immunostaining with monoclonal antibodies (mAbs) detected PD-L1 between 30% to 53% of BTCs [146,147]. Whether the expression level of PD-L1is related to prognosis and ICI response remains controversial. Results from the KEYNOTE-028 and KEYNOTE-158 basket studies indicated that PD-L1 status was not correlated with outcomes and ORRs [135]. Surprisingly, a nivolumab-related Phase-II study showed a statistically significantly superior mPFS in PD-L1-postive BTCs, with an objective response rate (ORR) of 50% compared to negative group. Clinically superior mOS was observed in PD-L1-positive patients, but showed no statistical significance [137]. Overall, the putative role of PD-L1 expression level in predicting the ICI response and outcome in BTC remains unclear and additional results from multiple studies are needed.

The tumor immune microenvironment could modify and modulate a state of immune tolerance in part by tumor-associated macrophages called Kupffer cells and myeloid-derived suppressor cells in BTCs [148–150]. Tumor microenvironments exhaust T cells by up-regulating immune checkpoints such as PD-1, and CTLA-4 expressed by Kupffer cells and dendritic cells [151]. In tumor microenvironments, CD8+ T cell density and immune checkpoint expression could affect responsiveness to ICIs. According to the density of CD8+ T cells and expression of immune checkpoint molecules, BTCs could be divided into immune ‘hot’ and ‘cold’ tumors. As regards the former, higher CD8+ T cell density and expression of enhanced immune checkpoint molecules lead to superior response rates to ICIs. Conversely, subgroups without T-cell infiltrated tumor microenvironment and low expression of immune checkpoint molecules can be classified as immune ‘cold’ tumors which are associated with sub-optimal response rates to ICIs [152]. Collectively, the heterogeneity of BTCs and tumor microenvironment result in differential responses to ICIs. Whole-exome sequencing of tumor and subgroup analyses of tumor microenvironment is essential when assessing response rates to ICIs.

Pembrolizumab as a highly selective, humanized monoclonal PD-1 inhibitor has been approved by the U.S. Food and Drug Administration for high TMB ($\geq$10 Mut/Mb) non-colorectal malignancies [153]. With regard

to BTCs, data from the pembrolizumab-related Phase-Ib KEYNOTE-028 and the Phase-II KEYNOTE-158 trials have been mentioned above [135,136]. Data showed a safe profile for pembrolizumab with infusion reactions record and few Grade 4–5 immune-mediated adverse events. 8% patients presented immune-mediated hypothyroidism in both trials; 6% patients presented immune-mediated pneumonitis in KEYNOTE-158.

Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody which can bind to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2. Based on the results of the CheckMate-040 trial, nivolumab is approved for use in hepatobiliary cancers. A single-group, multi-center nivolumab-related Phase-II study (NCT02829918) with 45 patients indicated that 27 patients achieved stable disease and a partial response rate was 22.2%. Among the intention-to-treat population, median PFS was 3.68 months (95% CI, 2.30–5.69 months) and mOS was 14.24 months (95% CI, 5.98 months to not reached) [137]. In an open-label, single-arm, Phase-II trial, patients with unresectable or metastatic BTCs received a regimen of nivolumab in combination with gemcitabine and cisplatin. The mPFS and mOS were 6.1 and 8.5 months, respectively, with 55.6% of ORR [154]. Furthermore, regimen of nivolumab alone or in combination with gemcitabine and cisplatin provides a controlled safety profile for patients with advanced BTCs. The most common nivolumab-related Grade 3 or 4 AEs were hyponatremia (3 of 54 [5.6%]) and increased alkaline phosphatase (2 of 54 [3.7%]). For combinations with gemcitabine and cisplatin, the most common Grade 3 or worse AEs were thrombocytopenia (18 of 32 [56.3%]) and neutropenia (7 of 32 [21.9%]). Other AEs included hypertension, elevated lipase and immune-related elevated aspartate aminotransferase or alanine aminotransferase, rash, diarrhea, and pruritus.

Durvalumabis a human IgG1 monoclonal antibody that selectively binds to Programmed cell-death 1 ligand 1 (PD-L1) [155]. Based on the results observed in urothelial carcinoma and non-small cell lung cancer, durvalumab is currently under investigation in advanced BTCs [156]. Tremelimumab is a human monoclonal IgG2 antibody targeting CTLA-4, a co-inhibitory receptor that represses effector T-cell activity in tumor [157]. Preliminary results indicated that mOS values for durvalumab and durvalumab + tremelimumab were 8.1 (95% CI 5.6–10.1) months and 10.1 (95% CI 6.2–11.4) months, respectively. Treatment-related AEs (trAE) of any grade occurred in 64% and 82% of patients in the durvalumab and durvalumab plus tremelimumab cohorts: incidence of grade $\geq$3 trAEs was19% and 23%, respectively. In addition, a randomized Phase-II trial (IMMUNOBIL PRODIGE 57) relates to combinational strategies with durvalumab plus tremelimumab or triple combinations of durvalumab, tremelimumab, and chemotherapy are on-going [158]. Published clinical trials involved immunotherapy alone or combination therapies in BTCs are listed in Table 3.

Immune related adverse events (irAEs) are defined as tissue damage induced by the interruption of immune tolerance to autoantigens. Various organs may be affected by irAEs, and the sites include skin (mainly rash and pruritus), endocrine organs (hypothyroidism), gastrointestinal tract (diarrhea), liver (liver dysfunction and jaundice), and lung (pneumonia). Immune-mediated hepatitis occurs in 3–9% of patients treated with CTLA4 inhibitors (ipilimumab) and 1–4% of patients with PD-1 inhibitors (nivolumab) [159,160]. Although hepatic irAEs and autoimmune hepatitis demonstrate some common characteristics, increasing evidence suggests that the two are histologically and immunologically distinct. Immunostaining showed the presence of many CD3+ and CD8+ lymphocytes in checkpoint-inhibitor-induced hepatic irAEs, while CD20+ B cells and CD4+ T cells were significantly less than those in autoimmune hepatitis [161]. Similar to common drug-induced liver injury, grading hepatic irAEs are based on the Common Terminology Criteria for Adverse Events. Management recommendations of hepatic irAEs are referred to a colitis model (Table 4) [162–164].

In some tumor immune microenvironments of BTCs, immunosuppressive cells such as tumor-associated macrophages, tolerant dendritic cells, and myeloid-derived inhibitory cells predominate. To overcome the harsh tumor microenvironment, adoptive cell therapy (ACT) was attempted by transplanting *in vitro* amplified tumor-responsive T cells into patients. Some cases successfully describe the application of adoptive cell therapy to BTCs. A single case study of an iCCA patient with lymph node metastasis and portal vein invasion treated with surgery and subsequently underwent immunotherapy with CD3-activated T cells and tumor peptide or lysate-pulsed dendritic cells. Surprisingly, the patient had no sign of recurrence for three years and six months since undergoing surgery [165]. In another case, a 43-year-old patient extensively metastatic cholangiocarcinoma first received adoptive cell therapy containing CD4+ ERBB2 interacting protein mutation-reactive T cells. After this therapy, lung and liver tumors continued to shrink, reaching a maximum reduction of 30% at 7 months. After approximately 13 months of disease stabilization, only lung lesions progressed. Subsequently, the patient received adoptive transfer of >95% of the mutation–reactive T helper 1 cells, resulting in tumor regression [166]. Based on the early encouraging results, a case-control adjuvant study was conducted to investigate the efficacy of dendritic cell vaccine plus activated T-cell transfer in achieving long-term survival and preventing recurrence in patients with postoperative iCCA [167]. mPFS and mOS were 18.3 and 31.9 months, respectively, in the 36 patients who received adjuvant immunotherapy, while in the 26 patients who underwent surgery alone, mPFS and mOS were 7.7 and 17.4 months, respectively. In addition, Kai-Chao *et al.*

Table 3. Clinical trials evaluating immunotherapy alone or in combination with molecular targeted agents or chemotherapy in BTCs.

Trial number	Phase	Pathway targets	Treatment	Outcome(months)
NCT02628067(KN-158)	II	PD-1	Pembrolizumab	ORR 5.8%; DCR 22.1%; mPFS 2.0, mOS 7.4
NCT02829918	II	PD-1	Nivolumab	ORR 22%; DCR 59%; mPFS 3.68, mOS 14.24
JapicCTI-153,098	I	PD-1, chemotherapy	Nivolumab (Arm A)	Arm A: ORR 3%; mPFS 1.4, mOS 5.2
			Nivolumab + GemCis (Arm B)	Arm B: ORR 37%; mPFS 4.2, mOS15.4
NCT03101566	II	PD-1, CTLA-4, chemotherapy	Nivolumab + GemCis (Arm A)	Arm A: ORR n.a.; mPFS 7.4, mOS 10.6
			Nivolumab + Ipilimumab(Arm B)	Arm B: ORR n.a.; mPFS 4.1, mOS 8.3
NCT03046862	II	PD-1, CTLA-4, chemotherapy	GemCis (Arm A)	Arm A: DCR 96.7%; mPFS 13, mOS 15
			GemCis + Durvalumab (Arm B)	Arm B: DCR 100%; mPFS 11, mOS 18.1
			GemCis + Durvalumab + Tremelimumab (ArmC)	Arm C: DCR 97.8% mPFS 11.9, mOS 20.7
NCT02443324	I	PD-1, VEGR	Pembrolizumab plus Ramucirumab	ORR 4%; DCR 78.1%; mPFS 1.6, mOS 6.4
NCT03895970	II	PD-1, TKI	Pembrolizumab plus Lenvatinib	ORR 25%; mPFS 4.9, mOS 11.0

Table 4. General guidance for the management of hepatic immune-related adverse events.

Grade of hepatic irAE	FAD recommendations	Additional management
G1 AST or ALT >1–3 × ULN and/or Total bilirubin >1–1.5 × ULN	Continue ICI therapy Monitoring liver function	-
G2 AST or ALT >3–5 × ULN and/or Total bilirubin >1.5–3 × ULN	Delaying ICI therapy Prednisone 0.5–1.0 mg/kg/day Continuing to perform ICI therapy once ≤Grade 1 and off prednisone	-
G3 AST or ALT >5–20 × ULN and/or Total bilirubin >2–10 × ULN	Discontinue ICI therapy investigation for potential alternative hepatitis Prednisone 1.0–2.0 mg/kg/day (or equivalent corticosteroid)	Intravenous administration of corticosteroids and proton pump inhibitorsis considered for the treatment of gastrointestinal diseases. Providing supportive treatment
G4 AST or ALT > 20× ULN and/or Total bilirubin >10× ULN	Discontinuing ICI therapy Investigation of for potential alternative hepatitis Provid ING supportive treatment Prednisone 1.0–2.0 mg/kg/day (or equivalent corticosteroid)	Intravenous administration of corticosteroids and proton pump inhibitorsis considered for the treatment of gastrointestinal diseases. Providing supportive treatment Mycophenolate mofetil (500–1000 mg BID) can be administered if no improvement after corticosteroid therapy 2–3 days
G5 liver failure	Not applicable	-

[149] reported a case of Chimeric antigen receptor-modified T cell (CART) cocktail immunotherapy, targeting epidermal growth factor receptor (EGFR) and CD133 in a patient with advanced unresectable CCA. The patient had a partial response to each infusion (OS and PFS were 8.5 and 4.5 months, respectively),but treatment-related AEs such as epidermal or endothelial damages need emergent medical intervention [168]. Another Phase-I clinical trial (NCT01869166) evaluated the activation of adoptive cell therapy that transferred epidermal growth factor receptor (EGFR)-specific chimeric antigen receptor-engineered autologous T (CART) cell into EGFR-positive advanced unresectable, relapsed or metastatic BTCs. In the 17 evaluable patients, one achieved complete response and 10 achieved stable disease [169]. The CART-EGFR cell immunotherapy has proven to bea safety option for EGFR-positive advanced BTCs. A Grade $\geq$3 acute fever or chill occurred in three patients. Grade 1/2 AEs occurred in some patients after cell infusion, including gastrointestinal hemorrhage, pruritus, desquamation, oral mucositis, and oral ulcer. All AEs could be reversed. Two Phase-I/II clinical trials include NCT04426669 and NCT01868490, and a Phase-III trial (NCT02482454) remains incomplete.

6. Conclusions

Distinct BTCs have significant differences in epidemiology, past history, clinical manifestations, anatomical location, and gene heterogeneity, leading to the differences in surgical efficacy, responses to chemotherapy, targeted therapy, immunotherapy, and prognosis. Surgery or liver transplantation is potentially curative treatment of early-stage tumors. Although gemcitabine in combination with cisplatin is the standard first-line systemic therapy for advanced CCA, targeted therapy representing precision medicine is recommended if genetic aberrations are identified through genomic profiling analysis. The role of immunotherapy is still in the early stage, but the ongoing study of stratification of patients according to tumor subtype and genetic drivers will help identify subgroups who have sustained response to treatment. The combination of targeted therapy and immunotherapy may be an effective therapy for BTCs by targeting the various interactions and crosstalk of signaling pathways in tumor and tumor microenvironment. Other immunotherapeutic strategies including adoptive cell therapy and tumor vaccines remain at the early development stage and may have beneficial effect in certain patients.

Author contributions

LZ and WC made the study concepts, designed and drafted manuscript. ZH has been involved in drafting the manuscript or revising it critically for important intellectual content. JS, YW, BZ made substantial contributions to conception and design, or acquisition of data, and interpretation of data. LZ were the guarantor of integrity of the entire study.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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