Journal of Clinical and Translational Hepatology **2023** vol. 11(2) | 490–501
DOI: 10.14218/JCTH.2022.00277

Review Article

Combination Therapies for Advanced Biliary Tract Cancer

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Received: 5 June 2022 | **Revised:** 9 August 2022 | **Accepted:** 18 August 2022 | **Published:** 6 September 2022

Abstract

Biliary tract cancers (BTCs) are a group of malignant neoplasms that have recently increased in incidence and have a poor prognosis. Surgery is the only curative therapy. However, most patients are only indicated for palliative therapy because of advanced-stage disease at diagnosis and rapid progression. The current first-line treatment for advanced BTC is gemcitabine and cisplatin chemotherapy. Nonetheless, many patients develop resistance to this regimen. Over the years, few chemotherapy regimens have managed to improve the overall survival of patients. Accordingly, novel therapies such as targeted therapy have been introduced to treat this patient population. Extensive research on tumorigenesis and the genetic profiling of BTC have revealed the heterogenicity and potential target pathways, such as EGFR, VEGF, MEK/ERK, PI3K and mTOR. Moreover, mutational analysis has documented the presence of *IDH1*, *FGFR2*, *HER2*, *PRKACA*, *PRKACB*, *BRAF*, and *KRAS* gene aberrations. The emergence of immunotherapy in recent years has expanded the treatment landscape for this group of malignancies. Cancer vaccines, adoptive cell transfer, and immune checkpoint inhibitors have been extensively investigated in trials of BTC. Therefore, patient stratification and a combination of various therapies have become a reasonable and important clinical strategy to improve patient outcomes. This review elaborates the literature on combined treatment strategies for advanced BTC from the past few years and ongoing clinical trials to provide new inspiration for the treatment of advanced BTC.

Citation of this article: Zeng W, Mao R, Zhang Z, Chen X. Combination Therapies for Advanced Biliary Tract Cancer. J Clin Transl Hepatol 2023;11(2):490–501. doi: 10.14218/JCTH.2022.00277.

Keywords: Biliary tract cancer; Drug therapy, Combination; Immunotherapy; Molecular targeted therapy; Clinical trials.

Abbreviations: AKT, protein kinase B; ALK, anaplastic lymphoma kinase; BTC, biliary tract cancer; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CAR-T, chimeric antigen receptor-modified T cell; CCA, cholangiocarcinoma; c-MET, c-mesenchymal-epithelial transition factor; CTLA-4, cytotoxic T lymphocyte antigen 4; DCR, disease control rate; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; FGFR, fibroblast growth factor receptor; FOLFOX, folinic acid, 5-FU, and oxaliplatin; GemCis, cisplatin plus gemcitabine; GEMOX, gemcitabine plus oxaliplatin; HAIC, hepatic arterial infusion chemotherapy; HER, human epidermal growth factor receptor; HDAC, histone deacetylases; ICI, immune checkpoint inhibitor; IDH, isocitrate dehydrogenase; KRAS, KRAS proto-oncogene; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NACCRT, neoadjuvant concurrent chemoradiotherapy; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PDGFR, platelet-derived growth factor receptor; PDT, photodynamic therapy; PFS, progression-free survival; PI3K, phosphoinositide-3-kinase; PRKACA, protein kinase cAMP-activated catalytic subunit alpha; PRKACB, protein kinase cAMP-activated catalytic subunit beta; PTEN, phosphatase and tensin homolog; RCT, randomized clinical trial; RFA, radiofrequency ablation; ROS1, c-ros oncogene 1; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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Introduction

Biliary tract cancers (BTCs) are malignant neoplasms with high heterogenicity that arise from the epithelial cells of the bile ducts and gallbladder. Anatomically, BTCs are classified as intrahepatic, perihilar, distal cholangiocarcinoma (CCA), gall bladder cancer, and carcinoma of the ampulla of Vater. The subtypes exhibit distinct clinical symptoms and histomorphological and molecular characteristics. According to epidemiological studies, BTCs represent 3% of gastrointestinal malignancies and 15% of all primary liver cancers, and have a poor prognosis and 5-year survival of 5–15%.[1,2] In recent years, the increased incidence and mortality of BTC have been largely attributed to CCA.[3]

At present, surgery is the only curative treatment for BTC. However, only a small group of patients are indicated. Most patients with BTC present with metastasis or local progression at diagnosis, leaving palliative therapy as the only option. The current treatment option for patients with advanced BTC is cisplatin plus gemcitabine (GemCis), demonstrating huge therapeutic potential in the ABC-02 and BT22 trials.[4,5] The ABC-02 trial comprising 410 patients with advanced BTC reported a median progression-free survival (PFS) of 8.0 months and a median overall survival (OS) of 11.7 months, which were encouraging at that time. The BT22 trial reported a median PFS and median OS of 5.8 months and 11.2 months. Since then, GemCis has become the first-line treatment for advanced BTC. Promising results were announced for another gemcitabine-based chemotherapy, gemcitabine plus oxaliplatin (GEMOX), which has

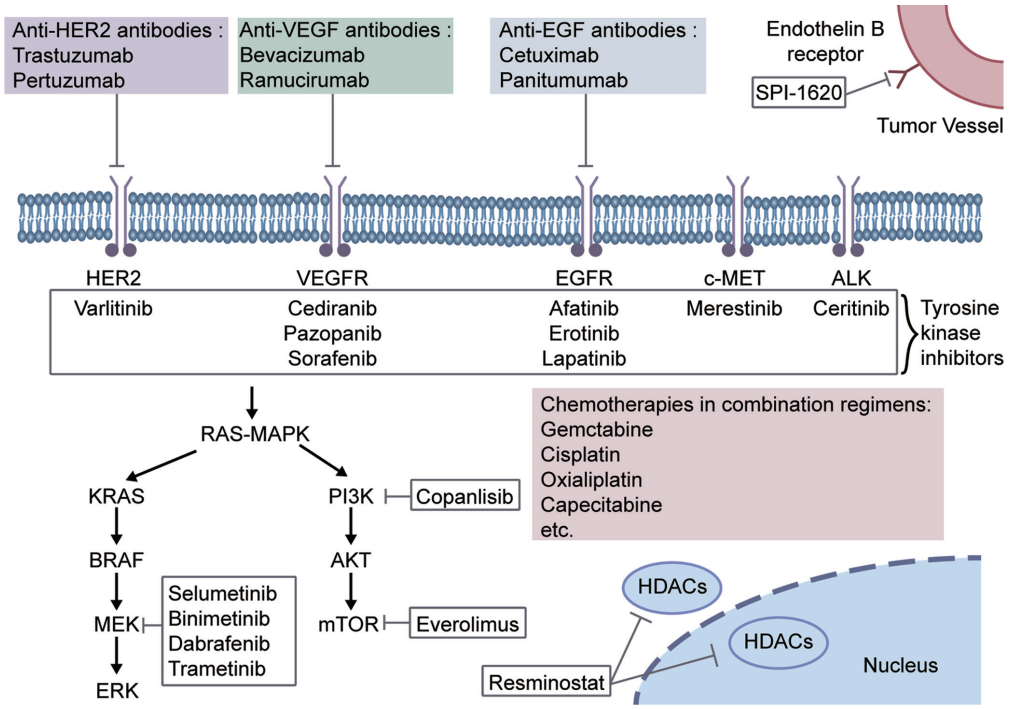

Fig. 1. Targeted therapies used in combination therapies. The figure shows various targets and corresponding medicine that draw attention in clinical trials of advanced biliary tract cancer. ALK, anaplastic lymphoma kinase; AKT, protein kinase B; BRAF, B-Raf proto-oncogene; serine/threonine kinase; c-MET, c-mesenchymal-epithelial transition factor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; KRAS, KRAS proto-oncogene; GTPase; PI3K, phosphoinositide-3-kinase; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

become widely used clinically.[6] FOLFOX (folinic acid, 5-FU, and oxaliplatin) is a commonly used second-line treatment for advanced BTC and has been associated with improved OS rates of 6 and 12 months.[7] However, the results observed after clinical use were below expectations as some patients relapsed after chemotherapy.

A growing understanding of the heterogenicity of BTC and the emergence of targeted therapies and immunotherapy have raised the expectations for enhanced treatment efficacy in advanced BTC. Many ongoing clinical trials have emphasized the combined application of various approaches. This review will elaborate on these combinations including targeted therapy, immunotherapy, radiotherapy, microwave ablation, photodynamic therapy, and discuss the challenges that researchers encounter.

Combination therapies focusing on targeted therapies

The increased incidence of chemoresistance has prompted researchers to explore genes, molecules, and pathways related to tumorigenesis. Classic pathways in other tumors, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), mitogen-activated protein kinase (MEK)/extracellular signal-related kinase (ERK), and phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), have been explored. Moreover, genetic analysis of BTC found a series of gene mutations in BTCs, including fibroblast growth factor receptor (*FGFR*) 2 fusion and isocitrate dehydrogenase (*IDH*) ½ mutation in intrahepatic CCA, protein kinase cAMP-activated catalytic subunit alpha (*PRKACA*) or protein kinase cAMP-activated catalytic subunit beta (*PRKACB*) fusion in extrahepatic CCA, B-Raf proto-oncogene, serine/threonine kinase (*BRAF*), and phosphatase and tensin homolog (*PTEN*) mutation in gallbladder cancer. Other genetic aberrations involving *EGFR*, tumor protein p53, KRAS proto-oncogene, GTPase (*KRAS*) and human epidermal growth factor receptor (*HER*) 2 were also found in common.[8] Hence, it is reasonable to develop strategies targeting these specific mutations or amplified genes. Figure 1 shows combination regimens that include targeted therapies in completed clinical trials.

EGFR pathway

EGFR regulates epithelial cell homeostasis under physiological conditions. However, EGFR is also related to tumor proliferation, migration, angiogenesis, invasion, metastasis, and apoptosis. Interestingly, 38–100% of intrahepatic BTCs exhibit EGFR overexpression, suggesting that targeting EGFR may yield significant clinical benefits.[9] Encouraging results were found when cetuximab, a monoclonal antibody against EGFR, was combined with gemcitabine and oxaliplatin in a phase 2 study. Response to treatment was observed in 19 of 30 patients with unresectable advanced or metastatic BTCs with a median OS of 22.0 months. Nine patients underwent secondary resection after cetuximab plus GEMOX therapy, and eight of them experienced major tumor shrinkage by at least 40%. However, the median PFS of all patients was not improved in contrast with the ABC-02 trial.[10] Malka *et al*.[11] re-evaluated the same therapy in the BINGO trial. Consistently, the median OS was not prolonged when compared with GEMOX. A multicenter phase 2 trial involving cetuximab with gemcitabine demonstrated antitumor activity with a median PFS of 5.8

months and median OS of 13.5 months. *KRAS* mutation status was not associated with PFS.[12] The combination of cetuximab, gemcitabine, and capecitabine was also studied in another phase 2 trial. The best overall response rate was 17.6%, with a favorable median PFS (34.3 weeks) and median OS (62.8 weeks), suggesting the feasibility of the combination.[13] Cetuximab plus GEMOX was evaluated in another study that enrolled 122 patients, which showed that the PFS was numerically improved but the difference was not statistically significant.[14] The findings from these studies suggested that cetuximab plus chemotherapy may not be a better option for all patients. Chiang *et al*.[15] stratified patients by the expression levels of c-ros oncogene 1 (ROS1), anaplastic lymphoma kinase (ALK), and c-mesenchymal-epithelial transition factor (c-Met). Interestingly, patients with low expression of these genes exhibited better median PFS when treated with cetuximab plus GEMOX compared to GEMOX alone (7.3 vs. 4.9 months). An EGFR tyrosine kinase inhibitor (TKI), erlotinib, was evaluated in a randomized phase 3 study including 268 patients with advanced BTC. Patients who received erlotinib and GEMOX dual therapy had a better overall response rate (40.8% vs. 18.6%). Nevertheless, no significant differences in median PFS and median OS were found between the two groups. Interestingly, subgroup analysis revealed that dual therapy improved median PFS in patients with CCA (5.9 vs. 3.0 months), which suggested the demand for patient classification.[16] This phase 3 study reported that early tumor shrinkage predicted outcomes and may be an indication for adding erlotinib to chemotherapy in BTC patients with wild-type *KRAS*.[17] A subsequent analysis of mutational status showed a better PFS in *KRAS* wild-type patients (6.2 vs. 2.7 months) and no improvement in patients with *KRAS* mutation between treatment groups. Accordingly, *KRAS* mutation status has significant value as a predictive biomarker for the response to erlotinib in BTCs.[18] Panitumumab is well established as the first monoclonal antibody directed against EGFR. Some phase 2 studies reported combinations of panitumumab with different chemotherapies showed encouraging results.[19,20] Hezel *et al*.[21] conducted a phase 2 study of GEMOX combined with panitumumab in *KRAS* wild-type unresectable or metastatic BTC, and reported significantly prolonged median PFS (10.6 months) and median OS (20.3 months). The response rate was 45%, and the DCR reached 90%. The phase II TACTIC trial recently investigated the efficacy of panitumumab plus GemCis in patients with *KRAS* wild-type advanced BTC. Twenty-one of 48 participants had a complete or partial response. The median PFS was 8.0 months, and the median OS was 11.9 months. The above trials emphasize the need for stratification and substantiate the tolerability and feasibility of combination therapy involving panitumumab and,[22] However, the Vecti-BIL study involving panitumumab and GEMOX yielded similar results and highlighted the limited improvement of OS for patients with wild-type KRAS.[23] Similar conclusions were drawn from the PICCA study. The median PFS and median OS of panitumumab plus GemCis were inferior to those of GemCis therapy (6.6 vs. 8.3 months and 12.8 vs. 20.1 months, respectively).[24] The reason for the limited efficacy may be attributed to panitumumab itself rather than anti-EGFR approaches as erlotinib and cetuximab had promising effects. Afatinib is an irreversible ErbB family blocker that can inhibit EGFR and HER2 with established antitumor activity in CCA cells *in vitro* and in patients with solid tumors.[25] A phase 1 study reported that afatinib combined with GemCis failed to have a survival benefit in patients with advanced CCA and EGFR overexpression. Meanwhile, patients experienced adverse effects, including diarrhea, thrombocytopenia, and sepsis.[26] In recent years, therapies targeting EGFR did not achieve substantial progress, which warrants further investigation.

VEGF pathway

VEGF is widely acknowledged to play an important role in promoting tumor angiogenesis. Clinically, targeted therapy against VEGF has been applied to solid tumors such as colorectal cancer, cervical cancer, and ovarian cancer. Bevacizumab, a monoclonal antibody against VEGF, in combination with erlotinib exhibit antitumor activity against BTC according to a phase 2 trial with a median OS of 9.9 months and time to progression of 4.4 months.[27] The results from a recent phase 2 study of capecitabine, irinotecan, gemcitabine, and bevacizumab in advanced BTC were below expectations, with a response rate of 6%, a median PFS of 3.6 months, and a median OS of 6.4 months.[28] A retrospective study reported that bevacizumab plus GEMOX achieved a better median PFS than GEMOX alone (6.48 vs. 3.72 months) but no significant improvement in median OS.[29] Amin *et al*.[30] compared the efficacy between bevacizumab and panitumumab in a chemotherapy-based phase 2 trial. No significant differences were found, though panitumumab resulted a higher response rate (45% vs. 20%). Similarly, no significant improvement was observed with ramucirumab combined with GemCis in unstratified patients with locally advanced or metastatic BTC. In the ramucirumab group, 51% of patients experienced serious adverse events, and 49% developed neutropenia.[31] A pan-VEGF receptor (VEGFR) TKI, cediranib, was evaluated in a phase 2 randomized clinical trial (RCT). The regimen of cediranib and GemCis did not improve the median PFS and resulted in a higher prevalence of adverse events such as hypertension, diarrhea, and decreased platelet count.[32] Pazopanib is a multi-TKI of VEGFR, platelet-derived growth factor receptor (PDGFR) and FGFR. Its tolerance was investigated together with paclitaxel and lapatinib (an EGFR/HER2 TKI) in a phase 1 study including six patients with BTC.[33] A subsequent study of pazopanib plus the MEK inhibitor trametinib in patients with advanced CCA reported a median PFS of 3.6 months, a median OS of 6.4 months, an objective response rate (ORR) of 5%, and a DCR of 75%.[34] In addition, a phase 2 study of gemcitabine plus pazopanib revealed a low response rate of 13.8% in the intent-to-treat analysis.[35] Sorafenib is another TKI targeting VEGFR. In a phase 2 study, sorafenib monotherapy showed therapeutic benefit but no objective response improvement.[36] In another study, sorafenib plus GemCis presented a median PFS of 6.5 months and median OS of 14.4 months in patients with advanced biliary adenocarcinoma. With a stricter patient selection, this single-center study demonstrated that the combination did not improve efficacy but the toxicity was increased compared with that in the ABC-02 study.[37] Subsequently, sorafenib and erlotinib were tested in the S0941 study. The therapeutic effect of the combination was not significant, with a median PFS of 2 months and median OS of 6 months.[38] A multicenter phase II study including 102 unresectable or metastatic BTC patients exhibited no difference in median PFS or median OS between gemcitabine-plus-sorafenib group and gemcitabine-plus-placebo group. The in group analysis revealed that patients with hepatic metastases significantly benefited from sorafenib, and patients in the sorafenib group who developed hand-foot syndrome had a better median OS (7.2 vs. 1.9 months).[39] A VEGFR2/EGFR inhibitor, vandetanib, was found to be effective in an animal CCA model.[40] A phase I trial of vandetanib combined with gemcitabine and capecitabine reported antitumor activity in patients with advanced solid tumors, including CCA.[41] Santoro *et al*.[42] reported that vandetanib did not improve the PFS in patients with BTCs. In that multicenter phase II study, the median PFS was 105 days in the vandetanib monotherapy group, 114 days in the vandetanib-gemcitabine group, and 148 days in the gemcitabine-placebo group. A total of 96.6% of patients in

the monotherapy group experienced adverse events. Overall, combination therapies targeting the VEGF pathway did not significantly improve the median PFS, median OS, or response rate. Accordingly, the role of the VEGF pathway in BTC requires further investigation. The pressing need for molecular analysis and stringent selection of patients were also emphasized in the studies above.

HER2

HER2 amplification has been reported in 10–19% of gallbladder cancers, 11–17% of extrahepatic CCA, and 1% of intrahepatic CCA. Kawamoto *et al*.[43] found that the HER2 monoclonal antibody, pertuzumab, inhibited BTC cell growth, suppressed the growth of subcutaneous tumors and induced cancer cell apoptosis *in vivo*. A synergistic inhibitory effect on tumor growth *in vivo* was found with trastuzumab and pertuzumab. A retrospective study found that eight of nine patients with gallbladder cancer featured *HER2* gene amplification or overexpression. Interestingly, four of the nine had a partial response to HER2-directed therapy (trastuzumab, lapatinib, or pertuzumab), and one had a complete response.[44] A phase 2a study of trastuzumab and pertuzumab displayed promising results in patients with *HER2*-positive metastatic BTC. Nine of 39 patients had a partial response with an ORR of 23%, and disease control was achieved in 51% of patients after 4 months. The median PFS was 4.0 months, and the median OS was 10.9 months.[45] Varlitinib is a small molecule pan-HER inhibitor targeting EGFR, HER2, and HER4. *In vitro* and *in vivo* experiments revealed that varlitinib suppressed cell growth in CCA cell lines with high *EGFR* and *HER2* expression. It has been suggested that a targeted or cytotoxic drug should be recommended in combination with varlitinib.[46] In contrast, a recent phase 2 study reported that, compared with capecitabine alone, varlitinib plus capecitabine failed to improve ORR, PFS, or OS in patients with advanced BTC.[47] Indeed, targeted therapies against HER2 in biliary cancer still face many challenges. Interestingly, unlike *FGFR* and IDH, the incidence of *HER2* amplification in BTC patients is not particularly high. Thus only a small portion of these patients can be assigned to clinical trials. Therefore, further research is required to provide novel insights for HER2 therapy in clinical practice.

RAS/RAF/MEK/ERK pathway and c-MET

The RAS/RAF/MEK/ERK pathway is frequently activated in biliary cancer and associated with tumorigenesis and chemotherapy resistance. Selumetinib, a potent and selective MEK inhibitor, was found to be efficient in combination with gemcitabine in animal experiments.[48] In addition, selumetinib monotherapy exhibited antitumor activity and tolerability in patients with metastatic BTC.[49] The ABC-04 study reported a median PFS of 6.4 months in patients with advanced or metastatic BTC who received selumetinib and GemCis.[50] Unfortunately, there were no further results from studies of combination therapies including selumetinib. Another MEK inhibitor, binimetinib, was evaluated in some phase 1 studies and identified as a tolerable approach in patients with advanced BTC.[51,52] A phase 1b study of binimetinib plus capecitabine reported a median PFS of 4.1 months and an OS of 7.8 months. The ORR and DCR were 20.6% and 76.5%, respectively. Moreover, a better tumor response, PFS (5.4 vs. 3.5 months) and OS (10.8 vs. 5.9 months) was found in patients with RAS/RAF/MEK/ERK pathway mutations, indicating good antitumor activity in specific patient populations.[53] In the absence of mutation analysis, a phase ½ study of binimetinib plus GemCis reported no significant improvement in patient outcomes, with a median PFS of 6 months and a median OS of 13.3 months.[54] Recent studies have documented *BRAF* mutations in 5% of biliary tract tumors. The combination of dabrafenib and trametinib, two MEK inhibitors, exhibited antitumor activity in several $BRAF^{V600E}$-mutated cancers. A phase 2 trial of this combination enrolled 43 patients with $BRAF^{V600E}$-mutated BTC and reported an ORR of 20% by independent assessment with a manageable safety profile. The median PFS by investigator assessment was 9 months, and the median OS was 14 months, which were promising results.

c-Met has been associated with therapeutic resistance, tumor progression, and poor prognosis. It is overexpressed in 50–60% of BTC. Merestinib, a c-Met inhibitor, was evaluated in a phase 2 study. The addition of merestinib to first-line chemotherapy resulted in no significant improvement of the median PFS (7.0 vs. 6.6 months) or median OS (14.0 vs. 13.0 months) compared with that in the placebo group. The ORR of merestinib was lower than that of the placebo (19.6% vs. 32.7%), and the DCR was not different.[31] Another oral selective c-Met inhibitor, tivantinib, showed promising results when administered with gemcitabine in patients with advanced CCA.[55] Such outcomes substantiate the significance of mutation analysis and routine surveillance in patients with advanced BTC.

PI3K/AKT/mTOR, ALK/ROS1 and other targets

Upregulation of the PI3K/AKT/mTOR pathway due to *PIK3CA* mutations has been identified in BTC. Copanlisib is a PI3K inhibitor that has been reported to have potential antitumor activity combined with GemCis in patients with BTC in a phase 1b study.[56] Recently, a phase 2 study evaluated the safety and efficacy of copanlisib with GemCis in advanced BTC. The PFS rate at 6 months was 51%, with median OS of 13.7 months and median PFS of 6.2 months. In group analysis of revealed a better prognosis in patients with low *PTEN* expression, which indicated precise administration of such therapy.[57] The mTOR inhibitor everolimus has also attracted much interest in recent years. Importantly, phase 2 studies of everolimus monotherapy reported clinical activity in BTC.[58,59] The combination of everolimus and chemotherapy exhibited a synergistic antiproliferative effect on CCA *in vitro* but has not been utilized clinically.[60] Although *ALK/ROS1* aberrance has been detected in CCA, few studies have been designed to target it. A recent phase 1 study of ceritinib, an ALK inhibitor, combined with gemcitabine-based chemotherapy reported clinical benefits in advanced CCA patients.[61] Further antitumor activity of this approach remains largely unknown.

In addition to the well-known signaling pathways above, combination therapies involving unconventional targets have been investigated in advanced BTC. Resminostat has been established as an inhibitor against histone deacetylases (HDAC), which is overexpressed in BTC and regulates the expression of various genes related to cell survival, growth, differentiation, and apoptosis.[62] In a phase 1 study of resminostat and S-1, the combination was well tolerated and resulted in a DCR of 84.6%, a median PFS of 5.5 months, and a median OS of 10.2 months in advanced BTC, indicating good efficacy.[62] SPI-1620 is an investigational, highly selective peptide agonist of endothelin B receptors that can reportedly enhance chemotherapy. However, when combined with docetaxel, SPI-1620 failed to improve antitumor activity with a poor median PFS and median OS.[63]

Immunotherapy

Immunotherapy generally includes cancer vaccines, adop-

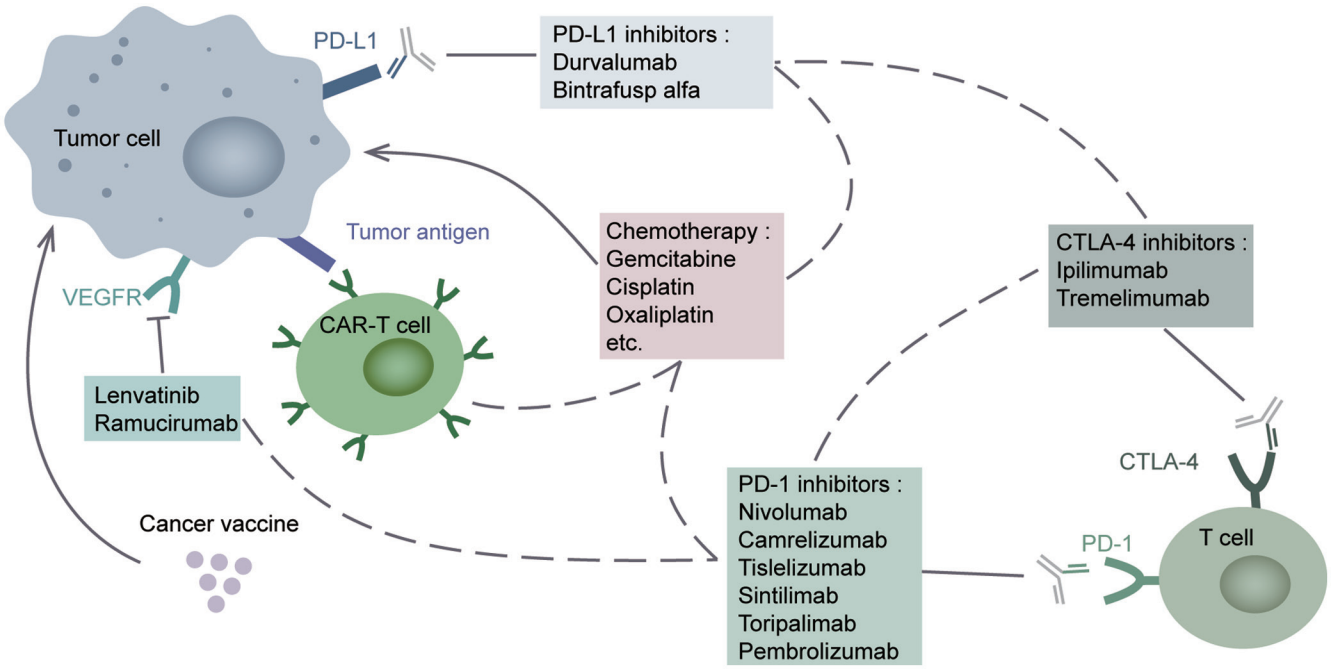

Fig. 2. Immunotherapies used in combination regimens. The figure shows immunotherapy approaches in clinical trials of advanced biliary tract cancer and combinations include them. CAR-T, chimeric antigen receptor-modified T cell; CTLA-4, cytotoxic T lymphocyte antigen 4; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; VEGFR, vascular endothelial growth factor receptor.

tive cell transfer and immune checkpoint inhibitors (ICIs) against programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), which have been widely applied to many solid tumors, including non-small cell lung cancer, colon cancer, and breast cancer. Currently, immunotherapy is a focus of attention. Promising results have been observed in immunotherapy trials involving monotherapy with pembrolizumab, nivolumab, or durvalumab in advanced BTC. Consequently, immunotherapy combined with other therapies is a potentially valuable approach for enhancing antitumor efficacy. Figure 2 shows combination regimens that include immunotherapies in a spectrum of clinical trials.

ICI combinations and in synergy with chemotherapy

Nivolumab is a PD-1 inhibitor that blocks the immunosuppressive signaling pathway triggered by PD-1 and restores the antitumor function of T cells. Nivolumab alone or combined with GemCis was assessed in 30 patients with unresectable or recurrent BTC. In the monotherapy cohort, the median OS was 5.2 months, the median PFS was 1.4 months, and only one of 30 patients achieved an objective response. In the combined therapy cohort, the median OS was 15.4 months, the PFS was 4.2 months, and 11 of 30 patients had an objective response. This study validated the safety of nivolumab in advanced CCA patients and suggested that combination therapy may improve the effectiveness of nivolumab.[64] Based on these findings, the same combination was evaluated in a phase II study in 2019, where patients in cohort B, consisting of patients sensitive to cisplatin and gemcitabine, achieved a median OS of 8.6 months and median PFS of 6.2 months. Thirteen of 21 patients had an objective response, and the DCR was 95.2%. An objective tumor response was observed in two of six patients in cohort A, consisting of patients resistant to cisplatin and gemcitabine, indicating that nivolumab was able to resensitize patients to cisplatin and gemcitabine chemotherapy. The study also suggested that nivolumab, when combined with gemcitabine and cisplatin, had promising antitumor efficacy and a manageable safety profile in advanced unresectable or metastatic BTC.[65] Klein *et al*.[66] evaluated the combination of nivolumab and ipilimumab, an anti-CTLA-4, in patients with advanced BTCs. The ORR was 23%, disease control was observed in 17 out of 39 patients, the median PFS was 2.9 months, and the median OS was 5.7 months. Interestingly, intrahepatic cholangiocarcinoma (iCCA) and gallbladder cancer patients had an ORR of 31%, but no response was observed in patients with extrahepatic CCA, indicating that the efficacy of ICIs differed based on the anatomic sites. Camrelizumab, a PD-1 antibody, was evaluated together with GEMOX in a phase 2 study of advanced BTC. The most common treatment-related adverse events were fatigue (73%) and fever (73%). The combination resulted in an objective response in 54% of patients. The median of 11.8 months, and median PFS of 6.1 months were prolonged compared with those for GEMOX alone.[67] A phase 2 study in China assessed camrelizumab plus oxaliplatin-based chemotherapy in patients with advanced BTC. The reported ORR was 16.3%, the median PFS was 5.3 months, and the median OS was 12.4 months, similar to the ABC-02 trial. These promising results suggest that camrelizumab plus oxaliplatin-based chemotherapy could be a new first-line therapy for advanced BTC.[68] Another phase 1 study of durvalumab (an anti-PD-L1) and tremelimumab, an anti-CTLA-4, reported similar, promising results. Durvalumab monotherapy cohorts achieved a median OS of 8.1 months and dual therapy achieved 10.1 months, suggesting its potential as a novel second-line therapy.[69] A phase 2 study investigated the combination of gemcitabine and cisplatin plus durvalumab with or without tremelimumab in chemotherapy-naïve advanced BTC patients. All patients received chemotherapy and immunotherapy in three regimens, and the doses of drugs in different regimens were consistent.

The overall median PFS was 12.1 months and the median OS was 18.4 months, which was promising compared with classical chemotherapy.[70] However, the study did not include a control group. The differences of OS or PFS in the three regimens were not significant. The investigators found that decreased PD-L1 expression in tumor cells and immune cells after one cycle of treatment was associated with shorter PFS. Median OS was associated with PD-L1 expression in immune cells. The results indicated that changes in PD-L1 expression after treatment may predict clinical outcomes. Given the encouraging prospect of chemotherapy plus durvalumab in advanced BTC, a subsequent phase 3 study (TOPAZ-1) evaluated durvalumab plus GemCis. In that randomized, double-blind, multicenter study, 685 patients were enrolled to receive GemCis plus durvalumab or placebo. Durvalumab plus GemCis improved the median OS (12.8 vs. 11.5 months) and median PFS (7.2 vs. 5.7 months) compared with placebo plus GemCis. The 18-month OS was 35.1% in patients treated with durvalumab and 25.6% in patients treated with placebo. The gap was also apparent in the 24-month OS (24.9% vs. 10.4%). The ORR was 26.7% with durvalumab and 18.7% with placebo. The occurrence of grade ¾ treatment-related adverse events showed no differences. In general, the TOPAZ-1 study may describe a novel first-line therapy for advanced BTC. However, the treatment in clinical practice may differ from clinical trials for various reasons. The optimal dose for most patients warrants additional study as the dose of durvalumab in TOPAZ-1 was not consistent with that reported in a previous study.[71] Furthermore, triplet combination including durvalumab, tremelimumab, and paclitaxel is being assessed by Boilève *et al*.[72] in an ongoing trial that preliminarily reported a high incidence of adverse events. Half of the patients experienced grade 3 or 4 toxicities, and different grades of colitis, diarrhea, and anaphylaxis were observed. Consequently, the trial proceeded without paclitaxel.

Cancer vaccine and chimeric antigen receptor-modified T cells

Peptide vaccination was first studied in patients with advanced BTCs in 2005.[73] Another study conducted a few years later demonstrated no significant clinical efficacy with the Wilms tumor 1 peptide vaccine and gemcitabine combination therapy.[74] Subsequently, combination therapy with gemcitabine plus antiangiogenic vaccination of elpamotide in patients with advanced or recurrent BTC achieved a median survival of 10.1 months in a phase II trial, and 44.4% of patients survived after 12 months.[75] Shirahama *et al*.[76] also developed personalized peptide vaccination (PPV) that triggered an antitumor immune response and achieved a relatively short median PFS of 2.9 months and a median OS of 5.9 months.. In contrast, combining cyclophosphamide, a cytotoxic agent that suppresses regulatory T cells, with PPV treatment significantly prolonged the median OS (6.1 months) and median PFS (12.1 months). A phase 1 study investigated a four-peptide cancer vaccine in advanced BTC. It was well tolerated; no grade ¾ events occurred. The median PFS and OS were 156 and 380 days, indicating the potential of such therapy.[77] Generally, vaccine-dominant therapies have potential clinical benefits. However, they need to be optimized for the target population. Recently, chimeric antigen receptor-modified T cell (CAR-T) therapy has been extensively studied. A case report documented the efficacy of EGFR-specific and CD133-specific CAR-T cells in a 52-year-old patient with CCA.[78] Subsequently, the same research group investigated CAR-T cells in EGFR-positive advanced BTCs in a phase 1 study that established the safety and feasibility of that approach.[79] Another study evaluated CAR-T cells plus nab-paclitaxel and cyclophosphamide in patients with HER2-positive advanced BTCs, which validated its safety and yielded a median PFS of 4.5 months.[80] CAR-T therapy has been applied in other tumors, and its limitations in BTC remain unclear, warranting further studies.

Immunotherapy plus targeted therapy

Studies of immunotherapy and targeted therapy have had encouraging outcomes, but few have confirmed the clinical benefits of their combinations. A phase 1 trial of ramucirumab plus pembrolizumab did not report satisfactory outcomes. The median OS in patients with PD-L1 positivity and PD-L1 negativity was 11.3 months and 6.1 months, respectively. Nonetheless, a median PFS of 1.5 months in all patients decreased the feasibility of this therapy.[81] Bintrafusp alfa, a bifunctional fusion protein composed of human TGF-β receptor II and anti-PD-L1, was investigated in a phase 1 study. A median PFS and OS of 2.5 months and 12.7 months were observed respectively, suggesting the potential of bintrafusp alfa as an effective approach for patients with advanced BTCs.[82] Lenvatinib, a multi-TKI targeting VEGFR and PDGFR with established efficacy in hepatocellular carcinoma, was also found to be effective against advanced BTCs.[83] In that regard, lenvatinib plus pembrolizumab yielded promising outcomes with a decrease in tumor size in 68.8% patients, a partial response in 25%, and stable disease in 53%. The median PFS was 4.9 months and the median OS was 11.0 months. Gene expression analysis also revealed that patients with positive PD-L1 expression had a longer median PFS (6.3 vs. 4.5 months) and median OS (20.7 vs. 8.4 months).[84] Zhang *et al*.[85] assessed the antitumor efficacy of lenvatinib plus PD-1 inhibitors in a phase 2 study in which 38 patients with unresectable BTC received lenvatinib and PD-1 inhibitors, including pembrolizumab, tislelizumab, sintilimab, camrelizumab, or toripalimab, based on patient preference. Thirteen patients achieved downstaging and underwent surgery. The primary study endpoints were an ORR of 42.1%, DCR of 76.3%, median event-free survival of 8.0 months, and a median OS of 17.7 months. These encouraging results suggested the feasibility of lenvatinib plus PD-1 inhibitors as a potential first-line therapy in unresectable BTC. Two case reports, with treatments including lenvatinib plus sintilimab dual therapy and systemic sequential therapy comprising GemCis, tislelizumab, and lenvatinib, had promising results in patients with advanced intrahepatic CCA.[86,87] These pieces of evidence highlight the need for more trials involving ICIs and targeted therapies to improve patient prognosis.

Radiotherapy, microwave ablation, and photodynamic therapy

Research on different types of radiotherapy in advanced BTCs can be traced back decades. The combination of chemotherapy and radiotherapy has been the focus of research in recent years. Figure 3 demonstrates the combination approaches include radiotherapy and ablation in different clinical trials. Autorino *et al*.[88] reported a 2-year OS of 27% in patients with unresectable extrahepatic CCA who received adjuvant concurrent chemoradiation therapy, including gemcitabine. Interestingly, a boost of intraluminal brachytherapy resulted in better local control but failed to improve the median OS. A retrospective study of the benefit of neoadjuvant concurrent chemoradiotherapy (NACCRT) for locally advanced perihilar CCA found that NACCRT did not prolong OS and DFS but did downstage tumors.[89] A phase II single-arm study (MISPHEC trial) of patients with

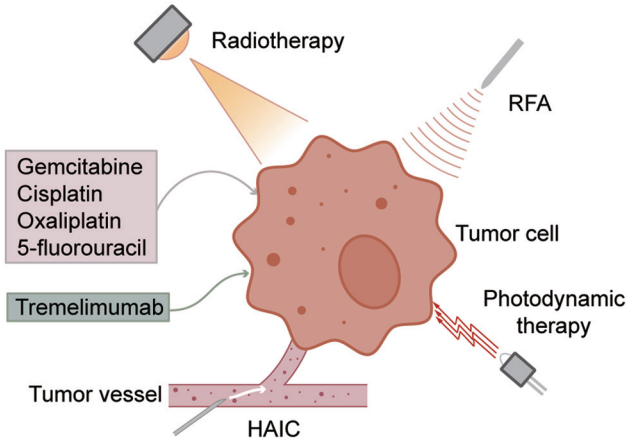

Fig. 3. Interventional therapies in combination regimens. The figure shows interventional therapies such as radiotherapy, RFA, HAIC, photodynamic therapy, and their combinations with other therapies in clinical trials. HAIC, hepatic arterial infusion chemotherapy; RFA, radiofrequency ablation.

locally advanced intrahepatic CCA reported that the combination of selective internal radiation therapy plus GemCis was feasible. The ongoing correlative phase 3 trial is worthy of attention.[90] A study demonstrated that the median OS was improved after endoscopic radiofrequency ablation (RFA) plus S-1 compared with RFA treatment alone (16.0 vs. 11.0 months).[91] Gou *et al*.[92] reported that stent placement combined with intraluminal RFA and hepatic arterial infusion chemotherapy (HAIC) might be a promising strategy for advanced BTC patients with and biliary obstruction. Similarly, gemcitabine and cisplatin plus HAI with floxuridine was effective for disease control, yielding a median OS of 23.9 months and a median PFS of 10.1 months in patients with unresectable CCA.[93] Another study that combined tremelimumab with microwave ablation supported the feasibility of this novel strategy, but it remains investigational.[94] Taken together, radiotherapy and microwave ablation have advantages including targeting lesions, producing less damage than surgery, and reducing drug dosage. However, the challenge is that efficacy is strongly related to the equipment used and clinician experience. Accordingly, the comprehensive application of radiotherapy or microwave ablation with other therapies remains worthy of investigation. Photodynamic therapy (PDT) is another nonsurgical approach for BTC treatment and is generally tolerable when combined with chemotherapy. The PCS Nordic study, a phase 2 RCT of temoporfin photodynamic therapy with or without chemotherapy, found no difference in patient quality of life between the two cohorts.[95] In a prospective phase 2 RCT, compared with PCT alone, PDT plus S-1 significantly improved median OS and PFS.[96] A comparative study of 68 patients with unresectable hilar CCA reported a significantly higher 1-year survival rate in the PDT plus chemotherapy group than in the PDT monotherapy group (88% vs. 58%).[97] A study by Gonzalez-Carmona *et al*.[98] reported that combining photodynamic therapy with chemotherapy resulted in longer OS with a median survival of 20 months in the combination group, 15 months with PDT alone, and 10 months with chemotherapy alone. A phase 2 pilot study showed that PDT combined with chemotherapy down-staged tumors and led to curative resection.[99] Accordingly, systemic chemotherapy plus PDT is a feasible approach to treat unresectable CCA. Overall, PDT has the advantages of noninvasive and precise positioning like radiotherapy while avoiding radiation-related adverse events. Figure 3 summarizes clinical trials including PDT, which has great prospects for treating advanced CCA when combined with other therapies. The underlying mechanisms behind its efficacy remain largely unknown given the lack of related studies, emphasizing the need for further exploration.

Challenges

It has been established that various elements may affect the results and reliability of clinical trials. From the point of view of experimental design, many trials are single-arm, which means control groups are absent. The comparison is made between the experiment group and historical data, thus the study error is inevitably larger than that of double-arm studies. In addition, historical data should be reviewed. It has been a decade since the ABC-02 and BT22 trials, and the improvement of health care systems and nursing techniques have had a positive influence on OS. Consequently, the results of monocenter, single-arm, open-label studies may not be universally applicable and need to be viewed with caution.

A frequently mentioned challenge is that the number of patients who complete trials is insufficient. In most cases, rapid disease progression is observed and patients cannot tolerate the intervention during clinical trials. It should thus be borne in mind that although the incidence of BTC is increasing, it still comprises a group of relatively rare and highly malignant tumors. Rather than admitting more new patients, motivating existing patients to participate in clinical trials is a more rational option. Interagency collaboration is a better approach, as multicenter studies usually enroll more patients and increase credibility.

The inclusion and exclusion criteria were not identical among studies. The age range, Eastern Cooperative Oncology Group scores of the patients, Child-Pugh scores, different approaches to tumor confirmation, and others differed among studies. In addition, doses of the same drug differed among studies. For example, the dose of nivolumab was 240 mg every 2 weeks in a Japanese study[64] and 3 mg/kg every 3 weeks in a phase 2 study.[65] It is difficult to define the optimal dose without conducting larger trials. One aspect that all researchers should not neglect is adverse events. Combination therapies contain more drugs, which have more adverse effects. Although early-phase studies have evaluated safety, some unexpected events such as a high incidence of grade 3 or 4 toxicities still occur.[72] Furthermore, the process of treatment is often long and painful for patients with advanced BTC, and few studies have included quality of life as an outcome. Currently, quality of life is defined by questionnaires, scales or scores, and it is relatively subjective. Nevertheless, it will influence patient compliance.

Another important challenge is patient stratification. Many studies involving targeted therapies and immunotherapies have now realized the importance of gene sequencing. By stratification, researchers can predict prognosis and find patients who will benefit most from a given therapy. As mentioned above, gene expression, such as *ALK, KRAS and PD-L1,* are associated with the prognosis of patients given corresponding therapies. Consequently, future clinical trials require deeper analysis in addition to basic variables such as tumor site. It is possible that new evidence may be found by re-evaluating completed trials.

Conclusions

As mentioned above, combination therapy for BTC has developed rapidly in recent years. Our growing understanding of BTC has been translated into new therapies. Table 1 lists

Table 1. Ongoing trials of combination therapies of biliary tract cancer

Combination therapy	Phase	Pathway	Primary outcome	Secondary outcome	Trial number
TS-1, Gemcitabine, Nivolumab	2	PD-1, chemotherapy	ORR	–	NCT04172402
Atezolizumab, Bevacizumab, Cisplatin, Gemcitabine	2	PD-L1, VEGF, chemotherapy	PFS	OS, ORR, DOR, DCR, TTCD, AE	NCT04677504
Sym021, Sym022, Sym023, Irinotecan Hydrochloride	1	PD-1, LAG-3, TIM-3, chemotherapy	Safety and ORR	Pharmacokinetics, DOR, DCR, OS	NCT04641871
Lenvatinib, Toripalimab, or GEMOX	2	PD-1, chemotherapy, VEGF	ORR	OS, PFS, AE	NCT04361331
DKN-01, Nivolumab	2	DDK1, PD-1	ORR	PFS, OS	NCT04057365
Toripalimab, S1, Albumin Paclitaxel	2	PD-1, chemotherapy	ORR	PFS, DCR, OS	NCT04027764
JS001, Lenvatinib, Oxaliplatin, Gemcitabine,	2	PD-1, VEGF, chemotherapy	ORR	OS, PFS, AE	NCT03951597
Nivolumab, Nanoliposomal-Irinotecan, 5-FU, Leucovorin	1b/2	PD-1, chemotherapy	DLTs, PFS	ORR, OS, AE	NCT03785873
Guadecitabine, Durvalumab	1	PD-L1, chemotherapy	AE, Tumor response	OS, PFS	NCT03257761
Toripalimab, Lenvatinib	2	PD-1. VEGF	ORR, AE	OS, PFS, SD, CBR	NCT04211168
Sitravatinib, Tislelizumab	2	RTKs, PD-1	DCR	ORR, PFS, OS	NCT04727996
Durvalumab, Tremelimumab	2	PD-L1, CTLA-4	OS	PFS, OS, AE, Tumor response, HRQoL	NCT03704480
Ceralasertib, Durvalumab, Olaparib	2	ATR, PD-L1, PARP	DCR	OS, PFS, DOR, Safety and tolerability, QoL	NCT04298021
Ipilimumab, Nivolumab	2	CTLA-4, PD-1	PFS at 6 months, CBR	OS, PFS, AE	NCT04969887
Pembrolizumab, Cisplatin, Gemcitabine	3	PD-1, chemotherapy	OS	PFS, ORR, DOR, AE	NCT04003636
Nivolumab, Ipilimumab, Radiotherapy	2	PD-1, CTLA-4, radiotherapy	CR, PR, SD at 6 months	AE, ORR	NCT02866383
Surufatinib, Capecitabine	2b/3	VEGF, chemotherapy	OS	PFS, ORR, DCR, DOR	NCT03873532
SC-43, Cisplatin	1/2	STAT3, chemotherapy	Pharmacokinetics, ORR	Pharmacokinetics, DOR, DCR, PFS, OS	NCT04733521
Levamisole, Anlotinib	3	VEGF, chemotherapy	PFS	OS, DCR, ORR, AFP	NCT03940378
mFOLFOX6, Atezolizumab, and Bevacizumab	1b/2	PD-L1, VEGF, chemotherapy	ORR	DCR, DOR, BORR, PFS, ORR, TTF, tumor size, OS, AEs	NCT05052099
Camrelizumab, Apatinib, or FOLFOX4 or GEMOX	2	PD-1, VEGF, chemotherapy	Safety and tolerability	OS, PFS, DCR, DOR	NCT03092895
Afatinib Dimaleate, Capecitabine	1	EGFR, HER2, HER4, chemotherapy	Safety and pharmacokinetics	DOR, OS, PFS, RR, duration of stable disease	NCT02451553

AE, adverse event; AFP, alpha fetoprotein; ATR, serine/threonine-protein kinase; BORR, best overall response rate; CBR, clinical benefit rate; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DCR, disease control rate; DKK1, Dickkopf-related protein 1; DLT, dose-limiting toxicity; DOR, duration of response; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; HRQoL, health-related quality of life; LAG-3, lymphocyte activation gene 3; ORR, objective response rate; OS, overall survival; PARP, poly (ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PD-1, programmed cell death protein ligand 1; PFS, progression-free survival; QoL, quality of life; RTK, receptor tyrosine kinase; STAT3, signal transducer and activator of transcription 3; TACE, transarterial Chemoembolization; TDP, time to disease progression; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; TTCD, time to confirmed deterioration; TTF, time to treatment failure; VEGF, vascular endothelial growth factor.

some ongoing clinical trials of combination therapies and their targeted pathways. Some monotherapies have yielded promising results. In the ClarIDHy study, ivosidenib targeting *IDH1*-mutant CCA achieved significant improvement of median PFS (6.9 vs. 1.6 months) and median OS (10.3 vs. 5.1 months) compared with placebo.[100,101] Moreover, BGJ398, a selective pan-FGFR kinase inhibitor, had promising antitumor activity in patients with CCA with *FGFR2* fusion/rearrangement.[102,103] Other agents targeting *BRCA* mutations or NTRK are currently under investigation (NCT04042831, NCT04584008). Unfortunately, there are few clinical trials and corresponding results on the efficacy of those agents in combination therapies. The reason could be concern of increased adverse events, drug interactions, and drug toxicity. Meanwhile, significant efficacy has been observed in clinical trials of chemotherapeutic drugs. S-1, an oral fluoropyrimidine combination, was associated with fewer adverse events together with gemcitabine and was noninferior to GemCis, according to a recent phase 3 trial.[104] A Phase I clinical trial also demonstrated the feasibility of NUC-1031 in combination with cisplatin.[105] Encouragingly, a recent phase 2 trial reported that nab-paclitaxel plus gemcitabine-cisplatin prolonged median PFS (11.8 months) and OS (19.2 months) compared with current first-line chemotherapy.[106]

One of the top priorities is to improve the quality of clinical trials by better design, interagency collaboration, and unified criteria. The other top priority is patient stratification, which has not been well implemented. Proper stratification directly benefits the efficacy of targeted therapies and immunotherapies. Although current studies focus on targeted therapy, immunotherapy, and the combination of both. All kinds of therapies deserve attention. For example, interventional therapies mentioned above could have an auxiliary role and even downstage tumors to enable curative resection.

In summary, research on the heterogeneity of BTC has provided a foundation for developing novel therapeutic approaches. Effective treatment requires the combination of different approaches to minimize side effects and yield optimal clinical effectiveness while preserving patient quality of life. However, the optimal treatment for different patient groups remains to be determined, and warrants further study. The advents of new technologies, new agents, and new combinations has brought hope to researchers, clinicians, and patients. Combating BTC requires cooperation.

Funding

This study was funded by National Natural Science Foundation of China (No.81502530, No.82172976)

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (WZ, ZZ), supervision and administrative support (ZZ, XC), manuscript writing and revising (all authors), and final approval of manuscript (WZ, RM, ZZ, XC).

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