Review

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Exploring Molecular Mechanisms in the Second-Line Treatment of Biliary Tract Malignant Tumors

QianLin Liu[1,2], PengFei Zhang[1,2], Qiu Li[1,2,*]

[1]Department of Medical Oncology, Cancer Center, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China
[2]West China Biomedical Big Data Center, Sichuan University, 610041 Chengdu, Sichuan, China
*Correspondence: liqiu@scu.edu.cn (Qiu Li)
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Biliary tract malignant tumors account for about 3% of gastrointestinal malignancies. Based on anatomical location, biliary tract malignant tumors can be divided into gallbladder carcinoma, intrahepatic cholangiocarcinoma (ICC), hilar cholangiocarcinoma, and distal cholangiocarcinoma. Surgical treatment is the main treatment for early-stage biliary malignant tumors, the insidious nature of the disease often leads to late diagnoses, causing many patients missing the window for surgical intervention. Gemcitabine combined with cisplatin serves as a first-line treatment for patients with advanced or unresectable lesions, however, a definitive standard for second-line treatment has not yet been established. In recent years, many advances have occurred in the study of the molecular mechanisms contributing to the occurrence and development of biliary malignancies, providing a foundation for targeted treatments of the disease. This review summarizes the existing literature and explores potential second-line treatment options for advanced biliary malignancies based on our understanding of the molecular pathogenesis and tumor pathology.

Keywords: cholangiocarcinoma; second-line treatment; targeted therapy; molecular pathology

Introduction

Biliary tract cancer (BTC) originates from the epithelium of the bile duct and gallbladder wall, which are highly invasive and heterogeneous with poor prognosis. Different types of BTC have different epidemiological and molecular pathological characteristics [1]. The 5-year survival rate is less than 20% [2]. Unfortunately, about 60%–70% of patients with BTC are diagnosed at an advanced stage. This delay in diagnosis limits the treatment options, which mainly rely on medical system-based treatment. The combination of gemcitabine and cisplatin is now considered the standard first-line treatment for advanced BTC, based on the results of relevant tests [3,4]. In addition, based on the results of the Phase III trial in Japan, gemcitabine plus S-1, gemcitabine plus cisplatin and S-1 are also optional first-line treatments [5,6]. Beyond chemotherapy, the results from two phase 3 trials (KEYNOTE-966 and TOPAZ-1) suggest that immunotherapy alongside gemcitabine plus cisplatin shows promise as a potential first-line treatment option for patients with advanced, unresectable biliary malignancies [7–10]. However, the survival benefit of first-line treatment remains limited, and some patients will inevitably need second-line treatment after disease progression.

It is reported that about 15–25% of patients received second-line therapy [11]. However, a lack of recognized standards exists for the second-line treatment of patients with advanced BTC after receiving gemcitabine combined with cisplatin as standard first-line treatment. A systematic review containing a total of 761 patients in 25 studies evaluated the efficacy of second-line treatment in advanced BTC, exhibiting an average overall survival (OS) rate of 7 months and progression-free survival (PFS) of 3 months [11]. Moreover, the study reported an 8% effectiveness of second-line treatment, exemplifying the positive influence such treatment may have for some patients. In the same study, the fluorouracil plus leucovorin and oxaliplatin (FOLFOX) group exhibited a median overall survival (OS) of 6.2 months compared to 5.3 months in the active symptom control group, with respective 6-month survival rates of 50.6% and 35.5%. Additionally, their 12-month survival rates stood at 25.9% and 11.4%. Regarding safety, the incidence of grade 3–5 adverse events (AEs) was 69% in the FOLFOX group and 52% in the active symptom control group. The most common treatment-related adverse events were neutropenia (12%), fatigue and drowsiness (11%) and infection (10%). This study provides high-level medical evidence for second-line treatment supplementation. However, this study did not include a fluorouracil monotherapy group and a fluorouracil plus oxaliplatin treatment group to further evaluate the respective efficacy contributions of fluorouracil and oxaliplatin without leucovorin. Of note, another multicenter retrospective study of 196 patients with advanced BTC showed that FOLFOX was not superior to fluorouracil alone as a second-line treatment [12].

Table 1. Genetic variation spectrum of biliary tract cancers.

Variation sites		Prevalence
Intrahepatic cholangiocarcinoma	*FGFR1–3* fusions, amplifications, and mutations [16]	11–45%
	TP53 mutation [17]	2.5–44%
	ARID1A mutation [18]	15–36%
	CDKN2A or *CDKN2B* loss [19]	6–30%
	IDH1 or *IDH2* mutation [20,21]	23–28%
	EGFR expression [22–24]	11–27%
	KRAS mutation [17]	11–25%
	SMAD4 mutation [17]	4–17%
	MLL3 mutation [17]	15%
	BAP1 mutation [18]	13%
Extrahepatic cholangiocarcinoma	*KRAS* mutation	8–42%
	TP53 mutation	40%
	CDKN2A or *CDKN2B* loss	17%
	HER2 amplification [25]	11–17%
	ARID1A mutation	12%
Gallbladder carcinoma	*TP53* mutation	47–59%
	HER2 amplification	10–19%
	CDKN2A or *CDKN2B* loss	6–19%
	ARID1A mutation	13%
	PIK3CA mutation [26]	6–12.5%

TP53, Tumor Protein 53; *FGFR1–3,* fibroblast growth factor receptor1-3; *ARID1A*, AT-rich interaction domain 1A; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDKN2B*, cyclin-dependent kinase inhibitor 2B; *IDH1*, isocitrate dehydrogenase 1; *IDH2*, isocitrate dehydrogenase 2; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten; *SMAD4*, mothers against decapentaplegic homologs; *MLL3*, mixed-lineage leukemia 3; *BAP1*, BRCA1-associated protein 1; *HER2,* human epidermal growth factor receptor 2; *PIK3CA*, phosphoinositide-3-kinase,catalytic,alpha polypeptide.

In recent years, with the in-depth exploration of the molecular mechanism of tumors, immunotherapy and targeted therapy have also made progress in the application of BTC. In a comprehensive genomic analysis of a sizable sample of BTC patients, Tumor Protein 53 (*TP53*) emerged as the predominant gene variation, present in 38% of cases, followed by cyclin-dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) at 29% [13]. Of note, at least 43% of tumor samples detected potentially meaningful genetic abnormalities, suggesting that targeted therapy has a place in the posterior treatment of cholangiocarcinoma. Initially, the benefits of targeted therapy were not apparent in unscreened patients. Erlotinib, a tyrosine kinase inhibitor that targets the epidermal growth factor receptor (*EGFR*) pathway, demonstrates modest efficacy although *EGFR* is overexpressed in both intrahepatic (11–27%) and extrahepatic (5–9%) BTC. Erlotinib brings only mild benefits as a second-line treatment [14]. Not all of the identified targets are potential therapeutic targets, however, precise targeted therapy has yielded exciting therapeutic effects in patients with treatment-related specific targets such as fibroblast growth factor receptor (*FGFR2*) fusion, human epidermal growth factor receptor 2 (*HER2*), and isocitrate dehydrogenase (*IDH*) mutations [15]. A brief summary of the BTC targets is provided in Table 1 [16–26].

Analyzing Patient Profiles and Prognosis for Second-Line Treatment Potential in Advanced BTC

The high heterogeneity of cholangiocarcinoma suggests that individual differences must be considered between patients in clinical practice [27]. A multicenter trial showed that patients with low carbohydrate antigen 19-9 (CA19-9) concentration, PFS exceeding 6 months after first-line treatment, and subsequent surgical resection of the primary tumor had a better prognosis with second-line treatment [28]. Patients in the high-risk group mentioned in the study had three or four factors. A study in Korea showed that less albumin concentration, higher carcinoembryonic antigen (CEA) concentration, higher CA19-9 concentration, and a neutrophil/lymphocyte ratio greater than three in patients with BTC may lead to a poor prognosis [29]. In a separate single-center study involving 144 patients with advanced BTC who received second-line chemotherapy, findings showed that patients with elevated CEA concentrations exceeding 3 µg/L and leukocytosis and cholinesterase concentrations below 5 kU/L exhibited a lower OS rate [30]. A multicenter study with a large sample size constructed a prognostic model to predict the survival benefit of treatment [31]. The model contained four independent prognostic fac-

tors: baseline functional status, reasons for first-line treatment termination, surgical treatment of primary tumors, and presence of peritoneal metastasis. The advantage of this model is that it incorporates a large patient sample size and has been validated in three countries. Another study [32] identified several circulating proteins such as Interleukin 6 (IL-6) and Interleukin 15 (IL-15) as potential biomarkers for patient prognosis, especially OS, but future studies are needed to determine the best combination and threshold.

The results highlight differences in patient OS rates, suggesting the need for defined screening criteria and the creation of a universal model. Such initiatives should aim to facilitate global multicenter studies of second-line BTC treatment and explore more convenient, rapid, and accurate prognostic evaluation tools for patients with advanced BTC.

Common Types of Genetic Variation and Targeted Therapy Model of Advanced BTC

This section introduces the common types of gene mutations in BTC and related targeted therapeutic drugs. The development of related clinical studies has also laid the foundation for the development of subsequent drugs.

FGFR2 Fusions

FGFR is a subfamily of tyrosine kinase receptors, which plays an important role in embryonic development, angiogenesis, and other physiological processes. *FGFR* can activate downstream signaling pathways after binding to ligands and drive tumor formation [33]. *FGFR2* fusion is detected in approximately 13–20% of patients with intrahepatic cholangiocarcinoma, with *FGFR2-BICC1* being the most common form of fusion [33,34]. In addition to gene fusion, *FGFR1* and *FGFR3* gene mutations may also promote the occurrence of intrahepatic biliary tract cancer [35]. Patients with cholangiocarcinoma carrying *FGFR2* fusion exhibit distinct characteristics: they are typically younger, female, and demonstrate a relatively prolonged median overall survival of 123 months compared to 37 months [34]. Conversely, the presence of coexisting mutations involving *TP53*, cyclin-dependent kinase inhibitor 2A (*CDKN2A*), and cyclin-dependent kinase inhibitor 2B (*CDKN2B*) has been linked to a shorter median overall survival [36]. Targeted therapy targeting *FGFR2* has become a potential therapeutic drug for the treatment of advanced BTC. Since the *FGFR2* gene contains a tyrosine kinase domain, the efficacy of related drugs has been observed in clinical trials (Table 2) [12,37–59].

Pemigatinib (approved by the European Medicines Agency) and infigratinib are selective reversible *FGFR2* inhibitors targeting *FGFR1* to *FGFR3*, but are less potent *FGFR4* inhibitors [43,45]. Of note, manufacturers will no longer produce Infigratinb. Futibatinib is an irreversible *FGFR* inhibitor that targets *FGFR1* to *FGFR4* and was approved by the Food and Drug Administration (FDA) in September 2022 [60]. Pemigatinib is a potent selective oral inhibitor for *FGFR* subtypes 1–3. The FIGHT-202 study, a sizable single-arm, open, multicenter phase 2 trial [61], investigated its efficacy among patients with advanced cholangiocarcinoma who had undergone prior treatments. The results of this study indicate that, among patients in the cohort with *FGFR2* fusion or rearrangement, the treatment achieved an objective response rate (ORR) of 37.0%, a disease control rate (DCR) of 82.4%, a median progression-free survival (mPFS) of 7.0 months, and a median overall survival (mOS) of 17.5 months. Moreover, treatment responders exhibited an extended median overall survival of 30.1 months. According to the data presented at the 2023 American Association for Cancer Research (AACR) annual meeting, the Chinese population also showed excellent efficacy, with ORR of 60%, mPFS of 9.1 months, and mOS of 23.9 months. Therefore, Pemigatinib was approved by the FDA and the National Medical Products Administration (NMPA) for the treatment of adult patients with advanced, metastatic, or unresectable cholangiocarcinoma who have previously received at least one systemic treatment and have been confirmed to have *FGFR2* fusion or rearrangement.

FOENIX-CCA2 was an international multicenter, open-label, single-arm phase 2 clinical trial. That included 103 intrahepatic cholangiocarcinoma (ICC) patients with an *FGFR2* gene mutation (78% for gene rearrangement, 22% for gene fusion) [44]. The enrolled patients had previously received gemcitabine plus platinum chemotherapy (but had not received other *FGFR* inhibitors) and exhibited disease progression. After a median follow-up of 17.1 months, 43 (42%) of the 103 patients reported for follow-up. Among them, 31 patients achieved sustained remission for more than six months. The median PFS was 9.0 months and the median OS was 21.7 months. The most common AEs were nail toxicity, musculoskeletal pain, constipation, diarrhea, and fatigue. In this study, circulating-tumor DNA (ctDNA) was used to detect tumor mutation characteristics, presenting a less invasive alternative to biopsy. The detection rate of *FGFR2* mutant ctDNA reached 87%, proving beneficial in screening individuals sensitive to FGFR inhibitors among patients with ICC.

In addition, at the 2023 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancer Conference, Dr. Shubham Pant released the relevant data of the phase 2 RAGNAR study (NCT04083976). At the data cut-off (median follow-up of 20.4 months), 35 patients with cholangiocarcinoma received Erdafitinib. The trial included 35 patients, three of whom had less common *FGFR* mutations, while the remaining 32 had *FGFR* fusion. Among them, the Independent Review Committee-certified (IRC-certified) objective response rate (ORR) reached 60.0%, with a median time to response (mTTR) of 1.5 months. Notably, treatment response was observed across patients with *FGFR* mutation, *FGFR* fusion, and those with co-mutations. The median DCR and clinical benefit rate

(CBR) were 100.0% and 71.4%, respectively. The median duration of response (DOR), PFS, and OS were 5.6 months, 8.4 months, and 18.7 months, respectively. The efficacy endpoints assessed by the researchers were consistent with the results of IRC certification. At present, three phase 3 clinical trials of *FGFR* inhibitors Futibatinib, Infigratinib, and pemigatinib are currently being compared against standard first-line chemotherapy, and follow-up research results need to be reviewed.

IDH1 and IDH2 Mutations

IDH plays an important role in the tricarboxylic acid cycle and has three subtypes: *IDH1*, *IDH2*, *IDH3* [33,62–64]. Isocitric acid converts into α-ketoglutaric acid under the action of *IDH1/2* to form nicotinamide adenine dinucleotide. However, when *IDH1/2* is mutated, isocitrate converts into 2-hydroxyglutarate (2-HG), resulting in epigenetic changes and ultimately contributing to tumorigenesis and disease progression [33]. *IDH1* often mutates at the pR132 site, and *IDH2* mutations are concentrated at the pR172 site [16]. In approximately 14% of intrahepatic BTC, *IDH* genetic aberrations were related to increased D-2-hydroxyglutarate concentrations. *IDH* drugs produce effects by inhibiting dioxygenases, hypermethylating DNA, and enhancing D-2-hydroxyglutarate which alters cell TP53 proteins. The ClarIDHy study was a phase 3 randomized controlled clinical study that aimed to evaluate the efficacy and safety of the *IDH1* inhibitor Ivosidenib versus placebo in patients with advanced *IDH1* mutant cholangiocarcinoma [65]. Patients were randomly assigned to either the Ivosidenib group (IVO) or the placebo group (PBO). The median PFS was 2.7 months for the IVO group and 1.4 months for the PBO group. Additionally, the median OS of the IVO group was 10.3 months, exceeding that of the PBO group (5.1 months). Among the two groups, ascites emerged as the most common grade $\geq$3 adverse event.

Patients with advanced cholangiocarcinoma and *IDH* mutations are more likely to exhibit an immunosuppressive microenvironment characterized by reduced T cell infiltration and diminished T cell cytotoxicity [63]. In patients with intrahepatic cholangiocarcinoma (iCCA), those with *IDH1* mutations exhibited a lower CD8+ T cell count within their tumors compared to individuals with *IDH1* wild-type iCCA. Additionally, immune-related signals were attenuated in *IDH1* mutant patients [66,67]. Therefore, the therapeutic effect of immunotherapy on *IDH1* mutant CCA patients may be less effective. Basic research reported in recent years has shown that Ivosidenib can improve the tumor immunosuppressive microenvironment of patients with *IDH1* mutation by inducing tumor immune interaction and interferon pathway activation, and Ivosidenib plus anti-CTLA4 monoclonal antibody can significantly induce tumor cell death while fostering a sustained tumor-specific T cell response [66]. The above suggests that Ivosidenib combined with immune checkpoint inhibitors may have a synergistic effect, warranting further exploration. At present, a phase 2 clinical study (NCT04056910) of Ivosidenib combined with nivolumab in the treatment of *IDH1* mutant solid tumors is underway, and the results of the study are expected to be published to provide more new treatment strategies.

HER2 Amplifications

HER2 is a member of the epidermal growth factor receptor (*EGFR*) kinase family. Typically, *HER2* is not expressed or expressed at low levels. After it is transformed into an oncogene, *HER2* will induce abnormal cell proliferation and promote tumorigenesis. A study showed that the overexpression rate of *HER2* in BTC was 26.5%, and the amplification rate was 30.1% [68]. Previous basic studies have shown that drugs targeting *HER2* can produce antitumor effects [69]. Moreover, *HER2* clinical studies have been performed consistently, with anti-*HER2* therapy producing positive outcomes in some cases.

HERIZON-BTC-01 is a global phase 2b clinical study that includes patients with *HER2*-amplified, locally advanced unresectable or metastatic bile duct cancer who have previously received gemcitabine-containing treatment and have not received *HER2* targeted therapy [70]. Patients were divided into two cohorts based on tumor immunohistochemistry (IHC) status: cohort 1 was IHC 2+/3+ (*HER2* positive) patients, and cohort 2 was IHC 0/1+ patients. The primary endpoint of the study was the ORR confirmed by the independent central review (ICR) in cohort 1. At present, 87 patients have been enrolled (cohort 1, n = 80; cohort 2, n = 7). In cohort 1, the ORR was 41%, the median PFS was 5.5 months, and the median DOR was 12.9 months. Among the 33 remission patients at the data deadline (October 10, 2022), 49% had sustained remission and 82% had DOR $\geq$16 weeks. The median TTR in this study was 1.8 months. Comparatively, no remission was observed in cohort 2. In both cohorts, 72% of patients experienced treatment-related adverse events (TRAE). The most common TRAEs, observed in $\geq$10% of patients, were diarrhea (37%) and infusion-related reactions (33%). In general, Zanidatamab showed a positive effect on *HER2*-positive patients (2 + or 3 +) with BTC. The drug not only works quickly, but also lasts for a very long time. Similar attempts have been made in other previous studies.

The MyPathway trial is a phase 2 “basket” study [52], which included 39 *HER2*-amplified/overexpressed BTC patients treated with trastuzumab and paltuzumab. The treatment was well tolerated, yielding an ORR of 23%, a mOS of 10.9 months, and a mPFS of 4.0 months, indicating that anti-HER2 therapy positively affects *HER2*-amplified/overexpressed cholangiocarcinoma. Trastuzumab deruxtecan (T-DXd) is a targeted *HER2* antibody and topoisomerase inhibitor conjugate. Based on data from the HERB study published by the ASCO in 2022 [71], patients with *HER2*-positive cholangiocarcinoma ex-

hibited an ORR of 36.4%, a DCR was 81.8%, a mOS was 7.1 months, and a mPFS of 4.4 months. It also shows that T-DXd has a therapeutic prospect in the treatment of *HER2*-positive cholangiocarcinoma. It is worth noting that efficacy was observed in one patient with low *HER2* expression in this study, but additional detailed studies are still needed to determine the befit of anti-*HER2* therapy on individuals with low *HER2* expression. However, there are still many challenges in future HER2-related clinical trials, such as insufficient sample size due to the small proportion of *HER2*-positive tumors in biliary tract tumors and the ambiguous definition of *HER2*-positive amplification.

NTRK Fusions

The neurotrophin receptor kinase (*NTRK*) gene encodes the tropomyosin receptor kinase. Fusion events involving the uncommon *NTRK* gene result in persistent activation of the receptor and subsequent activation of downstream signaling pathways, including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and Protein Kinase C (PKC). These mechanisms collectively contribute to the promotion of tumorigenesis [72]. The overall frequency of *NTRK* gene fusion was about 0.25% in cholangiocarcinoma [73] and about 3.5% [19] in intrahepatic cholangiocarcinoma. *NTRK* inhibitors larorectinib and entrectinib are FDA-approved drugs that can be used to treat cholangiocarcinoma patients with NTRK fusion. In previous reports, larorectinib demonstrated an ORR of 75%, while entrectinib showed a total ORR of 57%. Of note, partial remission was exhibited in two out of three CCA patients in key trials of these drugs [74,75].

RET Fusions

The presence of proto-oncogene tyrosine-protein kinase receptor Ret *(RET)* fusion in cholangiocarcinoma is very rare, with a prevalence of 0.15% in ICC and a prevalence of 0.11% in hilar and distal cholangiocarcinoma [76]. *RET* gene fusion leads to ligand-independent dimerization and continuous activation of *RET* kinases. Activation of downstream signaling pathways such as rats sarcoma viral oncogene homolog (RAS)/MARK, phosphatidylinositol 3-kinase (PI3K)/Protein Kinase B (PKB). Janus Kinase (JAK)/signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase γ (PLCγ) can cause excessive cell proliferation, which may lead to tumorigenesis [77]. The phase 1/2 ARROW trial evaluated the efficacy and selectivity of the *RET* inhibitor pralsetinib in 29 patients with advanced *RET*-altered solid tumors [78]. Of the three patients with cholangiocarcinoma included in the basket trial, two had partial remission and one had stable disease [78]. In the ongoing phase 1/2 LIBRETTO-001 basket trial [79], selpercatinib (another highly selective *RET* inhibitor) is evaluating the safety and efficacy of *RET* fusion-positive cancer patients (including two CCA patients). One patient with cholangiocarcinoma was included in the trial and the efficacy was partial response (PR).

$BRAF^{V600E}$ Mutations

The vrafmurine sarcoma viral oncogene homolog B (*BRAF*) gene encodes a serine/threonine protein kinase of the RAF protein family, which stimulates cell growth and survival through the mitogen-activated protein kinase (MAPK) signaling pathway. Mutations in $BRAF^{V600E}$ can lead to kinase activation, triggering a sustained activation of signaling pathways, thereby promoting tumorigenesis [80]. The frequency of *BRAF* gene mutation in biliary tract malignant tumors is generally low, and the current report is 1–7% [81–84]. The RORA study is a multicenter, single-arm, basket phase 2 study [85], which aims to explore the efficacy and safety of dabrafenib combined with trimetazidine in rare tumors with $BRAF^{V600E}$ mutation. The results showed that the ORR was 47% and the median PFS was 9.0 months. Based on data from ROAR and several other trials, the combination was approved by the FDA and can be used in previously treated patients with non-colorectal solid tumors, including patients with cholangiocarcinoma containing $BRAF^{V600E}$ mutation. Data supporting *BRAF* inhibitors as BTC monotherapy is limited. In the VE-BASKET study, a multi-cohort basket trial investigating the treatment of various *BRAF* mutant pan-cancer types with vemurafenib monotherapy, only 9 cases of BTC were included. Among these, merely 1 case showed nearly optimal shrinkage close to a partial response (PR), highlighting limited efficacy in this specific cohort. Despite the small sample size, the single target treatment is not beneficial to $BRAF^{V600E}$ mutation BTC, aligning with previous applications to colon cancer [86].

Homologous Recombination Deficiency

DNA damage is usually repaired by homologous recombination, and defects in this pathway lead to the accumulation of DNA damage, thereby promoting tumorigenesis. The molecular spectrum analysis of 1288 cases of BTC showed that Breast Cancer Susceptibility Genes 2 (*BRCA2*) mutations accounted for 3% of the samples analyzed, while *BRCA1* mutations accounted for 0.6% [87]. A multicenter retrospective study showed that the use of poly adenosine diphosphate (ADP)-ribose polymerase (*PARP*) inhibitors had better survival outcomes than platinum-based chemotherapy in 18 patients with *BRCA* mutant BTC [88]. The *PARP* inhibitor has been observed to be effective in *BRCA*-mutated ovarian, breast, and prostate cancers, but the efficacy and safety of the drug in patients with advanced BTC with *BRCA* mutations remain to be evaluated (NCT04042831, NCT03639935).

Table 2. Some trials in advanced biliary tract cancers.

Therapies		Study	Sample size (n)	Target	Complete response (%)	Partial response (%)	Stable disease (%)	Progressive disease (%)	Progression-free survival (months)	Median overall survival (months)
Chemotherapy	Capecitabine and Oxaliplatin	Kim ST *et al*. (2019) [37]; 32 phase 2	50	..	2%	12%	28%	32%	5.1	8.1
	FOLFOX	Lamarca *et al*. (2021) [12]; 16 phase 3	162: FOLFOX (n = 81); active symptom management (n = 81)	..	1%	4%	28%	37%	4	6.2 (FOLFOX); 5.3 (active symptom management)
	Liposomal Irinotecan Plus Fluorouracil and Leucovorin	Hyung J *et al*. (2023) [38]; 33 phase 2b	174: nal-IRI plus FU/LV (n = 88); FU/LV (n = 86)	..	0%	19% (nal-IRI plus FU/LV); 2% (FU/LV)	..	..	3.9 months (nal-IRI plus FU/LV); 1.6 Months (FU/LV)	8.6 months (nal-IRI plus FU/LV); 5.3 Months (FU/LV)
	FOLFIRINOX	Belkouz A *et al*. (2020) [39]; 34 phase 2	30	..	0%	10%	57%	33%	6.2	10.7
Targeted therapy	Surufatinib	Xu J *et al*. (2021) [40]; 35 phase 2	39	*EGFR*	0%	0%	81.50%	14.80%	3.7	6.9
	regorafenib	Sun W *et al*. (2019) [41]; 36 phase 2	43	*VEGFR*	0%	11%	44%	29%	3.9	7.9
	regorafenib	Demols A *et al*. (2020) [42]; 37 phase 2	33	*VEGFR*	0%	0%	74%	..	3	5.3
	Infigratinib	Javle M *et al*. (2021) [43]; 38 phase 2	108	*FGFR*	1%	22%	61%	10%	7.3	12.2
	Futibatinib	Goyal L *et al*. (2023) [44]; 39 phase 2	103	*FGFR*	1%	41%	41%	15%	9	21.7
	Pemigatinib	Abou-Alfa GK *et al*. (2020) [45]; 40 phase 2	107:*FGFR2* fusions or rearrangements	*FGFR*	3%	32%	46%	15%	6.9	21.1
	Derazantinib	Mazzaferro *et al*. (2019) [46]; 41 phase1/2	29	*FGFR*	0%	21%	62%	17%	5.7	..
	Erdafitinib	Bahleda *et al*. (2019) [47]; 42 phase1	11	*FGFR*	0%	27%	27%	45%	5.1	..
	Ivosidenib	Abou-Alfa GK *et al*. (2020) [48]; 43 phase3	185	*IDH1*	0%	2%	51%	33%	2.7	10.8
	Ramucirumab	Lee S *et al*. (2022) [49]; 44 phase 2	61	*VEGFR-2*	0%	2%	42%	43%	3.2	9.5
	Apatinib	Zhang G *et al*. (2021) [50]; 45 phase 2	26	*VEGFR-2*	4%	17%	42%	37%	3.2	8.3
	Apatinib	Wang C *et al*. (2021) [51]; 46 phase 2	20	*VEGFR-2*	0%	15%	45%	40%	2.7	4.8
	Trastuzumab and Pertuzumab	Javle M *et al*. (2021) [52]; 47 phase 2a	39	*HER2*	0%	23%	51%	26%	4	10.9
Immunotherapy	Pembrolizumab	Piha-Paul SA *et al* (2020) [53]; 48 phase 2	104	PD-1	0%	5.80%	16.30%	62.50%	2	7.4
	bintrafusp alfa	Yoo C *et al*. (2023) [54]; 49 phase 2	159	PD-1, TGF-β	1.90%	8.80%	11.90%	66.00%	1.8	7.6
	Nivolumab	Kim *et al*. (2020) [55]; 50 phase 2	54	PD-1	0%	22%	38%	..	4	14.2

Table 2. Continued.

Therapies		Study	Sample size (n)	Target	Complete response (%)	Partial response (%)	Stable disease (%)	Progressive disease (%)	Progression-free survival (months)	Median overall survival (months)
Combination therapies	Allogeneic NK Cell ("SMT-NK") and Pembrolizumab	Leem G *et al.* (2022) [56]; 51 phase 1/2a	8	PD-1	0%	50%	12.50%	37.50%	4.1	..
Pembrolizumab	Pembrolizumab in Combination with Capecitabine and Oxaliplatin	Monge C *et al.* (2022) [57]; 52 phase 2	11	PD-1	0%	27%	55%	18%	4.1	9.9
	Varlitinib and capecitabine	Javle MM *et al.* (2022) [58]; 53 phase 2	127: Varlitinib and capecitabine (n = 81); Placebo and capecitabine (n = 81)	*EGFR*, *HER-2*, *HER-4*	0%	9%	45%	37%	2.8	7.8
	MEK162 and Capecitabine	Kim JW *et al.* (2019) [59]; 54 phase 1b	34	*MEK1/2*	0%	20%	56%	24%	4.1	7.8

FOLFOX, fluorouracil plus leucovorin and oxaliplatin; FOLFIRINOX, fluorouracil, irinotecan, leucovorin, and oxaliplatin; EGFR, epidermal growth factor receptor; nal-IRI, irinotecan liposome injection; FU/LV, fluorouracil and leucovorin; VEGFR, vascular endothelial growth factor receptor; PD-1, programmed cell death protein 1; TGF-β, transforming growth factor-β; MEK1/2, mitogen-activated protein kinase kinase1/2.

Table 3. Some ongoing clinical studies evaluating second-line treatment for advanced biliary tract cancers.

Therapies		Phase	Pathways targeted	Primary outcomes	Secondary outcomes	Trial number
Chemotherapy	Cisplatin and nab-paclitaxel	2	Chemotherapy	ORR	PFS, OS, DCR, AE	NCT04111380
	Raltitrexed and oxaliplatin	2	Chemotherapy	PFS	ORR, OS, DCR, QoL, AE	NCT05148143
	Nanoliposomal, Irinotecan, eucovorin and Fluorouracil	2	Chemotherapy	The efficacy	ORR, mPFS, mOS, mTTP, DCR, DDC, CA19-9	NCT04005339
	TRIFLURIDINE	2	Chemotherapy	PFS	Safety and tolerability, DCR, DOR	NCT04076761
Targeted therapy	Fruquintinib	2	VEGFR	PFS	ORR, DCR, OS, Safety and Tolerability	NCT04156958
	Surufatinib vs capecitabine	2–3	MAPK	OS	PFS, DCR, duration of response	NCT03873532
	Tivozanib	1–2	VEGFR	Phase1: Determine safety and establish the recommended Phase II dose Phase2: ORR	OS, DCR	NCT04645160
	Olaparib	2	*IDH1* or *IDH2*	ORS	PFS, OS, duration of response	NCT03212274
	Larotrectinib	2	Neurotrophic tyrosine receptor kinase fusions	ORR by radiologic Criteria	PFS, OS, duration of response	NCT02576431
	Entrectinib	2	Neurotrophic tyrosine receptor kinase fusions	ORR	PFS, duration of response, time to response	NCT02568267
	E7090	2	*FGFR2*	ORR	PFS, DOR, TTR, OS, DCR, CBR	NCT04238715

Table 3. Continued.

Therapies		Phase	Pathways targeted	Primary outcomes	Secondary outcomes	Trial number
Immunotherapy	Tumor-infiltrating lymphocytes	2	Cell therapy targeting tumor microenvironment	ORR	Duration of response, DCR, OS, PFS	NCT03801083
Combination therapies	TQB2450 and Anlotinib	3	VEGFR, PDGFR, *FGFR*, PD-1	OS	PFS, ORR, DCR, DOR, Progression-free survival at 6 months, Overall survival at 6 months, Overall survival at 12 months	NCT04809142
	Toripalimab and Lenvatinib	2	VEGFR, *FGFR*, PD-1	ORR, AE	OS, PFS, SD, CBR, 6-month Progression-free survival rate and 1-year mortality rate	NCT04211168
	Axitinib and Toripalimab	2	VEGFR, PD-1	ORR, PFS	DCR, OS, DOR, SD, Progression-free survival rate, Rate of 6 months and 1-year overall survival	NCT04010071
	Nal-irinotecan, fluorouracil, leucovorin, and nivolumab	1b-2	hemotherapy and PD-1	C Phase 1b: DLT; phase 2: PFS	Adverse events, ORR, OS	NCT03785873
	mFOLFOX6, Atezolizumab and Bevacizumab	1b-2	Chemotherapy, *VEGF* and PD-L1	ORR	DCR, DOR, BORR, PFS, TTF, ORR, OS, AE, Best percentage change from baseline in tumor size	NCT05052099
	Binimetinib and FOLFOX	2	*MEK1/2*	OS	ORR, PFS, DOR, Clinical benefit, adverse events	NCT05564403
	AZD6738 and Durvalumab	2	*ATR*, PD-L1	DCR	ORR, PFS, DOR, OS, Safety and tolerability quality of life measurement	NCT04298008
	Pembrolizumab and Lenvatinib	2	*VEGF*, PD-1	ORR	safety and tolerability, DOR, PFS, OS	NCT04550624
	Rucaparib and nivolumab	2	*PARP*, PD-1	4-month survival, clinical and radiological response at 4 months	Treatment response (CR, PR, SD), PFS, OS	NCT03639935
	Nivolumab and ipilimumab	2	PD-1, CTLA-4	ORR	Safety, OS, PFS, DCR	NCT02834013
	Durvalumab, tremelimumab, and Radiotherapy	2	PD-L1, CTLA-4, radiation	ORR (CR, PR)	Adverse events, OS, DCR, PFS, duration of response	NCT03482102
	Nal-irinotecan, fluorouracil, leucovorin, and nivolumab	1b–2	Chemotherapy and PD-L1	Phase 1b: DLT; phase 2: PFS	Adverse events, ORR, OS	NCT03785873
	Bevacizumab, floxuridine	2	*VEGF*, chemotherapy	PFS	Use of dynamic contrast MRI, correlate expression of VEGFR markers and response and dexamethasone	NCT00410956

PFS, progression-free survival; mPFS, median PFS; OS, overall survival; mOS, median OS; FOLFOX, oxaliplatin, fluorouracil, and leucovorin; DCR, disease control rate; ORR, overall response rate; BORR, best ORR; PR, partial response; CR, complete response; SD, stable disease; AE, adverse event; QoL, quality of life; mTTP, median time to progression; DDC, duration of disease control; DOR, duration of response; TTR, time to response; CBR, clinical benefit rate; TTF, time to failure; DLT, dose-limiting toxicities; PD-L1, Programmed cell death-ligand 1.

KRAS Mutations

In patients with cholangiocarcinoma, the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation rate can be as high as 26% [89]. A study assessing adagrasib's efficacy and safety in patients with solid tumors with the KRAS G12C mutation [90] included 12 subgroups with refractory biliary cancer and this specific mutation. Results demonstrated an ORR of 41.7%, a DCR of 91.7%, a median PFS of 8.6 months, and a median OS of 15.1 months. Other related studies are ongoing.

Mismatch Repair Deficiency, Microsatellite Instability, Checkpoint Ligands and Immunotherapies

Some evidence suggests that high tumor mutational burden (TMB) is a predictive marker of immunotherapy response or a prognostic factor. A study of 119 patients who received gemcitabine combined with cisplatin showed that there was no significant difference between TMB status and clinical outcomes such as median PFS, and whether TMB-H had no effect on median OS [91–93]. The prevalence of mismatch repair (MMR)-deficient (MMR-d) or microsatellite instability (MSI) in BTC is less than 5% [94]. One study showed that more than 10 mutations could be detected in less than 5% of the samples [95]. High microsatellite instability (MSI-H) is rare (1%) and occurs mostly in tumors with many mutations. Programmed cell death-ligand 1 (PD-L1) was positive in 9% of tumors, and PD-L1 amplification was found in 0.27% of tumors. Based on similar studies on other tumor types, checkpoint inhibitors may be a reasonable choice for advanced BTC containing MSI and mismatch repair defects [96–98]. Immunotherapy still occupies a place in second-line treatment. According to the results of Clinical Trials registration study NCT02829918 [53]. For unresectable or advanced biliary malignant tumors with positive PD-L1 expression and failed first-line treatment, nivolumab monotherapy is recommended as a potential option. According to the results of Clinical Trials registration study NCT03797326 (LEAP-005 trial) [99] and Clinical Trials registration study NCT03895970 [100], it is recommended that pembrolizumab combined with lenvatinib can be used as a post-line exploratory treatment for unresectable or advanced BTC. Although numerous related phase 1 and 2 clinical trials have proven the high safety of immune checkpoint inhibitors, the adverse events related to immune checkpoint inhibitors may involve any organ or system of the body, among which gastrointestinal tract, skin, liver, endocrine, and lung are more common [101–104]. Considering that BTC is commonly associated with abnormal liver function, it is necessary to pay close attention to the risk of adverse events with immune checkpoint inhibitors.

Potential Challenges and Development Direction of Second-Line BTC Treatment

First of all, tumor heterogeneity limits the efficacy of second-line treatments to specific patient groups. Additionally, drug resistance can develop during tumor treatment. Tumor cells often develop new mutations or change signaling pathways to resist drug action, making previously effective targeted drugs lose efficacy. Therefore, it is particularly important to study the mechanism of drug resistance in the treatment of biliary tract tumors. At present, the research on the resistance mechanism of targeted therapeutic drugs mainly focuses on epigenetic regulation, apoptosis, DNA repair, and transcriptional regulation. Studies have found various drug resistance mechanisms in biliary tract tumors, including gene mutation, epigenetic regulation changes, and abnormal expression of drug-metabolizing enzymes [105]. Therefore, the study of these drug resistance mechanisms will help to further improve the targeted treatment strategy of biliary tract tumors. Finally, since targeted therapy is based on the molecular feature selection of tumor cells, targeted therapy may be ineffective or inapplicable for tumors without specific target changes. In addition, due to the relatively low incidence of biliary tract malignant tumors, it is difficult to recruit enough large-scale clinical trial participants, which limits the research progress of second-line treatment.

Researchers can continue to explore the molecular mechanisms and signaling pathways of tumors and find more targeted therapeutic targets to provide more individualized treatment options. Due to the heterogeneity and drug resistance of tumors, the combination of multiple targeted drugs may interfere with multiple key signaling pathways simultaneously, reducing the possibility of tumor escape. Therefore, the development of multi-target combination therapy strategies is also one of the future development directions of targeted therapy [106]. Additionally, identifying and validating clinical markers for targeted therapy enhances understanding of patient response to specific drugs, improving treatment precision and customization for each individual patient [107]. Immunotherapy has been applied to the treatment of BTC, and combined targeted therapy may have a favorable therapeutic effect [108]. However, there are few studies on targeted therapy combined with immunotherapy, and related research may be needed in the future. Researchers have tested new treatments such as cell therapy in BTC patients. Cell therapy refers to the use of bioengineering methods to obtain and/or process some cells with specific functions, so that these cells can enhance immunity and kill tumor cells, with the purpose of treating tumors. A certain therapeutic effect was observed in a patient with gallbladder cancer who received dendritic cell vaccination combined with sequential anti-angiogenesis therapy, including a three-year survival period [109]. The emergence of new treatment methods has brought new hope to patients with advanced stage BTC.

Conclusions

In summary, clinical trials of traditional chemotherapy and new targeted therapy and immunotherapy alone or in combination have been carried out in patients with advanced BTC who have previously received treatment (Table 3).

The core principle of customizing treatments based on patient characteristics relies on understanding the pharmacological mechanisms of new drugs, predictive biomarkers in the tumor microenvironment, and effectively managing drug-related toxicities. This approach aims to optimize patient care and maintain their quality of life during treatment. In the future, the use of the most advanced next-generation sequencing technology and teamwork with clinicians, basic science, and translational science researchers will determine more detailed potential therapeutic targets and possible drug resistance mechanisms to further benefit patients.

Author Contributions

Conceptualization was contributed by QL and PFZ; methodology was contributed by QLL; writing—original draft preparation was contributed by QLL and PFZ; writing—review and editing were contributed by QL and PFZ; funding acquisition was contributed by QL and PFZ; supervision was contributed by QL and PFZ. All authors have read and agreed to the published version of the manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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