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REVIEW

Promising Molecular Targets for the Targeted Therapy of Biliary Tract Cancers: An Overview

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Abstract: Biliary tract cancer (BTC) is a leading cause of cancer-related death, due to the limited benefits of current systematic therapies and the heterogeneity of the tumor itself. High heterogeneity means that the clinical and molecular features vary between different subtypes of BTC, while the underlying molecular mechanisms remain unclear. Targeted therapy, where inhibitors are developed to selectively combine with targeted molecules in order to block abnormal signaling pathways in BTC, has shown promise as an emerging form of treatment for various types of cancer. In this article, a comprehensive review is conducted to examine potential molecular targets for BTC targeted therapy and their mechanisms. Furthermore, preliminary data published from clinical trials is utilized to analyze the main drugs used to combat BTC. The collective information presented in this article has provided useful insights into the current understanding of BTC.

Keywords: biliary tract cancer, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder carcinoma, targeted therapy

Introduction

Biliary tract cancer (BTC), originating from the epithelium of biliary duct systems, is the second most common hepatobiliary cancer and the fifth most common malignancy of digestive system cancers. Based on anatomy, BTC is mainly classified into gallbladder cancers (GBCs), cholangiocarcinomas (CCAs) and the ampullary carcinoma (also called carcinoma of the ampulla of Vater). Cholangiocarcinomas include intrahepatic cholangiocarcinomas (iCCAs) and extrahepatic cholangiocarcinomas (eCCAs), and eCCAs can be further divided into perihilar and distal CCAs.[1] Histologically, BTCs include several types: adenocarcinomas, papillary carcinoma, mucinous carcinoma and squamous cancers. Particularly, adenocarcinomas are the most common histologic types (more than 95%) with poor differentiation.[1,2]

These tumors are rare but malignant with a poor prognosis. The incidence of BTCs varies among different areas of the world. BTCs are relatively common in Southeast Asia and South America, with up to 96 cases per 100,000 people, while their incidence is lower in western countries such as Canada (0.5 to 1.5 cases per 100,000 people).[3,4] This phenomenon can be explained by the uneven distribution of risk factors. Moreover, pathogenesis, clinical manifestation and management of different subtypes of BTCs are distinct. Based on some studies, the incidence of iCCAs is increasing, while eCCAs have been in decline.[4–6]

According to previous studies, a number of risk factors might be significant in biliary tract carcinogenesis. Primary sclerosing cholangitis, choledochal cysts,

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Caroli's disease, cirrhosis, congenital fibropolycystic liver disease, Hepatitis B (HBV) and hepatitis C (HCV) – which could cause chronic injury of the hepatobiliary system – are prominent risk factors for BTCs.[7,8] Bile duct adenomas, biliary papillomatosis and intrahepatic biliary stones are also demonstrated factors which contribute to BTCs.[9] Patients with Lynch syndrome and breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) genetic aberrations might also be at higher risk for BTCs.[10,11] In northern Thailand, liver fluke infestation, particularly the *Opisthorcis viverrini* (OV), is considered an enhanced risk of CCA.[12] Some other potential contributing factors may include chemicals (eg, Thorotrast), excess alcohol, obesity and smoking.[8,9]

Patients with BTCs are characterized by weight loss, fever, jaundice and pain, and these tumors aggressively lead to a quick deterioration of patient performance status.[13] However, in early stages, most patients with BTCs are asymptomatic with no sensitive biomarker for biliary tract tumors, so it is difficult for the disease to be assessed and treated in time. Accordingly, the global five-year survival rate is only about 10%.[14]

Current treatments for BTCs mainly include surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy. Surgery is the first choice for early-stage BTCs. Radical surgery with lymphadenectomy is the only potential treatment to cure localized BTCs. However, less than 35% of BTC patients are diagnosed at an early enough stage to be amenable to surgery.[15] Furthermore, even when the early-stage tumors are resected, their relapse rate is very high and the rate of prolonged survival is low.[16] Tumor location, pathological type, lymph node invasion and vascular invasion all affect survival after surgical resection. The 5-year overall survival rate for patients after iCCA resection ranges from 39.8% to 48.6%.[17,18] Patients with localized biliary tract tumors can also be treated by radio-embolization, chemoembolization and radiotherapy, even though they are not adopted in standard treatment procedures.

Most new cases of BTC are diagnosed at an advanced stage, where the tumors are unresectable and the main treatment option is chemotherapy. Biliary tract cancer is chemotherapy responsive. For first-line treatment, the combination of gemcitabine and cisplatin (GEMCIS) is the standard of care. The superiority of GEMCIS was proved by a Phase III randomized clinical trial, ABC-02. BTC patients in the GEMCIS group had prolonged mOS (11.7 vs 8.1 months, P<0.001) and median progression-free survival (mPFS) (8.0 vs 5.0 months, P<0.001) compared to gemcitabine monotherapy with tolerant toxicity. The rate of tumor control of the GEMCIS group was 81.4%, which was higher than that of the gemcitabine monotherapy control group (71.8%) (NCT00262769).[19] In another Phase II study, encouraging antitumor activity suggests gemcitabine plus capecitabine might be an alternative treatment for BTC patients - the mOS was 14 months, the mPFS was 7 months, and patients achieved a disease control rate (DCR) of 73%.[20] Gemcitabine plus oxaliplatin (GEMOX) regimen was also assessed in a phase II study as first-line chemotherapy showing marginal improvement.[21] Recently, active antitumor activity of oral fluoropyrimidine, S-1, plus gemcitabine (GS) was confirmed for advanced BTC in a phase II clinical trial. The one-year survival, OS, PFS and response rate (RR) were all superior in the experimental arm (S-1 plus gemcitabine) compared to the S-1 monotherapy group.[22] Consequently, a phase III randomized clinical trial was conducted to assess and compare the efficacy and safety of the GS and GEMCIS regimens for BTC patients.[23] Through March 2016, 354 patients were recruited. The reported mOS was 13.4 months for GEMCIS and 15.1 months for GS therapy, and median PFS also showed the superiority of the GS regimen compared with GEMCIS (6.8 vs 5.8 months). Both regimens had good safety profiles.[24] Therefore, S-1 plus gemcitabine might become an emerging standard of care for advanced BTC patients who cannot be treated with platinum agents. A new combination chemotherapy regimen, GEMCIS plus nab-paclitaxel, was tested in a phase II study as first-line treatment in patients with advanced BTC. Based on the published data, nab-paclitaxel plus GEMCIS therapy achieved prolonged mPFS (11.8 months) and mOS (19.2 months) compared to data from previous studies where BTC patients were treated with GEMCIS only. To confirm these findings, a phase III trial will be carried out.[25]

Currently, there is no standard second-line chemotherapy for BTCs. Due to the quickly worsening performance status after first-line setting, the effectiveness of second-line treatments are limited.[26] A randomised phase II study showed prolonged median overall survival (mOS) and median progression-free survival (mPFS) with well-tolerated toxicity indicated an obvious advantage for the second-line XELIRI regimen (irinotecan and capecitabine) compared with irinotecan monotherapy (NCT02558959).[27] ABC-06 is a completed phase III clinical trial (NCT01926236) which aimed to determine

whether patients with advanced BTC could benefit from chemotherapy (Oxaliplatin, L-folinic acid plus 5 FU) in the second-line treatment. The experimental arm (active symptom control plus chemotherapy) showed an improved mOS (6.2 months vs 5.3 months) and 12-month OS-rate (25.9% vs 11.4%) compared to the control arm (active symptom control only).[28]

Although chemotherapy is a mainstay of treatment for advanced BTCs, its marginal benefits and relatively severe toxicity may cause adverse effects and diminish the life quality of cancer patients. In the last ten years, targeted therapy has grown increasingly popular due to its better safety profiles and efficiency. The existence of next-generation sequencing and genetic studies shed insight on the molecular mechanism of pathogenesis and its relative molecular signaling pathways in BTCs.

Based on several studies, various genetic aberrations are considered exclusive to the anatomical location of the BTC. In iCCA, the most frequent genomic alterations are TP53 (27%), CDKN2A/B (27%), K-Ras (22%), ARID1A (18%), and IDH1/2 (19%).[29,30] K-Ras (42%), TP53 (40%), CDKN2A/B (17%), and SMAD4 (21%) gene aberrations are most common in eCCA, and TP53 (59%), CDKN2A/B (19%), ARID1A (13%), and ERBB2 (16%) are the top four genomic alterations in GBC.[29] Particularly, IDH1/2 and FGFR 2 fusion are almost limited to iCCA, with BAP1 gene alteration also being relatively common. On the contrary, ERBB2 and TP53 mutations are more common in eCCA and GBC than in iCCA. PRKACA or PRKACB fusion was exclusively identified in eCCA, and EGFR, ERBB3 and PTEN mutations specifically occurred in GBC.[30–32] TP53 and K-Ras mutations indicated poor prognosis of the BTC.[29,33]

The discovery of the genetic aberrations which might drive the pathogenesis of tumors has promoted the development and application of personalized medicine. Molecular target drugs' active efficacy has been tested for several kinds of cancers, either as monotherapy or in combination with other antitumor drugs, but a compelling targeted agent for treating BTCs with satisfactory clinical activity has not currently been found, though a large number of basic studies and clinical trials are ongoing.

In this article, we summarized the current targeted therapy of BTCs and reviewed the mechanisms and clinical trials of several promising therapeutic biomarkers which might be targetable in BTCs.

Therapeutic Targets in Biliary Tract Cancers

With targeted therapy becoming the mainstay treatment, identification of molecular alterations and the specific molecules expressed by cancer cells can guide research and treatment. The following represent the most promising targets for BTC targeted therapy.

Fibroblast Growth Factor Receptors (FGFRs)

Fibroblast growth factor receptors (FGFRs) are a family of receptor tyrosine kinases (RTKs), which carry out essential physiologic functions involving cell proliferation, differentiation, migration, and apoptosis. There are four members in the FGFR family: FGFR1, FGFR2, FGFR3 and FGFR4.[34,35] The four FGFRs share a high homologous structure, containing an extracellular ligand-binding domain (D1, D2 and D3 immunoglobulin (Ig)-like subunits), a single transmembrane helix and an intracellular tyrosine kinase domain.[36,37] There are over 48 receptor isoforms caused by alternative splicing of the four FGFR genes, which differ in ligand-binding and kinase domains.[38] FGFRs are located in the cell membrane and can be activated by extracellular signals. The ligand-binding domains can interact with fibroblast growth factors (FGFs), which drive the homodimerization or heterodimerization to subsequently activate the kinase domain and then induce the intracellular cascades. Consequently, FGFRs achieve their physiologic functions in human body.[39–41]

FGFR2 fusions, the major FGFR gene abbreviations, are frequently found in iCCAs with an incidence of 10–45%, but they are rare in eCCAs (less than 5%).[42,43] This phenomenon implies the different pathophysiological features between various anatomical parts in the biliary tract system. FGFR2 fusion proteins could be activated by the dimerization of their respective partners, thus inducing the activation of downstream oncogenic signaling pathways including RAS-RAF-MEK-ERK/MAPK, PI3K/AKT/mTOR and JAK/STAT pathways. Particularly, the MAPK signaling pathway is involved in increased cancer cell motility.[44–46]

In recent years, numerous studies have illustrated that the FGFR inhibitors play a role in suppressing the growth of biliary tract tumors in cancer patients with FGFR2 gene fusions, particularly inhibiting iCCA development. Several preclinical studies also demonstrated anti-tumor efficacy in

murine models, so many FGFR inhibitors have been tested in clinical trials (Table 1).

So far, the FGFR inhibitors that have entered clinical trials could be divided into three groups: selective tyrosine kinase inhibitors (TKIs), non-selective tyrosine kinase inhibitors (TKIs), and monoclonal antibodies (mAbs).[47]

Selective TKIs

BJG398 (Infigratinib)

BGJ398 (Infigratinib), a selective FGFR kinase inhibitor against FGFR 1–4, exhibited effective therapeutic activity against intrahepatic cholangiocarcinoma harboring FGFR2 fusions.[48,49] According to several preclinical studies, BGJ398 performed well in suppressing tumor growth in

Table 1 Clinical Trials Involving FGFR Targeted Therapy in BTC

Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
BGJ398 (Infigratinib)	NCT02150967	Recruiting	II	CCA with FGFR2 mutation	160	BGJ398
	NCT03773302	Recruiting	III	CCA with FGFR2 mutation	384	BGJ398 vs GEMCIS
	NCT04233567	Recruiting	II	CCA with FGFR2 fusion	50	BGJ398
ARQ087 (Derazantinib)	NCT01752920	Completed	II	iCCA with FGFR2 fusion, Other solid tumor types with FGFR alterations	119	ARQ087
	NCT03230318	Recruiting	II	iCCA, Combined hepatocellular and CCA	143	ARQ087
TAS-120 (Futibatinib)	NCT04093362	Not yet recruiting	III	CCA with FGFR2 rearrangements	216	TAS-120 vs GEMCIS
	NCT02052778	Recruiting	I II	CCA, Other solid tumor types with FGFR2 gene fusions	371	TAS-120
Pemigatinib (INCB054828)	NCT02924376	Active, not recruiting	II	CCA	147	Pemigatinib
	NCT03656536	Recruiting	III	CCA	432	Pemigatinib vs GEMCIS
	NCT04256980	Recruiting	II	CCA	54	Pemigatinib
	NCT04088188	Not yet recruiting	I	CCA	40	Pemigatinib plus GEMCIS vs Ivosidenib plus GEMCIS
	NCT02393248	Recruiting	I II	CCA, Other solid tumor types	325	Pemigatinib plus GEMCIS vs Pemigatinib plus Pembrolizumab vs Pemigatinib plus Docetaxel vs Pemigatinib plus Trastuzumab vs Pemigatinib plus INCMGA00012
Erdafitinib (JNJ-42756493)	NCT02699606	Recruiting	II	CCA, Other solid tumor types	63	Erdafitinib
Pazopanib	NCT01855724	Terminated	II	BTC	29	Pazopanib plus Gemcitabine
	NCT01438554	Completed	I	CCA Thyroid Cancer Soft-tissue Sarcoma	89	Pazopanib plus GSK1120212
Ponatinib	NCT02265341	Completed	II	BTC	12	Ponatinib
Dovitinib	NCT01497392	Completed	I	BTC Pancreatic Cancer	26	Dovitinib plus Gemcitabine plus Capecitabine

preclinical CCA models with a well-tolerated safety profile.[48] A single-arm phase II clinical trial assessed the therapeutic activity of BGJ398 in 61 patients with advanced cholangiocarcinoma containing FGFR alterations, including FGFR2 fusions, mutations and amplifications (NCT02150967). The results reported an overall response rate (ORR) of 14.8% and a disease control rate (DCR) of 75.4%. The mPFS was 5.8 months, which is comparable to first-line chemotherapy. BGJ398 had promising anti-tumor activity especially in patients with FGFR2 fusions.[50,51] Recently, based on this encouraging data, a phase III random controlled trial has started to recruit subjects with cholangiocarcinoma containing FGFR2 gene alterations to evaluate the efficacy and safety of BGJ398 versus chemotherapy (NCT03773302). A phase II clinical trial was also initiated to further explore the anti-tumor activity of BGJ398 in CCA patients with FGFR2 fusions (NCT04233567).

ARQ 087 (Derazantinib)

ARQ 087, also known as Derazantinib (DZB), is a multi-tyrosine kinase inhibitor targeting FGFR1 to 4.[52,53] According to preclinical research, ARQ 087 has displayed prominent inhibitory effects in vivo xenograft models[52,54] and in vitro CCA cell lines,[54,55] which indicates its potential therapeutic efficacy. The first phase I/II clinical trial for ARQ 087 has completed (NCT01752920). In the beginning, this study recruited 80 patients with advanced solid tumors, including 12 iCCA patients. Among the 12 iCCA patients with FGFR2 fusions, 2 patients had PRs and one confirmed a stable disease (SD). This study showed the tolerant toxicity of ARQ 087 and confirmed its therapeutic effect in advanced cancer patients with FGFR gene alterations, particularly iCCA patients.[53] Based on the promising preliminary data, more patients with FGFR2 gene fusion positive advanced iCCA were enrolled; they took derazantinib according to the recommended Phase 2 dose (RP2D). Mazzaferro et al reported the results: among 29 FGFR2 gene fusion-positive iCCA patients, the median PFS was 5.7 months, the ORR was 20.7%, and the DCR was 82.8%.[56] This data suggests that Derazantinib might be a good drug for treating iCCA patients. Another phase II study for Derazantinib targeting FGFR2 fusion positive iCCA is ongoing (NCT03230318).

Futibatinib (TAS-120)

Futibatinib (TAS-120) is an irreversible and highly selective inhibitor which targets all four FGFR subtypes.[57] A clinical study reported that TAS-120 showed therapeutic effects in four iCCA patients with FGFR2 fusions who were resistant to the other two FGFR inhibitors (BGJ398 and Debio1347).[58] TAS-120 is under phase I/II clinical trials (NCT02052778) investigating its safety and efficacy. According to the recently published data, the disease control rate (DCR) was 75%, indicating promising clinical benefits. The toxicity of TAS-120 is also manageable.[59] Another Phase 3 study will begin to assess the efficacy and safety of TAS-120 versus gemcitabine-cisplatin chemotherapy in advanced FGFR2-alteration-positive iCCA patients as first-line treatment (NCT04093362). Futibatinib is a highly selective irreversible FGFR antagonist, which means it has durable activity. Several trials have showed its meaningful benefit in patients with pretreated iCCA with FGFR2 gene aberrations, thus it might be a promising agent for BTCs treatment.[60]

Pemigatinib (INCB054828)

Pemigatinib (INCB054828) is a reversible and selective inhibitor of FGFR 1, FGFR2 and FGFR3.[35,61] Pemigatinib has potential in the treatment of cholangiocarcinoma. A preclinical cell-based study revealed that the cells harboring FGFR2-CLIP1 fusion responded noticeably to Pemigatinib, whereas cells with FGFR2-CLIP1 fusion and N549H mutation both were resistant to this drug.[62] There is a large-scale single-arm phase 2 trial (FIGHT-202) assessing the safety and therapeutic activity of Pemigatinib in cholangiocarcinoma patients with and without FGFR2 fusions or rearrangements (NCT02924376). One hundred and seven of the enrolled 146 patients harbored FGFR2 gene fusions or rearrangements, and this group of patients showed a remarkable objective response: 35.5% (95% CI: 26.5–45.4) of patients achieved objective response (3 had complete responses and 35 had partial responses), and the disease control rate (DCR) was 82% (95% CI: 74–89). Median PFS was 6.9 months (95% CI 6.2–9.6) and median OS was 21.1 months. On the contrary, the groups of patients with other FGFR alterations or without FGFR alterations did not achieve any response.[63] Based on these encouraging results, a phase 3 clinical trial (FIGHT-302; NCT03656536) is ongoing to compare pemigatinib with chemotherapy (gemcitabine plus cisplatin) for advanced CCA patients with FGFR2 rearrangements.

A host of selective TKIs for FGFR, including AZD4547, CH5183284 (Debio 1347), JNJ-42756493, BAY1163877, and dovitinib, are currently under examination in early-phase trials.

Nonselective TKIs

Apart from selective FGFR inhibitors, there are also several non-selective FGFR inhibitors entering clinical trials.

Pazopanib

Pazopanib is a multi-kinase inhibitor mainly targeting VEGFR, PDGFR, c-Kit, FGFR, and c-Fms.[64] The anti-tumor effect of pazopanib has been demonstrated in preclinical research. An in vitro study illustrated that the number of cells in gastric cancer cell lines containing FGFR2 gene amplifications would decrease significantly after being treated with pazopanib.[65]

A phase II multicenter trial was conducted to evaluate the therapeutic efficacy of a gemcitabine and pazopanib combination therapy in 29 advanced biliary tract carcinoma (BTC) patients. 13.8% of enrolled patients in the ITT (intent-to-treat) group and 19.1% in the per protocol (PP) population achieved complete response or partial response. The disappointing objective response rate terminated this trial and prevented more clinical trials from assessing this therapeutic regimen (NCT01855724).[66] A Phase I clinical trial evaluated the clinical benefits of the combination of pazopanib with trametinib (an MEK inhibitor) for several kinds of solid tumors including Cholangiocarcinoma (NCT01438554).

Ponatinib

Ponatinib is also defined as a multi-TKI because it can target many kinds of tyrosine kinase, such as FGFR 1 to 4, VEGFR, PDGFR, FLT3 and c-SRC.[67] In a study, a patient with CCA and FGFR2-MGEA5 fusion took ponatinib, finally achieving preliminary anti-tumor activity.[68] Furthermore, meaningful clinical benefits were also verified in another CCA patient with FGFR2-TACC3 fusion who took pazopanib and ponatinib.[68] According to the data published on clinical trials.gov, a completed phase II trial of 12 BTC patients with FGFR fusions reported a disease control rate of 45.5% (95% CI: 16.8 to 76.6), progression-free survival (PFS) of 2.4 months and overall survival (OS) of 15.7 months (NCT02265341).

Besides the drugs mentioned above, dovitinib and lenvatinib are also non-selective TKIs entering clinical trials. However, due to the non-selective activity, these drugs may lead to severe toxicities on the cardiovascular system related to VEGFR inhibition, which limits the long-term use of non-selective FGFR inhibitors.[69]

mAb

In addition to TKIs, monoclonal antibodies (mAbs) are another group of FGFR inhibitors. They can target FGFR with a higher specificity than TKIs, which may result in a better safety profile for patients. However, only a few mAbs have entered clinical trials.[47]

Bemarituzumab (FPA144)

Bemarituzumab (FPA144) is a humanized IgG1 monoclonal antibody specific to the FGFR2b isoform.[70] The specific targeting activity of Bemarituzumab could avoid adverse events like hyperphosphatemia, which occurred in patients treated with pan-FGFR TKIs.[71,72]

Up to date, there are no clinical trials specifically evaluating BTC patients treated with this drug. A Phase 1 trial demonstrated that bemarituzumab targeted FGFR2b and could be safely used to treat patients with advanced solid tumors (NCT02318329).[70]

Many FGFR inhibitors are under evaluation, the research about the mechanisms of resistance is ongoing at the same time. On a basis of several studies, secondary mutations in FGFR2 kinase domain, mutations in the TKI domain and emergence of new FGFR2 fusions might all be the reasons for resistance.[73–75] Further studies are needed to have a better understand of the mechanisms of resistance and find potential ways to overcome it.

Metabolic Pathway Linked to IDH1/2 Mutations

Isocitrate dehydrogenase (IDH), an essential enzyme for the citric acid cycle, can convert isocitrate to α-ketoglutarate (α-KG) by oxidative decarboxylation, and finally provides ATP and precursors for cellular metabolism.[76] In humans, there are 3 isoforms of IDH (IDH1, IDH2, and IDH3) which contribute to regulating cellular metabolism. Several studies have indicated that mutant IDH1 (mIDH1) and mutant IDH2 (mIDH2) are "gain of function" mutations, which means that they gain the ability to catalyze the conversion of α-KG to 2-hydroxyglutarate (2-HG).[77,78] The accumulation of 2-HG inhibits the αKG-dependent dioxygenases which play a part in epigenetic regulation, leading to cell proliferation, suppression of cellular differentiation, angiogenesis and invasion.[79–84] Therefore, mutations in IDH 1/2 genes are highly related to tumorigenesis. IDH gene mutations are heterozygous point mutations generally occurring in Arginine 132 of IDH1 and Arginine 140 or Arginine 172 of IDH2.[78,84] According to genomic profiling, IDH 1/2 mutations were more common in iCCA

than in eCCA or GBC, with an incidence ranging from 10% to 36%.[30,33,84–86] The occurrence of IDH1 mutation was higher than IDH2.[29,87]

To examine the mechanism of IDH1/2 gene mutation driving tumorigenesis, two preclinical studies were conducted. AGI-5198, as a tool compound, was proven to target IDH1-mutant glioma cells and suppress the growth of cells, but did not work in IDH1 wild-type glioma cells.[88] In another study, a compound named AGI-6780 and hematopoietic cell lines were used to assess the potential utility of mIDH2 inhibitors in treating leukemias with IDH2/R140Q mutations. This study also discovered that AGI-6780 could promote the differentiation of the human IDH2/R140Q mutant hematopoietic cells.[82]

Although these two compounds showed encouraging effects in preclinical tests, poor pharmacokinetics of AGI-5198 and the lack of in vivo tests to assess AGI-6780 prevent their use in clinical studies.[89,90] Several kinds of mIDH inhibitors with good safety and efficacy were developed and have entered clinical trials (Table 2).

mIDH1 Inhibitors

AG-120 (Ivosidenib)

AG-120 (Ivosidenib), a highly specific inhibitor of mutant IDH1 (mIDH1) enzymes, was developed through optimizing AGI-5198 to enable it to be applied to human therapy.[89,90] AG-120 was the first mIDH inhibitor studied in CCA.[90] An in vitro study confirmed the ability of AG-120 to selectively decrease the 2-HG levels and restore

Table 2 Clinical Trials for BTC Targeted Therapy Targeting IDH Mutations

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
IDH 1/2	**AG-120 (ivosidenib)**	NCT02073994	Active, not recruiting	I	CCA, Other solid tumors	170	AG-120
		NCT02989857	Active, not recruiting	III	CCA with IDH1 mutations	186	AG-120 vs Placebo
		NCT04088188	Not yet recruiting	I	CCA	40	Ivosidenib plus GEMCIS vs Pemigatinib plus GEMCIS
	Olutasidenib (FT-2102)	NCT03684811	Recruiting	I II	BTC, Hepatocellular Carcinoma	200	FT-2102 plus Nivolumab (Hepatobiliary tumors) FT-2102 plus GEMCIS (iCCA)
	IDH-305	NCT02381886	Active, not recruiting	I	Advanced malignancies with IDH1-R132 mutations	166	IDH-305
	AG-221	NCT02273739	Completed	I II	iCCA, Other solid tumor types with IDH2 mutations	21	AG-221
	Dasatinib	NCT02428855	Completed	II	CCA	8	Dasatinib
PARPi	**Olaparib (Lynparza)**	NCT03212274	Recruiting	II	CCA, Other solid tumor types with IDH1/IDH2 mutations	145	Olaparib
		NCT04306367	Recruiting	II	CCA	29	Olaparib plus Pembrolizumab
		NCT04298021	Not yet recruiting	II	BTC	74	Olaparib plus AZD6738 vs Durvalumab plus AZD6738
	Rucaparib	NCT03639935	Recruiting	II	BTC	35	Rucaparib plus Nivolumab
		NCT03337087	Active, not recruiting	I II	BTC Other solid tumor types	110	Rucaparib plus Nal-IRI plus Leucovorin plus Fluorouracil
	Niraparib	NCT03207347	Recruiting	II	CCA, Other solid tumor types	57	Niraparib

cell differentiation in mIDH1-positive AML cells by inhibiting the mutant IDH1 enzyme.[91] AG-120 also lowered 2-HG levels and showed significant mutant IDH1 enzyme inhibition ability in mice with IDH1-R132 mutations.[89] These preclinical studies supported further clinical research of this drug. The published data from a phase I dose escalation study which preliminarily explored the safety and activity of AG-120 in a group of CCA patients with IDH1 mutations was encouraging (NCT02073994). Among 73 pretreated CCA patients, 5% had a partial response and 56% experienced stable disease. Moreover, a 6-month PFS rate of 40.1% and a 12-month PFS rate of 21.8% were achieved, as well as a median OS of 13.8 months (95% CI: 11.1–29.3).[92] In addition, when studying the tumor biopsies collected from these patients, scientists found that mutant IDH1 cholangiocarcinoma with a post-dose cytoplasmic decrease upregulated several immune response-related genes such as CTLA4, CXCL10, and CD3G, implying that using AG-120 plus immunotherapies might be a potential regimen.[93] A phase III clinical trial named ClarIDHy is under development which compares the efficacy of AG-120 with a placebo in IDH1-mutation-positive CCA patients (NCT02989857). Compared with placebo, ivosidenib showed improved mPFS (2.7 vs 1.4 months) and mOS (10.8 vs 9.7 months). In addition, the group of patients treated with ivosidenib experienced a better quality of life.[94] However, 1.3 months of PFS benefit and 1.1 months of OS benefit are limited with a large cost of this drug.

Several other mIDH1 inhibitors that might be effective in CCA are still undergoing testing in clinical trials. Olutasidenib (FT-2102) is a potent inhibitor of mIDH1 whose clinical trials are ongoing in advanced malignancies including CCA (NCT03684811).[95] IDH305 is another selective mIDH1 inhibitor developed by Novartis.[96] Its activity has been tested by preclinical studies and has moved into clinical trials assessed in patients with advanced malignancies harboring IDHR132 mutations (NCT02381886).[97]

mIDH2

Enasidenib (AG-221)

Enasidenib is a first-in-class selective inhibitor which is specific to the mutant IDH2 enzyme. Its 2-HG suppression ability was demonstrated in multiple in vitro and in vivo preclinical studies. These studies explored the function of Enasidenib in several IDH2-mutant systems, such as cells taken from AML patients and mouse xenograft models. These studies verified that the inhibition of 2-HG led to cellular differentiation, and the research conducted in the AML xenograft mouse model achieved a dose-dependent survival benefit, which promoted the clinical development of Enasidenib.[98] Although it has been approved by the FDA, more studies need to be carried out to confirm its efficacy in BTC patients.[90] A phase I/II trial of AG-221 in subjects with IDH2-mutant advanced solid tumors, including CCA, was completed in 2018, but results are still unreported (NCT02273739).

Pan-Inhibitor

AG-881 (Vorasidenib)

AG-881 (Vorasidenib) is the first pan-inhibitor of both mIDH1 and mIDH2.[90,99,100] However, AG-881 has not been approved by the FDA and there have been no clinical trials evaluating it in biliary tract cancer patients.

Multi-TKIs

Dasatinib

Besides these mIDH inhibitors, a preclinical study discovered that two iCCA cell lines with IDH1 mutations were highly sensitive to multi-tyrosine kinase inhibitors (multi-TKIs), dasatinib and saracatinib. Both of these inhibitors belong to the SRC family of tyrosine kinases and the subsequent experiments suggested SRC inhibition was of great significance for dasatinib-mediated cytotoxicity. This sensitivity to dasatinib has not occurred in all tumor types with IDH mutations, only in mIDH-positive iCCA tissues.[101] A phase II clinical trial testing dasatinib in iCCA patients with IDH mutations was completed, but the results have not yet been published (NCT02428855).

PARPi

Moreover, there were some preclinical studies indicating that poly ADP ribose polymerase (PARP) inhibitors could kill the tumor cells with IDH mutations.[102,103] As mentioned before, mutant IDH can cause the accumulation of 2-HG, which can significantly decrease homologous recombination repair (HRR) activity by inhibiting the αKG-dependent dioxygenases and subsequently improving the sensitivity to PARP inhibitors.[102] Based on these promising results, several PARP inhibitors are being investigated in mIDH CCA patients, including olaparib (NCT03212274, NCT04306367, NCT04298021), rucaparib (NCT03639935, NCT03337087) and niraparib (NCT03207347).

Furthermore, a recent report studied tumor samples from 1292 BTC patients showed Breast Cancer Susceptibility Gene (BRCA) mutations with higher rate

in subjects with microsatellite instability high (MSI-H) and tumors with higher tumor mutational burden (TMB). PARP inhibitor is a possible treatment for BRCA-mutated cancers. Cancers with high TMB and MSI-H showed a better response to immunotherapy. Therefore, the combination of PARPi plus immune checkpoint inhibitors is of high interest in treatment of BTCs.[104]

In the future, the combination of IDH inhibitors and other agents (eg, chemotherapy, targeted therapy, immunotherapy) may become the first-line treatment. However, the mechanism of resistance is still unclear and how to overcome the resistance needs to be explored.

Epidermal Growth Factor Receptor (EGFR)/HER2

Epidermal growth factor receptor (EGFR) and HER2, members of the ErbB family, are two common receptors involved in the tumorigenesis of BTCs. The ErbB family consists of four members: ERBB1 (EGFR), ERBB2 (HER2), ERBB3 and ERBB4, which are all receptor tyrosine kinases. They have a similar molecular structure composed of an intracellular tyrosine kinase domain, a single transmembrane lipophilic region and an extracellular ligand-binding domain.[105,106]

Besides epidermal growth factors (EGFs), transforming growth factor-α (TGF-α) and amphiregulin specifically bind to EGFR. Binding of the ligands to EGFR is followed by dimerization, which successively stimulates its tyrosine kinase domain autophosphorylation and activates downstream signal transduction cascades. Specifically, none of the EGFs can interact with HER2. Although there is no soluble ligand for HER2, it is the preferential partner of another member of the same family during heterodimerization, which subsequently induces the activation of its tyrosine kinase domain and downstream signaling pathways.[105] The main signaling pathways activated by ErbBs are the MAPK, PI3K/AKT/mTOR, and JAK/STAT pathways that control and regulate cell proliferation, differentiation, metabolism, stress reaction and migration.[106–109]

In various human cancers, EGFR gene amplification commonly takes place, resulting in EGFR overexpression and making tumor cells sensitive to epidermal growth factors. This phenomenon enables the downstream signaling pathway to be continuously activated, causing cancer cells to gain proliferative and metastatic advantage.[105,110] In many tumors, EGFs and cytokines can be produced by tumor cells, stromal cells or macrophages that interact with tumor cells inducing constitutive EGFR activation and tumor cell metastasis.[111] Amplification of HER2 also leads to HER2 overexpression in several kinds of tumors, which is highly related to tumorigenesis, tumor cell invasion and metastasis. Regarding genetic mutations, EGFR mutations are rare, and HER2 mutations have only been identified in a small number of cancers.[105]

Based on a previous study, EGFR expression occurs in iCCAs with an incidence of 100%, followed by eCCAs with an expression level of 52.6% and GBCs at 38.5%. HER2 is mainly overexpressed in eCCAs (ranging from 5.1% to 26.3%) and GBCs (ranging from 5.1% to 10%).[112–114] The EGFR mutations were tested in up to 15% of BTCs and the incidence of HER2 mutations in iCCAs was only 0.9%.[106,114,115]

To date, several preclinical studies tested and confirmed the potential therapeutic effect of EGFR or HER2 inhibitors for BTCs. A study carried out by Weidmann et al demonstrated that NVP-AEE788, a dual EGFR/HER2 inhibitor, could more effectively suppress the proliferation of human CCA cell lines in vitro compared to gefitinib and erlotinib (EGFR inhibitors). Furthermore, this team also tested the antitumor activity of NVP-AEE788 in vivo. In the experiment group, NVP-AEE788 was administered in nude mice which were injected with EGl-1 eCCA cell lines, significantly reducing the volume of tumors compared with the control group.[116] Another preclinical study examined the effect of gefitinib (a selective EGFR inhibitor) and GW2974 (a dual EGFR/HER2 inhibitor) in mice with gallbladder carcinoma. The results showed that both two inhibitors acted as promising chemopreventive and therapeutic agents for GBCs in mice models.[117] These results from preclinical studies suggest that EGFR and HER2 might be targetable and promising receptors in BTC targeted therapy.

EGFR Inhibitors

The selective EGFR inhibitors primarily include Erlotinib, Cetuximab, Panitumumab and Gefitinib.

Erlotinib

Erlotinib is a selective and reversible EGFR inhibitor, which has been under clinical evaluation for a long time. A Phase II clinical trial preliminarily evaluating the efficacy of erlotinib in patients with unresectable BTCs revealed that the disease control rate was 50% with 52%

of patients achieving 6-month overall survival.[118] Based on the modest benefits showed in phase II studies, a large phase III trial comprised of 268 patients compared the efficacy and safety of erlotinib plus gemcitabine plus oxaliplatin (GEMOX) regimen with GEMOX regimen alone in patients with metastatic BTC (NCT01149122). The group treated with chemotherapy plus erlotinib achieved a higher objective response rate (30% vs 16%, p=0.005). However, there was no survival benefit in either group with a median OS of 9.5 months in both groups (p=0.611) and an mPFS slightly longer in the GEMOX plus erlotinib group (5.8 vs 4.2 months). Particularly, in subgroup analysis, patients with CCAs achieved significantly longer mPFS after taking the erlotinib plus GEMOX regimen (5.9 months vs 3.0 months, p=0.049).[119]

Cetuximab

Cetuximab is another monoclonal antibody selectively targeting EGFR, which has been assessed in combination with chemotherapy in several phase II studies with BTC patients. A phase II trial compared the efficacy and safety of GEMOX with and without Cetuximab in patients with advanced BTCs (NCT01216345). For patients taking GEMOX plus cetuximab, the overall response rate was 63% and the disease control rate was 80%, which indicated the encouraging antitumor activity of the GEMOX plus cetuximab regimen. Compared with results from other studies, cetuximab plus GEMOX demonstrated a better overall response rate.[120] However, data from another phase II trial, BINGO study, suggested that the potential antitumor activity of cetuximab did not provide any clinical benefit when used in combination with GEMOX in patients with biliary cancer compared with the GEMOX regimen alone (NCT00552149). mOS was 11.0 months in the chemotherapy plus cetuximab arm, which is lower than that of the chemotherapy alone arm (12.4 months).[121]

K-Ras mutations are regarded as a negative predictive factor for cancer prognosis and the therapeutic efficacy of EGFR inhibitors in colorectal cancer patients.[122] In a phase II trial, BTC patients stratified by K-Ras status were administered with GEMOX with or without cetuximab (NCT01267344). GEMOX plus cetuximab only achieved marginal therapeutic benefits, and the overall survival of the GEMOX plus cetuximab group did not improve significantly (10.6 vs 9.8 months, P=0.91). The data also suggested that K-Ras mutations did not affect the survival of BTC patients.[123]

All in all, most of the clinical trials failed to verify any compelling therapeutic effect of the addition of cetuximab to GEMOX.

Panitumumab

Panitumumab, a selective EGFR inhibitor, has been tested in combination with chemotherapy in several phase II trials. Vecti-BIL study was designed to compare the therapeutic efficacy of GEMOX with and without panitumumab in chemotherapy-naïve BTC patients possessing a wild-type K-Ras status (NCT01389414). The addition of panitumumab did not improve mPFS significantly (5.3 vs 4.4 months), and no survival benefit was observed (9.9 vs 10.2 months).[124] Similarly, data from the PICCA study also confirmed that there was no survival benefit gained from the addition of panitumumab to gemcitabine and cisplatin chemotherapy (GEMCIS) in K-Ras wild-type BTC patients (NCT01320254).[125]

So far, though results from a meta-analysis indicated that anti-EGFR inhibitors could prolong PFS and response rate, several completed randomized clinical trials all failed to confirm the therapeutic effects of EGFR inhibitors in BTC patients with little clinical benefit. Therefore, further exploration in this field is needed.

HER2 Inhibitors

HER2 overexpression and gene amplification are the common occurrences in BTCs, leading to the development of specific HER2 inhibitors.

Trastuzumab

Trastuzumab is an antibody specifically targeting HER2. Although trastuzumab has not been approved for the treatment of BTC, its anti-proliferative activity in HER2-overexpressing BTC cell lines was verified in a preclinical study.[126] Another preclinical study demonstrated the antitumor effect in a mouse xenograft model through increasing apoptosis.[127] A retrospective analysis conducted by Javle et al found that trastuzumab had a disease control rate (including partial response, stable disease, or complete response) of 100% in gallbladder cancer group patients. On the contrary, there were no responses in CCA patients after taking trastuzumab.[128] These promising results promoted further investigation of HER2 inhibitors in BTCs. This drug is under clinical research and exploration, with several phase II studies in progress.

Pertuzumab

On the basis of preclinical studies, the inhibitory effect of pertuzumab was confirmed both in BTC cell lines which overexpressed HER2 and HER3 and in vivo.[129] Two case reports showed that dual-anti-HER2 therapy pertuzumab and trastuzumab significantly improved the survival benefits of BTC patients. Therefore, dual anti-HER2 therapy might become a potent treatment option against BTC.[130,131] Currently, a phase II trial evaluating a trastuzumab plus pertuzumab regimen in patients with advanced solid tumors, including BTCs, is ongoing (NCT02091141).

There is still some disagreement on the therapeutic effects of HER2 inhibitors, so further data from clinical studies is expected.

EFGR and HER2 Double Inhibitors

Dual EFGR and HER2 tyrosine kinase inhibitors are inhibitors of both EGFR and HER2, including lapatinib, afatinib, neratinib, AEE788, varlitinib, and dacomitinib.

Lapatinib

So far, two phase II clinical trials assessing the therapeutic efficacy of lapatinib in BTC patients have been completed. A phase II study beginning in 2004 evaluated lapatinib in 17 advanced BTC patients and 40 hepatocellular cancer (HCC) patients (NCT00101036). Results were poor: no objective response, as well as PFS and mOS for BTC patients of only 1.8 months and 5.2 months, respectively.[132] Later, similar poor data were obtained from another phase II study, and this study was terminated early (NCT00107536).[133]

Afatinib

A phase I study investigated the efficacy of afatinib in combination with gemcitabine and cisplatin (GEMCIS) in patients with advanced BTC (NCT01679405). Only 9 participants enrolled and this study was discontinued due to futility.[134] Afatinib combined with capecitabine is currently under evaluation in a phase I trial in patients with bile duct carcinoma and pancreatic cancer (NCT02451553).

Neratinib

So far, there are few clinical studies evaluating neratinib. SUMMIT, a basket trial, explored the efficacy of neratinib in EGFR/HER2 mutation-positive cancer patients, including BTC patients (NCT01953926). The preliminary data presented at American Association for Cancer Research's Annual Meeting 2017 indicated promising antitumor activity of neratinib in BTC patients with an ORR of 22%.[135]

Others

For AEE788, varlitinib and dacomitinib, though they lack clinical trials to verify their therapeutic efficacy, preclinical studies have implied bright prospects for them as treatments for biliary tract cancers. The preclinical study for AEE788 has been mentioned before. Varlitinib (ASLAN001) is a new promising therapeutic inhibitor for CCA treatment. Its anti-tumor effect was confirmed both in vitro and in vivo, and the effect was improved when used in combination with the PI3K inhibitor BKM-120.[136] Currently there are several ongoing phase I and phase II clinical trials with varlitinib in BTC patients. The efficacy of dacomitinib (PF00299804) was assessed in eight BTC cell lines. As monotherapy, dacomitinib showed good inhibitory effects in two of the eight cell lines. Furthermore, dacomitinib in combination with gemcitabine showed improved anti-tumor effects in seven of the eight cell lines.[137]

These promising results support further studies to be carried out for the treatment of BTCs (Table 3).

Neurotrophic Tropomyosin Receptor Kinase (NTRK)

Recently, neurotrophic tropomyosin receptor kinase (NTRK) gene fusion has become a promising avenue for cancer targeted therapy. NTRK genes encode for tropomyosin receptor kinase (TRK) receptors, which are transmembrane receptors structured with an extracellular ligand-binding domain, a transmembrane region and an intracellular kinase domain. There are three TRK receptors in the TRK receptor family: TRK A, TRK B and TRK C receptors, encoded by NTRK1, NTRK2 and NTRK3 genes, respectively.[138,139] TRK receptors play an essential role in nervous system development and function. The ligands for TRK receptors, neurotrophins (NTs), activate downstream signaling pathways regulating cellular proliferation, differentiation and survival when they bind to TRK receptors. However, when NTRK gene fusion occurs, chimeric TRK proteins are produced which are constitutively activated conferring an oncogenic potential.[140,141]

With the development of next-generation sequencing (NGS) and fluorescence in situ hybridization (FISH)

Table 3 Clinical Trials for BTC Targeted Therapy Targeting ErbB Family

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
EGFR	**Erlotinib**	NCT01149122	Completed	III	BTC	268	Erlotinib plus GEMOX vs GEMOX
		NCT03110484	Not yet recruiting	II	BTC	38	Erlotinib plus Pemetrexed
		NCT00987766	Completed	I	BTC, Other solid tumor types	28	Erlotinib plus GEMOX
		NCT00266097	Completed	I	BTC, Pancreatic Cancer	23	Erlotinib + GEMOX + Radiation vs GEMOX + Radiation
		NCT02091141	Recruiting	II	BTC, Salivary cancer, Bladder Cancer	765	Erlotinib
		NCT00033462	Completed	II	BTC	78	Erlotinib
		NCT00350753	Completed	II	BTC	126	Erlotinib plus Bevacizumab
		NCT01093222	Completed	II	BTC	40	Erlotinib plus Sorafenib
		NCT00955149	Completed	I	CCA Primary Sclerosing Cholangitis	6	Erlotinib
		NCT00356889	Completed	II	BTC	56	Erlotinib plus Bevacizumab
		NCT00397384	Completed	I	BTC Other solid tumors	43	Erlotinib plus Cetuximab
	Cetuximab	NCT01216345	Completed	II	BTC	30	Cetuximab plus GEMOX
		NCT00552149	Completed	II	BTC	150	Cetuximab plus GEMOX vs GEMOX
		NCT01267344	Completed	II	BTC	122	Cetuximab plus GEMOX vs GEMOX
		NCT01247337	Unknown	II	CCA	100	Cetuximab plus GEMOX plus Capecitabine
		NCT00397384	Completed	I	BTC, Other solid tumors	43	Cetuximab plus Erlotinib
		NCT03768375	Recruiting	II	BTC	150	Cetuximab plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Cetuximab plus GEMOX vs GEMOX
		NCT00747097	Completed	II	BTC	43	Cetuximab plus Gemcitabine
	Panitumumab	NCT01389414	Completed	II	BTC	89	Panitumumab plus GEMOX vs GEMOX
		NCT01320254	Completed	II	CCA	93	Panitumumab plus GEMCIS vs GEMCIS
		NCT01206049	Completed	II	CCA	88	Panitumumab plus Combination chemotherapy vs Bevacizumab plus Combination chemotherapy
		NCT00948935	Completed	II	BTC	35	Panitumumab plus Gemcitabine plus Irinotecan
		NCT00779454	Completed	II	CCA	72	Panitumumab plus GEMOX plus Capecitabine (Kras WT) vs GEMOX plus Capecitabine (Kras mutation)
		NCT01308840	Completed	II	BTC	31	Panitumumab plus GEMOX
	Varlitinib (ASLAN001)	NCT02609958	Completed	II	CCA	32	Varlitinib
		NCT03231176	Active, not recruiting	II	BTC	68	Varlitinib plus Capecitabine
		NCT03093870	Active, not recruiting	II	BTC	490	Varlitinib plus Capecitabine vs Placebo plus Capecitabine
		NCT02992340	Recruiting	I II	BTC	204	Varlitinib plus GEMCIS
		NCT03082053	Completed	I	BTC	24	Varlitinib vs Varlitinib plus Capecitabine
	Gefitinib	NCT03768375	Recruiting	II	BTC	150	Gefitinib plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Gefitinib plus GEMOX vs GEMOX

(*Continued*)

Table 3 (Continued).

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
HER2	**Trastuzumab**	NCT03613168	Recruiting	II	BTC	15	Trastuzumab plus GEMCIS
		NCT02999672	Completed	II	CCA, Pancreatic cancer	20	Trastuzumab
		NCT00004074	Completed	I	Malignancies with HER2-Neu overexpressing	15	Trastuzumab plus IL12
		NCT03768375	Recruiting	II	BTC	150	Trastuzumab plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Trastuzumab plus GEMOX vs GEMOX
		NCT02393248	Recruiting	I II	CCA, Other solid tumor types	325	Trastuzumab plus Pemigatinib vs Pemigatinib plus GEMCIS vs Pemigatinib plus Pembrolizumab vs Pemigatinib plus Docetaxel vs Pemigatinib plus INCMGA00012
		NCT02465060	Recruiting	II	Malignancies with HER2 amplification	6452	Trastuzumab
		NCT03185988	Recruiting	II	BTC	100	Trastuzumab plus Chemotherapy
		NCT02091141	Recruiting	II	BTC, Salivary Cancer, Bladder Cancer	765	Trastuzumab plus Pertuzumab
	Pertuzumab	NCT02091141	Recruiting	II	BTC, Salivary Cancer, Bladder Cancer	765	Pertuzumab plus Trastuzumab
		NCT02465060	Recruiting	II	Malignancies with HER2 amplification	6452	Pertuzumab plus Trastuzumab
EGFR&HER2	**Lapatinib**	NCT00101036	Completed	II	BTC	57	Lapatinib
		NCT00107536	Completed	II	BTC	26	Lapatinib
		NCT03768375	Recruiting	II	BTC	150	Lapatinib plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Lapatinib plus GEMOX vs GEMOX
	Afatinib	NCT02451553	Recruiting	I	BTC, Pancreatic Cancer	48	Afatinib plus Capecitabine
		NCT02465060	Recruiting	II	Malignancies with EGFR mutations	6452	Afatinib
	Neratinib	NCT01953926	Recruiting	II	Solid tumors with somatic HER2 or EGFR exon 18 mutations	650	Neratinib vs Neratinib plus Paclitaxel vs Neratinib plus Trastuzumab vs Neratinib plus Fulvestrant plus Trastuzumab

techniques, NTRK fusions have been detected in various types of tumors, such as salivary gland carcinoma, sarcoma, and thyroid carcinoma.[142] Ross et al identified NTRK fusion (RABGAP1L-NTRK1) in one of the 28 iCCA patient samples (3.5%).[86] Another study reported an incidence rate of 0.25% in 787 CCA patients.[142] A recent report presented at ESMO World Congress on Gastrointestinal Cancer 2020 studied the incidence of NTRK gene fusions in biliopancreatic malignancies, which showed the percentage of NTRK gene fusions was only 0.67% among patients with BTC.[143] Moreover, TRK inhibitors can also suppress the abnormal activity induced by ROS1 and ALK fusions, which also occur in CCA patients.[144] Although their incidence in BTCs is still low,

selective TRK inhibitors have been developed and the concept of precision medicine has gradually become popular, implying that NTRK fusions might become a promising target for biliary tumor treatment.

The efficacy of a few selective TRK inhibitors for BTCs are under evaluation in preclinical and clinical studies (Table 4).

Two preclinical studies used mouse models to verify that TRK inhibitors effectively control tumor growth and confirm that NTRK gene fusions (Etv6-NTRK3 fusion and Bcan-Ntrk1 fusion) can initiate tumorigenesis.[145,146]

Entrectinib (RDX-101, NMS-P626) is an oral inhibitor against the activity induced by the TRK family, C-ros oncogene 1 (ROS1) and anaplastic lymphoma kinase (ALK). It has proven effective in several clinical trials involving patients with NTRK gene fusions.[147–149] Larotrectinib (VITRAKVI) is the first pan-TRK inhibitor approved by the US Food and Drug Administration for treating patients with solid tumors harboring NTRK gene fusions. Promising data published from three multicenter clinical trials (NCT02122913, NCT02637687, NCT02576431) contributed to the accelerated approval of larotrectinib. The efficacy was assessed in 55 participants covering 12 cancer types, including 2 with CCA. The reported ORR was 75%, including 22% CR and 53% PR. A phase II MATCH trial also evaluated the efficacy of Larotrectinib in cancer patients with NTRK1, NTRK2, or NTRK3 gene fusions (NCT02465060).

Additional TRK inhibitors are under preclinical and clinical development, but few of them have been tested in BTC patients. ONO-7579 is a pan-TRK inhibitor whose anti-tumor effect was demonstrated in a preclinical study using two GBC cell lines: NOZ (harboring K-Ras mutant) and TYGBK-1 (wild-type K-Ras). The results indicated that ONO-7579 could effectively suppress proliferation in the TYGBK-1 cell line, but not in the NOZ cell line, suggesting that ONO-7579 may have a potent anti-tumor effect on GBC cells without K-Ras mutation.[150] However, the only phase I clinical study of ONO-7579 for patients with NTRK gene fusion-positive solid tumors was terminated for commercial reasons (NCT03182257).

Even though the incidence of NTRK gene fusion is low, the testing of NTRKgene fusion is of high interest due to the development of several specific TRK inhibitors. Further studies evaluating TRK inhibitors specific to BTC treatment are expected.

Ras/Raf/MEK/Erk Signaling Pathway Inhibitors

The Ras/Raf/MEK/Erk signaling pathway is one of the main signaling pathways for BTC carcinogenesis. Ras is a kinase which is encoded by the Ras gene, a proto-oncogene. When the Ras gene mutates, it expresses abnormal Ras oncoproteins, which leads to consecutive activations of itself and its downstream signaling pathways. Consequently, the unlimited proliferation and suppressed apoptosis of cells occurs.[151,152]

There are at least three downstream signaling pathways of the Ras oncoprotein. Raf kinase was the first discovered Ras effector. This signaling pathway has a generic name: mitogen-activated protein kinase (MAPK) pathway. In this signal transduction cascade, Ras combines with a GTP molecule to activate the Raf kinase (a MAPKKK); Raf then activates MEK (a MAPKK), which subsequently activates ERK1/2 (MAPKs). Erk1 and Erk2 are able to phosphorylate some transcription factors (eg, Ets, Elk-1, SAP-1) and kinase which is responsible for protein synthesis (eg, Mnk1 kinase). Besides activating several growth-promoting genes, this pathway also causes cells to lose anchorage and contact inhibition properties. Furthermore, it plays an important role in Ras oncogene-associated cell shape changing.[153,154] B-Raf is a homology of the Raf protein and its mutant form is observed in about 66% of human melanomas.[155,156]

K-Ras, N-Ras and B-Raf mutations have been commonly detected in various cancer types such as gastrointestinal cancers, lung cancers and melanomas. For biliary tract carcinomas, studies in different countries show a variance in the frequency of K-Rasmutations, ranging from 15.3% to 67% in eCCAs and from 9% to 45% in iCCAs. Moreover, in a Japanese test group, the incidence of K-Ras mutations in GBC was relatively higher than in other regions. The presence of K-Ras mutations is correlated to worse prognosis.[157–159] By contrast, N-Ras mutations exist in 3.6% of iCCAs and 2.6% of eCCAs, while B-Raf mutations are only found in 3% to 5% of iCCAs.[46,157,160]

These mutated proteins and their downstream signal proteins have gradually become the new targets for BTC targeted therapy. Many novel inhibitors targeting the Ras–Raf–MEK–ERK pathway have been evaluated by a large number of studies (Table 4).

K-Ras

Currently, there are no specific inhibitors targeting the K-Ras mutated form, so this pathway can only be suppressed by inhibiting the downstream functional proteins.

Table 4 Clinical Trials for BTC Targeted Therapy Targeting the Ras/Raf/MEK/Erk Signaling Pathway

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
BRAF	**Vemurafenib**	NCT01524978	Completed	II	CCA with BRAF V600 mutation Other solid tumors with BRAF V600 mutation	208	Vemurafenib
	PLX8394	NCT02012231	Terminated	I II	CCA, Other malignancies	5	PLX8394
		NCT02428712	Active, not recruiting	I II	Advanced solid tumors with BRAF mutations	75	PLX8394
	Dabrafenib	NCT02465060	Recruiting	II	Malignancies with BRAF V600E/R/K/D mutation	6452	Dabrafenib plus Trametinib
		NCT02034110	Active, not recruiting	II	Rare cancers (including BTCs) with BRAF V600E mutations.	206	Dabrafenib plus Trametinib
	Regorafenib	NCT02162914	Active, not recruiting	II	CCA	66	Regorafenib vs Placebo
		NCT02053376	Completed	II	CCA	43	Regorafenib
		NCT02115542	Active, not recruiting	II	CCA	39	Regorafenib
		NCT03475953	Recruiting	II	BTC, Other solid tumor types	362	Regorafenib plus Avelumab
	Sorafenib	NCT00661830	Completed	II	BTC	103	Sorafenib plus Gemcitabine vs Gemcitabine plus Placebo
		NCT00919061	Completed	II	BTC	39	Sorafenib plus GEMCIS
		NCT00634751	Completed	I II	BTC, Pancreatic Neoplasms	48	Sorafenib plus Oxaliplatin plus Capecitabine
		NCT00238212	Completed	II	BTC	50	Sorafenib
		NCT01093222	Completed	II	BTC	40	Sorafenib and Erlotinib
		NCT03768375	Recruiting	II	BTC	150	Sorafenib plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Sorafenib plus GEMOX vs GEMOX
MEK	**Selumetinib**	NCT00553332	Completed	II	BTC	29	Selumetinib
		NCT01242605	Completed	I	BTC	13	Selumetinib plus GEMCIS
		NCT02151084	Active, not recruiting	II	BTC	57	Selumetinib plus GEMCIS vs GEMCIS
		NCT02586987	Completed	I	BTC, Other solid tumor types	58	Selumetinib plus MEDI4736 vs Selumetinib plus MEDI4736 plus Tremelimumab
	Refametinib **Trametinib (GSK1120212)**	NCT02346032	Completed	II	BTC	4	Refametinib
		NCT01438554	Completed	I	CCA, Thyroid cancer, Soft-tissue sarcoma	89	Trametinib plus Pazopanib
		NCT02042443	Completed	II	BTC	53	Trametinib vs Chemotherapy (Leucovorin calcium plus Fluorouracil or Capecitabine)
		NCT02465060	Recruiting	II	Malignancies with BRAF V600E/R/K/D mutation	6452	Trametinib plus Dabrafenib
		NCT01943864	Completed	II	BTC	20	Trametinib
		NCT01324258	Completed	II	BTC, Other solid tumor types	19	Trametinib vs Trametinib plus Gemcitabine
	MEK162 (binimetinib)	NCT02773459	Completed	I II	BTC	31	MEK162 plus Capecitabine
		NCT00959127	Completed	I	BTC, Colorectal cancer	93	MEK 162
		NCT01828034	Completed	I	BTC	42	MEK162 plus GEMCIS

(*Continued*)

Table 4 (Continued).

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
		NCT02465060	Recruiting	II	Malignancies with NRAS mutation in codon 12, 13, or 61	6452	MEK 162
RAS	**Tipifarnib (R115777)**	NCT00005842	Completed	I	Advanced Cancer	24	Tipifarnib plus Trastuzumab
ERK	**JSI-1187**	NCT04418167	Recruiting	I	Advanced solid tumors with MAPK pathway mutations	124	JSI-1187 vs JSI-1187 plus Dabrafenib

B-Raf

As mentioned before, the Raf protein has a homology called B-Raf which exists in various types of cancer.

Vemurafenib is a specific inhibitor of the B-Raf V600 mutated protein. Up to date, only one published clinical trial has evaluated the therapeutic effects of vemurafenib in BTC patients (NCT01524978). This phase II study assessed the clinical benefits of vemurafenib in multiple nonmelanoma cancers with B-Raf V600 mutations, including 8 CCA patients. The released results showed that, among the 8 CCA patients, only one patient had partial response (12%) and 4 patients experienced stable disease (50%).[161] A reported case showed that a CCA patient with B-Raf V600 mutations achieved complete response after taking vemurafenib, panitumumab, and irinotecan therapy.[162]

Another B-Raf inhibitor, PLX8394, was investigated by two phase I/II clinical trials in patients with advanced solid tumors including CCA (NCT02428712, NCT02012231). However, one study was terminated while the other has not yet released any data.

Dabrafenib is also a specific B-Raf V600 inhibitor. According to a published case report, a patient with B-Raf V600-mutated iCCA performed exceptional response, including symptomatic and radiological improvement, to dabrafenib plus trametinib (an MEK1/2 inhibitor) dual therapy, causing more clinical trials to be conducted.[163] An ongoing phase II clinical trial, Rare Oncology Agnostic Research (ROAR) basket trial, has provided extremely important results for dabrafenib and trametinib. Among 43 patients with B-Raf V600E-mutated BTCs, this regimen achieved an investigator-assessed ORR of 51% and an independent reviewer-assessed ORR of 47%, with a tolerant safety profile. Therefore, the authors suggested that B-Raf V600E mutation testing should be considered in all patients with BTCs.[164]

Additionally, regorafenib (BAY 73-4506) and sorafenib (Bay 43-9006) are both multi-kinase inhibitors against Raf-1 protein kinase and B-Raf kinase. However, a phase II trial described an intolerant toxicity and no achievable survival benefit by adding sorafenib to GEMCIS in BTC patients.[165]

MEK

MEK is another contributor to the MAPK pathway, and several kinase inhibitors for MEK are under investigation.

Selumetinib

A phase II multicenter trial led by The Ohio State University was designed to determine the safety and efficacy of selumetinib monotherapy, a MEK1/2 inhibitor, in metastatic biliary tract cancer. The results revealed that 12% of the participants had an objective response and another 68% experienced stable disease. Furthermore, the mPFS of 3.7 months and mOS of 9.8 months both compare favorably with previously published data. The encouraging response and well-tolerated safety profile indicate that selumetinib might be a promising inhibitor for BTC treatment.[166] Another phase Ib trial, the ABC-04 trial, evaluated the combination of selumetinib with GEMCIS in patients with advanced BTC (NCT01242605). A median PFS of 6.4 months and acceptable toxicity profile indicated this regimen could achieve a modest efficacy in BTC patients.[167] A phase II trial evaluating the combination of GEMCIS and selumetinib versus GEMCIS alone is ongoing (NCT02151084).

Trametinib (GSK1120212)

Trametinib, another MEK inhibitor, has been tested in a group of Japanese patients, showing a 12-week stable

disease rate of 10%, mPFS of 10.6 weeks and a rate of 1-year OS of 20% (NCT01943864).[168] Trametinib has also been tested with pazopanib, a VEGFR TKI, though this combination did not offer any survival benefit (NCT01438554).[169] Other clinical trials of trametinib monotherapy or combination therapy have not shown any priorities, so this drug warrants further research.

Binimetinib (MEK162)

Binimetinib (MEK162) is a potent MEK 1/2 inhibitor whose preliminary antitumor activity has been demonstrated in several Phase I clinical trials in patients with BTC.[170,171] A phase I/II trial assessing the combination of binimetinib and capecitabine showed promising antitumor efficacy in BTC patients with MAPK pathway mutations (NCT02773459).[172] However, another phase I/II trial assessing binimetinib in combination with chemotherapy (GEMCIS) in BTC patients did not show any priority compared to chemotherapy alone (NCT01828034).[173]

Refametinib

A phase II trial of refametinib assessed in BTC patients has completed, but the results are still unknown (NCT02346032).

ERK

Up to date, there have not been any Erk inhibitors approved in the world. JSI-1187 is an oral, highly selective Erk 1/2 inhibitor which is mainly used to treat tumors with MAPK pathway mutations. Other Erk inhibitors including LY3214996, LTT462 and Ulixertinib are under clinical evaluation.

PI3K/Akt/mTOR Signaling Pathway Inhibitors

A second important downstream signaling pathway driven by the Ras protein is the PI3K/Akt/mTOR pathway, which is involved in BTC tumorigenesis and progression.

Phosphatidylinositol 3-kinase (PI3K) is a direct downstream effector of Ras. It is an essential kinase adding phosphates to phospholipids, which contributes to the formation of phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 subsequently combines with Akt, a serine/threonine kinase also known as protein kinase B (PKB), and activates it.[174] Once activated, Akt can phosphorylate several proteins which affect the cells. Firstly, Akt can prolong the cell life cycle by inactivating the pro-apoptotic proteins, such as Bad and Caspase-9. Secondly, activated Akt promotes cell proliferation by inactivating the anti-proliferative proteins GSK-3β, FOXO4 and p21Cip1. Finally, Akt is able to activate mammalian target of rapamycin (mTOR), a protein promoting protein synthesis and stimulating cell growth in size.[175–177]

Normally, activation of the PI3K/Akt/mTOR pathway is under tight control. The PTEN gene is a tumor suppressor gene playing an essential role in regulating the activity of the PI3K/Akt/mTOR pathway. The PTEN gene encodes the PTEN protein, a phosphatase, which then reverses the actions of PI3K to control the level of PIP3, thus controlling the activation of the PI3K/Akt/mTOR pathway.[178] However, in various types of tumors, the hyperactivation of PI3K or inactivation of PTEN occurs, which deregulates this signaling pathway and confers the cells oncogenic potential.[179]

According to several studies, PI3K mutations were detected in 4.4% of iCCA patients and 6.5% of eCCA patients. PTEN mutations were observed in 4.4% of iCCAs and 3.9% of eCCAs.[157] Blocking this signaling pathway by the use of specific inhibitors might inhibit tumor growth, including biliary tract cancers. As a result, many inhibitors targeting the effector proteins in the PI3K/Akt/mTOR pathway are under development and investigation (Table 5).

PI3K Inhibitors

Copanlisib

Copanlisib (BAY 80–6946) is a selective pan-class I PI3K inhibitor which has been evaluated in a phase I study with 50 patients with advanced solid tumors, including 23 BTC patients (NCT01460537). Response rate was 6.3% in the copanlisib with gemcitabine and 12% in the copanlisib with cisplatin plus gemcitabine (GEMCIS) arm. Among the 23 BTC patients, response rate was 17%. The safety profile of copanlisib is acceptable.[180] Currently, the therapeutic effects of the copanlisib plus GEMCIS regimen is being assessed in a phase II trial with CCA patients (NCT02631590), and the results are highly anticipated.

Buparlisib (BKM120)

The first study demonstrating the anti-tumor effects of BKM120, also named Buparlisib, in BTC cells was conducted by Jin et al. They found that BKM120 could suppress the proliferation and migration of BTC cells in vitro. Furthermore, inhibiting both PI3K and MEK by BKM120 plus MEK162 showed inhibitory effects in BTC cells with both K-Ras mutations and PI3K mutations, which was not

Table 5 Clinical Trials for BTC Targeted Therapy Targeting the PI3K/Akt/mTOR Signaling Pathway

Target	Drugs	ClinicalTrials.gov Identifier	Status	Phase	Disease	Number of Patients	Design
PI3K	**Copanlisib**	NCT01460537	Completed	I	BTC, Other advanced solid malignancy	50	Copanlisib plus Gemcitabine vs Copanlisib plus GEMCIS
		NCT02631590	Active, not recruiting	II	CCA	25	Copanlisib plus GEMCIS
		NCT02465060	Recruiting	II	Malignancies with PIK3CA mutation	6452	Copanlisib
	Buparlisib (BKM120)	NCT01297452	Completed	I	Solid tumors	45	Buparlisib plus Paclitaxel plus Carboplatin
AKT mTOR	**MK-2206 Everolimus (RAD001)**	NCT01425879	Completed	II	BTC	8	MK-2206
		EUDRACT: 2008–007152-94	Completed	II	BTC	39	Everolimus
		NCT02449538	Completed	II	CCA, Other solid tumor types	10	Everolimus
		NCT00973713	Unknown	II	CCA	27	Everolimus
		NCT01525719	Unknown	II	CCA	40	Everolimus
		NCT00949949	Completed	I	BTC	38	Everolimus plus Gemcitabine vs Everolimus plus GEMCIS
		NCT03768375	Recruiting	II	BTC	150	Everolimus plus GEMOX vs GEMOX
		NCT02836847	Recruiting	II	BTC	152	Everolimus plus GEMOX vs GEMOX
	Sirolimus	NCT01888302	Completed	I	Patients at high risk for CCA recurrence after liver transplant or surgery	1	Sirolimus plus GEMCIS

achievable by BKM120 alone.[181] However, a phase I trial of BKM120 plus mFOLFOX6 (5-FU/LV plus oxaliplatin) did not show a tolerant safety profile in advanced gastrointestinal tumors, including 4 CCA patients (NCT01571024). Specifically, 13 of the 17 participants experienced grade 3/4 adverse events.[182] On the contrary, another phase I study demonstrated that BKM120 is safe with a clear evidence of antitumor activity in patients with advanced cancers, including GBC.[183] In addition, a phase I trial verified the good safety profile and therapeutic efficacy of buparlisib in combination with paclitaxel and carboplatin in solid tumors (NCT01297452). For patients without PTEN expression, the preliminary antitumor activity was notable.[184]

LY3023414 (Samotolisib)

Sakamoto et al used BTC cell lines as a preclinical model to verify that LY3023414, a PI3K/mTOR dual inhibitor, possessed anti-proliferative activity, marking it as a potential new agent for BTC treatment.[185]

AKT Inhibitors

The main Akt inhibitors, including MK-2206, FPA124 and A-443654, were confirmed to inhibit cell proliferation and migration in various BTC cell lines.[186] Moreover, in a preclinical study, genistein was found to suppress CCA cell growth by inhibiting the activation of Akt.[187]

MK-2206

Among these Akt inhibitors, MK-2206 is considered the most promising, as it is the only one to have entered clinical trials so far. Wilson et al confirmed that MK-2206 could suppress the CCA cell growth by inducting apoptosis in vitro.[188] A phase II clinical trial recruiting 8 BTC patients evaluated the safety and efficacy of MK-2206 (NCT01425879). However, only 2 patients achieved stable disease (25%), which was the best observed response. Even though the toxicity was tolerant, there were no notable clinical benefits existing in this small group of participants.[189] Further research needs to be conducted for MK-2206 development.

mTOR

Everolimus, sirolimus (also called rapamycin), and temsirolimus are all first-generation inhibitors of mTOR. The superiority of everolimus and sirolimus has been evidenced by preclinical researches in CCA, promoting the commencement of clinical studies.

Everolimus (RAD001)

Several clinical studies of everolimus in BTC have been carried out. A retrospective study showed that, among 22 BTC patients, everolimus achieved a DCR of 50% with a higher incidence of adverse events (64%).[190] A phase I study evaluated the safety and antitumor activity of everolimus in combination with gemcitabine and everolimus plus GEMCIS regimen in patients with solid tumors. Among 37 participants, 10 participants enrolled in Cohort III were all BTC patients, with 6 patients achieving stable disease (60%). The toxicities of both regimens were manageable.[191] A phase II clinical trial (I.T.M.O. study) was conducted in Italy (EUDRACT: 2008-007152-94) to evaluate the therapeutic efficacy and safety of RAD001 (everolimus) in 39 advanced BTC patients who were previously treated with chemotherapy. It reported a DCR of 44.7%, ORR of 5.1% and mOS of 7.7 months with tolerable drug toxicity.[192] Another relevant phase II clinical trial of everolimus in cancer patients with PI3K abbreviation or PTEN loss did not show any general clinical benefit, though the only CCA patient achieved stable disease (NCT02449538).[193]

In recent years, the data of the RADiChol study, a phase II clinical trial, was published (NCT00973713). Twenty-seven patients with advanced BTC were enrolled in this study. The primary endpoint DCR at 12 weeks was 48%, with an mPFS of 5.5 months and mOS of 9.5 months. Generally, everolimus monotherapy was well tolerated and did show a clinical benefit in advanced BTC patients.[194] Presently, the therapeutic efficacy of everolimus in BTC treatment has not been confirmed, so more clinical data are expected to be released.

Sirolimus

Sirolimus is another mTOR inhibitor under clinical investigation. Pilot studies have not demonstrated obvious clinical activity of sirolimus, but partial participants also achieved partial response and stable disease.[195,196] The only clinical study evaluating sirolimus in combination with gemcitabine plus cisplatin for patients at high risk for CCA after liver transplant or surgery has finished, but results are unknown so far (NCT01888302).

In conclusion, mTOR inhibitors achieved modest clinical benefits in advanced BTC patients, and they should be validated by more randomized controlled trial (RCT) studies.

Wnt Signaling Pathaway

Wnt signaling pathway is an intracellular signaling pathway. A study indicated that, in human CCA, the expression of Wnt signaling was significantly increased. Therefore, suppression of Wnt signaling pathway might be an option for inhibition of CCA growth.[197]

RNF43 is a RING domain E3 ubiquitin ligase, which could suppress p53-mediated apoptosis and inhibit Wnt signaling. When this gene mutated, Wnt signaling was increased.[198,199] Recently, RNF43 mutations have been highlighted in BTC patients, with an incidence of 9.3% in CCA cases.[200] Therefore, the blockades of this signaling pathway were developed and their activity and safety are assessed in clinical trial.

Other several Wnt signaling pathway inhibitors, including DKN-01, ICG-001, C-59 and CGX1321, are also under development and research. We are looking forward to unfolding this area in the next years.

Future Prospects

Biliary tract cancer is a highly fatal disease and a challenge for clinical treatment due to its "silent" symptoms, fast progression and high recurrence rate. As for systematic therapy, the options and therapeutic effects are limited.

Despite great advances made to uncover the molecular mechanism of BTC tumorigenesis, many obstacles remain. The major roadblock is that there are different entities included in BTC (ie, iCCA, eCCA, GBC) with different clinical and molecular features, which is also termed heterogeneity. Different signaling pathways and complex molecular interactions underlie the cancer heterogeneity and individual's susceptibility to different drugs. Heterogeneity is a limitation for studies with targeted agents and BTC targeted therapy, since various activated or inhibited pathways are strongly influenced by the molecular features of the tumor, which vary among the entities. In addition, as most clinical trials are currently in Phase II, more credible phase III randomized clinical trials are warranted to verify therapeutic efficacy and safety. Another tricky problem is that these promising targets only exist in a small proportion of patients; therefore, many approved

inhibitors cannot be used in the majority of patients. The identification of novel targets is required to carry out individualized treatment in most patients.

Gene aberrations are regarded as the drivers of tumors. With the concept of "precision medicine" and the continuing development of sequencing technology, molecular targeted therapy can offer new ideas. Since oncogenesis and tumor progression are regulated and controlled by a large number of signal molecules and signaling pathways, there are many promising targets for targeted therapy. Numerous preclinical studies and clinical trials are ongoing to develop and evaluate new inhibitors, and some have achieved an encouraging therapeutic efficacy. IDH1/2 mutations and FGFR2 fusions are some of the most promising current targets for BTC targeted therapy, and more data are expected to verify their efficacy in the future. Furthermore, immunotherapy has also been applied in the treatment of BTCs. Immune checkpoint inhibitors nivolumab and pembrolizumab have been approved by the FDA for microsatellite instability-high (MSI-H) tumors.

With the coming era of big data and the emergence of next-generation sequencing, the implementation of individualized treatment becomes possible. Timely diagnosis (eg, liquid biopsy) and targeted therapy will significantly improve the prognosis of cancer patients. Liquid biopsy is an emerging tool for earlier cancer diagnosis with minimal invasiveness. It tests circulating tumor DNA (ctDNA), a tumor-derived fragmented DNA which exists in blood, to diagnose cancers. The difficulty in obtaining sufficient biopsy samples to confirm the diagnosis is still challenging in BTC, therefore, ctDNA could play an important role in BTC patients.[201,202] Targeted therapy has become one of the mainstay treatments for cancer patients. More therapeutic strategies, such as immunotherapy, focusing on common molecular targets and epigenetic alterations have emerged, and even the non-coding RNA and miRNA may eventually become new targets for BTC treatments.

In the future, precision treatment may become a reality for patients with malignant biliary tract cancer through the combination of clinical therapy with the molecular profile of tumors.

Abbreviations

BTC, biliary tract cancer; GBCs, gallbladder cancers; CCAs, cholangiocarcinomas; iCCAs, intrahepatic cholangiocarcinomas; eCCAs, extrahepatic cholangiocarcinomas; HBV, hepatitis B; HCV, hepatitis C; BRCA1, breast cancer gene 1; BRCA2, breast cancer gene 2; OV, *Opisthorcis viverrine*; mOS, median overall survival; mPFS, median progression-free survival; DCR, disease control rate; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine plus oxaliplatin; GS, S-1 plus gemcitabine; FGFRs, fibroblast growth factor receptors; RTKs, receptor tyrosine kinases; FGFs, fibroblast growth factors; TKIs, tyrosine kinase inhibitors; mAbs, monoclonal antibodies; ORR, overall response rate; DZB, derazantinib; SD, stable disease; IDH, isocitrate dehydrogenase; PARP, poly ADP ribose polymerase; HRR, homologous recombination repair; EGFR, epidermal growth factor receptor; EGFs, epidermal growth factors; TGF-α, transforming growth factor-α; HCC, hepatocellular cancer; NTRK, neurotrophic tropomyosin receptor kinase; TRK, tropomyosin receptor kinase; NTs, neurotrophins; NGS, next generation sequencing; FISH, fluorescence in situ hybridization; ROS1, C-ros oncogene 1; ALK, anaplastic lymphoma kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PKB, protein kinase B; mTOR, mammalian target of rapamycin; RCT, randomized controlled trial; MSI-H, microsatellite instability-high.

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