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REVIEW

# Second-Line Treatment in cholangiocarcinoma - Current State of the Art and Future Perspectives

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**Opinion Statement**

The current landscape of second-line treatment for cholangiocarcinoma (CCA) appears to be evolving, with emerging strategies offering potential improvements over traditional approaches. Although conventional chemotherapy demonstrates limited efficacy following progression after first-line therapy, the development of targeted therapies and immunotherapies is creating new possibilities for enhancing clinical outcomes. The increasing focus on molecular biomarkers—such as isocitrate dehydrogenase (IDH), B-raf kinase (BRAF), and neurotrophic receptor tyrosine kinase (NTRK) mutations, as well as fibroblast growth factor receptor (FGFR2) fusions and HER2/neu overexpression—represents a significant advancement in enabling precise, targeted treatment options. Evidence from recently completed and ongoing clinical trials evaluating these therapies, both as monotherapy and in combination with chemotherapy and/or immunotherapy, supports this shift. Continued research aimed at refining treatment selection and advancing personalized therapeutic strategies is essential, with the goal of achieving longer survival and improved quality of life for patients undergoing second-line therapy for CCA.

**Keywords** Cholangiocarcinoma · CCA · Treatment · Immunotherapy · Targeted therapy

## Introduction

Cholangiocarcinoma (CCA) is a group of rare, malignant, and aggressive tumors that originate in different parts of the biliary tract [1]. Based on their anatomical location, they can be divided into intrahepatic (iCCA) and extrahepatic (eCCA). eCCAs are further divided into perihilar (pCCA) and distal (dCCA). iCCAs develop within bile ducts closer to the interior of the liver, in front of second order (i.e., segmental) ducts. pCCAs arise in the right and/or left hepatic ducts or at the junction of the two, the so-called hepatic hilar region. In contrast, dCCAs originate in the epithelium of bile ducts located further away than the cystic duct [2]. Approximately 60% of all biliary tract cancers are pCCAs, while iCCAs account for less than 10% of primary biliary tract cancers [3]. They are different in etiology, risk factors, prognosis, and clinical and treatment management. CCA is the second most common primary liver cancer after hepatocellular carcinoma (HCC). However, it accounts for only 2% of all cancers and about 15% of primary liver cancers [4].

Although CCA is a rare cancer, its incidence is steadily increasing worldwide. Overall, CCA has an incidence rate of about 0.3–6.3/100,000 population per year and a mortality rate of 1–6/100,000 population per year. In contrast, the highest incidence rate is found in East Asian countries, such as Thailand, where it reaches 85/100,000 inhabitants per year. By comparison, the

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same rate in Western countries fluctuates between 0.8 and 2 per 100,000. The disease is most common in people aged 50–70, with men more often affected than women. CCA is usually detected only at an advanced stage, when treatment options are severely limited. Although medicine has advanced in recent decades, the prognosis for patients remains unfavorable, mainly due to late diagnosis, a high risk of recurrence, and an extremely aggressive course of the disease. According to some studies, only about 5% of patients survive five years after diagnosis, and as many as 75% die within the first year [1, 4].

iCCA and eCCA have both shared and differing risk factors. One of the most significant for both types is primary sclerosing cholangitis (PSC), which significantly increases the likelihood of disease and identifies a group of patients at particular risk. In Western countries, iCCA is mainly associated with chronic liver disease, such as viral hepatitis, excessive alcohol consumption, or non-alcoholic steatohepatitis. In contrast, eCCA is more often associated with biliary tract diseases, including gallstones [3].

Treatment of CCA depends on the stage of the disease and includes various therapeutic approaches. For cancers confined to a specific area, the standard of care for iCCA and eCCA is surgical removal of the lesion. For iCCA, hepatectomy assisted by preoperative chemotherapy is beneficial, while treatment of pCCA and dCCA often requires more extensive procedures involving both the liver and biliary tract. Because CCA can be asymptomatic for a long time, the disease is sometimes diagnosed at an advanced stage, making only about 25% of patients eligible for radical surgery [1, 3]. As of 2010, the treatment of choice for advanced and unresectable CCA is gemcitabine and cisplatin (GP) combination chemotherapy [5]. Molecular analysis of the tumor should be performed before or alongside first-line treatment to enable the selection of appropriate therapies for subsequent treatment of advanced cancer. Approximately 40% of patients with biliary tract cancer are found to have genetic alterations that may provide a target for molecularly targeted therapy [6].

This review provides an overview of current treatment guidelines for CCA, with a focus on second-line treatment, including clinical trials. Useful biomarkers of response to 2nd line treatment are also discussed.

## Current Guidelines for Advanced or Metastatic CCA

### First-Line Treatment

More than 60% of patients with CCA are diagnosed with advanced disease (locally advanced or metastatic), and radical surgery is not a possibility for them. Therefore, the standard of care for 1 st line treatment is chemotherapy consisting of gemcitabine and cisplatin (GP). This regimen has been in effect since 2010, when the results of the ABC-02 trial were published [5, 7]. After a median follow-up time of 8.2 months and 327 deaths recorded, median overall survival (mOS) was 11.7 months among patients treated with the combination of cisplatin and gemcitabine (204 patients), compared to 8.1 months in patients receiving gemcitabine alone (206 patients). The risk ratio was 0.64 (95% CI: 0.52–0.80; $P < 0.001$), indicating a significant benefit of combination treatment. In contrast, the median time free from disease progression was 8.0 months in the GP group and 5.0 months in the gemcitabine monotherapy group ($P < 0.001$) [8]. Another study confirming the benefit of combining gemcitabine with cisplatin was the Japanese BT22 study. The mOS time was 11.2 months when treated with the combination of gemcitabine and cisplatin, compared to 7.7 months when treated with gemcitabine alone. The median progression-free survival (mPFS) time was 5.8/3.7 months with a benefit for the GP combination. The hazard ratios reported in this trial for OS were (0.69, 95% CI: 0.42, 1.13) and for PFS (0.66, 95% CI: 0.41, 1.05) [9]. If we are concerned about the patient's renal function, then cisplatin can be replaced by oxaliplatin. On the other hand, it is reasonable to use gemcitabine as monotherapy in patients with a performance status (PS) of 2 and other frailty factors [2, 6].

The TOPAZ-1 trial added durvalumab, which is the programmed death ligand 1 (PD-L1) immune checkpoint inhibitor (ICI), to gemcitabine and cisplatin [2]. This combination significantly improves response rate, PFS, and median survival (12.8 months) compared to placebo with chemotherapy (11.5 months; HR, 0.76; 95% CI, 0.64–0.91) [10]. The combination of gemcitabine and cisplatin with durvalumab should be considered as first-line treatment [2]. Another study that used immunotherapy in combination with chemotherapy was the KEYNOTE-966 trial. Pembrolizumab was added to gemcitabine and cisplatin, resulting in a treatment benefit [6, 11]. mOS was 12.7 months (95% CI 11.5–13.6) in the pembrolizumab group versus 10.9 (9.9–11.6) months in the placebo group (hazard ratio 0.83 [95% CI 0.72–0.95]. Given the statistically significant and clinically meaningful prolongation of OS compared to standard therapy with gemcitabine and cisplatin, and the lack of new safety signals, the combination of pembrolizumab with gemcitabine and cisplatin may represent a new therapeutic option for patients with metastatic or unresectable CCA who have not previously received treatment [12].

## Second-Line Treatment

Patients with advanced CCA often experience rapid deterioration in overall health and PS after failure of first-line chemotherapy. As a result, only 15–25% of patients qualify for second-line therapy, most often younger patients and those with a longer progression-free time after first-line treatment [11]. As mentioned earlier, molecular profiling of the tumor should be performed before or during the first line of treatment. The test should encompass genes such as FGFR2, IDH1, HER2/neu, BRAF and NTRK. Genes such as RET, BRCA1/2, PALB2 and c-MET can also be determined. An estimated 40% of cholangiocarcinoma patients have genetic lesions that could be potential targets for directed therapy [2]. The iCCA and eCCA are differentiated from each other in terms of their genetic profile. Multiple FGFR genetic alterations and IDH mutations are more frequent in iCCA, while KRAS mutations and HER2 overexpression/amplification are more commonly observed in eCCA (Fig. 1) [13].

## IDH

Approximately 10–30% of iCCA patients have an IDH mutation, while in eCCA, it is observed in only about 7% of patients [13]. About 90% of the mutations occur in IDH1, while about 10–20% are IDH2 mutations. The most frequently clinically meaningful mutations in IDH1 and IDH2 occur at amino acid positions 132 (R132) and 172 (R172) [2]. Ivosidenib is an oral IDH1 inhibitor that was approved by the European Medicines Agency (EMA) in May 2023 and by the U.S. Food and Drug Administration (FDA) in August 2021 for the treatment of adult patients with pretreated, locally advanced, or metastatic CCA with a current IDH1 mutation [2, 13]. The randomized phase 3 ClarIDHy study demonstrated the efficacy of ivosidenib. In this study, 187 patients were randomly divided into two groups. 92% of them had metastatic disease, and 43% had received two lines of prior chemotherapy. The first received ivosidenib ($n = 126$), while the second group received a placebo ($n = 61$). 43 patients crossed over from placebo to ivosidenib. PFS was significantly better with ivosidenib relative to placebo (2.7 months [95% CI, 1.6–4.2 months] vs.

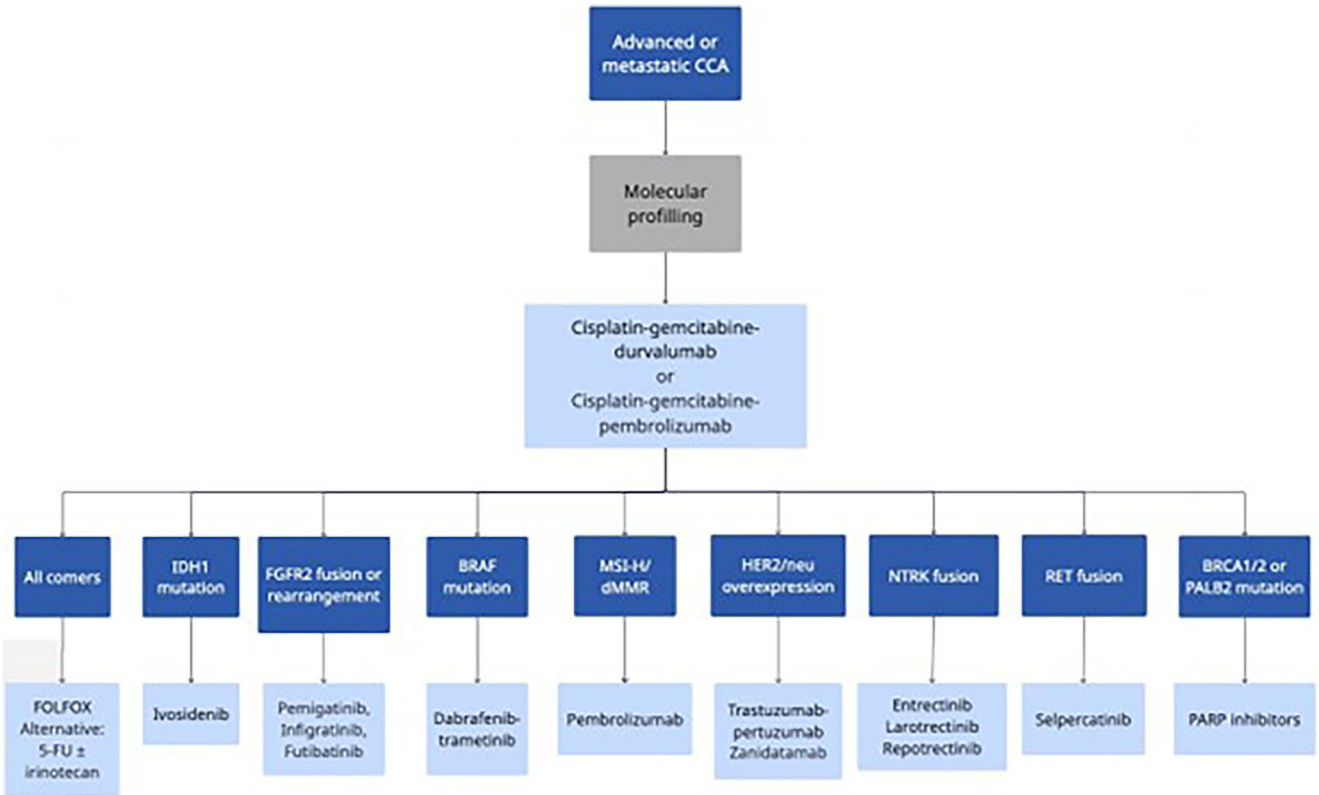



**Fig. 1** Treatment algorithm for advanced or metastatic CCA [2]. CCA – Cholangiocarcinoma; FOLFOX – Folinic Acid (Leucovorin), Fluorouracil (5-FU), and Oxaliplatin; 5-FU – 5-Fluorouracil; IDH1 – Isocitrate Dehydrogenase 1; FGFR2 – Fibroblast Growth Factor Receptor 2; BRAF – v-Raf Murine Sarcoma Viral Oncogene Homolog B1; MSI-H – Microsatellite Instability–High; dMMR – Deficient DNA Mismatch Repair; HER2/neu – Human Epidermal Growth Factor Receptor 2, NTRK - Neurotrophic Receptor Tyrosine Kinase, RET - Rearranged during Transfection, BRCA1/2 – Breast Cancer gene 1/2, PALB2 – Partner and Localizer of BRCA2, PARP – Poly-ADP ribose polymerase

1.4 months [95% CI, 1.4–1.6 months]; HR 0.37; 95% CI, 0.25–0.54; one-sided $p < 0.0001$). The final OS analyses showed a statistically insignificant improvement in OS with ivosidenib compared to placebo, even though there was a 70% transition rate from the placebo group. mOS was 10.3 months (95% CI, 7.8–12.4 months) with ivosidenib vs. 7.5 months (95% CI, 4.8–11.1 months) with placebo (hazard ratio, 0.79 [95% CI, 0.56–1.12]; 1-sided $P = 0.09$). When adjusted for crossover, mOS with placebo was 5.1 months (95% CI, 3.8–7.6 months; hazard ratio, 0.49 [95% CI, 0.34–0.70]; 1-sided $P < 0.001$) [2, 13–16]. Currently, ivosidenib is recommended in European Society for Medical Oncology (ESMO) guidelines for the treatment of patients with cholangiocarcinoma who have an IDH1 mutation and with progressive disease after at least 1 line of systemic therapy [2].

## BRAF

The BRAF gene encodes serine/threonine kinase B-Raf and functions as a protooncogene that, in mutated form, plays an important role in the development of malignancies. In CCA, the BRAF V600E mutation occurs in about 3–5% of patients, mostly in the iCCA form. BRAF is an essential component of the MAPK signaling pathway, which depends on activation by EGFR, and affects processes such as cell division, growth, differentiation, migration, and apoptosis. Mutations at codon 600 lead to sustained, uncontrolled activation of this pathway [17]. A few BRAF inhibitors are available for the treatment of cancer, but only a therapy combining dabrafenib (BRAF inhibitor) with trametinib (mitogen-activated protein kinase (MEK) inhibitor) has so far been evaluated in patients with biliary tract cancer [2, 13]. The Rare Oncology Agnostic Research (ROAR) study included 43 patients with biliary tract cancer with the BRAFV600E mutation, who were treated with combination therapy with dabrafenib (150 mg twice daily) and trametinib (2 mg once daily). The objective response rate (ORR) was 53% by investigator assessment (95% CI: 37.7–68.8) and 47% by independent assessment (95% CI: 31.2–62.3). The mPFS was 9.0 months, and the mOS was 13.5 months [17, 18]. Combination therapy with dabrafenib and trametinib is recommended for patients with the BRAFV600E mutation who have experienced disease progression after at least one prior line of systemic therapy [2].

## MSI-H/dMMR

Cholangiocarcinoma belongs to a group of solid tumors that may lack expression of unpaired base repair (MMR) proteins, resulting in an excessive number of mutations during DNA replication or leading to microsatellite instability (MSI). MMR protein expression is assessed by immunohistochemistry (IHC), and MSI or microsatellite stability status is confirmed by molecular testing. It is worth noting that the terms MSI-high and MSI-low are no longer used; MSI-low cases are now classified as stable. Samples that lack normal expression of one or more MMR proteins and are positive on MSI testing are considered MMR-deficient (dMMR). In contrast, samples with normal MMR protein expression on IHC are considered to have intact MMR function [13]. dMMR in CAA is rare, in less than 1% of patients. However, in cases with high microsatellite instability (MSI-H), the use of ICIs has provided significant clinical benefit. In the non-randomized, prospective phase II KEYNOTE-158 trial, 22 patients with CCA and confirmed MSI-H/dMMR received pembrolizumab. Pembrolizumab is an IgG4-class humanized monoclonal antibody that interacts with the PD-1 receptor found on T lymphocytes. By blocking the interaction of this receptor with its ligands - PD-L1 and PD-L2 - the drug restores the activity of T lymphocytes, enabling them to re-recognize and destroy cancer cells. A response rate of 40.9%, an mPFS of 4.2 months, and a mOS of 24.3 months were achieved. These results support the use of pembrolizumab in patients who do not have other effective therapies available. Pembrolizumab is also recommended for patients with MSI-H or dMMR whose disease has progressed despite previous therapy or who are unable to continue it due to intolerance [2, 19].

## HER2/neu Overexpression

HER2/ERBB2 overexpression is present in 3–8% of iCCA and 1.3–11% of eCCA [17]. The phase II MyPathway trial analyzed the efficacy of anti-HER2 targeted therapy with pertuzumab and trastuzumab in patients with cholangiocarcinoma who had amplification of the ERBB2 (HER2) gene and/or overexpression of the HER2 protein [13]. The study included 39 patients who received intravenous pertuzumab at an initial dose of 840 mg, followed by 420 mg every three weeks, and trastuzumab at an initial dose of 8 mg/kg, continuing with a dose of 6 mg/kg every three weeks. A promising ORR of 23% (95% CI: 11–39), mPFS of 4 months, and mOS of 10.9 months was achieved [2, 20]. Preliminary data from phase I-II trials indicate that cancers with amplification of the HER2 gene may respond better to treatment than CCA cases with mutations in this gene. Current evidence supports the use of HER2-targeted therapies in patients with HER2 amplification, especially when they have no other treatment options. However, it should be noted that none of these therapies have yet been approved by the EMA or FDA for this specific use. However, according to ESMO guidelines,

HER2-targeted therapies should be considered for use in patients who have had disease progression or intolerance to previous treatment [2].

## FGFR2 Fusions

The FGFR is a member of the receptor tyrosine kinase (RTK) family, which plays a key role in embryonic development, tissue regeneration, blood vessel formation in cancer, and regulation of cellular growth [21]. In humans, the FGFR receptor family consists of four active receptors: FGFR1, FGFR2, FGFR3, and FGFR4. There is also a fifth member, FGFR5, which, however, lacks intracellular tyrosine kinase activity [22]. The most common genetic alterations involve the FGFR2 gene, while FGFR1 or FGFR3 aberrations are observed in about 1% of patients with CCA. FGFR2 alterations are much more common in iCCA than in eCCA. FGFR2 fusions are present in 5–7% of all patients with CCA and in 10–20% of patients with iCCA [13].

Pemigatinib is a selective competitive inhibitor of FGFR1, FGFR2, and FGFR3 receptors. The FIGHT-202 trial (phase II) included 146 patients, divided into three groups: cohort A - with FGFR2 gene rearrangements or fusions, cohort B - with other FGF/FGFR gene alterations, and cohort C - without mutations in these genes. In cohort A, the mPFS was 6.9 months (95% CI: 6.2–9.6), and mOS reached 21.1 months (range: 14.8-not reached). The ORR was 35.5% (95% CI: 26.5–45.4). In contrast, no treatment response was observed in cohorts B and C. The FDA and EMA have both approved pemigatinib for the treatment of previously treated, locally advanced or metastatic CCA with FGFR2 gene rearrangements or fusions, based on favorable results from the FIGHT-202 trial [17, 23].

Futibatinib is a strongly selective, non-reversible inhibitor targeting FGFR1, FGFR2, FGFR3, and FGFR4. It was initially evaluated in the Phase I FOENIX-101 dose-escalation study, which enrolled 86 patients with previously treated, advanced cancers. Once the optimal dose was determined, 20 mg of futibatinib once daily was considered the recommended dose for further study. This was followed by the PHOENIX-CCA2 trial (Phase II), which enrolled 103 patients with unresectable or metastatic intrahepatic cholangiocarcinoma with positive fusion or rearrangement of the FGFR2 gene, whose disease had progressed despite prior systemic therapy. In the overall population of the PHOENIX-CCA2 trial, a response to treatment was observed in 43 patients (95% confidence interval, 32–52), with a median duration of response (mDOR) of 9.7 months. The mPFS was 9.0 months, while mOS reached 21.7 months after a median follow-up period of 17.1 months [17, 24]. Futibatinib received FDA and EMA approval for the treatment of previously treated, locally advanced, or metastatic CCA with FGFR2 gene rearrangements or fusions. The latest analysis, conducted after a median follow-up of 25 months, showed a mDOR of 9.5 months. In this revised review, the mPFS was 8.9 months, and mOS reached 20.0 months [17, 25].

There have also been promising results in the phase II CBGJ398 × 2204 trial with the FGFR inhibitor, infigratinib. In a multicenter, open-label phase II study, infigratinib achieved an ORR of 14.8% and a disease control rate (DCR) of 75.4% in 61 patients with advanced or metastatic cholangiocarcinoma with FGFR gene mutations whose disease had progressed after previous treatment. In the group of patients with FGFR2 gene fusions or rearrangements ($n$ = 108), results showed an ORR of 23.1% (95% CI, 15.6–32.2) after a median follow-up of 10.6 months, and a median duration of response of 5.0 months (range 3.7–9.3 months) in patients receiving infigratinib [13, 17, 26]. Based on these findings, infigratinib received FDA authorization for the treatment of previously treated, locally advanced or metastatic CCAs with FGFR2 gene rearrangements or fusions [17].

## All Comers

There is clinical evidence for the efficacy of the FOLFOX regimen as second-line therapy, as evidenced by the ABC-06 trial, which showed that FOLFOX has benefits overactive symptom control alone in patients with advanced biliary tract cancer [17]. The study included 162 patients who were randomly assigned to one of two groups: receiving active symptom control (ASC) in combination with FOLFOX ($n$ = 81) or ASC alone ($n$ = 81). The median follow-up time was 21.7 months (IQR 17.2–30.8). Patients treated with the ASC plus FOLFOX regimen had significantly longer overall survival compared to the ASC-only group, with median OS of 6.2 months (95% CI: 5.4–7.6) vs. 5.3 months (95% CI: 4.1–5.8), respectively, with an adjusted risk ratio of 0.69 (95% CI: 0.50–0.97; $p$ = 0.031). The overall survival rate at 6 months was 35.5% (95% CI: 25.2–46.0) in the ASC group and 50.6% (95% CI: 39.3–60.9) in the ASC plus FOLFOX group, while at 12 months it was 11.4% (95% CI: 5.6–19.5) and 25.9% (95% CI: 17.0–35.8.0.8), respectively [27]. Therefore, the FOLFOX regimen is recommended as second-line therapy after prior treatment with cisplatin and gemcitabine [2].

Selpercatinib is a first-in-class, highly selective RET kinase inhibitor, demonstrating efficacy in the treatment of lung and thyroid cancers with RET fusions. The LIBRETTO-001 trial is an ongoing phase 1/2, single-group, open-label, open basket study involving patients aged 18 years and older (or 12 years and older, if local regulations

permit) with RET tumors. Among the 41 patients in whom the efficacy of the therapy was evaluated, an independent review board found an objective response in 43.9% of patients (95% confidence interval: 28.5–60.3; 18 of 41 patients). Selpercatinib is included in the latest ESMO guidelines for the 2nd-line treatment of cholangiocarcinoma for patients with RET fusion [28, 29].

As in other cancers, patients with cholangiocarcinoma are found to have pathogenic variants in genes responsible for repairing DNA damage through homologous recombination, which may increase their sensitivity to treatment with DNA-damaging drugs such as platinum compounds or PARP (poly(ADP-ribose) polymerase) inhibitors. Although the exact definition of homologous recombination defects in BTC has not yet been established, patients with mutations in the BRCA1, BRCA2 or PALB2 genes who respond positively to platinum-based treatment may be candidates for PARP inhibitor therapy and should be considered for clinical trials. In such patients, treatment with PARP inhibitors is being considered as a potentially effective therapeutic option [2].

## Second-Line Treatment in Clinical Trials

### Chemotherapy

An alternative for second-line treatment is irinotecan-based chemotherapy. For the NALIRI regimen (liposomal irinotecan in combination with fluorouracil and leucovorin), however, there are inconsistent clinical data on its anti-tumor efficacy. In the NIFTY trial (Phase IIb, NCT03524508), with a median follow-up of 33.2 months, there was a significant improvement in mPFS - as assessed by an independent central review - in favor of NALIRI compared to 5-FU alone in patients with advanced cholangiocarcinoma previously treated with gemcitabine and cisplatin (mPFS: 4.2 months, 95% CI: 2.8–5.3 vs. 1.7 months, 95% CI: 1.4–2.6). mOS was 8.6 months (95% CI: 5.4–10.5) in the NALIRI group, vs. 5.3 months (95% CI: 4.7–7.2) in the control group. In contrast, the NALIRICC trial (phase II, NCT03043547) found no advantage for NALIRI over 5-FU - the reported median OS was 6.9 months in the NALIRI group versus 8.21 months in the group receiving only 5-FU. Therefore, the efficacy of NALIRI as a second-line therapy remains to be determined [17, 30, 31].

### Targeted Therapy

Several clinical trials of targeted therapies are currently underway to confirm their safety during use and efficacy (Table 1). A synopsis of the mechanisms of action of the substances utilized in targeted therapy for cholangiocarcinoma is presented in Fig. 2. Phase I and II trials of several small molecules are ongoing to target therapies for patients with IDH1, IDH2, and pan-IDH1/2 mutations (see Table 2 for details). In a completed phase II study with dasatinib (NCT02428855) in patients with advanced or metastatic biliary tract cancer (CCA) with IDH1/2 mutations, mPFS was 8.7 months and mOS was 37.9 months. Analyses of the results of phase I/II trials with olutasidenib (NCT03684811) and enasidenib (NCT02273739) in patients with advanced or metastatic CCA with IDH1 or IDH2 mutations are ongoing.

For CCAs without the BRAF V600E mutation, the phase II BEAVER trial (NCT03839342) is analyzing the combination of binimetinib with encorafenib. On the other hand, the phase II BELIEVE study evaluated the efficacy of dabrafenib plus trametinib therapy in patients with advanced solid tumors with or without BRAFV600E/R mutation. The study reported a clinically relevant response rate (ORR) of 28%. Among the 77 participants, 94% had the BRAFV600E mutation, and 3 had other BRAF variants. In the entire group of patients, mPFS was 6.5 months (95% CI: 4.2–7.2), while mOS reached 9.7 months (95% CI: 7.5–12.2). Participants included 2 patients with gallbladder tumor or extrahepatic bile duct cancer (GB/eCCA) and 4 with intrahepatic cancer. Survival data in these subgroups are still being analyzed [17, 32].

NTRK type 1–3 gene fusions are among the driving mutations that cause sustained activation of the kinase signaling pathway. In response to these findings, the U.S. FDA granted accelerated marketing approval in 2018 and 2019 to two targeted drugs - entrectinib (which acts on tyrosine receptor kinase (TRK), ROS protooncogene (ROS1), and anaplastic lymphoma kinase (ALK)) and larotrectinib - for patients with solid tumors containing NTRK gene fusions. These decisions were based on the results of a basket-type Phase I study (STARTRK-1 and ALKA-372-001), which demonstrated entrectinib's significant antitumor efficacy (ORR of 57%) and its favorable safety profile - Grade 1 and 2 side effects, mainly fatigue and taste disorders, predominated. The three basket trials - NAVIGATE (Phase II, NCT02576431), SCOUT (Phase I/II, NCT02637687), and the Phase I safety study (NCT02122913) - enrolled a total of 55 patients with solid tumors containing the NTRK gene fusion who received larotrectinib 100 mg twice daily. Participants included patients with 17 different types of TRK-positive tumors, including 2 patients with biliary tract cancer (CCA). The ORR was 75% (95% CI: 61–85) by independent assessment and 80% (95% CI: 67–90) by investigators. Importantly, treatment responses were durable, with a median of 10 months for entrectinib, while the median for larotrectinib has not yet been reached. Further studies are currently underway, including

**Table 1** Completed clinical trials of targeted Inhibition in CCA. ORR – Objective response Rate; mPFS – median Progression-Free Survival; mOS – median overall survival

| Target | Agent | Phase | Clinical Trial | ORR | mPFS (months) | mOS (months) |
|---|---|---|---|---|---|---|
| IDH | Ivosidenib + nivolumab | II | NCT04056910 | - | 1.94 (1.61–3.68) | - |
| | Ivosidenib | III | ClarIDHy trial NCT02989857 | - | 2.7 (1.6–4.2) | 10.3 |
| | Dasatinib | II | NCT02428855 | - | 8.7 (6.4- NA) | 37.9 (20.7-NA) |
| | Enasidenib (AG-221) | I/II | NCT02273739 | Ongoing | | |
| | Olutasidenib (FT-2102) | I/II | NCT03684811 | Ongoing | | |
| | Niraparib | II | NCT03207347 | - | 7.2 (1.8–12.5) | 7.2 (2.9–15.5) |
| BRAF | Dabrafenib + trametinib | II | ROAR trial NCT02034110 | 47% (20/43; 95% CI 31.2–62.3%) | - | - |
| NTRK | Entrectinib | I | STARTRK-1 NCT02097810 | 57% (31/54; 95% CI 43.2–70.8%) | - | - |
| HER2 | Trastuzumab + pertuzumab | II | MyPathway NCT02091141 | 21.0% (95% CI 16.9–25.6) | 2.8 (2.7–3.4) | 10.1 (8.7–11.7) |
| FGFR2 | Pemigatinib | III | FIGHT-202 NCT02924376 | 37% (95% CI 27.94–46.86%) | - | - |
| | Infigratinib | II | NCT02150967 | 23.1% (95% CI 15.6–32.2) | - | - |
| | Futibatinib | I/II | NCT02052778 | - | 8.9 (6.7–11.0) | 20.0 (16.4–24.6) |
| | Derazantinib | II | FIDES-01 NCT03230318, Substudy 1 – patients with FGFR2 fusions Substudy 2 – patients with FGFR2 mutations or amplifications | ORR2: 9.3% (95% CI 2.6–22.1) | - | mOS1: 17.2 (12.5 to 22.4) mOS2: 11.9 (8.4 to 15.9) |

the international, open-label, phase II STARTRK-2 basket study (NCT02568267), evaluating the efficacy of entrectinib in patients with solid tumors containing NTRK1/2/3, ROS1, or ALK fusions. Due to the rare occurrence of NTRK gene fusions in CCA, estimated at approximately 0.75%, and the small number of CCA patients included in the basket studies to date, further studies are needed to further define the clinical efficacy of larotrectinib and entrectinib in patients with advanced or metastatic CCA with the presence of an NTRK gene fusion [17, 33].

A multicenter, non-randomized phase II study (NCT02999672) aimed at evaluating the efficacy, safety, and pharmacokinetics of trastuzumab emtansine in patients with solid tumors overexpressing HER2, including CCA, after exhausting available treatment options, was prematurely terminated. The decision was made after five of seven patients with advanced pancreatic cancer, or CCA, experienced disease progression. Due to the limited efficacy of HER2-targeting monotherapy, phase I and II trials are currently underway to evaluate the safety and potential efficacy of trastuzumab in combination with other anti-cancer drugs, such as pembrolizumab, FOLFOX, or CAPOX (NCT04430738). In addition, a phase I study (NCT03219268) is underway to evaluate the safety and tolerability of the immune checkpoint inhibitor, tebotelimab, both as monotherapy and in combination with margetuximab, a chimeric IgG monoclonal antibody targeting HER2, in patients with advanced HER2-positive solid tumors [31]. The bispecific antibody zanidatamab, which targets the HER2 receptor, showed promising anti-tumor activity in the phase 2b study HERIZON-BTC-01 (NCT04466891), conducted among patients with locally advanced or metastatic cholangiocarcinoma (CCA) with HER2 amplification. The study included 87 subjects divided into two cohorts: cohort 1 (HER2-positive, IHC 2 + or 3+) and cohort 2 (IHC 0 or 1+). Participants received zanidatamab at a dose of 20 mg/kg intravenously every two weeks. Cohort 1 had an objective response rate (ORR) of 41.3% (95% CI: 30.4–52.8). It is also noteworthy that a beneficial effect of therapy was observed in patients with gallbladder cancer [34]. In a phase II study (NCT04722133), a treatment regimen based on mFOLFOX6 in combination with trastuzumab for second- or third-line therapy showed encouraging anti-tumor efficacy. mPFS was 5.1 months (95% CI: 3.6–6.7), and mOS reached 10.7 months (95% CI: 7.9- not reached). The KAMELEON trial (phase

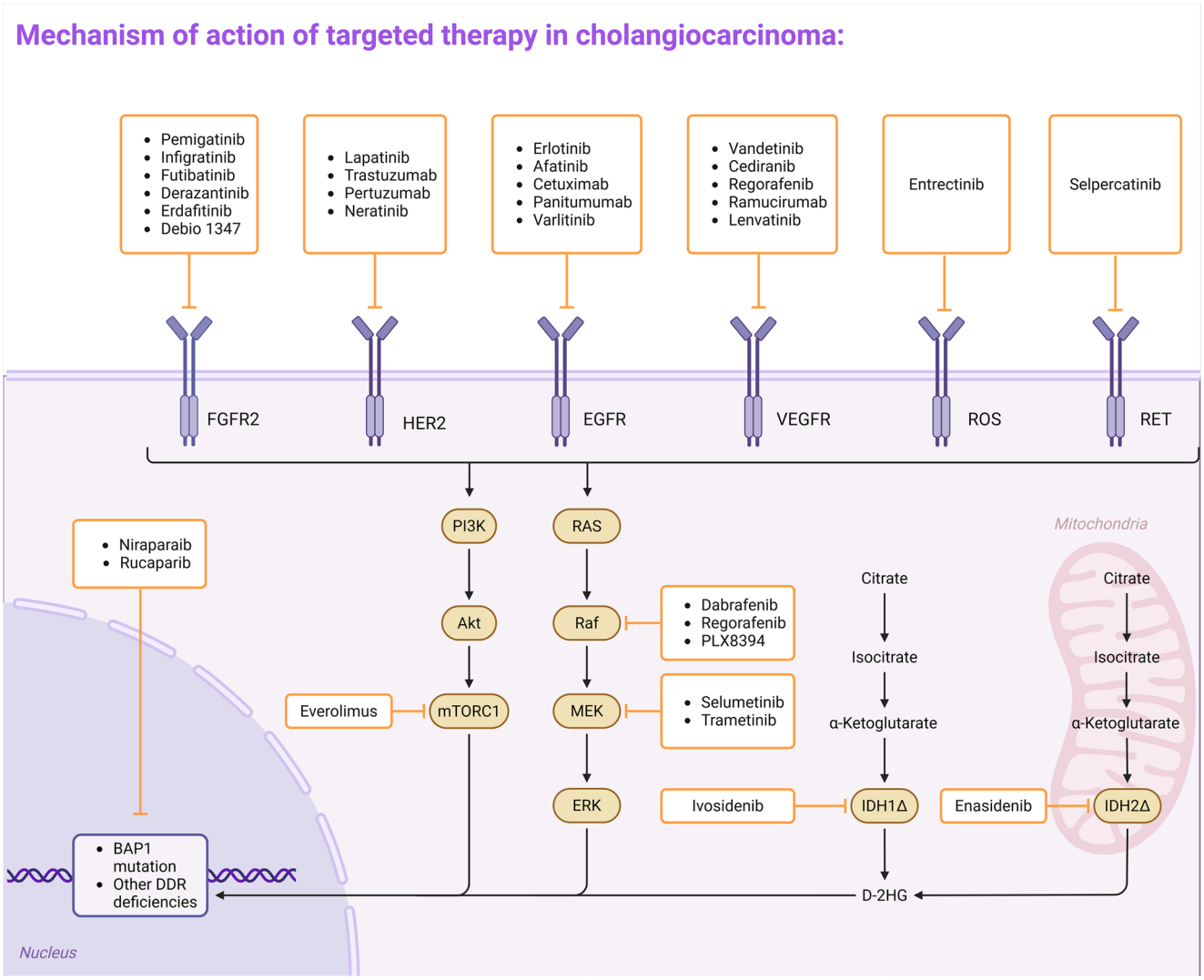



**Fig. 2** The mechanism of action of targeted therapies discussed in the above chapters. FGFR2 – Fibroblast Growth Factor Receptor 2; HER2 – Human Epidermal Growth Factor Receptor 2; EGFR – Epidermal Growth Factor Receptor; VEGFR – Vascular Endothelial Growth Factor Receptor; ROS – Reactive Oxygen Species (ROS1 receptor tyrosine kinase); RET – Rearranged during Transfection; PI3K – Phosphoinositide 3-Kinase; Akt – Protein Kinase B; mTORC1 – Mechanistic Target of Rapamycin Complex 1; RAS – Rat Sarcoma virus oncogene homolog; Raf – Rapidly Accelerated Fibrosarcoma kinase; MEK – Mitogen-Activated Protein Kinase Kinase; ERK – Extracellular signal-Regulated Kinase; IDH1 – Isocitrate Dehydrogenase 1; IDH2 – Isocitrate Dehydrogenase 2; D-2HG – D-2-Hydroxyglutarate; BAP1 – BRCA1-Associated Protein 1; DDR – DNA Damage Response

II, NCT02999672), which was designed to evaluate the efficacy of trastuzumab emtansine in patients with advanced HER2-positive CCA, encountered recruitment difficulties, thus not obtaining representative data on this group of patients. At the same time, another phase II study (NCT02675829) is underway that is analyzing the effect of trastuzumab emtansine in the treatment of solid tumors with HER2 overexpression [17]. Trastuzumab deruxtecan (T-DXd), which is a conjugate of an antibody and a cytotoxic drug, was evaluated in the DESTINY-PanTumor02 trial in patients with advanced solid tumors. The phase II study (NCT04482309) administered T-DXd at a dose of 5.4 mg/kg every three weeks to patients with locally advanced or metastatic HER2-expressing disease who had previously undergone at least one systemic treatment or had no other therapeutic options. Forty-one patients with biliary tract cancer were included in the study. Of these, 16 had high HER2 expression (IHC 3+) and 25 had moderate expression (IHC 2+). The ORR was 56.3% in the IHC 3 + group, while no response was observed in the IHC 2 + group (ORR 0%). The median progression-free survival was 7.4 months (95% CI: 2.8–12.5) in patients with HER3 + expression and 4.2 months (95% CI: 2.8–6.0.8.0) in HER2 + patients. In contrast, median overall survival was 12.4 months (95% CI: 2.8-none) and 6.0 months (95% CI: 3.7–11.7), respectively [35].

While more specific and more powerful FGFR2 inhibitors are being developed, current FGFR inhibitors continue to be studied both as monotherapy and in conjunction with chemotherapy. For example, the FIGHT-302 trial (NCT03656536) is evaluating the efficacy of pemigatinib compared to chemotherapy as first-line treatment in patients with advanced biliary tract cancer. At the same time, the FIGHT-101 trial (NCT02393248) is underway to analyze the safety, tolerability, and pharmacological effects of pemigatinib in monotherapy and in combination with drugs such as gemcitabine and cisplatin in patients with advanced cancers, including CCA. Currently available FGFR2 inhibitors show limited selectivity toward this receptor, resulting in a broader side effect profile. In addition, the development of FGFR2 resistance mutations poses a significant therapeutic problem. RLY-4008, which is the first highly selective FGFR2 inhibitor, is being tested in the ReFocus trial (Phase I/II, NCT04526106). Preliminary results from 17 patients treated with the recommended phase II dose showed high efficacy, achieving an ORR of 88% (95% CI: 63.6–98.5). In addition, teninotinib showed strong activity against resistance mutations in the initial CCA clinical trials [17, 33]. Erdafitinib is a tyrosine kinase inhibitor that potently inhibits FGFR1-4 receptors in a reversible manner. In addition to its action on FGFR, it also shows activity against other kinases such as ret protooncogene (RET), platelet-derived growth factor receptor (PDGFR) α/β, fms-related tyrosine kinase (FLT) 4, receptor tyrosine kinase (KIT), and vascular endothelial growth factor (VEGFR) 2. In a phase II study (NCT02699606) conducted in an Asian population, erdafitinib 8 mg orally once daily was administered to patients with FGFR abnormalities in whom previous systemic treatment had failed. The therapy yielded promising results – the ORR was 50%, the DCR was 83.3%, and the mPFS was estimated at 5.59 months (95% CI: 1.87–13.67) [17, 36, 37]. Derazantinib is a reversible multi-kinase inhibitor that potently blocks FGFR1, FGFR2, and FGFR3 receptors, in addition to affecting other tyrosine kinases such as RET, DDR2, VEGFR1, and KIT [36]. In the second cohort of the FIDES-01 trial (Phase II, NCT03230318), patients with cholangiocarcinoma had a mPFS of 7.8 months (95% CI, 5.5–8.3), an ORR of 6.8% (95% CI, 1.4–18.7) and a DCR of 63.6% (95% CI, 47.8–77.6). In contrast, cohort 1 of the same study, which included patients with iCCA and FGFR2 mutation or amplification,

**Table 2** Ongoing targeted therapy trials in CCA

| Target | Study | Phase | Treatment Arm A | Treatment Arm B | Primary Endpoint | NCT |
|---|---|---|---|---|---|---|
| IDH1 | - | I | AG-120 (oral Ivosidenib) | - | Safety | NCT02073994 |
| | - | I | LY3410738 ± (Gemcitabine + cisplatin) or durvalumab | - | Safety | NCT04521686 |
| | - | I | IDH305 | - | Safety | NCT02381886 |
| | ProvIDHe | III | Ivosidenib | - | Safety | NCT05876754 |
| | - | I/II | Ivosidenib + nivolumab + ipilimumab | - | Safety, OR | NCT05921760 |
| | - | II | Ivosidenib | - | 6-month PFS | NCT06081829 |
| | - | Obser--ational | Ivosidenib | - | PFS | NCT06607302 |
| | - | I/II | Ivosidenib + durvalumab + gem-citabine + cisplatin | - | Safety, ORR | NCT06501625 |
| | - | I | AB-218 | - | Safety | NCT05814536 |
| | - | II | Olaparib | - | ORR | NCT03212274 |
| | - | II | Olaparib + ceralasertib (AZD6738) | - | ORR | NCT03878095 |
| | - | I | BAY-1,436,032 | - | Safety | NCT02746081 |
| | - | I | Vorasidenib (AG-881) | - | Safety | NCT02481154 |
| BRAF | PiCCA | II | Panitumumab + cisplatin + gemcitabine | Cisplatin + gemcitabine | 6-month PFS | NCT01320254 |
| | - | II | Atezolizumab | Atezolizumab + cobimetinib | PFS | NCT03201458 |
| | - | II | Selumetinib + gemcitabine + cisplatin | Gemcitabine + cisplatin | Response | NCT02151084 |
| | BEAVER | II | Binimetinib + encorafenib | - | ORR | NCT03839342 |
| | TAPISTRY | II | Belvarafenib | - | ORR | NCT04589845 |
| | - | - | Ulixertinib | - | - | NCT04566393 |
| MSI-h/ MMR-d | KEYNOTE-158 | II | Pembrolizumab | - | ORR | NCT02628067 |

**Table 2** (continued)

| Target | Study | Phase | Treatment Arm A | Treatment Arm B | Primary Endpoint | NCT |
|---|---|---|---|---|---|---|
| HER2 | - | I/II | Zanidatamab + evorpacept | - | Safety, ORR | NCT05027139 |
| | - | II | Zanidatamab + cisplatin/5-FU or mFOLFOX6 or XELOX or mFOLFOX6 + bevacizumab or gemcitabine/ Cisplatin | - | Safety, ORR | NCT03929666 |
| | - | I | Tipifarnib + trastuzumab | - | Safety | NCT00005842 |
| | DPT02 | II | Trastuzumab deruxtecan (T-DXd) | - | ORR | NCT04482309 |
| | - | I | ZN-A-1041 (dose finding) | ZN-A-1041 plus TDxd or TD-M1 or pertuzumab/ trastuzumab | Safety | NCT05593094 |
| | - | II | Ado-Trastuzumab Emtansine | - | ORR | NCT02675829 |
| | TAPISTRY | II | Trastuzumab emtansine | - | ORR | NCT04589845 |
| | - | I/II | Tucatinib + trastuzumab ± (pembrolizumab or FOLFOX or CAPOX) | - | Safety | NCT04430738 |
| | - | I/II | A166 | - | Safety | NCT03602079 |
| | - | I | CT-0508 | - | Safety | NCT04660929 |
| | - | I | Tebotelimab ± margetuximab | - | Safety | NCT03219268 |
| | HERIZON-BTC-01 | II | Zanidatamab | - | ORR | NCT04466891 |
| | - | I/II | DB-1303/ BNT323 | - | Safety, ORR | NCT05150691 |
| | BILHER | II | Trastuzumab | - | ORR | NCT03613168 |
| | - | I/II | IAH0968 + Gemcitabine + Cisplatin | - | Safety, ORR | NCT05991518 |
| | TRAP-BTC | II | Gemcitabine/ cisplatin + trastuzumab + pembrolizumab | - | ORR | NCT06178445 |
| | - | II | Neratinib | - | ORR | NCT06519110 |
| | - | I | ZW49 | - | Safety | NCT03821233 |
| | - | II | Tucatinib + trastuzumab ± pembrolizumab ± FOLFOX or CAPOX | - | Safety | NCT04430738 |
| | - | II | Disitamab Vedotin + cadonilimab | - | ORR | NCT06383533 |
| | - | II | Varlitinib | - | ORR | NCT02609958 |
| | - | III | Zanidatamab + Cisplatin + Gemcitabine + Pembrolizumab + Durvalumab | Cisplatin + Gemcitabine + Pembrolizumab + Durvalumab | PFS | NCT06282575 |
| | - | I/II | TT-00420 | - | Safety | NCT04742959 |
| | - | I | Felmetatug Vedotin | Felmetatug Vedotin + pembrolizumab | Safety | NCT05194072 |
| | - | I/II | TT-00420 | TT-00420 + atezolizumab | Safety | NCT05253053 |
| NTRK | - | II | Entrectinib | - | ORR | NCT02568267 |
| | - | II | Larotrectinib Sulfate | - | ORR | NCT04879121 |
| | TAPISTRY | II | Entrectinib | - | Safety | NCT04589845 |
| | STARTRK-2 | II | Entrectinib | - | ORR | NCT02568267 |
| | NAVIGATE | II | Larotrectinib | - | ORR | NCT02576431 |
| | SCOUT | I/II | Larotrectinib | - | Safety, ORR | NCT02637687 |
| | - | I | Larotrectinib | - | Safety | NCT02122913 |

**Table 2** (continued)

| Target | Study | Phase | Treatment Arm A | Treatment Arm B | Primary Endpoint | NCT |
|---|---|---|---|---|---|---|
| FGFR2 | FIGHT-302 | III | Pemigatinib | Gemcitabine/ cisplatin | PFS | NCT03656536 |
| | FOENIX-CCA4 | II | Futibatinib 20 mg | Futibatinib 16 mg | ORR | NCT05727176 |
| | ADVANCE_2020 | II | Atezolizumab + derazantinib | - | Safety | NCT05174650 |
| | REFOCUS | I/II | RLY-4008 | - | Safety | NCT04526106 |
| | - | II | TT-00420 (tinengotinib) | - | ORR | NCT06057571 |
| | FIRST-308 | III | Tinengotinib | Physician's Choice (FOLFOX or FOLFIRI) | Safety, PFS | NCT05948475 |
| | - | II | E7090 | - | ORR | NCT04238715 |
| | FORTITUDE-301 | I/II | Bemarituzumab | - | Safety, ORR | NCT05325866 |
| | - | II | Erdafitinib | - | ORR | NCT02699606 |
| | - | I | TYRA-200 | - | Safety | NCT06160752 |
| | - | I/II | CGT4859 | - | Safety, ORR | NCT06777316 |
| | - | II | Pemigatinib | - | ORR | NCT04256980 |
| | - | II | TT-00420 (tinengotinib) | - | ORR | NCT04919642 |
| | - | II | Pemigatinib + durvalumab | - | ORR | NCT06728410 |
| | - | II | HMPL-453 | - | ORR | NCT04353375 |
| | - | II | 3D185 | - | ORR | NCT05039892 |
| | - | II | ICP-192 | - | ORR | NCT05678270 |
| | - | II | E7090 | - | ORR | NCT04238715 |
| | - | II | Pemigatinib + durvalumab | - | ORR | NCT06530823 |
| | - | I | Afatinib + pemigatinib | - | Safety, ORR | NCT06302621 |

reported a mPFS of 8.3 months (95% CI, 3.5–16.7), an ORR of 22.3% (95% CI, 14.7–31.6) and a DCR of up to 75.7% (95% CI, 66.3–83.6). Currently, derazantinib has no registration for the treatment of cholangiocarcinoma with the presence of FGFR2 mutations or amplifications [17, 38]. A summary of pending clinical trials with targeted therapies is shown in Table 2.

## Immunotherapy

Immune checkpoint inhibitors (ICIs) promote the body's adaptive immune response against tumor cells by blocking the interaction of PD-L1 ligand with the PD-1 receptor or CTLA-4 protein with its receptors on antigen-presenting cells. These drugs are widely used to treat a variety of solid tumors, especially those characterized by positive PD-L1 expression, high microsatellite instability, deficient mismatch repair mechanism, or high mutational burden (TMB-H). In patients with CCA, PD-L1 expression ranges from 9.1% to 72.2% [39, 40]. Also, a phase 2 study involving 54 patients (NCT02829918) reported moderate efficacy of nivolumab, with an ORR of 47.8%, mPFS of 3.68 months, and mOS of 8.9 months [33].

Due to the limited efficacy of immunotherapy used alone, many completed and ongoing studies have focused on comparing its safety and efficacy with combination therapy, combining immunotherapy with chemotherapy (e.g., gemcitabine and cisplatin). It is assumed that such a combination has a synergistic effect - chemotherapy can increase the immunogenicity of tumor cells by stimulating the release of cytokines, damage-associated molecules (DAMPs), and enhancing the expression of human leukocyte antigens (HLA) class I molecules, as well as through other mechanisms [33, 41]. A phase 2 study (NCT03111732) completed in November 2021 evaluated the efficacy of the combination therapy of pembrolizumab with capecitabine and oxaliplatin (CAPOX) in 11 patients with advanced biliary tract cancer. The mPFS was 4.54 months, while the mOS was 43 weeks. There were no cases of complete response, while partial response was observed in 3 patients (27.3%).

The NCT03092895 trial evaluated the efficacy of the combination therapy of camrelizumab with gemcitabine and oxaliplatin (Cam-GEMOX) or with FOLFOX (Cam-FOLFOX) in Asian patients. Among the 92 patients analyzed, the overall response rate (ORR) was 16.3% (95% CI: 9.4–25.5), and disease control was achieved in 75.0% of patients (95% CI: 64.9–83.4). The mPFS was 5.3 months (95% CI: 3.7–5.7), and the mOS was 12.4 months (95% CI: 8.9–16.1). Comparing the two regimens, Cam-FOLFOX had a slightly lower ORR (10.3% vs. 19%),

with similar median PFS and OS values [42]. The phase 2 study (NCT03796429), conducted among an Asian patient population, evaluated the efficacy of the PD-1 inhibitor toripalimab in combination with gemcitabine and the oral anticancer drug S-1 (containing tegafur, a prodrug of 5-FU, along with gimeracil and oteracil). Among the 48 participants, the ORR was 27%, mPFS was 7 months (95% CI: 5.5–9.1), and mOS was 16.0 months (95% CI: 12.1- no data available for the upper limit) [43]. Durvalumab, a PD-L1 inhibitor, was tested in combination with gemcitabine and cisplatin in the NCT03046862 trial, showing promising efficacy. The ORR of 73% was achieved, with a mOS of 18.1 months and a mPFS of 11 months. Interestingly, the addition of tremelimumab, a CTLA-4 inhibitor, to this regimen for dual blockade of PD-L1 and CTLA-4 yielded comparable results: ORR of 73.4%, mOS of 20.7 months and mPFS of 11.9 months [44].

The combination of durvalumab and tremelimumab, administered without chemotherapy, was evaluated in a phase 1 study (NCT01938612) in previously treated patients - the median was two lines of chemotherapy. Results were moderate, with ORRs of 10.8% and 8%, respectively, and mOS of 10.1 months (95% CI: 6.2–11.4) versus 8.1 months (95% CI: 5.6–10.1). Another approach based on simultaneous blockade of PD-1 and CTLA-4, the combination of nivolumab and ipilimumab, was evaluated in a phase 2 study (NCT02923934, CA209-538) in 39 patients with cholangiocarcinoma, 33 of whom were previously treated. The ORR was 24% - a response was achieved in 3 of 14 patients with iCCA and 1 of 10 with eCCA. Importantly, none of the responders had disease with microsatellite instability. Median OS and PFS were 6.1 and 3.1 months, respectively [45, 46].

A summary of completed clinical trials in CCA with immunotherapy is provided in Table 3. Ongoing studies using immunotherapy, meanwhile, are included in Tables 4 and 5.

## Biomarkers for prognosis/prediction

Cancer biomarkers are useful because they support early detection of the disease, help assess individual prognosis and risk of recurrence, and support selection of the most appropriate treatment strategy - surgical, local, or systemic. The ideal biomarker for cholangiocarcinoma, as for other cancers, should be readily available, inexpensive, have high sensitivity (to detect the disease at an early, potentially curable stage), and high specificity (i.e., the ability to distinguish CCA from other diseases, both malignant and benign). Unfortunately, such a perfect marker has not yet been discovered. Currently, serum markers such as carcinoembryonic antigen (CEA), carbohydrate antigens 19 − 9 (CA 19 − 9) and 125 (CA 125) are used in the diagnosis of CCA, although their limited sensitivity and specificity make them unsuitable for early diagnosis of the disease. CA 19 − 9, despite its widespread use, does not meet the criteria of an ideal biomarker - its levels can also increase in cases of mild biliary obstruction. As a result, its sensitivity and specificity in diagnosing CCA are 62% and 63%, respectively [5, 47]. Other biomarkers present in the blood, such as CYFRA 21 − 1 (a fragment of cytokeratin-19) and CA 242, have shown greater specificity than CA 19 − 9 in detecting CCA, although this has so far been confirmed in only a limited number of studies and is not routinely used in everyday clinical practice [5].

Tumor markers can provide important data to support diagnosis and prognostic evaluation and serve as an adjunctive tool in the analysis of solid tumors and post-surgical follow-up, especially in the context of disease recurrence. However, it is important to remember that biomarkers alone cannot be the basis for diagnosis [5, 48, 49]. Their usefulness depends largely on their ability to distinguish CCA from HCC. For example, CA 19 − 9 - one of the most used biomarkers for cholangiocarcinoma - is a Lewis blood group antigen produced by epithelial cells of the pancreas, bile ducts, stomach, and colon. It is worth mentioning that about 7% of people do not produce CA 19 − 9 due to the lack of the appropriate genes, which prevents its detection. Elevated CA 19 − 9 values are more often associated with CCA, while elevated AFP levels are more suggestive of the presence of hepatocellular carcinoma (HCC) [5, 50].

Although mutations in the KRAS gene are not specific to intrahepatic cholangiocarcinoma (iCCA), as they are found in many types of cancer, it remains one of the most frequently altered genes in iCCA and may be a potential biomarker. Of equal interest are BRAF mutations, which are found in about 10% of iCCA patients. In addition, up to 30% of cases show abnormalities in the phosphoinositide 3-kinases (PI3K)/phosphatase and tensin homolog (PTEN)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway, further highlighting the genetic diversity of factors influencing the development of iCCA. An interesting result came from a study in which several markers - CA19-9, CEA, MMP-7, and CYFRA 21 − 1 - were combined to form a panel with high diagnostic performance. The use of this set enabled a sensitivity of 92%, specificity of 96% and overall accuracy of 94% in diagnosing iCCA. Importantly, such a panel of biomarkers can not only improve the accuracy of genetic diagnosis, but also effectively distinguish iCCA from other cancers, such as HCC and pancreatic cancer [5, 51, 52].

**Table 3** Completed immunotherapy clinical trials in CCA

| Target | Agent | Phase | Clinical Trial | ORR | mPFS (months) | mOS (months) |
|---|---|---|---|---|---|---|
| PD-1, MSI-high, MMR-deficient, or TMB-H | Pembrolizumab (MK-3475) | I | KEYNOTE-028 (NCT02054806) | 17.4% (95% CI, 5.0–38.8.0.8) | 1.8 (95% CI, 1.4–2.1) | 5.7 (95% CI, 3.1–9.8) |
| | Nivolumab | II | NCT02829918 | 47.8% | 3.68 (95% CI, 2.3–5.69) | 8.9 (95% CI, 5.2–17) |
| PD-L1 and CTLA4 | Nivolumab + ipilimumab | II | CA209-538 | ORR: 36% (12/33) | - | - |
| PD-L1 and TGF-β RII | Bintrafusp alfa (M7824) | II | NCT03833661 | 10.7% | 1.8 (95% CI, 1.7–1.8) | 7.6 (95% CI, 5.8–9.5) |
| PD-1 and PARP | Pembrolizumab + Olaparib | II | NCT04306367 | 15.4% (2/13) | 5.45 (95% CI, 1.25–7.82) | 7.21 (95% CI, 4.5–13.77.5.77) |
| PD-1 | Pembrolizumab + oxaliplatin + capecitabine (CAPOX) | II | NCT03111732 | - | 4.54 (95% CI, 2.5–9.6) | 43 (95% CI, 22–66) weeks |
| | Arm A: Pembrolizumab + gemcitabine + cisplatin<br>Arm B:<br>Placebo + gemcitabine + cisplatin | III | KEYNOTE-966 NCT04003636 | arm A: 28.7% (95% CI, 24.9–32.8)<br>arm B: 28.5% (95% CI, 24.8–32.6) | arm A: 6.5 (95% CI, 5.7–6.9)<br>arm B: 5.6 (95% CI, 5.1–6.6) | arm A: 12.7 (95% CI, 11.5–13.6)<br>mOS arm B: 10.9 (95% CI, 9.9–11.6) |
| PD-1 and VEGFR2 | Pembrolizumab + ramucirumab | I | NCT02443324 | 3.8% | 1.6 (95% CI, 1.4–2.8) | 6.4 (95% CI, 4.2–13.3) |
| PD-1 and FGFR2 | Pembrolizumab + pemigatinib | I/II | NCT02393x Primary endpoint: dose escalation | - | - | - |
| PD-1 and benzamide histone deacetylase | Entinostat + nivolumab | II | NCT03250273 | 0% | - | 0.077 (95% CI, 0.012–0.506 at 36 months) |

**Table 4** Ongoing clinical trials for immunotherapy in monotherapy or combination in CCA

| Target | Study | Phase | Treatment Arm A | Treatment Arm B | Primary Endpoint | NCT |
|---|---|---|---|---|---|---|
| PD-L1 | - | I | Durvalumab | - | Safety | NCT01938612 |
| | - | II | AZD6738 + Durvalumab | - | DCR | NCT04298008 |
| PD-L1 and CTLA4 | - | I | Durvalumab ± tremelimumab | - | Safety | NCT01938612 |
| | IMMUNO-BIL | II | Durvalumab + tremelimumab | - | OS | NCT03704480 |
| | - | II | Durvalumab | Durvalumab + tremelimumab | ORR | NCT04238637 |
| | KEYNOTE-158 | II | Pembrolizumab | - | ORR | NCT02628067 |
| PD-1 and CTLA-4 | DART | II | Nivolumab + ipilimumab | Nivolumab | ORR | NCT02834013 |
| | - | II | Nivolumab + ipilimumab | - | Efficacy | NCT02923934 |
| | - | II | PD-L1 antibody + CTLA-4 antibody | - | ORR | NCT04634058 |

CCA is often characterized by mutations in genes such as FGFR2, IDH1, IDH2, RB1, ERBB2 and BAP1. Mutations in the TP53 gene are important because of their association with the aggressive course of the disease and resistance to conventional treatments. Alterations in FGFR2, often found in iCCA, are becoming a promising target for targeted therapies. Inhibitors targeting FGFR pathways offer new treatment options and are part of the development of precision medicine. IDH1 and IDH2 mutations also play an important prognostic and therapeutic role, and their presence can indicate specific molecular subtypes, enabling a more personalized treatment approach. Additionally, the detection of mutations in the ERBB2 gene underscores the molecular diversity of iCCA and the need to individualize therapy - hence the growing interest in the use of ERBB2 inhibitors, already proven in the treatment of other cancers, including in the context of biliary tract cancer [53, 54].

Tumor markers not only have a diagnostic function but can also provide prognostic information, such as predicted OS or risk of recurrence, and support therapeutic decision-making

**Table 5** Ongoing clinical trials for immunotherapy in combination with cytostatic chemotherapy or other agents in CCA

| Study | Phase | Treatment Arm A | Treatment Arm B | Primary Endpoint | NCT |
|---|---|---|---|---|---|
| - | II | Durvalumab + tremelimumab + gemcitabine/cisplatin | - | ORR | NCT03046862 |
| - | II | Tremelimumab + durvalumab + Radiation | - | ORR | NCT03482102 |
| - | II | SHR-1210 + GEMOX | - | 6-month PFS, safety | NCT03486678 |
| - | II | SHR-1210 + Apatinib | SHR-1210 + FOLFOX4 or GEMOX | Safety | NCT03092895 |
| - | II | Toripalimab + gemcitabine/S1 | - | PFS, OS | NCT03796429 |
| - | II | Pembrolizumab + cisplatin + gemcitabine | - | 6-month PFS | NCT03260712 |
| - | II | Lenvatinib + Toripalimab + GEMOX | Toripalimab + GEMOX | ORR | NCT05215665 |
| - | III | Toripalimab + lenvatinib + GEMOX or GC | Toripalimab + placebo + GEMOX or GC (Arm C: Placebo + Lenvatinib + GEMOX or GC) | OS | NCT05342194 |
| - | III | Envafolimab (KN035) + GEMOX | GEMOX | OS | NCT03478488 |
| ADJUBIL | II | Capecitabine + durvalumab + tremelimumab | Durvalumab + tremelimumab | 12-month RFS | NCT05239169 |
| - | I | Durvalumab + guadecitabine | - | Safety, response | NCT03257761 |
| - | II | Pembrolizumab + lenvatinib | - | ORR | NCT04550624 |
| - | I/II | SD-101 | SD-101 + pembrolizumab (Arm C: SD-101 + ipilimumab + nivolumab) | Safety, MTD, ORR | NCT05220722 |
| - | II | Lenvatinib + pembrolizumab | - | ORR, DCR, PFS | NCT03895970 |
| - | I/II | Pembrolizumab + MIV-818 | Lenvatinib + MIV-818 | Safety | NCT03781934 |
| - | I | NGM831 | NGM831 + pembrolizumab (Arm C: NGM831 + NGM438 + pembrolizumab | Safety | NCT05215574 |
| - | I/II | NGM707 ± pembrolizumab | - | Safety, PFS, OS | NCT04913337 |
| - | I | XmAb®22841 | XmAb®22841 + pembrolizumab | Safety | NCT03849469 |
| KEY-VIBE-005 | II | Pembrolizumab ± Vibostolimab ± (5-Fluorouracil + Cisplatin) ± Paclitaxel | - | ORR, PFS | NCT05007106 |
| - | I | CDX-1140 (CD40) ± (Pembrolizumab or CDX-301) | - | Safety | NCT03329950 |
| - | I | TRK-950 + gemcitabine/cisplatin | TRK-950 + pembrolizumab | Safety | NCT03872947 |
| - | I | TPST-1120 ± Nivolumab | - | Safety | NCT03829436 |
| IMMUCHEC | II | Durvalumab + Tremelimumab + Gemcitabine ± Cisplatin | - | ORR | NCT03473574 |
| - | II | AZD6738 + Durvalumab | AZD6738 + Olaparib | DCR | NCT04298021 |
| - | I/II | Atezolizumab + Tivozanib | - | ORR | NCT05000294 |

and evaluation of the effectiveness of adjuvant treatment. In the case of intrahepatic cholangiocarcinoma, the small bile duct type is more often associated with IDH1/2 mutations and FGFR2 rearrangements, while large bile duct cancers, including PHCC and dCCA, mainly exhibit alterations in the KRAS and/or TP53 genes. Advances in genetic analysis and growing knowledge of biomarkers have led to the development of targeted therapies that increase treatment efficacy and improve survival outcomes. In particular, IDH1/2 mutations, FGFR2 fusions, and NTKR gene rearrangements are found mainly in iCCA patients and have attracted considerable interest as potential therapeutic targets for new drug trials [5, 55].

It is estimated that up to 70% of iCCA patients have mutations that may provide a target for targeted therapies. In summary, molecular analysis of iCCA reveals a complex and diverse genetic profile that has a significant impact on the formation and development of this cancer. The identification of these alterations not only supports the assessment of prognosis but also enables the implementation of targeted therapies, which in turn sets the stage for more precise and effective treatment [5].

## Future Perspectives and Conclusions

With the development of molecular biology and next-generation sequencing (NGS) technology, second-line cholangiocarcinoma treatment is undergoing a major transformation - from a non-selective approach to precision medicine targeting specific genetic mutations. The discovery of aberrations such as FGFR2 fusions, IDH1/2 mutations, as well as alterations in ERBB2, BAP1, or TP53 creates real opportunities to implement targeted therapies that improve the prognosis of selected patient groups. The results of clinical trials to date indicate that although classical chemotherapy in the second line of treatment remains a therapeutic option, its effectiveness is limited. Inhibitors targeting specific molecular targets and immunotherapy, especially in combination with chemotherapy, show much greater potential. However, the effectiveness of these strategies depends on the availability of predictive biomarkers and the ability to identify them in a specific patient. In the future, the development of personalized oncology care will be key to improving treatment outcomes in CCA. Further larger clinical trials involving biomarkers are needed to better stratify patients and select optimal therapy. In conclusion, second-line CCA treatment is entering the era of precision treatment. Although the road to fully personalizing therapy remains a challenge, current and upcoming clinical trials offer real hope for improving the prognosis for patients with this aggressive cancer.

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  This reference provides a comprehensive review focusing on targeted therapies for CCA.

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**Data Availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing Interests** The authors declare no competing interests.

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