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**Novel targeted treatment options for advanced cholangiocarcinoma**

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**Key words:** Targeted therapy, cholangiocarcinoma, FGFR, IDH, gemcitabine
Abstract:

Introduction: Surgical resection remains the mainstay of potentially curative treatment in the early stages of cholangiocarcinoma, whereas for the advanced-stage, systemic chemotherapeutics and experimental targeted therapies are the primary treatment options. The molecular heterogeneity of the tumor is based on location, liver dysfunction and relative rarity of the disease and confers challenges for clinical trial enrollment. The advancements in the understanding of molecular pathogenesis of cholangiocarcinoma have led to the development of targeted therapies that are currently being evaluated in the clinical trials.

Areas covered: This review summarizes the current understanding and future directions of targeted therapeutic options in the management of advanced cholangiocarcinoma.

Expert opinion: Advanced cholangiocarcinoma has a dismal prognosis; improved understanding of the molecular pathogenesis and advancements in development of targeted therapy offers hope that we may improve outcomes in this rare, but highly lethal cancer. Among the newly discovered molecular alterations, targeting FGFR2 fusions, IDH1/2 mutations and HER2 receptors hold great promise for improving the future management of cholangiocarcinoma. Immunotherapy in combination with targeted agents and chemotherapy may improve outcomes. In addition, drugs targeting the MEK, EGFR, KRAS, BRAF and ROS1 pathways and neo-angiogenesis may also provide new horizons in the management of cholangiocarcinoma.
**Article highlights:**

- Most of the data on the chemotherapy regimens in advanced cholangiocarcinoma are primarily based on phase II trials except for gemcitabine and cisplatin combination, which remains the standard of care in advanced cholangiocarcinoma.

- Understanding the key signaling pathways and genetic alterations in cholangiocarcinoma play a prominent role in identifying novel drug targets.

- Among the newly discovered molecular alterations, targeting FGFR2 fusions, IDH1/2 mutations and HER2 receptors hold great promise for improving the future management of advanced cholangiocarcinoma.

- Advanced cholangiocarcinoma patients should be encouraged to participate in ongoing clinical trials.
1. Introduction:

Cholangiocarcinoma, an aggressive epithelial neoplasm of biliary tract constitutes approximately 3% of the gastrointestinal malignancies. Cholangiocarcinomas are anatomically classified into intrahepatic, perihilar and distal biliary tree cancer. There exist notable differences between the intrahepatic and extrahepatic subtypes in terms of incidence and molecular pathogenesis. Several epidemiological studies have shown that the incidence of intrahepatic cholangiocarcinoma is on the rise whereas the incidence of extrahepatic cholangiocarcinoma has decreased or stabilized [1]. Although a specific risk factor may not be identified in the majority of cholangiocarcinoma patients, common risk factors associated in the tumorigenesis of cholangiocarcinoma include primary sclerosing cholangitis, inflammatory bowel disease, fibro-polycystic liver disease (choledochal cysts), chronic intrahepatic stone disease, liver fluke infestation and chronic liver disease such as viral hepatitis and cirrhosis [2]. Genetic conditions, including Lynch syndrome and biliary papillomatosis, have also been implicated in the pathogenesis of cholangiocarcinoma [3, 4].

The prognosis of cholangiocarcinoma is highly dependent on the tumor stage at presentation. In the early stage disease, surgical resection is the sole curative treatment option. Unfortunately, most patients present with unresectable or advanced stages requiring systemic therapy depending on the extent of the disease and baseline functional status. Considerable progress has been made in
understanding the molecular pathogenesis of cholangiocarcinoma thereby opening doors for the development of novel targeted therapies.

The objective of this work is to review the differences in the molecular pathogenesis of intrahepatic and extrahepatic disease with a focus on the recent advancements in novel targeted therapies in the management of cholangiocarcinoma.

2. Overview of molecular pathogenesis of cholangiocarcinoma:

Understanding the key signaling pathways and genetic alterations in cholangiocarcinoma play a prominent role in identifying novel drug targets. Each subtype including intrahepatic, perihilar and distal cholangiocarcinoma has a distinct molecular pathogenesis. In a study that evaluated the genetic spectra among biliary tract cancers/cholangiocarcinoma using the comprehensive transcriptome and whole exome sequencing, distinct genetic alterations among different cholangiocarcinoma subtypes were noted [5]. In this analysis, recurrent mutations in IDH1, IDH2, FGFR1, FGFR2, FGFR3, EPHA2, and BAP1 genes were noted predominantly in intrahepatic subtype, whereas ARID1B, ELF3, PBRM1, PRKACA, and PRKACB genetic mutations occurred preferentially in perihilar and distal cholangiocarcinoma [5]. Given this distinctive molecular pathogenesis among different subtypes of cholangiocarcinoma, we reviewed the individual therapeutic biomarkers and potential drug targets of each subtype of cholangiocarcinoma individually.

2.1 Intrahepatic cholangiocarcinoma:

The advent of next-generation sequencing has paved the way for better understanding of molecular pathogenesis of intrahepatic cholangiocarcinoma[6]. Pre-clinical studies have identified the roles of IDH (IDH 1/ 2) mutation, chromatin-remodeling genetic mutations (BAP1) and fibroblast growth factor
receptor 2 (FGFR2) mutations as potential driver mutations in the pathogenesis of the disease[6]. Key signaling pathways involved in the carcinogenesis of intrahepatic subtype are summarized below.

2.1.1 IDH mutations:

Isocitrate dehydrogenase (IDH, isoforms 1 and 2) facilitates the conversion of isocitrate to α-ketoglutarate in Krebs cycle. Approximately 14% of intrahepatic cholangiocarcinoma tumors are known to harbor IDH genetic mutations. Epigenetic alterations of IDH1 and IDH2 genes lead to increased levels of D-2-hydroxyglutarate, an oncometabolite that is thought to inhibit α-ketoglutarate dependent dioxygenases; increased levels of TP53 proteins and DNA hypermethylation resulting in altered cell differentiation [7, 8].

2.1.2 Tyrosine kinase (FGFR) genetic aberrations:

FGFR2 genetic aberrations have been reported in approximately 13-20% of intrahepatic cholangiocarcinoma tumors [9, 10]. Molecular immunology studies have identified yes-associated protein, a transcriptional co-activator mediated upregulation of FGFR 1, 2 and 4 in cholangiocarcinoma cell lines[11]. FGF has also been shown to activate the phosphorylation of MEK 1/2 further contributing to migration of cholangiocarcinoma cells[12]. Majority of FGFR aberrations in cholangiocarcinoma are chromosomal fusions of FGFR2 with other genes such as BICC1, PPHLN1, MGEA5, TACC3, CCDC6 leads to autophosphorylation and activation of downstream signaling pathways [6, 13, 14]. In addition, a significant association of FGFR2 fusions (FGFR2-AHCYL1, FGFR2-KIAA158) and KRAS mutations was noted in the signaling pathway activation in the carcinogenesis of the intrahepatic subtype [13, 15]. Activating mutations in KRAS were seen in 2-57% of intrahepatic cholangiocarcinoma tumors, whereas activating mutations in BRAF gene were seen in 1-22% of tumors of intrahepatic subtype [16]. Mutations in FGRF1 and FGRF3 have also been encountered in the carcinogenesis of intrahepatic subtype [5]. Cytological review of formalin fixative specimens of intraductal papillary neoplasms of the bile duct showed that
tumors with FGFR2 mutations had either prominent intraductal growth or anastomosing tubular glands with desmoplasia [10]. Interestingly, patients with FGFR2 genetic aberrations were more commonly seen in females (13% vs 4%), younger age (52 years vs 65 years) and had a significantly better survival (123 months vs 37 months)[10]. In addition, a recent analysis has found that mutations in BAP1 gene were the most common coexisting mutation not affecting the OS[17]. On the contrary, co-existing mutations in TP53 (p=.04) and CDKN2A/B genes (P =.04) were correlated with a shorter OS [17].

2.1.3: HGF/c-MET signaling:

MET overexpression has been encountered in the carcinogenesis of intrahepatic subtype (12–58%) and perihilar/distal subtypes (16%) [18, 19]. The interaction of hepatocyte growth factor (HGF) and c-MET is known to activate the tyrosine kinase signaling pathways such as MAPK, PI3K/AKT and JAK-STAT3[20]. MET overexpression has been associated with a shorter disease-free survival [9]. In addition to MET overexpression, MET amplification has also been implicated in the pathogenesis of intrahepatic cholangiocarcinoma. Pathological evaluation of intrahepatic cholangiocarcinoma tumor cells has shown that MET amplification in the tumors were associated with larger tumor size (more than 5 cm) and shorter overall survival, whereas MET overexpression was associated with shorter disease-free survival [21].

2.1.4: Interleukin 6 mediated JAK-STAT signaling:

Interleukin-6 (IL-6) mediated activation of JAK-STAT signaling pathway accounts for half of the cases of intrahepatic cholangiocarcinoma. IL-6 overexpression has been encountered in chronic inflammation and was shown to cause epigenetic silencing of cytokine signaling suppressor gene, SOCS-3 [22]. Overexpression of IL-6 is also known to cause gp-130-mediated activation of JAK-STAT3 pathway [23]. In addition, enhanced IL-6 activity increases the telomerase activity and alters the methylation patterns of growth factor receptors such as EGFR [24].
2.1.5: EGFR signaling:

Epidermal Growth Factor Receptor (EGFR) is known to play key role in cell cycle, migration and angiogenesis[9]. EGFR overexpression has been implicated in the carcinogenesis of cholangiocarcinoma with highest level of expression in intrahepatic subtype (38-100%) [25]. Aberrant phosphorylation and overexpression of EGFR activates MAPK/ERK and p38 pathways triggering the tumor growth and inhibition of apoptosis thereby promoting the tumorigenesis [26].

2.1.6: Loss of BRCA1 associated protein 1 (BAP1) expression:

BAP1 is a tumor suppressor and chromatic remodeling gene. About 9-26% of intrahepatic cholangiocarcinoma tumors are known to harbor BAP1 mutations[27, 28]. Though the exact mechanism of carcinogenesis due to BAP1 mutation is yet to be determined, it is known to cause aggressive disease with poor response to standard therapies used in the management of cholangiocarcinoma [29].

Other signaling pathways such as BRAF (3-5% in intrahepatic subtype), NOTCH (upregulation of NOTCH1, NOTCH4) and WNT (overexpression of WNT7B and WNT10A) signaling pathways have been implicated in the carcinogenesis of intrahepatic cholangiocarcinoma [30, 31].

2.2 Perihilar and distal (extrahepatic) cholangiocarcinoma:

Though intrahepatic and extrahepatic subtypes of cholangiocarcinoma share some of the above mentioned molecular pathogenetic pathways, next generation sequencing studies showed molecular heterogeneity among the different subtypes of cholangiocarcinoma [32, 33].

2.2.1 HER2/neu (ERBB2) and HER3 receptor tyrosine kinase pathway (s) signaling:

HER2/neu overexpression has been implicated in approximately 5-9% of extrahepatic cholangiocarcinoma tumors [32, 34]. In contrast to extrahepatic subtype, HER2 overexpression was noted in about 1% of the tumors in intrahepatic cholangiocarcinoma tumors [9]. Activation of HER2/neu
receptors triggers the downstream signaling pathways such as RAS-RAF-MEK-ERK or PI3k-AKT-mTOR that results in the proliferation and survival of cancer cells [35].

In addition to HER2 aberrations, in cholangiocarcinoma, other biomarkers such as HER3 or HER3 gene amplification were identified. Furthermore, the co-expression of HER2/HER3 (resulting in phosphorylation of HER2) in cholangiocarcinoma ranged from 9-36% and has been implicated as a poor prognostic factor in extra-hepatic cholangiocarcinoma [36].

2.2.2 Cyclin-dependent kinase Inhibitor 2A (CDKN2A):

CDKN2A gene controls the cell cycle by inhibiting the phosphorylation of pRb protein, a protein that plays a key role in the arrest of the cell cycle. Mutations in CDKN2A gene were seen in about 42% and 28% of intrahepatic and extrahepatic subtypes, respectively [35].

Other key signaling pathways that have been encountered in the pathogenesis of cholangiocarcinoma are aberrations in PI3K-AKT-mTOR pathway, KRAS, TP53, BRAF, MET (overexpression and amplification), PD-L1 and tumor mutation burden (TMB) [5, 9, 37]. About 25% and 40% of intrahepatic and extrahepatic subtypes were known to harbor mutations in PI3K-AKT-mTOR pathway [38].

3. Approved therapies in the management of advanced cholangiocarcinoma:

Systemic treatment remains the mainstay of treatment for patients with advanced or metastatic disease. The data for systemic therapy is limited to handful of large randomized clinical trials. Gemcitabine and fluorouracil-based therapies are commonly evaluated systemic therapies.

Gemcitabine, either alone or in combination has been extensively studied in patients with advanced cholangiocarcinoma. Three phase II trials that evaluated the gemcitabine alone therapy in advanced cholangiocarcinoma concluded that the drug was well tolerated with response rate ranging from 0-30% [39, 40, 41]. Gemcitabine was also evaluated in combination with capecitabine in three phase II trials of
metastatic, unresectable cholangiocarcinoma [42, 43, 44]. Results of the three trials showed a response rate of approximately 30% with a median overall survival (OS) of 13-14 months. This combination regimen was well tolerated, with neutropenia and thrombocytopenia as the most significant toxicities. Gemcitabine was also evaluated in combination with 5-fluorouracil; and carboptatin. Patients who received the combination with 5-fluorouracil had modest improvements in progression free survival months but no overall survival improvement [63, 64].

In addition, gemcitabine was also evaluated in combination with cisplatin or oxaliplatin in phase II and III trials and the results (response rates and median OS) were encouraging. In gemcitabine plus cisplatin combination, phase II trials, response rates of about 21%–35% and median OS of 9.3–11 months were noted [45, 46, 47]. The combination was also evaluated in a phase III randomized trial, which also demonstrated the similar encouraging results making it the current choice of therapy for cholangiocarcinoma [48]. Compared to gemcitabine monotherapy, the combination therapy resulted in a better progression free survival (8 months vs 5 months, p <0.001) and OS (11.7 vs 8.1 months; HR: 0.67; CI: 0.54–0.84; p <0.001). The combination therapy was tolerated well without significant added toxicity. In addition, the rate of tumor control was significantly increased among patients in the combination therapy group (81.4% vs. 71.8%, P=0.049). Adverse events were similar in both the cohorts. Though the neutropenia was noted more in the cisplatin–gemcitabine group; the number of neutropenia-associated infections was similar in the two groups. Gemcitabine and oxaliplatin combination was evaluated in phase II trials and yielded comparable results in terms of efficacy and tolerability as gemcitabine and cisplatin combination [49, 50, 51]. The most common side effects included myelosuppression and peripheral neuropathy.

It is important to note that most of the data on the regimens that we use currently is mostly based on phase II trials except for gemcitabine and cisplatin combination, which remains the standard of care for first line treatment of advanced cholangiocarcinoma. Gemcitabine and oxaliplatin combination is also a
reasonable choice for these patients. Other alternative regimens include single agent gemcitabine, gemcitabine and carboplatin combination or 5-fluorouracil based regimens. Nonetheless, it is important to note that the prospective trials on these alternative regimens are lacking, which underscores the high unmet need of systemic therapeutic agents in the management of advanced cholangiocarcinoma.

4. Novel targeted therapeutic options in the management of advanced cholangiocarcinoma:

4.1 Agents targeting FGFR fusion/mutation targets:

FGFR2 mutations, particularly FGFR2 chromosomal fusions have been identified as potential novel targets in the management of advanced cholangiocarcinoma. As FGFR2 fusions are known to harbor tyrosine kinase domains, tyrosine kinase inhibitors are being evaluated in phase I and phase II trials in the management of cholangiocarcinoma. A preliminary study that evaluated the role of ponatinib and pazopanib in the management of cholangiocarcinoma in a patient with FGFR2-MGEA5 fusion showed promising results [26]. Similar encouraging results were seen in a patient who had FGFR2-TACC3 fusion gene. Currently, many active clinical trials are enrolling patients to further evaluate the role of FGFR2 inhibitors in the management of cholangiocarcinoma[15]. Ponatinib and BGJ398 are the two-small molecule tyrosine kinase inhibitors currently being evaluated in phase II trials (NCT02150967, NCT02272998, NCT02150967). BGJ398 demonstrated encouraging results in advanced cholangiocarcinoma patients with FGFR genetic alterations who progressed on gemcitabine based therapy in a multi-institutional phase II trial[52]. BGJ398 resulted in an overall response rate and disease control rate of 14.8% and 75.4%, respectively. Median progression-free survival was 5.8 months.
Hyperphosphatemia (72.1%), fatigue (36.1%), stomatitis (29.5%), and alopecia (26.2%) were the most common adverse events reported. Other tyrosine kinase inhibitors being evaluated in clinical trials include but not limited to are E7090 (FGFR 1, 2 and 3 receptor inhibitor), Rogaratinib (NCT01976741) and TAS-120 (NCT02052778) [15, 53].

BGJ398, a pan-FGFR tyrosine kinase inhibitor that was evaluated in a global phase I trial in solid tumors that harbor FGFR2 aberrations that included 3 patients with cholangiocarcinoma (2 patients with FGFR2 fusions and 1 with FGFR2 mutation) [54]. Reduction in tumor size (about 5-20%) was noted in the three patients who received BGJ398. The drug was also evaluated in a phase II trial in 61 gemcitabine refractory cholangiocarcinoma patients who had FGFR gene aberrations such as fusion (n=48), mutations (n=8) and amplification (n=3)[55]. The drug showed encouraging results with overall response rate of 14.8% in all the patients who received the therapy and 18.8% response rate in patients who had FGFR2 fusion. Responses were only observed in patients with FGFR2 fusions with disease control rate of 83.3%. The cohort had median progression free survival of 5.8 months (95% CI: 4.3-7.6 months). Most common adverse events noted were hyperphosphatemia (72.1%), fatigue (36%) and stomatitis (29%).

Ponatinib is another non-selective pan-FGFR inhibitor that is currently being evaluated in advanced cholangiocarcinoma. In a preclinical study, ponatinib therapy resulted in tumor shrinkage and decrease in CA 19-9 levels in an advanced cholangiocarcinoma patient with the \textit{FGFR-MGEA5} fusion [26]. Similar encouraging result of stabilization of disease was seen in another patient with \textit{FGFR-TACC3I} translocation whose disease had progressed on pazopanib [26]. Given the promising results, the drug is currently being evaluated in phase II clinical trials involving patients with advanced biliary tract cancers and other malignancies harboring FGFR aberrations (NCT02265341, NCT02272998, Table 1).

ARQ-087 is another oral pan-FGFR tyrosine kinase inhibitor that has been evaluated in cholangiocarcinoma in phase I and II trials. In a phase I trial involving 12 cholangiocarcinoma patients,
partial responses were seen in a couple of patients. In then extension phase I/II study involving 29 patients with cholangiocarcinoma, promising results were seen with a significant reduction in tumor burden (10-29%) in 25% (n=3) of patients with FGFR2 aberrations (n=12) [56]. About half of the patients (n=6) had durable disease control for ≥ 16 weeks. On an extension phase study in 29 cholangiocarcinoma patients with FGFR2 fusions, disease control was seen in about 83% of cases with objective response of 21% [57]. ARQ-087 is currently being evaluated in a single arm phase III trial for patients with FGFR2 gene fusion positive advanced cholangiocarcinoma who have failed one or more systemic therapy agents (FIGURE trial) (NCT03230318).

TAS-120 is the first irreversible, covalent inhibitor of FGFR 1-4 being tested in humans. TAS-120 was evaluated in 19 patients with FGF/FGFR abnormalities harboring cholangiocarcinoma (17 intrahepatic and 2 extrahepatic). Fifty percent of the patients with FGFR fusions had partial responses and a couple of more patients had tumor size reduction [58]. Interestingly, among 3 patients with FGFR2 fusions who had been previously treated with an FGFR inhibitor, one patient had partial response and another patient had stable disease. It is also being tested in patients who have previously progressed on other FGFR inhibitors (NCT02052778).

INCB054828 is a selective FGFR-1, 2, and 3 inhibitor that was evaluated in a phase I/II safety and tolerability study in patients with advanced solid tumors[59]. In phase 2 of the study, the drug was evaluated in 4 patients with cholangiocarcinoma and one patient who had FGFR2-CCDC6 translocation showed partial response. INCB054828 13.5 mg (21-day cycle) is currently being evaluated in a phase II open label multicenter study in advanced cholangiocarcinoma patients. The patients will be enrolled into three cohorts – cohort A enrolls the patients with FGFR2 translocations, cohort B enrolls the patients with other FGF/FGFR alteration and cohort C enrolls the patients with no FGF/FGFR alterations [60]. Currently, a phase III trial is being developed to evaluate the efficacy of INCB054828 compared to gemcitabine and cisplatin combination in first line setting. Another pan-FGFR inhibitor, erdafitinib is
also currently being evaluated in a phase 2 trial in Asian subjects with advanced solid malignancies harboring FGFR aberrations such as non-small cell lung cancer, urothelial cancer, or esophageal cancer (NCT02699606).

Apart from small molecule tyrosine kinase inhibitors, antibodies to the FGFR2-IIIb isoform are expected to be attractive targets for managing cholangiocarcinoma as they have less possible side effects as compared to tyrosine kinase inhibitors[15]. The isoforms of FGFR2-IIIb are known to selectively bind to FGF7 and FGF10 ligands and so targeting the isoforms of FGFR2-IIIb is thought to be better tolerated [61]. The other potential therapeutic agents of interest are heat shock protein Hsp90 inhibitors [15, 62]. Hsp90 has been shown to act as a chaperon to FGFR family and targeting Hsp90 would be a promising strategy in the tumors that harbor FGFR aberrations. AZD4547 is another orally administered small-molecule selective FGFR (FGFR 1-3) inhibitor that was extensively evaluated in pre-clinical models, phase I and phase II trials in cancers that harbor FGFR genetic aberrations[63].

4.2 Agents targeting IDH1 mutations:

IDH mutations have been implicated in epigenetic, metabolic reprogramming and in the alteration of cancer cell differentiation. About 25% of intrahepatic cholangiocarcinoma patients are known to harbor IDH mutations leading to the production of D-2-hydroxyglutarate, an oncometabolite leading to tumorigenesis. AG-120 (ivosidenib) is an oral IDH1 inhibitor that was evaluated in patients with solid malignancies. In a phase I trial, the drug was evaluated in 73 cholangiocarcinoma patients in the dose escalation and expansion cohorts [64]. The drug was tolerated well and partial response and stable disease was seen in about 6% and 56% of the patients. On molecular analysis of morphologic and gene expression profile changes in cholangiocarcinoma cells, AG-120 was shown to cause morphologic changes in cholangiocytes and increased liver-specific gene expression in IDH1 mutant cells[65]. A phase III, double blind, randomized multicenter trial (ClariDHy trial) is currently recruiting advanced, non-
resectable or metastatic cholangiocarcinoma patients to evaluate the progression free survival (primary end point), overall survival, safety and tolerability of the drug, which hopefully provides us more information (NCT02989857)[66]. Other IDH2 inhibitor-AG221 (NCT022273739) was evaluated in a phase II trial involving subjects with solid tumors with IDH2 mutation. This study included advanced cholangiocarcinoma patients and results are awaited. Table 2 summarizes clinical trials involving targeted therapy against IDH1 receptors.

4.3 Agents targeting HER2 (ERBB2) receptors:

Although ERBB2 gene aberrations are seen less frequently in cholangiocarcinoma as compared to that of breast cancer and upper gastrointestinal malignancies, data from retrospective case series on gallbladder cancer show some hope on HER2 targeted therapies[35]. However, in the same case series, HER2/neu receptor blocker, trastuzumab, failed to show any clinically meaningful response in HER2/neu mutations positive cholangiocarcinoma patients. All the cholangiocarcinoma patients had progressive disease with trastuzumab alone or in combination with concurrent systemic therapy. It is important to note that HER2/neu targeting agents such as trastuzumab or lapatinib may be helpful in case of extracellular domain mutations and their role in kinase domain mutations is questionable. For example, functional characterizations of HER2 mutations have shown that the presence of V777L mutation was associated with negative expression of ERBB2 conferring resistance to lapatinib[67]. Clinical trials that are evaluating the role of small molecule tyrosine kinase inhibitors in the management of cholangiocarcinoma may provide us more information on the role of ERBB2 targeting agents in this cancer (NCT00101036, NCT02836847) (Table 3).

4.4 Sphingosine kinase 2 inhibitors:

Sphingosine kinase 2 was found to be overexpressed in human cholangiocarcinoma cells as compared to normal cholangiocytes [68]. In cholangiocarcinoma cell lines, ABC294640, a sphingosine kinase 2
inhibitor was found to exert anti-proliferation activity on cholangiocarcinoma cells [68]. The drug also induced autophagy, caspase dependent apoptosis of cancer cells and inhibited STAT3 autophosphorylation, which plays a significant role in cholangiocarcinoma tumorigenesis. In addition, ABC294640 when combined with sorafenib synergistically inhibited proliferation of cholangiocarcinoma cells [68]. The sphingosine kinase pathway and the mechanism of action of ABC294640 is summarized in Figure 1. ABC294640 was evaluated in solid tumors (including advanced cholangiocarcinoma) in a phase I trial to determine the maximum tolerable dose (primary end point), safety and anticancer activity (secondary end points) (NCT01488513)[69]. The drug at the dose of 250 mg once daily has shown partial response (n=1) and stable disease (n=1) in patients with cholangiocarcinoma (n=3). Most common adverse events noted in the entire cohort were nausea, fatigue and vomiting. Nervous system and neuro-psychiatry related adverse events of grade 1-2 severity were noted, which were resolved on the discontinuation of the drug. ABC294640 (Yeliva®) is currently being evaluated in a phase II trial involving advanced cholangiocarcinoma patients (NCT03377179, NCT03414489) (Table 3).

4.5 Agents acting on tyrosine kinase, \(\text{EGFR}, \text{VEGF, c-MET, MAPK/ERK}\) pathways:

Tyrosine kinase inhibitor, erlotinib that act by blocking the \(\text{EGFR}\) pathway was evaluated in advanced cholangiocarcinoma [70]. Erlotinib resulted in modest benefit (~17% progression free survival) and all responding patients had mild skin toxicity (grades 1 and 2). A multicenter, randomized phase II trial evaluated the combination of cetuximab (an \(\text{EGFR}\) inhibitor) with gemcitabine-oxaliplatin in unresectable biliary tract cancers (76% had cholangiocarcinoma). The addition of cetuximab resulted in improved rates of 4-month progression free survival compared to gemcitabine-oxaliplatin group (61% vs 44%). However, the median OS did not differ much between the two groups (11 vs 12.4 months)[71]. The toxicity is comparable in both the groups with slightly higher rash/hypersensitivity reactions in cetuximab group. Panitumumab is a fully human monoclonal antibody specific to the \(\text{EGFR}\) that was evaluated in a phase II trial in combination with gemcitabine, and irinotecan in patients with advanced
cholangiocarcinoma showing promising results (5-month progression free survival of 69%)[72]. However, these encouraging results were not seen in advanced cholangiocarcinoma with wild type KRAS mutations (Vecti-BIL study) [73]. The drug has been evaluated in combination with other chemotherapeutic agents such as oxaliplatin or gemcitabine in advanced, inoperable cholangiocarcinoma patients in a phase 2 trial (GOC-B-P trial) and the results are awaited (NCT01206049).

Sorafenib, a tyrosine kinase inhibitor that efficaciously targets VEGF R-2, 3 and platelet derived growth factor, and less potently BRAF kinases was evaluated in a phase II clinical trial involving 31 patients with unresectable or metastatic cholangiocarcinoma or gallbladder cancer patients [74]. About 29% of the patients had stable disease and median progression free survival of the cohort was 2 months. Grade 3 or 4 toxicities were significant, affecting about two thirds of the patients (1 patient died of supraventricular tachycardia and thromboembolism). Regorafenib is another multi-kinase inhibitor that was evaluated in a phase II trial in the management of advanced cholangiocarcinoma who failed first line therapy with gemcitabine and cisplatin[75]. About 11% (n=3/28) had partial response, 64% (n=18/28) had stable disease and remaining 25% (n=7/28) had progressive disease. Median survival rate was 42% at 1 year and 38% at 18 months. Most common side effects noted were high blood pressure, hypophosphatemia, and hand-foot skin toxicities. There is a similar phase II, multi-institutional trial using regorafenib in refractory cholangiocarcinoma patients who failed up to 2 lines of chemotherapy (NCT02115542). Though several studies demonstrated the role of angiogenesis in the carcinogenesis of cholangiocarcinoma, studies evaluating the role of sorafenib or regorafenib did not yield enthusiastic results thus far. The role of these agents will better be elucidated in the ongoing multi-institutional studies involving selected patients.

Bevacizumab, a humanized monoclonal antibody against VEGF, was evaluated in a phase II trial combination with gemcitabine and oxaliplatin in unresectable cholangiocarcinoma (28% [n=10] of
enrolled patients had GBC) [76]. In the subset of GBC patients, the median progression free survival and OS were 6.1 and 8.5 months, respectively. Most common side effects noted were grade 3 or 4 hypertension (n=5), proteinuria (n=1), thrombosis (n=2), and cardiac ischemia (n=1). Bevacizumab was also evaluated in combination with erlotinib in a phase II trial in biliary tract cancers (n=53; 10 patients with GBC and 43 with non-GBC).[77] This biologic combination regimen resulted in stable disease in about 51% of the patients with median OS of the entire group was 9.9 months. Another study evaluated the addition of bevacizumab, to gemcitabine and oxaliplatin (GEMOX) [78]. The combination therapy of bevacizumab and GEMOX was associated with a better progression free survival as compared to that of GEMOX therapy (6.48 vs 3.72 months, p=0.049). No significant difference in terms of adverse events was noted between the groups. A retrospective analysis of intrahepatic cholangiocarcinoma patients that received irinotecan concurrently with folinic acid followed by fluorouracil then fluorouracil (FOLFIRI) and bevacizumab every 2 weeks showed similar encouraging results[79]. Tyrosine kinase inhibitors of the MEK pathway have also been studied in this disease. Selumetinib, a MEK 1/2 inhibitor, demonstrated a median overall survival of 9.8 months and 80% of the 28 patients had either confirmed objective response or experienced stable disease in a small phase II study [80]. A phase 1 dose-escalation and expansion study evaluated the role of binimetinib (MEK162), a potent and selective oral MEK 1/2 inhibitor in biliary tract cancers [81]. The drug was found to have a manageable safety profile and was effective in target inhibition. In addition, three patients with biliary tract cancers had objective response (2 partial and 1 complete responses). In a case study, trametinib, a MEK 1/2 inhibitor was evaluated in combination with dabrafenib, a BRAF inhibitor in a patient with advanced, metastatic extrahepatic cholangiocarcinoma with \textit{BRAF V600E} mutation who failed conventional systemic therapy [82]. The combination resulted in dramatic response with 12-week follow up radiologic evaluation showed regression of metastatic lesions. Similar encouraging results were seen with the combination therapy in a couple of other patients with intrahepatic cholangiocarcinoma harboring \textit{BRAF V600E} mutation [83].
However, in a randomized phase II study, trametinib monotherapy when compared to standard combination therapy (5-FU with leucovorin or capecitabine) in patients with refractory, advanced cholangiocarcinoma showed lack of response in unselected patient population[84].

Although some of the novel targeted therapeutic agents showed modest results (as most of the studies discussed in section 4.5 were performed in molecularly unselected population), more definitive randomized studies are required to define the role of antiangiogenic, tyrosine kinase pathway blocking agents in advanced cholangiocarcinoma, particularly among those harboring KRAS and BRAF mutations.

4.6 Agents acting on phosphatidylinositol 3-kinase (PI3K) pathway:

Dysregulation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway has been implicated in the tumorigenesis of cholangiocarcinoma- increased expression of phosphorylated Akt is present in >60% of intrahepatic cholangiocarcinoma and >80% of extrahepatic disease [85]. Given the involvement of the pathway in cell proliferation, angiogenesis and metastasis, the pathway has been an attractive target in the management of several cancers including cholangiocarcinoma [86, 87]. MK2206, an allosteric Akt inhibitor, alone or in combination with other agents demonstrated promising efficacy in vitro and in an early clinical trial involving solid tumors with Akt pathway aberrations [88, 89, 90]. MK2206 was shown to reduce cellular viability by inducing the apoptosis of tumor cells. Despite these encouraging results in pre-clinical studies involving cholangiocarcinoma cell lines, no objective clinical activity of MK2206 was noticed in a phase II trial involving 8 advanced cholangiocarcinoma patients[91]. Although the trial was stopped prematurely as none of the patients showed objective response, it is important to note that a couple of patients had stable disease (> 12 weeks). Future larger studies involving MK2206 alone or in combination with other targeted therapies may provide us more information on other response parameters such as progression free survival.

4.7 Alternative or combination options:
4.7.1 Immunotherapy:

Given the background of chronic inflammation in the pathogenesis of cholangiocarcinoma, immunotherapy may be a potential attractive therapy either alone or in combination with other therapies [92]. A couple of tumor-related antigens were also identified in cholangiocarcinoma: Wilms tumor 1 (WT1) and mucin-1 (MUC-1) in 68-80% and 59-77% of cholangiocarcinoma tumors, respectively[93]. In addition, recent studies have identified the expression of PD-L1 expression by neoplastic cells in about 9% of cholangiocarcinoma neoplastic cells and about 46% had PD-L1 positive inflammatory cell aggregates [94]. Another study on extrahepatic cholangiocarcinoma showed the expression of PD-L1 in about 12% of cases and tumor associated macrophages in about 30% of cases [95]. Expression of PD-L1 in cholangiocarcinoma cells was found to be associated with poor prognosis [5]. Evaluation of surgical specimens of 44 extrahepatic cholangiocarcinoma patients showed that the presence of CD8+CD45RO+ tumor infiltrating lymphocytes resulted in prolonged overall survival [96]. On the contrary, presence of CD8+ tumor infiltrating lymphocytes and expression of PD-L1 on tumor cells did not show any significant correlation with overall survival [96]. Another study evaluated the effect of pembrolizumab, a PD-L1 blocker in treatment-refractory progressive, metastatic, mismatch repair-deficient cholangiocarcinoma patients (n=4) in a phase II trial [63]. Out of the 4 patients enrolled in the study, 1 patient (25%) had complete response whereas 3 patients had stable disease. The study opened a new insight that testing for mismatch repair-deficiency might be considered in patients’ refractory to other conventional therapies in order to identify those who may benefit from PD-1 pathway blockade [63].

In vitro and preclinical in vivo evaluation of measles vaccine (oncolytic vaccine) against cholangiocarcinoma cell lines showed encouraging results [97]. Trials of both a dendritic-based cell vaccine against WT-1 and MUC-1 antigens as well as a randomized trial of chemotherapy and a WT1 vaccine in patients with advanced cholangiocarcinoma have been described [98]. Another trial evaluated
the combination of dendritic cell pulsed vaccine and ex vivo activated T-cells in the postoperative setting [98]. In addition, in the interim analysis of a phase II trial (KEYNOTE-028, NCT02054806) that evaluated the role of pembrolizumab in advanced cholangiocarcinoma, about 34% (n=8) of patients with positive PD-L1 expression had partial response or stable disease [99]. Interim analysis of a single institution study that evaluated the combination of lenvatinib (multi-kinase inhibitor) with either pembrolizumab (n=10) or nivolumab (n=4) in advanced cholangiocarcinoma who failed prior anti-cancer therapy showed promising results with a median progression free survival of 5 months [100]. Overall response rate was 21.4% with 3 patients having partial response and 79% (n=11) patients having stable disease.

Nivolumab is currently being studied in a multi-institutional, phase II single-arm trial, which is evaluating the role of nivolumab monotherapy on the overall response rate in advanced refractory disease (NCT02829918). Furthermore, there is a separate study with nivolumab in combination therapy with gemcitabine/cisplatin or ipilimumab as first-line therapy in advanced, unresectable biliary tract cancer [101]. These trials will hopefully provide us with more details about the role of immune-targeted therapy in cholangiocarcinoma.

5. Conclusion:

While many different therapeutic regimens have been evaluated in cholangiocarcinoma, only few studies have shown promising results. With the better understanding of molecular pathogenesis and tumor biomarkers with next generation sequencing, clinical trials now days are primarily focused on targeted therapies. In addition to molecular-targeted therapies, immunotherapeutic agents such as peptide-based oncolytic vaccines, dendritic cell-based vaccines, and PD-L1 antibodies have been tried with mixed results. Although some of the novel targeted therapeutic agents targeting FGFR2, IDH1/2 and other tyrosine kinase pathways showed encouraging results, clinical trials involving EGFR inhibitors such as cetuximab and panitumumab and erlotinib in combination with GEMOX showed disappointing
results. Hence, more definitive randomized studies are required to define the role of these antiangiogenic, tyrosine kinase pathway blocking agents in advanced cholangiocarcinoma, particularly among those harboring \textit{KRAS} and \textit{BRAF} mutations.

6. Expert opinion:

Cholangiocarcinoma is a rare gastrointestinal malignancy typically presenting at advanced, inoperable stages. The median survival of advanced cholangiocarcinoma is approximately 6 months. The combination therapy with gemcitabine and cisplatin is currently used as first line therapy in patients who have good baseline functional status but since then there has been no other phase III studies in the first line setting. Furthermore, currently to this date there is no standard therapy for refractory cholangiocarcinoma as well. However, we are making progress. A detailed understanding of the molecular pathogenesis of the tumor coupled with more extensive genetic profiling of cholangiocarcinoma will help to assess the therapeutic relevance of targeting a specific pathway by using targeted therapy. We now understand that cholangiocarcinoma tumors are anatomically distinct and genetically heterogeneous tumors based on molecular profiling. Emerging therapies that hold promises are IDH 1 inhibitors, FGFR inhibitors and immunotherapies, which are furthest along. Early studies have identified biomarkers, which are predictors of response to IDH1 and FGFR inhibitors with promising results that are described in this review. Ongoing randomized biomarker driven studies will hopefully demonstrate overall survival benefits with these therapies. MEK inhibitions at first appeared to be effective in cholangiocarcinoma patients based on two small prospective studies. However, negative results seen in a randomized phase II trial (SWOG S1310) that evaluated a single agent MEK inhibitor, trametinib vs. 5-fluorouracil or capecitabine in refractory advanced biliary cancer dampens the enthusiasm for MEK inhibition [84]. The trial was stopped early than planned due to lack of measurable overall survival benefit in trametinib arm. However, it is important to note that the S1310 trial included unselected cholangiocarcinoma patients and it is still unclear if subset of cholangiocarcinoma patients
will still benefit from MEK inhibitions. Furthermore, there is a possibility that combining MEK inhibitors with other molecular targeted or immunotherapy will still have activity in cholangiocarcinoma.

Concerning immunotherapies, provocative data in patients with MMR deficient cholangiocarcinoma underscores the importance of identifying the biomarker even though the incidence is low at 5-10% [102]. It is unclear if PD-L1 or that mutational tumor burden (TMB) is a good predictive biomarker in cholangiocarcinoma. However, at this time we have limited data on efficacy with immunotherapy in unselected cholangiocarcinoma patients, yet we are cautiously optimistic and eagerly anticipating the results from KEYNOTE -158 and the single arm open label nivolumab study (NCT02829918). If the immunotherapy trials demonstrate efficacy, then the next step would be to use the immunotherapy drugs in combination with chemotherapy or possibly with other molecularly targeted agents.

Additionally, targeting biomarkers such as BAP1, CDKN2A, MET amplifications (cabozantinib), and BRAF in clinical trials alone or in combination can produce a meaningful subset in the context of targeted therapy. Even though the incidence of these biomarkers maybe low, when considered together, the actionable mutations can potentially lead to an effective therapy to patients. Hence, whenever applicable, patients are to be highly encouraged to participate in clinical trials that are evaluating the novel targeted therapy in this dismal cancer. Though the rarity of cholangiocarcinoma, the complex interactions of the signaling pathways and anatomical heterogeneity are major limitations to design robust, randomized clinical trials, this diverse genomic landscape makes it an ideal cancer for targeted therapies. Tailoring the clinical trials to tumor biomarkers will be more informative to better determine the efficacy of these agents. To achieve adequate power for well-designed clinical trials, extensive collaborative efforts will be required.

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Declaration of interest

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References:

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


https://www.nature.com/articles/6604129#supplementary-information.


60. Borad MJ, Davis SL, Lowery MA, et al. Phase 2, open-label, multicenter study of the efficacy and safety of INCB054828 in patients (pts) with advanced, metastatic, or surgically unresectable


73. Leone F, Marino D, Cereda S, et al. Panitumumab in combination with gemcitabine and oxaliplatin does not prolong survival in wild-type KRAS advanced biliary tract cancer: A


91. Ahn DH, Li J, Wei L, et al. Results of an abbreviated phase-II study with the Akt Inhibitor MK-2206 in Patients with Advanced Biliary Cancer [Article]. Scientific Reports. 2015 07/10/online;5:12122. doi: 10.1038/srep12122

https://www.nature.com/articles/srep12122#supplementary-information.


Annotations:

● Ref 9 discusses the molecular pathogenesis of intrahepatic cholangiocarcinoma.

●● Ref 15 details about the role of FGFR aberrations in the molecular pathogenesis of cholangiocarcinoma.

●● Ref 16 details about the Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways.

●● Ref 28 Discusses exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas.

●● Ref 29 Discusses BAP1 mutation in the pathogenesis of cholangiocarcinoma

●● Ref 34 Discusses the clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma.

●● Ref 48 illustrates the importance of gemcitabine and cisplatin in the management of advanced cholangiocarcinoma (Phase III trial).

● Ref 69 discusses the role of sphingokinase 2 in the management of cholangiocarcinoma.

● Ref 93 Discusses immunotherapeutic approaches of cholangiocarcinoma.

● Ref 94 discusses PD-1 expression in cholangiocarcinoma.

Figure 1 legend:
Schematic representation of Sphingolipid metabolism and mechanism of action of ABC294640.
In the presence of proliferative or inflammatory stimuli, sphingomyelin is hydrolyzed to produce ceramides that induce apoptosis in tumor cells. Dihydroceramide, simple sphingolipid is also converted to ceramide in the presence of dihydroceramide reductase. Ceramides are further hydrolyzed to produce sphingosine, which is phosphorylated by sphingosine kinases (SK1 and SK2) to produce sphingosine-1-phosphate, that has mitogenic and proinflammatory properties. ABC294640 acts by blocking these sphingosine kinases (type 2) and dihydroceramide reductase.
Table 1: Selected key clinical trials of novel targeted therapies targeting FGFR pathway in the management of advanced cholangiocarcinoma

<table>
<thead>
<tr>
<th>Targeted therapy agents</th>
<th>Study</th>
<th>Target</th>
<th>Significance/outcome</th>
<th>Ongoing clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGJ398</td>
<td>Nogova L et al., (n=3) Phase I</td>
<td>FGFR2</td>
<td>Reduction in tumor size by 5-20% in all the three patients</td>
<td>NCT02150967 NCT02160041</td>
</tr>
<tr>
<td></td>
<td>Javle M et al., (n=74) Phase II</td>
<td>FGFR2</td>
<td>Overall response rate of 14.8% in all the patients who received BGJ398. In patients with FGFR2 fusions, response rate was 18.8%. The cohort had progression free survival of about 5.8 months with overall disease control rate of 75.4%.</td>
<td></td>
</tr>
<tr>
<td>Ponatinib</td>
<td>Borad MJ et al., (n=2)</td>
<td>FGFR2</td>
<td>Ponatinib therapy resulted in tumor shrinkage in an advanced cholangiocarcinoma patient with the FGFR-MGEA5 fusion. Another advanced cholangiocarcinoma patient with FGFR-TACC3I translocation had stable disease</td>
<td>NCT02272998 NCT02265341</td>
</tr>
<tr>
<td>ARQ087</td>
<td>Papadopoulos KP et al., (n=12) Phase I</td>
<td>FGFR2</td>
<td>ARQ087 was evaluated in 12 patients with advanced disease and 2 out of 3 patients with FGFR aberrations had a partial response.</td>
<td>NCT01752920 NCT03230318</td>
</tr>
<tr>
<td></td>
<td>Mazzaferro V et al., (n=29) Phase II</td>
<td>FGFR2</td>
<td>On an extension phase study in 29 cholangiocarcinoma patients (with FGFR2 fusions), disease control was seen in about 83% of cases with objective response of 21%. Out of 29 patients, 6 (20%) had partial response (32-47% tumor reduction, all FGFR2 fusion positive), 17 has stable disease (7 had tumor reduction between 10 to 25%).</td>
<td></td>
</tr>
<tr>
<td>TAS120</td>
<td>Goyal L et al., (n=19) Phase I</td>
<td>FGFR2</td>
<td>Eight patients (42.1%) had FGFR2 fusions; 7 (36.8%) had FGFR mutations, and 4 (21.1%) had FGF amplifications. Of the 8 patients with FGFR2 fusions, 4 (50%) achieved a partial response, and 2 additional patients had tumor shrinkage.</td>
<td>NCT03278106</td>
</tr>
<tr>
<td>INCB054828</td>
<td>Saleh M et al., (n=4) Phase I/II safety and tolerability study</td>
<td>FGFR2</td>
<td>In phase 2 of the study, the drug was evaluated in 4 patients with cholangiocarcinoma and one patient who had FGFR2-CCDC6 translocation showed partial response.</td>
<td>NCT02924376 NCT02393248</td>
</tr>
</tbody>
</table>

FGFR2: Fibroblast growth factor 2 receptor
Table 2: Selected ongoing clinical trials of novel targeted therapies targeting IDH pathway in the management of advanced cholangiocarcinoma

<table>
<thead>
<tr>
<th>Targeted therapy agents</th>
<th>Target</th>
<th>Ongoing/completed clinical trials</th>
<th>Significance/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG120</td>
<td>IDH1 mutation</td>
<td>NCT02989857</td>
<td>Phase III trial evaluating AG120 in previously treated advanced cholangiocarcinoma patients having IDH1 mutations (ClarIDHy).</td>
</tr>
<tr>
<td>AG120</td>
<td>IDH1 mutation</td>
<td>NCT02073994</td>
<td>Phase I trial evaluating AG-120 in subjects with advanced solid tumors (including glioma) With an IDH1 Mutation</td>
</tr>
<tr>
<td>AG-221</td>
<td>IDH2 mutation</td>
<td>NCT02273739 (completed)</td>
<td>Phase I/II trial evaluating AG-221 in solid tumors and lymphomas harboring IDH2 mutations.</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>IDH, multiple kinases</td>
<td>NCT02428855</td>
<td>Phase II trial evaluating IDH mutant intrahepatic cholangiocarcinoma.</td>
</tr>
<tr>
<td>IDH305</td>
<td>IDH1R132 Mutations</td>
<td>NCT02381886</td>
<td>Phase I trial evaluating patients with advanced malignancies that harbor IDH1R132 mutations</td>
</tr>
</tbody>
</table>
Table 3: Selected ongoing clinical trials of novel targeted therapies targeting HER2, other tyrosine kinase pathways and immunotherapies in the management of advanced cholangiocarcinoma

<table>
<thead>
<tr>
<th>Targeted therapy agents</th>
<th>Target</th>
<th>Ongoing/completed clinical trials</th>
<th>Significance/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>HER2</td>
<td>NCT02999672</td>
<td>Phase II trial evaluating trastuzumab in solid organ malignancies having HER2 mutations (KAMELEON).</td>
</tr>
<tr>
<td></td>
<td>HER2</td>
<td>NCT02836847</td>
<td>Phase II trial evaluating trastuzumab in combination with gemcitabine + oxaliplatin (GEMOX) in subjects with recurrent or advanced cholangiocarcinoma and gallbladder cancer.</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>HER2 and EGFR</td>
<td>NCT00101036</td>
<td>Phase II trial evaluating lapatinib in locally advanced or metastatic cholangiocarcinoma or hepatocellular cancer.</td>
</tr>
<tr>
<td></td>
<td>HER2 and EGFR</td>
<td>NCT02836847</td>
<td>Phase II trial evaluating lapatinib in combination with gemcitabine in subjects with recurrent or advanced cholangiocarcinoma and gallbladder cancer.</td>
</tr>
<tr>
<td>ABC294640</td>
<td>Sphingokinase-2</td>
<td>NCT03377179</td>
<td>Phase 2 trial evaluating ABC294640 in advanced cholangiocarcinoma.</td>
</tr>
<tr>
<td></td>
<td>Sphingokinase-2</td>
<td>NCT03414489</td>
<td>Expanded access program who may not qualify for NCT03377179 trial.</td>
</tr>
<tr>
<td>Dabrafenib and Trametinib</td>
<td>BRAF-V600E</td>
<td>NCT02034110</td>
<td>Efficacy and Safety of the combination regimen in subjects with BRAF V600E- Mutated Rare Cancers including biliary tract cancer.</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>PD-1 receptor</td>
<td>NCT03260712</td>
<td>Phase II trial evaluating the combination of pembrolizumab and gemcitabine/cisplatin in advanced cholangiocarcinoma.</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>PD-1 receptor</td>
<td>NCT03101566</td>
<td>Phase II trial evaluating the combination of nivolumab in combination with gemcitabine/cisplatin or ipilimumab in advanced unresectable biliary tract cancers.</td>
</tr>
<tr>
<td></td>
<td>PD-1 receptor</td>
<td>NCT03250273</td>
<td>Phase II trial evaluating the combination of entinostat (histone deacetylase inhibitor) with nivolumab in previously treated unresectable/metastatic Cholangiocarcinoma patients</td>
</tr>
<tr>
<td>Treatment</td>
<td>Description</td>
<td>Trial ID</td>
<td>Summary</td>
</tr>
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</tr>
<tr>
<td>Atezolizumab monoclonal antibody of IgG1 isotype against PD-L1</td>
<td>NCT03201458</td>
<td>Phase II trial evaluating the atezolizumab alone or in combination with cobimetinib (MEK inhibitor) in unresectable/metastatic Cholangiocarcinoma patients</td>
<td></td>
</tr>
<tr>
<td>Durvalumab + Tremelimumab  PD-1 receptor and CTLA4 receptor</td>
<td>NCT02821754</td>
<td>Phase II pilot study evaluating the combination of durvalumab with tremelimumab (CTLA4-inhibitor) along with procedures such as TACE, RFA or cryoablation in unresectable/metastatic Cholangiocarcinoma patients</td>
<td></td>
</tr>
</tbody>
</table>

PD-1 receptor: Programmed cell death-1 receptor; HER2: Human epidermal growth factor receptor 2; EGFR: Epidermal receptor growth factor receptor; MEK: mitogen-activated protein kinase kinase.
Figure 1