Review Article

Novel biomarkers and the future of targeted therapies in cholangiocarcinoma: a narrative review

Nishant Munugala[1], Shishir K. Maithel[2], Rachna T. Shroff[3]

[1]Department of Medicine, University of Arizona, Tucson, AZ, USA; [2]Division of Surgical Oncology, Emory University, Winship Cancer Institute, Atlanta, GA, USA; [3]Division of Hematology and Oncology, Department of GI Medical Oncology, University of Arizona Cancer Center, Tucson, AZ, USA

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Correspondence to: Shishir K. Maithel, MD. Division of Surgical Oncology, Emory University, Winship Cancer Institute, 1365C Clifton Road NE, 2nd Floor, Atlanta, GA 30322, USA. Email: smaithe@emory.edu.

Background and Objectives: Cholangiocarcinoma is a highly aggressive and heterogenous group of biliary malignancies arising from any site in the biliary tree, comprising 15% of all primary liver cancers. The nature of the disease and nonspecific presentation leads to late diagnosis and ultimately poor outcomes for patients. Combination gemcitabine and cisplatin has been the standard of care for cholangiocarcinoma (CCA) since 2010, with a median overall survival of 11.7 months. The five-year survival for CCA remains 5–10%, revealing a clear need for improved treatment options.

Methods: This targeted review highlights the role of next generation sequencing in CCA and the clinically relevant tumor biomarkers that have become the focus of therapeutic development.

Key Content and Findings: These tumor biomarkers or actionable mutations hold the potential to enable earlier diagnosis, provide prognostic information, and guide treatment decisions for patients with CCA. Specifically, the *FGFR2* fusion and *IDH1* mutation have shown considerable promise in development of targeted therapies. Clinical trials with inhibitors targeting *FGFR2* fusion and *IDH1* mutation have created expectations that these drugs will soon enter clinical practice. Other biomarkers including *KRAS* and *B-raf* protooncogenes, *Her2/neu* genes, and *BRCA1* and *2* tumor-suppressor genes have also been touted as potential targets for future therapies, with early data showing promise for new drug development.

Conclusions: The discovery of these actionable mutations and identification of targeted therapies have challenged the notion of a "one-size fits all" for treatment of CCA, and generated optimism that these novel treatments will soon be available for patients with CCA.

Keywords: Cholangiocarcinoma (CCA); targeted therapies; *IDH*; *FGFR2* fusion; tumor biomarkers

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Introduction

Cholangiocarcinoma (CCA) is a highly aggressive and diverse group of biliary malignancies arising from any site in the biliary tree and comprises 15% of all primary liver cancers (1). CCA can be classified into three distinct subtypes intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), or distal cholangiocarcinoma (dCCA) based on anatomical location. The nature of the disease, challenging surrounding anatomy, and nonspecific presentation, particularly for iCCA, leads to late diagnosis and ultimately poor outcomes for patients. Since 2010, the standard of care in treating all advanced, inoperable biliary tract cancers has been combination gemcitabine and cisplatin (gem-cis). Valle *et al.* demonstrated in the phase III, randomized ABC-02 trial, a median overall survival (OS) of

Table 1 Major studies with systemic therapies in CCA

Reference	Center	Study type	Number of patients	Study question	Findings & comments
Valle *et al*., 2010 (2)	University College, London	Randomized, multicenter	324	Phase III study comparing cisplatin plus gemcitabine with gemcitabine alone in patients with unresectable, recurrent, or metastatic biliary tract cancers	Reported median OS of 11.7 months with combination gem-cis compared to 8.1 months with single-agent gemcitabine. The rate of tumor control among patients in the gemcitabine-cisplatin group was significantly increased (81.4% *vs.* 71.8%, P=0.049). Study established gem-cis as standard of care for patients with advanced biliary cancer
Shroff *et al*., 2019 (3)	University of Texas MD Anderson Cancer Center, Mayo Clinic Arizona	Prospective, multicenter	62	Phase III study that evaluated the association between PFS and the addition of nab-paclitaxel to gemcitabine-cisplatin for the treatment of patients with advanced biliary tract cancer	Showed the addition of nab-paclitaxel with gem-cis notably prolonging median PFS 11.8 (95% CI: 6.0 to 15.6) months when compared to historical control of gem-cis alone in patients with advanced biliary tract cancers. These findings are being tested in a phase III randomized clinical trial

CCA, cholangiocarcinoma; PFS, progression-free survival; gem-cis, gemcitabine and cisplatin; nab, nanoparticle albumin-bound; OS, overall survival; CI, confidence interval.

11.7 months with this combination compared to 8.1 months with single-agent gemcitabine in patients with unresectable, recurrent, or metastatic biliary tract cancers. The rate of tumor control among patients in the gemcitabine-cisplatin group was significantly increased (81.4% *vs.* 71.8%, P=0.049) (2).

Unfortunately, since gem-cis was established as first-line therapy, progress in systemic therapies reaching clinical practice has been minimal. The reality is the 5-year survival for advanced CCA remains poor at 5–10% (3). There is a dire need for earlier diagnosis and improved treatment options for patients with CCA in both the first line and second-line settings, particularly given the known toxicity profile of gemcitabine-cisplatin (3). CCA has various etiologies and is clinically heterogenous, differing in presentation and complexity depending on tumor location, which presents a unique challenge (4,5). This has made development of universal treatments challenging, raising the question if targeted therapies will yield more promising results. Identifying clinically relevant biomarkers has come to the forefront of this discussion, with the hope for earlier diagnosis, prognostic information (i.e, overall survival or tumour recurrence), and ability to guide treatment decisions (6). Currently, carcinoembryonic antigen (CEA), CA 19-9, and CA 125 are the only biomarkers used in clinical practice with limited utility for diagnosis or monitoring of CCA given its poor sensitivity and specificity (1). However, the advent of next generation sequencing has started to elucidate the genetic landscape of CCA and helped to identify tumor biomarkers or "actionable mutations", raising the possibility of novel treatment approaches with targeted therapies. This focused review of the literature highlights the role of NGS in CCA and identifies the clinically relevant tumor biomarkers that have become the focus of ongoing therapeutic development (*Table 1*). We present the following article in accordance with the Narrative Review reporting checklist (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-20-475/rc).

Section 1: the role of molecular profiling

Next-generation sequencing (NGS) in CCA

NGS involves several different modern sequencing technologies, allowing for the sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing (7). Significant advances in genomics and molecular biology are directly attributable to NGS, which has become increasingly reliable in terms of sequencing, chemical analyses, and data interpretation. In recent years, NGS has become increasingly useful in clinical practice with applications ranging from mutation detection in inherited cancer syndromes, cancer somatic mutation analysis, pharmacogenetics and liquid biopsy (7). NGS has been central in advancing the understanding of

cancer biology with the identification of genetic variances contributing to tumor growth, development and metastasis, driver genes or mutations and passenger mutations (7). This has added relevance in the clinical setting through improved patient classification, prognostication, targeted treatments, drug-resistance, and pharmacogenetics. Recent advances in the understanding of the pathogenesis of CCA are tied to molecular insights provided by NGS.

Jusakul *et al.* was one of the first to explore the molecular and epigenomic landscape in CCA, studying a cohort of nearly 500 CCAs from distinct geographical regions with NGS (8). Four distinct etiological clusters were recognized, classified by differences in gene expression, mutation and copy number changes, and epigenetic changes. The specific mutations reported were *ERBB2* amplification, *TP53* mutations, levels of *PD-1/PD-L2* expression, *IDH1/2* and *BAP1* epigenetic mutations, and *FGFR/PRKA* related gene rearrangements (8). Classifying CCA's by these driver genes, noncoding promoter mutations, and structural variants, revealed distinct molecular and clinicopathologic features. In other words, molecular profiling of these CCAs provided insight beyond the anatomical location of the tumor. Interestingly, CCAs in different anatomical locations did not differ in their survival trends, while CCA's stratified by molecular clusters showed significant differences in survival were noted (8). This points to distinct cancer subtypes from the same organ arising from varying oncogenic processes. Given this, NGS offers the potential to identify disease subsets with differing prognostic and therapeutic implications, while further informing the development of targeted therapies.

NGS has facilitated the profiling and analysis of the tumor microenvironment in CCA and helped identify novel biomarkers ranging from extracellular vesicles, circulating nucleic acids, and circulating tumors cells (e.g, OPN, LOXL2, EPCAM) (4). Expression of non-coding RNA, including lncRNA, has shown some promising prognostic value in CCA (5). However, before these biomarkers can be utilized in the clinical setting, validation with large biopsy proven cohorts is needed (4). Accompanying this growing understanding of the molecular profile of CCA has prompted the search for actionable mutations to serve as targets for drug development. Among these potential biomarkers, the *FGFR2* fusion and *IDH1* and *IDH2* mutations, which are the two most common genetic alterations seen in iCCA, have been identified as mutations holding considerable promise for development of targeted therapies (9).

Isocitrate dehydrogenase (IDH1/IDH2) mutations

IDH1 and *IDH2* mutations were first discovered using NGS, and efforts to characterize *IDH* mutations have noted a higher mutation rate in iCCA, with mutations seen in about 20–25% of iCCA (8,9). However, the prognostic significance of *IDH* mutations remains unclear.

IDH1 and *IDH2* are metabolic enzymes involved in the tricarboxylic acid cycle, catalyzing the interconversion of isocitrate and alpha-ketoglutarate. The mutant *IDH* loses its normal enzymatic activity and instead produces increased amounts of the oncometabolite 2-hydroxyglutarate (2-HG), which can be detected in increased amounts in both the tumor and blood of *IDH* mutated patients (9). 2-HG can competitively inhibit dioxygenases, which play a role in DNA demethylation. Furthermore, *IDH* and *KRAS* mutations can cooperate to drive the expansion of liver progenitor cells, development of premalignant biliary lesions, and progression to metastatic iCCA (9).

Several highly specific IDH-inhibitors have been developed, working by blocking the function of mutant *IDH1* or *IDH2*, and ultimately reducing the levels of oncometabolite 2-HG (10). One such inhibitor, Ivosidenib, an oral, reversible inhibitor of mutant *IDH1* (*mIDH1*) was well tolerated among patients with advanced solid tumors with *IDH1* mutations in a phase I trial (NCT02073994). Ivosidenib is currently approved in the US for the treatment of mIDH1 acute myeloid leukemia in newly diagnosed patients ineligible for intensive chemotherapy and patients with relapsed or refractory disease. Seventy-three patients with mIDH1-CCA were enrolled in the phase I study with Ivosidenib (9). These patients had a median of 2 prior treatments (range, 1–5), pointing to a refractory study population. The median progression-free survival was 3.8 months (95% CI: 3.6–7.3), 6-month progression-free survival was 40.1% (28.4–51.6%), and 12-month progression-free survival was 21.8% (12.3–33.0%) (10). Median overall survival was 13.8 months (95% CI: 11.1–29.3); however, data were censored for 48 patients (66%) (10). No dose-limiting toxicities were reported and maximum tolerated dose was not reached (10). Given these encouraging results, 500 mg daily dose of Ivosidenib was selected for expansion in a phase III trial (10).

The ClarIDHy study is the randomized phase 3 clinical trial that evaluated ivosidenib (IVO) versus placebo (PBO) in patients with previously treated nonresectable or metastatic mIDH1-CCA. As of January 2019, 185 patients were randomized to IVO (n=124) or PBO (n=61) (11). Crossover

from placebo group to intervention (IVO) was permitted when progressive disease was documented. The primary endpoint was progression free survival, with IVO showing clear benefit with a hazard ratio (HR) 0.37 (95% CI: 0.25–0.54; P<0.001) (11). Initial results are promising, showing a PFS of 2.7 *vs.* 1.4 months (intervention *vs.* placebo). PFS rates at 6 and 12 months were 32.0% and 21.9% in IVO arm. On the other hand, no PBO patients were progression-free for ≥6 months at data cutoff (11). Ivosidenib's efficacy was seen across all sub-groups. Intention to treat analysis showed median OS was 10.8 months for IVO compared to 9.7 months for placebo (HR 0.69; one-sided P=0.06) with 57% of PBO patients crossed over to IVO (10). The Rank Preserving structural failure time (RPSFT)-adjusted median OS was 6 months for PBO (HR 0.46; P=0.0008) (11). Grade ≥3 adverse events reported in 46% IVO *vs.* 36% PBO and there were no treatment-related deaths. This study demonstrated significant improvement in PFS with Ivosidenib and a favorable OS trend compared to placebo (11). This is the first pivotal study demonstrating the clinical benefit of targeting *mIDH1* in patients with advanced mutant *IDH1* CCA.

Several other *IDH1* and *IDH2* inhibitors are being tested in ongoing clinical trials (NCT02746081, NCT02273739, NCT02381886, NCT02481154) (9). The potential of these therapies targeting *IDH1* and *IDH2* mutations raises questions on how these treatments should be used—as first-line therapy in selected populations or whether these inhibitors should be combined with chemotherapy or other treatment modalities. Additionally, the utility of these inhibitors in the adjuvant and neoadjuvant settings for patients with resectable disease will need to be explored. Regardless, the potential of these inhibitors offers optimism for improved therapies and a direction for future drug development.

Fibroblast growth factor receptor 2 (FGFR2) fusions

The *FGFR2* fusion, seen in about 15–20% of iCCA, is another actionable mutation showing considerable promise (9). Genome-wide structural analyses first revealed recurrent translocation events involving the *FGFR2* locus (9). The mechanism by which *FGFR2* fusions drive oncogenesis has yet to be fully understood, however, it is thought that genetic alterations of *FGFR* can alter *FGFR* kinase activity and result in constitutively active *FGFR* signaling that promotes cell proliferation and inhibits apoptosis (12). Jain *et al.* reported on clinical characteristics and treatment outcomes of biliary tract cancers with *FGFR* genetic alterations in a retrospective analysis and found this subtype to be associated with an indolent disease course and prolonged survival. It was additionally noted this subtype affected a disproportionate number of young women (12). Since the initial discovery of recurrent *FGFR2* fusions being present in multiple tumor types and particularly in iCCA, it has become the focus of developing targeted therapies.

The oral agent infigratinib (BGJ398) had the earliest reported data of selective *FGFR* inhibition in CCA. Infigratinib is an orally bioavailable, selective pan-*FGFR* kinase inhibitor that has shown preliminary clinical activity against tumors with *FGFR* alterations (13). A multicenter, open-label, phase II study (NCT02150967) evaluated infigratinib antitumor activity in patients age ≥18 years with advanced or metastatic CCA containing *FGFR2* fusions or other *FGFR* alterations whose disease progressed on prior therapy (13). Sixty-one patients (median age, 57 years) with *FGFR2* fusion (n=48), mutation (n=8), or amplification (n=3) participated. At the prespecified data cutoff (June 30, 2016), 50 patients had discontinued treatment. All responsive tumors contained *FGFR2* fusions. The overall response rate was 14.8% (18.8% in *FGFR2* fusions population), disease control rate was 75.4% (83.3% *FGFR2* fusions population) and estimated median progression-free survival was 5.8 months (95% CI: 4.3 to 7.6) (13). Infigratinib is a first-in-class *FGFR* kinase inhibitor with a manageable toxicity profile, showing meaningful clinical activity against refractory CCA containing *FGFR2* fusions. This promising antitumor activity supports continued development of Infigratinib in this patient population and further suggests *FGFR* inhibitor therapy as a viable therapeutic option in advanced biliary tract malignancies. The PROOF trial is currently ongoing, evaluating the efficacy of Infigratinib *vs.* gem-cis in first line treatment of patients with unresectable locally advanced or metastatic CCA with *FGFR2* fusions or translocations (NCT03773302). It is a multicenter, open-label, randomized, controlled phase III clinical trial aiming to enroll 384 patients (13). Patients will be randomized 2:1 to infigratinib versus standard of care, with the ability for patients who are unresponsive to gem-cis to crossover and receive infigratinib (14). In January 2020, Infigratinib (BGJ398) received fast-track and orphan drug designations by the FDA, highlighting the optimism surrounding the drug's potential to treat CCA in this select patient population (14).

TAS-120 is another irreversible *FGFR*-inhibitor showing promise in clinical trials. A phase I study of TAS-120

(8–24 mg daily) in adult patients with advanced solid tumors (NCT02052778) found the maximum tolerated dose to be 20 mg daily (15). Meric-Bernstam *et al.* specifically analyzed patients with CCA enrolled in this study. 45 patients with CCA (intrahepatic n=41) with *FGF/FGFR* aberrations were treated at 16 (n=24), 20 (n=14), and 24 mg (n=7) QD [median age 53 years (range, 29–73)]. Of note, 76% of the patients were female. Twenty-eight patients (62%) had tumor *FGFR2* gene fusions, while 17 (38%) had other *FGF/FGFR* aberrations (15). All patients received prior systemic therapy, 28.9% with reversible *FGFR*-inhibitor, revealing a highly refractory patient population with prior *FGFR* inhibitor exposure. Of the 28 patients with *FGFR2* gene fusions, 20 (71%) experienced tumor shrinkage and 7 achieved confirmed partial responses (cPRs) (15). The objective response rate was 25%. Fifteen of 28 (54%) patients had stable disease as best response (15). The disease control rate was 79%. Of the 17 patients with other *FGF/FGFR* aberrations, 3 had cPRs (all had *FGFR2* rearrangements; 1 also had *FGFR2* amplification). Median treatment time was 7.4 months. Of the 13 patients with prior *FGFR*-inhibitor treatment, 4 (3 with *FGFR2* gene fusions, 1 with *FGFR2* amplification) had cPRs (15). Grade ≥3 treatment related adverse events were reported in 23 of 45 (51%) patients, with the most common being hyperphosphatemia (22%) (15). TAS-120 showed notable clinical activity with manageable toxicities in patients with CCA and *FGFR2* fusions. The drug further showed efficacy in patients who had progressed on prior FGFR inhibitors. A phase II study testing TAS-120 in patients with *FGFR2* gene fusions is ongoing (16). Interim analysis as of May 2020, reported data for 67 patients with a minimum of 6 months of follow up and found the ORR was 37.3% (1 CR 1.5%; 24 PR 35.8%) (17). Median duration of response was 8.31 months. The most common treatment-related adverse events (all grades, grade 3) at the time of analysis were hyperphosphatemia (80.6%; 26.9%), diarrhea (37.3%; 0%), and dry mouth (32.8%; 0%) (17). There were no grade 4 treatment related adverse events. This initial anlaysis is encouraging that TAS-120 may having clinically meaningful benefit in patients with refractory iCCA with FGFR2 gene fusions.

Pemigatinib is an orally bioavailable inhibitor of *FGFR* 1, 2, and 3 currently being investigated. The Fight 202 trial is a phase II, open-label, single-arm, multicenter study evaluating the efficacy of pemigatinib in patients with advanced or surgically unresectable CCA with *FGFR2* translocation who have failed at least 1 previous treatment (NCT02924376). Patients were enrolled in three cohorts—those with *FGFR2* translocations (A), those with other *FGF/FGFR* genetic alterations (B), or those with neither (C) (18). Each cohort received pemigatinib 13.5 mg daily on a 21-day cycle until disease progression or intolerable toxicity. Interim data presented in September 2019 showed an objective response rate (ORR) of 35.5% in cohort A (18). Median duration of response in this group was 7.5 months. No response was seen in cohort B or C. Median PFS was 6.9 months in cohort A compared with 2.1 months in cohort B and 1.7 months in cohort C (19). This data supports *FGFR* inhibition being a meaningful treatment for this subset of patients with CCA. Based on the data from FIGHT-202, a phase III study of pemigatinib versus gemcitabine plus cisplatin chemotherapy in first-line treatment of patients with unresectable or metastatic CCA with *FGFR2* rearrangements is underway (NCT03656536). As of April 2020, pemigatinib became the first FDA approved targeted therapy for treatment of adults with previously treated, unresectable locally advanced or metastatic CCA with a fibroblast growth factor receptor 2 (*FGFR2*) fusion or other rearrangement (19).

Other selective *FGFR* inhibitors including Debio1347 (Debiopharm, NCT01948297) are currently being evaluated in early phase trials in patients with advanced solid tumors, including iCCA (9). A third non-selective tyrosine kinase inhibitor, ARQ-087 (Arqule, NCT01752920) has shown some encouraging anti-tumor activity in advanced iCCA with *FGFR2* fusion as well (9). Overall, the preliminary data for *FGFR* inhibitors in advanced iCCA offers guarded hope. However, some debate exists regarding if these trends in survival can be attributed to these targeted therapies or to the natural history of the *FGFR* phenotype, given its favorable survival profile. Looking to the future, questions remain whether these drugs can evolve into front-line therapies or if they should be used in combination with chemotherapy or other treatment modalities.

Mechanisms of resistance

Initial clinical trials of targeted therapies in CCA have rightfully generated significant optimism. However, concerns of drug resistance have emerged as a real threat to the potential impact of these therapies (9). Mechanisms of resistance are not fully understood, and looking to the future, adequately addressing drug resistance will likely determine the durability of these targeted therapies.

Isoform switching is a mechanism for acquired resistance

to *IDH* inhibitors that was first described in four clinical cases (20). Harding *et al.* describe a selective pressure of inhibiting mutant *IDH* activity in one subcellular compartment, which in turn provides a growth advantage for malignant subclones with unchecked mutant *IDH* activity in another subcellular compartment. In other words, by "isoform switching" from mutant *IDH1* to mutant *IDH2* or vice versa, *IDH*-mutant cancers can develop resistance to isoform-selective *IDH* inhibitors and restore the production of oncometabolite 2-HG, ultimately promoting tumor progression (20). The frequency of mutant *IDH* isoform switching as a mechanism of resistance to *IDH* inhibition is currently unknown.

The development of acquired resistance to *FGFR* kinase inhibitors has been even more common. Secondary *FGFR2* kinase domain mutations has been observed in patients in the Infigratinib (BGJ398) clinical trials (13). Goyal *et al.* reported that the irreversible *FGFR* inhibitor TAS-120 demonstrated efficacy in 4 patients with *FGFR2* fusion-positive iCCAwho developed resistance to Infigratinib (BGJ398) or Debio 1347. After examining serial biopsies, circulating tumor DNA, and patient-derived iCCA cells it was found that TAS-120 was active against multiple acquired *FGFR2* mutations which conferred resistance to BGJ398 or Debio 1347 (21). Functional assessment and modeling of the clonal outgrowth of individual resistance mutations from polyclonal cell pools mirrored the resistance profiles observed clinically for each inhibitor (21). These findings suggest that strategic sequencing of *FGFR* inhibitors, guided by serial biopsy and circuluating tumor DNA (ctDNA) analysis, may prolong the duration of benefit from *FGFR* inhibition in patients with *FGFR2* fusion-positive iCCA. Moving forward, therapeutic strategies to prevent or overcome resistance will be pivotal to sustained success of these developing targeted therapies.

Section 2: smaller molecular targets in CCA

KRAS gene

KRAS is the proto-oncogene encoding for a small GTPase involved in several cell signaling pathways, including the *MAPK-ERK* pathway which regulates cell differentiation and proliferation. Mutations can disable the GTPase activity of *KRAS*, keeping it constitutively active in a GTP-bound state, which ultimately leads to continuous activation of downstream signal transduction pathways that drive tumor growth, remodeling, and migration (22).

The prevalence of *KRAS* mutations in iCCA is reported between 7–24%, with considerable variation between studies (23-26). However, despite the variable frequency of *KRAS* mutations, there is data suggestive that testing for *KRAS* mutations may provide prognostic value. Robertson *et al.* conducted DNA extraction and pyrosequencing of 54 iCCA cases and found 4 cases with *KRAS* mutations. The reported median OS was 13.5 months for *KRAS* mutant cases, compared to 37.3 months for wild type cases, suggesting a worse prognosis for *KRAS* mutant cases. Additionally, *KRAS* mutant cases were also associated with higher tumor stage and greater likelihood of lymph node involvement at time of resection (24). Javle *et al.* reported similar findings in a multicenter study using hybrid capture-based comprehensive genomic profiling of 412 iCCA samples. *KRAS* mutations were observed in 22% of iCCA samples, and among the 224 iCCA cases analyzed, *KRAS* mutated tumors were associated with poorer overall survival (P=0.048) in comparison with *KRAS* wild type tumors (27). Though these studies are suggestive of *KRAS* mutations having prognostic significance, specific treatments targeting this pathway are still in preliminary stages of development. An irreversible *KRAS p.G12C* inhibitor (AMG510) is currently being tested in Phase I/II trials (NCT03600883). However, the *G12C* mutation has not been frequently noted in CCA and AMG510 may have limited practical significance in the treatment of CCA presently.

B-raf gene

The *B-raf* proto-oncogene encodes a serine-threonine protein kinase which activates the MAPK/ERK proliferation signaling pathway. Mutations in *B-raf*, such as *BRAF V600E*, lead to constitutive activation of its downstream signaling molecules, resulting in cell cycle progression (28). The nucleotide sequence of the raf gene was first discovered from murine sarcoma virus 3611 (MSV-3611) in 1983 (29,30).

In 2002, researchers identified *B-raf* somatic missense mutations in 66% of malignant melanomas, bringing *B-raf* mutations to the forefront of the discussion on utilizing targeted therapies (31). Specifically, the *BRAF V600E* mutation, which accounted for 80% of these reported mutations, provided a promising new therapeutic target. *BRAF* inhibitors, dabrafenib, and vemurafenib, have significant results in *BRAF V600E* mutated metastatic melanoma, and initially there was hope for similar results in the treatment for *BRAF*-mutated CCA. However, the

fact that B-raf mutations occur at a notably lower frequency (3–5%) in CCA than in melanoma have hampered these expectations (32-34). There are limited studies that evaluate the efficacy of Vemurafenib in *BRAF*-mutated CCA cases. In a phase II basket study of Vemurafenib in *BRAF* V600E-mutated non-melanoma cancers, one patient from a cohort of eight patients with CCA achieved a durable partial response of over 1 year (35). ROAR, a phase II trial investigating combination therapy with *B-raf* and *MEK* inhibition (Dabrafenib and Trametinib) in subjects with *BRAF* V600E- mutated rare cancers is ongoing (NCT02034110). Preliminary data for the ROAR trial was presented at the 2019 Gastrointestinal Cancers Symposium (36). Of the thirty-three biliary tract cancer patients enrolled in the study, thirty patients harbored *BRAF V600E* mutations. The objective response rate was 41% (13/32; 95% CI: 24–59%), with 6 of 13 responses ongoing at data cutoff, and 7 of 13 (54%) patients with a duration of response ≥6 months. The median progression-free survival was 7.2 months (95% CI: 4.6–10.1), and median overall survival was 11.3 months (95% CI: 7.3–17.6).

BRCA1 and BRCA2, PARP inhibition

The *BRCA1* and *BRCA2* tumor suppressor genes encode proteins that repair double-stranded DNA breaks (DSBs) through homologous recombination. *BRCA1/2* mutated cells are unable to carry out homologous recombination and perform DNA repair by alternative error-prone mechanisms, predisposing them to genomic instability (37). Inhibiting both *BRCA* and poly ADP-ribose polymerase (*PARP*) proteins is one approach that has been utilized to target *BRCA*-mutated tumors. Normally, *PARP* enzymes repair single-stranded DNA breaks via base excision repair. However, in *PARP*-deficient cells, single-stranded DNA breaks remain unrepaired, and when encountered by a replication fork, progress into double-stranded breaks. *BRCA*-mutated cells are unable to repair these DSBs and overcome the collapsed replication fork (38). Essentially, *PARP* inhibition exploits the inherent vulnerability of *BRCA*-mutated tumors and other DNA repair deficient cells.

In a retrospective analysis of patients with *BRCA*-mutated CCA (n=18), one of four patients who received *PARP* inhibitors had a sustained disease response with a progression-free survival duration of 42.6 months (39). However, given the limited number of patients in this study, more research is needed to establish *PARP* inhibition as a viable treatment option for *BRCA*-mutated CCA. A Phase II trial of *PARP* inhibitor, Niraparib, is underway at the University of Florida (NCT03207347). A basket phase II trial of *PARP* inhibitor, Olaparib, is also ongoing and will include patients with metastatic solid tumors harboring *IDH1* or *IDH2* mutations (NCT03212274).

Her2/neu gene

The *Her2/neu* gene encodes for the *Her2* (human epidermal growth factor receptor-2) protein, a transmembrane receptor with tyrosine kinase activity which is an upstream driver of multiple signal transduction pathways that promote tumorigenesis. The *Her2/neu* gene was first discovered when a series of neuroglioblastomas from carcinogen treated rats were found to have the same oncogene (40). *Her2* overexpression has notably been used as a predictive marker in breast and gastric cancers. However, the prevalence of *Her2* overexpression in biliary tract cancers was previously undetermined.

Galdy *et al.* were the first to delve into this question, performing a systematic review and meta-analysis in 2017 of 40 studies (including 3,839 patients in total) which revealed that extrahepatic biliary tumors have higher *Her2* overexpression compared to iCCA: 19.9% (95% CI: 12.8–27.1%) *vs.* 4.8% (95% CI: 0–14.5%), respectively (41). In addition to the low prevalence of *Her2* mutations in iCCA, previous Phase II trials of Lapatinib (n=17), a dual inhibitor of *EGFR* and *Her2/neu*, have shown poor results with a reported median progression-free survival duration of 1.8 months with a zero percent response rate (42). A similar phase II study of Lapatinib in nine biliary tract cancer patients reported zero responses and a median progression-free survival duration of 2.6 months (42,43). In comparison to Lapatinib, monoclonal antibodies such as Trastuzumab may be a better alternative for Her2 overexpressing biliary tract cancers. Varlitinib, a small molecule *Her-1/2/4* inhibitor, is also being investigated in a phase II/III study in combination with Capecitabine as second line treatment for unselected advanced biliary tract cancers (44). Preliminary results from an ongoing clinical trial (NCT02091141) has shown that pertuzumab plus trastuzumab has activity in *HER2* mutated biliary cancers, further supporting *HER2* as a potential therapeutic target for these cancers. Additionally, data from a Phase I study showed single agent ZW25, a bispecific antibody, induced anti-tumor activity in heavily pretreated patients with a variety of *HER2*-expressing cancers, including CCA (45). Phase II clinical trials are currently underway testing ZW25 in breast, gastric, and other *Her2* expressing cancers (*Table 2*).

Table 2 Major studies with targeted therapies in CCA

Reference	Center	Study type	Number of patients	Study question	Findings & comments
Lowery *et al.*, 2019 (10)	12 clinical sites in US, 1 in France	Prospective, multicenter	73	Phase 1 dose-escalation and expansion study of ivosidenib monotherapy in mIDH1 solid tumours	The median PFS was 3.8 months (95% CI: 3.6–7.3), 6-month PFS was 40.1% (28.4–51.6%), and 12-month PFS was 21.8% (12.3–33.0%) (10). Median OS was 13.8 months (95% CI: 11.1–29.3)
Abou-Alfa *et al.*, 2020 (11)	Global	Prospective, multicenter	185	Phase III study that evaluated ivosidenib (IVO) versus placebo (PBO) in patients with previously treated nonresectable or metastatic mIDH1-CCA	The primary endpoint was PFS, with IVO showing clear benefit with a HR 0.37 (95% CI: 0.25–0.54; P<0.001). Initial results are promising, showing a PFS of 2.7 *vs.* 1.4 months (intervention *vs.* placebo). Study demonstrated significant improvement in PFS with Ivosidenib and a favorable OS trend compared to placebo
Javle *et al.*, 2018 (13)	Global	Prospective, Multicenter	61	Phase II study evaluated infigratinib antitumor activity in patients age ≥18 years with advanced or metastatic CCA containing *FGFR2* fusions/alterations whose disease progressed on prior therapy	The overall response rate was 14.8% (18.8% in *FGFR2* fusions population), disease control rate was 75.4% (83.3% *FGFR2* fusions population) and estimated median PFS was 5.8 months (95% CI: 4.3–7.6). Infigratinib is a first-in-class *FGFR* kinase inhibitor with a manageable toxicity profile, showing meaningful clinical activity against refractory CCA containing *FGFR2* fusions
Meric-Bernstam *et al.*, 2018 (15)	Global	Prospective, Multicenter	45	Phase I study of TAS-120 (irreversible *FGFR1-4* tyrosine kinase inhibitor) in adult patients with advanced solid tumors; Specifically looked at CCA cases	Of the 28 patients with *FGFR2* gene fusions, 20 (71%) experienced tumor shrinkage and 7 achieved confirmed partial responses. The objective response rate was 25%. Fifteen of 28 (54%) patients had stable disease as best response. The disease control rate was 79%. Study showed TAS-120 had notable clinical activity with manageable toxicities in patients with CCA and *FGFR2* fusions
Goyal *et al.*, 2019 (16)	Global	Prospective, Multicenter	103	Phase 2 study evaluating (TAS-120) in patients with iCCA harboring *FGFR2* gene fusions or other rearrangements, who have failed at least one line of therapy	Interim analysis reported data for 67 patients (65%) with a minimum of 6 months of follow up and found the ORR was 37.3% (1 CR 1.5%; 24 PR 35.8%). Median duration of response was 8.31 months, demonstrated that treatment with the covalently-binding *FGFR* inhibitor futibatinib may lead to meaningful clinical benefit in patients with refractory iCCA with *FGFR2* gene fusions or other rearrangement
Vogel *et al.*, 2019 (18)	Global	Prospective, multi-center	147	Phase II study evaluating the efficacy of pemigatinib (*FGFR* 1, 2, 3 inhibitor) in patients with advanced or surgically unresectable CCA with *FGFR2* translocation failing prior treatment	Interim data shows an objective response rate of 35.5% in patients with *FGFR2* translocation. Median duration of response in this group was 7.5 months. Median PFS was 6.9 months in this group compared to 1.7 months in patients without *FGFR* alterations. This data supports *FGFR* inhibition being a meaningful treatment for this subset of patients with CCA
ROAR Trial (36) (NCT02034110	Global	Prospective, multicenter	206	Phase II trial investigating combination therapy with *B-raf* and *MEK* inhibition (Dabrafenib and Trametinib) in subjects with *BRAF V600E*- mutated rare cancers	Preliminary data notable for objective response rate was 41% (13/32; 95% CI: 24–59%), with 6 of 13 responses ongoing at data cutoff, and 7 of 13 (54%) patients with a duration of response ≥6 months. The median progression-free survival was 7.2 months (95% CI: 4.6–10.1), and median overall survival was 11.3 months (95% CI: 7.3–17.6)

CCA, cholangiocarcinoma; *FGFR*, fibroblast growth factor receptor; OS, overall survival; PFS, progression free survival; CR, complete response; PR, partial response; iCCA, intrahepatic cholangiocarcinoma.

Section 3: role of immunotherapy in CCA

Immunotherapy is a burgeoning field within oncology, particularly gaining popularity as a viable treatment option for cancers such as melanoma and colorectal cancer. Studies investigating the role of immunotherapy in CCA are also emerging. Immunotherapies such as programmed cell death protein 1 (PD-1) inhibitors enable tumor recognition by blocking the PD-1 and programmed cell death protein 1 ligand (PD-L1) interaction that allows tumor cells to evade T-cell recognition. The presence of mismatch repair (MMR) deficiency further enhances tumor recognition since MMR deficiency results in a high mutation burden with subsequent creation of neoantigens and microsatellite instability. As a result, tumors with MMR deficiency have been shown to be especially sensitive to PD-1 inhibitors such as Pembrolizumab (46). Unfortunately the prevalence of MMR deficiency in CCA is low as Goyal *et al.* report a 9% rate of MMR protein loss, with only 4.5% patients being microsatellite instability-high (MSI-H) (47).

The phase Ib KEYNOTE-028 trial evaluated 24 patients with biliary tract cancers, all of whom were PD-L1 positive but negative for MSI-H. The reported overall response rate (ORR) to pembrolizumab was 13.0% (3/23, all partial response; 95% CI: 2.8–33.6%) and median duration of response was not reached (range, 21.5 to 29.4+ months). Median overall survival and progression-free survival were 6.2 months (95% CI: 3.8–10.3) and 1.8 months (95% CI: 1.4–3.7), respectively (48). In the larger KEYNOTE-158 Phase II basket trial, 104 patients were enrolled in the biliary tract cancer cohort and treated with Pembrolizumab; 58% were PD-L1 positive and none harbored MSI-H. Here the ORR was disappointing at only 5.8% (6/104, all partial response; 95% CI: 2.1–12.1%) though median duration of response was not reached (range, 6.2 to 23.2+ months). Results from these trials, especially the low overall response rate in the larger KEYNOTE-158 trial, suggest that pembrolizumab is not an effective single-agent treatment option for CCA. Although there is no existing literature for immunotherapy combination regimens in CCA, combination therapy with Atezolizumab and Cobimetinib is being studied in the treatment of metastatic colorectal cancer. Results from a phase III, randomized controlled trial with 363 metastatic colorectal cancer patients showed a median overall survival of 8.8 months (95% CI: 7.00–10.61) with Atezolizumab plus Cobimetinib, compared to 7.1 months (95% CI: 6.05–10.05) with Atezolizumab, and 8.5 months (95% CI: 6.41–10.71) with Regorafenib (49). Although these results do not demonstrate that immunotherapy combinations are superior, this trial presents the possibilities for studying immunotherapy combinations. The first randomized trial of immunotherapy in BTC was the phase II trial of Atezolizumab plus Cobimetinib in metastatic, unresectable CCA in 2017. Eighty-six patients were enrolled in 23 centers across the Unites States (NCT03201458). Results were notable for a median PFS of 3.65 months (cobimetinib + atezolizumab) *vs.* 1.87 months (atezolizumab monotherapy) (P=0.027) (50). OS data was still pending at the time of analysis. Treatment was well tolerated in both groups as grade 3–4 treatment-related adverse events were similar in both arms, and there were no treatment-related deaths (50). Ultimately, the combination of atezolizumab + cobimetinib significantly prolonged PFS as compared to atezolizumab monotherapy in BTC without significant toxicity, warranting further investigation in treatment of biliary tract cancers.

A single-center, prospective cohort study, conducted between May 2018 and February 2019 in Korea, evaluated the efficacy and safety of pembrolizumab in patients who were positive for PD-L1 and progressed on first-line gemcitabine plus cisplatin. A total of 40 patients were enrolled and Pembrolizumab 200 mg was administered intravenously every 3 weeks. The objective response rate was 10% and 12.5% by Response Evaluation Criteria in Solid Tumor (RECIST) v1.1 and immune- modified RECIST (imRECIST) and median duration of response was 6.3 months (51). The median PFS and OS were 1.5 months (95% CI, 0.0 to 3.0) and 4.3 months (95% CI: 3.5 to 5.1), respectively, and objective response per imRECIST was significantly associated with PFS (P<0.001) and OS (P=0.001) (51). The data presented in this study suggests Pembrolizumab having modest anti-tumor activity in heavily pretreated PD-L1-positive BTC patients, with a durable response seen in patients who showed objective response.

In a recent phase II trial, Kim *et al.* investigated the efficacy of nivolumab, a PD-1 inhibitor in refractory biliary tract cancers. Fifty-four patients were enrolled in this multicenter study between October 2016 and December 2018 (52). Of these patients, 46 were examined for objective response via radiologic imaging. Investigator assessed objective responses (all partial responses) were observed in 10 (22%) of 46 patients. Additionally, it was noted 17 (37%) patients had stable disease, yielding DCR of 59%. Of note, all responses were observed in patients with mismatch repair protein-proficient tumors. Among all 54

Table 3 Selected ongoing trials with targeted and systemic therapy in CCA

Study name	Center	Study type	Number of patients	Purpose
PROOF Trial (14) (NCT03773302)	Global, QED Therapeutics	Prospective, multicenter	350	Phase III trial evaluating the efficacy and safety of the investigational agent oral infigratinib *vs.* standard of care chemotherapy (gemcitabine plus cisplatin) in first-line treatment of participants with unresectable, recurrent, or metastatic CCA with *FGFR2* gene fusions/translocations
S1815 Study (50) (NCT03768414)	Global	Prospective, multicenter	268	Phase III study evaluating efficacy of gem-cis given with or without nab-paclitaxel in treating patients with newly diagnosed biliary tract cancers with metastases. Primary objective is to compare OS in patients with untreated, advanced biliary cancers treated with gem-cis vs. those treated with gemcitabine, cisplatin, and nab-paclitaxel
MK-3475-966 Trial (NCT04003636)	Global	Prospective, multicenter	788	Phase III randomized, double-blind study of pembrolizumab plus gemcitabine/cisplatin versus placebo plus gemcitabine/cisplatin as first-line therapy in participants with advanced and/or unresectable biliary tract carcinoma
NuTide:121 Study (NCT04163900)	Global	Prospective, multicenter	828	Phase III, randomized study comparing NUC-1031 plus cisplatin to gemcitabine plus cisplatin in patients with previously untreated locally advanced or metastatic biliary tract cancer. Primary objectives are OS and ORR. Secondary objectives include further measurements of efficacy, safety, pharmacokinetics, and patient-reported quality of life

CCA, cholangiocarcinoma; OS, overall survival; ORR, objective response rate.

patients, median PFS was 3.68 months and median OS was 14.24 months (52). Patients with PD-L1 positive tumors had significantly prolonged PFS (median 10.4 *vs.* 2.3 months, HR 0.23, P<0.001) and nonsignificantly prolonged overall survival (median not reached *vs.* 10.8 months, P=0.19) *vs.* patients with PD-L1 negative tumors (52). This study found nivolumab was well tolerated and showed modest efficacy and durable response with patients with refractory BTC. The median OS of 14.24 months was impressive given the refractory patient population. Additionally, the ORR of 22% was a strikingly higher ORR than what has been previously reported with other checkpoint inhibitors, such as pembrolizumab. However, it is important to note centrally reviewed ORR was lower at 11% and more in line with previously published results. Interestingly, although all the patients were noted to be mismatch repair–proficient, there appeared to be a correlation between PD-L1 positivity and response. Moving forward, further studies to both verify these findings and to evaluate biomarkers for improved treatment selection are needed.

One such ongoing study is a randomized, multi-institutional, phase 2 study investigating the role of combinational immunotherapy, using nivolumab with chemotherapy (gemcitabine/cisplatin) or as dual immunotherapy (nivolumab and ipilimumab) in patients with advanced BTC (NCT03101566). The primary objective is to evaluate the PFS rate at 6 months. Secondary objectives include evaluation of ORR, median PFS and OS and safety. Exploratory objectives include identification of biomarker predictors of response and mechanisms of resistance through serial biopsies and blood collection, including sequential whole exome/transcriptomic analysis and immune cell analysis of tissue and blood (53). Patient accrual was completed as of January 2020 and final data analysis is pending. These results will not only provide information regarding efficacy of nivolumab in combination with gem-cis and ipilumamab but may also elucidate the role of NGS data in guiding treatment selection for patients (*Table 3*).

Conclusion: the future of treatment in CCA

The discovery of prognostic and predictive biomarkers in CCA has expanded the understanding of CCA as well as informed the development of novel therapies. Identifying actionable mutations suited for targeted therapies has been a point of emphasis of recent research in CCA. Since Gemcitabine-cisplatin combination therapy was established as first-line therapy in the treatment of advanced biliary cancers in 2010, advances in systemic therapies extending to standard clinical practice have been limited. However, a step forward was made with recent data showing the addition

of nab-paclitaxel with gem-cis notably prolonging median progression-free survival when compared to historical control of gem-cis alone in patients with advanced biliary tract cancers (3). These findings are being tested in a phase III randomized clinical trial (S1815 Study, NCT03768414).

Next generation sequencing has been central in elucidating the pathogenesis of CCA from a molecular level, bringing hope of identifying actionable mutations for the development of targeted therapies. The paradigm that "one-size fits all" for the treatment of CCA is likely obsolete, given the varying etiologies and heterogeneity of the disease. Looking to the future, next generation sequencing will likely become essential to clinical and therapeutic decision-making. Novel treatments for CCA are closer than ever to entering clinical practice, with the *IDH1* inhibitor, ivosidenib showing considerable promise in the ClarIDHy study. Similarly, *FGFR* inhibitors BGJ-398, TAS-120, and pemigatinib have also shown meaningful activity in phase II clinical trials, with pemigatinib becoming the first FDA approved therapy for CCA patients with *FGFR2* fusions, as of April 2020. The expectation is that more targeted therapies will follow pemigatinib's lead and enter the front-line of care in the near future.

The search to identify more actionable mutations is ongoing, with molecular targets such as *B-raf* and *BRCA* touted as potential targets. The obvious challenge is that many of these molecular targets occur at lower frequencies in CCA than *IDH1/IDH2* mutations and the *FGFR2* Fusion. Nevertheless, these targets may still have a role in the treatment of CCA, given several of these targets have approved therapies in other cancer types, raising the question whether these agents have a therapeutic role in select subsets of CCA patients. Further investigation is needed to evaluate the efficacy of targeting these mutations in CCA, which has proven challenging given the rarity of these cancers. One practical approach to increasing these investigations is through basket trials, which allow for evaluation of rare tumor subsets with shared controls. Introducing more basket trials could provide researchers with more opportunities to study some of the less common mutations seen in rare cancers such as CCA.

While immunotherapy has shown promising results for malignancies such as melanoma and colorectal cancer, results for pembrolizumab treatment in CCA have been mixed. Kang *et al.* (51) reported modest efficacy of pembrolizumab in PD-L1 positive patients, but the applicability of this study remains unknown given all patients were enrolled were Asian. Additionally, the results of Kim *et al.*'s (52) study are intriguing given reported ORR 22%, which was notably higher than prior studies, namely the large KEYNOTE-158 study. The data was certainly suggestive of durable response in patients who responded to nivolumab. However, it is important to note no molecular profiling data was reported in patients to this study. Looking to the future, identifying biomarkers beyond microsatellite instability or PD-L1 and integrating this information into clinical trials will be essential to elucidating the role of immunotherapy in the treatment of CCA.

The application and integration of these novel therapies and targeted approach in patients with resectable disease represents an opportunity for investigation. The difficulty in obtaining adequate tissue for diagnosis and NGS preoperatively in patients with hilar or dCCA poses an obstacle for neoadjuvant trials for these two disease sites. iCCA, on the other hand, is amenable to core biopsy and the prevalence of actionable mutations makes this ripe for neoadjuvant trials to assess the feasibility and efficacy of introducing targeted therapies in the preoperative space. Adjuvant trials for CCA utilizing these novel therapies are under development as well. Additioannly, the field of transplant oncology within CCA may benefit from advances in this targeted approach to therapy, but requires further investigation.

The development of targeted therapies has generated significant optimism for expanded treatment options for patients with CCA, however, a new set of challenges accompany these novel treatments. Questions remain on the ideal use of targeted therapies—specifically whether they should be used in combination with systemic therpies, surgery, or other treatment modalities. Furthermore, issues with resistance to *IDH* and *FGFR* inhibitors in clinical trials have emerged as a real challenge, threatening the potential impact of targeted therapies. Dedicated research is needed to better understand mechanisms of resistance and to ultimately address concerns of resistance threatening the durability of these therapies. Additionally, approaching CCA from a molecular level has made it clear that not all CCAs are created equal. However, the ideal means of stratifying CCA, likely with prognostic and therapeutic implications, remains a mystery. Despite the unknowns that remain in the treatment of CCA, the clinical use of tumor biomarkers and the development of targeted therapies have provided a tangible hope of a future in which patients with CCA will have improved survival.

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Footnote

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