Review Article

Molecular pathology for cholangiocarcinoma: a review of actionable genetic targets and their relevance to adjuvant & neoadjuvant therapy, staging, follow-up, and determination of minimal residual disease

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Abstract: Cholangiocarcinoma (CCA) represents a group of epithelial cell tumors classified based on their anatomic location along the biliary tree. This rare malignancy is often diagnosed at an advanced stage and deemed unresectable. Even for those patients who are surgical candidates, recurrence rates are high and survival rates low. The mainstay of therapy for advanced CCA remains cisplatin plus gemcitabine, with a median overall survival (mOS) under 12 months, although the TOPAZ-1 trial showed a survival benefit with the addition of programmed cell death ligand 1 (PD-L1) blockade. In recent years, molecular profiling has revealed a wealth of potentially targetable genetic alterations, including fibroblast growth factor receptor (*FGFR*) fusions, isocitrate dehydrogenase 1 (*IDH1*) mutations, human epidermal growth factor receptor 2 (*HER2*) amplification and overexpression, and microsatellite instability (MSI). These discoveries have prompted numerous clinical trials employing drugs against these specific genetic changes. The foundation laid by early clinical studies and the landscape of ongoing trials are both summarized here. While the role of adjuvant therapy has yet to be defined in this disease, we emphasize the importance of employing targeted therapies in trials in the adjuvant and neoadjuvant spaces and discuss ways to overcome challenges due to low incidence of targetable mutations. Personalized medicine for this disease promises significant clinical benefit to patients, but further investigation is needed.

Keywords: Cholangiocarcinoma (CCA); biliary tract cancer (BTC); targeted therapy; molecular profiling

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Introduction

Cholangiocarcinoma (CCA) represents a group of epithelial cell tumors classified into intrahepatic CCA (iCCA) or extrahepatic based on anatomic location along the biliary tree (1). Extrahepatic CCA is further divided into perihilar (pCCA) and distal CCA (dCCA). The term biliary tract cancer (BTC) is a more inclusive term used to incorporate gallbladder cancer (GBC) into this group. These malignancies are relatively rare, and unfortunately, the majority of patients are diagnosed at later stages and with unresectable disease (2). Even in the small subset of patients who are candidates for curative-intent surgical resection, recurrence rates are high and 5-year survival rates are less than 30% (3,4). The current standard of care for patients with advanced stage CCA remains cisplatin plus gemcitabine based on the ABC-02 trial published in 2010,

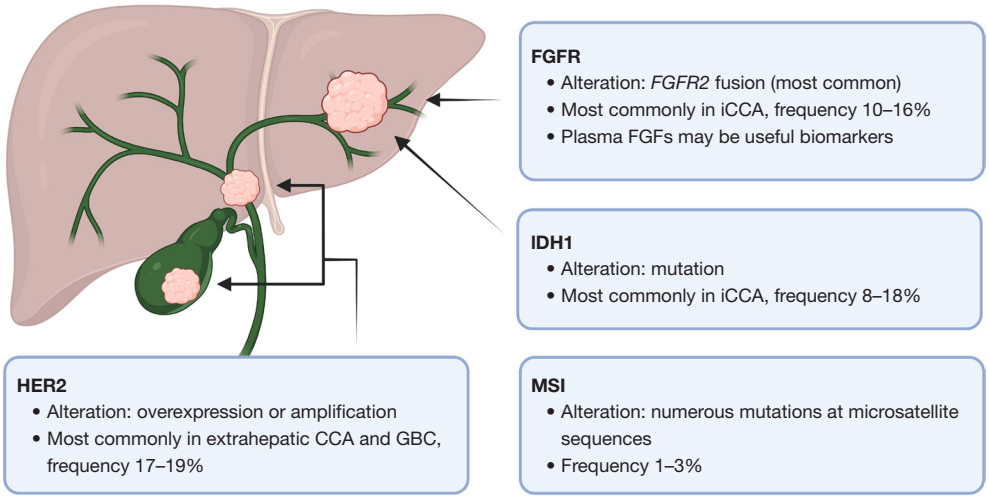

Figure 1 Four major molecular sites contain targetable genetic alterations in BTC: FGFR, IDH1, HER2, and MSI. Created with BioRender.com. FGFR, fibroblast growth factor receptor; iCCA, intrahepatic cholangiocarcinoma; FGF, fibroblast growth factor; IDH1, isocitrate dehydrogenase 1; MSI, microsatellite instability; HER2, human epidermal growth factor receptor 2; CCA, cholangiocarcinoma; GBC, gallbladder cancer; BTC, biliary tract cancer.

which improved the median overall survival (mOS) to just under 12 months compared to 8 months with gemcitabine alone (5). It is important to note, however, that although all are considered BTCs, each site of disease is unique in its presentation, surgical management, and molecular make-up. In recent years, molecular profiling of BTCs has revealed a wealth of potentially targetable genetic alterations, prompting numerous translational and clinical studies (6). The likelihood that a tumor will harbor a specific "actionable mutation" is unique to the site of disease. As demonstrated in other malignancies, personalized treatment strategies are instrumental in decreasing the burden of drug toxicity and improving patient outcomes (7,8). In this review, we illustrate the current status of targeted therapies by focusing on four examples, address their role in dictating staging and follow-up, and discuss potential applications to adjuvant and neoadjuvant therapy in order to push the field forward.

Discussion

Main actionable genetic targets

Here we present the current body of knowledge regarding the main molecular sites containing targetable genetic aberrations in BTC: fibroblast growth factor receptor (*FGFR*), isocitrate dehydrogenase 1 (*IDH1*), human epidermal growth factor receptor 2 (*HER2*), and microsatellite instability (MSI) (*Figure 1*). While this list is not comprehensive of all potential actionable mutations that have been uncovered in this malignancy, we chose these four to illustrate the overall status of this field.

FGFR

Any *FGFR* genetic aberration is most commonly found in iCCA, and *FGFR2* fusions, with over 50 different partners described, represent the vast majority (9,10). The earliest description of *FGFR* fusions in iCCA was published in 2013, based on two cases of *FGFR2-BICC1* fusions (11). The incidence of *FGFR2* fusion in iCCA ranges from 10–16%, although it has been reported up to 45% based on RNA sequencing (12,13) and as low as 5% based on the screening of patients for first-line clinical trials. Based on retrospective analysis, presence of an *FGFR* genetic aberration is associated with presentation at earlier stage, an indolent disease course, and longer overall survival (OS) (9). Thus, the presence of an *FGFR2* fusion is believed to be a favorable prognostic biomarker, and given its association with earlier stage disease, its presence is believed to be more common in patients undergoing curative-intent resection.

Several phase II studies in locally advanced or metastatic CCA patients with *FGFR* alterations have demonstrated durable objective responses to an FGFR inhibitor, with an overall response rate (ORR) ranging from 14–42% and a median progression-free survival (mPFS) of 5.6–9 months (*Table 1*) (14-21). The largest of these studies were limited

Table 1 Overview of phase II/III clinical trials targeting four major genetic alterations found in CCA

Molecular target	Trial identifier(s)	Phase	Treatment	Control arm	Genetic alteration included in selection criteria?	Number of patients eligible for treatment	Median OS, mos. (95% CI)	Median PFS, mos. (95% CI)	ORR %, DCR %	No. of patients with response
FGFR	NCT02924376 (FIGHT-202)	II	Pemigatinib (INCB054828)	None	No	146 (107 with FGFR2 rearrangement/ fusion)	21.1 (14.8–NR)^	6.9 (6.2–9.6)	35.5, 82	3 CR, 35 PR
	NCT02150967 (–)	II	Infigratinib (BGJ398)	None	Any FGFR alteration	122 (108 with FGFR2 rearrangement/ fusion)	12.2 (10.7–14.9)	7.3 (5.6–7.6)	23.1, 84.3	1 CR, 24 PR
	NCT02052778 (FOENIX-CCA2)	II	Futibatinib (TAS-120)	None	FGFR2 fusion/ rearrangement	103	20 (not reported)	8.9 (not reported)	41.7, 82.5	–, –
	NCT03230318 (FIDES-01)	II	Derazantinib (ARQ 087)	None	FGFR2 fusion	103	15.5 (11.8–21.9)	7.8 (5.5–8.2)	21.4, 74.8	22 PR
	NCT02699606* (LUC2001)	IIa	Erdafatinib (JNJ-42756493)	None	Any FGFR alteration	22	40.2 (9.9–NR)	5.6 (3.6–12.7)	40.9, 81.8	1 CR, 8 PR
	NCT03656536 (FIGHT-302)	III	Pemigatinib	Gemcitabine/ cisplatin	FGFR2 rearrangement	Study ongoing, no results have been published				
	NCT03773302 (PROOF 301)	III	Infigratinib	Gemcitabine/ cisplatin	FGFR2 fusion/ rearrangement	Study ongoing, no results have been published				
	NCT04093362 (FOENIX-CCA3)	III	Futibatinib	Gemcitabine/ cisplatin	FGFR2 rearrangement	Study ongoing, no results have been published				
IDH1	NCT02989857 (ClarIDHy)	III	Ivosidenib	Placebo	IDH1 mutation	187 (126 in treatment group)	10.3 (7.8–12.4)#	2.7 (1.6–4.2)	2.4, 53.2	3 PR
HER2	NCT02091141* (MyPathway)	IIa	Pertuzumab + trastuzumab	None	HER2 overexpression or amplification	39	10.9 (5.2–15.6)	4.0 (1.8–5.7)	23, 51	9 PR
	NCT04466891 (HERIZON-BTC-01)	II	Zanidatamab	None	HER2 amplification	Study ongoing, no results have been published				
MSI	NCT02628067* (KEYNOTE-158)	II	Pembrolizumab	None	MSI-H/dMMR	22	24.3 (6.5–NR)	4.2 (2.1–NR)	40.9, –	2 CR, 7 PR
	NCT03875235 (TOPAZ-1)	III	Durvalumab + gemcitabine/cisplatin	Gemcitabine/ cisplatin	No	685 (451 in treatment group)	12.8 (11.1–14.0)#	7.2 (6.7–7.4)	26.7, 85.3	7 CR, 84 PR

*, these trials also included patients with other malignancies; only the CCA cohorts are reported here; ^, the results reported in this row are for the subset of patients with *FGFR2* fusion; #, the results in these rows represent the treatment group. CCA, cholangiocarcinoma; OS, overall survival; CI, confidence interval; mos, months; PFS, progression-free survival; ORR, overall response rate; DCR, disease control rate; FGFR, fibroblast growth factor receptor; IDH1, isocitrate dehydrogenase 1; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MSI-H, microsatellite instability-high; dMMR, DNA mismatch repair deficiency; NR, not reached; CR, complete response; PR, partial response;

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to patients with *FGFR2* fusions or rearrangements. In the case of the FIGHT-202 trial of pemigatinib, results were stratified by patients with *FGFR2* fusions or rearrangements versus any other *FGFR* mutation. While the former group had an ORR of 35.5% with a mPFS of 6.9 months, no patients in the latter group experienced a complete or partial response and the mPFS was only 2.1 months (19). Therefore, the presence of an *FGFR2* fusion is currently considered the most "actionable" of the *FGFR* family. Food and Drug Administration (FDA) accelerated approval has since been granted for pemigatinib, infigratinib and futibatinib, pending confirmatory studies, for the treatment of patients with chemorefractory CCA that harbors an *FGFR2* fusion or rearrangement.

Post-hoc analysis of the FIGHT-202 trial revealed that patients who received second-line treatment with pemigatinib had longer mPFS and better response rates compared to patients who received second-line systemic therapy prior to trial enrollment, suggesting that earlier treatment is key for optimizing clinical efficacy (22). Three phase III trials are currently underway to compare futibatinib (FOENIX-CCA3, NCT04093362), infigratinib (PROOF 301, NCT03773302), and pemigatinib (FIGHT-302, NCT03656536) as first-line monotherapy against current standard of care gemcitabine/cisplatin. Enrollment in each of these trials has been challenging given the rarity of this disease, competing trials, and the lower observed incidence of an FGFR2 fusion compared to what was previously thought based on the phase II second-line studies.

Despite these promising results, primary and secondary resistance to FGFR inhibitors remains a concern. Resistance is thought to be influenced by co-occurring or acquired genetic alterations. For example, in the FIGHT-202 trial, a significantly shorter mPFS was seen in patients with a concomitant mutation in *TP53*, *CDKN2A/B* or *PBRM1* (23). FOENIX-CCA2 showed a similar trend with *TP53* co-mutation, although one patient did have a complete response (24). In four patients who progressed on either infigratinib or zoligratinib (debio-1347), new *FGFR2* kinase domain mutations were found. Two of these patients achieved partial response and two had stable disease after treatment with futibatinib, highlighting the ability of this irreversible FGFR inhibitor to overcome such acquired resistance (25).

Currently, the presence of an *FGFR* genetic aberration has no impact on staging or follow-up recommendations. There is, however, preliminary evidence that plasma fibroblast growth factors (FGFs) may be useful biomarkers. In the FIDES-01 study of derazantinib, levels of FGF23 were increased on cycle 2 day 1, which was consistent with FGFR inhibition as previously published in other malignancies (26,27). A similar increase was seen in FGF19 and 21. These plasma biomarkers may serve as an adjunct to assessing response in addition to the typical methods of radiography and serum tumor markers.

IDH1

Similar to *FGFR2* fusions, *IDH1* mutations (mIDH1) are more commonly present in iCCA, with a reported average frequency of 8–18% (28). There is no clear association between mIDH1 and OS or time to progression, so it is not considered a prognostic biomarker. After promising results in a phase I study, the randomized, double-blind phase III trial ClarIDHy was designed to compare ivosidenib (AG-120), a small molecule inhibitor of mDH1, against placebo in patients with advanced CCA with mIDH1 who had progressed on previous therapy (NCT02989857). There was a significant improvement in mPFS (2.7 *vs.* 1.4 months, P<0.0001) and a favorable benefit in mOS (10.3 *vs.* 7.5 months, P=0.09) with the targeted therapy (*Table 1*) (29,30). After adjusting for crossover at the time of progression, the mOS for placebo was only 5.1 months, further emphasizing the improvement in OS seen with administering ivosidenib to those patients whose tumors harbored an mIDH1. In the last year, the FDA approved ivosidenib for the treatment of chemorefractory mIDH1 CCA. Interestingly, comprehensive genomic profiling of over 3,000 advanced iCCA revealed that there are significantly fewer co-occurring targetable genetic aberrations, as well as lower TMB, MSI-high, and PD-L1 expression, in mIDH (1 or 2) than in wild-type IDH (31).

HER2

HER2 overexpression and gene amplifications are found most commonly in extrahepatic CCA and GBC, at a rate of 17–19% (32). Although case reports suggest that patients with *HER2* overexpression respond to HER2-targeting antibodies (33,34), initial phase I and II clinical trials employed broad inhibitors of EGFR and HER2 (lapatinib or afatinib) and failed to demonstrate any significant benefit (35-37). These trials were performed in an unselected group of patients, two of which later found no evidence of *HER2* overexpression in any participant. There are currently two active phase II clinical trials in *HER2*– overexpressing BTCs, both of which employ HER2– specific antibodies

(*Table 1*). Zanidatamab (ZW25) showed efficacy in a phase I trial with an ORR of 47% and is now being employed in a global phase IIb study (NCT04466891) (38). The MyPathway phase IIa basket trial employs a dual anti-HER2 regimen of pertuzumab and trastuzumab, and in the BTC cohort a 23% ORR was seen, with 9 of 39 patients achieving a partial response (NCT02091141) (39).

MSI/mismatch repair deficiency (MMR)

DNA mismatch repair deficiency (dMMR) leads to the accumulation of numerous mutations at repetitive (termed "microsatellite") sequences, leading to high levels of MSI. The reported incidence of MSI in BTC has varied widely, largely due to the diversity of methods used for detection, but the true frequency is believed to be low: 1–3% in CCA and only 1.4% in GBC (40-42). A study in liver-fluke-related CCA in endemic Thailand, however, suggested an incidence up to 27% in iCCA and an association with poor survival (43). In cancers with dMMR and MSI, there is a known upregulation of checkpoint proteins such as programmed cell death ligand 1 (PD-L1) and cytokine T-lymphocyte-associated protein 4 (CTLA-4) (44,45) and an association with favorable response to checkpoint inhibition (46,47). The KEYNOTE-158 phase II trial enrolled patients with MSI-high/dMMR cancers who failed prior therapy and treated them with anti-programmed cell death protein 1 (anti-PD-1) antibody pembrolizumab. The CCA cohort had an ORR of 40.9%, with 2 patients exhibiting a complete response and 7 with a partial response (*Table 1*) (48). In the TOPAZ-1 phase III trial, an unselected group of patients received standard of care gemcitabine/cisplatin plus the PD-L1 inhibitor durvalumab or placebo (NCT03875235). The patients receiving durvalumab had an ORR of 26.7%, with a significant improvement in mOS to 12.8 *vs.* 11.5 months with placebo (*Table 1*) (49). PD-L1 expression was associated with better PFS but not OS in patients who received checkpoint blockade. MSI was high in 1.5% of patients, but over 50% of the patients in each group had an unknown status. While no associations with survival were able to be made based on the small sample size, the authors concluded that the low incidence of MSI-high tumors makes it unlikely to be the sole driver behind significant survival benefits seen with PD-L1 blockade. Repeat studies with comprehensive MSI data are needed, but currently the addition of durvalumab to gemcitabine/cisplatin is considered a standard of care approach for patients with advanced stage disease regardless of MSI status.

Adjuvant and neoadjuvant therapy

Similar to the ongoing evolution of the standard of care regimen for patients with unresectable CCA, the role of and optimal adjuvant therapy regimen for resected disease has yet to be defined (50). There is evidence that adjuvant chemoradiation, based on a single-arm phase II study (S0809), may positively impact local control in margin-positive resections and node-positive disease (51,52). The BILCAP trial recently published the results of 447 patients who underwent curative intent resection and were randomized to six months of oral capecitabine versus observation (53). The per-protocol analysis showed a significant improvement in mOS (53 *vs.* 36 months, P=0.028) and median recurrence-free survival (26 *vs.* 17 months, P=0.0093) in the capecitabine group compared to observation, while the intent-to-treat analysis did not meet statistical significance for the primary outcome of OS. Still, extrapolating from the ABC-02 data, gemcitabine/cisplatin is often utilized in the adjuvant setting. A phase III study (ACTICCA-1) is currently underway comparing gemcitabine/cisplatin to capecitabine (NCT02170090).

No studies to date have included genetic profiling as a basis for targeted adjuvant therapy. Further large-scale randomized trials in the adjuvant space that employ this personalized approach are warranted, but there are several factors which make this undertaking a challenge. Only about 20% of patients who present with CCA are candidates for resection (54), and actionable genetic alterations (as discussed in the previous section) are even rarer. Post-operatively, not all patients are candidates for or would be able to tolerate adjuvant therapy. If enrollment is limited to one particular mutation, the feasibility of such a study is threatened. Instead, we propose an umbrella study as a possible solution, in which the control arm receives capecitabine (per the BILCAP protocol) and the treatment arms receive targeted therapy based on the presence of an actionable genetic mutation. In this scenario, there would be multiple treatment arms based on the specific mutation or alteration that is present, such as *FGFR2* fusion, *mIDH1*, *HER2* amplification, MSI-high, etc. The feasibility, safety, and efficacy of combining targeted therapy with cytotoxic chemotherapy remains unknown at this time and is under investigation in the advanced disease setting.

Given the natural history of this disease with high

recurrence rates after resection, optimizing preoperative therapy is the logical next step, much as we have done for esophageal, gastric, pancreas, and rectal cancers. Treatment in the neoadjuvant setting represents a completely underutilized space, as it harbors the potential to eradicate micrometastatic disease, downsize tumors, improve patient selection for resection, and ultimately improve survival. Furthermore, when treating in the neoadjuvant setting (as opposed to the adjuvant), there are opportunities to assess pathologic and radiologic response, as well as perform blood- and tissue-based correlative studies. The transition to the neoadjuvant setting needs to follow a stepwise approach, establishing feasibility and safety along the way, while considering the cytostatic versus cytotoxic nature of the drug. This transition can be accomplished by administering standard of care chemotherapy (i.e., gemcitabine/cisplatin) in the preoperative setting, which is being tested in EA2197 (OPT-IN) for incidentally-diagnosed GBC (NCT04559139). We can also employ a chemotherapy augmentation approach as was done in the NEO-GAP study where patients received preoperative therapy with gemcitabine/cisplatin/nab-paclitaxel prior to resection of oncologically high-risk iCCA (NCT03579771) (55). To push the field further and incorporate personalized targeted therapy in the neoadjuvant space, we have proposed an IRB -approved phase II study named OPT-IC (Optimal Preoperative Therapy for Intrahepatic Cholangiocarcinoma, NCT05514912), designed to assess the feasibility of administering neoadjuvant targeted therapy based on next-generation sequencing (NGS) performed on a preoperative core biopsy. While patients await their NGS results, both treatment arms will receive one cycle of nab-paclitaxel, gemcitabine and cisplatin. Patients bearing *FGFR2* fusions or translocations will transition to receive an oral FGFR inhibitor for two cycles while those without an *FGFR2* fusion will continue with chemotherapy for two more cycles. Patients with response, stable disease, or persistently resectable disease will then undergo surgery. We envision a myriad of further correlative studies that would be possible given collection of both pre- and post-treatment blood and tissue: from the identification of predictive factors for response, to examination of the effect that targeted therapy has at the cellular level. Future trials in the neoadjuvant space would likely incorporate an umbrella study design to include targeted therapies for other actionable mutations in multiple treatment arms as described for adjuvant therapy above. This personalized approach has the potential to improve the treatment strategy for patients with localized resectable disease.

Follow-up and minimal residual disease (MRD)

Tumor molecular profiling has yet to make an impact on patient follow-up or detection of MRD. Although not part of standard of care, one current option for assessing MRD is Guardant Reveal™, a blood test that detects circulating tumor DNA (ctDNA) based on genomic alterations and DNA methylation. Its use has demonstrated favorable sensitivity and specificity for identifying recurrence in colon and breast cancer (56,57). This method is "tumor-uninformed", in that it detects a standardized panel of known genomic alterations. An alternative is Signatera™, which detects ctDNA based on tumor whole-exome sequencing and is personalized for each patient. It has been shown to reliably predict recurrence in multiple cancer types (58-60). This "tumor-informed" approach, however, may be restricted when there is limited tissue or when tumor cellularity or DNA yield is low. In the phase II trial of infigratinib (BGJ398) in CCA, ctDNA was collected in three patients who experienced a short interval disease progression despite initial tumor regression (61). Analysis of ctDNA showed the presence of an *FGFR2* V565F gatekeeper mutation at progression in all three patients, and additional *FGFR2* kinase domain mutations in two patients. These findings represent ctDNA as a promising strategy for detecting therapeutic resistance in CCA, but its relevance to MRD remains largely unknown.

Conclusions

An increasing proportion of patients with biliary tract malignancies undergo molecular profiling at the time of diagnosis or at some point during their treatment sequence. There is an increasing body of evidence supporting that discrete genetic alterations in CCA are targetable with novel therapeutic agents and that such treatments offer significant clinical benefit for patients in the second-line setting. None of these genetic mutations yet dictate staging, follow-up or assessment of MRD and the future of their role in these settings is unclear. We stress that for patients with localized disease, focus should be turned to trials in the neoadjuvant setting, in addition to continued efforts in the first-line for unresectable disease. Ultimately, treating each patient with a personalized drug through targeted molecular therapy has the promise of improved outcomes with minimized side effects. Collaboration, at the national and international level, is crucial to success.

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Footnote

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