



Biliary tract cancer prognostic and predictive genomics

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Abstract: Biliary tract cancer (BTC) is comprised of intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (EHC) and gallbladder cancer (GBC). These tumors arise in the biliary epithelium, share histological characteristics and are associated with grim prognosis even when diagnosed at early stages. Moreover, its relatively low incidence in developed countries has precluded the development of clinical trials addressing specific differences among BTC subgroups in terms of their biology, treatment response and clinical outcomes. In this scenario, the development of effective treatment strategies for patients has been rather modest. To date, the combination of cisplatin plus gemcitabine remains as the standard first line therapy in advanced disease and after progression to this regimen there are limited treatment options. Next generation sequencing (NGS) studies have assessed the distribution of driver genes and potentially actionable genomic alterations among ICC, EHC and GBC. Here, we outline genomic differences among these subsets and describe key milestones in order to develop novel targeted drugs against BTCs. Although the early results of several studies are promising, international collaboration is critical to conduct adequately-powered trials, enrolling patients from high-incidence countries.

Keywords: Gallbladder cancer (GBC); biliary tract cancer (BTC); genomics; next generation sequencing (NGS)

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Introduction

Biliary tract cancer (BTC) is the second most common type of hepatobiliary cancer and is comprised of intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (EHC) and gallbladder cancer (GBC). EHC can be further divided into perihilar, proximal or distal cholangiocarcinoma (1). In Western countries, ICC and EHC incidence are modest, ranging from 0.35 to 2 per 100,000/year. However, there are considerable regional differences associated with risk factors such as endemic liver fluke infection (*Opisthorchis viverrini*) in Asia (2). The highest incidence rates of GBC (up to 7.5 per 100,000 for men and 23 per 100,000 for women) are found among populations living in the Andean region (Chile, Bolivia),

North-American Indians, Mexican Americans (3). As such, in Chile GBC is the second leading cause of death for women after breast cancer. Similarly, India accounts for a high burden of GBC worldwide with an age-standardized rate of 2.1 and 1.8 per 100,000 for incidence and mortality in women, respectively (4). GBC is closely associated with cholelithiasis and chronic gallbladder inflammation (cholecystitis). Other risk factors that might explain these regional differences in GBC incidence include chronic *Salmonella* or *Helicobacter bilis* infection, Native American ancestry and aflatoxin exposure (5-7).

All BTCs arise in the biliary epithelium; they are associated with poor outcomes even when diagnosed at early stage (8,9). Although BTCs share some histological characteristics and features, there are important differences

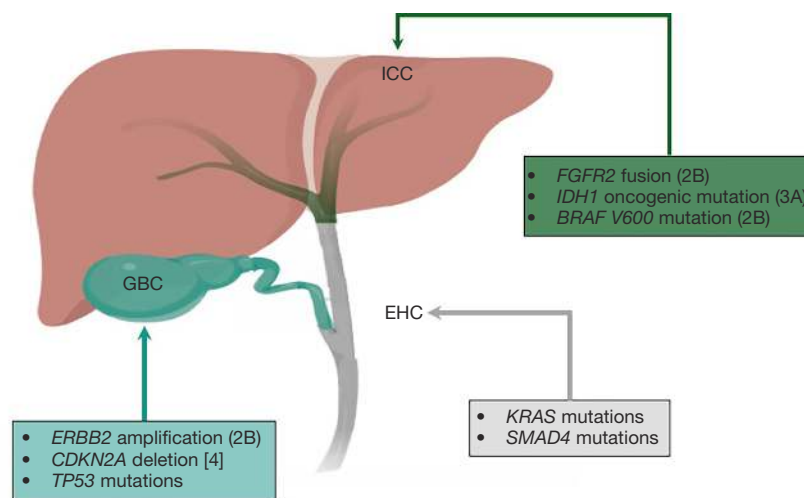


Figure 1 Frequent genomic alterations in biliary tract cancer subtypes. Actionability of alterations is described according to the OncoKB precision oncology knowledge base.

among them in terms of disease behavior, molecular profiles, and sensitivity to therapy (10). For example, GBC has been associated with worse prognosis compared to cholangiocarcinoma, however it has a greater response rate to chemotherapy (11). Historically, BTCs have been grouped together for the purpose of clinical trial accrual, however, genetic profiling has opened the opportunity to view them as molecularly distinct entities and enabled the targeting of specific genomic alterations. Herein we describe the genomic landscape of BTCs with a special comparative focus on relevant differences across BTC subgroups and biomarkers with potential clinical implications. *Figure 1* summarizes the most prevalent genomic alterations observed across different BTC subtypes and their current actionability according to the OncoKB precision oncology database (12).

Molecular landscape of BTC

Prevalent genomic alterations in BTC

Early BTC studies defined relevant oncogenes and tumor suppressor genes using polymerase chain reaction, Sanger sequencing, single-strand confirmation polymorphism and fluorescent *in situ* hybridization among other techniques. However, the actual frequency of these events and their relative distribution in different subgroups was difficult to assess. This can be explained by several reasons including sample heterogeneity, the application of a

variety of different methodologies and a high stromal cell content in samples that may dilute out cancer-cell specific nucleic acids (10). Following the introduction of next generation sequencing (NGS), most of these issues have been surpassed (*Table 1*) (13-20). Indeed, NGS assays are highly sensitive, able to analyze a large panel of genes, and detect novel mutations, small insertions or deletions (indels), copy number alterations, and select gene fusions and rearrangements from small amounts of sample (21). A pioneer study used NGS to characterize 54 ICC *Opisthorchis viverrini*-related cholangiocarcinoma cases and found mutations in *TP53* (44.4%), *KRAS* (16.7%), and *SMAD4* (16.7%) (22). Subsequent studies have applied a variety of NGS platforms allowing a thorough comparison among BTC subgroups. A larger study analyzed 412 ICC, 57 EHC and 85 GBC cases that underwent genomic sequencing with the hybrid capture-based NGS platform FoundationOne (14). Interestingly, this study demonstrated significant differences among these 3 subtypes. Potentially actionable mutations were found in *FGFR2* and *IDH1/2*, however, these were mostly limited to ICC with a prevalence of 11% and 20%, respectively. Also, *ERBB2* amplification was more frequently observed in GBC (16%) and EHC (11%) versus ICC (3%). Similarly, a prospective study analyzed 195 cholangiocarcinomas using the targeted MSK-IMPACT platform (15) and found that *FGFR2* fusions and *IDH1*, *BAP1* and *TP53* mutations occurred with greater frequency among ICCs, whereas *KRAS*, *SMAD4*,

Table 1 Main studies reporting molecular alterations associated with biliary tract cancers

First author	Platform/genes analyzed	N of patients	Genomic alterations (%)
Nakamura <i>et al.</i> (13)	WES	260	ICC: <i>KRAS</i> 25%, <i>FGFR2</i> 8%, <i>IDH1</i> 6%; EHC: <i>TP53</i> 26%, <i>KRAS</i> 12%, <i>SMAD4</i> 10%; GBC: <i>TP53</i> 43%, <i>ARID2</i> 18%, <i>EGFR</i> 18%
Javle <i>et al.</i> (14)	HC NGS 182	ICC 412; EHC 57; GBC 85	ICC: <i>TP53</i> 27%, <i>KRAS</i> 22%, <i>IDH1</i> 16%; EHC: <i>KRAS</i> 42%, <i>TP53</i> 40%, <i>SMAD4</i> 21%; GBC: <i>TP53</i> 59%, <i>CDKN2A/B</i> 19%, <i>ERBB2</i> 16%
Lowery <i>et al.</i> (15)	HC NGS 341/410	ICC 158; EHC 37	ICC: <i>IDH1</i> 30%, <i>BAP1</i> 20%, <i>FGFR2</i> 14%; EHC: <i>KRAS</i> 38%, <i>TP53</i> 49%, <i>SMAD4</i> 30%
Narayan <i>et al.</i> (16)	HC NGS 341/410	GBC 81	<i>TP53</i> 58%, <i>SMAD4</i> 31%, <i>ARID1A</i> 25%
Churi <i>et al.</i> (17)	AB NGS 46	ICC 55; EHC 20	ICC: <i>TP53</i> 29%, <i>KRAS</i> 24%, <i>IDH</i> 24%; EHC: <i>KRAS</i> 40%, <i>TP53</i> 45%, <i>SMAD4</i> 25%
Li <i>et al.</i> (18)	WES	GBC 57	<i>TP53</i> 47%, <i>ERBB3</i> 12%, <i>KRAS</i> 8%
Ross <i>et al.</i> (19)	HC NGS 182	ICC 28	<i>ARID1A</i> 36%, <i>IDH1/2</i> 36%, <i>TP53</i> 36%
Zou <i>et al.</i> (20)	WES	ICC 102	<i>TP53</i> 38%, <i>KRAS</i> 17%, <i>ARID1A</i> 7%

WES, whole-exome sequencing; HC NGS, hybrid capture next generation sequencing; AB NGS, amplicon-based next generation sequencing; ICC, intrahepatic cholangiocarcinoma; EHC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer.

and *STK11* alterations were more commonly seen in EHCs.

Molecular subtypes BTC

Additionally, NGS studies have contributed to refine etiologically distinct BTC subtypes. A study by the International Cancer Genome Consortium included a group of 489 ICC and EHC patients who underwent genomic, epigenomic, and transcriptomic analyses. Integrative clustering was primarily based on 71 patients who were assessed by whole-genome sequencing. This analysis found 4 clusters characterized by different clinical features including proportion of ICC and EHC and genomic alterations (23): Cluster 1 comprised mostly fluke-associated tumors that also displayed hypermethylation of CpG island-promoters, and enrichment of *ARID1A* and *BRCA1/2* mutations. Cluster 2 was characterized by a mix of fluke-positive and fluke-negative tumors, and upregulated *CTNNB1* and *AKT1* expression. Clusters 1 and 2 were significantly enriched in *TP53* mutations and *ERBB2* amplifications. Clusters 3 and 4 comprised almost exclusively fluke-negative tumors. Cluster 3 displayed the highest level of copy number alterations, including enrichment of amplifications at 2p and 2q; these tumors also exhibited upregulation of immune checkpoint genes such as PD-1 and PD-L2. Cluster 4 was characterized by

upregulation of *FGFR* family and PI3K pathways signatures along with frequent *BAP1* and *IDH1/2* mutations. Clusters 1 and 2 were enriched in EHC, whereas Clusters 3 and 4 were mostly ICC cases. Similarly, a Chinese study used a whole-exome sequencing approach and included 102 ICC patients. This study found that *TP53*-defective patients were more likely to be HBsAg-seropositive (P=0.021), also *KRAS* mutations were very infrequent in this group (20).

Germline alterations in BTC

Studies have determined that 10–20% of BTC patients display germline mutations on cancer-prone genes. Indeed, a recent study analyzed germline variants of selected genes in a cohort of 146 Japanese BTC patients. Germline variants were confirmed by the Sanger method and found a 11% of deleterious mutations in several genes including *BRCA1*, *BRCA2*, *RAD51D*, *MLH1*, or *MSH2* (24). A similar study found a higher prevalence (20%) of germline pathogenic or likely pathogenic genomic alterations (25). In this study, a total of 78 patients (ICC =52, EHC =13 and GBC =13) were analyzed using the NGS MSK-IMPACT platform. This platform includes 76 genes associated with hereditary cancer predisposition. The most frequent germline mutations were found in *BRCA2* (5.1%), *APC* (3.8%) and *MUTYH* (3.8%) genes. These findings support

germline analysis in BTC patients in view of screening and therapeutic implications. Series of BTC patients with germline mutations in mismatch repair (MMR) genes such as *MLH1* or *MSH2* suggest that this phenomenon is infrequent and represent a small minority of patients with MMR-deficient tumors (26).

Potential prognostic and predictive genomic markers in BTC

FGFR

The FGFR family comprises four transmembrane tyrosine kinase receptors (namely FGFR1, -2, -3, and -4). Downstream signaling of activated FGFRs involves activation of the mitogen-activated protein kinase (MAPK) and/or phosphoinositide-3 kinase—Akt pathways. FGFR gene-fusions (particularly *FGFR2*) are considered a promising target for therapeutic inhibition. In contrast, the predictive role of *FGFR1/2/3* mutations is more uncertain. As mentioned, *FGFR2* fusions that involve its kinase domain are commonly seen in ICCs ranging from 8% to 14%. The most frequent fusion partners for *FGFR2* is *BICC1*. However, a number of partners have been described in the literature, including *KIAA1217*, *AHCYL1*, *TACC1*, *MGEA5*, and *PPHLN* (15,27).

In a recent phase II study, infogatinib, a selective FGFR1-3 kinase inhibitor, was studied in cholangiocarcinoma patients who harbored *FGFR2* fusions or other *FGFR* alterations (28). The response rate among patients with *FGFR2* fusions was 19%, however no responses were seen in patients with other genetic *FGFR* alterations. *FGFR* plays an important role in phosphate homeostasis. Consequently, hyperphosphatemia has been a relevant on target toxicity of this class of drugs. Indeed, this trial found a 16% of patients were affected by grade 3–4 hyperphosphatemia. A phase III trial evaluating first-line infogatinib versus chemotherapy in ICC patients with *FGFR2* fusions has started accrual (NCT03773302). Erdafitinib is another oral pan-FGFR inhibitor that has also been evaluated in ICCs. The LUC2001 phase II trial (NCT02699606) evaluated 11 ICC patients with different genetic alterations in *FGFR2* or *FGFR3* (29). Given the abovementioned risk of hyperphosphatemia, investigators applied a dosing schedule based on serum phosphate levels. Within this group, 5 out of 11 patients showed partial responses. Interestingly, all responders had *FGFR2* alterations. Even though investigators used an up-titration protocol, hyperphosphatemia was frequent (10/11 patients). Despite

this, all patients continued their treatments. Interestingly, this drug has been recently approved by American Food and Drug Administration (FDA) for metastatic urothelial carcinoma, with susceptible *FGFR3* or *FGFR2* genetic alterations based on the results of the BLC2001 trial (30). A recent phase I trial evaluated the FGFR inhibitor DEBIO 1347 in biliary tract malignancies (31). Eight BTC patients were treated in the dose escalation cohort of this study and they reported a 12.5% response rate. Grade 3–4 hyperphosphatemia was seen in 21% of patients. The maximum tolerated dose was not reached; however, dermatologic toxicity became sometimes dose-limiting beyond the dose-limiting toxicity period. TAS-120 is highly selective, irreversible FGFR1–4 inhibitor that was also studied in a phase I trial including BTC patients harboring *FGFR* alterations. The outcomes of the subgroup of patients with *FGFR2* gene fusions (n=28) were reported showing an objective response rate of 25% (32). This drug has also demonstrated preclinical activity against multiple *FGFR2* mutations shown to confer resistance to other FGFR inhibitors such as *FGFR2* V565F gatekeeper mutation. A recent study showed that TAS-120 was active in four patients with *FGFR2*-fusion-positive ICC who developed resistance to infogatinib or Debio 1347; two of these patients achieved a partial response and two achieved stable disease by RECIST v1.1 criteria (33). In 3 of these patients *FGFR2* mutations associated with resistance to FGFR inhibitors were detected in tissue or circulating tumor DNA before starting TAS-120. Currently, the FOENIX-101 phase II trial is evaluating the efficacy of TAS-120 in patients with ICC harboring *FGFR2* gene rearrangements (NCT02052778). The FGFR inhibitor pemigatinib has also shown promising results in a phase II trial that included a cohort with tumors with *FGFR2* translocations (mostly ICC) (34). These results have provided foundation for a phase III trial assessing the efficacy of this drug versus gemcitabine plus cisplatin chemotherapy in first-line treatment of patients with cholangiocarcinoma with *FGFR2* rearrangement (NCT03656536). Likewise, the FGFR inhibitor derazantinib had an encouraging 21% response rate in patients with advanced ICC with *FGFR2* fusion in a phase I/II trial (35). A phase II trial assessing the efficacy of this drug in this population is ongoing (NCT03230318).

IDH1/2

Somatic mutations in IDH enzymes are observed in 5–36% of ICCs (27). These enzymes are part of the tricarboxylic

acid cycle and catalyze the interconversion of isocitrate to alpha-ketoglutarate (36). Cancer-associated mutations in *IDH1/2* result in high levels of (R)-2-hydroxyglutarate, an onco-metabolite that seems to be associated with alterations in several cell processes including redox state and DNA repair mechanisms, also modulating the activity of epigenetic tumor suppressor enzymes (37-39). In addition to its functional impact on cancer progression *IDH1/2* mutations have been implicated in ICC prognosis. Some studies have found an association between *IDH1/2* mutations and improved clinical outcomes (40,41). However, this is a controversial issue since other studies have not found significant differences (42). Both *IDH1* and *IDH2* inhibitors have shown robust activity in preclinical models for solid tumors and leukemia (43,44). Ivosidenib is a first-in-class mutant *IDH1* inhibitor that has been evaluated in a phase I study in *IDH1*-mutant advanced cholangiocarcinoma (45). This trial included 73 BTC patients (dose escalation n=24 and expansion n=49) and reported an objective response rate of 5% and a 12-month progression-free survival of 22%. Also, 3% of patients displayed grade 3-4 toxicity. A randomized phase III trial (ClarIDHy) of ivosidenib in this population is currently ongoing (NCT02989857) and recently it has been reported in a press release that it has achieved its primary endpoint (46). ClarIDHy did not include GBC patients since *IDH-1* mutations are very infrequent in this group. BAY 1436032 is another oral inhibitor against mutant *IDH1*, whose safety, tolerability and preliminary anti-tumor activity are under evaluation in a phase I basket trial (NCT02746081) including mutant *IDH1* BTC patients (47). *IDH2* mutations are found less frequently in ICC (3% to 5%) and they also have been described as an acquired resistance mutation to *IDH1* inhibition in this disease (48). Enasidenib is a mutant *IDH2* inhibitor that has been approved for *IDH2*-mutant acute myeloid leukaemia, however, there is not data supporting activity of this drug in *IDH2*-mutant BTC.

HER2

Amplification of *ERBB2* (also known as *HER2*) occurs in 3% to 19% of BTCs and is more frequent in GBC and EHC versus ICC (14,49). The study of *HER2* as an actionable target in BTC has been challenging. A phase II trial led by the American National Cancer Institute (NCT00478140) tried to assess the efficacy of trastuzumab in *HER2*-overexpressed BTC, however, this study was closed due to slow accrual. Subsequently, two phase II studies evaluating

the activity lapatinib in BTC did not show any responses, however these studies included an unselected population (50,51). Currently, a multi-cohort phase II trial in China is set to evaluate the combination of gemcitabine and oxaliplatin with trastuzumab in *ERBB2*-amplified BTC (NCT02836847). A limited number of BTC patients with *ERBB2* amplification have been recruited in basket trials demonstrating the efficacy of anti-*HER2* therapies. For example, the MyPathway trial included seven *HER2* overexpressed/amplified BTC patients treated with the combination of the anti-*HER2* antibodies trastuzumab and pertuzumab. In these patients the objective response was 29% (52). A similar response rate (17%) was recently shown for trastuzumab emtansine in this population (1 in 6 patients) (53). The antibody drug conjugate trastuzumab deruxtecan and the bispecific *HER2*-targeted antibody ZW25 have shown interesting activity in non-breast non-gastric *HER2*-amplified tumors in phase I trials (54,55). *HER2*-amplified BTCs have not been adequately represented in these studies, however, it is an interesting population to be included in future trials.

HER2 activating mutations have been described in between 1-8% of BTC and the potential therapeutic significance of these mutations is controversial. Interestingly, in a recent basket trial evaluating the efficacy of the pan-*HER* tyrosine kinase inhibitor neratinib in *HER2*-mutant tumors, nine BTC patients were enrolled. The reported response rate in this subgroup was 22% providing preliminary evidence of actionability (56).

BRAF

BRAF encodes a serine/threonine protein directly downstream from *RAS* in the *MAPK* cascade. The most frequent *BRAF* mutation is located in the V600 codon. In BTC patients *BRAF* mutations occur in 1% to 4% of ICC patients, while they are extremely infrequent in EHC or GBC (13,57). The phase II VE-BASKET trial included 8 *BRAF*-mutant BTC patients and reported 1 partial response (12%) (58). Subsequently, results of the ROAR Basket Trial demonstrated a 42% of response rate to the *RAF* inhibitor dabrafenib and *MEK* inhibitor trametinib in V600E *BRAF*-mutant BTC (59).

DDR

Alterations in DNA repair genes are relatively frequent in BTC including *MSH6*, *BAP1*, *ATM*, *MLH1*, *MSH2*, *BRCA1*

Table 2 Clinical trial efficacy data for targetable genomic alterations in biliary tract cancers

Alteration	Therapy	N, patients	ORR (%)	Median PFS (months)	Reference
<i>FGFR2</i>	Infigratinib	61	15	5.8	(28)
	Erdafitinib	11	46	5.6	(29)
	Debio 1347	8	12.5	NR	(31)
	TAS-120	28	25	NR	(32)
	Derazantinib	29	21	5.7	(35)
<i>IDH1/2</i>	Ivosidenib	73	5	3.8	(45)
<i>HER2</i>	Trast + P	7 (amp)	29	NR	(52)
	T-DM1	6 (amp)	17	NR	(53)
	Neratinib	9 (mut)	22	NR	(56)
<i>BRAF</i>	Vemurafenib	8	12	NR	(58)
	D + T	33	42	7.2	(59)
<i>EGFR</i>	Erl + Bev	49	12	4.4	(71)
	GEMOX + Erl	135 vs. 133	30 vs. 16 [‡]	5.8 vs. 4.2 [†]	(72)
	Gem + C	44	20	5.8	(73)
	GEMOX + C	76 vs. 74	24 vs. 23 [†]	6.1 vs. 5.5 [†]	(74)
	GEMOX + P	46	33	8.3	(75)

[†], not significant; [‡], statistically significant difference. Trast, trastuzumab; P, pertuzumab; T-DM1, trastuzumab emtansine; D, dabrafenib; T, trametinib; Erl, erlotinib; GEMOX, gemcitabine and oxaliplatin; C, cetuximab; P, panitumumab; NR, not reported; amp, amplified; mut, mutant.

and *BRCA2*. Indeed, studies demonstrate that up to 16% of ICC and 45% of EHC patients display mutations in these genes (17). DNA-repair deficient cells (particularly *BRCA1* or 2 mutants) can accumulate double-strand DNA breaks that result in genomic instability, leading to an increased sensitivity to DNA damaging therapies such as platinum compounds or poly ADP-ribose polymerase (PARP) inhibitors (60,61). Several studies have demonstrated the efficacy of PARP inhibitors in *BRCA1/2* mutant breast and ovarian cancer (62-66). A recent series showed that 4 *BRCA1/2*-mutant cholangiocarcinoma patients who received PARP inhibitors had a favorable response. In fact, one of these patients had a progression free survival of 42 months (67). This specific patient had a known pathogenic germline *BRCA2* mutation, however, the predictive features of germline versus somatic mutations remains to be elucidated. Anecdotal responses to PARP inhibitors have also been reported in metastatic *BRCA1* mutant GBC (68). Interestingly, a recent study demonstrated that the accumulation of 2-Hydroxyglutarate associated with IDH mutations can suppress homologous recombination and

thereby induces sensitivity to PARP inhibitors (69). These findings provide the foundations for a trial exploring the anti-tumoral activity of olaparib in solid tumors that harbor *IDH1/2* mutations (NCT03212274).

EGFR

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor that plays a key role in the cancer cell proliferation and is overexpressed in several solid tumors. Receptor-specific EGF ligands can bind onto EGFR (Erb1), Erb3, or Erb4 (70). Preclinical studies support the relevance of EGFR in the carcinogenesis of BTC, however results from early trials using anti-EGFR therapies have been disappointing (Table 2) (71-75), and to date no benefit has been demonstrated even in trials that have excluded *RAS*-mutant tumors.

Microsatellite instability (MSI)

MSI results from defects in the MMR system including

MLH1, *PMS2*, *MSH2*, and *MSH6* genes. The dysfunction of this repair system affects DNA proofreading after replication, leading to variable lengths of short tandem repeats, called microsatellites, within noncoding and coding regions (76). This phenomenon is known as MSI and can be attributed to germline mutations in one or several MMR genes that render a nonfunctional protein or to somatic hypermethylation of the *MLH1* promoter that leads to epigenetic silencing. Studies show that 1–10% of BTCs can be categorized as MSI-high. These are more frequent in ICC compared to EHC or GBC (77,78). In localized disease, MSI-H GBC might be associated with better outcomes as it has been demonstrated in localized colorectal and gastric cancer, however, this needs further confirmation (79–81). MMR-deficiency has been shown to predict response of solid tumors to immunotherapy with PD-1 checkpoint inhibitors (82). Consequently, the FDA has provided a tissue/site-agnostic approval for pembrolizumab for this indication. Interestingly, in the pivotal KEYNOTE16 phase II study, a total of 4 MSI-high cholangiocarcinoma patients received pembrolizumab: 1 had a partial response and 3 had stable disease (83).

CDKN2A

Cyclin-dependent kinase inhibitor 2A (*CDKN2A*, also known as P16INK4A) is a tumor suppressor gene that encodes two proteins via an alternative splicing: p16INK4A and p14ARF. These proteins can act as inhibitors of cyclin-dependent kinase 4 (CDK4) and/or 6 (CDK6) thereby regulating the cell cycle. Altered *CDKN2A* is observed in many malignancies, however, its prognostic relevance varies by tumor type (84). In BTCs, *CDKN2A* is frequently silenced by hypermethylation, by homozygous deletion, or by inactivating mutations (10). Studies demonstrate that *CDKN2A* alterations are more frequently seen in GBC versus ICC or EHC (14) and have been associated with decreased survival (85). Although *CDKN2A* alterations are not considered actionable *per se*, homozygous *CDKN2A* loss is usually concomitant with methylthioadenosine phosphorylase (*MTAP*) gene deletions, which might be targetable. *MTAP* encodes a key enzyme in the methionine salvage pathway (86) and *MTAP*-deficient cells rely on protein arginine methyl-transferase 5 (PRMT5) for purine biosynthesis. Preclinical studies demonstrate that *MTAP*-deficient cancers depend on methionine adenosyltransferase II alpha (*MAT2A*) to produce S-adenosylmethionine, an essential PRMT5-substrate for survival of these cells (87).

Currently, a phase I trial is set to evaluate the maximum tolerated dose, safety and efficacy of AG-270, a *MAT2A* inhibitor in solid tumors or lymphoma with homozygous loss of *CDKN2A* or *MTAP* (NCT03435250).

NTRK

The NTRK family includes three genes, *NTRK1*, *NTRK2*, and *NTRK3*, which encode three tropomyosin receptor kinase (TRK) proteins—TRKA, TRKB, and TRKC, respectively. Studies have identified *NTRK* fusions at low frequencies across various tumor types, including BTCs (88). Larotrectinib is now a FDA-approved selective small-molecule inhibitor of all three TRK proteins. Its use is approved for adult and pediatric patients with solid tumors that harbor *NTRK* gene fusions. Approval was based on the results of three studies: a phase I study in adults, a phase I/II study in children, and a phase II basket study in adolescents and adults. Altogether these studies included 55 *NTRK* fusion cases and reported a response rate of 80% (89). Two cholangiocarcinoma patients were included in these studies; one had a partial response and the other had progression of disease.

Conclusions

A deeper understanding of the molecular landscape and activated pathways involved in the carcinogenesis of BTCs has enabled to recognize BTCs subtypes as different entities. Furthermore, it has also guided the development of several novel drugs, which has derived into multiple ongoing phase II and III trials. Some of these trials have already shown preliminary efficacy on clinical outcomes and represent one step further towards becoming a standard treatment. Interestingly, some of the abovementioned targetable genomic alterations such as *FGFR2* fusions, *IDH1* and *BRAF* mutations, and *ERBB2* amplifications have already been identified in circulating-tumor DNA (ctDNA) from ~20% of BTC patients (90). As has been discussed, BTCs are more prevalent in developing countries. One significant barrier to conduct adequately-powered trials to demonstrate definitive efficacy of these targeted therapies in BTC, is that the access to tissue and ctDNA NGS is limited in these countries. Consequently, international collaboration in coming years will play a key role in order to provide the expertise and resources to develop such studies. Hopefully, these initiatives, along with lowering costs of sequencing technologies, will increase the enrollment of BTC cases

into molecularly-designed trials eventually leading to more treatment options for these patients in need.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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