Molecular profiling of biliary cancers reveals distinct molecular alterations and potential therapeutic targets

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Background: Biliary tract cancers (BTCs) are a heterogeneous group of aggressive, rare malignancies with limited standard chemotherapeutic options for advanced disease. Recent studies have demonstrated potential novel biliary cancer targets and a possible role for immunotherapy in the treatment of patients with this disease. Intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC), and gallbladder carcinoma (GBC) are frequently grouped together in clinical trials despite differences in tumor biology.

Methods: To further investigate tumor biology differences, we profiled 1,502 BTCs using next-generation sequencing (NGS), immunohistochemistry, *in situ* hybridization, and RNA sequencing.

Results: IHCCs had higher rates of *IDH1*, *BAP1*, and *PBRM1* mutations and *FGFR2* fusions; *EHCCs* had higher rates of *KRAS*, *CDKN2A*, and *BRCA1* mutations; and GBCs had higher rates of homologous recombination repair deficiency and Her2/neu overexpression and amplification. IHCCs and GBCs had higher rates of potential positive predictive biomarkers for immune checkpoint inhibition (PD-L1 expression, high microsatellite instability, and high tumor mutational burden) than EHCCs.

Conclusions: These findings support clinical molecular profiling of BTCs to inform potential therapeutic selection and clinical trial design based on the primary tumor's site of origin within the biliary tree.

Keywords: Biliary cancer; intrahepatic cholangiocarcinoma (IHCC); extrahepatic cholangiocarcinoma (EHCC); gallbladder carcinoma (GBC)

Introduction

Biliary tract cancer (BTC) is an uncommon cancer type with an overall poor prognosis and limited therapeutic options (1). BTCs arise from epithelial cells from one of three distinct locations, producing a total of three BTC subtypes: intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC), and gallbladder carcinoma (GBC).

While BTC subtypes have varying clinical presentations, they are often treated similarly (2,3). Treatment relies on surgery for resectable disease; however, BTCs are

frequently unresectable or recur following resection (4). For resectable BTCs, fluoropyrimidine- or gemcitabine-based chemotherapy remains the primary therapeutic option in the adjuvant setting, and there is a role for adjuvant chemoradiation with capecitabine in EHCC and GBC treatment (5-8).

For unresectable or metastatic BTC, the ABC-02 trial demonstrated that the combination of cisplatin and gemcitabine as first-line treatment prolonged overall survival over gemcitabine alone, and there were no significant differences in survival between IHCC, EHCC, and GBC subgroups (9). Despite being responsive to multiple chemotherapy agents, there is no established second-line treatment regimen for advanced BTC, and patients are strongly encouraged to enroll in clinical trials. This patient population is in dire need of novel, effective treatment options.

IHCCs, EHCCs, and GBCs have different molecular profiles (10), reflecting differences in underlying tumor etiology (11). In particular, Jusakul and colleagues carried out an integrated genomic, epigenomic, and transcriptomic analysis of BTCs, during which they identified 4 clusters, each with individual genetic, epigenetic, and clinical features (12). Cluster 1 is associated with increased Her2 amplification, *Her2* gene expression, and CpG island hypermethylation; cluster 2 is associated with *TP53* mutations and increased Her2 amplification and gene expression; cluster 3 is associated upregulated immune-related pathways (e.g., programmed cell death protein 1, PD-1); and cluster 4 is mostly IHCCs and is associated with *BAP1* and *IDH1/2* mutations, *FGFR* alterations, CpG shore methylation, and the best prognosis of the 4 clusters. These clusters are driven by different etiologies; for example, clusters 1 and 2 are frequently associated with liver fluke infections, whereas clusters 3 and 4 are not. A greater understanding of the genomic landscape of BTC has resulted in the identification of promising therapeutic targets including *FGFR2* fusions (10), Her2/neu (13), and IDH1 (14), Many of these targets are being actively investigated in both basket and BTC-specific clinical trials.

Molecular profiling may also predict response to immunotherapy. The efficacy of immune checkpoint blockade across tumor types led to the first site-agnostic FDA approval of the anti-PD-1 antibody pembrolizumab for microsatellite instability high (MSI-H) and mismatch repair deficient (MMRd) cancers (15). In addition, high tumor mutational burden (TMB-H) and programmed death-ligand 1 (PD-L1) expression are potential positive predictive biomarkers for immune checkpoint blockade with anti-PD-1 and anti-PD-L1 antibodies (16-21).

We report the molecular characterization of a large cohort of BTCs, comparing IHCCs, EHCCs, and GBCs in order to explore potential therapeutic opportunities.

Methods

Biliary tract tumors profiled by Caris Life Sciences between 2009 and July 2017 were de-identified and retrospectively analyzed for molecular alterations. Tumor histology and diagnoses were taken from submitted pathology reports and confirmed by board certified pathologists.

Next-generation sequencing (NGS) using Miseq or NextSeq platforms (Illumina, Inc., San Diego, CA, USA) was performed on genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples, and no matched normal tissue was sequenced. A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA, USA). All variants were detected with >99% confidence based on allele frequency and amplicon coverage with an average sequencing depth of coverage of >500 times and an analytical sensitivity of 5%. Tumor enrichment was achieved by manual microdissection of harvested target tissue prior to molecular testing in all cases.

TMB was measured in each BTC by counting the number of non-synonymous, somatic mutations found per megabase (MB). The 592 genes sequenced comprised 1.4 MB of total genomic space. Tumors were considered to be TMB-H if they had greater than or equal to 17 mutations per megabase. This threshold had been previously established in colorectal cancer (CRC) studies: TMB was compared with MSI by fragment analysis, based on reports of TMB having concordance with MSI in CRC (22). MSI was examined at over 7,000 target microsatellite loci and compared to the reference genome hg19 from the UCSC Genome Browser database (23). Copy number variation (CNV) was tested by NGS and was determined by comparing the depth of sequencing of genomic loci to a diploid control as well as the known performance of these genomic loci. Calculated gains of 6 copies or greater were considered amplified.

For gene fusion detection, anchored multiplex polymerase chain reaction (PCR) was performed for targeted RNA sequencing using the ArcherDx fusion assay (Archer FusionPlex Solid Tumor panel). Unidirectional gene-specific primers were used to enrich

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Table 1 Patient and tumor characteristics of age and gender by tumor location

Characteristic	IHCC (n=825)	EHCC (n=249)	GBC (n=428)	Total (n=1,502)
Tumors analyzed by NGS, n (%)	200 (24.2)	51 (20.5)	105 (24.5)	356 (23.7)
Age (years), mean [range]	59.8 [20–90]	63.0 [27–88]	63.6 [32–90]	61.4 [20–90]
Gender (female), %	52.1	39.8	62.1	52.9
Primary/local, n (%)	387 (46.9)	82 (32.9)	124 (29.0)	593 (39.5)
Metastatic, n (%)	137 (16.6)	61 (24.5)	150 (35.0)	348 (23.2)
Unknown (primary/local or metastatic), n (%)	301 (36.5)	106 (42.6)	154 (36.0)	561 (37.4)

IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma; NGS, next-generation sequencing.

for target regions, followed by NGS (Illumina MiSeq platform). Targets included 593 genes selected for known associations with various carcinomas (the complete panel of tested gene fusions is available at: https://www.carismolecularintelligence.com/tumor-profiling-menu/mi-profile-usa-excluding-new-york/). Fusions among the >11,000 fusions known to be found in normal tissues were excluded (24). The detection sensitivity of the assay allows for detection of a fusion that is present in at least 10% of the cells in the samples tested.

Immunohistochemistry (IHC) was performed on full FFPE sections of glass slides. Slides were stained using automated staining techniques per the manufacturer's instructions and were optimized and validated per CLIA/CAP and ISO requirements. Staining was scored for intensity (0: no staining; 1+: weak staining; 2+: moderate staining; 3+: strong staining) and staining percentage (0–100%). Results were categorized as positive or negative by defined thresholds specific to each marker based on published clinical literature that associates biomarker status with patient response to therapeutic agents. A board-certified pathologist evaluated all IHC results independently. The primary antibodies used were PD-L1 (SP142), ERCC1 (8F1), RRM1 (polyclonal), TS (TS106), TOPO2A (3F6), Her2 (4B5), and cMET (SP44).

Fluorescence in situ hybridization (FISH) was performed to detect TOP2A (TOP2/CEP17 probe) gene amplification (Abbott Molecular/Vysis). Chromogenic in situ hybridization (CISH) was also used for Her2/neu (INFORM HER-2 Dual ISH DNA Probe Cocktail) and cMET (Ventana). HER2 amplification was defined as Her2/chr17 ratio ≥2.0; TOP2A amplification as TOP2A/CEP17 ratio ≥2.0, and cMET as >5 copies per tumor cells.

Human subjects had been de-identified prior to analysis.

The study was deemed exempt by the Western Institutional Review Board (IRB registration number IRB00000533, WIRB work order #1-895778-1), and no informed consent was obtained for this retrospective analysis.

Results

Tumor characteristics

A total of 1,502 BTCs, including 825 IHCCs, 249 EHCCs, and 428 GBCs that underwent Caris molecular profiling were included in the analysis (*Table 1*). Three hundred and fifty-six BTCs were tested with NGS using the MiSeq platform, and 149 of these were also tested with RNA sequencing for genetic fusions. Of the 1,502 BTCs analyzed, 39.5% were from primary/localized tumors, 23.2% were from metastatic tumors, and 37.4% were from unknown sites (primary/localized or metastatic, *Table 1*).

Patient characteristics

The median age was similar across tumor subtypes (59.8 years in IHCC; 63.0 years in EHCC; and 63.6 years in GBC). GBC (62.1%) was significantly more prevalent in females compared to IHCC (52.1%, P=0.0008) or EHCC (39.8%, P<0.0001).

Molecular alterations

Among the 592 genes sequenced, 356 genes were mutated in at least one tumor (*Figure 1*). The most prevalent mutations were in *TP53* (42.7%), *ARID1A* (21.7%), *KRAS* (15.7%), *IDH1* (8.7%), *CDKN2A* (7.8%), *BAP1* (6.7%), *SMAD4* (6.5%), and *PIK3CA* (6.0%). Mutation rates in all other genes were seen in fewer than 5% of tumors. Of the
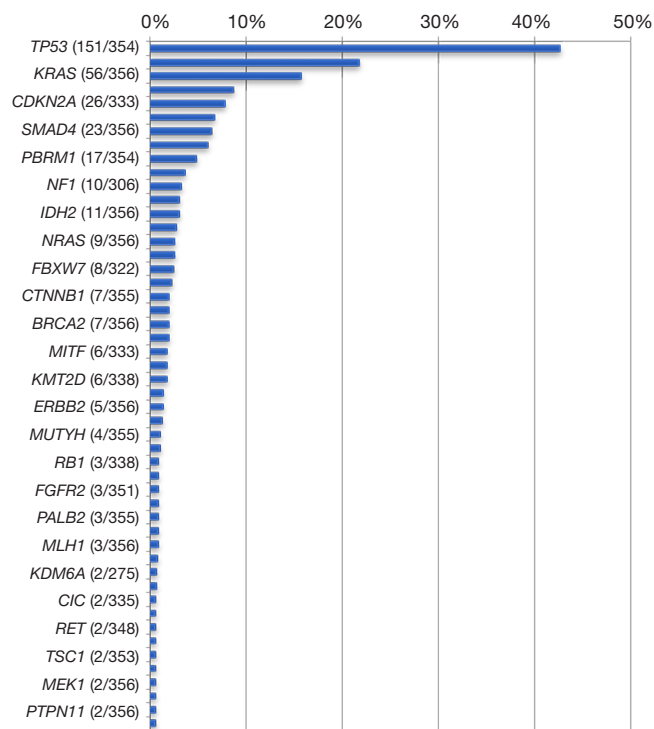
Figure 1 Gene mutation rates across all biliary cancers (n=356).

Table 2 Copy number variations in all biliary cancers

Gene	CNV+	CNV total number	CNV, %
Her2/Neu	16	342	4.7
MYC	11	342	3.2
MDM2	11	342	3.2
cMET	8	342	2.3
CCND1	7	342	2.0
CCNE1	7	342	2.0
HMGA2	6	325	1.8
KRAS	6	325	1.8
FGF4	6	342	1.8
FGF3	6	342	1.8
WIF1	5	325	1.5
FGF19	5	325	1.5
MLLT6	5	325	1.5
PDCD1	4	325	1.2
RARA	4	325	1.2
MCL1	4	342	1.2
CDK4	4	342	1.2
FGFR3	4	342	1.2

CNV, copy number variation.

443 genes interrogated for CNVs, 119 genes were amplified in at least one tumor (*Table 2*). The highest amplification rates were in *Her2/neu* (4.7%), *MYC* (3.2%), *MDM2* (3.2%), *cMET* (2.3%), *CCND1* (2.0%), and *CCNE1* (2.0%). Her2/neu amplifications were identified in 6.4% of tumors by CISH, 4.7% had CNVs by NGS read depth analysis, and overexpression was observed in 3.7% of tumors by IHC. cMET amplifications were seen in 1.8% of tumors by CISH, 2.3% of tumors by NGS, and was overexpressed in 40.9% of tumors by IHC. Protein expression of TS, ERCC1, and RRM1 by IHC was seen in 27.8%, 27.3%, and 14.2% of tumors, respectively.

RNA sequencing was performed on 149 tumors in total (*Table 3*), of which 10 had a detectable fusion (6.7%). *FGFR2* fusions were seen in 7 tumors (all with different fusion partners), one EHCC, one GBC, and 5 IHCCs. A single *FGFR3* fusion was seen in one GBC (*FGFR3-TACC3*). *BRAF* fusions were identified in one GBC (*CUX1-BRAF*) and one IHCC (*SND1-BRAF*).

Comparison of molecular profiles in IHCC, EHCC and GBC

Significant differences were seen when comparing gene mutation rates between the 3 different BTC subtypes (*Figure 2A*). IHCCs had significantly higher *IDH1* (14.5%), *BAP1* (9.5%), and *PBRM1* (7.5%) mutation rates than GBCs ($P < 0.05$). On the other hand, EHCCs were characterized by significantly more frequent mutations in *KRAS* (37.3%), *CDKN2A* (22.0%), and *BRCA1* (3.9%, all $P < 0.05$). GBCs were characterized by significantly higher *Her2/neu* overexpression (9.2%) and amplification (14.4%), as well as high TOP2A expression (78.3%) and amplification (25.0%, all $P < 0.05$). EHCCs and GBCs carry some similar molecular features when compared to IHCCs, including higher mutation rates of *TP53*, *SMAD4*, *APC*, and *ERBB2*. Grouping the mutations in homologous repair genes together (*ATM*, *ATRX*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *PTEN*, and *NBN*), 13.8% of EHCCs, 19.1% of GBCs, and 7.4% of IHCCs demonstrated evidence of homologous repair deficiency (*Figure 2B*). Looking at mutations or rearrangements in BRAF and receptor tyrosine kinases HER2 and FGFR, 15.7% of EHCCs, 23.8% of GBCs, and 12.0% of IHCCs had potentially therapeutic targets.

Table 3 Fusion genes detected by RNA sequencing

Gene	Fusions	Total tested	Frequency, %	IHCC fusions	EHCC fusions	GBC fusions
BRAF	2	131	1.5	*SND1-BRAF*	–	*CUX1-BRAF*
FGFR2	7	149	4.7	*FGFR2-AHCYL1 FGFR2-AXDND1 FGFR2-FMR1 FGFR2-PAWR FGFR2-MYLK*	*FGFR2-ADAM9*	*FGFR2-BICC1*
FGFR3	1	149	0.7	–	–	*FGFR3-TACC3*

IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma.
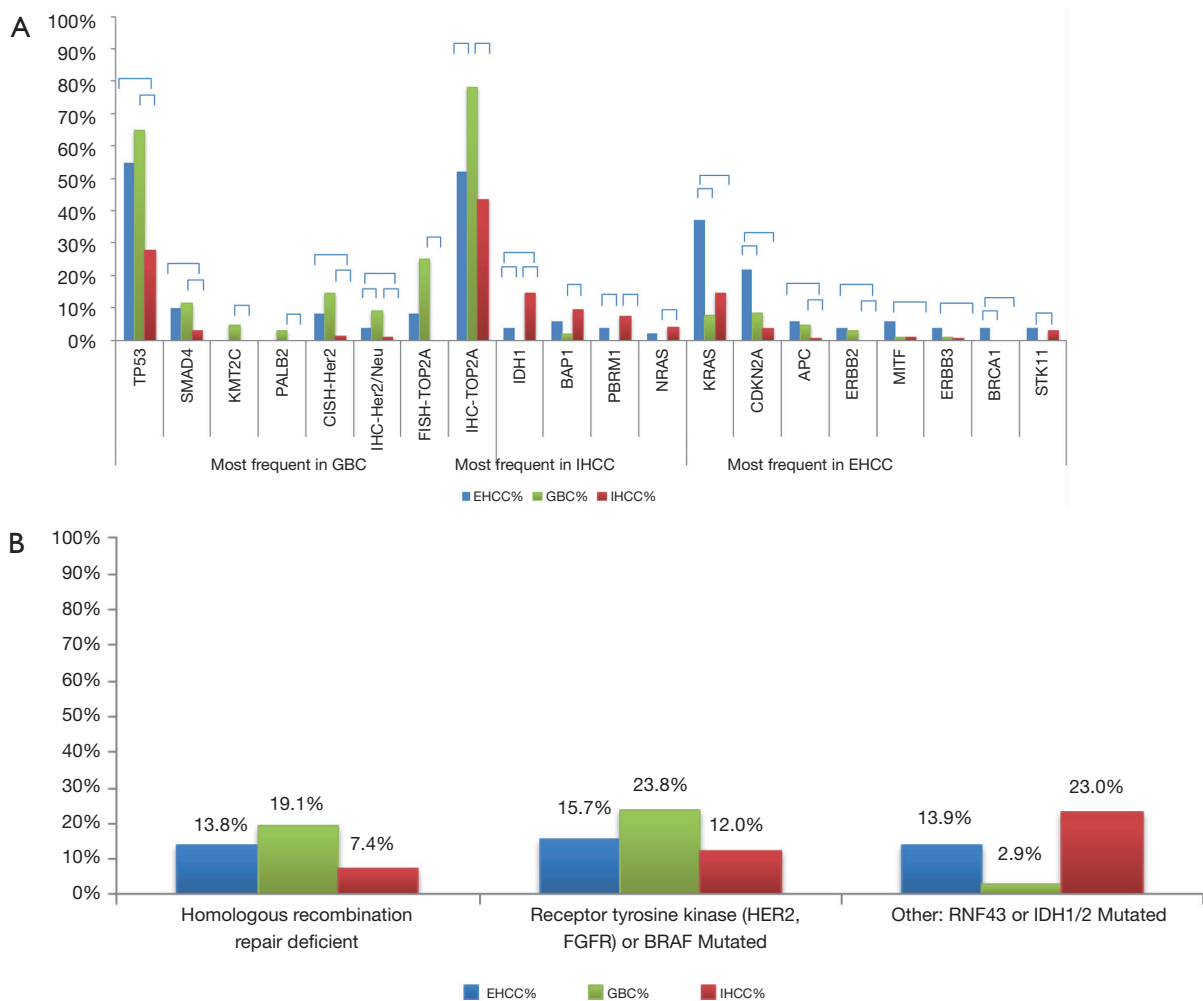

Figure 2 Difference in mutation rates between extrahepatic cholangiocarcinoma, intrahepatic cholangiocarcinoma, and gallbladder carcinoma. (A) Significant differences in a range of gene mutation rates between extrahepatic cholangiocarcinoma, intrahepatic cholangiocarcinoma, and gallbladder carcinoma. Relative gene mutational frequencies across subtypes of biliary tract cancer. Horizontal brackets indicate significant differences by Chi-squared tests (P<0.05); (B) rates of homologous recombination repair deficiency, receptor tyrosine kinase mutations, and other significant actionable mutations across tumor subtypes. IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma; CISH, chromogenic in situ hybridization; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

Table 4 Biomarkers associated with response to immune checkpoint inhibitors

BTC subtype	PD-L1 by IHC	MSI-H	TMB-H	Overall percent positive
All BTCs	7.9% (63/798)	2.0% (7/352)	4.0% (14/352)	11.4% (39/342)
IHCC	8.1% (36/444)	2.5% (5/198)	3.5% (7/198)	13.0% (25/193)
EHCC	6.9% (8/116)	2.0% (1/50)	2.0% (1/50)	6.9% (2/29)
GBC	8.0% (19/238)	1.0% (1/104)	5.8% (6/104)	12.0% (12/100)

Overall percent positive is calculated in the subgroups of tumors with results from all 3 available biomarkers. PD-L1, programmed death-ligand 1; MSI-H, microsatellite instability high; TMB-H, high tumor mutational burden; BTC, biliary tract cancer; IHCC, intrahepatic cholangiocarcinoma; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma.

Immune checkpoint inhibitor-associated biomarkers

PD-L1 overexpression was seen on tumor cells from 63 of 798 (7.9%) tumors using IHC. Increased microsatellite instability (MSI-H) was seen in 7 out of 352 tumors (2.0%) using NGS (*Table 4*). Four percent of all BTCs (all subtypes) were TMB-H based on a cutoff of 17 somatic missense mutations per MB of targeted sequence. Of note, gene amplification of *CD274* (the PD-L1-encoding gene) was only seen in one of 325 tumors. Overall, when all 3 markers (PD-L1, MSI-H, and TMB-H) were considered, 11.4% of tumors carried at least one favorable biomarker for immune checkpoint blockade. When the 3 BTC subtypes were investigated individually, IHCCs and GBCs had a similar prevalence of predictive markers to immune checkpoint blockade (13.0% and 12.0%, respectively), whereas EHCCs had a prevalence of only 6.9% (P>0.05).

Discussion

BTCs are an uncommon group of cancers with a generally aggressive clinical course and poor prognosis. Molecular characterization of BTCs provides potentially actionable clinical information, especially because EHCC, IHCC, and GBC are anatomically close and histologically similar cancer entities (13). In order to improve patient outcomes for BTC, it is imperative to better tailor therapies for patients, selecting who will benefit from immune checkpoint inhibitors and specific targeted therapies. BTCs are a good candidate for molecular analysis because they are rich in therapeutic targets and are highly heterogeneous (13,14). The targetable alterations in BTCs vary significantly with etiology, patient geographical origin, and different anatomical locations despite similar histology (12).

Chemotherapy resistance and sensitivity

High expression of TS, RRM1, and ERCC1 has been shown to confer resistance to fluoropyrimidines, gemcitabine, and platinum-based agents, respectively (25-30). We found that over one-quarter of all BTCs had increased expression of at least one of these markers. The high prevalence of TS, RRM1, and ERCC1 may indicate resistance to standard chemotherapies (*de novo* resistance) or reflect previous exposure to those cytotoxic agents (acquired resistance). TOP2A overexpression was also very common, especially in GBCs (78.3% positive). TOP2A is often associated sensitivity to anthracyclines, more so in breast cancer than in hepatocellular carcinoma (31), and its predictive power in BTCs is unknown. Anthracyclines have been previously used in salvage regimens in chemorefractory BTCs (32-35), although their use has declined due to their associated cardiotoxicity and effective alternative agents. However, anthracyclines may be still appropriate in a specific subset of patients, conceivably those with TOP2A amplification by FISH (9.0% of our cohort), although this should be further evaluated in prospective clinical trials.

Homologous recombination repair deficiency

An increasing number of malignancies have been associated with defects in the homologous recombination DNA repair pathway including mutations in *BRCA1*, *BRCA2*, *PALB2*, and others (see above). Although many of these genes are mutated relatively infrequently, they are a sizable subset when grouped together and were seen in 19.1% of GBCs in our study. This finding has potential therapeutic implications, as tumors driven by homologous recombination repair deficiency may be more sensitive to poly ADP-ribose polymerase (PARP) inhibitors and
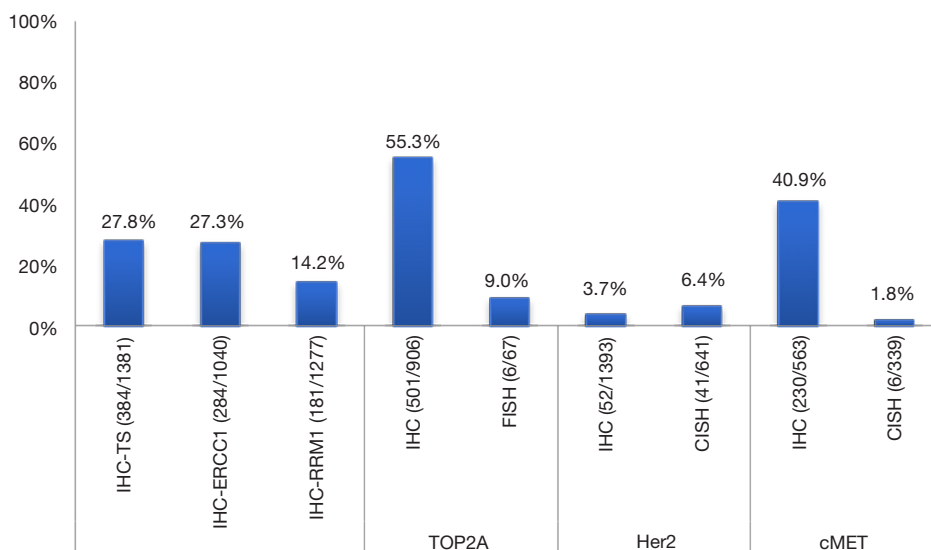
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Figure 3 IHC and CISH protein overexpression and amplification rates across all biliary cancers. IHC, immunohistochemistry; CISH, chromogenic in situ hybridization.

platinum-based chemotherapy (36-39).

cMET

In our cohort, cMET expression was seen in 40.9% of BTCs, with no significant difference between subgroups. Although cMET expression has been associated with an unfavorable prognostic marker in some studies, conflicting results have also been reported (40-42). Agents that specifically target cMET have shown promise in various cancer types, and although BTC-specific trials are lacking, early preclinical studies in cholangiocarcinoma cell-lines that overexpress cMET have shown promising results (43). In contrast to the high cMET overexpression rate observed in BTCs (40.9%; *Figure 3*), cMET amplification was seen in only 1.8% of tumors, and unsurprisingly, all 6 cMET amplified tumors showed strong positive IHC staining. While cMET inhibitors are being investigated in various solid tumors, these data suggest that BTCs should be included. The best predictive biomarker (overexpression by IHC *vs.* amplification by CNV *vs.* other) for cMET-targeted therapies in BTC remains undetermined and warrants further investigation.

Her2/neu

Her2 overexpression and amplification were seen in 4–6%

of BTCs profiled. Consistent with previous observations, the extent of Her2 overexpression and amplification in GBCs (9–14%) is greater than in EHCCs (4–8%), which is greater than in IHCCs (1%). Large, randomized, controlled trials of Her2-targeted therapies have demonstrated benefit for patients with Her2-positive breast and gastroesophageal cancers, but similar trials in BTCs have not been carried out. This is because the prevalence of Her2 overexpression and amplification is more common in breast and gastroesophageal cancers. However, case reports and series have shown Her2 to be an effective target in GBCs (44,45). The MyPathway basket trial included 7 patients with Her2 amplified or overexpressed BTC treated with Her2-targeted therapy (trastuzumab and pertuzumab); 2 patients had a partial response and another 3 patients had stable disease beyond 120 days (46). In addition, the SUMMIT trial using the pan-Her kinase inhibitor neratinib included 9 patients with Her2-mutated BTC; 2 patients had a partial response and a third patient had stable disease (47). Currently, a clinical trial (NCT03093870) is underway to investigate the use of varlitinib, a poly-tyrosine kinase inhibitor (including epidermal growth factor receptor (EGFR), HER2, and HER4), in BTCs.

EHCCs and GBCs had higher rates of *ERBB2* and *TP53* mutations than IHCCs. Jusakul and colleagues observed a similar tendency for *ERBB2* and *TP53* mutations in EHCCs (clusters 1 and 2) (12). Their study found these mutations

were associated with fluke infection, *O. viverrini* and *C. cinensis*, which is relatively uncommon in the United States. In general, Her2-targeted therapy may be the best hope for EHCCs since they tend to lack other viable targets.

FGFR

Gene fusions are often oncogenic driver mutations. However, only about 10% of our cohort had fusions assayed, and they were found in all 3 tumor-subtypes. *FGFR2* fusions were seen most often in IHCCs. This is noteworthy because of ongoing clinical trials of FGFR inhibitors in *FGFR2* fusion-positive cholangiocarcinomas (NCT02150967; NCT03230318; NCT02924376) and various tumors with FGFR2-related abnormalities (basket trials; NCT02052778; NCT01948297).

IDH1

We found a high frequency of chromatin-remodeling gene mutations in our patient cohort, which is consistent with other reports identifying mutations in *IDH1*, *BAP1*, *ARID1A*, and *PBRM1* (12,14,44,48). IHCCs are frequently not associated with liver fluke infection (cluster 4), and mutations in these chromatin modifying genes often serve as the primary epigenetic driver mutation leading to DNA hypermethylation and tumor growth (12). IDH1 is particularly significant because there are several ongoing clinical trials (NCT02073994; NCT02989857; NCT02746081; NCT03212274) that are investigating small molecule IDH1 inhibitors in solid tumors, including cholangiocarcinomas.

RNF43

RNF43 was rarely mutated in our study, seen in 2.0% of EHCC, 1.0% of GBCs, and 1.5% of IHCCs. Ring finger protein 43 (RNF43) mutations can promote Wnt/β-catenin pathway signaling which causes tumorigenesis, and novel Wnt inhibitors are currently being studies in ongoing clinical trials (NCT02675946, NCT025221844, NCT01351103). Therefore, although this is a rare subset of BTCs, patients with *RNF43*-mutated tumors should be referred to these ongoing clinical trials as appropriate.

Immune checkpoint inhibition

Although only a single gene amplification of *CD274* was observed in the 352 tumors analyzed for *CD274* CNV by NGS, 7.9% of all tumors overexpressed PD-L1 by IHC. Abundant somatic mutations in solid tumors have been associated with a greater responsiveness to immune checkpoint inhibitors (20,21,49). TMB-H and MSI-H are potentially useful for assessing neoantigen presentation and viability of immune checkpoint inhibition. In this cohort, 11.4% of all BTCs had a predictive biomarker for immune checkpoint inhibitors, particularly in IHCC and GBC types. Our findings support the notion that immune checkpoint blockade may be used to treat BTCs, and this is currently being tested in multiple ongoing clinical trials (NCT03267940, NCT02703714, NCT03101566, NCT03111732).

Limitations to our study include the lack of clinical outcome information and the use of diagnostic testing platforms that are commonly used for clinical purposes. Also, tumors did not undergo identical diagnostic testing (many underwent analysis by IHC only). In addition, we do not have data on previous exposure of patients to liver flukes, incidence of primary sclerosing cholangitis, or incidence of choledochal cysts, all of which would help elucidate the etiology of individual BTCs.

Conclusions

Our characterization of 1,502 BTCs has led to insights into molecular identities of IHCCs, EHCCs, and GBCs, which may be helpful in understanding treatment and improving outcomes. Patients with IHCCs may benefit from enrollment in clinical trials targeting *FGFR2* and *IDH1*. GBCs in particular may be suitable for agents targeting homologous recombination repair deficient tumors (e.g., PARP inhibitors). IHCCs and GBCs may warrant immunotherapeutic approaches due to increased levels of PD-L1 expression, TMB-H, and MSI-H. EHCCs might have the fewest prospects of future treatments based on current clinical trials and the molecular targets assessed by our study. While no targeted therapies or immunotherapies (except for MSI-H tumors) are currently FDA approved for BTC, several promising agents are in development, and comprehensive molecular profiling will enable optimal therapeutic selection for patients.

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