Biliary cancer: intrahepatic cholangiocarcinoma vs. extrahepatic cholangiocarcinoma vs. gallbladder cancers: classification and therapeutic implications

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Abstract: Biliary cancers (BCs) are a diverse group of tumors that arise from the bile duct epithelium and are divided into cholangiocarcinomas of the intrahepatic and extrahepatic cholangiocarcinoma (EHCC) and cancer of the gallbladder. Despite improvements in treatment and diagnosis, BCs are often diagnosed at an advanced stage and associated with poor prognosis and limited treatment options. Recent discoveries have allowed us to have a better understanding of the genomic diversity in BC, and identify genes that are likely contributing to its pathogenesis, proliferation and treatment resistance. Additionally, these advances have allowed us to reason that each anatomic group within BC behave as distinct diseases, with differences in prognosis and outcomes. Based on this knowledge, recent advances have allowed us to identify actionable mutations that form rational therapeutic targets with novel agents, where their relevance will be better understood through the completion of prospective clinical trials.

Keywords: Next generation sequencing; targetable mutations; biliary cancers (BCs)

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Introduction

Biliary cancers (BCs) are rare but heterogeneous, and comprised of intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC) and gallbladder cancers. They may arise along any portion of the bile duct system, from the neoplastic proliferation of cholangiocytes, the epithelium of the bile ducts. While all three anatomic groups fall under BC, our increased understanding has allowed us to rationalize that each may be in fact distinct diseases, with differences in patterns of recurrence and prognosis (1). Pre-clinical studies have reinforced this thought process, where immunohistochemical studies from BC samples revealed phenotypic traits of cholangiocytes and progenitor cells consistent with their anatomic sites of origin, confirming the heterogeneity between the three groups (2). Progenitor cells from the canals of Hering have

been identified in IHCC, while those within the peribiliary glands have been identified in EHCC and gallbladder cancers, respectively (2-5). Furthermore, the increased availability of next-generation sequencing panels has facilitated this thought process by allowing us to understand the tumor somatic variants and genomic heterogeneity between the three groups. Herein, we will the current treatment in BC, genomic landscape in BC and its role in treatment selection and integration in clinical trials.

Therapeutic regimens for biliary tract cancer

Chemotherapeutic treatment options

In patients who are diagnosed with early stage disease, surgical resection, regardless of anatomic location, is the sole curative treatment option (6). Unfortunately, most

Table 1 Clinical trials for patients refractory to prior gemcitabine/platinum therapy

Author	Treatment	Phase	No. of patients	PFS (months)	OS (months)	ORR (%)
He <i>et al.</i> (8)	FOLFOX-4	II	37	3.1	NR	21.6
Paule <i>et al.</i> (9)	Gemcitabine/oxaliplatin + cetuximab	II	9	4.0	7.0	22.0
Sasaki <i>et al.</i> (10)	Irinotecan	II	13	1.8	6.7	7.7
Suzuki <i>et al.</i> (11)	S-1	II	40	2.5	6.8	7.5
Croitoru <i>et al.</i> (12)	Gemcitabine/5-FU	II	17	3.2	13.2	17.6
Fornaro <i>et al.</i> (13)	Gemcitabine combination	Retrospective	174	3.0	6.6	10.2

PFS, progression-free survival; OS, overall survival; ORR, overall response rate.

patients present with advanced disease at diagnosis, where no curative treatments exist and are primarily supportive. Based on ABC-02, a large, randomized stage III trial conducted by Valle *et al.*, the combination of gemcitabine and platinum based chemotherapy has become the standard approach in treating advanced BC, for all anatomic groups. The combination demonstrated superior clinical efficacy when compared to single-agent gemcitabine (7). Patients who received the combination experienced an absolute 3.6 months survival benefit in comparison to gemcitabine monotherapy with similar rates of adverse events. Eighty percent of patients experienced disease control. Despite this, the vast majority of patients develop treatment resistance after few months on therapy and the overall survival (OS) remains at less than 1 year (7). Outcomes in second-line therapies after gemcitabine platinum-based therapy failure result in dismal outcomes with progression-free survival (PFS) only several months, highlighting the need to develop new and effective therapies in BTC (*Table 1*) (8-14).

Targeted therapies and the role of cancer genomics in its treatment selection process

Over the past two decades, the discovery and successful targeting of genes involved in oncogenesis has helped increase our understanding of targeted management of solid tumor malignancies. Pre-clinical studies revealing a high proportion of genomic alterations in critical signaling pathways involved in tumor proliferation, growth and treatment resistance has led to an interest in developing clinical trials investigating novel targeted therapies in BC.

While early studies investigating novel agents targeting relevant signaling pathways demonstrated interesting anti-tumor activity in a small proportion of patients, the overall

results have been disappointing, with outcomes largely similar to chemotherapy agents in the refractory setting (*Table 2*) (15-25). This may be in part due to the utilization of non-selected trials, where patients are eligible for enrollment regardless of their tumor genomic alterations. Furthermore, the challenge to have sufficient enrollment and timely completion of clinical trials has led to lumping all sites of BC into its eligibility, irrespective of anatomic subtype.

Through the availability and implementation of next generation sequencing technologies, recent reports have allowed us to understand the genomic landscape present in BC, as well as understand and identify actionable molecular alterations in this disease. While studies have identified common mutations in *KRAS* (up to 20%), *BRAF* (5%) and epidermal growth factor receptor (*EGFR*) (3%) in gallbladder, IHCC and EHCCs, targetable mutations are enriched in IHCC that have only been identified in this anatomic group, suggesting differing influences in pathogenesis and reinforcing the thought process that BC is comprised of three heterogeneous diseases. Herein, we briefly discuss common targetable mutations, while focusing on recently identified genes that may be treated with novel, targeted therapies.

ErbB family signaling pathway in BC

Four receptors belong to the ErbB family of receptor tyrosine kinases: *EGFR*, ErbB-2 (*HER-2* or *HER-2/neu*), ErbB-3 (*HER-3*) and ErbB-4 (*HER-4*). ErbB receptors and the binding to its ligands are integral to biliary carcinogenesis. Anti *EGFR* and *HER-2* therapies have demonstrated interesting anti-tumor activity in preclinical studies (27), resulting in their investigation in clinical trials.

Table 2 Results from clinical trials with select molecularly targeted agents

Author	Treatment	Target	Percent of patients in refractory setting (%)	PFS (months)	OS (months)	ORR (%)
Bekaii-Saab <i>et al.</i> (15)	Selumetinib	MEK	39	3.70	9.80	12.0
Finn <i>et al.</i> (16)	Binimetinib	MEK	43	2.14	4.78	7.0
Ahn <i>et al.</i> (17)	MK2206	Akt	100	0.50–6.60	2.20–20.20	0
Ramanathan <i>et al.</i> (18)	Lapatinib	HER-2	65	1.80	5.20	0
Peck <i>et al.</i> (19)	Lapatinib	HER-2	100	2.60	5.10	0
Philip <i>et al.</i> (20)	Erlotinib	EGFR	57	2.60	7.60	8.0
Lubner <i>et al.</i> (21)	Erlotinib + bevacizumab	EGFR + VEGFR	0	4.40	9.90	11.0
El-Khoueiry <i>et al.</i> (22)	Sorafenib	VEGFR, PDGFR, Raf	0	3.00	9.00	0
Bengala <i>et al.</i> (23)	Sorafenib	VEGFR, PDGFR, Raf	56	2.30	4.40	2.0
El-Khoueiry <i>et al.</i> (24)	Erlotinib + sorafenib	EGFR, VEGFR, PDGFR, Raf	0	2.00	6.00	6.0
Yi <i>et al.</i> (25)	Sunitinib	VEGFR, PDGFR, RET	100	1.70	4.80	8.9
Buzzoni <i>et al.</i> (26)	Everolimus	mTOR	100	3.20	7.70	5.1

PFS, progression-free survival; OS, overall survival; ORR, overall response rate; EGFR, epidermal growth factor receptor.

EGFR signaling pathway

Preclinical work has identified *EGFR* to be aberrantly activated in BC, across all three anatomic groups (28,29). The *EGFR* signaling pathway is integral in epithelial cell growth and proliferation, where preclinical studies have shown tumor regression with *EGFR* inhibition (28,30), forming the rationale for targeting *EGFR* for treatment. While initial small studies demonstrated promising anti-tumor activity with anti-*EGFR* therapy in combination with gemcitabine-based chemotherapy (31,32), these findings were unable to translate into a significant clinical benefit in larger randomized clinical trials (33,34).

One rationale for the limited clinical activity seen with anti-*EGFR* therapies may be due to the activation of downstream effectors, including the *PI3K/Akt* and mitogen activated protein kinase (*MAPK*) pathways. The *MAPK* pathway is a downstream signaling pathway, where upstream receptor tyrosine kinases (*EGFR*, *TGF-β*, etc.) result in the phosphorylation, activation and signal transduction via *Ras*, *Raf*, *MEK* and *ERK*. Activated *ERK* (pERK) translocates to the nucleus and affects many cellular responses. In the *PI3K/Akt* pathway, *PI3K* activates *Akt* (pAkt), which mediates its effects through various downstream substrates. Activation of *Akt* and *ERK* contribute to tumor proliferation, invasiveness and chemotherapy resistance, representing rational therapeutic targets against these pathways (35,36).

Both pathways are frequently activated, often concomitantly in all subtypes of BC (37–39). While the overall results of several studies investigating were unimpressive, interesting clinical activity was observed in several patients with advanced, refractory BC who received *MEK* and *Akt* inhibitors (15,16,40). The clinical benefit from targeting single signaling pathways is often short lived due to mechanisms of resistance including communication between parallel signaling pathways, activation of downstream effectors and negative loop feedback inhibition.

HER-2/neu gene

Human growth factor receptor 2, also known as *HER-2/neu* or *ERBB2*, is an oncogene encoded by the *ERBB2* gene, and is a member of the human *EGFR* family. Its amplification or overexpression has been shown to play an important role in the development and progression of several solid tumor malignancies, and is an effective therapeutic target in breast and gastric cancer (41–43). Studies evaluating *HER-2* overexpression in BC have identified *HER-2* mutations in gallbladder (about 10%) and EHCCs (up to 25%), and have been associated with a more aggressive phenotype (27,44). While small case series demonstrated anti-tumor activity with anti *HER-2* directed therapy in patients whose tumors expressed *HER-2* overexpression or amplification, these findings did not translate in BC patients

Table 3 Ongoing clinical trials with select molecularly targeted agents

Target	Agent	Trial design	NCT number	Comment
<i>IDH1</i>	AG-120	Phase I	NCT02073994	Tumors harboring <i>IDH1</i> mutations with failure of prior standard therapy
<i>IDH2</i>	AG-221	Phase I/II	NCT02273739	Tumors harboring <i>IDH2</i> mutations including glioma and angioimmunoblastic T-cell lymphoma
<i>FGFR2</i>	BGJ398	Phase II	NCT02150967	<i>FGFR2</i> fusions or other <i>FGFR</i> mutations
	Ponatinib	Phase II	NCT02265341	<i>FGFR2</i> fusions
<i>EGFR</i> or <i>VEGF</i>	Panitumumab or bevacizumab with chemotherapy	Phase II	NCT01206049	<i>KRAS</i> wild type
<i>ALK/ROS1</i>	LDK378	Phase II	NCT02374489	<i>ROS1</i> or <i>ALK</i> overexpression
<i>BRAF + MEK</i>	Dabrafenib + trametinib	Phase II	NCT02034110	<i>BRAF</i> V600E mutated cancers

IDH, isocitrate dehydrogenase; FGFR, fibroblast growth factor receptor; EGFR, epidermal growth factor receptor.

(18,19,45,46). However, these studies were not limited to *HER-2* overexpressing tumors and allowed all comers. Improved patient selection, in addition to dual anti *HER-2* targeted therapies may result in improved clinical efficacy, and are a consideration for future clinical trials.

Vascular endothelial growth factor (VEGF) pathway in BC

Angiogenesis is a mechanism by which tumor cells can proliferate and is controlled by VEGF. In addition to angiogenesis and vascular permeability, VEGF mediated signaling facilitates signaling in tumor cells that contribute to cholangiocarcinogenesis, including facilitating the function of tumor stem cells, tumor invasiveness and modulate regulatory T cells that can contribute to treatment resistance (47). It is highly expressed in BC, in up to 75%, and is associated with a more aggressive phenotype and poorer prognosis (48). Therapies targeting VEGF, including monoclonal antibodies against VEGF (bevacizumab) or multi-targeted small molecule inhibitors with activity against VEGFR have failed to demonstrate any significant clinical activity in randomized clinical trials in BC (21,49-52). While there is no indication for anti-VEGF therapies in BC at this time, given its activity in other solid tumors, it remains a therapeutic target of interest. The identification of prognostic and predictive biomarkers may improve patient selection and identify patients who are likely to benefit from anti-VEGF therapies.

Novel genomic alterations identified in IHCC

BRAF mutations

As described above, the *MAPK* pathway regulates cellular proliferation, survival and migration and is constitutively activated in BC. Mutation of the *BRAF* gene is a mechanism of aberrant activation of the *MAPK* pathway and occurs in many solid tumors, including melanoma, colorectal cancer and non-small cell lung cancer (53-56). The most common *BRAF* mutation results in a single amino acid substitution of glutamic acid to valine at residue 600 (V600E). Larger studies suggest *BRAF* V600E mutations are exclusive to IHCC, with an incidence of 3–5% (57,58). The presence of a *BRAF* mutation has been associated with a more aggressive phenotype, with a higher tumor stage and likelihood of lymph node involvement at diagnosis (59). While inhibition of *BRAF* with small molecule inhibitors have demonstrated anti-tumor activity in various other *BRAF*-mutated malignancies and have become a standardized treatment in *BRAF*-mutated melanoma, its therapeutic relevance in IHCC is unknown, but represents an intriguing therapeutic option for this disease (60-66). Ongoing early clinical trials are investigating the role of *BRAF* mutations as a potential targetable mutation in BC (Table 3).

Isocitrate dehydrogenase 1/2 (*IDH1/2*)

IDH1 and *IDH2* function to encode metabolic enzymes

that normally convert isocitrate, a metabolic intermediate, to α -ketoglutarate. Somatic mutations in *IDH1/2* can occur in upwards of 20% of IHCC and result in abnormal enzymatic activity, allowing them to reduce α -ketoglutarate to 2-hydroxyglutarate, which has been identified as an “oncometabolite” that inhibits enzymatic activity dependent on α -ketoglutarate (67-69). This results in altered cell maturation, differentiation and survival. Pre-clinical studies have confirmed the role of *IDH* mutations in the pathogenesis of intrahepatic BC, where their mutations inhibit hepatocyte differentiation and induce proliferation of hepatic progenitors and the development of premalignant biliary lesions (70). Based on early promising results in preclinical studies, specific inhibitors for *IDH1* and *IDH2* are currently under investigation in clinical trials (Table 3).

Fibroblast growth factor receptor (FGFR)

The *FGFR* comprise a family of highly conserved tyrosine kinase receptors consisting of four members (*FGFR1*, 2, 3 and 4). These receptors bind to one of 18 secreted glycoprotein ligands, or fibroblast growth factors (FGFs), to their extracellular domain (71). Several *FGFR2* chromosomal fusions, with several genomic partners, have been identified in IHCC (72-77). Preclinical studies have identified several *FGFR2* fusions (*FGFR2-PPHLN1*, *FGFR2-BICC1*, *FGFR2-TACC3*, *AHCYL1*) specifically in intrahepatic BC, where genomic sequencing identified an incidence ranging up to 50% of intrahepatic BC patients. Upon its fusion, activation of the tyrosine kinase protein results in the activation of the *FGFR2* receptor with autophosphorylation and activation of downstream signaling pathways, including *Ras/MAPK*, *PI3K/Akt* and the signaling transducer and activator of transcription (STAT) pathway, which play a critical role in the regulation of cell proliferation, differentiation, and survival (78). Additionally, identification of an association between the presence of *KRAS* mutations and *FGFR2* fusions suggest a mutual role in driving oncogenesis.

While pre-clinical studies and isolated case reports have demonstrated anti-tumor activity with *FGFR* inhibition in intrahepatic BC exhibiting *FGFR2* fusions, its actual role in oncogenesis and its sensitivity as a targetable actionable mutation is unknown. Ongoing phase II studies, specifically in BC patients with *FGFR2* mutations are ongoing and may reveal the potential therapeutic benefit from *FGFR* inhibition (Table 3).

ROS1 gene

ROS1 fusions, a proto-oncogene, have been identified in up to 9% of IHCC (79). Its role as an oncogenic driver and potential therapeutic target has been recently validated in preclinical studies, where its inactivation led to a potent anti-tumor effect (80). While its role as a potential therapeutic target has been confirmed in other solid tumor malignancies, notably non-small cell lung cancer (81), further validation will be required to assess its frequency in BC in addition to assessing its relevancy as a potential targetable mutation in patients exhibiting *ROS1* fusions.

Notch pathway

The Notch signaling pathway plays an important role in embryogenesis and the proper structural development of the liver. It is also plays an important role in oncogenesis, where its dysregulation results in increased inflammation and development in intrahepatic BC. Mouse models with increased *Notch1* expression resulted in the formation of intrahepatic BC, reinforcing its importance in tumor development and growth (82). Notch receptor 1-4 upregulation has been identified in all anatomic subsets of BC, and in up to 80% of intrahepatic BC (83,84). In addition to its role in BC, Notch expression has also been implicated in the development of primary hepatocellular carcinomas (HCC). Interestingly, a study utilizing mouse models driven by *Ras*, demonstrated that the inhibition of specific Notch receptors resulted in reducing HCC like primary tumors, while leading to the development of primary BC like tumors (85). Thus, while targeting Notch represents an intriguing therapeutic option in BC, further studies are needed to validate its role in this disease.

Conclusions

BCs are a rare, heterogeneous disease group that have limited treatment options and are associated with universally poor outcomes. Recent advances in sequencing technologies have resulted in an increased understanding of the genomic alterations present in BC, and understand the differences in the genomic makeup in each anatomic subgroup. Through these efforts, the identification of targetable mutations has allowed us to develop and tailor novel therapeutic agents against these genes of interest. The completion of ongoing prospective trials may result in the shift in the treatment paradigm in BC, where patients will be able to receive

treatment tailored specifically based on each individual's tumor genomic profile. Prospective correlative studies that include the utilization of a biorepository will allow us to understand mechanisms of treatment efficacy, study the process of treatment resistance, and importantly, identifying biomarkers of secondary resistance that may allow for development of alternate therapeutic options.

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Footnote

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