REVIEW

New Horizons for Precision Medicine in Biliary Tract Cancers

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ABSTRACT

Biliary tract cancers (BTC), including cholangiocarcinoma and gallbladder cancer, are poor-prognosis and low-incidence cancers, although the incidence of intrahepatic cholangiocarcinoma is rising. A minority of patients present with resectable disease but relapse rates are high; benefit from adjuvant capecitabine chemotherapy has been demonstrated. Cisplatin/gemcitabine combination chemotherapy has emerged as the reference first-line treatment regimen; there is no standard second-line therapy. Selected patients may be suitable for liver-directed therapy (e.g., radioembolization or external beam radiation), pending confirmation of benefit in randomized studies. Initial trials targeting the epithelial growth factor receptor and angiogenesis pathways have failed to deliver new treatments. Emerging data from next-generation sequencing analyses have identified actionable mutations (e.g., FGFR fusion rearrangements and IDH1 and IDH2 mutations), with several targeted drugs entering clinical development with encouraging results. The role of systemic therapies, including targeted therapies and immunotherapy for BTC, is rapidly evolving and is the subject of this review.

Significance: The authors address genetic drivers and molecular biology from a translational perspective, in an intent to offer a clear view of the recent past, present, and future of BTC. The review describes a state-of-the-art update of the current status and future directions of research and therapy in advanced BTC. Cancer Discov; 7(9): 943–62. © 2017 AACR.

INTRODUCTION

Biliary tract cancers (BTC), including cholangiocarcinoma [both intrahepatic (ICC) and extrahepatic (ECC)] and gallbladder cancer, are low-incidence cancers carrying a poor prognosis (1). BTCs account for approximately 3% of all adult cancers (2). Incidence and mortality are increasing, largely due to an increase in ICC (3–5). Most patients (>65%) are diagnosed with nonresectable disease (1), and there is a high relapse rate in the minority of patients who undergo potentially curative surgery (6, 7). The five-year survival rate is around 5% to 15% when considering all patients (8, 9); estimated five-year survival rate varies with stage: 50% for American Joint Committee on Cancer (AJCC) stage I, 30% for stage II, 10% for stage III, and 0% for stage IV (6, 10).

It is widely accepted by the BTC community that BTC malignancies are not one unique disease only, but a group of different diseases with distinct demographics, molecular characteristics, and treatment options (Fig. 1). Such differences are worth taking into account at time of treatment planning, research, and clinical trial design. BTCs are more frequent in patients between ages 50 and 70 years, with a male preponderance for cholangiocarcinoma and female for gallbladder cancers (2); >90% are adenocarcinomas (1). Several risk factors, mainly associated with chronic gallbladder or biliary tract inflammation, have been identified (11–13). Opisthorchis viverrini is one of the three major liver trematodes (flukes) that infect humans. It is endemic in Thailand, Vietnam, Cambodia, and Laos and accounts for a global “hotspot” of intrahepatic cholangiocarcinoma in this region. Adult flukes can remain in the bile ducts for years, stimulating a host immune response, leading to chronic biliary tract inflammation. This results in an up to 15-fold increase in the risk of developing intra/extrahepatic cholangiocarcinoma (14). There are also differences in risk factors (15–17) and symptoms at presentation between the different BTCs [gallbladder patients (in advanced stages) are less likely to present with jaundice and usually present with abdominal pain; refs. 15, 18].
Clinicl Context

Patients with tumors arising in proximity to the bile ducts present with biliary obstruction, due to local infiltration of the biliary tract. A minority of patients will be diagnosed with early (resectable) disease, in which case treatment will be surgical with curative intent. For patients diagnosed with advanced disease (often presenting with nonspecific, nonbiliary obstructive symptoms), treatment options are noncurative and mainly chemotherapy-based.

Despite potentially curative resection for localized disease, relapse rates are high (19), highlighting the need to optimize adjuvant strategies. The role of adjuvant treatment for BTC has been unclear for many years (20). A systematic review and meta-analysis found that adjuvant treatment did not improve survival when compared with surgery alone when considering all patients (21). However, there appeared to be benefit for patients with microscopically involved margins (R1-resection) versus clear resection margins [R0-resection; odds ratio (OR), 0.36; 95% confidence interval (CI), 0.19–0.68] and lymph node–positive disease (OR, 0.49; 95% CI, 0.30–0.80). Two randomized phase III clinical trials exploring adjuvant chemotherapy were reported in 2017. First, the results from the PRODIGE-12/ACCORD-18 clinical trial assessing the benefit of adjuvant combination chemotherapy compared with observation alone were reported in January 2017 (22). This multicenter phase III trial randomized 196 patients within 3 months of resection of a localized BTC (intrahepatic, perihilar, or extrahepatic cholangiocarcinoma, or gallbladder cancer), to receive either adjuvant gemcitabine and oxaliplatin or surveillance; there was no significant difference in relapse-free survival between the arms [HR of 0.83 (95% CI, 0.58–1.19); P = 0.31]. Second, the BilCap clinical trial exploring the role of adjuvant capecitabine compared with observation alone was reported at ASCO 2017. A total of 447 patients with BTC were randomized to capecitabine (n = 223) or observation (n = 224; ref. 23). Sensitivity analyses by intention-to-treat were adjusted to nodal status, grade of disease, and gender (447 patients). This trial demonstrated benefit from capecitabine in terms of overall survival [OS;

**Figure 1.** BTCs are a group of different diseases which includes ICC, ECC, and gallbladder cancer. They differ in many aspects, such as anatomical location, demographics, clinical presentations, and treatment options.

### Biliary tract cancer
- >90% of cases are adenocarcinoma
- Level 1 evidence for adjuvant chemotherapy: capecitabine
- Palliative 1st-line chemotherapy: cisplatin/gemcitabine
- No 2nd-line palliative chemotherapy with a demonstrated survival benefit over active symptom control
- Median overall survival: ~12 months

### Intrahepatic cholangiocarcinoma
- Risk factors: primary sclerosing cholangitis, cirrhosis, *Opisthorchis viverrini* or *Clonorchis sinensis*, obesity, diabetes, chronic hepatitis B and C, hepatolithiasis, Lynch syndrome, biliary papillomatosis, biliary duct morphologic anomalies
- Typically presents as incidental hepatic lesion(s)
- Radioembolization or radiation can be considered for liver-predominant disease

### Extrahepatic cholangiocarcinoma
- Males > females
- Risk factors: primary sclerosing cholangitis, gallstones, Lynch syndrome, *Opisthorchis viverrini* or *Clonorchis sinensis*, bile duct morphologic anomalies
- Typically presents with obstructive jaundice

### Gallbladder cancer
- Females > males
- Risk factors: gallstones, gallbladder polyps, chronic cholecystitis, *Salmonella typhi*, obesity, diabetes
- Typically presents as an incidental finding following cholecystectomy (localized stage) or with abdominal pain (advanced stage)
Precision Medicine in BTC

HR, 0.71 (95% CI, 0.55–0.92); P < 0.01; median OS 51 months (95% CI, 35–59) and 36 months (95% CI, 30–45) for capcitabine and observation arms, respectively. There was also benefit from adjuvant capcitabine in terms of relapse-free survival [median 25 months (95% CI, 19–37) and 18 months (95% CI, 13–28) for capcitabine and observation arms, respectively]. Based on these results, adjuvant capcitabine is likely to be considered standard of care following surgery for BTC.

Around 60% to 70% of patients will be diagnosed with advanced disease, which is defined as inoperable or metastatic disease. For these patients, palliative treatment, usually in the form of systemic chemotherapy, is the only treatment option. Selected patients with liver-predominant disease may benefit from liver-directed therapies such as external beam radiation (24, 25) or radioembolization (26, 27). Unfortunately, data suggesting such benefit are based on retrospective series or small phase II trials rather than randomized studies; further data are awaited to confirm the incremental benefit of approaches involving liver radioembolization. Liver transplant has been suggested as a potential treatment option for patients with small perihilar cholangiocarcinoma (28, 29).

However, prospective studies are needed to ensure the most suitable patient selection and benefit due to challenges for organ allocation and living donation policies; currently, liver transplant remains controversial in this setting. New options for systemic treatment are an urgent area of unmet need for this patient population.

SYSTEMIC THERAPY OF ADVANCED DISEASE

First-Line Chemotherapy

The most active cytotoxic chemotherapy agents in the management of BTCs are gemcitabine and platinum agents (30, 31). The first study to suggest that palliative chemotherapy could improve survival and quality of life was reported in 1996 and established gemcitabine as a treatment option for patients with advanced disease (32). These results increased the interest in the treatment of BTCs, and over the past 20 years many studies have been performed (30) that have been negative or lacked the statistical power and rigor to change clinical practice.

In 2010 we showed, in the 410-patient, randomized, phase III, ABC-02 study, a benefit from cisplatin/gemcitabine chemotherapy over single-agent gemcitabine; the doublet conferred an advantage in OS over gemcitabine alone (11.7 vs. 8.1 months; HR, 0.64; 95% CI, 0.52–0.80; P < 0.001) (33). These results were replicated in the Japanese randomized phase II (BT22) study (34). Based on these results, cisplatin/gemcitabine has become the recognized reference regimen for first-line treatment of patients with advanced BTC. Although patients with jaundice were excluded from the ABC-02 study, we have since shown that the doublet may safely be used in patients with jaundice secondary to obstructing endoluminal disease (although not the case in patients with jaundice due to parenchymal replacement by metastatic disease; ref. 35). The lack of further practice-changing trials since these studies highlights the desperate need for new therapies for patients with advanced BTC.

Second-Line Chemotherapy

Patients progressing on first-line chemotherapy often have a rapidly worsening performance status, and only a small number of patients may be suitable for further treatment. In addition, patients often have the inherent problems of biliary obstruction and sepsis associated with BTC, which may preclude further treatment.

Currently, no quality evidence is available supporting the use of second-line chemotherapy (36). For most patients, active symptom control (ASC, e.g., biliary stenting and antibiotics, as appropriate) is considered the standard of care after progression on first-line chemotherapy. However, small prospective and retrospective studies have shown signals of potential benefit in selected patients (37–41). Based on the previously reported benefit from 5-fluorouracil (5-FU) in BTCs (42, 43), the ongoing UK ABC-06 study is a randomized phase III study comparing oxaliplatin and 5-FU (FOLFOX regimen) with ASC versus ASC alone following progression on or after first-line cisplatin/gemcitabine; recruitment is ongoing (NCT01926236; ref. 44).

In summary, robust (phase III) evidence is available for the use of first-line chemotherapy in patients presenting with advanced disease. Cisplatin and gemcitabine have become the reference regimen; other regimens are sometimes considered by individual clinicians based on phase II studies. The role of second-line therapy is unclear; no single regimen has emerged. Ongoing trials are trying to address this lack of treatment options, highlighting the need for development of novel targeted therapy approaches.

CURRENT GENETIC LANDSCAPE AND ACTIONABLE SIGNATURES

Tumor Sequencing Data

There has been a great effort to apply new parallel sequencing technologies to gather more insight about the molecular biology of these malignancies. As with other studies with next-generation sequencing (NGS) in cancer, the starting questions were if these malignancies share common anomalies with other cancers and what is the idiosyncratic pattern of anomalies associated with BTCs.

Main New Findings for ICC and ECC

Several studies have identified different genetic alterations that occur in cholangiocarcinoma using various approaches, from whole-exome sequencing (WES; refs. 45–47) to a focused approach on specific pathways (48–53). A WES study of 8 cases of Opisthorchis viverrini–related cholangiocarcinoma revealed mutations in TP53 (44.4%), KRAS (16.7%), and SMAD4 (16.7%; ref. 45). Loss-of-function mutations of tumor suppressor genes have been reported in cholangiocarcinoma with an overall frequency of 15% (54–59). Activating mutations of KRAS (22%, range 5%–57%), mainly located in codon 12 hotspots, have been associated with a worse prognosis after radical surgery. The tumor suppressor gene SMAD4, located in the long arm of chromosome 18q 21.1, encodes a nuclear transcription factor of TGFβ (60, 61) and is usually inactivated when mutated. In the infection-driven cases, mutations in TP53 and SMAD4 were more common,
39.8% and 19.4% versus 9.3% and 5.8%, respectively. Mutations in CDKN2A/B (p16) were identified in 7% of patients with ICC (52).

In a sequencing study of 102 patients with ICC from China, Zou and colleagues found TP53 mutations are more likely to be HBsAg-seropositive, whereas KRAS mutations are nearly exclusively found in HBsAg-seronegative patients with ICC (62).

Interesting findings include inactivating mutations in MLL3 (14.8%), ROBO2 (9.3%), RNF43 (9.3%), and PEG3 (5.6%), and activating mutations in GNAS (9.3%; ref. 45). RNF43 (encoding a RING domain E3 ubiquitin ligase) interacts with p53 and suppresses p53-mediated apoptosis (63). In this study, RNF43 was an independent prognostic factor in the multivariable analysis. Interestingly, RNF43 is also a key molecule in the WNT–β-catenin pathway and can inhibit WNT signaling by interacting with the WNT receptors of the Frizzled family (64). RNF43 mutations may predict sensitivity to porcine inhibitors (65). PEG3 is an imprinted gene that regulates apoptosis; when it is inhibited, it blocks p53-induced apoptosis (66). Loss of PEG3 activates WNT signaling, leading to chromosomal instability (67). MLL3 encodes a histone–lysine N-methyltransferase that is one of the histone modifiers implicated in numerous cancers such as pancreatic cancer (68–70). Most of the tumors harboring a mutation in MLL3 did not contain mutations in TP53, KRAS, or SMAD4, despite the fact that these three genes were mutated together (68). The Johns Hopkins study also found four somatic mutations in FGFR2 (13%; ref. 47).

Nakamura and colleagues conducted a comprehensive genomic analysis of 260 BTCs and found that 40% of cases harbored targetable genetic alterations (88). They found that gene fusions involving FGFR2 and PRKACA or PRKACB preferentially occurred in ICC and ECC, respectively.

**Chromatin Modifiers**

The Singapore group subsequently analyzed cases of infection-related versus non–infection-related cholangiocarcinoma (46). A new set of 15 non–infection-related cases were sequenced identifying mutations in chromatin-remodeling genes: ARID1A (19%) and BAPI (25%). ARID1A encodes an accessory subunit of the SWI/SNF chromatin remodeling complex. In cell lines, silencing ARID1A results in a significant increase in the proliferation of cholangiocarcinoma-derived cell lines compared with cells expressing the wild-type form (46), showing mechanistically the involvement of this alteration in cholangiocarcinoma proliferation. BAPI1 is a deubiquitinate protein of the ubiquitin C-terminal hydrolase (UCH) family (89–91). Increased proliferation was shown after BAPI1 knockdown using an RNA-interference approach.

The Johns Hopkins group identified mutations in several of the chromatin-remodeling genes, including the ones described by Chan-On and colleagues (46), ARID1A and BAPI. They also found alterations in PBRM1 (17%), a gene that encodes a subunit of the ATP-dependent SWI/SNF chromatin-remodeling complexes. These findings reinforced the idea of a major role of the chromatin remodeling process in the carcinogenesis of this tumor (Fig. 3; ref. 92).

**New Gene Fusions: NTRK**

New gene fusions have also been identified in ICC, such as RABGAP1L–NTRK1 (52). NTRK1 encodes a protein of the neurotrophic tyrosine kinase receptor (NTRK) family; this kinase is a membrane-bound receptor that, on neurotrophin binding, phosphorylates itself and members of the MAPK pathway (93). Gene fusions involving the NTRK1, NTRK2, and NTRK3 genes result in constitutively active TRKA, TRKB,
and TRKC kinases. The presence of these kinases leads to cell differentiation and may play a role in specifying sensory neuron subtypes. The TRK inhibitor LOXO-101 has shown early promise in a phase I trial of patients with advanced solid tumors where 5 out of 6 (83%) patients evaluable for response and harboring NTRK fusions achieved a partial response (although no patients had a diagnosis of cholangiocarcinoma/gallbladder cancer; ref. 94). Other compounds targeting an NTRK1/2/3, ROS1, or ALK gene rearrangement have shown positive responses in the selected population (95). TRK inhibition is being explored in cholangiocarcinoma in a selected cohort in the STARTRK-2 phase II basket study of entrectinib (RXDX-101) in patients with solid tumors harboring an NTRK1/2/3, ROS1, or ALK gene rearrangement (NCT0256867). Sequencing studies have identified the presence of NTRK fusions in 1 of 28 (3.5%) of patients diagnosed with ICC (52). ROS1 and ALK fusions are also rare targets ICC, with a frequency of 0% to 8.7% (96, 97) and 2.6% (98), respectively.

### Protein Tyrosine Phosphatases

Protein tyrosine phosphatases (PTP) are a structurally diverse family of tightly regulated enzymes. Gao and colleagues (49) found frequent mutations in PTPN3 in ICC that were significantly correlated with tumor recurrence.

### Gene Profiling

Gene expression profiles, high-density single-nucleotide polymorphism arrays, and mutation analyses using formalin-fixed ICC samples from patients diagnosed with ICC identified two main biological classes of ICC (54). The first group, the inflammation class (38% of ICCs), was characterized by activation of inflammatory signaling pathways, overexpression of cytokines, and STAT3

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**Figure 2.** IDH1 and IDH2 are metabolic enzymes found in the cytoplasm and mitochondria, respectively, and catalyze the decarboxylation of isocitrate to alpha-ketoglutarate (α-KG), resulting in the reduction of NADP⁺ to NADPH. The oncometabolite 2-hydroxyglutarate (2-HG) can competitively inhibit one or more members of the family of over 60 dioxygenases which require α-KG as a cofactor. The dioxygenases include the JmjC family of histone demethylases and the TET family of methylcytosine dioxygenase enzymes that catalyze the demethylation of DNA. IDH and KRAS mutations can cooperate to drive the expansion of liver progenitor cells, development of premalignant biliary lesions, and progression to metastatic ICC. Agents targeting IDH1 and IDH2 are under development. m, mutant.
identified (12.5%; ref. 103).

PIK3CA

Activating mutations in the PI3K–AKT–mTOR pathway, activating mutations in PIK3CA, have been identified in bladder cancer (102). Regarding the classic PI3K–AKT–mTOR pathway and activating mutations in the ERBB family of proteins (including ERBB3, the majority of mutations were found in a hotspot in codon 104 (101). This pattern is not shared with cholangiocarcinomas (107). Interestingly, a more recent mouse model expressing the IDH2-mutant variants R140Q or R172K in adult mouse hepatocytes generated ICCs when combined with KRASG12D mice, suggesting the need for additional hits after the mutation of the IDH genes (76). IDH1/2 mutants reduced the expression of HNF4α, which is a master transcriptional regulator of hepatocyte differentiation. Notch1 activation as a transdifferentiating factor has also been observed in an animal model by Guest and colleagues (108).

In summary, the recent targeted and WES genomic analyses have enriched our understanding of the genetic landscape of BTCs and informed us on the most actionable signatures (Fig. 4). They have highlighted that (i) the genomic spectra vary significantly in different subtypes of BTCs; (ii) IDH mutations and FGFR2 fusions are the most common genetic alterations in ICC; (iii) frequent mutations occur in chromatin-remodeling genes: ARID1A, BAP1, and PBRM1; and (iv) mutation frequency in different genes varies by etiology and geographic regions.

Animal Models

Some of the efforts to generate animal models were primarily focused on well-known oncogenes such as KRAS and TP53 (104). The Notch and IDH pathways seem to have an oncogenic role in cholangiocellular malignancies. A transgenic mouse model (NotchIC::AlbCre) expressing the intracellular domain of Notch receptor-1 (NICD) in the liver was able to generate cholangiocarcinomas (a similar approach was previously used by Sekiya and colleagues, ref. 105; and Fan and colleagues, ref. 106) derived from hepatic progenitors. This model describes a subtype characterized by the overexpression of the Notch pathway with a different genetic background from bile-duct derived cholangiocarcinomas (107). Interestingly, a more recent mouse model expressing the IDH2-mutant variants R140Q or R172K in adult mouse hepatocytes generated ICCs when combined with KRASG12D mice, suggesting the need for additional hits after the mutation of the IDH genes (76). IDH1/2 mutants reduced the expression of HNF4α, which is a master transcriptional regulator of hepatocyte differentiation. Notch1 activation as a transdifferentiating factor has also been observed in an animal model by Guest and colleagues (108).

Gallbladder Subset Findings

A later study sequenced gallbladder carcinoma and cholangiocarcinoma separately. The analysis of 57 tumor-normal pairs with a double approach using WES and ultra-deep sequencing of a 283-gene panel gave a striking result; mutations in the ERBB family of proteins (including their downstream genes) were found in 35.8%, and, in the multivariable analysis, these cases had a worse outcome (P = 0.001). Among the 11.8% of mutations in ERBB3, the majority of mutations were found in a hotspot in codon 104 (101). This pattern is not shared with cholangiocarcinoma, suggesting that although both tumors originate from the biliary epithelium, they are genetically distinct. No IDH mutations have been identified in gallbladder cancer (102). Regarding the classic PI3K–AKT–mTOR pathway, activating mutations in PIK3CA have been identified (12.5%; ref. 103).
A model of cholangiocarcinoma using zebrafish was generated by inducing the coexpression of viral hepatitis-B and hepatitis-C core proteins, the first animal model showing the involvement of these viral proteins in the pathogenesis of cholangiocarcinoma (109). Classic models in rodents have used a carcinogen-induced model, usually diethylnitrosamine (DEN) and thioacetamide (TAA) and infection with *Opisthorchis viverrini* (110). Genetically engineered mouse models of cholangiocarcinoma were generated by targeting TP53, NF2, PTEN, SMAD4, and KRAS (110–112). In addition, in a transgenic mouse model, constitutive overexpression of ERBB2 in the basal layer of biliary tract epithelium led to the development of gallbladder adenocarcinoma (113).

Unfortunately, some of the key features of human disease, such as the genetic landscape, chronic inflammation, and cholestasis, are clearly underrepresented in these models. The latest published model, consisting of combining an activating mutation in *KRAS* and *PTEN* deletion, has not incorporated the new knowledge from NGS information yet (114). Little information about the involvement of the microenvironment
has been generated in animal models. Furthermore, there is no model of non-gallbladder extrahepatic cholangiocarcinoma.

**EMERGING THERAPIES**

**Targeting the Molecular Biology of BTC**

A deeper understanding of the natural behavior and activated pathways involved in BTCs is required to guide the development of new drugs, aiming to improve patient outcomes. A summary of the main pathways and potential targeted therapies is shown in Fig. 5 and Table 1.

**IDH Metabolism**

Results from the collective efforts of several groups to characterize IDH mutations have shown that (i) IDH1 mutation is more common than mutation of IDH2; (ii) the “hotspot” IDH1/2 mutations are point mutations located in the arginine 132 (R132) residue in IDH1 or the arginine 172 (R172) residue in IDH2 (47, 102, 115–118); (iii) these mutations are
**Table 1. Heat map summary of the status of evidence supporting known molecular biology involved in BTCs**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Supported by basic/preclinical research (including sequencing or animal models)</th>
<th>Supported by translational research (including pathway status analysis by immunohistochemistry or other techniques)</th>
<th>Clinical research with available results or ongoing clinical trials pending data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell proliferation (FGFR)</td>
<td>Yes ++</td>
<td>Yes ++</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell metabolism (IDH)</td>
<td>Yes ++</td>
<td>Yes ++</td>
<td>Yes</td>
</tr>
<tr>
<td>Angiogenesis (VEGF)</td>
<td>Yes +</td>
<td>Yes ++</td>
<td>Yes ++</td>
</tr>
<tr>
<td>Inflammation (IL6, TGFβ)</td>
<td>Yes +</td>
<td>Yes ++</td>
<td>No</td>
</tr>
<tr>
<td>Stroma and stemness (Wnt/β catenin pathway)</td>
<td>Yes +</td>
<td>Yes +</td>
<td>Yes</td>
</tr>
<tr>
<td>Stroma and stemness (c-MET/HGFR)</td>
<td>Yes +</td>
<td>Yes +</td>
<td>Yes</td>
</tr>
<tr>
<td>Stroma and stemness (Hedgehog pathway)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stroma and stemness (Notch pathway)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cell proliferation (KRAS–BRAF–MEK–ERK pathway)</td>
<td>Yes</td>
<td>Yes +</td>
<td>Yes +</td>
</tr>
<tr>
<td>Cell proliferation (HER family growth factor receptors: EGFR, HER2)</td>
<td>Yes</td>
<td>Yes +</td>
<td>Yes +</td>
</tr>
<tr>
<td>Cell proliferation (PI3K–AKT–mTOR pathway)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tumor suppressor genes [p53, p16 (CDKN2A/B), SMAD4]</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Chromatin remodeling (ARID1, BAP1, PBRM1)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

NOTE: Available evidence is classified according to the type of research: basic/preclinical, translational, or clinical research. The table indicates if there is any evidence available (yes, gray; no, white); moreover, the evidence is ranked as follows: + (light gray, poor quantity/quality; retrospective data, absence of prospective/randomized studies), ++ (mid-dark gray color, medium quantity/quality; prospective clinical trials), and +++ (dark gray, high quantity/quality; randomized trials). Please refer to the main text for references applicable for each pathway.

ubiquitously higher in ICC than in ECC (118); (iv) the prognostic significance of IDH mutations remains conflicting in cholangiocarcinoma (47, 115–117); and (v) the mutant IDH loses its normal enzymatic activity and gains a new ability to produce the oncometabolite 2-hydroxyglutarate (2-HG), which can be detected in the tumor and blood (102, 119).

Pharmacologic inhibitors highly specific to the individual IDH-mutant alleles (e.g., to IDH1R132 and IDH2R172) have been developed. These block the function of mutant IDH1 or IDH2 at nanomolar concentrations, leading to reduced 2-HG levels (Fig. 2). Rohle and colleagues found that a selective IDH1R132H inhibitor (AGI-5198) impeded the growth of IDH-mutant glioma cells (120). Similarly, Wang and colleagues demonstrated that AGI-6780 could selectively inhibit leukemic cells harboring mutant IDH2R140Q (121). In a phase I trial, AG-120 (IDH1 inhibitor; Agios) was well tolerated among patients with advanced solid tumors with IDH1 mutations (NCT02073994). There were no dose-limiting toxicities, and anemia was the most frequent grade 3 adverse event (5%). Of the 20 patients with advanced ICC, 1 patient (5%) achieved a partial response and 11 patients (55%) had stable disease. In all patients responding to AG-120, a reduction in circulating 2-HG levels ranging from 73% to 99% and a reduction in Ki67 staining ranging from 22% to 96% from baseline were observed. The expansion phase with 500 mg once daily is under way. Other IDH1...
and IDH2 inhibitors have entered clinical trials recently (NCT02273739, NCT02381886, and NCT02481154) and are enrolling patients with ICC. Through a high-throughput drug screen of a large panel of cancer cell lines, including 17 BTCs, we recently found that ICC cells harboring IDH mutations exhibited a striking response to the multi-TKI dasatinib (122). In addition, dasatinib-treated IDH-mutant xenografts demonstrated pronounced apoptosis and tumor regression. A trial with dasatinib in patients with IDH-mutant advanced ICC is ongoing (NCT02428855).

**FGFR**

The recent discovery of recurrent FGFR2 fusions in 11% to 45% of patients with ICC, described previously, has opened a promising therapeutic avenue (52, 81–83, 123, 124). In genomic profiling studies, FGFR2 fusions are found concurrently with mutations in genes such as ARID1A, PBRM1, and TP53, among others (79). Histologically, FGFR2 fusions are associated with prominent intraductal growth and anastomosing tubular glands (123); prognostically, they appear to be associated with an indolent disease course and prolonged survival (83, 123).

The discovery of FGFR aberrations in multiple tumor types has stimulated pharmaceutical and scientific interest in the development of FGFR inhibitors. The earliest reported data of selective FGFR inhibition in cholangiocarcinoma are the oral agent BGJ398 (infigratinib; Novartis), which is currently being evaluated in a phase II trial of previously treated patients with FGFR-aberrant tumors, including FGFR2 fusion–positive advanced ICC. Preliminary data from the phase I/II basket trial indicate that 3 of the 12 patients with FGFR2 fusion–positive advanced ICC treated with ARQ 087 had a partial response (with a disease control rate of 75%; ref. 127).

FGFR2 fusions appear to be driver alterations that predict sensitivity to FGFR inhibition in ICC, but the impact of the fusion partner and the sensitivity of FGFR mutations and amplifications to FGFR inhibition in ICC is yet unknown. Circulating levels of FGF ligands such as FGF19, FGF21, and FGF23 showed some correlation with response in the ARQ 087 trial (128), but further investigation into these biomarkers and others is warranted.

The safety profile of FGFR inhibitors is manageable, with hyperphosphatemia being one of the most common toxicities. This is a class effect due to on-target blockade of FGF23 in the bone and kidney. FGFR2 is a phosphaturic hormone that regulates phosphorus excretion in the proximal tubule of the kidney, and inhibition of this hormone leads to phosphate reabsorption (129). FGFR inhibitors can also cause nail changes with onycholysis, mucosal dryness, ocular toxicity, nausea, anorexia, diarrhea, and constipation, and adequate management of the toxicities will be key to further development of this class of drugs.

**Angiogenesis**

Not only are the ligands regulating angiogenesis (particularly VEGF) commonly present (40%–75%) in BTCs (130–132), but their expression is colocated with their receptors VEGFR1 and VEGFR2 in endothelial cells adjacent to the tumors (133). This appears to be most evident at the invasive edge of the tumor (134). VEGF expression is associated with a number of adverse prognostic features, including the presence of metastases in ICC (132) and increased microvascular density (MVD) in both cholangiocarcinoma (131) and gallbladder cancer (130). MVD is an independent prognostic factor for disease-free survival following resection of ECC (134) and for OS in lymph node–negative ICC (135) and gallbladder cancer (134). MVD is also an independent negative predictor of OS in ECC (136). Consequently, a number of clinical trials have evaluated VEGF inhibition.

In a phase II trial of bevacizumab combined with gemcitabine and oxaliplatin, our group demonstrated a significant decrease in standardized uptake values on FDG-PET scans after two treatment cycles, particularly in patients with a partial response or stable disease (137). However, the 6-month progression-free survival (PFS; 63%) was below the target rate of 70%. Combining bevacizumab with erlotinib (an anti-EGFR TKI) achieved partial responses in 12% of patients and stable disease in 51%, with a median OS of 9.9 months, notably in the absence of concurrent chemotherapy (138).

Cediranib is an oral VEGFR1, VEGFR2, and VEGFR3 TKI, with activity against PDGF receptors and c-KIT. In the randomized phase II, placebo-controlled ABC-03 study, we...
observed an improved response rate in patients receiving cisplatin/gemcitabine with cediranib (44%) versus placebo (19%; \( P = 0.0036 \)) and improved 6-month PFS (70.5% vs. 61.3%; \( P > 0.05 \)). However, the study did not meet its primary endpoint (improvement in median PFS), in part due to the relatively poor tolerability of cediranib (139).

Forays into VEGF inhibition with other TKIs have been disappointing. Single-arm, phase II studies of sorafenib as monotherapy (140, 141) or combined with erlotinib (142) or cisplatin/gemcitabine (143) have all failed to demonstrate sufficient activity in BTC. Most recently, sorafenib failed to improve PFS when added to gemcitabine in a randomized phase II, placebo-controlled study (144). A phase II clinical trial of sunitinib including 56 patients with BTC reported a median time to progression of only 1.7 months, an objective response rate of 8.9% and a disease control rate of 50% (145).

In addition, the VanGogh study failed to show an improvement in PFS in a 3-arm randomized phase II study exploring the role of vandetanib in 173 patients (146). Results of ongoing studies with pazopanib (NCT01855724), regorafenib (NCT02053376 and NCT02115542), and ramuciruban (NCT02711553) are awaited.

**HER Family**

EGFR amplifications and mutations have been described in around 6% and 13.6% to 15% of BTCs, respectively. However, the biological implication of these mutations is unclear (147–149).

Several phase II clinical trials have combined cetuximab, a monoclonal antibody targeting EGFR, with chemotherapy in the treatment of BTCs, most of them with gemcitabine and oxaliplatin (150–153). Initial promising results reporting high tumor response rates (63%) from a small study (\( n = 30; \) ref. 151) were not confirmed in the randomized phase II BINGO study, in keeping with results from other negative phase II studies combining cetuximab with chemotherapy (153).

KRAS wild-type patients with advanced BTC were treated with panitumumab combined with gemcitabine, capecitabine, and oxaliplatin (46 patients; ref. 154) and with gemcitabine and oxaliplatin (31 patients; ref. 155) in two separate phase II clinical trials. In each study, the primary endpoint was achieved [6-month PFS of 74% (95% CI, 58–84; ref. 154) and response rate of 45% (155), respectively]. A phase II study (panitumumab with gemcitabine and irinotecan in nonselected patients) also supported further development of this compound in BTC with no difference in OS by KRAS status (7 of 31 patients harbored a KRAS mutation; ref. 156). Unfortunately, these signals have not been confirmed in other studies (157, 158), including the largest randomized phase II study combining panitumumab with gemcitabine and oxaliplatin (the Vecti-BIL study; ref. 158) which showed no differences in survival in 85 randomized patients.

Erlotinib, a TKI targeting EGFR, has also shown varying results (159–161). In the largest (phase III) study, 133 patients were randomized to receive gemcitabine and oxaliplatin chemotherapy with or without erlotinib (160); there were no differences in PFS or OS when all patients with BTC were analyzed together. However, the cholangiocarcinoma patient subgroup did appear to derive benefit from adding erlotinib to chemotherapy [median PFS 5.9 months (95% CI, 4.7–7.1) vs. 3.0 months (95% CI, 1.1–4.9); \( P = 0.049 \)], and further clinical trials are ongoing (NCT00832637 and NCT00987766).

**HER2** (v-ERBB2, erythroblast leukemia viral oncogene homolog-2) overexpression and gene amplification are also described in BTCs, with a higher incidence in gallbladder cancer (19%; ref. 162). The rate of HER2 overexpression was found to be higher in ECC (17.4%) than in ICC (4.8%) in a recent systematic review and meta-analysis published by Galloy and colleagues in 2016 (162). Good correlation between overexpression and gene amplification (75%) has been shown (148). Two phase II trials have yielded disappointing results of first-line/second-line lapatinib monotherapy in an unselected population of patients with advanced BTC (163, 164). Case reports using trastuzumab in patients with gallbladder carcinoma with HER2 overexpression have suggested activity (165, 166), and a phase II clinical trial is under way (NCT00478140). Afatinib has shown activity in one patient diagnosed with cholangiocarcinoma in a phase I clinical trial (167); a phase I study of afatinib combined with cisplatin and gemcitabine in patients with BTC is ongoing (NCT01679405).

**Targeting WNT/β-catenin, Hedgehog, and HGF/c-MET**

The WNT/β-catenin pathway is involved in the regulation of cell invasion and migration. High nuclear expression with low membranous expression of β-catenin expression has been described in ICC (15%; ref. 168); WNT signaling seems to be most relevant in hilar cholangiocarcinoma (169). WNT pathway activation was associated with chemoresistance and metastatic spread in a cholangiocarcinoma xenograft tumor model (170), and WNT inhibition reversed chemoresistance in cell lines (171). In contrast, its impact and the mutational status of this pathway’s components are not completely understood in BTC (172, 173). Whole-exome sequencing of *Opisthorchis viverrini*-related cholangiocarcinoma identified mutations in the WNT pathway [**RFN43** (9.3%; refs. 45, 174)]. β-catenin expression is associated with decreased apoptosis in gallbladder cancers (175), and Yadav and colleagues showed that most of the genetic variants of WNT signaling pathways that were evaluated influenced gallbladder cancer susceptibility (176). Although multiple WNT pathway inhibitors are currently under development (177), only a few clinical trials have been reported for BTC as of yet. Eads and colleagues explored the safety of DKK1, an inhibitor of the canonical WNT/β-catenin pathway, in combination with gemcitabine and cisplatin in a phase I clinical trial enrolling patients with BTCs (178). The combination was found to be safe, suggesting possible prolonged disease stabilization; further development is awaited.

The Hedgehog pathway may also be involved in the development of BTC (179–182). Among gallbladder cancer specimens, expression of Hedgehog pathway components by IHC has been described [**SHH** (81.7%), **PTCH1** (75.3%), and **GLI1** (70.0%) with impact on stage and lymph node, venous, and hepatic infiltration; patients with activated Hedgehog...
pathway had more aggressive tumors and worse outcome (183). Similar findings have been described in cholangiocarcinoma [SHH (87.8%), PTC1H (89.2%), GLI1 (85.4%); ref. 184]. Suppression of the Hedgehog pathway in gallbladder (185) and cholangiocarcinoma (186) cell lines implanted in mouse xenografts inhibited epithelial–mesenchymal transition and reduced tumor volume, suggesting this pathway as a potential new target.

c-MET tyrosine kinase plays an integral role in carcinogenesis by promoting angiogenesis, tumor invasion, and metastasis. Binding of this receptor by the ligand hepatocyte growth factor (HGF) activates multiple downstream signal transduction pathways, including the GRB2–RAS–MAPK cascade and the PI3K, EGFR, VEGF, and RAC1–CDC42 pathways (187). c-MET overexpression, associated with a poor prognosis in cholangiocarcinoma (188), is seen in 12% to 58% of ICCs (151, 188, 189) and 0% to 16% of ECCs (151), a wide variation likely accounted for by differences in the c-MET antibody used, definition of positivity, analysis of resection versus late-stage biopsy samples, and sample size per study. c-MET amplification is rare in cholangiocarcinoma, but has been reported at a frequency of 7% in one study of 26 cases of ICC analyzed by NGS (52). In addition to the above-mentioned effect in cholangiocarcinoma, the HGF–c-MET pathway promotes proteolytic activity and induces cellular motility, essential for the invasive progression of gallbladder carcinoma cell lines (190). In human tissue, c-MET expression is higher in cancer cells than in normal gallbladder tissue (191), up to 74% in some series (192).

Significant cross-talk has been demonstrated between the c-MET pathway and other pathways such as the EGFR and VEGF pathways in other tumor types. c-MET amplification has been shown to drive resistance to EGFR inhibitors via ERBB3-dependent activation of PI3K (193). Similarly, tumor hypoxia, which can be a consequence of VEGF pathway inhibition, has been shown to upregulate c-MET and enhance scatter and invasiveness (194). Thus, dual inhibition of c-MET with other pathways may be a strategy in cholangiocarcinoma. Cabozantinib, which has potent activity against both c-MET (IC50 = 1.3 nmol/L) and VEGFR2 (IC50 = 0.035 nmol/L), was tested in a phase II trial in patients with advanced cholangiocarcinoma; preliminary data showed minimal activity, with early trial discontinuation after 12 of 19 patients failed to be progression-free at 16 weeks (195). A randomized phase II study with merestinib in addition to cisplatin/gemcitabine as first-line therapy is ongoing (NCT02711553).

**KRAS–BRAF–MEK–ERK Pathway**

As in many other cancers, the RAS–RAF–MEK–ERK signal transduction pathway is frequently dysregulated in cholangiocarcinoma (196). The binding of ligands, including EGF and PDGF, to the receptors triggers a cascade of activation of downstream signaling molecules. Activated RAS triggers phosphorylation and activation of RAF kinase, leading to end phosphorylation of MEK1 and MEK2. Activated MEK phosphorylates ERK1 and ERK2, the only known substrates. Phosphorylated ERK (pERK) then dimerizes and translocates to the nucleus (197), where it regulates several important cellular functions. Gain-of-function KRAS mutation with a frequency of 9% to 40% has been reported in cholangiocarcinoma (52, 83). KRAS mutation has been associated with perineural invasion, advanced stage, and poor prognosis (198). KRAS mutations have also been found in up to 7.8% of gallbladder cancers (101, 199). BRAF mutations seem not to be significant in gallbladder cancer and appear to be restricted to ICC only (199, 200). However, other groups’ results show mutation rates between 5.9% (101) and 33% (201).

Despite the recognized frequency of KRAS mutations, targeting this pathway remains challenging. BRAF is a proto-oncogene and is a key component of the RAS–RAF–MEK–ERK proliferation signaling pathway. The most common BRAF gene mutation found in human cancers is V600E, with varying frequency reported in cholangiocarcinoma (202). In a recent phase II basket study of vemurafenib in BRAFV600E-mutated non-melanoma cancers, one patient with cholangiocarcinoma achieved a durable partial response of over a year (203).

MEK is also an attractive target, as ERK1 and ERK2 are the only known MEK substrates (204). Early evidence of the efficacy of a MEK inhibitor was reported in a single-arm study of selumetinib in advanced BTCs (205). Of 29 patients enrolled, 25 were evaluable for response: 3 patients (12%) had confirmed partial responses and 17 (68%) had stable disease. The median PFS was 3.7 months (95% CI, 3.5–4.9), and median OS was 9.8 months (95% CI, 5.9–not available). In this study, no BRAFV600E mutations were found, but the absence of pERK staining appeared to be associated with a lack of response to selumetinib. Recently, in the ABC-04 phase Ib study, we assessed the safety and tolerability of selumetinib in combination with cisplatin/gemcitabine in advanced BTC; 3 of 8 patients evaluable for response had partial responses. Selumetinib-related toxicities were manageable and included grade 1 to 2 edema and rash (206).

Given well-known redundancy and cross-talk in this pathway, novel combined strategies targeting different molecules within this pathway or different pathways represent attractive approaches in cholangiocarcinoma.

**The PI3K–AKT–mTOR Pathway**

This pathway is upregulated in cholangiocarcinoma cells; moreover, activation of this pathway is associated with adverse prognosis in some patients with BTC (207) and good prognosis in others. Some studies have shown that somatic PIK3CA mutations contribute to the frequent activation of the PI3K–AKT pathway in BTC (208).

In a study of 212 ECC cases, patients with high pAKT expression had shorter survival than those with low pAKT expression (P = 0.06). Cases with high p-mTOR expression also showed shorter survival (P = 0.06). Patients with low PTEN expression (median survival, 18 months) had a significantly worse survival time than those with high PTEN expression (median survival, 39 months; log-rank test P = 0.004; ref. 209). Conversely, a study on 101 ICCs showed the aberrant expression of pAKT1 and p-mTOR was associated with a favorable prognosis regardless of PTEN (210). PTEN overexpression was found as an independent favorable prognostic factor for patients with ICC. In addition, the overexpression of p-mTOR was more frequently observed in well-differentiated to moderately differentiated tumors.
and in tumors without metastasis. The comparison of these two studies underlined the difficulties in comparing different BTCs due to biological differences depending on their primary location and, ultimately, cell of origin. In addition, the redundancy and cross-talk involving this intracellular pathway makes targeting a single point/level unlikely to be a successful approach.

MK-2206 is an oral selective allosteric inhibitor of AKT that targets all three isoforms of human AKT (AKT1, AKT2, and AKT3) with IC_{50} values of 8, 12, and 65 nmol/L, respectively. An abbreviated phase II study using this compound in 8 patients with at least one prior systemic treatment was disappointing, with a median PFS of 1.7 months and median OS of 3.5 months (211).

A first-line phase II study with everolimus showed evidence of antitumor activity, with 14 out of 27 patients (56%, 95% CI, 35–76) achieving tumor control at 12 weeks and 2 of them achieving partial response. The median PFS was 6.0 months (95% CI, 2.1–11.2), and median OS was 9.5 months (95% CI, 5.5–16.6). Correlative studies suggest that KRAS mutational status and basal pAKT levels might be associated with resistance to everolimus treatment (212).

A phase II trial using a PI3K inhibitor, copanlisib (BAY 80-6946), as first line in combination with gemcitabine and cisplatin is ongoing (NCT02631590).

**CURRENT STATUS OF EMERGING TARGETED THERAPIES**

Currently, the most promising targets under development due to a more solid preclinical research background are IDH inhibitors for IDH-mutant BTC and molecules targeting FGFR2 gene fusions (Fig. 4 and Fig. 5). A window of opportunity is open, with new drugs in development targeting chromatin remodeling gene mutations (ARID1, BAP1, and FBRM1) such as bromodomain and extra-terminal (BET) inhibitors (213). Most of the remaining molecular targets that have been tested in clinical trials have been somewhat disappointing, with conflicting data and negative trials, underlining the need for new models and new approaches to unravel the complex molecular biology of BTC (Table 1).

**Is Precision Medicine Regarding Targeted Therapies in BTC Ready for the Clinic?**

As in other malignancies, the meaningful decrease in the cost of NGS technologies has opened the door for more sophisticated trials in which different molecular subtypes of a malignancy can be matched to targeted inhibitors. Obtaining tumor molecular profiling on patients who are fit to enroll in clinical trials beyond first-line systemic therapy may offer these patients additional promising treatment options. However, obtaining sufficient tissue for such analyses in BTC can be difficult, making this approach more challenging. For this scenario, the use of liquid biopsies [circulating tumor cells (CTC), cfDNA, exosomes, etc.], when validated, may lead the way to such approaches in these neoplasms.

**Role of Immunotherapy**

The relationship between chronic inflammation and the development of BTC has led investigators to harness the immune response through vaccination, adoptive immunotherapy, and checkpoint inhibition. Immune cells (both innate and adoptive) are present in BTCs; this appears to be stage dependent (for macrophages), and the presence of dendritic cells, CD4+ helper T-lymphocytes, CD8+ cytotoxic T-lymphocytes, and B-lymphocytes/plasma cells is associated with improved survival (214).

Vaccination studies have yielded modest results in monotherapy; the commonest targets are Wilm’s tumor-1 (WT1) and mucin protein 1 (MUC1). WT1, a transcription factor, is also a tumor suppressor through interaction with PDGFR, EGFR, c-MYC, and BCL2. A phase I study in combination with gemcitabine showed that patients demonstrating a T-cell response to WT1 vaccination had a longer OS than patients treated with gemcitabine only (215). MUC1, a glycoprotein forming a hydrophilic barrier to hydrophobic cytoytic agents and immune surveillance, is highly overexpressed in gallbladder cancers (90%), and less so in cholangiocarcinoma (59%–77%), and is associated with advanced stage and impaired survival. An early study showed that MUC1 vaccination did not translate into clinical benefit despite achieving an IgG response (216). A dendritic cell-based vaccine targeting MUC1 in patients with resected pancreatic cancer and BTC (with adjuvant chemotherapy or radiotherapy as appropriate) saw 4 of 12 patients disease free at four years (217). Expanding vaccination to target two (218), three (219), or four (220) peptides, or even “personalizing” the vaccination (221), holds promise but remains investigational. Defining the optimal target among heterogeneous entities within BTC, vaccination against single versus multiple targets, and definition of optimal adjuvants are required.

Shimizu and colleagues vaccinated patients with resected ICC with autologous tumor lysate–pulsed dendritic cells plus ex vivo–activated T-cell transfer (adoptive immunotherapy). These patients had a near-double OS (31.9 vs. 17.4 months, P = 0.022) compared with surgery-alone patients, most marked in patients with prominent skin reactions (222).

Mutational load is known to be high in tumors in which immunotherapies have been shown to be effective, such as melanoma and lung cancer (223). Based on a similar rationale, efficacy of checkpoint inhibitors in tumors with mismatch-repair (MMR) deficiency was proven to be successful in a phase II study, achieving up to 40% objective responses (224). Mutational load has shown to be high in BTCs (88). In addition, MMR and microsatellite instability (MSI) have been explored in BTCs. MMR and MSI have been suggested to be frequent in BTCs without hereditary nonpolyposis colorectal cancer (225). Results vary between series; high-level MSI has been shown in 5% of gallbladder carcinoma (226), 5%–13% of ECC (226, 227), and up to 10% of ICC (226). MMR status (hMLH1 and hMSH2 negativity) was shown in 51.3% and 59% of gallbladder carcinoma and 57.1% and 65.7% of ECC, respectively (228). In addition, O(6)-Methylguanine–DNA methyltransferase (MGMT) methylation was identified in 59% of gallbladder carcinoma and 60% of ECC (228). Both MGMT methylation and MMR status correlated with poor prognosis in gallbladder carcinoma and ECC (228).

A case report of tumor-infiltrating lymphocytes from a patient with metastatic cholangiocarcinoma containing CD4+ T-helper-1 cells recognizing a mutation in ERBB2-interacting
protein induced an impressive and durable response; moreover, this effect was reproduced after subsequent disease progression (229). Adoptive immunotherapy studies in Thailand (NCT018668490) and the United States (NCT01174121) are ongoing.

Holcombe and colleagues explored a cohort of BTC samples (126 ECC, 434 ICC, 244 gallbladder cancer, and 11 not specified) and identified high PD-1 (40%) and PD-L1 in (15%) expression (50). In the BTC cohort of KEYNOTE-28 (NCT02054806), 37 of 89 patients (42%) were PD-L1-positive (defined as ≥1% staining of cells in tumor nests or PD-L1-positive bands in stroma by IHC). Four of 24 patients (17%) treated with pembrolizumab, a highly selective humanized monoclonal antibody targeting PD-1, had a partial response, with another 4 achieving stable disease; 5 patients entered long-term treatment, including all 4 responders (230). These encouraging results suggest that this strategy is worth pursuing (a phase I study in combination with FOLFOX chemotherapy, with an expanded phase II cohort in BTC, is under way, NCT02268825), along with validation of PD-L1 expression as a predictive biomarker, evaluation of the role of PD-L2 expression, and assessment of efficacy in the various BTC subgroups as well as in patients with MMR-deficient tumors (224).

CONCLUSION

The treatment paradigm for patients with advanced BTC is evolving; through international collaboration, BTCs are no longer considered “too rare” for adequately powered clinical studies. Emerging evidence suggests that BTC encompasses subgroups with discrete driver mutations, some of which are targetable with novel therapies. The role of conventional therapies (chemotherapy and radiotherapy) has yet to be fully defined, particularly in the adjuvant and second-line settings. In addition, investigation of a number of pathway-targeted therapies, as well as modulation of the immune environment, holds promise for patients with these diseases. Given the low prevalence of BTC, clinical development must go hand-in-hand with sound basic and translational research.

Disclosure of Potential Conflicts of Interest

The authors would like to thank Stephanie L. Reyes for her assistance with Figs. 2 and 4.

Grant Support

J.W. Valle received research funding from Cancer Research UK (CRUK) and The Alan Moremen Memorial Fund (AMMF). A. Lamacra received funding from the Spanish Society of Medical Oncology (SEOM) Fellowship, the Cholangiocarcinoma Foundation Research Fellowship, and the Conquer Cancer Foundation Young Investigator Award. J. Barriuso is partly funded by the Spanish Society of Medical Oncology (SEOM) Fellowship. L. Goyal is supported by the MGH Executive Committee on Research Fund for Medical Discovery Award, the Cholangiocarcinoma Foundation Research Fellowship, the NIH Loan Repayment Program Grant, the Jonathan Kraft Translational Award, and NIH/NCI Gastrointestinal Cancer SPOREPS0 CA127003. A. Zhu is supported by the TargetCancer Foundation, a Translational Research Grant Award from the V Foundation for Cancer Research, and the Jonathan Kraft Translational Award.

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