New Horizons for Precision Medicine in Biliary Tract Cancers

Juan W. Valle[1,2], Angela Lamarca[1], Lipika Goyal[3], Jorge Barriuso[1,4], and Andrew X. Zhu[3]

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 a rise 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). *Opistorchis 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].

[1]Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Manchester, UK. [2]Institute of Cancer Sciences, University of Manchester, Wilmslow Road, Manchester, UK. [3]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts. [4]Faculty of Medical, Biological and Human Sciences, University of Manchester, Rumford Street, Manchester, UK.

Corresponding Authors: Juan W. Valle, Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Manchester M20 4BX, UK. Phone: 44-161-446-8106; Fax: 44-161-446-3468; E-mail: juan.valle@christie.nhs.uk; and Andrew X. Zhu, Massachusetts General Hospital Cancer Center, Harvard Medical School, 55 Fruit Street, Boston, MA 02114. Phone: 617-726-2000; Fax: 617-724-1137; E-mail: azhu@mgh.harvard.edu

doi: 10.1158/2159-8290.CD-17-0245

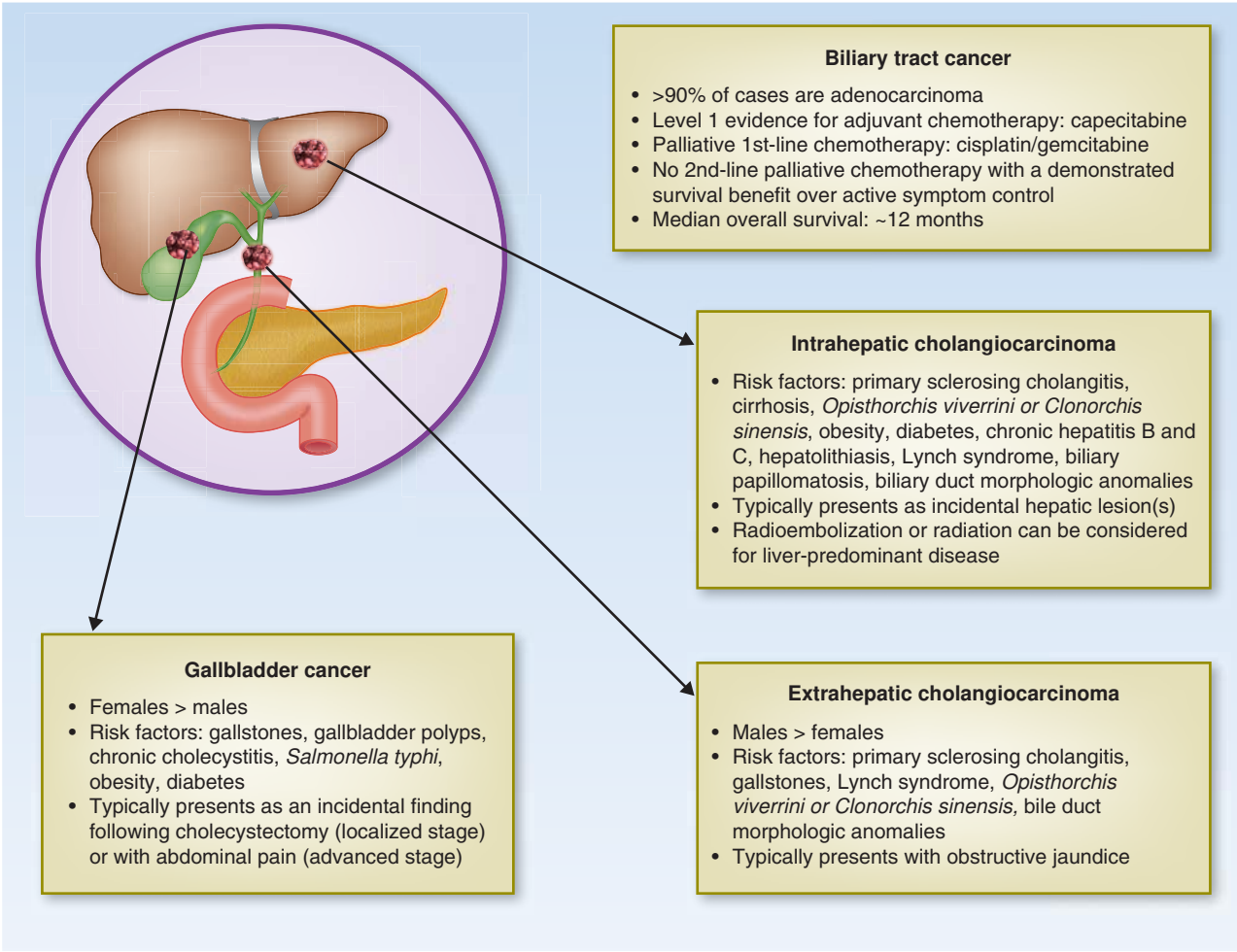

Figure 1. BTCs are a group of different diseases which includes ICC, ECC, and gallbladder cancer. They differ in many aspects, such as anatomical location, demographics, clinical presentations, and treatment options.

CLINICAL 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;

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 capecitabine and observation arms, respectively]. There was also benefit from adjuvant capecitabine in terms of relapse-free survival [median 25 months (95% CI, 19–37) and 18 months (95% CI, 13–28) for capecitabine and observation arms, respectively]. Based on these results, adjuvant capecitabine 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 porcupine 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 in 57% of cases. This finding suggested the possibility of a particular subtype of cholangiocarcinoma where alterations in chromatin packaging have driven the development of the disease.

ROBO2 is a receptor protein involved in activating the SLIT–ROBO pathway. These proteins are key components of the axon guidance signaling and have been recently implicated in pancreatic adenocarcinoma (71).

GNAS encodes a guanine nucleotide-binding protein alpha subunit (72), which is frequently mutated in intraductal papillary mucinous neoplasms of the pancreas and villous adenoma of the colon (73, 74).

IDH Alterations

Mutations in the isocitrate dehydrogenase genes (*IDH1* and *IDH2*; Fig. 2; refs. 75, 76) were found more often in noninfectious cholangiocarcinomas (76). *IDH1* and *IDH2* mutations were also found (19%) by the Johns Hopkins group. These mutations were clustered in previously identified hotspots (codons 132 and 172, respectively; ref. 77, 78) and were associated with a worse prognosis in contrast to the previously published report (47). These differences in prognosis may be accounted for by the differing sample size and baseline characteristics of the two studies. In a Chinese study, only five (4.9%) patients with ICC harbored *IDH1* mutations (62).

FGFR Pathway

Genome-wide structural analyses showed recurrent translocation events involving the *FGFR2* locus (48). Wu and colleagues published the first report of *FGFR* fusions in ICC in 2013 with a description of 2 cases of *FGFR2–BICC1* fusions (79). BICC is a negative regulator of WNT signaling (80). Tumor profiling studies of ICC have reported multiple additional fusion partners with *FGFR2,* including *AHCYL1, TACC1, MGEA5*, and *PPHLN* (48, 81–83), all of which fuse at a consistent breakpoint within the *FGFR2* gene on chromosome 10 (48). The mechanism by which *FGFR2* fusions drive oncogenesis is under active investigation. Arai and colleagues showed that in clones expressing *FGFR2–BICC1* and *FGFR2–AHCYL1*, the MAPK pathway was activated but not AKT or STAT, suggesting that FGFR2 fusion proteins activate canonical signaling events downstream of FGFR (81). A mutation in *ERRFI1*, a negative regulator of the EGFR family, was found that was not present in cases having alterations of *FGFR* (81). ERBB receptor inhibitor-1 has a role as a negative regulator of the EGFR family of receptors (84–87). Thus, patients harboring this mutation may be suitable for an anti-EGFR treatment approach.

Another cooperative effort of sequencing intrahepatic cholangiocarcinoma tumor samples confirmed the presence of *FGFR2* fusions in 3 of 28 tumor samples (10.7%). 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 *BAP1* (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. BAP1 is a deubiquitinase protein of the ubiquitin C-terminal hydrolase (UCH) family (89–91). Increased proliferation was shown after *BAP1* 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 *BAP1*. 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 (NTKR) 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,

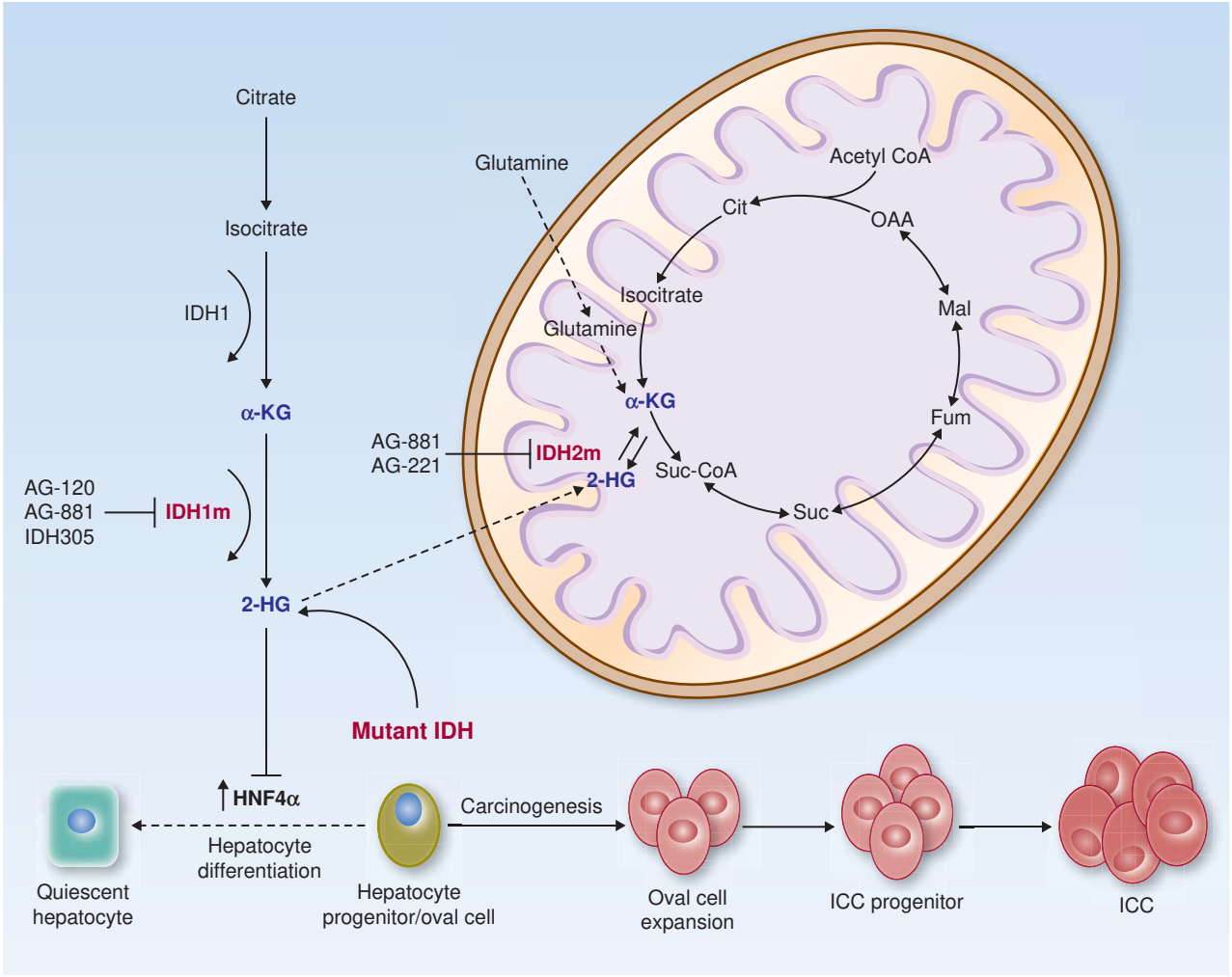

Figure 2. IDH1 and IDH2 are metabolic enzymes found in the cytoplasm and mitochondria, respectively, and catalyze the decarboxylation of isocitrate to alpha-ketoglutarate (α-KG), resulting in the reduction of $NADP^+$ to NADPH. The oncometabolite 2-hydroxyglutarate (2-HG) can competitively inhibit one 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.

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

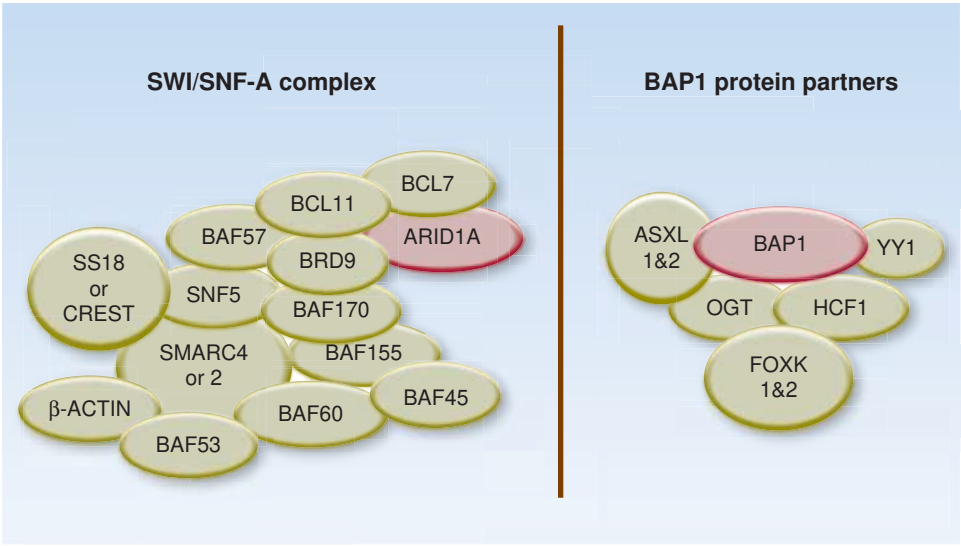

Figure 3. Chromatin remodeling complex: DNA is packaged in chromatin to allow the 1.8 m–long human genome to fit in a single cell of the body. SWI/SNF complexes are evolutionarily conserved, ATP-dependent, molecular machines that alter local chromatin structure. *ARID1A* encodes an accessory subunit of the SWI/SNF chromatin remodeling complex. ARID1A, AT-rich interactive domain-containing protein 1A; BAF, BRG1 associated factor; BRD, Bromo domain containing protein; SMARC, SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, also known as *BRG*: Brahma related gene; BAP1, BRCA1-associated protein-1 ASXL: additional sex combs-like; OGT, UDP-glucose-dependent O-glucosyltransferase; HCF1, host cell factor 1; YY1, Ying Yang 1; FOXK, Forkhead box protein K.

activation. In contrast, the proliferation class (62% of ICCs) was characterized by activation of oncogenic signaling pathways and was associated with shorter survival. Andersen and colleagues also characterized ICC based on the genomic as well as transcriptomic signatures and were able to classify ICC into subclasses with different prognoses (99). Recently The Cancer Genome Atlas (TCGA) study in ICC was published, and this integrated analysis of somatic mutations, RNA expression, copy number, and DNA methylation also led to a molecular classification scheme and identified an *IDH* mutant–enriched subtype with distinct molecular features, including low expression of chromatin modifiers, elevated expression of mitochondrial genes, and increased mitochondrial DNA copy number (100).

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).

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 $KRAS^{G12D}$ 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).

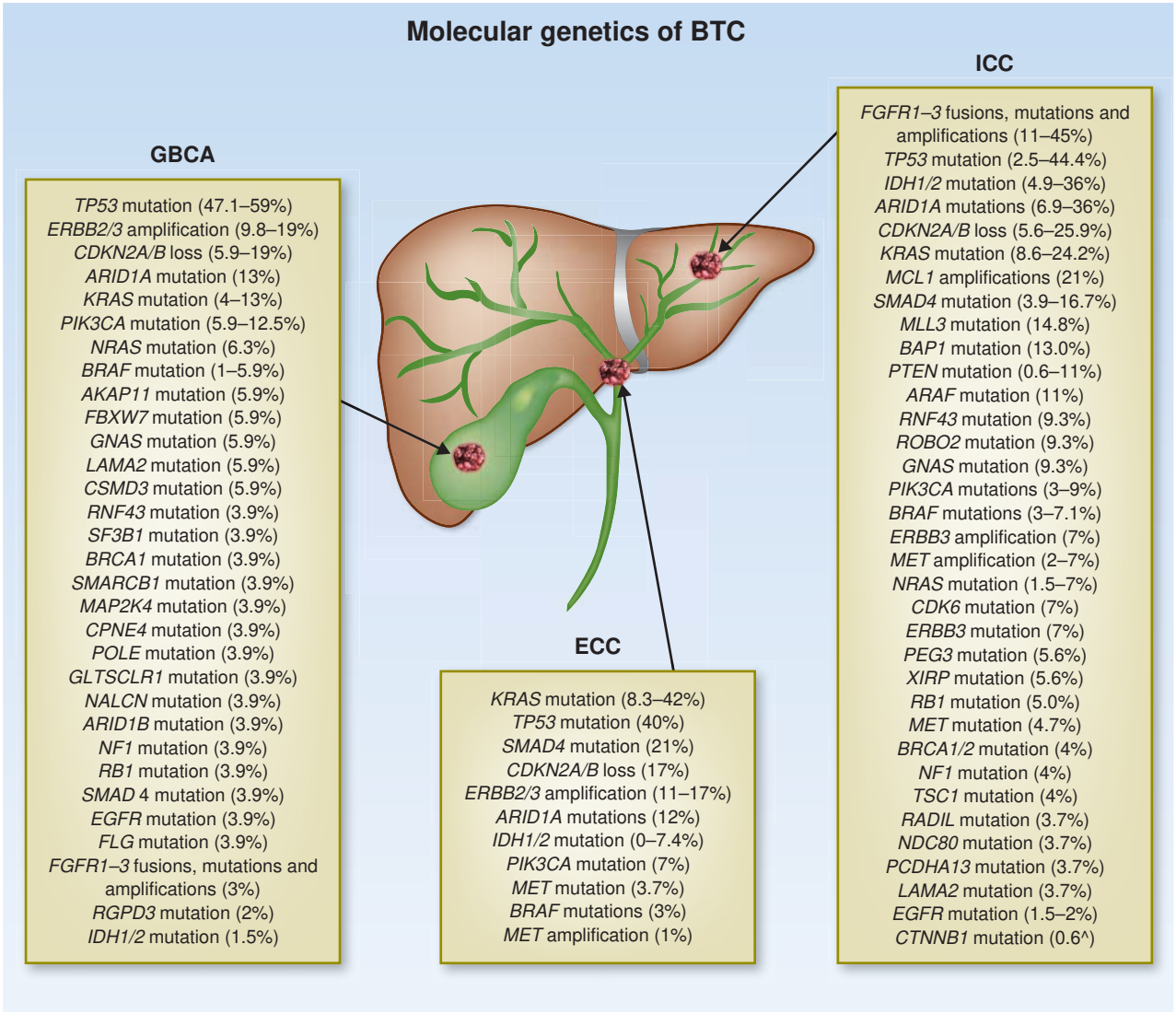

Figure 4. Genetic landscape of BTC. Most frequent genetic aberrations in targetable pathways of interest in BTC. The mutation is quoted as the highest to lowest with range from different reports on each mutation. Those without range come from single reports. Extracted from Desphande et al. BMC Cancer 2011 (103), Borger et al. The Oncologist 2012 (102), Voss et al. Human Pathology 2013 (204), Ross et al. The Oncologist 2014 (52), Ong et al. Nature Genetics 2012 (45), Graham et al. Human Pathology 2014 (123), Arai et al. Hepatology 2014 (81), Sia et al. Nature Communications 2015 (82), Javle et al. Cancer 2016 (53), Zou et al. Nature Communications 2014 (62), Li et al. Nature Genetics 2014 (101), Zhu et al. Annals of Surgical Oncology 2014 (116), Sia Gastroenterology 2013 (54), Jiao et al. Nature Genetics 2013 (47), Chan-On et al. Nature Genetics 2013 (46), Wang et al. Oncogene 2013 (115), Wu et al. Cancer Discovery 2013 (79), Ross et al. Journal of Clinical Oncology 2015 (51), Nakamura et al. Nature Genetics 2015 (89), Borad et al. PLoS Genetics 2014 (48), Randall et al. Journal of Clinical Oncology 2015 (50), Galdy et al. Cancer and Metastases Reviews 2016 (162), Churi et al. PlosOne 2014 (83), Turner et al. Nature Reviews in Cancer 2010 (124), Pai et al. European Journal of Cancer Prevention 2011 (199), Riener et al. Genes, Chromosomes and Cancer 2008 (208). GBCA, gallbladder cancer.

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 *Opistorchis 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

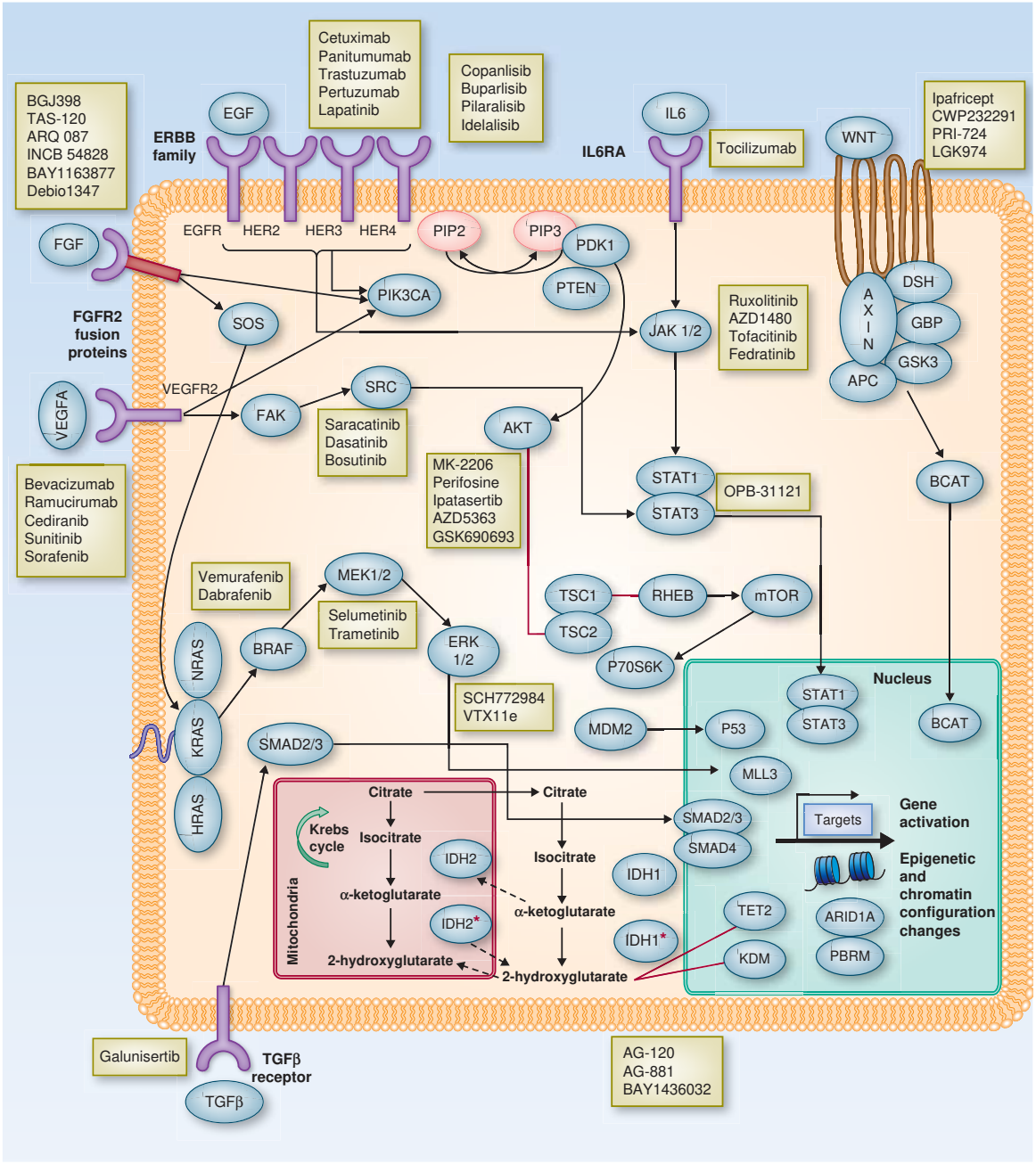

Figure 5. Summary of the relevant pathways for BTCs. Activation links are described with black arrows. Negative links are described as red lines. Red asterisk identifies the mutated variant of the protein.

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

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

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 $IDH1^{R132}$ and $IDH2^{R172}$) 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 $IDH1^{R132H}$ 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 $IDH2^{R140Q}$ (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 with the oral agent BGJ398 (infigratinib; Novartis), which has an IC_{50} for FGFR2 of 1.4 nmol/L. Preliminary results of 34 patients in the ongoing phase II trial of BGJ398 in advanced cholangiocarcinoma with *FGFR* aberrations after first-line chemotherapy (NCT02150967) included patients with *FGFR2* fusions (n = 28), *FGFR2* mutations (n = 2), *FGFR2* amplification (n = 3), or *FGFR3* amplification (n = 1); the median time on treatment was 188 days, and the objective response rate was 22% (all 8 patients with a partial response had an *FGFR2* fusion; ref. 125). As seen with other oncogene-addicted tumors treated with tyrosine kinase inhibitors (TKI), acquired resistance limited the durability of response in some patients. Goyal and colleagues reported the first evidence of clinically acquired resistance to FGFR inhibition in an analysis of three patients with *FGFR2* fusion–positive ICC who were treated with BGJ398 (126). Sequencing of cell-free DNA (cfDNA) and biopsy samples collected at baseline and postprogression revealed polyclonal secondary mutations in the FGFR2 kinase domain, including the gatekeeper mutation $FGFR2^{V564F}$ in all three patients. *In vitro* studies further identified structurally distinct FGFR inhibitors that may overcome the resistance, and these data may guide future treatment strategies in this scenario.

Other selective FGFR inhibitors, including INCB54828 (Incyte; NCT02924376), BAY1163877 (Bayer; NCT01976741), and the irreversible FGFR inhibitor TAS-120 (Taiho; NCT02052778), are currently being evaluated in early-phase trials in patients with advanced solid tumors, including ICC. Nonselective multi-TKIs that also target FGFR, including ponatinib and pazopanib, have demonstrated activity in individual patients with ICC who have developed resistance to chemotherapy (48). A third nonselective TKI, ARQ 087 (Arqule; NCT01752920), which inhibits RET, PDGFR, KIT, SRC, and FGFR1–3 (IC_{50} for FGFR2 = 0.68 nmol/L), 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. FGF23 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.

Overall, the preliminary data for FGFR inhibitors in advanced ICC are encouraging.

Angiogenesis

Not only are the ligands regulating angiogenesis (particularly VEGFA) 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 ramucirumab (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 third 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, erythroblastic leukaemia 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 Galdy 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 [*RNF43* (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%), PTCH1 (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 (IC_{50} = 1.3 nmol/L) and VEGFR2 (IC_{50} = 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 $BRAF^{V600}$-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, 5.97–not available). In this study, no $BRAF^{V600E}$ 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 *PBRM1*) 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 cytotoxic 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 infrequent 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 (NCT01868490) 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

J.W. Valle has received honoraria from the speakers bureau of Celgene and is a consultant/advisory board member for AstraZeneca, Lilly, Merck, and Agios. A.X. Zhu is a consultant/advisory board member for Bayer, BMS, Eisai, Merck, Novartis, and Sanofi. No potential conflicts of interest were disclosed by the other authors.

Acknowledgments

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 Morement Memorial Fund (AMMF). A. Lamarca 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 SPOREP50 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.

Received March 7, 2017; revised May 24, 2017; accepted July 10, 2017; published OnlineFirst August 17, 2017.

REFERENCES

1. Valle JW. Advances in the treatment of metastatic or unresectable biliary tract cancer. Ann Oncol 2010;21;Suppl 7:vii345–8.
2. Edge S, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual (Seventh Edition). New York: Springer-Verlag. 2010. Full book.
3. Patel T. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. Hepatology 2001; 33:1353–7.
4. Bertuccio P, Bosetti C, Levi F, Decarli A, Negri E, La Vecchia C. A comparison of trends in mortality from primary liver cancer and intrahepatic cholangiocarcinoma in Europe. Ann Oncol 2013;24:1667–74.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
6. Wang Y, Li J, Xia Y, Gong R, Wang K, Yan Z, et al. Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. J Clin Oncol 2013;31:1188–95.
7. Wang SJ, Lemieux A, Kalpathy-Cramer J, Ord CB, Walker GV, Fuller CD, et al. Nomogram for predicting the benefit of adjuvant chemoradiotherapy for resected gallbladder cancer. J Clin Oncol 2011;29:4627–32.
8. Anderson C, Kim R. Adjuvant therapy for resected extrahepatic cholangiocarcinoma: a review of the literature and future directions. Cancer Treat Rev 2009;35:322–7.
9. DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg 2007;245:755–62.
10. Bridgewater J, Lopes A, Wasan H, Malka D, Jensen L, Okusaka T, et al. Prognostic factors for progression-free and overall survival in advanced biliary tract cancer. Ann Oncol 2016;27:134–40.
11. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology 2011;54:173–84.
12. Zatonski WA, Lowenfels AB, Boyle P, Maisonneuve P, Bueno de Mesquita HB, Ghadirian P, et al. Epidemiologic aspects of gallbladder cancer: a case-control study of the SEARCH Program of the International Agency for Research on Cancer. J Natl Cancer Inst 1997;89:1132–8.
13. Chapman RW. Risk factors for biliary tract carcinogenesis. Ann Oncol 1999;10Suppl 4:308–11.
14. Chaiyadet S, Sotillo J, Smout M, Cantacessi C, Jones MK, Johnson MS, et al. Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J Infect Dis 2015;212:1636–45.
15. Gourgiotis S, Kocher HM, Solaini L, Yarollahi A, Tsiambas E, Salemis NS. Gallbladder cancer. Am J Surg 2008;196:252–64.
16. Gatto M, Alvaro D. Cholangiocarcinoma: risk factors and clinical presentation. Eur Rev Med Pharmacol Sci 2010;14:363–7.
17. Heinrich S, Clavien PA. Ampullary cancer. Curr Opin Gastroenterol 2010;26:280–5.
18. Valle JW, Borbath I, Khan SA, Huguet F, Gruenberger T, Arnold D. Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27:v28–37.
19. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, et al. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. Ann Surg 2008;248:84–96.

20. Zhu AX, Knox JJ. Adjuvant therapy for intrahepatic cholangiocarcinoma: the debate continues. Oncologist 2012;17:1504–7.
21. Horgan AM, Amir E, Walter T, Knox JJ. Adjuvant therapy in the treatment of biliary tract cancer: a systematic review and meta-analysis. J Clin Oncol 2012;30:1934–40.
22. Julien Edeline FBJMPea. Gemox versus surveillance following surgery of localized biliary tract cancer: Results of the PRODIGE 12-ACCORD 18 (UNICANCER GI) phase III trial. ASCO 2017 Gastrointestinal Cancer Symposium, J Clin Oncol 2017;35(suppl 4S; abstract 225).
23. Primrose JN, Fox R, Palmer D, Prasad R, Mirza D, Anthoney DA, et al. Adjuvant capecitabine for biliary tract cancer: The BILCAP randomized study. J Clin Oncol 2017;35(suppl; abstr 4006).
24. Tao R, Krishnan S, Bhosale PR, Javle MM, Aloia TA, Shroff RT, et al. Ablative radiotherapy doses lead to a substantial prolongation of survival in patients with inoperable intrahepatic cholangiocarcinoma: a retrospective dose response analysis. J Clin Oncol 2016;34:219–26.
25. Hong TS, Wo JY, Yeap BY, Ben-Josef E, McDonnell EI, Blaszkowsky LS, et al. Multi-institutional phase II study of high-dose hypofractionated proton beam therapy in patients with localized, unresectable hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Clin Oncol 2016;34:460–8.
26. Al-Adra DP, Gill RS, Axford SJ, Shi X, Kneteman N, Liau SS. Treatment of unresectable intrahepatic cholangiocarcinoma with yttrium-90 radioembolization: a systematic review and pooled analysis. Eur J Surg Oncol 2015;41:120–7.
27. Edeline J, Du FL, Rayar M, Rolland Y, Beuzit L, Boudjema K, et al. Glass microspheres 90Y selective internal radiation therapy and chemotherapy as first-line treatment of intrahepatic cholangiocarcinoma. Clin Nucl Med 2015;40:851–5.
28. Hibi T, Itano O, Shinoda M, Kitagawa Y. Liver transplantation for hepatobiliary malignancies: a new era of "Transplant Oncology" has begun. Surg Today 2017;47:403–15.
29. Rosen CB, Heimbach JK, Gores GJ. Surgery for cholangiocarcinoma: the role of liver transplantation. HPB (Oxford) 2008;10:186–9.
30. Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. Br J Cancer 2007;96:896–902.
31. Tsavaris N, Kosmas C, Gouveris P, Gennatas K, Polyzos A, Mouratidou D, et al. Weekly gemcitabine for the treatment of biliary tract and gallbladder cancer. Invest New Drugs 2004;22:193–8.
32. Raderer M, Hejna MH, Valencak JB, Kornek GV, Weinlander GS, Bareck E, et al. Two consecutive phase II studies of 5-fluorouracil/leucovorin/mitomycin C and of gemcitabine in patients with advanced biliary cancer. Oncology 1999;56:177–80.
33. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med 2010;362:1273–81.
34. Okusaka T, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, Funakoshi A, et al. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. Br J Cancer 2010;103:469–74.
35. Lamarca A, Benafif S, Ross P, Bridgewater J, Valle JW. Cisplatin and gemcitabine in patients with advanced biliary tract cancer (ABC) and persistent jaundice despite optimal stenting: Effective intervention in patients with luminal disease. Eur J Cancer 2015;51:1694–703.
36. Lamarca A, Hubner RA, Ryder WD, Valle JW. Second-line chemotherapy in advanced biliary cancer: a systematic review. Ann Oncol 2014;25:2328–38.
37. Walter T, Horgan AM, McNamara M, McKeever L, Min T, Hedley D, et al. Feasibility and benefits of second-line chemotherapy in advanced biliary tract cancer: a large retrospective study. Eur J Cancer 2013;49:329–35.
38. Bridgewater J, Palmer D, Cunningham D, Iveson T, Gillmore R, Waters J, et al. Outcome of second-line chemotherapy for biliary tract cancer. Eur J Cancer 2012;49:1511.
39. Oh SY, Jeong CY, Hong SC, Kim TH, Ha CY, Kim HJ, et al. Phase II study of second line gemcitabine single chemotherapy for biliary tract cancer patients with 5-fluorouracil refractoriness. Invest New Drugs 2011;29:1066–72.
40. Lee S, Oh SY, Kim BG, Kwon HC, Kim SH, Rho MH, et al. Second-line treatment with a combination of continuous 5-fluorouracil, doxorubicin, and mitomycin-C (conti-FAM) in gemcitabine-pretreated pancreatic and biliary tract cancer. Am J Clin Oncol 2009;32:348–52.
41. Sasaki T, Isayama H, Nakai Y, Mizuno S, Yamamoto K, Yagioka H, et al. Multicenter phase II study of S-1 monotherapy as second-line chemotherapy for advanced biliary tract cancer refractory to gemcitabine. Invest New Drugs 2012;30:708–13.
42. Rao S, Cunningham D, Hawkins RE, Hill ME, Smith D, Daniel F, et al. Phase III study of 5FU, etoposide and leucovorin (FELV) compared to epirubicin, cisplatin and 5FU (ECF) in previously untreated patients with advanced biliary cancer. Br J Cancer 2005;92:1650–4.
43. Lim JY, Jeung HC, Mun HS, Lee DK, Paik YH, Lee SJ, et al. Phase II trial of oxaliplatin combined with leucovorin and fluorouracil for recurrent/metastatic biliary tract carcinoma. Anticancer Drugs 2008;19:631–5.
44. Lamarca A, Palmer D, Wasan H, Ryder WD, Davies L, Flight H, et al. ABC-06: A randomised phase III, multi-centre, open-label study of active symptom control (ASC) alone or ASC with oxaliplatin/5-FU chemotherapy for patients with locally advanced/metastatic biliary tract cancer (ABC) previously treated with cisplatin/gemcitabine chemotherapy. Ann Oncol 2014;25(suppl_4):iv252.
45. Ong CK, Subimerb C, Pairojkul C, Wongkham S, Cutcutache I, Yu W, et al. Exome sequencing of liver fluke-associated cholangiocarcinoma. Nat Genet 2012;44:690–3.
46. Chan-On W, Nairismagi ML, Ong CK, Lim WK, Dima S, Pairojkul C, et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet 2013;45:1474–8.
47. Jiao Y, Pawlik TM, Anders RA, Selaru FM, Streppel MM, Lucas DJ, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet 2013;45:1470–3.
48. Borad MJ, Champion MD, Egan JB, Liang WS, Fonseca R, Bryce AH, et al. Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma. PLoS Genet 2014;10:e1004135.
49. Gao Q, Zhao YJ, Wang XY, Guo WJ, Gao S, Wei L, et al. Activating mutations in PTPN3 promote cholangiocarcinoma cell proliferation and migration and are associated with tumor recurrence in patients. Gastroenterology 2014;146:1397–407.
50. Holcombe RF, Xiu J, Pishvaian MJ, Millis SZ, Gatalica Z, Reddy SK, et al. Tumor profiling of biliary tract carcinomas to reveal distinct molecular alterations and potential therapeutic targets. ASCO Annual Meeting 2015, J Clin Oncol 33, 2015 (suppl 3; abstr 285).
51. Ross JS, Wang K, Catenacci DVT, Chmielecki J, Ali SM, Elvin JA, et al. Comprehensive genomic profiling of biliary tract cancers to reveal tumor-specific differences and genomic alterations. ASCO Annual Meeting 2015, J Clin Oncol 33, 2015 (suppl 3; abstr 231).
52. Ross JS, Wang K, Gay L, Al-Rohil R, Rand JV, Jones DM, et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. Oncologist 2014;19:235–42.
53. Javle M, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, et al. Biliary cancer: Utility of next-generation sequencing for clinical management. Cancer 2016;122:3838–47.
54. Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, et al. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. Gastroenterology 2013;144:829–40.
55. Furubo S, Harada K, Shimonishi T, Katayanagi K, Tsui W, Nakanuma Y. Protein expression and genetic alterations of p53 and ras in intrahepatic cholangiocarcinoma. Histopathology 1999;35:230–40.
56. Momoi H, Itoh T, Nozaki Y, Arima Y, Okabe H, Satoh S, et al. Microsatellite instability and alternative genetic pathway in intrahepatic cholangiocarcinoma. J Hepatol 2001;35:235–44.
57. Tannapfel A, Weinans L, Geissler F, Schutz A, Katalinic A, Kockerling F, et al. Mutations of p53 tumor suppressor gene, apoptosis, and proliferation in intrahepatic cholangiocellular carcinoma of the liver. Dig Dis Sci 2000;45:317–24.

58. Tannapfel A, Sommerer F, Benicke M, Weinans L, Katalinic A, Geissler F, et al. Genetic and epigenetic alterations of the INK4a-ARF pathway in cholangiocarcinoma. J Pathol 2002;197:624–31.
59. Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. Oncogene 2013;32:4861–70.
60. Lee KT, Chang WT, Wang SN, Chuang SC, Chai CY, Hu SW. Expression of DPC4/Smad4 gene in stone-containing intrahepatic bile duct. J Surg Oncol 2006;94:338–43.
61. Yan XQ, Zhang W, Zhang BX, Liang HF, Zhang WG, Chen XP. Inactivation of Smad4 is a prognostic factor in intrahepatic cholangiocarcinoma. Chin Med J (Engl) 2013;126:3039–43.
62. Zou S, Li J, Zhou H, Frech C, Jiang X, Chu JS, et al. Mutational landscape of intrahepatic cholangiocarcinoma. Nat Commun 2014;5:5696.
63. Shinada K, Tsukiyama T, Sho T, Okumura F, Asaka M, Hatakeyama S. RNF43 interacts with NEDL1 and regulates p53-mediated transcription. Biochem Biophys Res Commun 2011;404:143–7.
64. Loregger A, Grandl M, Mejias-Luque R, Allgauer M, Degenhart K, Haselmann V, et al. The E3 ligase RNF43 inhibits Wnt signaling downstream of mutated beta-catenin by sequestering TCF4 to the nuclear membrane. Sci Signal 2015;8:ra90.
65. Koo BK, van Es JH, van den Born M, Clevers H. Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43;Znrf3-mutant neoplasia. Proc Natl Acad Sci U S A 2015;112:7548–50.
66. Deng Y, Wu X. Peg3/Pw1 promotes p53-mediated apoptosis by inducing Bax translocation from cytosol to mitochondria. Proc Natl Acad Sci U S A 2000;97:12050–5.
67. Chiba T, Zheng YW, Kita K, Yokosuka O, Saisho H, Onodera M, et al. Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. Gastroenterology 2007;133:937–50.
68. Parsons DW, Li M, Zhang X, Jones S, Leary RJ, Lin JC, et al. The genetic landscape of the childhood cancer medulloblastoma. Science 2011;331:435–9.
69. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–6.
70. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 2011;476:298–303.
71. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399–405.
72. Hsu M, Sasaki M, Igarashi S, Sato Y, Nakanuma Y. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. Cancer 2013;119:1669–74.
73. Dal Molin M, Matthaei H, Wu J, Blackford A, Debeljak M, Rezaee N, et al. Clinicopathological correlates of activating GNAS mutations in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg Oncol 2013;20:3802–8.
74. Fecteau RE, Lutterbaugh J, Markowitz SD, Willis J, Guda K. GNAS mutations identify a set of right-sided, RAS mutant, villous colon cancers. PLoS One 2014;9:e87966.
75. Dang L, Jin S, Su SM. IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med 2010;16:387–97.
76. Saha SK, Parachoniak CA, Ghanta KS, Fitamant J, Ross KN, Najem MS, et al. Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer. Nature 2014;513:110–4.
77. Borger DR, Zhu AX. IDH mutations: new genetic signatures in cholangiocarcinoma and therapeutic implications. Expert Rev Anticancer Ther 2012;12:543–6.
78. Cairns RA, Mak TW. Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. Cancer Discov 2013;3:730–41.
79. Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, et al. Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov 2013;3:636–47.
80. Maisonneuve C, Guilleret I, Vick P, Weber T, Andre P, Beyer T, et al. Bicaudal C, a novel regulator of Dvl signaling abutting RNA-processing bodies, controls cilia orientation and leftward flow. Development 2009;136:3019–30.
81. Arai Y, Totoki Y, Hosoda F, Shirota T, Hama N, Nakamura H, et al. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. Hepatology 2014;59:1427–34.
82. Sia D, Losic B, Moeini A, Cabellos L, Hao K, Revill K, et al. Massive parallel sequencing uncovers actionable FGFR2-PPHLN1 fusion and ARAF mutations in intrahepatic cholangiocarcinoma. Nat Commun 2015;6:6087.
83. Churi CR, Shroff R, Wang Y, Rashid A, Kang HC, Weatherly J, et al. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. PLoS One 2014;9:e115383.
84. Ballaro C, Ceccarelli S, Tiveron C, Tatangelo L, Salvatore AM, Segatto O, et al. Targeted expression of RALT in mouse skin inhibits epidermal growth factor receptor signalling and generates a Waved-like phenotype. EMBO Rep 2005;6:755–61.
85. Ferby I, Reschke M, Kudlacek O, Knyazev P, Pante G, Amann K, et al. Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. Nat Med 2006;12:568–73.
86. Anastasi S, Fiorentino L, Fiorini M, Fraioli R, Sala G, Castellani L, et al. Feedback inhibition by RALT controls signal output by the ErbB network. Oncogene 2003;22:4221–34.
87. Zhang L, Jiang L, Sun Q, Peng T, Lou K, Liu N, et al. Prostaglandin E2 enhances mitogen-activated protein kinase/Erk pathway in human cholangiocarcinoma cells: involvement of EP1 receptor, calcium and EGF receptors signaling. Mol Cell Biochem 2007;305:19–26.
88. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, et al. Genomic spectra of biliary tract cancer. Nat Genet 2015;47:1003–10.
89. Pena-Llopis S, Vega-Rubin-de-Celis S, Liao A, Leng N, Pavia-Jimenez A, Wang S, et al. BAP1 loss defines a new class of renal cell carcinoma. Nat Genet 2012;44:751–9.
90. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 2010;330:1410–3.
91. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet 2011;43:668–72.
92. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv 2015;1:e1500447.
93. Amatu A, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open 2016;1:e000023.
94. Hong DS, Brose MS, Doebele RC, Shaw AT, Dowlati A, Bauer TM, et al. Clinical safety and activity from a phase I study of LOXO-101, a selective TRKA/B/C inhibitor, in solid-tumor patients with NTRK gene fusions. Mol Cancer Ther 2015(14) (12 Supplement 2; PR13).
95. Drilon A, Siena S, Ou SI, Patel M, Ahn MJ, Lee J, et al. Safety and antitumor activity of the multi-targeted Pan-TRK, ROS1, and ALK inhibitor entrectinib (RXDX-101): combined results from two phase 1 trials (ALKA-372-001 and STARTRK-1). Cancer Discov 2017;7:400–9.
96. Lee KH, Lee KB, Kim TY, Han SW, Oh DY, Im SA, et al. Clinical and pathological significance of ROS1 expression in intrahepatic cholangiocarcinoma. BMC Cancer 2015;15:721.
97. Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rimkunas V, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. PLoS One 2011;6:e15640.
98. Ruzzenente A, Fassan M, Conci S, Simbolo M, Lawlor RT, Pedrazzani C, et al. Cholangiocarcinoma heterogeneity revealed by multigene mutational profiling: clinical and prognostic relevance in surgically resected patients. Ann Surg Oncol 2016;23:1699–707.
99. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology 2012;142:1021–31.

100. Farshidfar F, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, et al. Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-mutant molecular profiles. Cell Rep 2017;18:2780–94.
101. Li M, Zhang Z, Li X, Ye J, Wu X, Tan Z, et al. Whole-exome and targeted gene sequencing of gallbladder carcinoma identifies recurrent mutations in the ErbB pathway. Nat Genet 2014;46:872–6.
102. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist 2012;17:72–9.
103. Deshpande V, Nduaguba A, Zimmerman SM, Kehoe SM, Macconaill LE, Lauwers GY, et al. Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. BMC Cancer 2011;11:60.
104. O'Dell MR, Huang JL, Whitney-Miller CL, Deshpande V, Rothberg P, Grose V, et al. Kras(G12D) and p53 mutation cause primary intrahepatic cholangiocarcinoma. Cancer Res 2012;72:1557–67.
105. Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest 2012; 122:3914–8.
106. Fan B, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S, et al. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest 2012;122:2911–5.
107. Zender S, Nickeleit I, Wuestefeld T, Sorensen I, Dauch D, Bozko P, et al. A critical role for notch signaling in the formation of cholangiocellular carcinomas. Cancer Cell 2013;23:784–95.
108. Guest RV, Boulter L, Kendall TJ, Minnis-Lyons SE, Walker R, Wigmore SJ, et al. Cell lineage tracing reveals a biliary origin of intrahepatic cholangiocarcinoma. Cancer Res 2014;74:1005–10.
109. Liu W, Chen JR, Hsu CH, Li YH, Chen YM, Lin CY, et al. A zebrafish model of intrahepatic cholangiocarcinoma by dual expression of hepatitis B virus X and hepatitis C virus core protein in liver. Hepatology 2012;56:2268–76.
110. Ko KS, Peng J, Yang H. Animal models of cholangiocarcinoma. Curr Opin Gastroenterol 2013;29:312–8.
111. Kalamarides M, Niwa-Kawakita M, Leblois H, Abramowski V, Perricaudet M, Janin A, et al. Nf2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. Genes Dev 2002;16:1060–5.
112. Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, et al. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. J Clin Invest 2006;116:1843–52.
113. Kiguchi K, Carbajal S, Chan K, Beltran L, Ruffino L, Shen J, et al. Constitutive expression of ErbB-2 in gallbladder epithelium results in development of adenocarcinoma. Cancer Res 2001;61:6971–6.
114. Ikenoue T, Terakado Y, Nakagawa H, Hikiba Y, Fujii T, Matsubara D, et al. A novel mouse model of intrahepatic cholangiocarcinoma induced by liver-specific Kras activation and Pten deletion. Sci Rep 2016;6:23899.
115. Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene 2013;32:3091–100.
116. Zhu AX, Borger DR, Kim Y, Cosgrove D, Ejaz A, Alexandrescu S, et al. Genomic profiling of intrahepatic cholangiocarcinoma: refining prognosis and identifying therapeutic targets. Ann Surg Oncol 2014;21:3827–34.
117. Goyal L, Govindan A, Sheth RA, Nardi V, Blaszkowsky LS, Faris JE, et al. Prognosis and clinicopathologic features of patients with advanced stage isocitrate dehydrogenase (IDH) mutant and IDH wild-type intrahepatic cholangiocarcinoma. Oncologist 2015;20: 1019–27.
118. Kipp BR, Voss JS, Kerr SE, Barr Fritcher EG, Graham RP, Zhang L, et al. Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. Hum Pathol 2012;43:1552–8.
119. Borger DR, Goyal L, Yau T, Poon RT, Ancukiewicz M, Deshpande V, et al. Circulating oncometabolite 2-hydroxyglutarate is a potential surrogate biomarker in patients with isocitrate dehydrogenase-mutant intrahepatic cholangiocarcinoma. Clin Cancer Res 2014;20:1884–90.
120. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science 2013;340:626–30.
121. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. Science 2013;340:622–6.
122. Saha SK, Gordan JD, Kleinstiver BP, Vu P, Najem MS, Yeo JC, et al. Isocitrate dehydrogenase mutations confer dasatinib hypersensitivity and SRC-dependence in intrahepatic cholangiocarcinoma. Cancer Discov 2016;6:727–39.
123. Graham RP, Barr Fritcher EG, Pestova E, Schulz J, Sitailo LA, Vasmatzis G, et al. Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma. Hum Pathol 2014;45:1630–8.
124. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116–29.
125. Javle MM, Shroff RT, Zhu A, Sadeghi S, Choo SP, Borad MJ, et al. A phase 2 study of BGJ398 in patients (pts) with advanced or metastatic FGFR-altered cholangiocarcinoma (CCA) who failed or are intolerant to platinum-based chemotherapy. 2016 Gastrointestinal Cancers Symposium, J Clin Oncol 34, 2016(suppl 4S; abstr 335).
126. Goyal L, Saha SK, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, et al. Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in patients with FGFR2 fusion-positive cholangiocarcinoma. Cancer Discov 2017;7:252–63.
127. Mazzaferro V, Shaib W, Rimassa L, Harris WP, Personeni N, El-Rayes B, et al. ARQ 087, an oral pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients with advanced and/or metastatic intrahepatic cholangiocarcinoma (iCCA). 18th ESMO World Congress on Gastrointestinal Cancer. 29 June -2 July, 2016, Barcelona, Spain. 2016. Abstract PD19.
128. Hall TG, Savage RE, Schwartz B, Yu Y, Wang Y, Kazakin J, et al. In FGFR2 driven tumors, preclinical pharmacokinetics (PK), pharmacodynamics (PD) and efficacy translate into clinical activity of ARQ 087. Mol Cancer Ther 2015;14(12 Suppl 2):Abstract nr B151.
129. Liu S, Quarles LD. How fibroblast growth factor 23 works. J Am Soc Nephrol 2007;18:1637–47.
130. Giatromanolaki A, Koukourakis MI, Simopoulos C, Polychronidis A, Sivridis E. Vascular endothelial growth factor (VEGF) expression in operable gallbladder carcinomas. Eur J Surg Oncol 2003;29:879–83.
131. Tang D, Nagano H, Yamamoto H, Wada H, Nakamura M, Kondo M, et al. Angiogenesis in cholangiocellular carcinoma: expression of vascular endothelial growth factor, angiopoietin-1/2, thrombospondin-1 and clinicopathological significance. Oncol Rep 2006;15: 525–32.
132. Yoshikawa D, Ojima H, Iwasaki M, Hiraoka N, Kosuge T, Kasai S, et al. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. Br J Cancer 2008;98:418–25.
133. Benckert C, Jonas S, Cramer T, Von MZ, Schafer G, Peters M, et al. Transforming growth factor beta 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells. Cancer Res 2003;63:1083–92.
134. Mobius C, Demuth C, Aigner T, Wiedmann M, Wittekind C, Mossner J, et al. Evaluation of VEGF A expression and microvascular density as prognostic factors in extrahepatic cholangiocarcinoma. Eur J Surg Oncol 2007;33:1025–9.
135. Shirabe K, Shimada M, Tsujita E, Aishima S, Maehara S, Tanaka S, et al. Prognostic factors in node-negative intrahepatic cholangiocarcinoma with special reference to angiogenesis. Am J Surg 2004;187:538–42.
136. Hida Y, Morita T, Fujita M, Miyasaka Y, Horita S, Fujioka Y, et al. Vascular endothelial growth factor expression is an independent negative predictor in extrahepatic biliary tract carcinomas. Anticancer Res 1999;19:2257–60.
137. Zhu AX, Meyerhardt JA, Blaszkowsky LS, Kambadakone AR, Muzikansky A, Zheng H, et al. Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. Lancet Oncol 2010;11:48–54.

138. Lubner SJ, Mahoney MR, Kolesar JL, Loconte NK, Kim GP, Pitot HC, et al. Report of a multicenter phase II trial testing a combination of biweekly bevacizumab and daily erlotinib in patients with unresectable biliary cancer: a phase II consortium study. J Clin Oncol 2010;28:3491–7.
139. Valle JW, Wasan H, Lopes A, Backen AC, Palmer DH, Morris K, et al. Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): a randomised phase 2 trial. Lancet Oncol 2015;16:967–78.
140. Bengala C, Bertolini F, Malavasi N, Boni C, Aitini E, Dealis C, et al. Sorafenib in patients with advanced biliary tract carcinoma: a phase II trial. Br J Cancer 2010;102:68–72.
141. El-Khoueiry AB,Rankin C, Lenz HJ, Philip P, Rivkin SE, Blanke CD. SWOG 0514: A phase II study of sorafenib (BAY 43-9006) as single agent in patients (pts) with unresectable or metastatic gallbladder cancer or cholangiocarcinomas. Journal of Clinical Oncology, 25;18_suppl (June 2007) 4639–9.
142. El-Khoueiry AB, Rankin C, Siegel AB, Iqbal S, Gong IY, Micetich KC, et al. S0941: a phase 2 SWOG study of sorafenib and erlotinib in patients with advanced gallbladder carcinoma or cholangiocarcinoma. Br J Cancer 2014;110:882–7.
143. Lee JK, Capanu M, O'Reilly EM, Ma J, Chou JF, Shia J, et al. A phase II study of gemcitabine and cisplatin plus sorafenib in patients with advanced biliary adenocarcinomas. Br J Cancer 2013;109:915–9.
144. Moehler M, Maderer A, Schimanski C, Kanzler S, Denzer U, Kolligs FT, et al. Gemcitabine plus sorafenib versus gemcitabine alone in advanced biliary tract cancer: a double-blind placebo-controlled multicentre phase II AIO study with biomarker and serum programme. Eur J Cancer 2014;50:3125–35.
145. Yi JH, Thongprasert S, Lee J, Doval DC, Park SH, Park JO, et al. A phase II study of sunitinib as a second-line treatment in advanced biliary tract carcinoma: a multicentre, multinational study. Eur J Cancer 2012;48:196–201.
146. Santoro A, Gebbia V, Pressiani T, Testa A, Personeni N, Arrivas BE, et al. A randomized, multicenter, phase II study of vandetanib monotherapy versus vandetanib in combination with gemcitabine versus gemcitabine plus placebo in subjects with advanced biliary tract cancer: the VanGogh study. Ann Oncol 2015;26:542–7.
147. Gwak GY, Yoon JH, Shin CM, Ahn YJ, Chung JK, Kim YA, et al. Detection of response-predicting mutations in the kinase domain of the epidermal growth factor receptor gene in cholangiocarcinomas. J Cancer Res Clin Oncol 2005;131:649–52.
148. Nakazawa K, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. J Pathol 2005;206:356–65.
149. Leone F, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Piacibello W, et al. Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. Clin Cancer Res 2006;12:1680–5.
150. Paule B, Herelle MO, Rage E, Ducreux M, Adam R, Guettier C, et al. Cetuximab plus gemcitabine-oxaliplatin (GEMOX) in patients with refractory advanced intrahepatic cholangiocarcinomas. Oncology 2007;72:105–10.
151. Gruenberger B, Schueller J, Heubrandtner U, Wrba F, Tamandl D, Kaczirek K, et al. Cetuximab, gemcitabine, and oxaliplatin in patients with unresectable advanced or metastatic biliary tract cancer: a phase 2 study. Lancet Oncol 2010;11:1142–8.
152. Borbath I, Ceratti A, Verslype C, Demols A, Delaunoit T, Laurent S, et al. Combination of gemcitabine and cetuximab in patients with advanced cholangiocarcinoma: a phase II study of the Belgian Group of Digestive Oncology. Ann Oncol 2013;24:2824–9.
153. Malka D, Cervera P, Foulon S, Trarbach T, de la FC, Boucher E, et al. Gemcitabine and oxaliplatin with or without cetuximab in advanced biliary-tract cancer (BINGO): a randomised, open-label, non-comparative phase 2 trial. Lancet Oncol 2014;15:819–28.
154. Jensen LH, Lindebjerg J, Ploen J, Hansen TF, Jakobsen A. Phase II marker-driven trial of panitumumab and chemotherapy in KRAS wild-type biliary tract cancer. Ann Oncol 2012;23:2341–6.
155. Hezel AF, Noel MS, Allen JN, Abrams TA, Yurgelun M, Faris JE, et al. Phase II study of gemcitabine, oxaliplatin in combination with panitumumab in KRAS wild-type unresectable or metastatic biliary tract and gallbladder cancer. Br J Cancer 2014;111:430–6.
156. Sohal DP, Mykulowycz K, Uehara T, Teitelbaum UR, Damjanov N, Giantonio BJ, et al. A phase II trial of gemcitabine, irinotecan and panitumumab in advanced cholangiocarcinoma. Ann Oncol 2013;24:3061–5.
157. Vogel A, Kasper S, Weichert W, Bitzer M, Block A, Riess H, et al. Panitumumab in combination with gemcitabine/cisplatin (GemCis) for patients with advanced kRAS WT biliary tract cancer: a randomized phase II trial of the Arbeitsgemeinschaft Internistische Onkologie (AIO). J Clin Oncol 33, 2015 (suppl; abstr 4082).
158. Leone F, Marino D, Cereda S, Filippi R, Belli C, Spadi R, et al. Panitumumab in combination with gemcitabine and oxaliplatin does not prolong survival in wild-type KRAS advanced biliary tract cancer: a randomized phase 2 trial (Vecti-BIL study). Cancer 2016;122:574–81.
159. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, et al. Phase II study of erlotinib in patients with advanced biliary cancer. J Clin Oncol 2006;24:3069–74.
160. Lee J, Park SH, Chang HM, Kim JS, Choi HJ, Lee MA, et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2012;13:181–8.
161. Chiorean EG, Ramasubbaiah R, Yu M, Picus J, Bufill JA, Tong Y, et al. Phase II trial of erlotinib and docetaxel in advanced and refractory hepatocellular and biliary cancers: Hoosier Oncology Group GI06-101. Oncologist 2012;17:13.
162. Galdy S, Lamarca A, McNamara MG, Hubner RA, Cella CA, Fazio N, et al. HER2/HER3 pathway in biliary tract malignancies; systematic review and meta-analysis: a potential therapeutic target? Cancer Metastasis Rev 2016;36:141–57.
163. Ramanathan RK, Belani CP, Singh DA, Tanaka M, Lenz HJ, Yen Y, et al. A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. Cancer Chemother Pharmacol 2009;64:777–83.
164. Peck J, Wei L, Zalupski M, O'Neil B, Villalona CM, Bekaii-Saab T. HER2/neu may not be an interesting target in biliary cancers: results of an early phase II study with lapatinib. Oncology 2012;82:175–9.
165. Sorscher S. Marked radiographic response of a HER-2-overexpressing biliary cancer to trastuzumab. Cancer Manag Res 2013;9:1–3.
166. Roa I, de Toro G, Schalper K, de A, X, Churi C, Javle M. Overexpression of the HER2/neu gene: a new therapeutic possibility for patients with advanced gallbladder cancer. Gastrointest Cancer Res 2014;7:42–8.
167. Suder A, Ang JE, Kyle F, Harris D, Rudman S, Kristeleit R, et al. A phase I study of daily afatinib, an irreversible ErbB family blocker, in combination with weekly paclitaxel in patients with advanced solid tumours. Eur J Cancer 2015;51:2275–84.
168. Settakorn J, Kaewpila N, Burns GF, Leong AS. FAT, E-cadherin, beta catenin, HER 2/neu, Ki67 immuno-expression, and histological grade in intrahepatic cholangiocarcinoma. J Clin Pathol 2005;58:1249–54.
169. Chen W, Liang J, Huang L, Cai J, Lei Y, Lai J, et al. Characterizing the activation of the Wnt signaling pathway in hilar cholangiocarcinoma using a tissue microarray approach. Eur J Histochem 2016;60:2536.
170. Wang W, Zhong W, Yuan J, Yan C, Hu S, Tong Y, et al. Involvement of Wnt/beta-catenin signaling in the mesenchymal stem cells promote metastatic growth and chemoresistance of cholangiocarcinoma. Oncotarget 2015;6:42276–89.
171. Shen DY, Zhang W, Zeng X, Liu CQ. Inhibition of Wnt/beta-catenin signaling downregulates P-glycoprotein and reverses multi-drug resistance of cholangiocarcinoma. Cancer Sci 2013;104:1303–8.
172. Sugimachi K, Taguchi K, Aishima S, Tanaka S, Shimada M, Kajiyama K, et al. Altered expression of beta-catenin without genetic mutation in intrahepatic cholangiocarcinoma. Mod Pathol 2001;14:900–5.
173. Tokumoto N, Ikeda S, Ishizaki Y, Kurihara T, Ozaki S, Iseki M, et al. Immunohistochemical and mutational analyses of Wnt signaling

components and target genes in intrahepatic cholangiocarcinomas. Int J Oncol 2005;27:973–80.
174. Jusakul A, Kongpetch S, Teh BT. Genetics of Opisthorchis viverrini-related cholangiocarcinoma. Curr Opin Gastroenterol 2015;31:258–63.
175. Hirata K, Ajiki T, Okazaki T, Horiuchi H, Fujita T, Kuroda Y. Frequent occurrence of abnormal E-cadherin/beta-catenin protein expression in advanced gallbladder cancers and its association with decreased apoptosis. Oncology 2006;71:102–10.
176. Yadav A, Gupta A, Yadav S, Rastogi N, Agrawal S, Kumar A, et al. Association of Wnt signaling pathway genetic variants in gallbladder cancer susceptibility and survival. Tumour Biol 2016;37:8083–95.
177. Rosenbluh J, Wang X, Hahn WC. Genomic insights into WNT/beta-catenin signaling. Trends Pharmacol Sci 2014;35:103–9.
178. Eads JRLipika G, stein S, El-Khoueiry A, Manji GA, Abrams TA, et al. Phase I study of DKN-01, an anti-DKK1 antibody, in combination with gemcitabine (G) and cisplatin (C) in patients (pts) with advanced biliary cancer. J Clin Oncol 201634:15 (suppl,abstract e15603).
179. Fingas CD, Mertens JC, Razumilava N, Sydor S, Bronk SF, Christensen JD, et al. Polo-like kinase 2 is a mediator of hedgehog survival signaling in cholangiocarcinoma. Hepatology 2013;58:1362–74.
180. Mertens JC, Fingas CD, Christensen JD, Smoot RL, Bronk SF, Werneburg NW, et al. Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. Cancer Res 2013;73:897–907.
181. Fingas CD, Bronk SF, Werneburg NW, Mott JL, Guicciardi ME, Cazanave SC, et al. Myofibroblast-derived PDGF-BB promotes Hedgehog survival signaling in cholangiocarcinoma cells. Hepatology 2011;54:2076–88.
182. Xie F, Xu X, Xu A, Liu C, Liang F, Xue M, et al. Aberrant activation of Sonic hedgehog signaling in chronic cholecystitis and gallbladder carcinoma. Hum Pathol 2014;45:513–21.
183. Li J, Wu T, Lu J, Cao Y, Song N, Yang T, et al. Immunohistochemical evidence of the prognostic value of hedgehog pathway components in primary gallbladder carcinoma. Surg Today 2012;42:770–5.
184. Tang L, Tan YX, Jiang BG, Pan YF, Li SX, Yang GZ, et al. The prognostic significance and therapeutic potential of hedgehog signaling in intrahepatic cholangiocellular carcinoma. Clin Cancer Res 2013;19:2014–24.
185. Matsushita S, Onishi H, Nakano K, Nagamatsu I, Imaizumi A, Hattori M, et al. Hedgehog signaling pathway is a potential therapeutic target for gallbladder cancer. Cancer Sci 2014;105:272–80.
186. Riedlinger D, Bahra M, Boas-Knoop S, Lippert S, Bradtmoller M, Guse K, et al. Hedgehog pathway as a potential treatment target in human cholangiocarcinoma. J Hepatobiliary Pancreat Sci 2014;21:607–15.
187. Gherardi E, Birchmeier W, Birchmeier C, Vande WG. Targeting MET in cancer: rationale and progress. Nat Rev Cancer 2012;12:89–103.
188. Miyamoto M, Ojima H, Iwasaki M, Shimizu H, Kokubu A, Hiraoka N, et al. Prognostic significance of overexpression of c-Met oncoprotein in cholangiocarcinoma. Br J Cancer 2011;105:131–8.
189. Terada T, Nakanuma Y, Sirica AE. Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. Hum Pathol 1998;29:175–80.
190. Li H, Shimura H, Aoki Y, Date K, Matsumoto K, Nakamura T, et al. Hepatocyte growth factor stimulates the invasion of gallbladder carcinoma cell lines in vitro. Clin Exp Metastasis 1998;16:74–82.
191. Yang L, Guo T, Jiang S, Yang Z. Expression of ezrin, HGF and c-met and its clinicopathological significance in the benign and malignant lesions of the gallbladder. Hepatogastroenterology 2012;59:1769–75.
192. Moon WS, Park HS, Lee H, Pai R, Tarnawski AS, Kim KR, et al. Co-expression of cox-2, C-met and beta-catenin in cells forming invasive front of gallbladder cancer. Cancer Res Treat 2005;37:171–6.
193. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
194. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3:347–61.
195. Goyal L, Yurgelun MB, Abrams TA, Kwak EL, Cleary JM, Knowles M, et al. A Phase II trial of Cabozantinib (XL-184) in Patients with Advanced Cholangiocarcinoma. J Clin Oncol 33, 2015 (suppl 3; abstr 800).
196. O'Neill E, Kolch W. Conferring specificity on the ubiquitous Raf/MEK signalling pathway. Br J Cancer 2004;90:283–8.
197. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, et al. Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell 1998;93:605–15.
198. Chen TC, Jan YY, Yeh TS. K-ras mutation is strongly associated with perineural invasion and represents an independent prognostic factor of intrahepatic cholangiocarcinoma after hepatectomy. Ann Surg Oncol 2012;19 Suppl 3:S675–81.
199. Pai RK, Mojtahed K, Pai RK. Mutations in the RAS/RAF/MAP kinase pathway commonly occur in gallbladder adenomas but are uncommon in gallbladder adenocarcinomas. Appl Immunohistochem Mol Morphol 2011;19:133–40.
200. Goeppert B, Frauenschuh L, Renner M, Roessler S, Stenzinger A, Klauschen F, et al. BRAF V600E-specific immunohistochemistry reveals low mutation rates in biliary tract cancer and restriction to intrahepatic cholangiocarcinoma. Mod Pathol 2014;27:1028–34.
201. Saetta AA, Papanastasiou P, Michalopoulos NV, Gigelou F, Korkolopoulou P, Bei T, et al. Mutational analysis of BRAF in gallbladder carcinomas in association with K-ras and p53 mutations and microsatellite instability. Virchows Arch 2004;445:179–82.
202. Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut 2003;52:706–12.
203. Hyman DM, Puzanov I, Subbiah V, Faris JE, Chau I, Blay JY, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. N Engl J Med 2015;373:726–36.
204. Voss JS, Holtegaard LM, Kerr SE, Fritcher EG, Roberts LR, Gores GJ, et al. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. Hum Pathol 2013;44:1216–22.
205. Bekaii-Saab T, Phelps MA, Li X, Saji M, Goff L, Kauh JS, et al. Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. J Clin Oncol 2011;29:2357–63.
206. Bridgewater J, Lopes A, Beare S, Duggan M, Lee D, Ricamara M, et al. A phase 1b study of selumetinib in combination with cisplatin and gemcitabine in advanced or metastatic biliary tract cancer: the ABC-04 study. BMC Cancer 2016;16:153–2174.
207. Herberger B, Puhalla H, Lehnert M, Wrba F, Novak S, Brandstetter A, et al. Activated mammalian target of rapamycin is an adverse prognostic factor in patients with biliary tract adenocarcinoma. Clin Cancer Res 2007;13:4795–9.
208. Riener MO, Bawohl M, Clavien PA, Jochum W. Rare PIK3CA hotspot mutations in carcinomas of the biliary tract. Genes Chromosomes Cancer 2008;47:363–7.
209. Chung JY, Hong SM, Choi BY, Cho H, Yu E, Hewitt SM. The expression of phospho-AKT, phospho-mTOR, and PTEN in extrahepatic cholangiocarcinoma. Clin Cancer Res 2009;15:660–7.
210. Lee D, Do IG, Choi K, Sung CO, Jang KT, Choi D, et al. The expression of phospho-AKT1 and phospho-MTOR is associated with a favorable prognosis independent of PTEN expression in intrahepatic cholangiocarcinomas. Mod Pathol 2012;25:131–9.
211. Ahn DH, Li J, Wei L, Doyle A, Marshall JL, Schaaf LJ, et al. Results of an abbreviated phase-II study with the Akt inhibitor MK-2206 in patients with advanced biliary cancer. Sci Rep 2015;5:12122.
212. Yeung YH, Chionh FJM, Price TJ, Scott AM, Tran H, Fang G, et al. Phase II study of everolimus monotherapy as first-line treatment in advanced biliary tract cancer: RADichol. J Clin Oncol 32:5s, 2014 (suppl; abstr 4101).
213. Brand M, Measures AR, Wilson BG, Cortopassi WA, Alexander R, Hoss M, et al. Small molecule inhibitors of bromodomain-acetyllysine interactions. ACS Chem Biol 2015;10:22–39.
214. Marks EI, Yee NS. Immunotherapeutic approaches in biliary tract carcinoma: current status and emerging strategies. World J Gastrointest Oncol 2015;7:338–46.

215. Kaida M, Morita-Hoshi Y, Soeda A, Wakeda T, Yamaki Y, Kojima Y, et al. Phase 1 trial of Wilms tumor 1 (WT1) peptide vaccine and gemcitabine combination therapy in patients with advanced pancreatic or biliary tract cancer. J Immunother 2011;34:92–9.
216. Yamamoto K, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res 2005;25:3575–9.
217. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther 2008;6:955–64.
218. Kobayashi M, Sakabe T, Abe H, Tanii M, Takahashi H, Chiba A, et al. Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. J Gastrointest Surg 2013;17:1609–17.
219. Aruga A, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, et al. Phase I clinical trial of multiple-peptide vaccination for patients with advanced biliary tract cancer. J Transl Med 2014;12:61.
220. Aruga A, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, et al. Long-term vaccination with multiple peptides derived from cancer-testis antigens can maintain a specific T-cell response and achieve disease stability in advanced biliary tract cancer. Clin Cancer Res 2013;19:2224–31.
221. Yoshitomi M, Yutani S, Matsueda S, Ioji T, Komatsu N, Shichijo S, et al. Personalized peptide vaccination for advanced biliary tract cancer: IL-6, nutritional status and pre-existing antigen-specific immunity as possible biomarkers for patient prognosis. Exp Ther Med 2012;3:463–9.
222. Shimizu K, Kotera Y, Aruga A, Takeshita N, Takasaki K, Yamamoto M. Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci 2012;19:171–8.
223. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
224. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.
225. Rashid A, Ueki T, Gao YT, Houlihan PS, Wallace C, Wang BS, et al. K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: a population-based study in China. Clin Cancer Res 2002;8:3156–63.
226. Silva VW, Askan G, Daniel TD, Lowery M, Klimstra DS, Abou-Alfa GK, et al. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. Chin Clin Oncol 2016;5:62.
227. Suto T, Habano W, Sugai T, Uesugi N, Kanno S, Saito K, et al. Infrequent microsatellite instability in biliary tract cancer. J Surg Oncol 2001;76:121–6.
228. Kohya N, Miyazaki K, Matsukura S, Yakushiji H, Kitajima Y, Kitahara K, et al. Deficient expression of O(6)-methylguanine-DNA methyltransferase combined with mismatch-repair proteins hMLH1 and hMSH2 is related to poor prognosis in human biliary tract carcinoma. Ann Surg Oncol 2002;9:371–9.
229. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4$^+$ T cells in a patient with epithelial cancer. Science 2014;344:641–5.
230. Bang YJ, Doi T, De Braud F, Piha-Paul S, Hollebecque A, Abdul Razak AR, et al. Safety and efficacy of pembrolizumab (MK-3475) in patients (pts) with advanced biliary tract cancer: Interim results of KEYNOTE-028. Eur Cancer Congress 2015, Vienna, Abstract 525.