Biliary tract cancers: current knowledge, clinical candidates and future challenges

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Abstract: Biliary tract cancers (BTCs) are rare with poor prognosis. Due to the advent of genomic sequencing, new data have emerged regarding the molecular makeup of this disease. To add to the complexity, various subtypes also harbor a varied genetic composition. The commonly mutated genes associated with this cancer are KRAS, EGFR, IDH, FGFR and BAP1. Various clinical studies are looking at targeting these genetic mutations. Another therapeutic area of note is the potential for the use of immunotherapy in patients with BTC. Although BTC may be a result of chronic inflammation, this does not necessarily translate into increased immunogenicity. This literature review discusses the diverse molecular and immune-related pathways in patients with BTC and their potential therapeutic implications.

Keywords: biliary tract cancer, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, genome sequencing, molecular targets, immunotherapy

Introduction

Biliary tract cancers (BTCs) constitute epithelial malignancies of the biliary tree and include the following: gallbladder cancer (GBC), ampulla of Vater cancer (AVC), (the extra-hepatic [EHC] and intra-hepatic [IHC] bile ducts). Historically, the term cholangiocarcinoma (CCA) encompasses EHC and IHC, excluding GBC and AVC.1 The anatomical subtypes of BTC are depicted in Figure 1.

BTC constitutes approximately 3% of all gastrointestinal malignancies2 and is the most common hepatobiliary cancer after hepatocellular carcinoma.3 Unfortunately, the mortality rate (3.58 per 100,000) is very high. This is comparable to the incidence rate (3.64 per 100,000) in England4 and equates to a 5-year survival of 2% in the metastatic setting.5,6 The global prevalence of BTC has risen by a factor of 22%, and 150,000 patients were diagnosed with BTC in 2015.7 Overall, there is a huge variation in incidence with certain areas depicting high prevalence (eg, Japan and South Korea). This can be accounted for by liver fluke (Opisthorchis viverrini [OV] and Clonorchiasis sinensis [CS]) infestation in zones (north-east Thailand and China), where CCA is more common.8,9 Areas with high prevalence of cholelithiasis correspond to a high prevalence of GBC, such as India and Chile.10–12 Geographical regions where the abovementioned risk factors are uncommon have less cases of BTC.11

Apart from the abovementioned risk factors, primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), cirrhosis due to other causes, hepatitis C and congenital malformations such as choledochal cysts and multiple biliary papillomatosis are also associated with an increased risk of developing BTC.13–15
Further to these, patients with germline mutations resulting in Lynch syndrome and \textit{BRCA1} and \textit{BRCA2} (breast cancer gene 1 and 2) genetic aberrations are also predisposed to BTC. There is a lifetime risk of 2% of developing BTC with Lynch syndrome and RR of 4.97% of developing CCA in carriers of \textit{BRCA2}.\textsuperscript{16,17}

Treatments for BTC are stratified according to the stage of the disease, where surgery remains the mainstay of cure in early stages, although this represents a small minority of patients (10%–40%).\textsuperscript{18} Recent data from the BILCAP study support the use of adjuvant capecitabine with an improvement in median overall survival (OS) from 36 (observation alone after surgery) to 53 months (HR 0.75, \textit{P}=0.028 in the sensitivity analysis).\textsuperscript{19} For locoregional disease, treatments such as radio-embolization, chemoembolization and external beam radiotherapy can be considered, although due to preliminary evidence these techniques have not yet been adopted in standard practice. For the first-line treatment of advanced disease, the Phase III ABC-02 clinical trial confirmed the superiority of the combination of gemcitabine and cisplatin (GC) over single-agent gemcitabine. Reported median OS was 11.7 months vs 8.1 months, respectively (HR 0.64; 95% CI 0.52–0.80; \textit{P}<0.001),\textsuperscript{20} and henceforth this has become a global standard of care for late-stage BTC. Although the modest survival benefit gained from this regimen has not yet been surpassed in a randomized Phase III trial, the combination of gemcitabine with an oral fluoropyrimidine S-1, in a Phase III study, reported a median OS of 15.1 months for the gemcitabine and S-1 arm vs 13.4 months in the GC arm (HR 0.95; 90% CI 0.78–1.15; \textit{P}=0.046 for non-inferiority).\textsuperscript{21} This regimen may be considered as an alternative treatment for appropriate patients where comorbidities restrict the use of platinum agents. A Phase II clinical trial evaluating the combination of gemcitabine, cisplatin and nab-paclitaxel in the first-line setting in patients with advanced BTC has reported a superior median progression-free survival (PFS) than that associated historically with the standard GC regimen (11.4 months vs 8.0 months) in the preliminary results with a median OS of 19.2 months. This study (NCT02392637) is estimated to be completed in April 2019.\textsuperscript{22,23}

There is no current defined standard-of-care regimen in the second-line setting in advanced BTC. The current ABC-06 randomized Phase III clinical study is analyzing the role of chemotherapy in this setting vs symptomatic management in patients who have received previous chemotherapy. This study is completed, and the results are expected (NCT01926236).\textsuperscript{24}

The advent of genomic sequencing has led to better understanding of pathogenesis of cancers. Studies in BTC have revealed not only germline and somatic mutations but also genetic aberrations exclusive to anatomical subtypes of BTC. These include \textit{KRASTP53ErhB2} in EHC; \textit{IDH1/2FGFR1/2} and \textit{BAP1} in IHC; and \textit{TP53}, \textit{ErbB2}, \textit{PIK3CAErhB1/EGFR} in GBC.\textsuperscript{25–28} These findings may potentiate the development of the use of personalized medicine in this disease group.

Further to the use of genomics and personalized medicine aiming at indubitable targets in this cancer, the in-depth
analysis of the immune microenvironment may uncover potential targetable pathways, as BTC has been associated with chronic inflammatory pathology.11

The aim of this review was to evaluate the various potential pathways implicated at the molecular level in the development and progression of BTC and also to address the immune microenvironment and its potential involvement. Localized therapy is beyond the remit of this review.

Methodology

A categorical review of electronic databases was performed, which included Embase, Medline, PubMed and clinicaltrials.gov. Full manuscripts as well as conference abstracts available in the English language and published up to July 2018 employing the following keywords were interrogated: biliary tract cancer, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gall bladder cancer, genome sequencing, KRAS, BRAF, FGFR, IDH, VEGFR, EGFR, BAP1, molecular targets and immunotherapy.

Significant potential targetable pathways in patients with BTC

Figure 2 shows the important signaling pathways that may be targetable in patients with BTC.

Significant potential targetable pathways in patients with BTC

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The Ras–Raf–MEK–ERK pathway, as shown in Figure 2, is one of the focal signaling pathways for the development of carcinogenesis of BTC,29,30 and one of its first links, KRAS, is a frequently mutated site31 in BTC. Studies in different geographical regions have shown a variance in the frequency of KRAS across the anatomical subtypes, demonstrating 67% in EHC, 45% in IHC and 84% in GBC by the Japanese group,32 42% in EHC, 22% in IHC and 11% in GBC by the Cambridge and MD Anderson groups33 and 22% in EHC and 9% in IHC by the Mayo clinic group.34 Further to differences in frequencies as per the anatomical variant, the KRAS mutation was evaluated in association with clinical outcomes in patients with BTC in different geographical regions by groups in Taiwan, India and USA.32–34

Table 1 summarizes the association of OS with the mutation status for KRAS in the various anatomical subtypes of BTC. The frequency of KRAS mutations ranges from 0% to 41% in these studies, and its presence is associated with worse OS in all the different analyses.27,35–38

Apart from KRAS, other links in this pathway have been evaluated. In Taiwan, the presence of mutations in EGFR, KRAS and BRAF genes was analyzed in relation to median OS. Although the rest of the mutations failed to reveal a significant association with OS, patients carrying EGFR mutations had a median OS of 6 months as compared to 16 months (P≤0.00001) in patients who did not have these mutations.39 A German study evaluated 69 patients with CCA, reporting the presence of BRAF mutations in 22% of the cases, but OS was not significantly correlated with its presence.40 Another study revealed the presence of BRAF mutations in 7.4% of patients with IHC with the OS for patients with wild-type (wt) tumors being 37.3 months as compared to 13.5 months in the population bearing the mutation.41

Potential therapeutic targets for BTC within the Ras–Raf–MEK–ERK pathway

Different novel treatments for targeting the Ras–Raf–MEK–ERK pathway have been analyzed in various studies, including therapeutic agents such as sorafenib, selumetinib, refametinib, trametinib and pazopanib.

Sorafenib is a multi-kinase inhibitor and angiogenesis blocker, which after showing activity in vitro42 was tested in patients with inoperable or advanced IHC in a pilot study reporting an OS of 5.7 months in these cases.43 Further to this, a Phase II study described an increase in toxicities without any benefit in survival outcomes by adding sorafenib to cisplatin and gemcitabine in patients with advanced BTC.44

Selumetinib is another molecule which targets MEK1/2 link by inhibition and has been tested in vitro and in xenografts, prepared from patients with CCA and GBC. It demonstrated activity through cell cycle arrest and delayed reinitiation of S-phase in the cell cycle.45 A Phase II study of selumetinib in monotherapy in patients with predominantly pretreated advanced BTC reported a median OS of 9.8 months.46 Another MEK inhibitor, refametinib and vemurafenib, which is a v-Raf murine sarcoma viral oncogene homolog B (BRAF) inhibitor, is being assessed in Phase II clinical studies (NCT01524978, NCT02346032).37,48 Trametinib, which is an MEK inhibitor, has been tested alongside a VEGFR TKI, pazopanib, resulting in dose-limiting toxicities in nearly all patients (96% [24/25]) in this study who had CCA, while the OS was only 6.4 months.49 Another negative study was closed to accrual after interim analysis, where patients with pretreated advanced BTC who received trametinib failed to show any responses.50
Figure 2 Important signaling pathways of potential therapeutic significance in patients with BTC.

**Abbreviations:** AKT/PKB, protein kinase B; ARID1A, AT-rich interactive domain containing protein 1A; BAP1, BRCA1-associated protein 1; BRAF, V-Raf murine sarcoma viral oncogene homolog B; BTC, biliary tract cancer; Dvl, disheveled protein; ErbB1, erythroblast leukemia viral oncogene 1; ErbB2, erythroblast leukemia viral oncogene 2; FGFR, fibroblast growth factor receptor; FZD, frizzled family; HDAC, histone deacetylase; IDH, isocitrate dehydrogenase; KRAS, Kirsten rat sarcoma viral oncogene homolog; MAPK/ERK pathway, mitogen-activated protein kinase/extracellular signal-regulated kinase pathway; mTOR, mammalian target of rapamycin; NADP, nicotinamide adenine dinucleotide phosphate; NADPH (reduced), nicotinamide adenine dinucleotide phosphate; PDGFR, plasma-derived growth factor receptor; PI3KCA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit A; PKA, protein kinase; PORC, serine O-palmitoyltransferase porcupine protein; PTEN, phosphatase and tensin homolog; RNF43, ubiquitin E3 ligase ring finger 43; TCF, T cell factor; VEGFR, vascular endothelial growth factor receptor; Wnt, Wingless-related integration; ZNRF3, E3 ubiquitin ligase zinc and ring finger 3.
Relevance of the phosphatidylinositol-4,5-bisphosphate 3-kinase–AKT–mammalian target of rapamycin (PI3K–AKT–mTOR) pathway in patients with BTC

The PI3K–AKT–mTOR pathway, as shown in Figure 2, is also known to be a pivotal link in carcinogenesis. Preclinical studies on human CCA cell lines, using MEK1/2 and PI3K inhibitors, showed that the CXC chemokine ligand-12/C-X-C motif chemokine receptor 4 (CXCL-12/CXC4) was blocked by these inhibitors. These receptors/ligands act as an activator for this pathway. Another key element of this pathway, mTOR, was assessed via an mTOR inhibitor, everolimus, in vitro showing dose-dependent inhibition of cell proliferation by this inhibitor. Further to this, a Phase II study investigated everolimus as a first-line treatment option in patients with advanced BTC, reporting a median OS of 9.5 months and was associated with everolimus resistance in patients with KRAS mutations (P=0.03), with a negative correlation seen between basal pAKT and tAKT with everolimus resistance (P=0.007), regardless of the KRAS status.

Relevance of the FGF pathway in BTC

The FGF pathway as shown in Figure 2 directly and indirectly upregulates the MAPK and PI3K pathway, and some studies have shown that fusions in this pathway in BTC have a positive correlation with OS, specifically in the CCA cohort. These include FGFR2-BICCI, FGFR2-AHCYL1, FGFR2-MGEA5, FGFR2-TACC3, FGFR2-KIAA1598 and FGFR2-CREB5. The frequency of FGFR genetic aberrations varied from 8% to 25% with some exclusiveness for the IHC subtype, where most studies reported a range of 13%–14%. These include mutations, insertions, deletions, gene fusions, and translocations, etc.

Table 2 summarizes the variance in FGFR genetic alterations in the anatomical subtypes and its association with survival in patients with BTC. These studies show a wide range of frequency of FGFR genetic aberrations from 0% to 100% with a positive correlation with survival in carriers of the genetic aberration and a more indolent course of disease, resulting in better outcomes. Again, exclusiveness of the presence of FGFR genetic aberrations in the IHC subtype was noted.

Preclinical studies used a multi-receptor inhibitor (including FGFR), pazopanib, to target cell lines with mutated FGFR2, which resulted in cell cycle arrest after which ponatinib and/or pazopanib in two patients with IHC carrying the FGFR fusion gene who achieved partial responses. Another highly selective pan-FGFR inhibitor, BGJ398, was assessed in an umbrella study which included patients with CCA and resulted in stable disease in patients with FGFR2 fusions and mutations, whereas one patient who had a KRAS mutation progressed rapidly on this study drug. Recently, a Phase II study that analyzed the efficacy of BGJ398 in patients with advanced CCA harboring FGFR2 fusions or other FGFR molecular alterations that were refractory to standard-of-care chemotherapies was reported. It reported an overall response rate (ORR) of 14.8% and a disease control rate (DCR) of 75.4%; however, there was exclusiveness of response in the population harboring FGFR2 fusions only with a DCR of 83.3% in these patients. On the other hand, the patients (n=4) harboring FGFR3 amplifications did not show any response to BGJ398. A Phase II basket study analyzed ARQ 087 which is a pan-FGFR inhibitor in patients with CCA, adrenocortical carcinomas and other solid tumors with FGFR1-3 or KIT/PDGFR genetic aberrations. Of the

| Table 1 Association of survival and frequency of KRAS mutations in patients with BTC |

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>Patient group</th>
<th>Frequency of KRAS mutations (%)</th>
<th>Impact on survival</th>
<th>P-value</th>
<th>OS in months in KRAS mutant type</th>
<th>OS in months in KRAS wt</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>111</td>
<td>EHC, GBC, AVC</td>
<td>41%</td>
<td>Worse OS</td>
<td>0.003</td>
<td>NA</td>
<td>NA</td>
<td>HR=2.94</td>
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<tr>
<td>36</td>
<td>86</td>
<td>IHC</td>
<td>22%</td>
<td>Worse OS</td>
<td>0.002</td>
<td>5.7</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>39</td>
<td>GBC</td>
<td>41%</td>
<td>Worse OS</td>
<td>0.003</td>
<td>12.5</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>75</td>
<td>CCA</td>
<td>24% in IHC</td>
<td>Worse OS</td>
<td>0.002</td>
<td>7.4</td>
<td>40.2</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>412</td>
<td>IHC, EHC, GBC</td>
<td>22% in IHC, 42% in EHC, 0% in GBC</td>
<td>Worse OS</td>
<td>0.04</td>
<td>38.2</td>
<td>49.2</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>80</td>
<td>AVC</td>
<td>35%</td>
<td>Worse OS</td>
<td>0.021</td>
<td>78</td>
<td>138</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AVC, ampulla of Vater cancer; BTC, biliary tract cancer; CCA, cholangiocarcinoma; EHC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; IHC, intrahepatic cholangiocarcinoma; KRAS, Kirsten rat sarcoma viral oncogene homolog; NA, not available; OS, overall survival; wt, wild type.
80 patients analyzed, nearly one-half (n=7) of the 16 patients who exhibited durable response had a genetic alteration in the FGFR pathway, highlighting the response exclusivity to FGFR pathway alterations. In the IHC subgroup, three of five patients with FGFR2 fusions had response (partial response + stable disease) to treatment. All the five patients with IHC, but without FGFR fusions/amplifications, progressed on treatment. In other solid tumors, patients with FGFR amplifications also had a response.66 The same agent, ARQ 087, was analyzed in another Phase I/II open-label study of patients with IHC who were carriers of FGFR2 genes, showing promising results, with a durable DCR in 67% in these patients, and nine patients were still having ongoing treatment at the time the study was published.67 A Phase II study on an FGFR 1–4 inhibitor, TAS-120, in patients with refractory CCA and FGFR2 gene fusions and FGFR2 translocations reported a durable DCR in 67% in these patients, and nine patients were still having ongoing treatment at the time the study was published.69 A Phase II study on an FGFR 1–3 inhibitor, INCB054828, in patients who are refractory to first line and have unresectable, advanced or metastatic CCA harboring the FGFR2 translocation, and it is estimated to be completed in December 2018.69

### Table 2 Frequency of FGFR mutations across various anatomical subtypes of BTC and their association with survival

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>Patient groups</th>
<th>Frequency of FGFR mutations/fusions (%)</th>
<th>Association with survival</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>41</td>
<td>IHC, GBC</td>
<td>13% in IHC</td>
<td>NA</td>
<td>FGFR2 mutations</td>
</tr>
<tr>
<td>58</td>
<td>4</td>
<td>MBC, MPC, CCA</td>
<td>100% in CCA</td>
<td>NA</td>
<td>In 2/2 patients of CCA</td>
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<tr>
<td>62</td>
<td>28</td>
<td>IHC</td>
<td>14%</td>
<td>NA</td>
<td>Three gene fusions identified</td>
</tr>
<tr>
<td>60</td>
<td>156</td>
<td>IHC, EHC, intraductal papillary BTC</td>
<td>8%</td>
<td>FGFR2 translocation OS =37 months vs no translocation OS =23 months</td>
<td>Most mutations in IHC =13% All FGFR2 translocations OS P-value =0.039</td>
</tr>
<tr>
<td>56</td>
<td>102</td>
<td>CCA</td>
<td>13.6%</td>
<td>NA</td>
<td>No difference in OS noted</td>
</tr>
<tr>
<td>59</td>
<td>319</td>
<td>Lung, breast, papillary thyroid, glioblastoma, CCA</td>
<td>NA</td>
<td>NA</td>
<td>Presence of FGFR fusion in CCA sample</td>
</tr>
<tr>
<td>26</td>
<td>75</td>
<td>IHC, EHC</td>
<td>13% in IHC</td>
<td>NA</td>
<td>Better outcome</td>
</tr>
<tr>
<td>27</td>
<td>412</td>
<td>IHC, EHC, GBC</td>
<td>11% in IHC</td>
<td>NA</td>
<td>Indolent course of disease in carriers</td>
</tr>
</tbody>
</table>

### Abbreviations:
- BTC, biliary tract cancer
- CCA, cholangiocarcinoma
- EHC, extrahepatic cholangiocarcinoma
- FGFR, fibroblast growth factor receptor
- GBC, gallbladder cancer
- IHC, intrahepatic cholangiocarcinoma
- MBC, metastatic breast cancer
- MPC, metastatic prostate cancer
- NA, not available
- OS, overall survival

### Relevance of the isocitrate dehydrogenase (IDH) pathway in patients with BTC

IDH acts as a key enzyme for the citric acid cycle,70 as shown in Figure 2, and thus far mutations in this enzyme, which may result in oncogenesis,71 have been exclusively linked to the IHC sub-variant of BTC.72 Studies have reported a frequency of 19%–36% in patients with IHC,73 where Borger et al72 reported it as not only a mutation exclusive to IHC subtype but also the most frequently mutated gene in this disease subtype.

A preclinical study analyzed response to 122 Food and drug administration (FDA)-approved drugs to 17 BTC cell lines including two IHC cell lines with mutations in IDH1, employing high-throughput drug screening to produce a unique drug-sensitivity profile for each individual cell line. This study showed high sensitivity of the IDH-mutant IHC cell lines to dasatinib and saracatinib; both of which are inhibitors of Src family of tyrosine kinases, whereas dasatinib also inhibits (segment of Abelson proto-oncogene and breakpoint cluster region) BCR/ABL family of tyrosine kinases. Interestingly, this response did not correlate with Src activity in the IDH mutant IHC cells, and neither cell lines with IDH mutation in other solid tumors showed such a striking sensitivity. Although this article provides helpful insight into this pathway, it needs to be verified in human studies.73 Another preclinical study employed high-throughput screening to evaluate cell inhibition with 484 small molecular targeting compounds on cell lines and organoids derived from patients with IHC and EHC. This study reported pathways of resistance through micro-RNA 21 (MIR21) to heat shock protein 90 (HSP90) inhibitors. All cell lines, irrespective of mutations, were sensitive to HSP90 inhibitors, but high levels of MIR21 conferred resistance to these molecules. Not only this study was able to identify a potential therapeutic agent...
but also a biomarker for the efficacy of these agents warrants further evaluation in studies.74

The IDH mutations in patients who had liver fluke infestation (OV and CV) leading to CCA were analyzed and found to be prevalent in cases of IHC which were not associated with OV. The non-OV-associated group had a higher prevalence of IDH mutations: 9.3% as compared to 2.8% in the OV-associated cases.75

Table 3 summarizes the frequency and correlation of survival in patients with BTC and IDH mutations.

After the promising results of the Phase I study of an IDH1 inhibitor, AG-120, in patients with previously treated advanced BTC which showed stable disease in 56% of patients76 with IHC and EHC carrying the IDH1 mutation, a Phase III randomized clinical trial (RCT), “ClarIDHy”, has been developed, comparing AG-120 with a placebo in patients with CCA who carry the mutation in IDH1.77 It is estimated to be completed in August 2020.

Relevance of the Wingless-related integration (Wnt) pathway in patients with BTC

The Wnt signaling cascade is a complex intracellular signaling pathway, as shown in Figure 2, and its dysfunctionality can lead to stimulation of genes, such as c-myc, c-jun, VEGF and cyclin D.78,79 A preclinical study reported increased expression of Wnt and its components in human CCA and IHC cell lines, whereby the blockage of the Wnt pathway resulted in increased apoptosis and cell cycle arrest.80 This pathway has also been studied in relation to liver fluke infestation and one of its components, ubiquitin E3 ligase ring finger 43 (RNF43), was found to be mutated in 9.3% of cases of CCA which were associated with OV, alongside a negative trend for survival in these patients (HR 7.775; P<0.001).31 However, despite the abovementioned findings, apart from a preclinical study evaluating an inhibitor of the Wnt pathway, ie, Dickkopf-1 (DKK1) in cells lines from various tumor sites, including BTC, which suppressed cell invasion and growth, especially in cell lines which had a high expression of DKK1 gene.82 Currently in this particular pathway there no current trails in BTC.

Relevance of the deoxyribonucleic acid damage response (DDR) pathway in patients with BTC

Functional BRCA1 and BRCA2 genes are essential for genomic stability and help the nuclei in resisting damage to deoxyribonucleic acid (DNA). These genes are one of the tumor suppressor genes, and defects in these have been associated with apoptosis and malignant cell transformation.83 The breast cancer linkage consortium reports an RR of developing CCA in carriers of BRCA2 mutations to be 4.97%.84 The combined data from six studies61,85–89 evaluating 142 patients with all four types of BTC stated

<table>
<thead>
<tr>
<th>References</th>
<th>Methods</th>
<th>Number of patients</th>
<th>Patient group</th>
<th>Frequency of IDH mutations (%)</th>
<th>Impact on survival</th>
<th>P-value</th>
<th>OS in IDH mutant type</th>
<th>OS in IDH wt type</th>
<th>Comments</th>
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<tbody>
<tr>
<td>61</td>
<td>Exome sequencing</td>
<td>41</td>
<td>IHC, GBC</td>
<td>19% in IHC</td>
<td>Worse OS</td>
<td>0.0034</td>
<td>33% 3-year survival</td>
<td>81% 3-year survival</td>
<td>Exclusive to IHC</td>
</tr>
<tr>
<td>27</td>
<td>Comprehensive genomic profiling</td>
<td>554</td>
<td>IHC, EHC, GBC</td>
<td>20% in IHC</td>
<td>Unable to show</td>
<td></td>
<td></td>
<td></td>
<td>Exclusive to IHC and 0% in EHC and GBC</td>
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<tr>
<td>143</td>
<td>Tumor mutational analysis</td>
<td>104</td>
<td>IHC</td>
<td>28.8%</td>
<td>Unable to report</td>
<td></td>
<td></td>
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<tr>
<td>144</td>
<td>Whole exome sequencing</td>
<td>326</td>
<td>IHC</td>
<td>7.5% in Chinese 25% in Caucasian</td>
<td>Worse relapse-free survival</td>
<td>45.3% 7-year relapse-free survival</td>
<td>81.3% 7-year relapse-free survival</td>
<td>Difference in frequency in races</td>
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<tr>
<td>142</td>
<td>Next-generation sequencing</td>
<td>412</td>
<td>IHC, EHC, GBC</td>
<td>22% in IHC 42% in EHC 0% in GBC</td>
<td>Worse OS</td>
<td>0.04</td>
<td>38.2</td>
<td>49.2</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** BTC, biliary tract cancer; EHC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; IDH, isocitrate dehydrogenase; IHC, intrahepatic cholangiocarcinoma; OS, overall survival; wt, wild type.
a frequency of 2.41% for BRCA2 and 1.81% for BRCA1 genetic aberrations by the CCA Cancer Genome Atlas 2018, reporting a median survival of approximately 24 months (all stages). A retrospective study evaluated 18 patients with CCA who either carried germline (five cases) or somatic (13 cases) mutations reporting a median OS of 25 months in advanced stages (III and IV) and 40.27 months in early disease (stage I and II). These patients were also evaluated for response to treatments, where a platinum-based chemotherapy agent was compared to a poly-ADP ribose polymerase inhibitor (PARPi). The later showed a better outcome, and patients who were treated with PARPi were reported to have an OS going up to 64.76 months. A Phase I/II study, which is estimated to start in August 2018, is going to analyze the dose and side effects of liposomal irinotecan alongside a PARPi (rucaparib) in various cancers including BTC and is expected to be completed in 2021. Another Phase II trial is analyzing the ORR with a PARPi (niraparib) in patients with BAP1 and other DNA damage response pathway-deficient cancers including CCA and is expected to be completed in 2021.

Clinical relevance of key targets identified in patients with BTC
After discussing individual potential implicated pathways, this review now evaluates some further mechanistic targets, including angiogenesis and their role in BTC.

Angiogenesis in tumors of patients with BTC
Although factors such as vascular endothelial growth factor (VEGF), FGF and EGF, which promote angiogenesis, have been identified in patients with BTC, BTCs are considered as hypovascularized tumors. This fact is further supported in patients with IHC, by the presence of low microvessel density (MVD) in tumors, which denotes areas of neovascularization. Another study reported a mean MVD of 30.5 vessels per ×200 optical field in a sample of 62 patients with GBC. Within this cohort, patients who had higher MVD had a worse median OS (2-year survival of 25%) as compared to patients with low MVD (2-year survival of 43%). The same group evaluated MVD in another sample of 60 patients with GBC and revealed an MVD of 20 per ×200 optical field. Another study on 118 patients with GBC confirmed a correlation of tumor stage and liver metastasis with MVD and classified MVD as an independent prognostic factor.

Role of VEGF in patients with BTC
VEGF, which has been reported as a pivotal angiogenesis factor, was found to be highly expressed at 75.6% in a study of 33 surgically resected cases of CCA. A larger analysis of tumors from 236 patients for molecular profiling revealed the presence of the VEGF gene in 53.8% (n=57/106) of IHC and 59.2% (n=77/130) of EHC cases. A study that assessed 60 cases of patients with GBC by immunohistochemistry revealed a high VEGF expression in 27 cases and a low VEGF expression in 33 cases, where no significant association between VEGF expression and survival was found.

Among the various VEGF inhibitors, bevacizumab and cediranib have been assessed in patients with BTC. A Phase II study that evaluated the addition of bevacizumab to gemcitabine and oxaliplatin reported a median OS of 14.2 months in patients with advanced IHC (n=22) and 8.5 months in patients with advanced GBC (n=10), whereas median OS was not given for patients with EHC (n=3). A Phase III study, ABC-03, evaluated the addition of cediranib (vs placebo) to GC chemotherapy in patients with advanced BTC. Although this study failed to reach its primary end point (improvement in PFS) or show a significant difference in OS, the response rate improved by 25% in the cediranib arm (P=0.0036). A Phase II study comparing ramucirumab (VEGF antibody) vs merestinib (MET inhibitor) vs placebo, in combination with GC in patients with advanced or metastatic BTC as a first-line treatment, has completed accrual and is awaiting results.

Role of EGFR in patients with BTC
The EGFR/HER2 receptor acts through targeting all the leading pathways including the Ras-Raf-MAP-ERK pathway, the PI3k-AKT-mTOR pathway, the phospholipase C, Ca2+/calmodulin-dependent kinase (CaMK/PKC), Janus-associated kinase (JAK) pathway and the STAT protein pathway which makes it a highly susceptible anti-tumorigenesis target. It was found to be present in 100% of IHC samples, 52.6% of EHC samples and 38.5% of GBC samples from treatment-naïve patients.

Table 4 summarizes the various EGFR antibodies such as erlotinib, cetuximab and panitumumab which have been analyzed in various combinations with gemcitabine in selective (KRAS wt) and nonselective patient groups with advanced (inoperable or metastatic) BTC in Phase II and III clinical studies. However, the largest Phase III study that analyzed samples from 268 patients who were diagnosed with all the four types of advanced BTC failed to show any
Table 4 Potential treatments for targeting EGFR in patients with BTC

<table>
<thead>
<tr>
<th>References</th>
<th>Phase</th>
<th>Agent investigated</th>
<th>Number of patients</th>
<th>Patient group</th>
<th>Survival</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>II</td>
<td>Erlotinib</td>
<td>42</td>
<td>aBTC</td>
<td>8% confirmed response rate</td>
<td>28% in the EGFR group were PF at 5.52 months</td>
</tr>
<tr>
<td>107</td>
<td>III</td>
<td>Erlotinib with GemOx</td>
<td>268</td>
<td>IHC, EHC, GBC, AVC</td>
<td>30% ORR in the erlotinib arm</td>
<td>No difference in OS</td>
</tr>
<tr>
<td>146</td>
<td>II</td>
<td>Cetuximab with GemOx</td>
<td>34</td>
<td>aBTC</td>
<td>Median PFS = 7.89 months</td>
<td>Median OS = 14.45 months</td>
</tr>
<tr>
<td>147</td>
<td>II</td>
<td>Cetuximab with GemCape</td>
<td>122</td>
<td>aBTC</td>
<td>Median PFS = 6.7 months vs 4.1 months (P=0.05)</td>
<td>No significant benefit in OS, no relation to KRAS mutation</td>
</tr>
<tr>
<td>148</td>
<td>II</td>
<td>Cetuximab with GemOx</td>
<td>150</td>
<td>aBTC</td>
<td>PFS = 6.1 months</td>
<td>Failed to reach PEP (improvement in PFS)</td>
</tr>
<tr>
<td>149</td>
<td>II</td>
<td>Panitumumab with GemOx</td>
<td>46</td>
<td>Unresectable KRAS wt BTC</td>
<td>Median PFS = 8.3 months</td>
<td>Median OS = 10.0 months</td>
</tr>
<tr>
<td>150</td>
<td>II</td>
<td>Panitumumab with CisGem</td>
<td>93</td>
<td>KRAS wt aBTC and aGBC</td>
<td>PFS at 6 months = 73% (P=0.24)</td>
<td>Failed to reach PEP. OS = 21.4 months (P=0.35)</td>
</tr>
<tr>
<td>151</td>
<td>II</td>
<td>Panitumumab with GemOx</td>
<td>31</td>
<td>KRAS wt aBTC</td>
<td>Median PFS = 10.6 months</td>
<td>Median OS = 20.3 months</td>
</tr>
</tbody>
</table>

**Abbreviations:** aBTC, advanced biliary tract cancer; aGBC, advanced gallbladder cancer; AVC, ampulla of Vater cancer; CisGem, cisplatin and gemcitabine chemotherapy; EHC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GemCape, gemcitabine and capecitabine; GemOx, gemcitabine and oxaliplatin; IHC, intrahepatic cholangiocarcinoma; KRAS, Kirsten rat sarcoma viral oncogene homolog; ORR, overall response rate; OS, overall survival; PEP, primary end point; PF, progression free; PFS, progression-free survival; wt, wild type.

The presence of aberrations in the BAP1 gene was associated with shorter OS in patients with EHC (8.9 months vs 19.9 months, P=0.007), when compared to patients with EHC, who did not have the BAP1 gene mutation.26 Another study that reviewed 22 patients with CCA who bore mutations in the BAP1 gene reported a mean time to progression of 3.8 months in these cases, and a patient who had undergone curative resection presented with recurrence 8 weeks after surgery, illustrating the aggressive nature of this disease.112

Histone deacetylase inhibitors (HDACI) have been used to target BAP1 mutations and have shown preclinical activity with 30% inhibitory effect in the CCA cell lines13 and in combination with cisplatin led to cytotoxicity, inhibition of growth and increased cell apoptosis in another preclinical study using CCA cell lines.114

**Role of BAP1 in patients with BTC**

Germline mutations in the BAP1 gene have been associated with cancers of the uvea, kidney, skin and mesothelium,109 and it has been identified as a tumor suppressor gene.110 A study of 64 patients has reported mutations in this gene in 20% of patients with IHC and 6% of patients with GBC.61 A larger study reported the presence of BAP1 mutations in 26% of cases from a cohort of 211 patients with IHC.111

This gene was also analyzed in 209 patients in association with OV-associated cases of CCA, and a 10.5% frequency of BAP1 genes in non-OV-associated cases was reported in comparison to 2.8% in OV-associated cases.75

The presence of aberrations in the BAP1 gene was associated with short time to recurrence in postsurgical patients with CCA in a study that included 75 patients. It was also associated with shorter OS in patients with EHC (8.9 months vs 19.9 months, P=0.007), when compared to patients with EHC, who did not have the BAP1 gene mutation.26 Another study that reviewed 22 patients with CCA who bore mutations in the BAP1 gene reported a mean time to progression of 3.8 months in these cases, and a patient who had undergone curative resection presented with recurrence 8 weeks after surgery, illustrating the aggressive nature of this disease.112

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**Differentiation of the proliferation and the inflammation class**

Further to the abovementioned details, a multinational study has presented an interesting concept of defining two unique classes of BTC to help understand the tumor biology of BTC. This study assessed 149 samples (including all stages) of IHC from Milan, Barcelona and New York. The study analyzed genomic mutations using high-density single-nucleotide polymorphism array and gene expression profiles. It classified the samples into two broad categories: the “inflammation class” and the “proliferation class”. The first class, ie, “the inflammation class”, constituted 38% of the total samples and
was found to have overexpression of cytokines and activation of STAT3. However, “the proliferation class”, accounting for 62% of the total samples, harbored activated oncogenic pathways with mutations expressed in MAPK, Ras pathways and KRAS, BRAF genes. A better median OS was associated with the inflammation class, ie, 47.2 months compared to 24.3 months in the proliferation class (P=0.048).115 This innovative concept needs further exploration to assess whether this work can be used translationally.

Table 5 summarizes the current trials in targeted treatments in BTC registered on clinicaltrials.gov (last accessed on August 25, 2018).

Potential for the use of immunotherapy in patients with BTC

BTCs have been associated with chronic inflammatory conditions and viral infections; therefore, there may be a role for immunomodulatory agents in this disease group. Understanding the underlying immune environment may yield a successful strategy to target this poor prognostic disease group.

The concept of immunosurveillance and immune editing has been supported by Dunn et al.116 They introduced the concept of elimination whereby the host immunity consisting of natural killer T cells (NKTC), natural killer cells (NKC), interferon γ (IFN-γ; initiates immune reactions) led to cytotoxic death of cancer cells. This was followed by equilibrium whereby the immune environment and the cancer cells lived in harmony. Eventually, leading to escape, whereby the cancer cells that survived the equilibrium phase form tumorigenic growths. Research was conducted on the development of carcinoma in situ leading to fulminant tumors in a large sample size of 375 patients with BTC. This study reported an increase in the number of macrophages as the precancerous lesion developed into carcinoma, whereas B-lymphocytes, CD8+ T cells, CD4+ T cells, regulatory T cells, mast cells and NKC steadily declined as the cancer formed.117 The same cells that reduced in number as the cancer formed were associated with better prognosis, highlighting a robust immunosurveillance mechanism led by these cells.

Inflammatory markers using neutrophil-to-lymphocyte ratio (NLR) and derived neutrophil-to-lymphocyte ratio (dNLR) have been evaluated as prognostic biomarkers in BTC. Higher values of NLR ≥3.0 in patients with BTC were associated with a poor OS of 12 months as compared to patients who had lower values of NLR <3.0 with an OS of 21.6 months (adjusted HR =1.26, P=0.01).118 Similarly, high dNLR was associated with poor prognosis.119

Although the role of cancer vaccines has been evaluated in Phase I clinical studies in BTC, the modest benefit has not sparked enough interest to lead to further trials.120–122 Another important component of the immune environment is the cytokines that were analyzed in a cohort of 54 patients with inoperable or advanced BTC who had stable disease after first-line chemotherapy followed by chemoradiation. This was followed by IL2 and retinoic acid (RA) infusion. Although only a small number completed treatment (seven patients), the median OS was not reached when the trial was reported (at 27.5 months).123 Apart from vaccines, adoptive cell therapy (ACT) is also being evaluated in BTC, albeit in small case series or case reports. This involves using the patient’s own cells, which are adapted after extrapolating them from the host. The reformed cells are then again infused into the host after the depletion of lymph in patients. A single case study of a locally advanced patient with IHC treated with ACT was reported as being disease-free 3.5 years after surgery at the time of the case report.124 In another case report, a patient with metastatic CCA who received ACT followed by IL2 was reported to have stable disease for 13 months.125 A case series that reported on the combination of the use of vaccine and ACT in the adjuvant setting reported a better OS of 31.9 months in the patients who received this adjuvant treatment strategy as compared to a median OS of 17.4 months in patients (P=0.022) who underwent surgery alone.126

Immune check point inhibitors are currently being used across various poor prognostic tumor groups with good results; however, there is some association of programmed cell death protein ligand-1 (PD-L1), expression and effectiveness of these treatments.127,128 Expression of PD-L1 in BTC shows a wide range from 29% to 100%,129,130 and the full analysis of the KEYNOTE-028 study is still awaited. This Phase Ib trial is evaluating the effects of treatment with a monoclonal antibody against human immune cell check point programmed death 1 (PD-1), pembrolizumab in patients with previously pretreated advanced BTC who have PD-L1 expression.131 BTCs are infrequently associated with Lynch syndrome, a genetic disorder that predisposes to microsatellite instability (MSI) and mismatch repair deficiency (MMR).132 With the food and drug association (FDA) approval of pembrolizumab132 for MSI- and MMR-deficient tumors, in patients who have BTC associated with Lynch syndrome, there may be an option for treating them with immune check point inhibitors, where available.133

Table 6 summarizes the current immune-mediated trials in BTC registered on clinicaltrials.gov (last accessed on August 25, 2018).
<table>
<thead>
<tr>
<th>NCT; references</th>
<th>Study</th>
<th>Phase</th>
<th>Status</th>
<th>Recruiting countries</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>NCT03144856</td>
<td>Apatinib as second-line treatment in aBTC</td>
<td>II</td>
<td>Recruiting</td>
<td>China</td>
<td>VEGFR2 TKI</td>
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<tr>
<td>NCT02579616</td>
<td>Lenvatinib (E7080) in unresectable BTC who have failed Gem-based combination</td>
<td>II</td>
<td>Active, not recruiting</td>
<td>Japan</td>
<td>VEGFR1/2/3 TKI</td>
</tr>
<tr>
<td>NCT02773459</td>
<td>To test the efficacy of MEK162 with Cape in Gem-pretreated aBTC, predict biomarkers</td>
<td>I/II</td>
<td>Recruiting</td>
<td>Korea</td>
<td>MEK inhibitor</td>
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<tr>
<td>NCT03093870</td>
<td>Multicentre double-blind, placebo-controlled study of varlitinib plus capcitabine vs placebo plus capcitabine in aBTC or mBTC as second-line treatment</td>
<td>I/III</td>
<td>Not yet recruiting</td>
<td>USA</td>
<td>EGFR, HER2, HER4 inhibitor</td>
</tr>
<tr>
<td>NCT03129074</td>
<td>Varlitinib and Cape in aBTC and mBTC patients</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>NA</td>
<td>EGFR, HER2, HER4 inhibitor</td>
</tr>
<tr>
<td>NCT03110484</td>
<td>Pemetrexed with erlotinib as salvage treatment in mBTC patients who have failed Gem</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>Korea</td>
<td>EGFR TKI</td>
</tr>
<tr>
<td>NCT02992340</td>
<td>Varlitinib with GC for treatment-naive aBTC or mBTC</td>
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<td>Recruiting</td>
<td>Korea, Singapore, Taiwan</td>
<td>EGFR, HER2, HER4 inhibitor</td>
</tr>
<tr>
<td>NCT02711553</td>
<td>Ramucirumab or merestinib or placebo with GC in aBTC or mBTC</td>
<td>II</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>Ramucirumab = VEGFR2 antibody</td>
</tr>
<tr>
<td>NCT02966821</td>
<td>Sulfatinib as second-line treatment in patients with BTC</td>
<td>II</td>
<td>Recruiting</td>
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<td>VEGFR and FGFR 1 TKI</td>
</tr>
<tr>
<td>NCT02520141</td>
<td>Ramucirumab for pretreated aBTC</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>VEGFR2 antibody</td>
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<tr>
<td>NCT02836847</td>
<td>Molecularly targeted therapy with GemOx in aEHC or rEHC and GBC</td>
<td>II</td>
<td>Recruiting</td>
<td>China</td>
<td>Precision target therapy</td>
</tr>
<tr>
<td>NCT02443324</td>
<td>Ramucirumab with pembrolizumab in gastric, GEJ adenocca, NSCLC, TCC in urothelium or BTC</td>
<td>I</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>Ramucirumab = VEGFR2 antibody</td>
</tr>
<tr>
<td>NCT02386397</td>
<td>Regorafenib dose for Phase II trial in combination with mGemOx in aBTC</td>
<td>I/II</td>
<td>Recruiting</td>
<td>France</td>
<td>Dual VEGFR-TIE2 TKI</td>
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<tr>
<td>NCT01825603</td>
<td>ADH-1 with GC in patients with irresectable BTC/pancreatic ca</td>
<td>I</td>
<td>Recruiting</td>
<td>USA</td>
<td>Alcohol dehydrogenase 1 blocks N-cadherin</td>
</tr>
<tr>
<td>NCT03082053</td>
<td>Varlitinib in Japanese subjects with advanced or MET solid tumors</td>
<td>I</td>
<td>Recruiting</td>
<td>Japan</td>
<td>EGFR, HER2, HER4 inhibitor</td>
</tr>
<tr>
<td>NCT00948935</td>
<td>Panitumumab with Gemr in aBTC and mBTC</td>
<td>II</td>
<td>Ongoing but not recruiting</td>
<td>USA</td>
<td>EGFR antibody</td>
</tr>
<tr>
<td>NCT02151084</td>
<td>Different dosing schedules of selumetinib with GC vs GC alone in BTC</td>
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<td>Recruiting</td>
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<td>MEK inhibitor</td>
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<td>NCT02265341</td>
<td>Ponatinib in aBTC patients with FGFR2 fusions</td>
<td>II</td>
<td>Recruiting</td>
<td>US</td>
<td>BCR–ABL inhibitor</td>
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<td>NCT02451553</td>
<td>Afatinib with Cape in advanced refractory solid tumors, pancreatic ca, BTC</td>
<td>I</td>
<td>Recruiting</td>
<td>USA</td>
<td>EGFR TKI</td>
</tr>
<tr>
<td>NCT02943031</td>
<td>Effect of individualized precision therapy programs in patients with BTC</td>
<td>IV</td>
<td>Not yet recruiting</td>
<td>China</td>
<td>Precision therapy</td>
</tr>
<tr>
<td>NCT03185980</td>
<td>Patients with metastatic disease of the digestive system</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>China</td>
<td>Anti-HER2 therapy</td>
</tr>
<tr>
<td>NCT02042443</td>
<td>Trametinib or combination chemotherapy in patients with refractory or advanced BTC or GBC that is irresectable</td>
<td>II</td>
<td>Active, not recruiting</td>
<td>USA</td>
<td>MEK inhibitor</td>
</tr>
<tr>
<td>NCT02586987</td>
<td>Study to assess the safety and tolerability of ascending doses of selumetinib with MEDI4736 and selumetinib, MEDI4736, treumelumab in advanced solid tumors</td>
<td>I</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>Selumetinib = MEK inhibitor</td>
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<thead>
<tr>
<th>NCT; references</th>
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<th>Phase</th>
<th>Status</th>
<th>Recruiting countries</th>
<th>Comments</th>
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<tr>
<td>NCT02128282174</td>
<td>CX-4945 in combination with GC as frontline treatment for CCA</td>
<td>I/II</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>CK2 protein kinase inhibitor</td>
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<tr>
<td>NCT02053376175</td>
<td>Phase II trial of regorafenib in aBTC and mBTC, CCA patients who have failed first-line chemotherapy</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>Dual VEGFR-TIE2 TKI</td>
</tr>
<tr>
<td>NCT02715089176</td>
<td>Precise treatment in hepatobiliary cancers</td>
<td>Observational</td>
<td>Recruiting</td>
<td>China</td>
<td>Precision treatment</td>
</tr>
<tr>
<td>NCT01752920177</td>
<td>Phase I/II study of ARQ087 in advanced solid tumors with FGFR genetic alterations</td>
<td>I/II</td>
<td>Active, not recruiting</td>
<td>USA</td>
<td>Pan-FGFR inhibitor</td>
</tr>
<tr>
<td>NCT01855724176</td>
<td>Clinical trial to investigate the efficacy of treatment with Gem and pazopanib in BTC</td>
<td>II</td>
<td>Recruiting</td>
<td>Greece</td>
<td>C-KIT, FGFR, PDGFR and VEGFR inhibitor</td>
</tr>
<tr>
<td>NCT02631590179</td>
<td>Copanlisib with GC in aCCA</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PI3K inhibitor</td>
</tr>
<tr>
<td>NCT02576341180</td>
<td>Study of LOXO-101 (larotrectinib) in subjects with NTRK fusion-positive solid tumors (NAVIGATE)</td>
<td>II</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>Tropomyosin receptor kinase inhibitor</td>
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<td>NCT03027284180</td>
<td>Merestinib in Japanese patients with advanced or metastatic CA</td>
<td>I</td>
<td>Recruiting</td>
<td>Japan</td>
<td>MET inhibitor</td>
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<tr>
<td>NCT01766219180</td>
<td>CPI-613 in irresectable, advanced or metastatic BTC</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PDH and αKGDH inhibitor</td>
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<tr>
<td>NCT02495896183</td>
<td>Recombinant EphB4-HSA fusion protein with standard chemo in advanced or metastatic solid tumors</td>
<td>I</td>
<td>Recruiting</td>
<td>USA</td>
<td>Recombinant fusion protein composed of full length extracellular domain soluble of human receptor TK ephrin type B receptor 4</td>
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<tr>
<td>NCT03639935180</td>
<td>Rucaparib in combination with nivolumab in patients with advanced or mBTC following platinum</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>USA</td>
<td>PARP inhibitor and PD-1 antibody</td>
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<tr>
<td>NCT02433639180</td>
<td>Study of TH-302 monotherapy as second-line treatment in aBTC</td>
<td>II</td>
<td>Unknown</td>
<td>South Korea</td>
<td>Hypoxia activated prodrug</td>
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<td>NCT03185989180</td>
<td>Anti-HER2 therapy in patients with metastatic disease and HER2-positive disease of the digestive system</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>China</td>
<td>HER2 antibody</td>
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<td>NCT02115542180</td>
<td>Single-agent regorafenib in refractory aBTC</td>
<td>II</td>
<td>Active</td>
<td>USA</td>
<td>Multikinase inhibitor</td>
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<td>NCT03337087180</td>
<td>Varglintinib in combination with capcitabine for metastatic and aBTC</td>
<td>II/III</td>
<td>Recruiting</td>
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<td>Precise treatment in hepatobiliary cancers</td>
<td></td>
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<td></td>
<td>Use of NGS to obtain genomic data and targeting specific mutations with precision medicines</td>
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<td>NCT02631590180</td>
<td>Copanlisib (BAY B0-6946) in combination with GC in aCCA</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PI3K inhibitor</td>
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<td>NCT03230318180</td>
<td>ARQ087 in subjects with FGFR2 gene fusion-positive inoperable or advanced IHC</td>
<td>II</td>
<td>Recruiting</td>
<td>USA, Canada and Italy</td>
<td>Pan-FGFR inhibitor</td>
</tr>
</tbody>
</table>

**Abbreviations:** aBTC, advanced biliary tract cancer; aCCA, advanced cholangiocarcinoma; αKGDH, α-ketoglutarate dehydrogenase; BTC, biliary tract cancer; Bcr-abl, bcr-abl fusion oncogene/Philadelphia chromosome; ca, carcinoma; chemo, chemotherapy; CCA, cholangiocarcinoma; cKIT, proto-oncogene c-Kit; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FGFR, fibroblast growth factor receptor; GEJ, gastro-esophageal junction; GBC, gallbladder cancer; GC, gemcitabine and cisplatin; gem, gemcitabine; GemCape, gemcitabine and capecitabine; GemOx, gemcitabine and oxaliplatin; HCC, hepatocellular carcinoma; HER, human EGF receptor; HER2, human EGF receptor 2; IHC, intrahepatic cholangiocarcinoma; mBTC, metastatic biliary tract cancer; MEK, mitogen-activated protein kinase; MET, MET proto-oncogene, mesenchymal to epithelial transition proto-oncogene; NGS, next generation sequencing; NA, not available; NSCLC, non-small-cell lung cancer; PARP, poly-ADP ribose polymerase; PD-1, programmed death 1; PDGFR, plasma-derived growth factor receptor; PDH, pyruvate dehydrogenase; PD-L1, programmed cell death ligand-1; PI3K, phosphoinositide 3-kinase; TIE2, TCC, transitional cell carcinoma; TIE family of angiopoietin receptor kinase; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
Discussion

BTCs are rare cancers which are poorly understood and have few treatment options, low response rates and bad prognosis. The rarity and difficulty in getting good diagnostic samples pose hurdles to effective development of translational research. The various complex issues that govern this disease group include the following: identification of a driver mutation, heterogeneity that exists within the tumor, difficulty in getting repeat samples on recurrence and difference in the behavior of cell lines from real-life patients.

Available information on genomic and somatic mutations in patients with BTC has expanded, but this comes with its own limitations. Various techniques used for molecular profiling yield different results, and there is a lack of global standardization. Much work is needed to reduce the variance in the results obtained across the use of different methodologies.

Intra-tumoral heterogeneity has been an area of debate from as early as 1976, further supplemented by work done in clonal evolution in 1990 by Fearon and Vogelstein. Further research has been completed recently in this area, where central and peripheral samples from the same tumorogenic mass, from four patients with surgically resected IHC, were evaluated for private and common mutations. Therefore, private mutations were defined as exclusive mutations found

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**Table 6 Current trials involving the immune system in BTC registered on clinicaltrials.gov**

<table>
<thead>
<tr>
<th>NCT</th>
<th>Study</th>
<th>Phase</th>
<th>Status</th>
<th>Recruiting countries</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02829918</td>
<td>Nivolumab in patients with aBTC which is refractory</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PD-1 antibody</td>
</tr>
<tr>
<td>NCT03110328</td>
<td>Pembrolizumab in mBTC as second-line treatment after failing one cytotoxic chemo</td>
<td>II</td>
<td>Not yet open</td>
<td>Korea</td>
<td>PD-1 antibody</td>
</tr>
<tr>
<td>NCT03046862</td>
<td>Durvalumab/tremelimumab in combination with GC in chemo-naive BTC patients</td>
<td>II</td>
<td>Recruiting</td>
<td>Korea</td>
<td>Durvalumab = PD-L1 antibody Tremelimumab = CTLA-4 antibody</td>
</tr>
<tr>
<td>NCT03101566</td>
<td>Nivolumab in combination with GC or ipilimumab in aBTC</td>
<td>II</td>
<td>Not yet open</td>
<td>USA</td>
<td>Nivolumab = PD-1 antibody Ipilimumab = CTLA4 antibody</td>
</tr>
<tr>
<td>NCT02632019</td>
<td>Immunotherapy using precision T cells specific to neo-antigens for the treatment of advanced BTC</td>
<td>I/II</td>
<td>Recruiting</td>
<td>China</td>
<td>Dendritic cell precision T cells against neoantigen</td>
</tr>
<tr>
<td>NCT02586987</td>
<td>Study to assess the safety and tolerability of ascending doses of selumetinib with MEDI4736 and selumetinib, MEDI4736, tremelimumab in advanced solid tumors</td>
<td>I</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>Selumetinib = MEK inhibitor MEDI4736 = PD-L1 antibody Tremelimumab = CTLA4 antibody</td>
</tr>
<tr>
<td>NCT01938612</td>
<td>Phase I, open-label, multicentre study to evaluate MEDI4736 in advanced solid tumors</td>
<td>I</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>PD-L1 antibody</td>
</tr>
<tr>
<td>NCT02628067</td>
<td>Pembrolizumab in patients with advanced solid tumors, KEYNOTE-158</td>
<td>II</td>
<td>Recruiting</td>
<td>Multinational</td>
<td>PD-L1 antibody</td>
</tr>
<tr>
<td>NCT02821754</td>
<td>Pilot study of combined immune check point inhibitors with ablative therapy in HCC and BTC</td>
<td>I/II</td>
<td>Recruiting</td>
<td>USA</td>
<td>Durvalumab = PD-L1 antibody Tremelimumab = CTLA4 antibody</td>
</tr>
<tr>
<td>NCT01853618</td>
<td>Tremelimumab with chemoembolization or ablation for liver ca</td>
<td>I</td>
<td>Active, not recruiting</td>
<td>USA</td>
<td>CTLA4 antibody</td>
</tr>
<tr>
<td>NCT02662348</td>
<td>T cell-mediated adaptive therapy for Her2-positive digestive system ca</td>
<td>I</td>
<td>Enrolling by invitation</td>
<td>China</td>
<td>HER2 Bi-armed T cells</td>
</tr>
<tr>
<td>NCT03482102</td>
<td>Durvalumab (MEDI4736) and tremelimumab and radiation therapy in HCC and BTC</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PD-L1 antibody and anti-CTLA4</td>
</tr>
<tr>
<td>NCT03111732</td>
<td>Pembrolizumab with CapeOx in aBTC</td>
<td>II</td>
<td>Recruiting</td>
<td>USA</td>
<td>PD-1 antibody</td>
</tr>
<tr>
<td>NCT03260712</td>
<td>Pembrolizumab in BTC</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>Germany</td>
<td>PD-1 antibody</td>
</tr>
<tr>
<td>NCT03358849</td>
<td>Evaluation of the safety of allogeneic NKC (SMT-NK) cell therapy in aBTC</td>
<td>I</td>
<td>Recruiting</td>
<td>South Korea</td>
<td>Human NKC therapy designated as SMT 01</td>
</tr>
</tbody>
</table>

Abbreviations: aBTC, advanced biliary tract cancer; aCCA, advanced cholangiocarcinoma; BTC, biliary tract cancer; ca, carcinoma; CapeOx, capecitabine and oxaliplatin; CCA, cholangiocarcinoma; chemo, chemotherapy; CTLA4, cytotoxic T-lymphocyte-associated antigen 4; GBC, gallbladder cancer; GC, gemcitabine and cisplatin; HCC, hepatocellular carcinoma; HER, human EGF receptor; mBTC, metastatic biliary tract cancer; MEK, mitogen-activated protein kinase; NKC, natural killer cell; PD-1, programmed death 1; PD-L1, programmed cell death ligand-1; TKI, tyrosine kinase inhibitor.
in only one region of one tumor for one patient. In contrast, common mutations were, as the name suggests, the mutations which were found in most of the patients. Overall, 75% of patients exhibited private mutations in the center as well as the periphery, whereas one patient had a high percentage (58%) of private mutations in the periphery. The average mean percentage of private mutations was 12% across all samples in all patients. Although exciting, this heterogeneity limits the use of personalized medicine in everyday clinical practice. In lung adenocarcinomas, these private mutations or “neoantigens” have been shown to increase sensitivity to immune check point inhibitors, such as anti-cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA4) and PD-1 inhibitors with resulting improved outcomes.

Another important facet of this cancer where limited research has been performed is the “tumor microenvironment” (TME). The dense collagenous stroma constitutes TME and contains important components such as cancer-associated fibroblasts (CAFs), α-smooth muscle actin (α-SMA+), which probably originate from activated hepatic stellate cells or hepatic portal fibroblasts. In a mouse study, it was reported that there was intrahepatic accumulation of extracellular matrix components, type III collagen and activated fibroblasts, which then resulted in CCA genesis. It was reported that there was intrahepatic accumulation of extracellular matrix components, type III collagen and activated fibroblasts, which then resulted in CCA genesis. Indeed, studies in pancreatic ductal adenocarcinoma have previously shown a role of the stroma in tumor growth.

Another point to note is that most of the molecular profiling studies include the analysis of surgical samples and therefore represent early-stage disease, which may not be a true representation of the patients who are seen in clinics. The role of immunomodulating treatments in BTC is still an area of exploration, and none of the current immune investigational drugs have been approved in this disease group. The expression of PD-L1 is a predictive biomarker in other tumor sites, such as non-small-cell lung cancer (NSCLC), for the efficacy of these immunotherapies. Apart from this biomarker, another recently emerging predictor of response is the human microbiome, where certain bacterial species are associated with clinical efficacy of immunotherapies.

The use of monotherapy vs combination treatment in advanced BTC is also an issue. Historically, clinical studies have used both novel agents as monotherapy as well as in combination with cytotoxic treatments. However, further research in combining treatments that potentiate cytotoxic effects and are at the same time tolerable is necessary. There is also a niche for developing prognostic and predictive biomarkers in BTC to better inform treatment choice.

Currently, there are still gaps in the understanding of the whole process that governs carcinogenesis and resistance to treatments in BTC, and future studies may be able to address this dilemma. In time, prospective studies may further identify novel therapies targeting this disease and lead to improvements in survival outcome.

**Disclosure**

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The authors report no other conflicts of interest in this work.

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