Targeted therapy in biliary tract cancers—current limitations and potentials in the future

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Abstract: Biliary tract cancers (BTC)/Cholangiocarcinoma (CCA) is an aggressive biliary tract epithelial malignancy from varying locations within the biliary tree with cholangiocyte depreciation., including intrahepatic cholangiocarcinoma (iCCA) (CCA), extrahepatic cholangiocarcinoma (eCCA) and gallbladder carcinoma (GBC). The disease is largely heterogeneous in etiology, epidemiology, and molecular profile. There are limited treatment options and low survival rates for those patients with advanced or metastatic disease. Systemic treatment is confined to cytotoxic chemotherapy with the combination of gemcitabine and cisplatin. Lack of a stereotype genetic signature makes difficult in identification of potential actionable target directly, which may also explain lack of obvious clinic benefit with target oriented agents from current studies. It is crucial to understand of BTC carcinogenesis, tumor-stroma interactions, and key molecular pathways, and herald to establish targeted, individualized therapies for the heterogeneous disease, and eventually to improve the survival and overall outcome of patients.

Keywords: Biliary cancer; hepatocellular carcinoma; abnormal liver function; chemotherapy

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Introduction

Biliary tract cancers (BTC)/Cholangiocarcinoma (CCA) are resulting from malignant transformation of epithelial cells within the bile system as related but distinct malignancies along the intrahepatic and extrahepatic biliary tree. Besides the gallbladder carcinoma (GBC), CCA is also refers to cancers of the entire biliary tree. CCA is commonly classified as intrahepatic cholangiocarcinoma (iCCA) and extrahepatic cholangiocarcinoma (eCCA), with extrahepatic further divided into perihilar cholangiocarcinoma (pCCA) and distal cholangiocarcinoma (dCCA) tumors based on anatomical location with the cystic duct as the point of distinction (1). There is increasing recognition that these subtypes separated by anatomic origins of BTCs distinct not only in desmoplasia markers of cholangiocytes, but also in biological and clinical characteristics (2). BTC has an aggressive natural course without early specific warning sign of the disease; therefore, potential curative surgery is only suitable for a limited number of patients. So far, systemic treatment is confined to the gemcitabine and cisplatin combination as the practice standard for patients with advanced and metastatic disease (3). The overall outcome is disappointing with limited response rate (RR) and low 5-year survival rate. It is crucial to understand BTC/CCA carcinogenesis, tumor-stroma interactions, and key molecular pathways, and herald to establish targeted, individualized therapies for the heterogeneous disease, and eventually to improve the survival and overall outcomes of patients. In this review, we are going to summarize the
results of systemic cytotoxic chemotherapy, and review the data from studies with ‘target-oriented’ agents. Since most of these studies are not targeting the distinguished subgroups of the disease specifically, they are more likely as ‘target intended’ rather than ‘target-oriented’. We will also discuss the current knowledge regarding the genetic basis of this disease, including molecular pathways involved in its carcinogenesis, and potential targeted therapies that hold promise in the future research and practice.

**Cytotoxic chemotherapy**

Cytotoxic chemotherapy remains the mainstay of treatment for patients with advanced unresectable or metastatic BTC. Given the rarity of this disease, clinical studies have been small and have almost always been combined with ‘lumping’ various BTCs with very few randomized trials have been conducted. The majority of trials have been performed with either fluoropyrimidine-based or gemcitabine-based combination. 5-flourouracil (5-FU) had been tested in small trials, both as monotherapy and in combinations. Overall RRs in these studies varied from 10% to 40%; median survival also varied notably, from 5 to 16 months (4-21).

A phase III study randomized 54 patients with previously untreated advanced biliary cancer between ECF (epirubicin, cisplatin, 5-FU) and FELV (5-FU/LV, etoposide) (14). The median OS was not significantly different between the two arm (9.02 months in ECF vs. 12.03 months in FELV, P=0.2059). Objective RRs were also similar (19.2% in ECF vs. 15% in FELV, P=0.72). The interesting point is greater than 60% of patients in each arm demonstrated resolution of pain, anorexia, weight loss, and nausea.

Based on the data of studies from advanced and metastatic pancreatic cancer, gemcitabine has also been evaluated biliary cancers at the similar setting (7,12,15,16,22-41) (**Table 1**). As a single agent, gemcitabine showed only moderate efficacy with RRs ranging from 0% to 30% at varied dosing schemes demonstrated. Efforts had been tried in combination of gemcitabine with multiple other cytotoxic agents, including 5-FU, capecitabine, cisplatin, oxaliplatin, and irinotecan with significant variations in RRs and survival.

In order to assess the overall efficacy of systematic chemotherapy, a pooled analysis was performed including 104 trials published between 1985 and 2006 (42). The overall RR was 22.6%; and the tumor control rate (TCR) was 57.3%. Significant correlations of RR and TCR with survival times were found. Subgroup analysis showed superior RRs for GBC compared with CCA (RR 35.5% vs. 17.7%, P=0.008), but shorter OS for GBC (median 9.3 in CC vs. 7.2 months in GBC, P=0.048).

Based on treatment type subgroup analyses, the analysis showed that regimens containing both gemcitabine and a platinum agent had significantly higher response and TCRs compared to either fluoropyrimidine or gemcitabine monotherapy or fluoropyrimidine-plus-platinum regimens. Based on this information, United Kingdom-based Advanced Biliary Care (ABC)-02 trials was conducted to validate the combination (3). In this study, 410 patients with non-resectable, recurrent, or metastatic BTC were randomized to receive either gemcitabine alone or gemcitabine and cisplatin combination. The trial included patients with CCA, gallbladder cancer, or ampullary cancer. Patients in the combination arm received cisplatin 25 mg/m² and gemcitabine 1,000 mg/m² on days 1 and 8 of a 3-week cycle, for eight cycles; patients in the gemcitabine monotherapy arm received gemcitabine 1,000 mg/m² on days 1, 8, and 15 of a 4-week cycle, for six cycles. After a median follow-up of 8.2 months, the median OS was 11.7 months in the gemcitabine/cisplatin combination arm, compared to 8.1 months in the gemcitabine arm (HR =0.64, P=0.001). The median progression-free survival (mPFS) was improved with the combination (8.0 vs. 5.0 months; HR =0.64; P<0.001) as well. Severe hematologic toxicities were seen more frequently in the combination arm. However, there were more severe liver toxicities reported in the gemcitabine-alone arm for unclear reason.

**Target intended therapy**

Although the combination of gemcitabine and cisplatin has achieved some advances in the treatment of advanced and metastatic biliary tract cancers and has been accepted as the standard treatment option as the first line therapy since 2010, the overall outcome is still disappointing. Clinic studies of target-oriented agents (most of them in combination with gemcitabine based regimen) have been attempted for improving the outcomes of the disease. Those target-oriented agents primarily are monoclonal antibodies and tyrosine kinase inhibitors against EGFR and vascular endothelial growth factor (VEGF) (43-57) (**Table 2**). However, no or only marginal benefits showed from those trials, which is likely because of a mixed cohort of BTC patients (iCCA, eCCA, and GBC), and the underlying genetic variability of the disease.

One phase III trial randomized 268 patients with
Table 1 Gemcitabine-based studies in advanced BTCs

<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>Treatment regimen</th>
<th>RR (%)</th>
<th>mPFS (mo)</th>
<th>mOS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raderer et al. (7)</td>
<td>19</td>
<td>Gem</td>
<td>16</td>
<td>2.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Penz et al. (22)</td>
<td>32</td>
<td>Gem</td>
<td>22</td>
<td>5.6</td>
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</tr>
<tr>
<td>Gabbia et al. (23)</td>
<td>18</td>
<td>Gem</td>
<td>22</td>
<td>3.4</td>
<td>8</td>
</tr>
<tr>
<td>Kuhn et al. (24)</td>
<td>22</td>
<td>Gem + 5-FU + LV</td>
<td>36</td>
<td>4.1</td>
<td>11</td>
</tr>
<tr>
<td>Bhargava et al. (25)</td>
<td>14</td>
<td>Gem + irinotecan</td>
<td>14</td>
<td>1.5</td>
<td>NR</td>
</tr>
<tr>
<td>Kornek et al. (12)</td>
<td>25</td>
<td>Gem + MMC</td>
<td>20</td>
<td>4.2</td>
<td>6.7</td>
</tr>
<tr>
<td>André et al. (26)</td>
<td>23</td>
<td>Gem + Ox</td>
<td>22</td>
<td>3.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Knox et al. (27)</td>
<td>27</td>
<td>Gem + 5-FU</td>
<td>33</td>
<td>3.7</td>
<td>5.3</td>
</tr>
<tr>
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<td>9.7</td>
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<tr>
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<td>Gem + Cis</td>
<td>28</td>
<td>4.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Knox et al. (30)</td>
<td>45</td>
<td>Gem + Cape</td>
<td>31</td>
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<td>14</td>
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<tr>
<td>Cho et al. (15)</td>
<td>44</td>
<td>Gem + Cape</td>
<td>32</td>
<td>6.0</td>
<td>14</td>
</tr>
<tr>
<td>Cho et al. (16)</td>
<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Gem + Cape</td>
<td>33</td>
<td>6.0</td>
<td>16</td>
</tr>
<tr>
<td>Lee et al. (31)</td>
<td>24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Gem + Cis</td>
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<td>5.0</td>
<td>9.3</td>
</tr>
<tr>
<td>Kim et al. (32)</td>
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<tr>
<td>Harder et al. (33)</td>
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<td>Gem + Ox</td>
<td>26</td>
<td>6.5</td>
<td>11</td>
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<tr>
<td>Manzione et al. (34)</td>
<td>34</td>
<td>Gem + Ox</td>
<td>41</td>
<td>NR</td>
<td>10</td>
</tr>
<tr>
<td>Alberts et al. (35)</td>
<td>58</td>
<td>Gem + Pem</td>
<td>NR</td>
<td>3.8</td>
<td>6.6</td>
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<td>6.2</td>
<td>12.7</td>
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<tr>
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<td>Gem + Cis</td>
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<td>3.2</td>
<td>8.6</td>
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<td>349</td>
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<tr>
<td>Meyerhardt et al. (39)</td>
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<td>Gem + Cis</td>
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<td>9.7</td>
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<tr>
<td>Kim et al. (40)</td>
<td>40</td>
<td>Gem + Ox</td>
<td>15</td>
<td>4.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Jang et al. (41)</td>
<td>53</td>
<td>Gem + Ox</td>
<td>19</td>
<td>4.8</td>
<td>8.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>, CCA patients only; <sup>b</sup>, gallbladder cancer patients only. RR, response rate; mPFS, median progression-free survival; mOS, median overall survival; Gem, gemcitabine; NR, not reported; 5-FU, 5-fluorouracil; LV, leucovorin; MMC, mitomycin-C; Cis, cisplatin; Cape, capecitabine; Ox, oxaliplatin; Pem, pemetrexed; BTC, biliary tract cancers; CCA, cholangiocarcinoma.

metastatic biliary tract adenocarcinoma to the combination of gemcitabine and oxaliplatin (GemOx) with erlotinib or chemotherapy (GemOx) alone (49). The combination showed increased RR (30% vs. 16%, P=0.005), however, there were no significant difference in mPFS (5.8 vs. 4.2 months, P=0.087) and median overall survival (mOS) (9.5 months for both arms). A randomized phase II study evaluated the GemOx with or without cetuximab in 150 patients with advanced and metastatic BTC (51). There were no differences between the two arms in RR, mPFS or mOS. The TCGO trial evaluated the same regimen in advanced and metastatic BTC patients with stratification of iCCA/eCCA/GBC (71.3%/16.4%/12.3%) and KRAS status (36.1% KRAS mutation). On the intent-to-treat analysis, it showed some benefit of mPFS in the arm of GemOx + cetaximab (7.1 vs. 4.0 months, P=0.0069), but no difference
in RR (27.3% vs. 15%, P=0.1223), and mOS (10.3 vs. 8.8 months, P=0.4057). Planned subgroup analysis showed that potential more benefits of cetaximab with GemOX in KRAS mutated patients with mPFS of 7.1 vs. 1.9 months (P=0.0351), however, no statistical significance in mOS (10.3 vs. 6.6 months, P=0.6924) (58). Interestingly, a pooled analysis with 161 trials comprising 6,337 patients (trials published in English between 1/2000 and 1/2014 as well as ASCO abstracts 2010 to 2013) showed some potential survival benefits of gemcitabine-based chemotherapy with targeted therapy (predominantly EGFR inhibitor as either monoclonal antibody or TKI) (59).

Table 2 Selected studies of targeted agents in advanced BTCs

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>RR (%)</th>
<th>mPFS (mo)</th>
<th>mOS (mo)</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>Bevacizumab (VEGF-A)</td>
<td></td>
<td></td>
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<tr>
<td>Bev + Gem + Ox</td>
<td>35</td>
<td>40</td>
<td>7.0</td>
<td>12.7</td>
<td>Zhu et al. (43)</td>
</tr>
<tr>
<td>Sorafenib (VEGFR-2/3, PDGFR, RAF)</td>
<td></td>
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<td></td>
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<tr>
<td>Sorafenib</td>
<td>46</td>
<td>2</td>
<td>2.3</td>
<td>4.4</td>
<td>Bengaia et al. (44)</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>31</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>El-Khoueiry et al. (45)</td>
</tr>
<tr>
<td>Sunitinib (VEGFR, PDGFR, cKit)</td>
<td></td>
<td></td>
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<tr>
<td>Sunitinib</td>
<td>56</td>
<td>9</td>
<td>1.7</td>
<td>4.8</td>
<td>Yi et al. (46)</td>
</tr>
<tr>
<td>Erlotinib (EGFR)</td>
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<td></td>
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</tr>
<tr>
<td>Erlotinib</td>
<td>43</td>
<td>8</td>
<td>2.6</td>
<td>7.5</td>
<td>Philip et al. (47)</td>
</tr>
<tr>
<td>Erlot + docetaxel</td>
<td>11</td>
<td>0</td>
<td>NR</td>
<td>5.7</td>
<td>Chiorean et al. (48)</td>
</tr>
<tr>
<td>Erlot + Gem + Ox</td>
<td>135</td>
<td>30</td>
<td>5.8</td>
<td>9.5</td>
<td>Lee et al. (49)</td>
</tr>
<tr>
<td>Gem + Ox</td>
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<td>4.2</td>
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<tr>
<td>Cetuximab (EGFR)</td>
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<tr>
<td>Cet + Gem + Ox</td>
<td>9a</td>
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<td>4</td>
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<tr>
<td>Gem + Ox</td>
<td>74</td>
<td>23</td>
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<td>Panitumumab (EGFR)</td>
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<td>46</td>
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<td>9.7</td>
<td>12.9</td>
<td>Sohal et al. (53)</td>
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<td>5.2</td>
<td>Ramanathan et al. (54)</td>
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<td></td>
<td></td>
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<tr>
<td>Selumetinib</td>
<td>28</td>
<td>12</td>
<td>3.7</td>
<td>9.8</td>
<td>Bekail-Saab et al. (55)</td>
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<td>Target-oriented agent combination</td>
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<tr>
<td>Erlot + Bev</td>
<td>49</td>
<td>12</td>
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<td>Lubner et al. (56)</td>
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<tr>
<td>Erlot + Soraf</td>
<td>34</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>El-Khoueiry et al. (57)</td>
</tr>
</tbody>
</table>

*a*, CCA patients only. RR, response rate; mPFS, median progression-free survival; mOS, median overall survival; EGFR, epidermal growth factor receptor; Erlot, erlotinib; NR, not reported; Gem, gemcitabine; Ox, oxaliplatin; Cet, cetuximab; Pan, panitumimab; VEGF, vascular endothelial growth factor; Bev, bevacizumab; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; RAF, rapidly accelerated fibrosarcoma; Soraf, sorafenib; HER2, human epidermal growth factor receptor 2; MEK, mitogen-activated protein kinase/extracellular-signal regulated kinase; BTC, biliary tract cancers; CCA, cholangiocarcinoma.
New insights into the molecular pathogenesis and therapeutic opportunities

Recent studies have begun to uncover the genetic and molecular processes underlying carcinogenesis using advanced technologies such as next-generation sequencing (NGS) in the BTC. The emerging knowledge and data generated from studies of epidemiology, genome profiling, and laboratory-based investigations provide new insights into risk factors, genomic composition, cellular origins and contribution of the tumor microenvironment to the pathogenesis of BTC. The remarkable genetic heterogeneity of BTC may be the result of a complex interplay among different factors—some of them are shared by most human cancers, while others may be unique for this malignancy. Emerging data supports that iCCA, eCCA, and GBC represent distinct tumors arising from different genetic backgrounds (60,61).

Cancer-associated fibroblasts (CAF) in the tumor stroma

CAF secondary to desmoplastic response is a prominent tumor microenvironment characteristic of biliary track cancers, especially iCCA. CAF may impede access of therapeutic agents to the tumor and pose therapeutic challenges further besides the genetic heterogeneity of this malignancy (1,62). Preclinical studies have demonstrated a reduction in fibrosis and carcinogenesis in BTC/CCA with 1D11, a transforming growth factor β (TGF-β) antagonist, as well as curcumin, a nutraceutical agent (63). A pre-clinic study with an orthotopic CCA model showed that the BH3 (BCL-2 family protein, pro-apoptosis) mimetic, Navitoclax, enhanced selective CAF apoptosis, decreased expression of α-SMA, and reduced tumor burden and metastasis while improving survival (64). Further preclinical and clinical studies are needed to explore the role of antifibrotic therapies in CCA chemoprevention.

Inflammation and carcinogenesis

Chronic inflammation plays a significant role in the development of BTC. Chronic inflammatory pathways not only are key components in carcinogenesis, but also promote tumor invasion and migration. Primary sclerosing cholangitis (PSC), hepatobiliary flukes Opisthorchis viverrini (O. viverrini) and Clonorchis sinensis characterized by chronic biliary tract inflammation and liver injury, are common predisposing conditions for BTC. In addition, prolonged hepatolithiasis may promote CCA development by calculi occurring proximal to the hepatic duct confluence (2,65). Inducible nitric oxide synthase (iNOS) activation by inflammatory cytokines contributes to nitrosative stress by generation of excess nitric oxide, which then results in inhibition of DNA repair proteins, and single-stranded, double-stranded, and oxidative DNA lesions. Oxidative stress via generation of oxysterols, cholesterol oxidation products present in human bile, creates a milieu favorable for tumor development and progression by activating Hedgehog signaling pathway. Oxysterols, bile acids, and iNOS all stimulate over-expression of cyclooxygenase-2, which has been implicated carcinogenesis of BTC (66–68). Therefore, inflammatory processing control may play a significant role in management of BTC, especially in the prevention.

Genomic profiling studies

Genomic profiling has demonstrated characteristic profiles for iCCA and eCCA. Next generation sequencing (NGS) of a BTC series showed key variations in certain mutations based on tumor location (69). Mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes have consistently been shown to be more frequent in iCCA versus eCCA or GBC. IDH mutations caused inhibition of HNF-4α (hepatocyte nuclear factor 4 alpha, a nuclear receptor; also known as NR2A1- nuclear receptor subfamily 2, group A, member 1), leading to impaired hepatocyte differentiation and increased cell proliferation, and associated with poorly differentiated histology. FGFR (Fibroblast growth factor receptor) mutations, specifically in FGFR2 are associated with the production of two fusion kinase genes, FGFR2-AHCYL1 and FGFR2-BICC1 that are mutually exclusive with KRAS/BRAF mutations. The median cancer specific survival is suggested significantly longer for patients whose tumors contained FGFR2 translocations (70).

It appears that mutational profiles may be influenced by the etiology of BTC. A study revealed the results of exome sequencing of 209 CCA samples from Asia and Europe with 108 cases of O. Viverrini infection related and the other 101 cases has non-O. viverrini-related etiologies (71). The study showed that TP53 was mutated in 40% of O. viverrini infection related CCA, and only in 9% of the non-O. viverrini-related cases (P<0.001). On the other hand, SMAD4 mutation was more frequently in non-O. viverrini-related CCA (6% vs. 19%, P=0.006). IDH1 and
IDH2 mutations were tested in 22.2% of non-O. viverrini iCCA and only 3.2% of O. viverrini-related iCCA.

Activating mutations in cell proliferation oncogenes lead to uncontrolled cell growth and survival. The Ras/MAPK signaling pathway plays a key role in cell growth, differentiation, survival, and migration. Gain-of-function mutations in KRAS are present in approximately 45–55% of iCCA and 10–15% of eCCA. One study showed that BRAF, an important downstream effector of KRAS, was found to be mutated in 22% of iCCA, no BRAF mutation was found in those cases with KRAS mutations; however, KRAS mutations were seen in 20% of tumors with BRAF mutations (72). The ErbB family consists of four receptor kinases, including ErbB1 (or EGFR) and ErbB2 [or human epidermal growth factor receptor 2 (HER2)]. Mutations in the EGFR gene were seen in 15% of CCA cases (73). MET is an oncogene that encodes for the hepatocyte growth factor (HGF) receptor. HGF/MET pathway is less established but may be important in development and progression of BTC/CCA. MET is a key regulator of invasive growth. Interaction of HGF and its receptor MET can activate many pathways including MAPK, PI3K and STAT. Overexpression of MET occurs in 12–58% of cases of iCCA and has been linked to overexpression of members of the EGFR family and shown the capacity of HGF to stimulate migration and invasion in CC cells (74).

Loss-of-function mutations in tumor suppressor genes also play a role in CCA. CDKN2A (p16INK4a) negatively regulates proliferation in normal cells and is capable of cell cycle arrest. This tumor suppressor gene was highly mutated in reports with 55% loss-of-function in iCCA and 83% in eCCA. TP53, a principal regulator of cell division, appears to be inactivated in approximately one-third of BTC, both iCCA and eCCA. SMAD4, in conjunction with the other SMAD proteins, is an end effector in the TGFβ pathway that promotes epithelial-mesenchymal transition, directly regulating the activity of genes controlling cell proliferation. Mutations in SMAD4 were described in up to 40% in iCCA with the relationship of disease staging (75). A recent large cohort study with 103 iCCA in identification of an iCCA-specific mutation signature that is associated with liver inflammation, fibrosis and cirrhosis (76). The study found that TP53-defection is more likely to be HBsAg-seropositive, whereas KRAS mutations are nearly exclusively in HBsAg-seronegative CCA patients. The study demonstrated that three pathways (Ras/PI3K, p53/cell cycle, and TGFβ/Smad), genes important for epigenetic regulations and oxidative phosphorylation are substantially affected in iCCA.

Potential role of stem and progenitor cells

Besides the different carcinogenetic mechanisms driven by risk factors in BTC, the distinct genetic profiles are also reflecting two distinct stem cell niches, the canals of Hering harboring hepatic stem cells (HpSCs) and the peribiliary glands harboring biliary tree stem/progenitor cells (BTSCs) (77). Cell populations from HpSCs and BTSCs lineages may represent distinct candidate cells of origin during CC carcinogenesis, susceptible to distinct risk factors and responsible for the development of the different iCCA and eCCA or GBC subtypes: e.g., BTSC lineage may be activated under pathological conditions affecting the large intrahepatic and extrahepatic bile ducts (including liver flukes, cholangitis, PSC, hepatolithiasis, etc.), giving rise to large bile duct pure mucin secreting iCCA and eCCA; conversely, the hHpSC lineage has been suggested to be activated in response to parenchymal liver diseases (such as chronic viral/non-viral liver disease, schistosomiasis and liver cirrhosis) and to be involved in the development of combined hepatocellular carcinoma-iCCA, bile ductular iCCA and mixed iCCA A (with a focal hepatocytic differentiation, ductular reaction and mucin-secreting adenocarcinoma). This stem cell compartment is probably activated also during nonalcoholic steatohepatitis (NASH) and asbestos exposure, as these two risk factors are exclusively associated with the development of ICC.

Summary and future directions

Despite some advances in treatment of BTCs, the overall outcomes of the disease remain poor. With learning from ‘target intended’ studies and emerging understanding of the heterogeneity and the molecular landscape of BTC, future target oriented research/studies should be based on the underlining etiology, the specific genetic profile of each subgroup, and cancer-stroma microenvironment of the selected particular disease population. A recent retrospective study showed the promising therapy with blockage of Her-2/neu in BTC (with both GBC and CCA) patients who with the gene amplification (78). Advances in immunotherapy may also provide new opportunities for treating BTC (79). A complete response (CR) was reported in a chemotherapy refractory metastatic iCCA patient with mismatch-repair deficiency (dMMR) after being treated with PD-1 inhibitor (80). Most current on-going ‘target-oriented’ studies are less distinctive regarding the specificity of the characteristic mechanism of the disease (Tables 3,4). However, we are certain that the future studies will be more precisely to meet our goal.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase</th>
<th>Disease</th>
<th>Target</th>
<th>Schedule</th>
<th>Start date</th>
<th>Complete date</th>
<th>Sponsor</th>
<th>Clinictrials.gov ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramucirumab</td>
<td>II</td>
<td>Advanced, pre-treated biliary</td>
<td>VEGFR II</td>
<td>8 mg/kg IV on day 1 of each 14 day cycle</td>
<td>12/2015</td>
<td>12/2019</td>
<td>M.D. Anderson Cancer Center</td>
<td>NCT02520141</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>II</td>
<td>Refractory advanced biliary</td>
<td>—</td>
<td>—</td>
<td>04/2014</td>
<td>10/2018</td>
<td>H. Lee Moffitt Cancer Center and Research Institute</td>
<td>NCT02115542</td>
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<tr>
<td>Selumetinib (AZD6244) with cisplatin/gemcitabine (Cis/Gem) versus Cis/Gem alone</td>
<td>Phase II/ randomized</td>
<td>Advanced, biliary, first line</td>
<td>MEK 1/2 inhibitor</td>
<td>Selumetinib, orally, BID from days 1–21 of every 28 day cycle. Cisplatin/gemcitabine, IV, on days 1 and 8 of every 28 day cycle</td>
<td>11/2014</td>
<td>05/2017</td>
<td>University Health Network, Toronto</td>
<td>NCT02151084</td>
</tr>
<tr>
<td>Afatinib dimaleate (BIBW 2992) and capecitabine</td>
<td>II</td>
<td>Advanced refractory pancreatic cancer or biliary cancer</td>
<td>EGFR/HER2 tyrosine kinase inhibitor afatinib</td>
<td>Afatinib dimaleate PO QD on days 1–28 and capecitabine PO BID on days 1–14. Courses repeat every 21 days</td>
<td>11/2015</td>
<td>11/2018</td>
<td>University of Washington</td>
<td>NCT02451553</td>
</tr>
<tr>
<td>Ponatinib hydrochloride</td>
<td>II</td>
<td>Advanced biliary cancer with FGFR2 fusions</td>
<td>FGFR2 blockage</td>
<td>Ponatinib hydrochloride PO QD on days 1–28</td>
<td>12/2014</td>
<td>10/2019</td>
<td>NCI</td>
<td>NCT02265341</td>
</tr>
<tr>
<td>Lenvatinib (E7080)</td>
<td>II</td>
<td>BTC, failed gemcitabine-based therapy</td>
<td>—</td>
<td>24 mg lenvatinib, orally</td>
<td>10/2015</td>
<td>10/2017</td>
<td>Eisai Co., Ltd.</td>
<td>NCT02579616</td>
</tr>
<tr>
<td>Ramucirumab (LY3009806) or merestinib (LY2801653) or placebo plus gem/cis</td>
<td>—</td>
<td>Advanced or metastatic BTC</td>
<td>—</td>
<td>Ramucirumab plus cisplatin and gemcitabine IV on days 1 and 8, merestinib orally each day, plus cisplatin and gemcitabine IV on days 1 and 8, placebo plus cisplatin and gemcitabine IV on days 1 and 8, every 21 days</td>
<td>04/2016</td>
<td>04/2018</td>
<td>Eli Lilly and Company</td>
<td>NCT02711553</td>
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Table 3 (continued)
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase</th>
<th>Disease</th>
<th>Target</th>
<th>Schedule</th>
<th>Start date</th>
<th>Complete date</th>
<th>Sponsor</th>
<th>Clinicaltrials.gov ID</th>
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</thead>
<tbody>
<tr>
<td>Regorafenib in combination with mGEMOX</td>
<td>II</td>
<td>–</td>
<td>–</td>
<td>Regorafenib: X mg/d, PO, from days 1 to 14, days 15 to 20; gemcitabine 900 mg/m² IV in 30 minutes; oxaliplatin 80 mg/m² IV in 120 minutes, days 1 and 8</td>
<td>09/2014</td>
<td>04/2019</td>
<td>Institut du Cancer de Montpellier - Val d’Aurelle</td>
<td>NCT02386397</td>
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<tr>
<td>ADH-1, gemcitabine hydrochloride and cisplatin</td>
<td>I</td>
<td>Metastatic pancreatic or BTC</td>
<td>–</td>
<td>ADH-1 IV over 20–80 minutes on days 1, 4, 8, 11, 15, and 18, cisplatin IV and gemcitabine hydrochloride IV over 30 minutes on days 1 and 8</td>
<td>04/201312/2018</td>
<td>–</td>
<td>NCI; Adherex Technologies, Inc.</td>
<td>NCT01825603</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>II</td>
<td>Advanced and metastatic biliary</td>
<td>–</td>
<td>Regorafenib 120 mg orally once daily 21 days on and 7 days off in the 28-day cycle</td>
<td>01/2014</td>
<td>02/2018</td>
<td>University of Pittsburgh</td>
<td>NCT02053376</td>
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<tr>
<td>BIBW 2992 with Gem/Cis</td>
<td>I/Ib</td>
<td>Advanced and metastatic biliary</td>
<td>–</td>
<td>–</td>
<td>08/2012</td>
<td>–</td>
<td>Johannes Gutenberg University Mainz</td>
<td>NCT01679405</td>
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<tr>
<td>Copanlisib (BAY 80-6946) in combination with gemcitabine and cisplatin</td>
<td>II</td>
<td>Advanced and metastatic CCA</td>
<td>–</td>
<td>Cisplatin (25 mg/m²) + gemcitabine (1,000 mg/m²) + copanlisib (60 mg) on days 1 and 8 with days 15 off to be administered on an every 21-days schedule</td>
<td>05/2016</td>
<td>12/2018</td>
<td>H. Lee Moffitt Cancer Center and Research Institute</td>
<td>NCT02631590</td>
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<tr>
<td>Refametinib</td>
<td>II</td>
<td>Advanced biliary, 2nd line</td>
<td>MEK inhibitor</td>
<td>Orally at the starting dose of 50 mg twice daily on a continuous daily dosing schedule</td>
<td>01/2015</td>
<td>07/2016</td>
<td>Ho Yeong Lim, Samsung Medical Center</td>
<td>NCT02346032</td>
</tr>
<tr>
<td>RRx-001</td>
<td>II</td>
<td>Advanced cholangiocarcinoma, 2nd line</td>
<td>Epigenetic agent</td>
<td>–</td>
<td>05/2015</td>
<td>05/2018</td>
<td>EpicentRx, Inc.</td>
<td>NCT02452970</td>
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</table>
Table 3 (continued)

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<thead>
<tr>
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<th>Target</th>
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<th>Start date</th>
<th>Complete date</th>
<th>Sponsor</th>
<th>Clinictrials.gov ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARQ 087</td>
<td>I/II</td>
<td>Advanced solid tumors with FGFR genetic alterations</td>
<td>FGFR inhibitor</td>
<td>–</td>
<td>12/2012</td>
<td>12/2016</td>
<td>ArQule</td>
<td>NCT01752920</td>
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<tr>
<td>CX-4945 in combination with gemcitabine and cisplatin</td>
<td>I/II</td>
<td>Advanced cholangiocarcinoma, 1st line</td>
<td>–</td>
<td>–</td>
<td>06/2014</td>
<td>12/2016</td>
<td>Senhwa Biosciences, Inc.</td>
<td>NCT02128282</td>
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<tr>
<td>Pazopanib/gemcitabine</td>
<td>II</td>
<td>Biliary tree cancer (CCA or GBC)</td>
<td>–</td>
<td>–</td>
<td>06/2013</td>
<td>11/2016</td>
<td>Hellenic Cooperative Oncology Group/GSK</td>
<td>NCT01855724</td>
</tr>
</tbody>
</table>

BTC, biliary tract cancers; CCA, cholangiocarcinoma; GBC, gallbladder carcinoma; IV, intravenously; NCI, National Cancer Institute.

Table 4 Immunotherapy of BTC (clinicaltrials.gov)

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Schedule</th>
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<th>Complete date</th>
<th>Sponsor</th>
<th>Clinictrials.gov ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab and GM-CSF</td>
<td>I/II</td>
<td>Advanced Biliary</td>
<td>–</td>
<td>–</td>
<td>04/2016</td>
<td>09/2019</td>
<td>University of California, San Francisco</td>
<td>NCT02703714</td>
</tr>
<tr>
<td>Precision T cells specific to personalized neo-antigen</td>
<td>II</td>
<td>Advanced Biliary</td>
<td>DC-PNAT combined with gemcitabine treatment: Gemcitabine: once a week with a total of 6 times before 60 days prior to the start of drawing blood. DC-PNAT: once per 3 weeks with a total of three periods</td>
<td>09/2015</td>
<td>09/2017</td>
<td>Second Military Medical University (China)</td>
<td>NCT02632019</td>
<td></td>
</tr>
</tbody>
</table>

BTC, biliary tract cancers; DC-PNAT, dendritic cell-precision T cell for neo-antigen.
Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References


49. Lee J, Park SH, Chang HM, et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-


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