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# Emerging therapies for the treatment of cholangiocarcinoma

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## ABSTRACT

**Cholangiocarcinoma (CCA) is a cancer arising from the epithelium of intrahepatic or extrahepatic bile ducts. Cholangiocarcinoma often has a poor prognosis due to late diagnosis and the incidence and mortality rate of intrahepatic CCA appear to be increasing. Current therapies include surgical resection, orthotopic liver transplantation, chemotherapy/chemoradiation and palliative care. Depending on the location, the 5-year survival for CCA ranges from 27–60%. Emerging new therapies are currently being developed for treating CCA include immunotherapy, altering the tumor microenvironment, targeting growth factor gene mutations and signal pathways and that control tumor growth, and targeting gene therapy. The objective of this paper is to summarize the research that is currently ongoing for treating this challenging disease.**

**Keywords: Cholangiocarcinoma, Immunotherapy, Molecular targeting, Mutation profiling, Tumor microenvironment**

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## INTRODUCTION

### Description

Cholangiocarcinoma (CCA) is a cancer arising from the epithelium of intrahepatic or extrahepatic bile ducts caused by the malignant transformation of hepatic biliary cholangiocytes [1, 2]. Cholangiocarcinoma can occur anywhere from the small peripheral hepatic ducts to the distal common bile duct [2]. The three primary types of CCA and their relative frequency are intrahepatic (10%), distal (40%) and perihilar (50%), the latter being confined to the larger bile ducts in the hepatic hilum. Mixed hepatocellular cholangiocarcinomas have recently been described in which CCA and hepatocellular carcinoma are found in the same nodule [2]. Cholangiocarcinoma primarily arises from the biliary epithelium in the case of extrahepatic cholangiocarcinoma while hepatic progenitors are believed to play a role in intrahepatic CCA [3]. Cholangiocarcinomas have a dense stromal component that result from the recruitment of fibroblasts, remodeling of the extracellular matrix, altered immune cell migration, and angiogenesis. The tumor stroma surrounds the malignant ducts and glands and comprises most of the tumor mass [4].

## Epidemiology

Cholangiocarcinoma is the second most common primary liver tumor with estimates ranging from 10–25% of all hepatobiliary malignancies [1]. The age-adjusted incidence of CCA ranges from a high of 2.8–3.3 per 100,000 among Hispanic and Asian populations to a low of 2.1 per 100,000 among non-Hispanic white and black people and is slightly more prevalent among men [2]. Several recent epidemiological studies have shown that the incidence and mortality rates of intrahepatic CCA are increasing [5].

In one series, five-year survival rates for intrahepatic, perihilar and distal cholangiocarcinoma were 60%, 30% and 27% respectively [6]. Median and five-year overall survival (OS) for intrahepatic CCA after surgical resection were 28 months (range: 9–53 months) and 30% (range: 5–56%), respectively [7]. Factors predicting shorter OS included large tumor size, multiple tumors, lymph node metastasis, and vascular invasion. Adjuvant chemotherapy or radiotherapy did not appear to be beneficial. With the exception of advanced patient age, factors associated with shorter OS are tumor-related and include larger tumor size, presence of multiple tumors, lymph node metastasis, vascular invasion, and poor tumor differentiation [7].

## Risk Factors

Cholangiocarcinoma frequently arises under conditions of chronic inflammation which is believed to contribute to pathogenesis [8]. In the Western world, primary sclerosing cholangitis (PSC) and fatty liver disease resulting in chronic inflammation of the biliary tree represent the most common predisposing conditions for CCA [8, 9]. Other proposed risk factors include alcohol consumption [10], diabetes mellitus [10, 11], opisthorchiasis (hepatobiliary flukes) [12, 13], choledochal cysts [14], chronic hepatitis B and C [10, 15], obesity [10, 15, 16], cirrhosis [10], hepatolithiasis [17], and chemical carcinogens [5].

## Diagnosis

The clinical presentation of CCA patients varies according to the clinical type. Patients with intrahepatic CCA present with abdominal pain, malaise, night sweats, weight loss and anorexia. Patients with extrahepatic CCA typically present with symptoms of obstructive jaundice and sometimes with complications such as cholangitis, which represents a major cause of morbidity in this population [18].

There are few approved serum biomarkers for detecting cancer and none are specific for CCA [19]. Fluorescence in situ hybridization (FISH) has high specificity for CCA and maybe useful for patients at high-risk for CCA, such as those with primary sclerosing cholangitis (PSC) [20, 21]. However, this test has relatively poor specificity, especially in the presence of jaundice. Better biomarkers are needed for the early detection of CCA. Immunohistochemical methods can be used to distinguish intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma [22]. The measurement of volatile organic compounds in biliary fluid is emerging as a possible method for diagnosing CCA in patients with PSC [23]. The lack of early diagnostic biomarkers results in late diagnosis and poor prognosis. Thus, 80% patients with CCA will present with unresectable or metastatic disease with poor prognosis [24].

Ultrasonography, computed tomography (CT) scan and magnetic resonance imaging (MRI) scan are the most common non-invasive imaging modalities used in the diagnosis and staging of hilar cholangiocarcinoma [25]. Endoscopic ultrasound is emerging as a useful tool for the diagnosis and staging of CCA [26].

# CURRENT THERAPIES

## Surgery

The treatment of choice for intrahepatic CCA is surgical resection. Surgical treatments are the only potentially curative therapeutic options for intrahepatic CCAs. Unfortunately, only a minority of patients qualify for surgical resection as most present with advanced unresectable disease [18]. Following surgical resection, the median time of disease-free survival is 26 months and five-year survival ranges from 30–60% [3]. There are no large randomized controlled trials demonstrating a survival benefit of combining neoadjuvant or adjuvant chemotherapy with surgical resection [18].

## Orthotopic liver transplantation

Liver transplantation has not yet been shown to be a viable option for intrahepatic CCA as disease recurrence was reported to be as high as 70% within five years [27]. However, liver transplantation has been shown to result in significant clinical benefit for select patients with hilar CCA following neoadjuvant chemoradiation [28, 29]. This modality typically involves preoperative chemoradiation and resulted in 75% five-year survival for these patients. The selection criteria for transplant as rigorous, however, and a few patients qualify for this therapy [30]. For early stage hilar CCA, surgical resection remains the standard of care and transplant is an option for selected small but unresectable cases of hilar CCA.

## Chemotherapy

Chemotherapy may be considered for patients who are not candidates for surgical resection; however, there currently is no established adjuvant chemotherapy for CCA [31]. The combination of gemcitabine and cisplatin remains the standard therapy for advanced CCA [32, 33]. No second line therapy has definitely demonstrated improved survival benefits [34]. Published studies for the treatment of CCA using a range of chemotherapeutic

agents, alone and in combinations, during the past five years are summarized in Table 1 [35–68].

## Radiation

There is limited evidence supporting the use of radiotherapy alone although some series demonstrate superior five-year survival, especially in perihilar CCA. Adjuvant radiotherapy may improve overall survival in patients undergoing resection for extrahepatic biliary tract carcinomas [69].

## Chemoradiotherapy

Chemoradiation can prolong survival in CCA, particularly in the cases of hilar CCA and mass-forming intrahepatic CCA. Capecitabine is commonly used concurrently with radiotherapy and the available modalities for radiation include photons, intensity modulated radiation therapy (IMRT), proton beams and stereotactic radiation [70]; however, a randomized clinical trial in this regard are lacking.

Table 1: Recent studies treating cholangiocarcinoma with chemotherapeutic agents

| Author | Treatment | Patient Population | Primary, Secondary Endpoints | Design | Outcome |
|---|---|---|---|---|---|
| **Phase 3 Studies** | | | | | |
| Rogers et al. [35] | First line gemcitabine + cisplatin (n = 36), gemcitabine + cisplatin + erlotinib (n = 8), gemcitabine (n = 1) or other (n = 11). | Advanced intrahepatic cholangiocarcinoma | Primary: PFS with second-line systemic treatments. Secondary: OS and disease control rate with second-line systemic regimens. | Retrospective chart review | Median PFS of 2.7 months, 50% disease control rate, and a potential survival benefit with second-line systemic therapy. No significant difference between groups. |
| Lee et al. [36] | Gemcitabine and oxaliplatin plus erlotinib (n = 135) vs. gemcitabine and oxaliplatin alone (N = 133) | Metastatic biliary-tract cancer (cholangiocarcinoma, gallbladder or ampulla of Vater cancer) | Primary: PFS | Open-label, randomized | Median PFS was 4.2 months in the gemcitabine and oxaliplatin group versus 5.8 months in the gemcitabine and oxaliplatin plus erlotinib group (p = 0.087). |
| **Phase 2 Studies** | | | | | |
| El-Khoueiry et al. [37] | Sorafenib (n = 36) | Metastatic or unresectable biliary cancers | Primary: Determine the confirmed objective response rate in patients treated with sorafenib 400 mg twice daily. Secondary: PFS, OS and AE profile. | Open-label | The study failed to meet the primary objective was terminated after the first stage of accrual. A confirmed response rate of 0% (0–11%) was observed; 39% demonstrated SD including two with unconfirmed PR. PFS was three months and OS nine months. |
| Ben-Josef et al. [38] | Adjuvant capecitabine and gemcitabine followed by radiotherapy and concurrent capecitabine (N = 79) | Extrahepatic cholangiocarcinoma or gallbladder carcinoma | Primary: Two-year survival (overall and after R0 or R1 resection), pattern of relapse, and toxicity | Open-label | Two-year survival was 65% (RO, 67%; R1, 60%). Median overall survival was 35 months (R0, 34 months; R1, 35 months). |

Table 1: *(Continued)*

| | | | | | |
|---|---|---|---|---|---|
| Malka et al. [39] | Gemcitabine and oxaliplatin with (N = 76) or without cetuximab (N = 74) | Non-resectable or metastatic cholangiocarcinoma, gallbladder carcinoma, or ampullary carcinoma | Primary: Proportion of patients who were PF at four months | Open-label, randomized | 48 (63%) treated with gemcitabine and oxaliplatin with cetuximab and 40 (54%) treated with gemcitabine and oxaliplatin alone were progression-free at four months. Cetuximab did not enhance the activity of chemotherapy. |
| Denlinger et al. [40] | Bortezomib (N = 20) | Locally advanced or metastatic Cholangiocarcinoma (n = 14) or gallbladder adenocarcinoma (n = 6) | Primary: Objective response rate. | Open-label | One unconfirmed partial response. Trial discontinued early because of lack of confirmed partial responses. |
| Park et al. [41] | PDT with (N = 21) or without S-1 (N = 22) | Unresectable hilar cholangiocarcinoma | Primary: OS; Secondary: PFS, complications, re-intervention rate, QOL | Open-label, randomized | PDT plus S-1 showed higher one-year survival rate versus PDT alone (76.2% versus 32%, p = 0.003) and prolonged OS (median 17 months versus 8 months, p = 0.005. PDT plus S-1 showed prolonged PFS versus PDT alone (median 10 months versus 2 months p = 0.009. |
| El-Khoueiry et al. [42] | Sorafenib and erlotinib (N = 34) | Unresectable or metastatic. Cholangiocarcinoma or gallbladder carcinoma | Primary: PFS; Secondary: response probability, OS and toxicity | Open-label | At fourth month patients had unconfirmed PR (n = 6; 6%), SD (n = 10; 29%), PD (n = 18; 53%) and symptomatic deterioration leading to treatment discontinuation (n = 1). Three patients were not evaluable. Median PFS was two months and four-month PFS was 29%. |
| Sohal et al. [43] | Panitumumab with gemcitabine and irinotecan (N = 35) | Unresectable or metastatic cholangiocarcinoma | Primary: Five-month PFS; Secondary: ORR and OS | Open-label | After a medial of seven cycles, 28 evaluable patients had CR (n = 2), PR (n = 9), SD (n = 15) for a disease-control rate of 74%. Two patients underwent surgical resection. The five-month PFS was 69%. The median PFS was 9.7 months and the median OS was 12.9 months. |
| Rubovszky et al. [44] | Cetuximab, gemcitabine and capecitabine | Intrahepatic (n = 16) and extrahepatic cholangiocarcinoma (n = 8) and gallbladder cancer (n = 10) | Primary: Assess RR; Secondary: PFS, OS, AEs | Open-label | The ORR 17.6% (two CR, four PR) and the CBR was 76.5%. After a median of 15.4 months, the median PFS was 34.3 weeks and the median OS was 62.8 weeks. |
| Borbath et al. [45] | Cetuximab and gemcitabine (N = 44) | Unresectable cholangiocarcinoma | Primary: PFS | | At sixth month, PFS reached by 47%. Median OS was 13.5 months. Nine patients (20.4%) had PR and disease-control rate was 79.5%. |
| Lee et al. [46] | Gemcitabine and cisplatin plus sorafenib (N = 39) | Advanced biliary adenocarcinomas | Primary: Improvement in six-month PFS6 from historical 57–77% | Open-label | Six-month PFS was 51%, median PFS was 6.5 months and OS was 14.4 months. |

Table 1: *(Continued)*

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| Yamanaka et al., [47] | Gemcitabine following surgical resection (n = 40) or resection alone (n = 158) | Biliary tract cancer including gemcitabine-treated patients with extrahepatic (n = 18) and intrahepatic cholangiocarcinoma (n = 15) | Primary: OS | Open-label | Among patients with intrahepatic CCA, the probability of two-year survival in the gemcitabine group was 91.7 %, versus 68.2 % in the resection alone group (p = 0.04). |
| Sasaki et al. [48] | Gemcitabine plus S-1 versus gemcitabine alone | Advanced cholangiocarcinoma or gallbladder cancer (N = 62) | Primary: RR | Open-label, randomized | RR for combination therapy was 20% versus 9.4% for monotherapy; however, the median time-to-progression and OS were nearly the same (5.6 versus 4.3 months; 8.9 versus 9.2 months). |
| Jensen et al. [49] | Panitumumab (N = 46) | Non-resectable biliary tract cancer | Primary: Patients alive without progression at sixth month. Secondary: Median PFS, RR, median OS | | Among evaluable patients (n = 42), PFS at 6 months was 31 (74%), 42 had measurable disease. RR was 33% and disease control rate 86%. Median PFS was 8.3 months and median OS was 10.0 months. |
| Roth et al. [50] | Imatinib mesylate (N = 9) | Unresectable advanced or metastatic intrahepatic and extrahepatic cholangiocarcinoma or gallbladder carcinoma unresponsive to prior treatment | Primary: CR, PR and PFS. Secondary: PK, SD, TTP and OS | Open-label | The study was stopped early due to poor recruitment but five patients treated for at least three months. Five patients experienced PD with a median TTP of 79 days (range, 38–116 days). Median OS was 4.9 months (range, 1.5–42.8 months). |
| Kemeny et al. [51] | Hepatic arterial infusion of floxuridine and dexamethasone plus bevacizumab (N = 22) | Unresectable intrahepatic cholangiocarcinoma or hepatocellular carcinoma | Primary: Effect of adding bevacizumab on PFS. Secondary: RR, OS and conversion to resectability. | Open-label | Seven (31.8%) had PR and 15 (68.2%) had SD. Median survival was 31.1 months, PFS 8.45 months and hepatic PFS 11.3 months. The trial was terminated early due to increased biliary toxicity. |
| Iqbal et al. [52] | Gemcitabine and capecitabine (N = 57) | Gallbladder cancer or cholangiocarcinoma | Primary: RR (CR and PR). Secondary: OS and toxicity. | Open-label | There were 52 evaluable patients with cholangiocarcinoma (n = 35) and gallbladder cancer (n = 17). There were seven confirmed PR, six unconfirmed PR, 12 SD. The six-month OS was 55% and median survival was seven months. |
| Oh et al. [53] | Gemcitabine (N = 32) | Intra-hepatic cholangiocarcinoma (n = 16), gallbladder cancer (n = 12), and extrahepatic cholangiocarcinoma (n = 4) with disease progression after 5-fluorouracil-based palliative chemotherapy | Primary: Efficacy and safety of gemcitabine as second-line | Open-label | Among evaluable patients (n = 29), two achieved PR with an overall response rate of 6.9%, six had SD, 21 showed disease progression. The median follow-up duration was 23.2 months. The median TTP was 1.6 months and the median OS time was 4.1 months. |

Table 1: *(Continued)*

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| Bekaii-Saab et al. [54] | Selumetinib (N = 28) | Histologically confirmed advanced biliary tract carcinoma including intrahepatic cholangiocarcinoma (n = 17). | Primary: ORR (CR and PR). Secondary: Toxicity, OS, PFS. | Open-label | Three patients had OR including confirmed PR (n = 3) and unconfirmed CR (n = 1) 17 patients (68%) had SD including 11 (44%) with SD duration ≥16 weeks and three (12%) with duration of more than one year. Median PFS was 3.7 months and median OS was 9.8 months. |
| **Phase 1/2 Studies** | | | | | |
| Iwahashi et al. [55] | Valproic acid (VPA) plus S-1 (N = 12) | Advanced pancreatobiliary tract cancers | Primary: Effectiveness of combination therapy with VPA and S-1 | Open-label | PR (n = 1), SD (n = 10), PD (n = 1). |
| **Phase 1 Studies** | | | | | |
| Costello et al. [56] | Everolimus plus gemcitabine (Cohort 1) and cisplatin (Cohort 2) | Cholangiocarcinoma or gallbladder carcinoma (Cohort 3; N = 10) | Primary: MTD | Open-label, dose escalation | Among patients in Cohort 3, six had stable disease and four had progressive disease. |
| Suder et al. [57] | Afatinib plus paclitaxel (N = 16) | Advanced solid tumors including cholangiocarcinoma | Primary: MTD of afatinib plus paclitaxel. Secondary: safety, PK, antitumor activity | Open-label, dose escalation | A confirmed partial response was observed in one patient with cholangiocarcinoma. |
| Isakoff et al. [58] | Bosutinib plus capecitabine (N = 32) | Locally advanced, treatment-resistant metastatic cancer (N = 32) including cholangiocarcinoma (n = 1) | Primary: MTD, safety and efficacy | Open-label, dose escalation | None of the patients with pancreatic cancer or cholangiocarcinoma achieved a response or SD. |
| Koido et al. [59] | Gemcitabine plus dendritic cells pulsed with a mixture of three types of WT1 peptides, including both MHC class I and II-restricted epitopes | Pancreatic ductal adenocarcinoma (n = 10), intrahepatic cholangiocarcinoma (n = 1) | Primary: safety and toxicity | Open-label | WT1-specific DTH-positive patients showed significantly improved OS and PFS vs. negative controls. All three patients with PDA with strong DTH reactions had a median OS of 717 days. |
| Pant et al. [60] | Tivantinib and gemcitabine, N = 74 (n = 29, dose escalation phase), (n = 45, expansion phase) | Advanced-stage solid tumors including cholangiocarcinoma (n = 8) | Primary: safety, tolerability and establish a phase 2 dose of tivantinib and gemcitabine | Open-label | The overall disease control rate (CR + PR + SD) was 66% (37 of 56). The median PFS was 129 days. PR was observed in 10 patients including one with cholangiocarcinoma. |
| Hickey et al. [61] | Radioembolization (yttrium-90) and capecitabine (N = 16) | Cholangiocarcinoma or metastases confined to the liver | Primary: MTD | Open-label | Criteria for establishing yttrium-90 MTD were not met. |
| Kobrinsky et al. [62] | Bortezomib with oxaliplatin (N = 30) | Solid tumors | Primary: MTD | Open-label, dose escalation | Among 25 evaluable patients, four had PR including one ampulla of Vater carcinoma and one cholangiocarcinoma. |

Table 1: *(Continued)*

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|---|---|---|---|---|---|
| Pant et al. [63] | ME-143 (N = 18) | Advanced solid tumors including cholangiocarcinoma (n = 2) | Primary: MTD | Open-label, dose escalation | SD achieved in three patients including one with cholangiocarcinoma. |
| Plummer et al. [64] | Pazopanib plus gemcitabine (N = 22) | Advanced solid tumors | Primary: MTD | Open-label, dose escalation | Prolonged disease stabilization (>12 cycles) was reported in three patients including one with cholangiocarcinoma. |
| Kurzrock et al. [65] | EZN-2208 (N = 39) | Metastatic or advanced malignancies including cholangiocarcinoma (n = 1) | Primary: MTD | Open-label, dose escalation | The patient with cholangiocarcinoma had a transient 32% tumor regression in target lesions. |
| Konner et al. [66] | KOS-862 (Epothilone D) (N = 32) | Advanced solid tumors or lymphoma | Primary: MTD | Open-label | Stable disease >3 months was achieved by five patients including one with cholangiocarcinoma |
| Gore et al. [67] | Trabectedin plus capecitabine (N = 40) | Advanced malignancies refractory to standard therapy | Primary: MTD | Open-label | One patient with cholangiocarcinoma achieved a sustained PR |
| Katayose et al. [68] | Neoadjuvant chemoradiation with gemcitabine and surgical resection (N = 12) | Resectable cholangiocarcinoma | Primary: MTD | Open-label, dose escalation | 600 mg/m² was determined to be the MTD and recommended dose for gemcitabine in a phase 2 study. |

AE: Adverse Event, CBR: Clinical Benefit Rate, CR: Complete Remission, MHC: *Major Histocompatibility Complex,* MTD: Maximum Tolerated Dose, ORR: Overall Response Rate, OS: Overall Survival, PD: Progressive Disease, PDT: Photodynamic Therapy, PK: Pharmacokinetics, PR: Partial Response, PSF: Progression-Free Survival, QOL: Quality of Life, RR: Response Rate, SD: Stable Disease, TTP: Time to Tumor Progression, WT1: Wilms' tumor 1.

Afatinib: Irreversible ErbB family blocker
Bosutinib: competitive Src/Abl tyrosine kinase inhibitor
S-1: Oral fluoropyrimidine derivative consisting of 5-fluorouracil
Tivantinib: Non-adenosine triphosphate competitive, selective c-MET inhibitor
Panitumumab: Monoclonal anti-EGFR antibody, with gemcitabine and irinotecan
ME-143: Second-generation tumor-specific NADH oxidase inhibitor
EZN-2208: Polyethylene glycol conjugate of SN38, the active moiety of irinotecan

### Palliative care for unresectable Cholangiocarcinoma

Nearly half of patients with CCA have unresectable disease and are candidates for palliative care [71]. Endoscopic biliary drainage is the gold standard treatment in advanced or inoperable hilar cholangiocarcinoma [72]. The main goal is to provide biliary drainage with long-term relief from pruritus, cholangitis, pain and jaundice [73]. In patients with inoperable disease, drainage of 50% or more of the liver parenchyma via stenting can improve patient survival [2]. The management of biliary obstruction with stents is obligatory in perihilar CCA [2]. This may be done endoscopically or percutaneously [74]. Possible complications to stenting include infections [75] and stent migration causing injury [76]. Other palliative measures may include oral nutritional supplements or parenteral nutritional support for patients with cachexia [77].

## EMERGING THERAPIES

### Adjuvant therapy

A recent phase 2 trial demonstrated gemcitabine and capecitabine followed by chemoradiotherapy with concurrent capecitabine is an effective and promising adjuvant regimen in extrahepatic cholangiocarcinoma and also gallbladder carcinoma [38]. Subjects with extrahepatic cholangiocarcinoma or gallbladder carcinoma and radical resection, stage pT2-4 or N+ or positive resection margins, M0, and performance status 0 to 1 were treated with four cycles of IV gemcitabine 1,000 mg/m² on day-1 and day-8 and daily capecitabine 1,500 mg/m² on days 1–14 followed by concurrent capecitabine 1,330 mg/m² daily and radiotherapy. With 80 evaluable patients, results would be promising if two-year survival 95% CI were >45% and R0 and R1 survival estimates were ≥65% and 45%, respectively. Among evaluable subjects

(n = 79), the median overall survival was 35 months and disease-free survival at second year was 52%. Local, distant, and combined relapse occurred in 14, 24, and 9 patients, respectively.

The superiority of cisplatin and gemcitabine chemotherapy over gemcitabine alone for treating advanced biliary tract cancer (ABC) was demonstrated in two randomized trials (ABC02 and BT-22 studies) [78]. Combined data from these trials was used to investigate the derived neutrophil-to-lymphocyte ratio which may predict clinical outcomes in some solid tumors including ABC. A total of 462 individual patient records were analyzed, 328 with baseline derived neutrophil-to-lymphocyte ratio <3 and 134 ≥3. All surviving patients (n = 19) had a derived neutrophil-to-lymphocyte ratio <3. There was strong evidence that derived neutrophil-to-lymphocyte ratio was closely associated with both overall survival (hazard ratio 1.62; 95% CI 1.32–2.01) and progression-free survival (hazard ratio 1.40; 95% CI 1.13–1.72). There was significant evidence of an association between low baseline derived neutrophil-to-lymphocyte ratio and long-term survival on a cisplatin and gemcitabine regimen.

## Immunotherapy

Advances in cancer immunotherapy have encouraged the development of new treatment options. This type of treatment strengthens the patient's immune system by priming it against tumor-specific antigens. Immunotherapy is based on the observation that tumor infiltration by the cellular mediators of the adaptive immune response such as CD8+ and CD4+ cells is generally correlated with improved outcomes in biliary tract cancers [79, 80]. Similarly, patients with higher total regulatory T lymphocyte counts have a significantly better prognosis when compared with those patients whose tumor tissues showed lower regulatory T-lymphocyte counts [81]. Such treatments are more selective against malignant cells and generally less toxic than traditional chemotherapy [82]. Conversely, antibodies against glycoprotein 2 are associated with more severe phenotype and poor survival due to cholangiocarcinoma [83].

Immunotherapy has recently made great advances in the field of oncology, such as the programmed death ligand 1 (PD-L1) inhibitors pembrolizumab for non-small cell lung cancer [84] and nivolumab for metastatic melanoma [85], non-small cell lung cancer [86] and renal cell carcinoma [87]. Elevated serum PD-L1 in patients with cholangiocarcinoma have been shown to have poorer overall survival [88], suggesting these checkpoint inhibitors may also be beneficial for this patient population. In one case report, a patient with extrahepatic cholangiocarcinoma demonstrated a strong and durable response to the immune checkpoint inhibitor pembrolizumab [89].

## Orthotopic liver transplantation

Several ongoing clinical trials are assessing new methods for OLT. One prospective, open-label, randomized, study will compare the use of capecitabine and radiotherapy or neoadjuvant radiochemotherapy and liver transplantation versus conventional liver and bile duct resection (ClinicalTrials.gov Identifier: NCT02232932). An observational study is designed to validate results of a previous study performed at the Mayo Clinic [90] where patients were treated with combination chemotherapy and radiation and maintained on oral capecitabine until they can receive a liver transplant (ClinicalTrials.gov Identifier: NCT00301379).

## Tumor microenvironment

Similar to other treatment-resistant cancers, intrahepatic CCA is characterized by the growth of fibrous or connective tissue, or desmoplasia, around the tumor [91]. This desmoplasia progresses during disease progression and includes stromal fibroblasts, immune cells, and excessive deposition of a complex extracellular matrix which is often rich in hyaluronan [92]. Proliferation of this tumor microenvironment forms an impediment to treating solid tumors such as CCA by increasing interstitial fluid pressure which compresses the surrounding vasculature and promotes tumor progression and the metastatic potential of cancer cells [93] and reduces the beneficial effects of systemic chemotherapeutic agents [91, 94]. As hyaluronan is a major component of the tumor microenvironment, reducing tumor hyaluronan with PEGylated human recombinant hyaluronidase decreased interstitial fluid pressure, improved vascular perfusion and increased the effectiveness of docetaxel and liposomal doxorubicin in a murine model of prostate cancer [94].

Large amounts of hyaluronan also exist in the tumor microenvironment of intrahepatic CCA [95] and may represent a target for future therapies [96]. A phase 1 study assessed the efficacy of PEGylated human recombinant hyaluronidase in combination with gemcitabine in patients with untreated stage IV metastatic pancreatic ductal adenocarcinoma [97]. Among patients evaluated for pretreatment tissue hyaluronan levels, median progression-free survival and overall survival rates were 7.2 and 13.0 months, respectively, for patients with high hyaluronan levels versus and 3.5 and 5.7 months for patients with low hyaluronan levels.

## Molecular targeted agents

Intrahepatic CCA exists as a range of genetic subtypes [98] with varying sensitivity to chemotherapeutics and molecular targeted agents [99]. Genetic studies of biliary tumors have identified known growth factor gene mutations [100, 101] and signaling pathways [102] that control tumor growth and survival. Target-specific monoclonal antibodies and small molecule inhibitors directed against the signaling pathways that promote

the growth and spread of cholangiocarcinoma are being developed [103]. Such targeted therapies are showing promise for treatment-resistant CCA.

Recently identified gene mutations include epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS), v-raf murine sarcoma viral oncogene homolog (BRAF) and tumor protein p53 (TP53). Other novel mutations include isocitrate dehydrogenase (IDH), BRCA1-associated protein 1 (BAP1) and AT-rich interactive domain-containing protein 1A (ARID1A), and novel fusions such as fibroblast growth factor receptor 2 (FGFR2) and ROS proto-oncogene 1 (ROS1) [100].

Several ongoing clinical trials using targeted therapy for cholangiocarcinoma are described in Table 2. In one example, folic acid was used as a targeting agent in CCA cells expressing folic acid receptors [104]. When 5-fluorouracil and folic acid were linked to gold nanoparticles, its cytotoxicity was correlated with folic acid receptor expression, suggesting the use of folic acid as a targeted therapy.

## Mutation profiling

Significant genetic differences between intrahepatic and extrahepatic CCA have been identified which may have implications for treatment and outcomes [105]. In one study, 75 samples undergoing next generation sequencing were from patients with intrahepatic (n = 55) and extrahepatic CCA (n = 20) [106]. Significant differences were found in these two groups with respect to the nature and frequency of the genetic aberrations. These are summarized in Table 3. IDH1 and DNA repair gene alterations occurred more frequently in intrahepatic CCA, while more ERBB2 genetic abnormalities occurred

Table 2: Clinical trials of molecular targeted agents for cholangiocarcinoma

| Target | Study title | ClinicalTrials.gov Identifier[a] | Sponsor | Outcome measures |
|---|---|---|---|---|
| EGFR | A phase II, single arm study of BGJ398 in patients with advanced cholangiocarcinoma | NCT02150967 | Novartis Pharmaceuticals | Primary: ORR. Secondary: OS, PFS, OR, DCR, AEs/ SAEs, PK |
| VEGF | A phase 2 trial of regorafenib as a single agent in advanced and metastatic biliary tract carcinoma/ cholangiocarcinoma patients who have failed first-line chemotherapy | NCT02053376 | University of Pittsburgh | Primary: PFS. Secondary: OR, OS, changes in biomarkers (CA19-9 and CEA) |
| FGFR | Open-label, dose-escalation study of INCB054828 in subjects with advanced malignancies | NCT02393248 | Incyte Corporation | Primary: MTD Secondary: ORR (RECIST), PK |
| BRAF | Afatinib dimaleate and capecitabine in treating patients with advanced refractory solid tumors, pancreatic cancer or biliary cancer | NCT02451553 | University of Washington | Primary: AEs, DLT, MTD (CTCAE v4.0), PFS, RP2D. Secondary: biomarker profile (EGFR, HER2 gene, etc.), DOR, SD, OS, RR (RECIST v1.1), TTP |
| PD-1/ PDL-1 | Study of pembrolizumab (MK-3475) in participants with advanced solid tumors (MK-3475-158/ KEYNOTE-158) | NCT02628067 | Merck Sharp & Dohme Corp. | Primary: ORR |
| Tyrosine kinase | Basket study of entrectinib (RXDX-101) for the treatment of patients with solid tumors harboring NTRK1/2/3, ROS1, or ALK gene rearrangements (fusions) (STARTRK-2) | NCT02568267 | Ignyta, Inc. | Primary: ORR (RECIST v1.1) Secondary: DOR, TTR, CBR, intracranial tumor response, CNS PFS, PFS, OS |
| IDH1 | Study of orally administered AG-120 in subjects with advanced solid tumors, including glioma, with an IDH1 mutation | NCT02073994 | Agios Pharmaceuticals, Inc. | Primary: AEs, MTD. Secondary: DLTs, PK, PD, clinical activity (RECIST v1.1) |

[a]Studies can be reviewed at: https://clinicaltrials.gov
AEs: Adverse events, AG-120: IDH1 mutant inhibitor, CA19-9: Cancer antigen 19-9, CEA: Carcinoembryonic antigen, CBR: Clinical benefit rate, DCR: Disease control rate (proportion of patients with a best overall response of CR or PR or SD), DOR: Duration of response, EGFR: Epidermal growth factor receptor, FGFR: *Fibroblast growth factor receptors*, IDH1: Isocitrate dehydrogenase-1, PD: Pharmacodynamics, PK: Pharmacokinetics, ORR: Objective response rate, RECIST: Response Evaluation Criteria in Solid Tumors, RP2D: Recommended phase 2 dose, RR: Response rate, PD-1 / PDL-1: Programmed cell death protein 1 / programmed death ligand 1, SAEs: Serious adverse events, SD: Stable disease, TTP: Time to progression, VEGF: Vascular endothelial growth factor.

Table 3: Genetic differences between Intrahepatic and extrahepatic cholangiocarcinoma

| Abnormality | Intrahepatic cholangiocarcinoma | Extrahepatic cholangiocarcinoma |
|---|---|---|
| **Genes** | | |
| TP53 | 16 (29.1%) | 9 (45%) |
| KRAS | 13 (23.6%) | 8 (40%) |
| ARID1A | 11 (20%) | 1 (5%) |
| ERBB2 | 1 (1.8%) | 5 (20%) |
| PBRM1 | 6 (10.9%) | 1 (5%) |
| BAP1 | 5 (9.1%) | 2 (10%) |
| FBXW7 | 3 (5.5%) | 3 (15%) |
| SMAD4 | 2 (3.6%) | 5 (25%) |
| IDH | 13 (23.6%) | 0 (0%) |
| **Pathways** | | |
| MAP-ERK | 19 (34.5%) | 11 (55%) |
| mTOR | 14 (25.5%) | 8 (40%) |
| DNA Repair | 9 (16.4%) | 8 (40%) |
| FGF Pathway | 7 (12.7%) | 1 (5%) |
| Chromatin Modification | 18 (32.7%) | 3 (15%) |

Modified from Churi et al., 2014.

among extrahepatic. In intrahepatic CCA, KRAS, TP53 or MAPK/mTOR genetic abnormalities were significantly associated with a worse prognosis while FGFR genetic abnormalities were correlated with slow-growing tumors. Based on these mutational profiles, many patients were referred to phase 1 or 2 clinical trials with targeted therapy for enrollment [106].

## Promoting the advancement of drug development

The cholangiocarcinoma Foundation (CF) was founded in 2006 with the mission of finding a cure and improving the quality of life for those affected by cholangiocarcinoma. In recent years, the CF has promoted the development of new treatments for CCA by sponsoring an annual conference where interested individuals come together to learn more about the latest advances in bile duct cancer research, treatment and care. Recognizing the unmet medical need for treating CCA, the CF is proactively working with the pharmaceutical and biotechnology companies to accelerate drug development by sponsoring an Industry Night at the annual conference. Representatives from industry are invited to present cholangiocarcinoma clinical trial concepts to a panel of more than 20 global biliary cancer experts. The objective is to accelerate drug development by providing immediate feedback at a considerable cost-savings. To date, this program has been very well received and will hopefully serve as a model for advancing the treatment of other challenging disorders.

## CONCLUSION

Cholangiocarcinoma has long been a challenging disease with poor outcomes. The development of emerging new therapies is currently underway that may improve the treatment outcomes of this devastating disease. In addition to providing research grants, disease foundations have an opportunity to work co-operatively with the pharmaceutical and biotechnology companies to advance emerging new therapies.

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## Author Contributions

Sean Turbeville – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important intellectual content, Final approval of the version to be published

Carl S. Hornfeldt – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important intellectual content, Final approval of the version to be published

Milind Javle – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important intellectual content, Final approval of the version to be published

Eric Tran – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important

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Marion Schwartz – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting of article, Revising it critically for important intellectual content, Final approval of the version to be published

## Guarantor
The corresponding author is the guarantor of submission.

## Conflict of Interest
Authors declare no conflict of interest.

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