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Bile Duct Cancer Treatment and Research

Latest Developments

Edited by Qiang Yan and Feng Cen

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Aims and Scope of the Series

The field of oncology has undergone extraordinary change and progress over the past several decades. Today, information is evolving at a rapid rate, with standards of management far different than standards of care applied during the training of most practicing oncologists. Oncology practitioners in all disciplines must remain current to optimize patient care. Basic and translational science remain critical in developing process improvements for patient care. The closer we understand the mechanism, the more we can improve targeted therapies and apply them to patient care. The pace of information is moving faster than at any previous time in history, and all oncology disciplines must remain current to provide excellent service to patients. The modern oncologist must be fluent in using big data and the volume of information generated in clinical trials. As we move closer to personalized patient care based on genomics and molecular biomarkers, the modern oncologist has to be nimble in assessing all available information and how this would be applied to each patient, balanced by the clinical status and medical co-morbidities of each patient. Targeted therapies can bring new and different sequelae, and oncology teams need to remain fluent in managing the consequences of therapy and primary management. In this book series, we will present how modern care has progressed in multiple disease areas and how modern oncology teams need to adapt in order to manage the cancer patients of today successfully. Surgery, radiation therapy, and medical oncology are practiced today with the support of exceptional modern technology, and in this series, we will review how these improvements are applied to each disease site to maintain excellence in patient care.

Meet the Series Editor

Dr. FitzGerald is the professor and chair of the Department of Radiation Oncology at UMass Chan Medical School in the USA. He serves as one of the principal investigators of the Imaging and Radiation Oncology Core (IROC) service for the National Cancer Institute clinical trials program and is directly involved in the quality assurance of clinical trials in the National Clinical Trials Network (NCTN). Dr. Fitzgerald manages NCTN clinical trials with a real-time pre-therapy review of imaging and radiation therapy treatment objects to ensure the care plan complies with study objectives and the patient stage has been assigned to the correct study. His basic science interest is in hematopoietic stem cell biology and cellular adhesion molecules as they pertain to therapeutic resistance and mitigation of injury from therapy.

Meet the Volume Editors

Qiang Yan, MD, FACS, is a Professor and Chief General Surgery Physician. He currently serves as Vice President of Huzhou Hospital, Zhejiang University School of Medicine, and Chairman of the National Clinical Key Specialty (Department of General Surgery). In 2019, he earned a certificate from the Surgical Leadership Program at Harvard Medical School in the United States. He became a Fellow of the American College of Surgeons in 2016 and was named a special member of the Japan-Germany Society for the Study of Liver Surgery in 2018.

Feng Cen is an Associate Chief Physician in Hepatobiliary and Pancreatic Surgery of Huzhou Hospital, Zhejiang University School of Medicine. He graduated from Wenzhou Medical University and received the degree of Chiurgiae Magister from Zhejiang University School of Medicine, as well as a Master's degree in teaching from Huzhou University. He is a member of the Minimally Invasive Surgery Branch of the Zhejiang Medical Association, as well as the Tumor Ablation Committee of the Zhejiang Cancer Association and the Minimally Invasive Committee for Hepatobiliary and Pancreatic Surgery of the Zhejiang Rehabilitation Medical Association.

Contents

Preface

Bile Duct Cancer is a common type of malignant tumor of the biliary tract, starting in the hilar region, characterized by an insidious onset, and is a prevalent malignant tumor in the biliary tract. Its diagnosis and treatment encompass multiple aspects, including epidemiology, clinical evaluation, imaging studies, pathological classification, staging, resectability assessment, perioperative management, surgical decision-making, systemic therapy, and follow-up monitoring. There are many links, which cause the most difficult diagnosis and treatment technology in Bile Duct Cancer.

The contents of the book include the following aspects:

1. *Bile Duct Cancer: From Bench to Bedside – Latest Developments* offers a comprehensive discussion on the latest treatment strategies and research developments for this challenging biliary tract cancer. This section serves as a bridge between cutting-edge research and real-world clinical application, offering actionable insights without overwhelming readers with technical details.

2. *Cholangiocarcinoma – A Comprehensive Review of Diagnostic Challenges and Novel Therapeutic Approaches* offers an engaging exploration of bile duct cancer research, blending scientific rigor with accessibility. This blend of depth and readability makes it an indispensable tool for anyone seeking to understand or contribute to the field of bile duct cancer management.

3. *Diagnosis, Imaging, and Prognostic Evaluation in Cholangiocarcinoma* provides a comprehensive exploration of Cholangiocarcinoma (CCA), a rare yet aggressive malignancy originating from the biliary epithelium. This section is invaluable for clinicians and researchers aiming to enhance the management and prognosis of CCA patients.

4. *Multiomics Approaches for the Identification of Biomarkers and Therapeutic Targets in Cholangiocarcinoma* delves into the integration of genomics, transcriptomics, epigenomics, proteomics, and metabolomics, unveiling a comprehensive molecular landscape of CCA. This section exemplifies how multiomics can reshape cancer care by prioritizing actionable knowledge and systemic understanding, rather than relying on incremental data. This approach empowers readers to engage with the material holistically.

5. *Optimising Therapeutic Options for Bile Duct Cancer in the Era of Molecular Profiling, Immunotherapy and Pharmacogenomics* captivates readers by exploring cutting-edge strategies to enhance cholangiocarcinoma treatment. The section's strengths lie in its comprehensive yet concise analysis of evolving treatment paradigms and its emphasis on patient-centric innovation.

This book provides a preliminary discussion on the diagnosis, treatment, and latest research developments of cholangiocarcinoma, aiming to offer some assistance in improving the treatment and prognosis of Bile Duct Cancer patients.

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Chapter 1

Bile Duct Cancer: From Bench to Bedside – Latest Developments

Govind Trivedi, Ashwani Anshu and Arnav Trivedi

Abstract

Cholangiocarcinomas are a heterogeneous group of malignancies that can arise from any location along the biliary tree. They are anatomically classified as intrahepatic, perihilar, and distal cholangiocarcinomas. Intrahepatic cholangiocarcinoma, the less common type, arises proximal to the second-order bile ducts. Several risk factors eventually lead to either chronic inflammation and/or cholestasis, which lead to the development of cholangiocarcinomas. E-cadherin under increased influence of TGF-β transforms into N-cadherin and loses its cell adhesion property and starts invading, causing metastases. The surgical approach is aimed at curative intent by achieving R0 resection. The standard surgery is liver resection for proximal cholangiocarcinoma. After the radical surgical excision, the available modalities are chemotherapy and radiotherapy, or a combination of the two. Resistance to the activity of chemotherapy can be overcome by addition of Acelarin. Acelarin (NUC-1031) is a first-class nucleotide analog that presents a good safety profile and is under trial. Ivosidenib (AG-120) is an oral, targeted, small-molecule inhibitor of IDH1-mutant. However, there have been reported incidences of acquired ivosidenib resistance. Derazantinib is an oral drug. It is an ATP-competitive, pan-FGFR inhibitor with potent activity against FGFR1–3 kinases. Pembrolizumab is a humanized, highly specific monoclonal antibody against PD-1, which blocks the interaction between PD-1 and its ligands, PD-L1, and PD-L2. FDA approved it for DNA mismatch repair (MMR) deficiency and/or MSI-H, high grade solid tumors, including cholangiocarcinomas. Tumor vaccines are also under trial.

Keywords: cholangiocarcinoma, biliary tract cancers, ALPPS, FGFR inhibitors, tumor vaccines, immunotherapy

1. Introduction

Cholangiocarcinomas are a heterogeneous group of malignancies that can arise from any location along the biliary tree [1]. They are anatomically classified as intrahepatic, perihilar, and distal cholangiocarcinomas. Intrahepatic cholangiocarcinoma, the less common type, develops proximally to the second-order bile ducts. Perihilar cholangiocarcinoma is the most common type, which arises between the second-order bile ducts and the insertion of the cystic duct into the common bile duct. Distal extrahepatic cholangiocarcinoma arises between the insertion of the cystic duct and

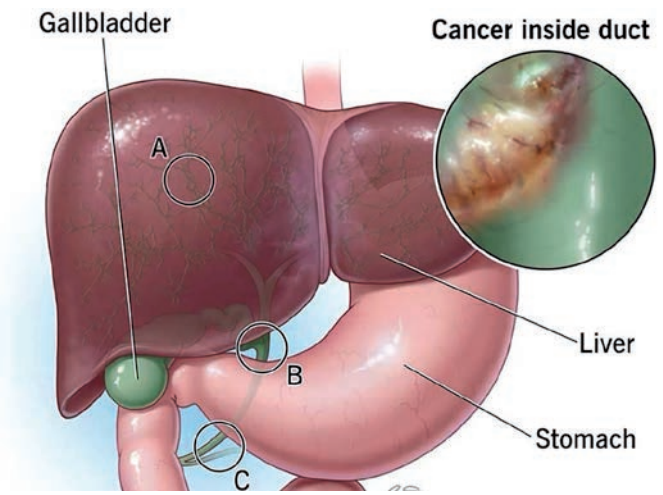

Figure 1.
Types of cholangiocarcinoma. A. Intrahepatic; B. Perihilar; C. Distal. (https://my.clevelandclinic.org/health/diseases/21524-cholangiocarcinoma).

the ampulla of Vater [2]. Besides this anatomical classification, other factors such as tumor growth pattern (mass-forming, periductal infiltrating, or intraductal) and the cell of origin (cholangiocytes, peribiliary glands, hepatic progenitor cells, or hepatocytes) provide alternative methods of classification that may better predict tumor behavior [3, 4].

The incidence of intrahepatic cholangiocarcinoma has increased over the past three decades while the incidence of perihilar and distal extrahepatic cholangiocarcinoma has remained stable [5]. The prognosis of this malignancy is not good because of its indolent character, difficulties in diagnosing early, and limited treatment approaches, due to which its median survival is even less than 24 months. Surgical management has the only potential to cure it, but only in early-stage disease (**Figure 1**).

2. Pathogenesis

Many different risk factors have been identified. The risk factors eventually lead to either chronic inflammation and/or cholestasis, which leads to development of cholangiocarcinomas. These changes form the basis of molecular pathogenesis of bile duct cancer by acting on a series of intercellular pathways (**Table 1**).

2.1 Inflammation

Inflammation is the main factor responsible for cholangiocarcinogenesis. High concentrations of inflammatory mediators cause progressive mutations in tumor suppressor genes, proto-oncogenes, and DNA mismatch-repair (MMR) genes, resulting in cell proliferation [6]. The role of IL-6 is well established in the development of cholangiocarcinomas by affecting multiple intracellular pathways, promoting carcinogenesis. This can also be highly overexpressed in both cultured cholangiocarcinoma cell lines and surgically resected specimens, supporting its role in carcinogenesis [7].

Cholestatic liver diseases	Primary Sclerosing Cholangitis (PSC) Fibropolycystic liver diseases Congenital hepatic fibrosis Caroli disease Choledochal cysts Biliary hamartomas
Liver cirrhosis (any etiology)	
Biliary stone disease	Cholecystolithiasis Hepatolithiasis Choledocholithiasis
Infections	Liver flukes Hepatitis B and C Chronic typhoid disease Recurrent pyogenic cholangitis Human Immunodeficiency Virus (HIV)
Inflammatory disorders	Inflammatory bowel disease Chronic pancreatitis Gout Thyrotoxicosis
Toxins	Alcohol Tobacco Thorotrast (contrast agent) Chemical toxins, e.g., dioxins, vinyl chloride, nitrosamines
Metabolic conditions	Diabetes Obesity Non-Alcoholic Fatty Liver Disease (NAFLD)
Genetic disorders	Lynch syndrome Bile salt transporter protein gene defects
Others	Intraductal Papillary Neoplasms of the Bile duct

Table 1.
Risk factors for development of cholangiocarcinoma.

In normal cholangiocytes, IL-6 activates the JAK-STAT pathway, which in turn increases transcription of the cytokine suppressor SOCS3 [6]. This is controlled by negative feedback under normal circumstances. In cholangiocarcinoma, there occurs epigenetic silencing of SOCS3, which in turn reduces the negative feedback [8]. DNMT1 alters gene expression by methylation of cytosine. IL-6 downregulates specific microRNAs, resulting in increased transcription of DNMT1 and decreased expression of tumor suppressor genes [9]. IL-6 upregulates Mcl-1 (an apoptosis inhibitor), which prevents cell death by activating STAT3 (a transcription factor in the STAT protein family) [10]. IL-6 also increases expression of progranulin (precursor protein for granulins), which is a family of peptides that regulate cell growth and result in activation of the AKT pathway, which mediates cell survival, mitosis, migration, and angiogenesis [6, 11]. Additionally, the liver fluke *Opisthorchis viverrini* secretes a granulin homolog (Ov-GRN-1) that can activate the AKT pathway directly, causing cell proliferation and angiogenesis [12–14]. IL-6 also decreases expression of p21, which is a mediator of cellular senescence, by activating p38 MAPK (a group of protein kinases responsible for cell differentiation and proliferation), resulting in

increased mitosis [15]. Lastly, IL-6 increases telomerase activity, causing telomere shortening during mitosis, further prolonging cell survival [16].

COX-2 is an enzyme important in prostaglandin production. High levels can stimulate cholangiocarcinoma. COX-2 inhibitors can induce apoptosis and inhibit proliferation by decreasing AKT pathway stimulation and activating p21 and other cyclin-dependent kinase inhibitors [17, 18].

There is also a role of macrophage in WNT pathway in cholangiocarcinogenesis. Macrophages upregulate the transcription of WNT7b and WNT10a which bind to its receptor FZD and its coreceptors LRP5/LRP6 present on cholangiocytes [19]. This in turn inhibits intracellular β-catenin degradation complex, which leads to the accumulation of β-catenin [20]. This in turn interacts with TCF/LEF family of transcription factors in the nucleus, causing increased cell viability and resistance to apoptosis [21].

2.2 Cholestasis

Cholestasis occurs due to obstruction of bile flow, resulting in increased retention of bile acids. This causes an acidic medium and promotes apoptosis. Normally conjugated bile acids can act as ligands for the G Protein-Coupled Bile Acid Receptor 1 (GPBAR1) and affect cell proliferation, chloride and bicarbonate excretion, and apoptosis of cholangiocytes [22, 23]. Studies have shown high expression of GPBAR1 in cholangiocarcinoma [23]. Conjugated bile acids also lead to activation of the ERK1/2, Akt, and Nuclear Factor-Kappa B (NF-κB) pathways, resulting in increased COX-2, cell proliferation, migration, and survival [24–26]. Decreased nuclear farnesoid X Receptor (FXR) also has a role in cholestasis [27]. A few specific bile acids (e.g., deoxycholic acid, taurocholic acid) stimulate abnormal cell growth. The bile salt glycochenodeoxycholate causes oxidative stress to cholangiocytes, which further causes genetic alterations [28].

2.3 MicroRNA changes

Multiple miRNAs are seen to be unregulated in cholangiocarcinoma, leading to impaired cell survival and carcinogenesis. Expression of some miRNAs is regulated by IL6, leading to carcinogenesis. IL6 increases expression of miR-let-7a and downregulates miR-148a and miR-152, resulting in increased carcinogenesis [9, 29].

2.4 Factors causing spread and invasion

E-cadherin under increased influence of TGF-β transforms into N-cadherin and loses its cell adhesion property and starts invading [30, 31]. The invading cells are supported by VEGF, which causes neoangiogenesis, increasing the survival of cancer cells [32]. The cell surface receptor tyrosine kinase c-Met is abnormally high in cholangiocarcinoma along with its only known ligand, Hepatocyte Growth Factor (HGF), promoting tumor growth, angiogenesis, and metastasis [33, 34]. VEGF, c-MET, COX-2, and IL-6 all have a complex interplay in promoting metastasis by activating p42/44MAPK and the Akt pathway [35]. Bcl-2, a potent anti-apoptotic protein, has also been found in high levels in cholangiocarcinoma [36]. In addition, sodium iodide symporter (NIS) and increased GLUT-1 also promote metastasis [37–39].

3. Treatment approaches

3.1 Surgical approach

Surgical approach is aimed at curative intent by achieving R0 resection. The standard surgery is liver resection for proximal cholangiocarcinoma [40]. The work-up for diagnosis and treatment of distal cholangiocarcinoma matches the cancer of the head of the pancreas. Important decision-making for the extent of resection depends on the future liver remnant (FLR). Surgical resection is the only potential to cure cholangiocarcinoma. Survival after resection ranges between 25% and 40% at 5 years [41]. The resectability is dependent on the location of primary lesion, the extent of invasion of intrahepatic biliary and vascular structures, and lastly, also on the quality and amount of parenchyma remaining after resection, known as the Future Liver Remnant (FLR). The intrahepatic cholangiocarcinomas require an extensive liver resection, sometimes hemihepatectomy or extended hemihepatectomy; additionally, resection of extrahepatic bile duct is required in perihilar cholangiocarcinoma.

Despite too many available preoperative diagnostic modalities, this is difficult to decide the extent of resection. The decision of stretch of resection or whether to even resect or not is generally made intraoperatively. So a staging laparoscopy is recommended before an extensive surgical exploration is carried out to look for obvious criteria of unresectability, such as liver or peritoneal metastases [42].

Besides extensive resection, an adequate FLR is equally important. In general, 25% is considered an adequate FLR for normal liver parenchyma. In cases of steatotic, cholestatic, cirrhotic, or post chemotherapy cases, at least 30–40% of FLR is considered to be adequate [43]. Inadequacy of FLR is compensated by procedures like portal vein embolization or associating liver partition and portal vein ligation (ALPPS) for staged hepatectomy, which ensures hypertrophy of normal parenchyma leading to increased FLR post resection. FLR can be assessed by hepatobiliary scintigraphy with indocyanine green [44].

It is equally important to perform a lymph node dissection of locoregional lymph nodes present in hepatoduodenal ligament. A minimum of six locoregional lymph nodes should be sampled [45]. Up to 45–65% of patients are found to be positive for metastasis in lymph nodes at the time of clinical diagnosis in intrahepatic cholangiocarcinoma, which significantly impacts the survival. Notably, 5-year overall survival (OS) is approximately 0–20% in pN1 patients versus 35–50% in pN0 patients [46]. Lymph node dissection has its own morbidity, and no clear-cut data exists suggesting its role in preventing local recurrence. A hepatobiliary multidisciplinary team should be assigned to decide upon it.

Prior to surgery, especially in cases of intrahepatic cholangiocarcinoma, there might exist biliary obstruction (about 15% cases) which needs to be relieved. Preoperative biliary drainage is highly recommended as biliary obstruction might impair liver regeneration post resection. Percutaneous transhepatic biliary drainage (PTBD) and endoscopic biliary drainage (EBD) are the two available procedures to drain the bile ducts, with both being equally effective [40]. Cholangitis is a possible complication of drainage affecting post-operative mortality, so decisions need to be taken wisely especially in immunocompromised group of patients [47]. Finally, in patients with non-resolving biliary obstruction, resection of the segment of biliary confluence is preferred, followed by reconstruction by roux-en-Y hepaticojejunostomy.

Neoadjuvant chemotherapy is also tried, although the survival benefit is doubtful. However, it can cause downstaging of tumor from unresectable to resectable disease in a few cases.

3.1.1 Liver transplantation

There is a limited role of liver transplantation in perihilar or distal disease. However, it can be curative for intrahepatic disease with no evidence of extrahepatic spread. In cases of inadequate FLR and where R0 resection is unlikely to be achieved, liver transplantation is the only option left. In addition, it is also curative for underlying cirrhosis or PSC.

According to retrospective studies, LT may offer satisfying outcomes for patients with unresectable very early intrahepatic cholangiocarcinoma (i.e., ≤ 2 cm) [41]. Sapisochin et al. [48], in their cohort of 2301 patients transplanted for end-stage liver disease or HCC, had 23 patients diagnosed with an intrahepatic cholangiocarcinoma on pathology examination; they reported a 5-year OS of 45% with far better results for very early intrahepatic cholangiocarcinoma (namely single tumor ≤2 cm) than multifocal and larger tumors in terms of 5-year risk of recurrence (18% versus 65%, P = 0.01) and 5-year OS (65% versus 45%, P = 0.02).

In a carefully selected group of patients with intrahepatic cholangiocarcinoma >2 cm but with favorable tumor biology (i.e., no evidence of extrahepatic disease, vascular invasion, and lymph node spread), at least 6 months of neoadjuvant chemotherapy with gemcitabine and cisplatin was shown to be beneficial [49].

3.1.2 ALPPS

It stands for Associating liver partition and portal vein ligation for staged hepatectomy. It is an emerging surgery intended to resect carcinomas which are classically unresectable. It consists of two stages:

Stage 1: The liver is surgically partitioned, and the portal vein is ligated. This induces rapid hypertrophy (growth) of the FLR.

Stage 2: After a short interval, typically around 9–11 days, the hypertrophied FLR is resected, along with the remaining tumor-bearing liver tissue.

This approach contrasts with conventional portal venous embolization, which requires a longer waiting period (4–8 weeks) for FLR growth. ALPPS can achieve FLR hypertrophy of 40–80% within 6–9 days, compared to 8–27% with Portal venous embolization (**Figure 2**) [48].

3.2 Chemotherapy

3.2.1 Adjuvant chemotherapy

After the surgical excision, the available modalities are radiotherapy and chemotherapy, or a combination. The ESPAC-3 trial studied the efficacy of 5-FU or gemcitabine compared to observation in periampullary carcinoma [50].

In the meta-analysis by Horgan et al. [51], which evaluated data from more than 6000 patients who underwent different types of post-surgical treatments, the results confirmed the benefit of adjuvant chemotherapy and chemoradiotherapy, in particular in the group with node-positive and surgical positive margin.

In accordance with results obtained from multiple studies, capecitabine is now considered the standard drug post curative resection.

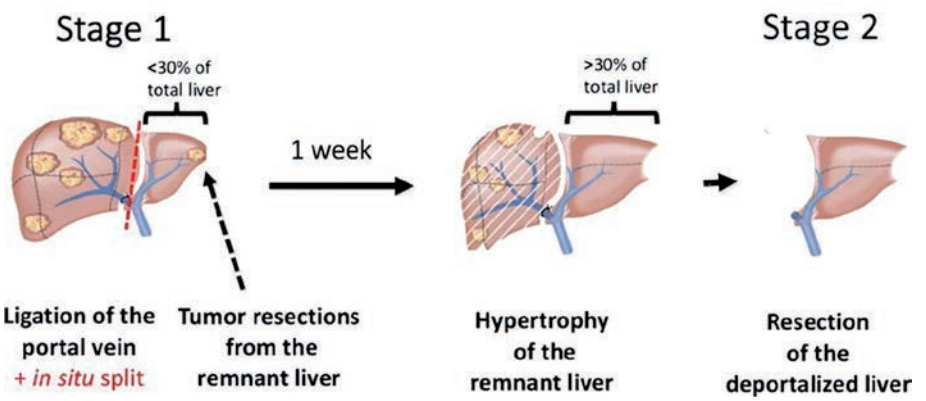

Figure 2.
Steps of ALPPS. (https://medizinonline.com/en/a-promising-innovation-in-liver-surgery/).

3.2.2 Chemotherapy for metastatic disease

Many trials for unresectable disease are ongoing. The AC-02 trial, in which the combination of gemcitabine and cisplatin is compared to gemcitabine alone, demonstrated a higher median OS (11.7 vs. 8.1 months, respectively; HR 0.64; 95% CI 0.52–0.8; $p < 0.001$) and better disease control rate (DCR) for the combination [52]. Addition of an immune checkpoint inhibitor was shown to be beneficial in an unselected population in the recent ASCO-Gastrointestinal Cancer Symposium's TOPAZ-1 study [53, 54]. Due to the results of this study, cisplatin along with gemcitabine and durvalumab might be the new standard medicine combination for high grade unresectable cholangiocarcinomas (**Figure 3**).

Resistance to the activity of chemotherapy can be overcome by the addition of Acelarin. Acelarin (NUC-1031) is a first-class nucleotide analog that presents a good

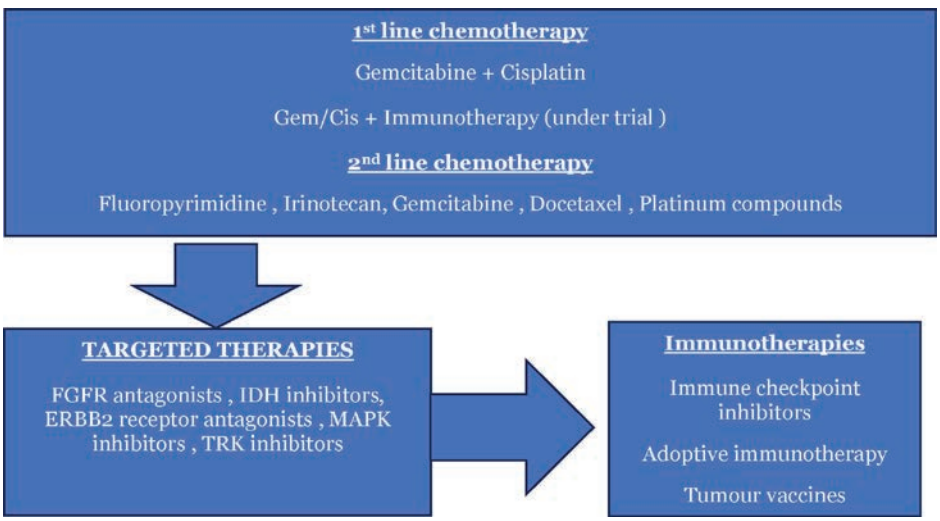

Figure 3.
Flow chart for treatment of unresectable metastatic disease.

safety profile in association with cisplatin for the first-line treatment in the phase Ib trial ABC-08 at the recommended dose of 725 mg/m^2 and is under trial [55].

For patients refractory to first-line chemotherapy, a good PS ECOG is the most important selection factor for the activation of second-line therapy [56].

The reported median survival benefit of the FOLFOX regimen over active symptom control was small (6.2 versus 5.3 months, adjusted HR 0.69), the FOLFOX regimen obtained a more significant survival rate at 6 (50.6% versus 35.5%) and 12 months (25.9% versus 11.4%), so the regimen is now considered the reference second-line treatment [57].

3.3 Target therapies

According to a landmark MOSCATO-01 trial by Massard C et al. [58], in a study comprising 43 biliary tract malignancies (29 intrahepatic cholangiocarcinoma, 10 extrahepatic cholangiocarcinoma, four gallbladder carcinoma), it was observed that specific molecule-directed targeted therapy was associated with a significantly improved proportion of patients with actionable alterations and overall better PFS.

3.3.1 FGFR antagonists

FGFRs are a family of tyrosine kinase receptors (FGFR1–4) triggered by ligand-dependent receptor dimerization. The binding of FGFR by its ligand at the cell surface results in multiple downstream signaling pathways activation, including JAK-STAT, RAS-BRAF-MEK-ERK, and PI3K-AKT-mTOR, leading to cell proliferation, angiogenesis, differentiation, and survival [59]. Any activating mutations, chromosomal translocations, gene fusions, or gene amplifications can result in ligand-independent signaling, which can cause pathologic cell proliferation (**Table 2**) [60].

3.3.1.1 Derazantinib

Derazantinib is an oral drug. It is an ATP-competitive pan-FGFR inhibitor. It has robust activity against FGFR1–3 kinases. It also inhibits a number of other kinases, including RET, DDR2, VEGFR1, and KIT [61].

A multicenter, phase I/II, open-label study (NCT01752920) enrolled 29 adult patients with unresectable intrahepatic cholangiocarcinoma with an FGFR2 fusion, who progressed on, were intolerant to, or not eligible for first-line chemotherapy. Derazantinib provided an overall response rate of 20.7% and the DCR was 82.8% with a median PFS of 5.7 months [62].

Reversible ATP-competitive FGFR inhibitors	Derazantinib [ARQ 087] Infigratinib [Truseltiq] Erdafitinib [JNJ-42756493] Pemigatinib [Pemazyre]
Irreversible non-ATP-competitive FGFR inhibitors	Futibatinib [TAS-120]

Table 2.
Classification of FGFR inhibitors.

3.3.1.2 Infigratinib

Infigratinib (BGJ398, Novartis AG) is also an oral drug. It is an ATP-competitive pan-FGFR inhibitor. It was tested in a phase II trial in patients with distinct FGFR alterations [FGFR2 fusions (n = 48), FGFR2 mutations (n = 8), FGFR2 amplification (n = 3)] post first-line chemotherapy. The overall response rate was 14.8%, almost all with FGFR2 fusions, and median PFS was 5.8 months, and interestingly, DCR was 75.4%; however, the durability of response was limited [63, 64].

3.3.1.3 Erdafitinib

Erdafitinib (JNJ-42756493, Jansenn) is a pan-FGFR inhibitor. It is currently being tested in clinical trials. In a phase I study, in patients with distinct high grade solid tumors, Erdafitinib was tried. It showed a positive response, with an ORR of 27.3% (3/11). Patients who responded positively to this drug carried FGFR fusions or mutations. The median response duration was 11.4 months for cholangiocarcinoma [65].

3.3.1.4 Pemigatinib

Pemigatinib (Pemazyre) is also an oral drug. It is a selective inhibitor of FGFR1–3. In FIGHT-202, 146 admitted patients were chosen according to FGFR2 fusions or rearrangements/alterations/no FGF/FGFR alterations. The primary checkpoint was decided to be ORR among patients with FGFR2 rearrangements or fusions. The median duration of response was 7.5 months [66]. In accordance with these studies, the US FDA approved pemigatinib as the first targeted drug for patients with high grade/advanced refractory disease with an FGFR2 rearrangement or fusion in April 2020.

3.3.1.5 Futinatinib

It is an oral, highly selective, irreversible FGFR1–4 [67] inhibitor which showed promising results in phase I trial (FOENIX-101; NCT02052778): among patients treated with futinatinib, 5.8 achieved partial response and 46% achieved stable disease. Responses were rapid (mostly occurring within 3 months) and lasted for >12 months in two of the five responders, indicating durable clinical benefit (**Table 3**) [68].

3.3.2 Inhibitors of IDH1/2 mutant

Normally, IDH performs the catalyzation of decarboxylation of isocitrate to form α-ketoglutarate. 2-hydroxyglutarate, an oncometabolite, is formed by mutated IDH. The accumulation of 2-hydroxyglutarate leads to epigenetic changes, impaired DNA repair, and aberrant cell metabolism, promoting carcinogenesis [69].

Ivosidenib (AG-120) is an oral drug. It is a targeted, small-molecule IDH1-mutant inhibitor. Incidence of acquired ivosidenib resistance have been reported recently. In the ClarIDHy trial, patients with advanced/high grade, not resectable IDH1-mutant disease, who underwent therapies previously, were randomized to ivosidenib versus placebo. The ORR was found to be 2.4% and the DCR to be 50.8%. Median progression-free survival was longer with this drug (2.7 months) versus placebo (1.4 months; HR 0.37 [95% CI 0.25–0.54]; $p < 0.0001$). Median OS was 10.3 months for ivosidenib versus 7.5 months for placebo ($p = 0.093$), which included 70% of patients who

Adverse effects of FGFR inhibitors	
Electrolyte abnormality	Hyperphosphatemia (55–81%)
Ophthalmologic	Retinal pigment epithelial detachment (RPED) (4%) Central serous retinopathy (CSR) (9%) Blepharitis Cataract development Increased lacrimation Trichiasis Trichomegaly Blurred vision
Nail toxicities	Onycholysis (5–7%) Paronychia (5–7%) Nail discoloration Nail disorder Nail dystrophy Nail hypertrophy Nail infection Onychalgia

Table 3.
Adverse effects of FGFR Inhibitors.

crossed over from placebo [70]. The most common side effects were nausea, followed by diarrhea and fatigue.

3.3.3 Agents targeting HER family (ERBB2) receptors

In a clinical study, 11 patients with formerly treated biliary cancer with HER2 amplification or mutations were subjected to trastuzumab and pertuzumab combination. ORRs of 7.5 and 33.3% were reported, respectively [71].

3.3.4 MAPK (mitogen-activated protein kinases) pathway inhibitors

Mutations of BRAF (1–3%) are seen in intrahepatic disease. BRAF inhibitors mostly target V600E. The inhibition of MEK could be an alternative strategy to target MAPK [72].

The RAS-ERK pathway is very effectively targeted by dual inhibition of BRAF and MEK. In two independent reports, the combination of Dabrafenib and Trametinib showed durable clinical responses [73, 74].

3.3.5 Tyrosine receptor kinase (TRK) inhibitors

There are three tyrosine kinase receptors, TRKA, B, and C, respectively encoded by the genes NTRK1, 2, and 3. Neurotrophin ligand binding and TRK activation result in activation of the receptor, activation of downstream signaling via the MAPK, PI3K, and/or PKC pathways, involved in cell cycle progression, cell proliferation, and cell survival [75].

The NTRK inhibitors larotrectinib (VITRAKVI; Loxo Oncology Inc., San Francisco, CA, USA) and entrectinib (Rozlytrek; Hoffmann-La Roche, Basel, Switzerland) are consistent with good response rates and long-lasting responses in early trials of NTRK-fusion-positive high grade solid tumors. The US FDA has

approved both the drugs larotrectinib and entrectinib for patients with NTRK-fusion-positive solid tumors who have no different therapy or progressed post treatment. In addition, in NTRK-fusion-positive biliary tract cancer, NTRK inhibitors are recommended as first or second-line treatment according to guidelines issued by NCCN.

3.4 Immunotherapy for metastatic biliary tract cancer

Immune checkpoint inhibitors consist of acting on programmed death 1 (PD-1), PD-L1, and cytotoxic T-lymphocyte antigen-4 (CTLA-4). It also includes newer novel treatment approaches like adoptive cell therapy (ACT) and cancer vaccines.

3.4.1 Immune checkpoint inhibitors

Most of the drugs in this category are under trial phase. They act on PD-1, PD-L1, and CTLA-4, and promote tumor destruction by T cells (**Table 4**).

Expression of PD-L1 has been found in 10–70% of patients with biliary tract cancer. Patients with a high expression of PD-L1 have a poor prognosis but can better respond to immune checkpoint inhibitors [76].

PD-L1 positive tumors are linked with: microsatellite instability, increased tumor mutational burden, and expression of biomarkers, e.g., BRCA2, TP53, BRAF, RNF43, TOP2A mutations [77].

Pembrolizumab is a humanized, highly specific, monoclonal antibody against PD-1. It blocks the interaction between PD-1 and its ligands, PD-L1 and L2. FDA approved it for DNA mismatch repair (MMR) deficiency and/or MSI-H, high grade solid tumors, including cholangiocarcinomas. Of note, MMR deficiency has been reported to occur in 5–10% of biliary tract cancers [78].

Nivolumab is also a monoclonal antibody. It blocks the interaction of PD-1 with PD-L1 and L2. It has been evaluated in a phase II trial, single-arm [79]. ORR was found to be 22% and the median OS was 14.24 months (95% CI: 5.98 months to not reached). All patients with a good response had MSS tumors.

Results of monotherapy were found to be unsatisfactory, so there was a need of combination therapy. The combination of checkpoint inhibitors with different checkpoint inhibitors, multi-target tyrosine kinase inhibitors (TKI), anti-angiogenic therapies, poly ADP-ribose polymerase inhibitors, and chemotherapy is being tried recently.

3.4.2 Adoptive immunotherapy

Chimeric antigen receptor (CAR) T cells [80, 81] provide T cells genetically modified to express CAR or tumor antigen-specific T cell receptors (TCR) in order to enhance their ability to specifically recognize and kill cancer cells.

Anti-CTLA-4	Ipilimumab Tremelimumab
Anti-PD-1	Pembrolizumab Nivolumab
Anti-PD-L1	Durvalumab

Table 4.
Major immune checkpoint inhibitors under investigation.

Over 50% of biliary tracts express CD133. Feng et al. [82] have tested in a patient with metastatic BTC CAR-T targeting CD133 and EGFR, with a PR of 8.5 months with CAR-T EGFR therapy and a partial response of 4.5 months with CAR-T 133 treatment.

Moreover, several studies have suggested that the addition of PD-1/PD-L1 blockade could improve the anti-tumor efficacy of CAR T cells in solid tumors but is under trial [83].

3.4.3 Tumor vaccines

Tumor vaccines are a newer approach to induce an immune response to specific oncogenes. Single peptide-based vaccines are shown to develop an immune response to Wilms Tumor 1 (WT1) and Mucin 1 (MUC1), which are expressed cell surface molecules in cholangiocarcinoma.

Multiple peptide-based vaccines improved the efficacy in patients with biliary tract cancers [84]. Several studies (NCT04853017, NCT03942328) are ongoing to study responses with tumor vaccines. Its role in the treatment of cholangiocarcinoma is under investigation. Immunotherapy alone has demonstrated unsatisfactory results, so there is a need of a combination of new therapeutic strategies to enhance efficiency.

4. Conclusion

The treatment of cholangiocarcinomas is a combination of surgery with chemotherapy\targeted therapies\immunotherapy. The overall survival has increased over the years with developing drugs and trials. Surgery is always intended with R0 resection. Newer drugs like FGFR inhibitors, a combination of anti-PD1/PD-L1 with CTLA4 inhibitors, might show promising results in the future. Larotrectinib and entrectinib are newer drugs approved by the FDA for patients with NTRK-fusion-positive solid tumors who have no different therapy or progressed post treatment. Surgical options include extensive liver resection, keeping in mind the FLR. Newer techniques include ALPPS, which ensures tumor removal with preservation of adequate FLR. Nevertheless, incidentally detected tumors have better survival rates than symptomatic cancers. Tumor vaccines are newer approaches under trial, aiming at improving the immunological response to tumor cells.

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Chapter 2

Cholangiocarcinoma – A Comprehensive Review of Diagnostic Challenges and Novel Therapeutic Approaches

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Abstract

Cholangiocarcinoma, also known as bile duct cancer, represents a rare yet aggressive form of cancer. It is a cancer of glandular cells lining the biliary tree that runs from the liver to the gallbladder, pancreas, and small intestine. As a result, cholangiocarcinoma encompasses a spectrum of invasive cancers, with adenocarcinoma as the most prevalent. Anatomically, cholangiocarcinoma can be divided into two main subtypes, intrahepatic or extrahepatic. According to the American Cancer Society, around 8000 patients are diagnosed each year in the United States; however, it is likely to be higher due to difficulty in diagnosing and to misclassification. Currently, surgery is the optimal approach; however, the disease is usually metastasized beyond the bile ducts by the time of diagnosis. Other treatment options include chemotherapy and radiotherapy. There have been novel developments in targeted therapy, immunotherapy, and molecular profiling to tackle this aggressive cancer through prevention and minimally invasive treatment. In this chapter, we present a complete overview of cholangiocarcinoma, including its classification, epidemiology, risk factors, diagnosis, and treatment.

Keywords: cholangiocarcinoma (CCA), biliary tract cancer (BTC), intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), chemotherapy, targeted therapy, immunotherapy, molecular profiling

1. Introduction

The biliary tract is a network of thin ducts that run within the liver (intrahepatic bile ducts) and outside it (extrahepatic bile ducts). These ducts are lined by glandular epithelium and their main function is to transport bile to the intestine.

Cholangiocarcinoma (CCA), also known as bile duct cancer, encompasses all tumors originating in the epithelium of the bile duct. It is characterized by late presentation, limited treatment options, and generally poor prognosis. There are

numerous different types of bile duct cancer, the most common type being adenocarcinoma which accounts for more than 90% of cases [1].

Originally, biliary tract cancers (BTC) were classified based on their anatomical position as cancers of the gallbladder, the extrahepatic ducts, and the ampulla of Vater, while intrahepatic biliary cancers were considered part of primary hepatic cancers. Recently, cholangiocarcinoma now refers to bile duct cancers originating within and outside the liver, separating them from gallbladder, ampulla of Vater, and hepatic cancers [2, 3].

2. Classification

According to the National Comprehensive Cancer Network (NCCN) guidelines, CCAs are classified according to their anatomic location into either intrahepatic cholangiocarcinoma (iCCA) or extrahepatic CCA (eCCA) (**Figure 1**) [4].

2.1 Extrahepatic CCA ~ 90%

Extrahepatic bile duct cancer starts in the extension of the bile duct network outside the liver, from the junction of the right and left hepatic ducts to the common bile duct, connecting to different target organs. They are much more common than intrahepatic CCAs. Extrahepatic CCAs are further subdivided into two subtypes: perihilar and distal CCAs.

Perihilar CCAs occur at or near the junction of the right and left hepatic ducts, and account for approximately 50% of CCAs, making it the most common [5]. They have been further classified based on the pattern of involvement of the hepatic ducts, using the Bismuth-Corlette classification (**Figure 2**). Of note, tumors that involve the common hepatic duct bifurcation are also referred to as Klatskin tumors (hilar CCA) [6].

On the other hand, lesions arising in the extrahepatic bile duct above the ampulla of Vater and below the confluence of the left and right bile ducts are referred to as

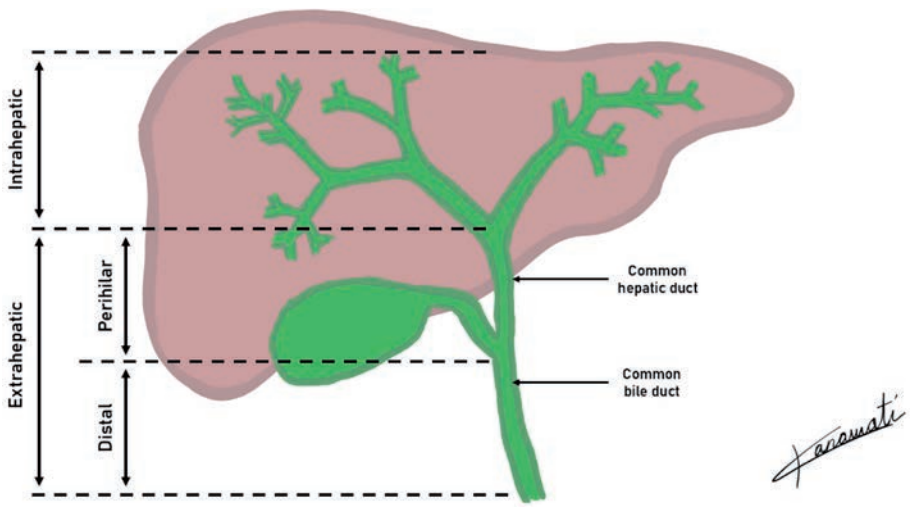

Figure 1.
Classification of cholangiocarcinoma.

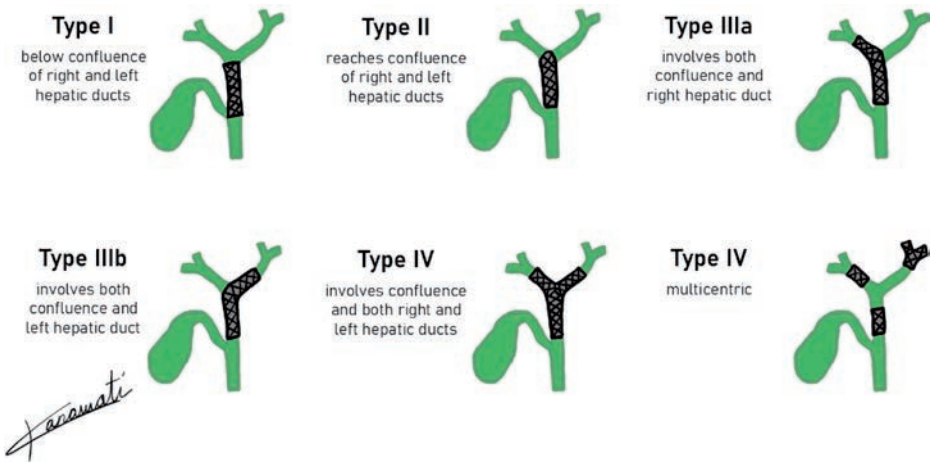

Figure 2.
The Bismuth-Corlette classification of perihilar cholangiocarcinoma.

distal CCAs [6]. They are slightly less common than perihilar CCAs, having a prevalence of 40% [5].

2.2 Intrahepatic CCA ~ 10%

Intrahepatic CCAs are located within the hepatic parenchyma, originating from small intrahepatic ductules, also known as peripheral CCAs, or large intrahepatic ducts proximal to the bifurcation of the right and left hepatic ducts. This subtype tends to be misclassified with other hepatic cancers, proving difficult to diagnose. Interestingly, it is the second most common intrahepatic malignancy after hepatocellular carcinoma (HCC) [7]. Conversely, they are far less common when compared to extrahepatic CCAs, accounting for only 10% of all bile duct cancers [5].

3. Epidemiology

Cholangiocarcinoma is considerably rare, accounting for only 3% of all gastrointestinal malignancies [8]. Nevertheless, the burden of gallbladder and other biliary tract cancers has been rising over the last 30 years around the world [9].

In the United States, approximately 42,000 cases of primary liver and intrahepatic bile duct cancers are diagnosed annually, with 15% of these being iCCA [10–12]. While the incidence of extrahepatic BTCs was approximately 12,600 cases, with one third of them being eCCA [10]. Furthermore, the American Cancer Society estimates around 8000 patients are diagnosed with CCA each year; however, the numbers tend to be underestimated due to difficulty in diagnosing and classifying [13]. It is also worth mentioning that in the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) program, data has been showing that the incidence of intrahepatic CCA is increasing dramatically, compared to a slower rate of increase in extrahepatic CCA. This could be due to advances in imaging, molecular diagnostics, and pathology that can accurately diagnose intrahepatic CCA [14, 15].

Internationally, incidence varies between CCA subtypes and across countries; however, there are regions which have been identified as endemic. When it comes to

high-income regions, the incidence can reach as low as 0.35 to 2 per 100,000 annually [11]. But with the case of endemic regions, such as China and Thailand, the incidence rises by up to 40 times which could be attributed to a higher incidence of parasitic infections that exacerbate the risk of cholangiocarcinoma [16].

4. Risk factors

There are many risk factors that have been recognized to be important in the development of CCA. Nevertheless, in most cases, not a single etiological factor could be identified [17]. According to the NCCN, these risk factors, like those for gallbladder cancer, are associated mainly with the presence of chronic inflammation [4].

Risk factors tend to have varying incidence depending on the geographic location, presumably due to differences in environmental and genetic factors. A systematic review and meta-analysis reported the strongest risk factors for both intrahepatic and extrahepatic CCA were biliary cysts and stones, liver cirrhosis, hepatitis B and hepatitis C. In the West, primary sclerosing cholangitis (PSC) is the most known risk factor. While in the East, especially in certain regions of Thailand for example, chronic infection with liver fluke is the driving risk factor. Other risk factors, such as diabetes, although less strong, are increasing globally and may be contributing [18].

Finally, since most CCA cases cannot be explained by the currently identifiable risk factors, it means there is a significant genetic component. This will require much more research in this field to identify such factors via whole genome sequencing [19].

5. Diagnosis

Cholangiocarcinoma is difficult to diagnose due to its location, presenting features, and lack of absolute diagnostic criteria. Majority of patients remain asymptomatic, developing symptoms only at an advanced stage of the disease. Diagnosis of such condition requires a high level of clinical suspicion as well as a constellation of clinical, laboratory, radiologic, and endoscopic data to confirm [20].

5.1 Clinical features

As mentioned earlier, most CCA patients do not present with any sign or symptom. In extrahepatic CCAs, symptoms usually become evident when the tumor obstructs the biliary drainage system which leads to jaundice, pruritus, clay-colored stools, and dark urine. Other symptoms include right upper quadrant (RUQ) abdominal pain, weight loss, and fever [20–22]. On physical examination, it is possible to palpate an enlarged gallbladder (Courvoisier sign) but is not specific to malignancies [23].

In contrast, patients with intrahepatic CCA are more likely to present with nonspecific symptoms such as fever, weight loss, and/or abdominal pain. Symptoms of biliary obstruction are uncommon because they do not necessarily involve the common hepatic or bile duct. This leads to a much more delayed diagnosis, and in some cases intrahepatic CCA may be detected incidentally as an isolated intrahepatic mass on imaging [24, 25].

5.2 Laboratory studies

Any patient presenting with jaundice or RUQ abdominal pain should include in their initial workup liver function tests, which entail aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). In eCCA, biliary stasis leads to elevated total and direct bilirubin, ALP, and GGT. However, ALT and AST are usually normal, unless in cases of chronic obstruction where there is hepatocellular damage as well. Alternatively, patients with iCCA usually have increased ALP, whereas serum bilirubin levels are normal or only slightly elevated [22].

Furthermore, other tests such as viral hepatitis serologies can be considered in the case of intrahepatic CCA. If hepatitis is diagnosed, it should be monitored and treated following the American Society of Clinical Oncology (ASCO) guidelines [26]. Some patients may also have elevated calcium levels associated with low parathyroid hormone and vitamin D levels due to malignancy [27].

As for tumor biomarkers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) testing can be considered. Although such markers can be elevated in CCA, they possess low specificity due to the possibility of other differentials or being falsely high [28, 29]. Alpha-fetoprotein (AFP) is another helpful marker as it can differentiate hepatocellular carcinomas from iCCA [30]. Keeping in mind that there are some cases of mixed HCC and iCCA in which AFP may be elevated. In such cases, imaging with the help of diagnostic criteria like Liver Imaging Reporting and Data System (LI-RADS) can help distinguish between the two entities [31].

5.3 Imaging studies

When suspecting CCA in a patient with jaundice, the first imaging of choice is a transabdominal ultrasound. This safe modality can detect biliary ductal dilatation and the possible site of obstruction and exclude other differentials such as gallstones. However, it has its own limitations if there is gas in any overlying bowel [32].

Further diagnostic imaging modalities include computed tomography (CT) scan or magnetic resonance imaging (MRI). They can detect mass lesions, if large enough, both within and outside the liver. Even if the lesion is small enough to be undetectable, a ductal dilation of more than 6 mm in the absence of stone can be suspicious for eCCA. Similarly, a focal area of bile duct dilation distal to an intrahepatic mass can be highly suggestive of iCCA. Another feature that differentiates iCCA from HCC on contrast-enhanced MRI is the presence of rim enhancement with a peripheral washout sign. Of note, a CT of the chest and abdomen with or without whole body positron emission tomography (PET) can be utilized for staging once the diagnosis has been established [32–35].

Finally, other studies include magnetic resonance cholangiopancreatography (MRCP) for better visualization of bile ducts and biliary trees, an endoscopic retrograde cholangiopancreatography (ERCP) for bile duct visualization, brush cytology/biopsy, and stent placement if needed. Not to mention, endoscopic ultrasound (EUS) is occasionally used to evaluate distal bile duct or ampullary tumors [22].

5.4 Pathological studies

The final step of the workup is taking a biopsy of the mass to obtain a histopathological confirmation and determine the next steps for initiating therapy [36]. The

method through which the tissue is sampled depends on the location of the lesion. For eCCA, it can be done through either ERCP or EUS with bile duct brushing or a core-needle biopsy, the latter being more preferred [33]. For iCCA, a percutaneous transhepatic biopsy can be done, although some centers defer from it and opt for complete excision if the disease is resectable [34, 35].

After confirmation, staging of the disease needs to be done. This is possible through laparoscopy, abdominal CT, or a PET-CT [30].

6. Treatment

Once a complete evaluation and staging is performed, treatment for patients is initiated. It requires a multidisciplinary team approach, especially those experienced in hepatobiliary malignancies [33].

6.1 Surgery

Complete resection of the tumor is currently the best available potentially curative treatment [4]. Nevertheless, patients who are eligible for such procedure are merely 10%, as contraindications such as locally advanced disease, distant metastasis, and decompensated cirrhosis are evident in majority of cases [34, 35, 37]. If a patient with distal eCCA is eligible, a pancreaticoduodenectomy can be performed [34]. While for patients with intrahepatic or perihilar CCA, a radical hepatic resection with regional lymphadenectomy approach is warranted, as there is a high risk of recurrence [33, 36, 37]. Other treatment options for cholangiocarcinoma include liver transplantation for select patients, and biliary decompression through stenting for palliation [33, 37].

6.2 Chemotherapy

After surgical resection of the tumor, if possible, adjuvant chemotherapy is important. The current recommendation is a complete course of capecitabine for 6 months. If it is unresectable, palliative chemotherapy is the next option, with a first-line regimen of gemcitabine, cisplatin, and durvalumab [34, 38]. Alternative second-line regimens include FOLFOX (folinic acid, fluorouracil, and oxaliplatin) or molecularly targeted therapy, such as IDH1 inhibitors *(discussed below)* [34, 37, 39].

6.3 Radiotherapy

Radiotherapy (RT) can also play a role in treatment. It can be done as adjuvant if there are positive lymph nodes or if the resection margins are not clear [38]. In advanced cases with poor prognosis, palliative RT can be offered to patients [34].

6.4 Targeted therapy

While rare, BTCs are known to harbor a disparity of clinically relevant mutations. ICCAs have been proven to have higher rates of isocitrate dehydrogenase 1(IDH1), BRCA1-associated protein 1 (BAP1), and polybromo 1(PBRM1) gene mutations. On the other hand, eCCAs have shown higher rates of Kirsten rat sarcoma viral oncogene homolog (KRAS), cyclin-dependent kinase inhibitor 2A (CDKN2A), and breast cancer 1 (BRCA1) gene mutations [40].

With new emerging evidence, a comprehensive molecular profiling is recommended for patients with advanced unresectable or metastatic cancer who are candidates for systemic therapy, such as young age at diagnosis or a strong personal or family history of cancer [4]. An international precision study, called the SAFIR-ABC 10 trial, matched patients into one of seven targeted therapies based on their tumor's genetic profile. Their results are promising as they have been able to shrink some tumors and turn some unresectable cases into operable [41].

Notable examples of targeted therapy drugs include: Ivosidenib for IDH1-mutated CCAs; pemigatinib or futibatinib for fibroblast growth factor receptor 2(FGFR2) fusion-positive tumors; larotrectinib or entrectinib for neurotrophic tyrosine receptor kinase (NTRK) fusion-positive solid tumors; adagrasib for CCAs with KRAS G12C mutation; and trastuzumab plus pertuzumab or tucatinib for human epidermal growth factor receptor 2 (HER2)-positive cancer [4]. In 2024, there was a breakthrough with the new FDA-approved drug zanidatamab, which is a bispecific humanized monoclonal antibody targeting HER2-positive biliary tract cancers [42, 43].

Recently, there have also been other therapeutic approaches that include the use of cold piezoelectric plasma (CPP) which is currently emerging to be a potential treatment option. CPP is a technology that uses piezoelectric materials to generate cold plasma. Vitro studies indicate that this plasma can induce DNA damage and apoptosis in cholangiocarcinoma cells, suggesting a non-invasive and promising antitumor therapy [44].

6.5 Immunotherapy and vaccine therapy

Immunotherapy is a rising novel treatment approach. It uses the patient's own immune system to target and fight cancerous cells. Normally, immune cells can recognize foreign antigens on malignant cells and eliminate these cells early on in their development. However, tumors, such as CCA, may utilize a variety of mechanisms to evade the immune system. By incorporating immunotherapy in biliary tract cancers, it eliminates these mechanisms and enhances the host's immune response [45, 46].

The are two important immune checkpoint inhibitors used in CCA, durvalumab and pembrolizumab. Durvalumab is monoclonal antibody that targets programmed death-ligand 1 (PD-L1) on tumor cells, while pembrolizumab is monoclonal antibody that specifically targets programmed death-1 (PD-1) on T cells. These drugs are highly effective. Therefore, the NCCN now recommends combination therapy with durvalumab plus gemcitabine and cisplatin, as well as combination therapy with pembrolizumab plus gemcitabine and cisplatin, as first-line systemic treatments of unresectable or metastatic BTCs [4].

Cancer vaccines are also gaining traction as they have been showing ability to generate an immune response against cancer cells. The immune system can become sensitized through either whole cell vaccines, antigen specific peptide vaccines, or dendritic cells preloaded with antigen. Two antigens, Wilm's tumor protein 1 (WT1) and mucin protein 1 (MUC1), are found in 80–90% of BTC cases, making them ideal targets. Studies mention that patients undergoing this treatment had positive outcomes [46].

6.6 Prognosis

BTCs are associated with a poor prognosis with patients commonly presenting at an advanced stage [4]. Even with curative resection, the 5-year survival rates for eCCAs and iCCAs were 20–30% and 16–44%, respectively [47, 48].

7. Conclusion

Cholangiocarcinoma remains a challenging and an aggressive form of biliary ductal malignancy with an overall poor prognosis due to its complex anatomical variations and subtle clinical manifestations which contribute to its late presentation. For non-resectable cases, the five-year survival rate is 0% and less than 5% in overall [49–51]. The mean duration of survival is less than 6 months in people with metastatic disease. Surgical resection remains the only potentially curative treatment, with better outcomes with those with intrahepatic disease, however most patients are diagnosed at an advanced stage, which limits the eligibility for surgical intervention [51].

Recent advances in chemotherapy, radiotherapy, targeted tumor therapy, and immunotherapy offer alternative therapeutic options for patients with surgically unresectable or metastatic disease. Molecular profiling is a cornerstone of tumor specific treatment, allowing the identification of actionable mutations and the application of novel targeted agents such as ivosidenib, pemigatinib, and zanidatamab.

More recent advances in immunotherapy and cancer vaccines have demonstrated promising clinical efficacy in enhancing anti-tumor immune responses. Ongoing clinical trials and technological innovations like cold piezoelectric plasma therapy continue to expand the therapeutic options available to the hepatobiliary surgeon and their patients.

A multidisciplinary approach is essential to achieve an optimal clinical outcome and improve quality of life for patients with cholangiocarcinoma.

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Conflict of interest

There are no conflicts of interest to disclose by any of the above-listed authors.

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Chapter 3

Diagnosis, Imaging, and Prognostic Evaluation in Cholangiocarcinoma

Yasemin Keskin

Abstract

Cholangiocarcinoma (CCA) is a rare but aggressive malignancy originating from the biliary epithelium, with distinct subtypes including intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) cholangiocarcinomas. Although it accounts for only a small proportion of gastrointestinal cancers, its incidence—particularly of iCCA—has been steadily increasing worldwide. Most patients present at advanced stages due to the lack of early symptoms, and prognosis remains poor despite recent advances in diagnostic and therapeutic approaches. This chapter provides a comprehensive overview of CCA, covering epidemiology, risk factors, pathogenesis, and histopathological classification. Major etiological factors such as primary sclerosing cholangitis, hepatolithiasis, biliary cysts, liver fluke infections, chronic liver diseases, and genetic predispositions are discussed. The pathophysiology is linked to chronic inflammation and bile stasis, with recurrent mutations identified in IDH1/2, KRAS, TP53, and BAP1. Current diagnostic strategies involve a multimodal approach including laboratory tests, imaging modalities such as ultrasonography, contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), positron emission tomography/CT (PET/CT), and histopathological confirmation. Imaging findings vary according to anatomical subtype and tumor morphology. Endoscopic and percutaneous techniques further aid in diagnosis and staging. Given its clinical heterogeneity, individualized assessment and molecular profiling are essential for optimizing diagnosis, prognostication, and therapeutic planning. This chapter emphasizes the need for early detection and personalized medicine in improving patient outcomes in CCA.

Keywords: cholangiocarcinoma, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, biliary tract malignancy, diagnosis

1. Introduction

Cholangiocarcinoma (CCA) is an aggressive malignancy arising from the biliary epithelium, which can develop at any point along the biliary tract, from the intrahepatic bile ducts to the ampulla of Vater. Based on its anatomical origin, CCA is classified into three subtypes: perihilar CCA (pCCA), intrahepatic CCA (iCCA), and extrahepatic CCA (eCCA) [1]. Among these, pCCA is the most common subtype, accounting for approximately 50–60% of all cases [2]. iCCA represents around 10% of all primary liver cancers and is the second most common primary hepatic malignancy following hepatocellular carcinoma (HCC) [1].

CCA primarily affects individuals in their seventies and shows a slight male predominance, although both sexes can be affected [3]. It accounts for approximately 3% of all gastrointestinal cancers [1].

Due to its aggressive nature and the tendency for late diagnosis, the prognosis of CCA is poor. At initial presentation, approximately 75% of patients have unresectable or metastatic disease [4]. Even in cases where CCA is suspected or confirmed, all patients should undergo evaluation for metastases. Notably, about 30% of patients who are initially thought to have resectable disease based on imaging are found to be unresectable during surgical exploration [5].

Carbohydrate antigen 19-9 (CA 19-9), a tumor marker secreted by the biliary epithelium, is frequently used for assessing disease severity and monitoring during follow-up. Surgical resection remains the only potentially curative treatment option; chemotherapy and radiotherapy are used as adjunctive therapies. Recent studies have increasingly focused on targeting molecular mechanisms to expand therapeutic options and improve clinical outcomes [1].

2. Epidemiology

CCAs, which account for approximately 3% of all gastrointestinal (GI) malignancies, represent the second most common type of primary liver tumors. They constitute about 10–15% of all hepatobiliary system malignancies [5]. pCCA is the most frequent subtype, comprising around 50% of all CCA cases. This is followed by dCCA with 20–30% and iCCA with 10–20% of cases [2].

The incidence of CCA increases with age, and the disease is most diagnosed between the ages of 50 and 70. It shows a slight male predominance, with a male-to-female ratio ranging from 1:1.2 to 1:1.5 [5].

Globally, the incidence of CCA is estimated at approximately 6 cases per 100,000 population [2]. However, this rate varies significantly by geographical region; for example, the incidence in Thailand has been reported to be up to 50 times higher than in the United States [4].

The subtypes of CCA differ not only in terms of their risk factors, clinical presentation, treatment approaches, and prognosis but also in their epidemiological patterns. Age-standardized incidence rates indicate a continuous rise in iCCA worldwide, whereas a decline is observed in eCCA [6]. Over the past decades, the incidence of CCA has increased in many countries, with iCCA showing an approximate 350% increase, while the incidence of eCCA has risen by about 20% [7].

When interpreting these epidemiological trends, several confounding factors must be considered, including improved diagnostic capabilities, increased clinical awareness, challenges in distinguishing CCA from HCC, and the presence of combined HCC–iCCA cases.

3. Etiology

CCA frequently develops in the absence of any clearly defined etiological factor or genetic predisposition. However, certain risk factors, which may vary among individuals, have been associated with increased susceptibility to CCA development [1]. Although the incidence of CCA exhibits notable geographical variation, these differences are largely attributed to region-specific exposure to various risk factors.

Recognized risk factors for CCA include primary sclerosing cholangitis (PSC), biliary tract cysts, parasitic infections, and hepatolithiasis. Less commonly, inflammatory bowel disease, chronic liver diseases, obesity, diabetes mellitus, alcohol consumption, smoking, and certain genetic conditions have also been implicated [2].

The majority of known etiological factors are associated with chronic biliary tract inflammation and bile stasis. Importantly, the global increase in the prevalence of these risk factors in recent years may contribute to the observed rise in CCA incidence [3].

3.1 Primary sclerosing cholangitis (PSC)

PSC is an autoimmune disease characterized by inflammation and subsequent obstruction of both intrahepatic and extrahepatic bile ducts. The risk of developing CCA in individuals with PSC is approximately 400 times higher than in the general population, with an estimated CCA incidence of 7% among PSC patients [1]. In this patient group, CCA typically arises during the fourth decade of life, and in approximately 50% of cases, the diagnosis of CCA is made within the first year following the diagnosis of PSC [8].

3.2 Cystic biliary tract diseases

Biliary tract cysts are congenital anomalies characterized by cystic dilatation of the intrahepatic and/or extrahepatic bile ducts. These cysts are more frequently observed in women from Asian countries, whereas their prevalence is lower in Western populations. In individuals with known biliary tract cysts, CCA typically develops at a mean age of 32 years [1].

Although the association between biliary tract cysts and CCA is not yet fully established, it is well recognized that in the absence of appropriate treatment, CCA may arise either from the cyst epithelium or from the dilated bile ducts. Patients with choledochal cysts, biliary mucinous cystic neoplasms (B-MCN), and intraductal papillary mucinous neoplasms of the bile duct are at significantly increased risk for developing CCA. Factors such as reflux of pancreatic enzymes into the bile ducts, bile stasis, and elevated bile acid concentrations are believed to contribute to malignant transformation of the biliary epithelium.

Biliary tract cysts are classified into five main types: type I, II, III, IV, and V (**Table 1**). Patients with choledochal cysts carry a 10- to 50-fold increased risk of developing CCA [5]. Among these, type I and type IV cysts are most strongly associated with malignancy risk, though the risk is elevated in all types [4]. In Caroli disease, iCCA and eCCA risks are increased by approximately 38-fold and 97-fold, respectively, due to chronic inflammation and bile stasis [9].

B-MCNs are located intrahepatically and are more frequently observed in women. These lesions are generally asymptomatic and may present with nonspecific findings secondary to liver capsule distension or mass effect.

Intraductal papillary mucinous neoplasms of the bile duct, which share similar characteristics with intraductal papillary mucinous neoplasms of the pancreas, are most located in the extrahepatic bile ducts, accounting for approximately 58% of cases [10]. The incidence of these neoplasms limited to the intrahepatic bile ducts is around 9%, while 33% of cases involve both intrahepatic and extrahepatic bile ducts [10]. A distinguishing feature of these tumors is their ability to secrete mucus. The five-year survival rate is markedly lower in patients with mucus-secreting tumors (19%) compared to those with non-mucinous tumors (52%) [10].

Type	Description
Type 1	The most common type (%80–90)
	Saccular or fusiform dilatation of the main bile duct without involvement of the intrahepatic bile ducts
Type 2	An isolated diverticulum arising from the main bile duct
Type 3	Choledochocele
	A cystic dilatation extending into the duodenum from the junction of the common bile duct and the pancreatic duct
Type 4a	Multiple dilatations involving the intrahepatic and/or extrahepatic bile ducts
Type 4b	Multiple dilatations confined to the extrahepatic bile ducts
Type 5	Multiple dilatations of the intrahepatic bile ducts without involvement of the extrahepatic biliary tree
	Caroli disease

Table 1.
Classification of biliary tract cysts [1].

3.3 Parasitic infections

Liver flukes strongly associated with the development of CCA include *Clonorchis* and *Opisthorchis* species. These parasitic infections are predominantly seen in the Southeast Asia region. The highest incidence rates of these parasites have been reported in northeastern Thailand [4]. These trematodes are transmitted through the consumption of undercooked or raw fish products, leading to chronic inflammation of the bile ducts and malignant transformation [11]. A study conducted in an area endemic for *Clonorchis sinensis* showed a CCA risk of 27.9% in men and 16% in women [12]. CCA associated with parasitic infection can develop at any site within the biliary tract and may occur in any anatomical subtype.

3.4 Bile duct stones

Although the exact malignant transformation pathway of CCA is not fully understood, it is hypothesized that CCA may develop secondary to chronic inflammation caused by bile duct stones [13]. Additionally, approximately 35% of patients with cholelithiasis experience subsequent episodes of cholecystitis or cholangitis over time, which may contribute to carcinogenesis [14].

Hepatolithiasis, defined as the presence of stones in the intrahepatic bile ducts, is particularly common in East Asia, with a prevalence of around 25% [15]. In this region, hepatolithiasis is often associated with parasitic infections [13]. The link between hepatolithiasis and iCCA is thought to be mediated by chronic inflammation, bile stasis, and bacterial infections, with an estimated incidence ranging between 5% and 13% [15].

Cholecystolithiasis and choledocholithiasis are more commonly associated with eCCA, and the risk increases with larger stone size, the presence of calcification, and longer disease duration.

3.5 Chronic liver disease

Liver cirrhosis, characterized by fibrosis of the hepatic parenchyma and the formation of regenerative nodules, is a well-established risk factor for HCC. However,

several studies have also demonstrated that cirrhosis is a significant risk factor for the development of both iCCA and eCCA [16, 17].

Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is also strongly associated with an increased risk of HCC, similar to cirrhosis. In addition to this known association, studies have shown that HBV and HCV infections are also important risk factors for iCCA in particular [5].

3.6 Lifestyle, environmental, and metabolic factors

There is evidence suggesting that exposure to toxins such as Thorotrast, inflammatory bowel disease, diabetes, obesity, excessive alcohol consumption, and cigarette smoking are risk factors for the development of CCA [5].

3.7 Genetic predisposition

The risk of developing CCA is increased in individuals with certain hereditary conditions, including Lynch syndrome (hereditary nonpolyposis colorectal cancer), biliary papillomatosis, BAP1 tumor predisposition syndrome, and cystic fibrosis [4].

4. Pathophysiology

CCA, whether or not preceded by premalignant lesions, typically arises in the setting of chronic inflammation. The molecular pathogenesis of CCA involves various intracellular signaling pathways, and some of the associated mutations share similarities with those seen in HCC [5]. Mutations in several proto-oncogenes and tumor suppressor genes play a role in the carcinogenic process. The most frequently identified mutations include RAS, BRAF, TP53, and SMAD4 [4].

In iCCA, KRAS mutations are particularly common [18]. Additionally, approximately 20% of patients with iCCA harbor mutations in the TP53 gene [5]. Mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 are found in about 10–23% of iCCA cases [19].

The development of pCCA often occurs spontaneously and is associated with mutations in KRAS, C-myc, p53, and Bcl-2. KRAS mutations are present in approximately 60% of pCCA patients, particularly among those with tumors larger than 3 cm and lymph node metastases—features indicative of poor prognosis [5].

Furthermore, in CCA cases related to parasitic infections, TP53 and ARID1A mutations are frequently observed, whereas BAP1, IDH1, and IDH2 mutations are more commonly seen in non-parasite-associated CCA [20].

5. Tumor location and morphology

The classification of iCCA, pCCA, and dCCA is based on anatomical localization. pCCA arises between the confluence of the right and left intrahepatic bile ducts and the cystic duct insertion; iCCA originates proximal to this confluence, while eCCA, particularly dCCA, arises between the common bile duct and the ampulla of Vater.

iCCA exhibits three distinct morphological growth patterns: mass-forming, periductal infiltrating, and intraductal growing. The mass-forming subtype is characterized by a firm, non-encapsulated, well-demarcated mass, often with satellite nodules,

and accounts for approximately 60% of all iCCAs [2]. Radiologically, these lesions appear as large, irregularly contoured but well-defined masses that may demonstrate peripheral contrast enhancement. This subtype tends to behave aggressively, with early invasion of portal vein branches and a propensity for intrahepatic metastases.

The periductal infiltrating type grows along the bile ducts without forming a distinct mass. These tumors typically follow a branching pattern along the biliary tree and can cause biliary obstruction due to their longitudinal extension. They are associated with poor prognosis and early lymphatic spread.

The intraductal growth pattern is characterized by papillary or nodular proliferation within the bile ducts. The tumor may spread superficially along the bile duct mucosa and can be multifocal. On imaging, these tumors present as intraductal masses involving the bile duct wall and if obstructive may cause proximal biliary dilatation. This subtype has the most favorable prognosis among iCCAs and is often diagnosed at an early stage [21].

eCCAs are pathologically classified into three types: sclerosing (70%), nodular (20%), and papillary (5–10%) [5]. The sclerosing and nodular types frequently involve the hepatic hilum. In particular, the sclerosing type leads to bile duct wall thickening and radial or longitudinal tumor extension, which can result in early lymphatic plexus involvement. The papillary type typically occurs in the distal bile ducts and, due to its lower tendency for deep tissue invasion, is associated with a better prognosis following surgical resection.

6. Histopathology

CCA originates from the biliary epithelium and is histologically classified as adenocarcinoma in approximately 90–95% of cases [22]. Rare histological subtypes include adenosquamous carcinoma and clear cell carcinoma [23].

The tumor cells typically exhibit a highly desmoplastic stroma composed of inflammatory cells and extracellular matrix components, forming a dense network known as the tumor immune microenvironment [24].

7. History and physical examination

The clinical presentation of CCA varies depending on the tumor's location and size. In some cases, patients may be asymptomatic, with the disease initially suspected due to incidental abnormalities in liver function tests.

iCCA is often diagnosed incidentally and may present with nonspecific symptoms such as abdominal pain, weight loss, and fatigue. If biliary obstruction develops, jaundice and signs of cholangitis may also occur. Symptoms typically emerge in advanced stages when the tumor has grown significantly, which often results in delayed diagnosis.

eCCA, in contrast, tends to present earlier due to its propensity to cause biliary obstruction. As a result, patients may experience symptoms such as jaundice, pruritus, acholic (pale) stools, and dark urine. On physical examination, patients with eCCA may sometimes exhibit jaundice, hepatomegaly, and a palpable mass in the right upper quadrant [25].

In advanced disease, findings such as a palpable abdominal mass or ascites may be present. Clinical evaluation should include a detailed abdominal examination, as

well as an assessment of the patient's nutritional status and liver function. Although rare, paraneoplastic syndromes such as Sweet's syndrome, erythema multiforme, or porphyria cutanea tarda may be observed in patients with CCA [2].

8. Diagnosis

8.1 Laboratory investigations

All patients should undergo a comprehensive panel of laboratory tests, including complete blood count, a broad biochemical assessment of liver function, and coagulation studies. In addition to these tests, tumor markers such as CA 19-9, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) should be measured. While tumor markers can be helpful in the diagnosis of CCA, they may be misleading in early-stage disease or in the presence of benign conditions.

In patients with eCCA, elevated levels of total bilirubin (typically >10 mg/dL), direct bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) are commonly observed. These elevations may range from 2- to 10-fold above the upper limit of normal [25]. Although aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels may be normal initially, hepatocellular injury secondary to cholestasis can eventually cause elevations in these enzymes as well.

In patients with iCCA, ALP levels are often within normal limits, and bilirubin levels may be normal or only mildly elevated.

CA 19-9, an epitope of the Sialyl-Lewis antigen, has a reported sensitivity ranging from 50 to 90% and specificity from 54 to 98% for the diagnosis of CCA [2]. Elevated CA 19-9 levels at diagnosis are associated with poor prognosis, and levels exceeding 1000 U/mL are indicative of unresectable disease [2]. One limitation of CA 19-9 is that it may also be elevated in cases of cholangitis or benign biliary diseases. Furthermore, its levels can be variably elevated in patients with CCA coexisting with benign inflammatory biliary conditions. In rare cases, individuals lacking the Lewis antigen do not exhibit elevated CA 19-9 levels [26].

CEA is a membrane glycoprotein that may also be elevated in certain benign conditions. Therefore, it lacks sufficient sensitivity and specificity when used alone. However, a CEA level > 5.2 ng/mL has been shown to have a sensitivity of 68% and specificity of 82% for diagnosing CCA [2].

AFP is primarily used to differentiate iCCA from HCC. Among patients diagnosed with iCCA, 19% have AFP levels >20 ng/mL, 10.3% have levels >200 ng/mL, and 6.3% have levels >1000 ng/mL [2].

8.2 Radiological investigations

8.2.1 Abdominal ultrasonography (USG)

Abdominal ultrasonography (USG) is generally preferred as the initial diagnostic modality due to its ease of use, non-invasive nature, and cost-effectiveness.

USG can detect the presence of a hepatic mass, dilatation of intrahepatic and extrahepatic bile ducts, and distension of the gallbladder. In patients without jaundice but presenting with right upper quadrant pain, ultrasonography demonstrates higher sensitivity than computed tomography (CT) for detecting gallstones and/or bile duct

dilatation. However, in cases with a high clinical suspicion of cholangiocarcinoma, cross-sectional imaging techniques are considered more appropriate for further evaluation.

8.2.2 Computed tomography (CT)

In addition to evaluating the hepatic parenchyma, CT is useful for diagnosing both local and metastatic disease. To improve diagnostic accuracy, a multiphase CT protocol—known as triple-phase CT—can be used, which includes imaging during the arterial, portal venous, and delayed phases.

In the peripheral type of iCCA, CT typically reveals an irregularly marginated mass, often associated with capsular retraction (in approximately 20% of cases) and intrahepatic bile duct dilatation. The mass demonstrates thin peripheral enhancement during the arterial and portal phases [2].

In the intraductal type of iCCA, low-density, irregularly shaped masses may be visualized, often accompanied by focal bile duct dilatation surrounding the tumor. These lesions may cause dilatation of the intrahepatic ducts, intraductal polypoid masses, or lobar atrophy.

pCCA generally presents as proximal bile duct dilatation. Imaging may suggest separation of the right and left hepatic ducts. A distinct mass is not always visible, and findings may instead reflect a stricture caused by mass effect.

eCCA may manifest as bile duct wall thickening in the extrahepatic tract, a mass in the portal region, or proximal ductal dilatation near the periampullary area. The level of biliary dilatation is often a useful indicator of the site of obstruction.

Dilatation of the bile ducts accompanied by gallbladder distension suggests a periampullary tumor, whereas isolated gallbladder enlargement without bile duct dilatation may indicate a stone or mass causing obstruction at the level of the cystic duct.

8.2.3 Magnetic resonance imaging (MRI)

On MRI, CCAs typically appear hypointense on T1-weighted images and hyperintense on T2-weighted images. The presence of central hypointensity on T2-weighted images is suggestive of intratumoral fibrosis [2]. Following contrast administration, the lesions demonstrate peripheral enhancement with slow, concentric filling.

When combined with magnetic resonance cholangiopancreatography (MRCP), MRI is among the best non-invasive methods for visualizing the biliary tree [4]. The use of gadolinium-based contrast agents allows for detailed evaluation of intrahepatic masses and their relationship with hepatic vascular structures [2]. MRCP provides information on disease extent and resectability comparable to that obtained with CT, endoscopic retrograde cholangiopancreatography (ERCP), and angiography [2].

8.2.4 Positron emission tomography (PET)

This imaging modality is primarily used for the evaluation of distant metastases and is not routinely recommended for the diagnosis and management of CCA according to the National Comprehensive Cancer Network guidelines [27].

Due to the high glucose uptake by the biliary epithelium in CCAs, imaging with fluorodeoxyglucose (FDG) PET is feasible. While it is capable of detecting even small nodular CCAs, its effectiveness is reduced in infiltrative tumors due to the potential absence of FDG accumulation [2].

8.3 Interventional procedures

8.3.1 Percutaneous transhepatic cholangiography (PTC)

PTC is a diagnostic and therapeutic technique that allows for the detection of biliary abnormalities through the administration of contrast material into the bile ducts. In the presence of obstruction, it enables planning for biliary drainage. It also allows for histopathological confirmation of biliary tract diseases.

In cases where a mass causes intrahepatic bile duct dilatation, percutaneous access to the bile ducts can be achieved under imaging guidance. This facilitates biliary decompression, thereby supporting liver regeneration in the postoperative period and minimizing the risk of hepatic failure [28].

In patients with eCCA, due to the longitudinal extension of the tumor along the bile ducts, residual tumor tissue may persist after surgery. In such cases, mapping biopsies can be performed using PTC.

8.3.2 Endoscopic techniques

ERCP can be used both for tissue diagnosis and for biliary drainage.

Endoscopic ultrasonography (EUS) is particularly useful for visualizing the portal structures and lymph nodes and offers the advantage of higher sensitivity for tissue sampling compared to ERCP [29]. It plays a key role in assessing the local extension of eCCA and regional lymph node involvement. Fine-needle aspiration performed under EUS guidance allows for the acquisition of tissue samples from both the tumor and lymph nodes.

8.4 Differential diagnosis

Due to the nonspecific clinical manifestations of CCA at presentation—such as jaundice, abdominal pain, and fatigue—a wide range of conditions must be considered in the differential diagnosis. Potential differential diagnoses include choledocholithiasis, cholecystitis, cholangitis, primary sclerosing cholangitis, primary biliary cirrhosis, HCC, and pancreatic cancer [4].

8.5 Prognosis

Despite advances in the diagnosis and treatment of CCA, it remains a disease with poor survival outcomes. Studies have shown that the one-year mortality rate is approximately 81.7%, with a median overall survival ranging between 4 and 8 months [7, 30]. The estimated 5-year survival rate is reported to be around 5% [13]. Although surgical resection represents the only potential curative treatment, many patients present at an advanced stage, rendering them ineligible for surgery.

9. Conclusions

Due to the rarity of CCA and its tendency to present at advanced stages, detailed characterization of this malignancy is of critical importance. Given the heterogeneity of these tumors, individual genomic and epigenetic profiling is essential for a clearer understanding of their pathogenesis, which in turn is crucial for improving diagnostic strategies, follow-up protocols, and therapeutic approaches.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

AFP	alpha-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
B-MCN	biliary mucinous cystic neoplasms
CA 19-9	carbohydrate antigen 19-9
CCA	cholangiocarcinoma
CEA	carcinoembryonic antigen
CT	computed tomography
eCCA	extrahepatic cholangiocarcinoma
ERCP	endoscopic retrograde cholangiopancreatography
EUS	endoscopic ultrasonography
FDG	fluorodeoxyglucose
GGT	gamma-glutamyl transferase
GI	gastrointestinal
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
IDH	isocitrate dehydrogenase
iCCA	intrahepatic cholangiocarcinoma
MRCP	magnetic resonance cholangiopancreatography
MRI	magnetic resonance imaging
pCCA	perihilar cholangiocarcinoma
PET	positron emission tomography
PSC	primary sclerosing cholangitis
PTC	percutaneous transhepatic cholangiography
USG	ultrasonography

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Chapter 4

Multiomics Approaches for the Identification of Biomarkers and Therapeutic Targets in Cholangiocarcinoma

Worachart Lert-Itthiporn

Abstract

Cholangiocarcinoma (CCA) is a biologically complex and aggressive malignancy of the bile ducts, with limited diagnostic and therapeutic options. Recent advances in omics technologies, including genomics, transcriptomics, epigenomics, proteomics, and metabolomics, have significantly enhanced our understanding of CCA pathogenesis. These approaches have provided a comprehensive molecular landscape of the disease, thus facilitating the identification of biomarkers and therapeutic targets. Integrative analyses have revealed subtype-specific genetic alterations, such as FGFR2 fusions and IDH1/2 mutations, epigenetic modifications, and metabolic changes associated with disease progression and treatment respon se. Artificial intelligence (AI) and machine learning (ML) have further expanded the potential of multiomics by facilitating data integration, molecular subtyping, prognostic modeling, and early detection strategies. These computational tools support precision oncology by stratifying patients, predicting treatment efficacy, and guiding personalized treatment decisions. The incorporation of multiomics profiling into clinical workflows will improve diagnostic precision, optimize treatment outcomes, and reduce adverse effects. Despite these advances, challenges remain in standardizing analytical pipelines, validating findings across diverse populations, and implementing multiomics approaches in routine clinical settings. Continued interdisciplinary collaboration and technological innovation are essential to fully harness the translational value of AI-driven multiomics to improve outcomes for CCA patients.

Keywords: cholangiocarcinoma, multiomics integration, biomarker discovery, precision oncology, transcriptomics, epigenomics

1. Introduction

Cholangiocarcinoma (CCA) is a malignant tumor arising from epithelial cells of the bile ducts, which are essential for bile transport from the liver to the intestine [1]. Although less common than other gastrointestinal cancers, CCA is a serious disease because of its aggressive progression, diagnostic challenges, and limited treatment

options. It is the second most frequent primary liver cancer following hepatocellular carcinoma, and accounts for 10–15% of all hepatic malignancies and approximately 3% of all gastrointestinal cancers [2].

CCA is anatomically categorized into three main subtypes: intrahepatic (iCCA), perihilar (pCCA), and distal extrahepatic (dCCA). Each subtype exhibits distinct molecular characteristics, etiological factors, and clinical management strategies. The incidence of iCCA has increased in recent decades, particularly in Southeast Asia, where liver fluke infections, such as *Opisthorchis viverrini*, are endemic [1, 2]. In contrast, dCCA and pCCA are collectively referred to as extrahepatic CCA (eCCA) and are more commonly associated with chronic biliary inflammation, primary sclerosing cholangitis, and choledochal cysts. Epidemiological patterns vary, with incidence ranging from 0.3 to 6 per 100,000 individuals and typically affecting those aged 60–70, with a slight male predominance. Prognosis is poor, as most cases are diagnosed at an advanced stage [2]. Curative surgery is usually not feasible, and the 5-year survival is less than 25%, with median survival for advanced disease rarely surpassing 7 months [3].

These challenges underscore the need for improved diagnostic and therapeutic strategies. Advances in multiomics, which include genomics, transcriptomics, epigenomics, proteomics, and metabolomics, offer insights into CCA biology. Integrative omics approaches have facilitated the discovery of biomarkers and molecular targets, paving the way for early detection, patient stratification, and precision medicine. This chapter highlights the role of multiomics in elucidating the pathogenesis of CCA and advancing individualized treatment regimens. The framework of multiomics integration applied in CCA research is illustrated in **Figure 1**, highlighting the complementary contributions of genomics, transcriptomics, epigenomics, proteomics, and metabolomics.

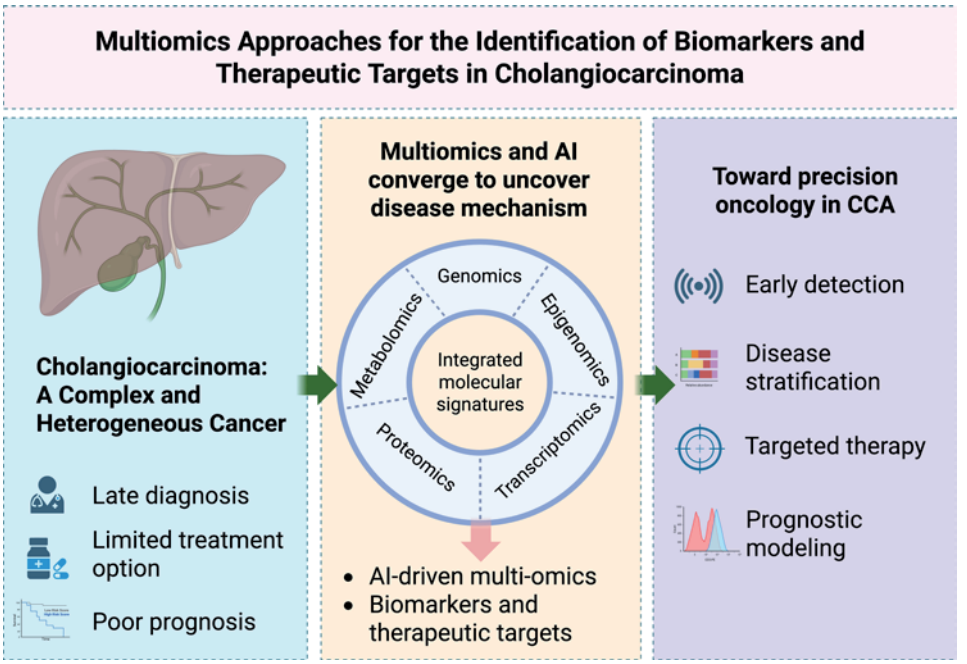

Figure 1.
Schematic representation of an integrated multiomics framework for biomarker and therapeutic target discovery in cholangiocarcinoma (Created with BioRender.com).

2. Need for reliable cholangiocarcinoma biomarkers

CCA is frequently diagnosed at an advanced stage because of the absence of sensitive and specific early detection tools. Current diagnostic approaches, including imaging, clinical assessment, and the assessment of serum tumor markers, such as CA19-9 and CEA, are limited by poor specificity [4]. These markers are frequently increased in benign biliary conditions (e.g., cholangitis, pancreatitis) and other gastrointestinal cancers, which results in diagnostic uncertainty and treatment delays [5].

Tissue biopsy remains the diagnostic gold standard; however, it is limited by the anatomical inaccessibility of tumors and procedural invasiveness. In many cases, sampling is contraindicated because of patient comorbidities and technical limitations. These obstacles underscore the need for minimally invasive, highly specific biomarkers that are capable of detecting early-stage disease, differentiating malignancy from benign conditions, and guiding prognosis and therapeutic decision-making.

Omics-based technologies, including genomics, transcriptomics, proteomics, and metabolomics, have transformed biomarker discovery. These platforms enable the identification of molecular signatures associated with tumor subtype, progression, and treatment response. Such biomarkers hold promise for improving diagnostic precision, personalized therapeutic strategies, and clinical outcomes in CCA.

3. Multiomics approaches to biomarker discovery

The advent of multiomics has transformed biological research by shifting research from single molecules to integrated systems analyses. Genomics, transcriptomics, epigenomics, proteomics, and metabolomics each interrogate distinct molecular layers, collectively reflecting cellular function. Anchored in the central dogma, DNA to RNA to protein, these approaches offer a comprehensive framework for examining disease etiology and therapeutic targets.

Integrated omics combines data across multiple platforms to elucidate complex biological interactions. This holistic perspective provides insight into disease mechanisms and the environmental effects of molecular pathways [6]. Compared with single-omic methods, integrated approaches yield greater insight into biomarker discovery, toxicity prediction, and disease classification, particularly in oncology, in which they refine prognostic models and identify disease-specific molecular profiles [6, 7]. The application of these methods also extends to environmental health and nutrition science, revealing how external factors modulate molecular biology. To illustrate the value of each omics layer and their integration. **Table 1** summarizes the key findings from multiomics studies on CCA and highlights their clinical relevance.

Integrating heterogeneous omics data poses technical and computational challenges. High-dimensional datasets require robust analytical pipelines and validation strategies to ensure reproducibility and interpretability [8]. Despite these challenges, integrated omics hold promise in improving predictive accuracy and personalizing healthcare strategies [9].

3.1 Expanding role of genomics in understanding cholangiocarcinoma

Genomics has significantly advanced our understanding of CCA, a highly aggressive malignancy of the bile ducts; however, therapeutic approaches are limited and are associated with poor outcomes. Genomic techniques, particularly high-throughput

Omics approach	Key findings	Clinical implications
Genomics	Gene mutations and fusions, such as FGFR2 fusions and IDH1/2 mutations	Molecular subtyping for targeted therapy
	ctDNA and liquid biopsy advances	Noninvasive tumor monitoring
Transcriptomics	Gene expression signatures for prognosis	Subtype-specific therapy
	Enhancer dysregulation	Prognostic stratification
Epigenomics	DNA methylation patterns	Identifies therapeutic vulnerabilities (e.g., mTOR, OxPhos inhibitors)
	Epigenetically defined subtypes	
Proteomics	Dysregulated signaling proteins	Biomarker discovery
	EMT and invasion-related markers	Informs about drug resistance and prognosis
Metabolomics	Altered bile acid, energy, and lipid metabolism	Diagnostic biomarker identification
		Predicts recurrence and treatment response
Integrated Multiomics	Harmonized insights from multiple layers	Enhanced precision diagnostics
	Subtype classification and modeling	Personalized treatment and risk prediction

Table 1.
Summary of multiomics insights and clinical implications for cholangiocarcinoma.

sequencing, have revealed key mutations involved in the pathogenesis of CCA, offering new avenues for precision oncology.

Genomic profiling revealed that CCA is molecularly heterogeneous, with distinct mutational landscapes between iCCA and eCCA subtypes and among populations. *KRAS*, *TP53*, *ARID1A*, and *SMAD4* mutations are frequently observed, with *KRAS* mutations present in approximately 44.2% of cases, predominantly in eCCA [10, 11]. In contrast, *IDH1/2* mutations and *FGFR2* fusions occur more frequently in iCCA, reflecting divergent oncogenic pathways [12, 13].

This molecular diversity underscores the need for subtype-specific genomic profiling. Nearly 79.1% of CCA cases harbor potentially actionable targets, enabling personalized therapeutic interventions [14]. Drugs, such as ivosidenib (IDH1 inhibitor) and infigratinib (FGFR2 inhibitor), have shown efficacy in mutation-specific subgroups, which represents a paradigm shift from traditional chemotherapy toward targeted therapy [15]. Beyond treatment selection, genomic data can improve diagnostic accuracy and guide patient stratification. Molecular markers help differentiate between CCA subtypes and predict therapeutic responses, which support biomarker-driven clinical trials [10, 13]. Certain mutations, such as *TP53*, *STK11*, and *ERBB3*, are associated with poor prognosis, thus enabling risk-based management strategies [16, 17]. Emerging alterations, such as *ROS1* rearrangements and *PBX1* mutations, further expand the list of therapeutic and prognostic biomarkers [18]. In autoimmune-related CCA, increased tumor mutational burden may enhance immunotherapy response, highlighting the value of integrating immunogenomic data [19].

Nevertheless, several challenges remain. Tumor heterogeneity across subtypes and regions complicates standardized treatment protocols, highlighting the need for individualized genomic strategies [20]. Although circulating tumor DNA (ctDNA) has potential as a noninvasive monitoring strategy, limitations to its sensitivity and

specificity have prevented its clinical utility [21, 22]. Finally, the immune-genomic interface remains underexplored. Integrating genomic, transcriptomic, and immunologic data will be important for developing multimodal treatment strategies and advancing CCA precision medicine.

3.2 Role of epigenetic modifications in the pathogenesis of cholangiocarcinoma

Epigenetic alterations are considered key contributors to the development of CCA, complementing genetic mutations that drive oncogenesis. These heritable, but reversible, modifications, including DNA methylation and histone modification, alter gene expression. By silencing tumor suppressor genes or activating oncogenes, epigenetic changes facilitate malignant transformation and disease progression. The reversibility of such mutations makes them attractive targets for biomarker discovery and therapeutic intervention.

DNA methylation, which involves the addition of methyl groups to cytosine in CpG islands, plays a central regulatory role in normal physiology. In CCA, abnormal promoter hypermethylation turns off tumor suppressor genes that control the cell cycle, DNA repair, and cell death. Genes, such as *MLH1*, *p14*, *p16*, and *DAPK,* are frequently involved [23]. Epigenetic repression also extends to tumor-suppressive microRNAs (miRNAs), including *miR-370* and *miR-376c*, which further disrupt gene regulation [24, 25]. DNA methylation inhibitors, such as azacitidine and decitabine, show promise for reactivating silenced genes and inducing antitumor immune responses through viral mimicry in other cancers [26]. These agents have a dual mechanism of action by modulating transcription and enhancing immunogenicity.

Histone modifications, including acetylation and methylation, regulate chromatin structure and gene accessibility. Disrupted histone acetylation can result in the repression of tumor suppressor genes, whereas aberrant methylation patterns may activate or silence genes, depending on the context. These alterations are also associated with chromatin remodeling, further attenuating transcriptional dynamics. Histone deacetylase (HDAC) inhibitors are effective at reversing pathological acetylation loss, restoring normal gene expression, and reducing tumor growth in preclinical models of CCA [27]. These drugs are being evaluated as monotherapies or in combination with chemotherapy and immunotherapy. Integrating epigenomic data with genomic and transcriptomic analyses is essential for addressing the full molecular complexity of CCA and developing personalized therapeutic strategies.

3.3 Transcriptomic approaches for biomarker and therapeutic target discovery in cholangiocarcinoma

Transcriptomics, particularly RNA sequencing (RNA-seq), has become a fundamental tool for elucidating the molecular landscape of CCA, a malignancy marked by profound heterogeneity and resistance to therapy. RNA-seq enables high-throughput, unbiased detection of differentially expressed genes (DEGs), splicing variants, and noncoding RNAs (ncRNAs), to provide a comprehensive view of the CCA transcriptome and facilitate biomarker and drug target discovery. Numerous studies have used RNA-seq to identify DEGs in tumor tissues compared with adjacent normal tissues [28, 29]. Transcriptomic profiling was used to classify CCA into immune "hot" and "cold" phenotypes, based on distinct immune and metabolic gene signatures, with implications for immunotherapy design [30].

Beyond coding transcripts, ncRNAs, including miRNAs, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), have emerged as key regulators of gene expression, immune escape, and metastasis. Dysregulated miRNAs that modulate the PI3K/AKT, MAPK, and Wnt/β-catenin pathways are detectable in serum and bile, thus facilitating noninvasive biomarker applications [31, 32]. LncRNAs, such as *H19*, *HOTAIR*, and *MALAT1,* influence chromatin remodeling, epithelial-mesenchymal transition (EMT), and therapeutic resistance, serving as biomarkers and therapeutic targets [33, 34]. Because of their covalently closed-loop structure, circRNAs exhibit remarkable stability and function primarily as miRNA sponges, which indirectly modulate oncogenic signaling [35]. In cholangiocarcinoma, circRNAs may affect key signaling pathways by modulating the availability of miRNAs that regulate genes involved in tumor growth and metastasis [36]. Gene expression signatures for the classification of CCA have been proposed through various studies, each focusing on different aspects of gene expression and their potential value for prognosis and treatment [37]. These studies identified multiple gene signatures that can classify patients with CCA into different risk groups, thereby aiding prognosis and potentially guiding therapeutic decisions.

To further resolve CCA heterogeneity, single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have been used to study CCA [38]. These methods reveal diverse cellular ecosystems, including malignant epithelial cells, immune infiltrates, and stromal components [39]. ScRNA-seq of the tumor's leading edge has revealed stromal–immune interactions that drive tumor progression [39]. Integrated single-cell and bulk RNA-seq analyses have identified telomere-related genes as prognostic markers [40]. Furthermore, fibroblast subtypes have been characterized, including LGALS1-positive fibroblasts that promote tumor proliferation and migration, which represent potential stromal targets [41].

3.4 Proteomics

Proteomic analyses have elucidated the key molecular alterations underlying the pathogenesis of CCA, particularly the metabolic reprogramming and oxidative stress responses. Several studies on iCCA have demonstrated the overexpression of redox-regulating enzymes, including catalase (CAT), superoxide dismutase (SOD), and peroxiredoxin 6 (PRDX6). These enzymes are integral to cellular antioxidant defense, functioning to neutralize reactive oxygen species and thereby mitigate oxidative damage. The upregulation of redox homeostasis suggests that it is critical for tumor survival, proliferation, and adaptation within the hypoxic tumor microenvironment [42]. A study in Thailand revealed marked inter- and intrapatient proteome variability, with only 18 proteins shared across all tumors [43]. These genes were primarily involved in metabolic and oxidative stress pathways, suggesting potential biomarkers and therapeutic targets. A significant clinical challenge in CCA management is the development of resistance to chemotherapeutic agents and targeted therapies. Proteomic studies have identified several resistance-associated proteins and signaling pathways. Notably, MET, LAMB1 (laminin subunit beta-1), and ITGA3 (integrin alpha-3) are consistently upregulated in drug-resistant CCA cell lines. These proteins contribute to enhanced cell motility, invasion, and EMT, features that are associated with aggressive tumor behavior and therapeutic resistance [44].

In large-duct-type iCCA, proteomic profiling has revealed the suppression of enzymes involved in glycolysis and gluconeogenesis. This metabolic shift is associated

with increased tumor proliferation and may serve as a compensatory mechanism that supports tumor growth under the stress of therapeutic intervention [45]. Such findings not only illuminate mechanisms of drug resistance but also reveal metabolic dependencies that may be exploited therapeutically. In addition to uncovering resistance mechanisms, proteomic studies have proven valuable for identifying prognostic biomarkers associated with disease progression and clinical outcomes. For instance, PPP3CA (protein phosphatase 3 catalytic subunit alpha), a calcium/calmodulin-dependent phosphatase, is overexpressed in highly aggressive forms of CCA and correlates with poor overall survival. As such, PPP3CA may serve as an independent prognostic indicator and potential therapeutic target [46].

3.5 Role of metabolomics in understanding and management of cholangiocarcinoma

Metabolomics involves the comprehensive profiling of small-molecule metabolites in cells, tissues, or biofluids. It has emerged as a valuable approach to study CCA. By capturing the downstream products of cellular processes, metabolomics provides a real-time view of physiological and pathological states. In CCA, a highly lethal biliary malignancy, metabolomic analyses have provided insight into tumor biology, diagnosis, prognosis, and treatment response. Advances in analytical platforms, including mass spectrometry and nuclear magnetic resonance, coupled with bioinformatics and machine learning, have enabled the identification of disease-specific metabolic signatures. These profiles help distinguish CCA from other hepatic malignancies, predict outcomes, and stratify patients for treatment. When integrated with genomics, transcriptomics, and proteomics, metabolomics enriches our understanding of the molecular complexity of CCA and supports personalized medicine.

A key contribution of metabolomics is the identification of dysregulated pathways during tumor development. Early-stage CCA is characterized by suppressed pyruvate metabolism and tricarboxylic acid (TCA) cycle activity, indicating a metabolic shift toward glycolysis (the Warburg effect) to support rapid growth under hypoxic conditions. In contrast, lipid metabolism is frequently upregulated in advanced or recurrent tumors and is associated with cancer stem-like traits, with an increased risk of recurrence [47, 48]. Metabolomic profiling of serum, urine, and bile has revealed promising noninvasive biomarkers. Urinary metabolomic profiling in a Thai cohort identified a distinct panel of ten metabolites that discriminated CCA with 93.4% diagnostic accuracy and an AUC of 98.8%, validating prior findings of disrupted acylcarnitine, bile acid, steroid, and purine metabolism. [49] Bile acid metabolism is another hallmark of CCA. A study on bile identified glycocholic acid (GCA) and taurochenodeoxycholic acid (TCDCA) as specific biomarkers for CCA [50].

Metabolomics also provides predictive insights into treatment response. Specific metabolic signatures are associated with chemosensitivity to gemcitabine and cisplatin, the standard chemotherapeutic regimen for advanced CCA. Increased ethanol levels and altered glucose metabolism correlate with enhanced drug sensitivity, suggesting a basis for stratifying patients for therapy [51]. In recurrence prediction, metabolomic studies have revealed consistent patterns: upregulated lipid metabolism and suppressed energy pathways characterize relapsed tumors, reflecting aggressive metabolic reprogramming [47]. Tracking these alterations posttreatment may inform surveillance and therapeutic adjustments.

4. Multiomics integration techniques

A major challenge in multiomics research is the integration of heterogeneous data types derived from genomics, transcriptomics, proteomics, and metabolomics. Canonical correlation analysis (CCorA) and its derivatives are among the most effective approaches for this task. For example, StabilityCCorA enhances traditional CCorA by incorporating stability selection, thereby improving the reproducibility of variable selection and showing particular success in linking metagenomics with metabolomics data to identify biomarkers in complex diseases [52]. Another powerful technique is Supervised Deep Generalized Canonical Correlation Analysis (SDGCCorA), which models complex nonlinear relationships across omics layers using deep learning. This approach integrates manifold learning with supervised classification to achieve superior phenotype prediction and feature ranking for standard models in cancer datasets [53].

Despite the promise of such techniques, multiomics data integration remains complex because of differences in scale, data quality, and biological context [54]. Integration often requires advanced computational methods, such as deep learning, to analyze high-dimensional data and uncover biologically meaningful patterns. Digital platforms, such as GraphOmics and OmicsAnalyst, offer interactive tools for multiomics visualization and analysis, although limitations in scalability and interoperability persist [55, 56]. Achieving reproducibility requires robust quality control, standardized workflows, and centralized repositories [57]. In addition, the field faces a shortage of researchers trained at the intersection of molecular biology, data science, and bioinformatics [58]. Nevertheless, advancements in sequencing, machine learning, and infrastructure are rapidly improving the feasibility of integrating multiomics data into clinical workflows [59].

In the context of CCA, such integrative approaches are particularly important because of its significant heterogeneity and poor prognosis. Multiomics profiling has enabled the molecular subtyping of CCA, with transcriptomic analyses revealing three major subtypes, each with distinct genetic dependencies that may be targeted [60]. This stratification has facilitated the development of personalized therapeutic regimens, shifting away from one-size-fits-all treatments. Biomarker discovery continues to play a pivotal role in early diagnosis and treatment. Novel serum and bile-based biomarkers, particularly noncoding RNAs, are being examined for their diagnostic and prognostic potential [5, 61, 62]. Enhancer activity profiling in cholangiocarcinoma revealed three molecular subtypes (ESTRO, OXPHO, and IMMUN), each with distinct etiologies and pathway activations, whereas ESTRO (fluke-related) exhibited estrogen signaling and mTOR pathway activation sensitive to mTOR inhibitors. OXPHO (BAP1/IDH-mutant) showed oxidative phosphorylation sensitivity, and IMMUN (immunogenic/AA-signature) involved immune-related pathways, collectively offering novel targets for subtype-specific therapies [63]. Advanced machine learning models that integrate multiomics and radiomics data are also under development, which will enable accurate risk stratification and outcome prediction [64].

The effective integration of data in multiomics research requires harmonizing datasets across platforms, omics layers, and sample sources, which often differ in scale, format, and biological context. Addressing batch effects and systematic nonbiological variations introduced during sample processing, sequencing, or measurement is essential to prevent spurious associations. Correction methods, such as ComBat, mutual nearest neighbors, and harmony algorithms, are commonly used to

enhance data comparability and model accuracy [65–68]. In addition, clinical translation remains limited because of challenges in data harmonization, molecular classification refinement, and the integration of omics with clinical and imaging data [69]. Future progress in CCA precision medicine will depend on innovations in molecular profiling, improved clinical trial design, and a complete understanding of the tumor microenvironment [70, 71]. Collaborative efforts across disciplines are necessary for ensuring that omics-based insights move from the bench to the bedside.

5. Future tools: AI/ML for multiomics analysis and predictive modeling

The integration of artificial intelligence (AI) and machine learning (ML) into multiomics has significantly advanced the study and clinical management of CCA. These computational frameworks provide powerful tools for analyzing high-dimensional heterogeneous datasets that encompass genetic, serological, histological, and radiological modalities. By synthesizing multiple omics layers, AI and ML can facilitate the identification of diagnostic and prognostic biomarkers with improved sensitivity and specificity, thereby enhancing early detection and individualized treatment regimens.

One notable application of this method in CCA is the use of high-throughput nanoassisted laser desorption ionization mass spectrometry, which enables the identification of molecular features with high diagnostic accuracy [72]. Furthermore, AI-driven integration of multiomics data supports the development of a precise prognostic and therapeutic model for intrahepatic iCCA patients predisposed to PANoptosis. This approach involved elucidating the molecular mechanisms of PANoptosis in iCCA cells and yielded a risk score with substantial prognostic and therapeutic predictive value [73]. The ability of AI and ML to detect subtle molecular signatures within integrated datasets also enables the characterization of tumor heterogeneity and promotes more refined treatment stratification [74]. In parallel, AI and ML technologies are increasingly being used for predictive modeling to inform clinical decision-making. These models aid in identifying prognostic factors, forecasting clinical outcomes, and guiding therapy selection. For example, ML algorithms have been used to generate intratumor heterogeneity signatures capable of predicting patient survival and immunotherapy responsiveness in CCA [75]. Similarly, in the context of primary sclerosing cholangitis (PSC), a recognized risk factor for CCA, AI-based models using clinical and laboratory data have surpassed traditional risk scores for early detection and risk stratification [76].

Despite the encouraging progress, many challenges remain in translating AI and ML into clinical practice for CCA. Most models require validation in large cohorts to ensure generalizability and reproducibility [64, 77], whereas the inherent complexity of CCA, driven by molecular subtypes, anatomical diversity, and immunological heterogeneity, further complicates predictive modeling [13]. Nonetheless, AI and ML applications include biomarker identification, molecular classification, drug response prediction, and noninvasive diagnostics, often requiring integrative analysis of multiomics, clinical, and imaging data [78, 79]. **Table 2** summarizes selected AI/ML-driven multiomics studies in CCA and highlights their contributions to precision oncology.

To fully harness the potential of AI and ML, sustained interdisciplinary collaboration is necessary, which includes the refinement of computational algorithms, seamless integration with clinical workflows, and the inclusion of patient-specific

Application area	Integrated omics/clinical layers	AI/ml techniques	Example of clinical or translational impact
Molecular Subtype Classification	Transcriptomics, proteomics, and epigenomics	Unsupervised clustering and supervised classification	Stratification of CCA into biologically distinct subtypes
Biomarker Discovery	Genomics, transcriptomics, and ncRNA profiles	Feature selection, Random forest, SVM	Identification of diagnostic and prognostic markers
Drug Response Prediction	Transcriptomics, proteomics, and cell line data	Deep learning, Network-based models	Prioritization of drug targets and prediction of therapy sensitivity
Prognostic Modeling	Genomics, Clinical parameters, Immune profiling	Cox regression and gradient boosting	Survival and disease progression prediction
Non-Invasive Diagnostics	Metabolomics, Circulating nucleic acids, Imaging	Ensemble learning, Neural networks	Early detection of CCA using urine, serum, or bile-based biomarkers
Immune Landscape Characterization	Single-cell RNA-seq, Spatial transcriptomics, Immune cell markers	Dimensionality reduction using graph-based models	Understanding immune evasion and response to immunotherapy
Risk Prediction in High-Risk Populations	Clinical data, Serologic markers, Imaging	Logistic regression and neural networks	CCA prediction in high-risk patients
Integrated Multiomics Profiling	Genomics, Epigenomics, Transcriptomics, Proteomics, and Metabolomics	Canonical correlation analysis and deep integration models	Holistic view of tumor biology and precision medicine frameworks

Table 2.
AI and multiomics applications in cholangiocarcinoma.

molecular data [79]. Equally important are standardized methodologies for data integration, normalization, and model evaluation to ensure reproducibility, alongside the adoption of explainable AI tools, such as SHAP and LIME, to support transparent biomarker discovery and informed clinical decision-making [80, 81].

6. Conclusion

Advances in multiomics research have significantly enhanced our understanding of CCA, a biologically heterogeneous and clinically aggressive malignancy. The integration of genomics, transcriptomics, proteomics, metabolomics, and epigenomics offers a comprehensive view of CCA pathobiology, enabling the discovery of actionable biomarkers and therapeutic targets.

Key genomic alterations, such as FGFR2 fusions and IDH1/2 mutations, define distinct molecular subtypes and guide targeted therapy [12, 13]. The advent of liquid biopsy and circulating tumor DNA (ctDNA) analysis has further improved noninvasive tumor monitoring [21, 22]. Epigenomic and transcriptomic analyses have revealed DNA methylation changes and enhancer dysregulation, which have identified CCA subtypes with therapeutic vulnerabilities, such as sensitivity to mTOR

and oxidative phosphorylation inhibitors [63]. Transcriptomic profiling also informs prognosis using molecular subtype classifiers [30]. Proteomic studies have revealed dysregulated signaling pathways and tumor-promoting proteins, whereas metabolomics has identified cancer-associated metabolites with diagnostic and therapeutic relevance [45, 51]. The results highlight the complexity of CCA and the value of integrative omics in precision oncology.

Multiomics integration reshapes CCA diagnosis and treatment by improving early detection, refining risk stratification, and enabling personalized therapeutic regimens [61]. These approaches can increase efficacy while reducing toxicity and enable the identification of novel targets in immune regulation and metabolic pathways [63]. However, clinical translation requires validation in large, diverse cohorts, standardized analysis pipelines, and integration into clinical workflows [77]. Advances in high-throughput omics platforms and computational analytics will continue to drive discovery. Despite the current data integration and regulation challenges, multiomics will revolutionize CCA diagnosis, treatment, and long-term patient care.

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Chapter 5

Optimising Therapeutic Options for Bile Duct Cancer in the Era of Molecular Profiling, Immunotherapy and Pharmacogenomics

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Abstract

This chapter explores the evolving landscape of bile duct cancer (cholangiocarcinoma, CCA) treatment, with a particular focus on the evolving landscape of systemic therapy driven by advances in molecular profiling, immunotherapy and pharmacogenomics. As new diagnostic technologies and precision therapies become available, there is increasing scope to personalise care based on tumour biology and individual patient characteristics. The chapter presents a critical review of current standard treatments and unmet needs, followed by an in-depth discussion of emerging therapies, including key molecular targets such as IDH1, FGFR2 and HER2, and the expanding role of immunotherapy. It also evaluates how pharmacogenomic tools and toxicity-monitoring innovations are helping to individualise treatment further. The chapter concludes with proposals for integrated treatment algorithms and strategies to optimise access, effectiveness, and patient outcomes in clinical practice.

Keywords: immunotherapy, molecular profiling, personalised, cancer genomics, pharmacogenomics

1. Introduction

Cholangiocarcinoma (CCA) is a highly heterogeneous malignancy arising from the epithelial cells of the bile duct. It is traditionally classified by anatomical location into intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) subtypes, each associated with distinct molecular profiles and clinical outcomes. Although uncommon, CCA accounts for approximately 3% of gastrointestinal cancers worldwide and has shown increasing incidence in recent decades, particularly in Western populations [1].

The prognosis for CCA remains poor, with overall five-year survival rates below 10% for advanced disease. Most patients are diagnosed at a late stage when curative resection is no longer feasible. For those eligible for surgery, the recurrence rate remains high, even with adjuvant therapy. Systemic treatment options have

historically provided limited benefit, with chemotherapy offering modest survival gains. The heterogeneity of the disease and the immunosuppressive tumour microenvironment (TME) have presented significant challenges in therapeutic development.

The rise of molecular diagnostics has been pivotal in changing the treatment paradigm for bile duct cancer -the advent of next-generation sequencing (NGS) has enabled more precise tumour characterisation, in turn uncovering a range of actionable mutations, and facilitating the use of targeted therapies. Similarly, checkpoint inhibitors and combination immunotherapies are beginning to demonstrate survival benefit in a growing number of patients. For those with CCA and other solid tumours, pharmacogenomic tools and wearable toxicity monitoring are supporting the development of truly personalised treatment regimens.

This chapter examines these developments and assesses how an integrated approach incorporating molecular biology, immunology, and pharmacogenomics can be harnessed to improve outcomes for patients with bile duct cancer.

2. Current therapeutic landscape in bile duct cancer

The current standard of care for CCA is determined by the stage at diagnosis. Surgery remains the only potentially curative intervention, however the majority of patients, over 70%—present with locally advanced or metastatic disease, making them ineligible for resection. For those who do undergo surgery, the recurrence risk remains significant, with rates exceeding 50% within two years postoperatively.

The BILCAP trial [2] confirmed capecitabine as the standard adjuvant therapy, with a median overall survival (mOS) of 51 months, compared to 36 months with observation alone. Although not statistically significant in the intention-to-treat analysis, the results were clinically meaningful and have been adopted in guidelines.

For unresectable or metastatic CCA, the ABC-02 trial [3] established the combination of cisplatin and gemcitabine (CisGem) as the first-line standard, demonstrating an mOS of 11.7 months compared to 8.1 months with gemcitabine alone. The recent TOPAZ-1 trial [4] added immunotherapy to this regimen with durvalumab, a PD-L1 inhibitor, combined with CisGem, led to a statistically significant improvement in survival (mOS 12.9 months vs. 11.3 months, HR 0.76) with evidence of durable response [5]. Resectability should be reassessed following a course of locoregional or systemic therapy—short and long-term outcomes in those receiving effective neoadjuvant treatment have been found to be similar to de novo resectable cases in small retrospective and phase II studies, though prospective phase III data is lacking [6, 7].

Second-line treatment has historically been limited. Retreatment with CisGem offers an option for patients who may have initially had benefit and is generally offered when relapse is at least 6 months following completion of initial therapy and patients do not demonstrate platinum resistance. The ABC-06 trial [8] demonstrated that modified FOLFOX (folinic acid, 5-fluorouracil and oxaliplatin) provided a modest benefit over best supportive care, with an mOS of 6.2 months. Critically, patient performance status remains a key determinant of eligibility for and outcomes of second-line chemotherapy. Chemoresistance commonly occurs and relapse after second-line treatment is frequently seen, particularly in those without an actionable target.

Radiation therapy, including chemoradiation, has a limited but important role in select patients with locally advanced, non-metastatic disease. Palliative radiation may also be employed for symptom control. The selected role of stereotactic ablative radiotherapy (SABR) for inoperable and/or oligometastatic disease may offer another

option for patients who have had poor responses to systemic anti-cancer therapy or have low burden of oligometastatic disease [9], though evidence is limited to retrospective analyses and case series.

Despite these advances, current systemic therapies are often associated with toxicity and limited durability of response. This underscores the need for better diagnostics, earlier detection, and innovations in new treatments including molecularly guided treatment strategies and clinical trials.

Current unmet needs include:

- Improved methods for early diagnosis, particularly in high-risk populations.
- Greater access to tumour tissue or liquid biopsy for genomic profiling.
- More effective, tolerable treatments for elderly or frail patients, and those with co-morbidity.
- Broader inclusion in clinical trials and more inclusive options for patients with rarer sub-types.

Lessons from pancreatic cancer research, such as the EUROPAC study, suggest that screening and stratification based on genetic risk may help identify patients earlier in the disease course. Similar approaches are needed in CCA, especially in individuals with primary sclerosing cholangitis, liver fluke infection, or hereditary cancer syndromes.

For patients with familial clustering or Lynch syndrome, studies are assessing the utility of periodic screening in identifying premalignant changes before progression. Individuals with Lynch syndrome, caused by germline mutations in DNA mismatch repair (MMR) genes such as MLH1, MSH2, MSH6, and PMS2, are at increased risk of a variety of malignancies, including cholangiocarcinoma. Although less common than colorectal or endometrial cancers, cholangiocarcinoma has been documented as part of the Lynch syndrome tumour spectrum. The estimated lifetime risk of developing biliary tract cancers in Lynch syndrome carriers ranges from 2 to 4%, significantly higher than the general population [1, 10]. These tumours often exhibit microsatellite instability-high (MSI-H) or mismatch repair deficiency (dMMR), which may inform the use of immune checkpoint inhibitors. Given their molecular features, routine surveillance and molecular profiling in Lynch syndrome patients with biliary symptoms may facilitate earlier detection and tailored therapeutic interventions. These efforts mirror initiatives in pancreatic cancer (e.g. EUROPAC), and exploration of how high-risk individuals can be identified and enrolled in surveillance protocols.

3. Molecular profiling: Changing the treatment paradigm

The rise of molecular diagnostics has been pivotal in transforming the therapeutic landscape for bile duct cancer. Cholangiocarcinoma (CCA) is increasingly recognised as a molecularly heterogeneous and genomically driven malignancy, drawing parallels to the evolution of precision medicine in lung adenocarcinoma [11]. The advent of next-generation sequencing (NGS) technologies and the widespread adoption of comprehensive genomic profiling have enabled the identification of a range of targetable alterations in CCA, including FGFR2 fusions, IDH1/2 mutations, BRAF

V600E, HER2 amplification, and others [1, 12]. These advances are most prominent in intrahepatic cholangiocarcinoma (iCCA), where molecular heterogeneity is particularly rich.

Liquid biopsy (of blood origin in the vast majority of cases) is becoming integral to the management of cholangiocarcinoma and solid tumours more broadly. ctDNA assays can non-invasively capture the evolving genomic landscape of the CCA tumour and identify emerging resistance mechanisms earlier than imaging, and more conveniently than repeat tissue biopsy [13, 14]. Testing platforms such as FoundationOne Liquid CDx and the Guardant360 suite deliver next-generation sequencing panels from a single blood test, with clinical validation demonstrating high analytic accuracy and rapid turnaround, thereby supporting serial sampling in routine practice [15, 16]. In a case of FGFR2-altered intrahepatic cholangiocarcinoma, serial liquid biopsies detected secondary kinase-domain mutations that arose on erdafitinib, prompting a switch to the covalent inhibitor futibatinib, and subsequent acquisition of compound gatekeeper mutations that were suppressed by the highly selective agent RLY-4008, achieving renewed tumour regression [12, 17, 18] demonstrating the importance of dynamic genomic reassessment as a strategy to achieve durable survival benefit in advanced cholangiocarcinoma.

International guidelines now recommend molecular profiling at diagnosis of advanced disease, ideally through enrolment in precision oncology programmes [7]. As the number of druggable targets increases, multidisciplinary tumour boards play a critical role in interpreting results and matching patients to clinical trials.

The genomic landscape of CCA is heterogeneous and subtype-specific. Intrahepatic cholangiocarcinoma (iCCA) tends to harbour FGFR2 fusions, IDH1/2 mutations, and BAP1 or ARID1A alterations, whereas extrahepatic and perihilar CCAs more frequently present with mutations in KRAS, TP53, and SMAD4 [19, 20].

Current key actionable mutations include:

- *FGFR2* fusions: Found in 10–15% of iCCA cases, these have emerged as one of the most clinically actionable targets. FGFR inhibitors such as pemigatinib, infigratinib, futibatinib, and erdafitinib have all demonstrated promising efficacy. The FIGHT-202 trial led to FDA approval of pemigatinib, with an overall response rate (ORR) of 35.5% and a median progression-free survival (PFS) of 6.9 months in previously treated patients with FGFR2 fusion-positive tumours [21].

- *IDH1* mutations: Occur in approximately 13–20% of iCCA and are associated with metabolic reprogramming. The ClarIDHy trial established the clinical benefit of ivosidenib, an *IDH1* inhibitor, with a PFS of 2.7 months compared to 1.4 months in the placebo arm [21].

- *BRAF* V600E mutations: Present in up to 5% of CCA, these mutations respond to dual inhibition with dabrafenib and trametinib. The ROAR basket trial showed an ORR of 47% in BRAF V600E-mutated biliary tract cancers [22].

- *HER2* amplification/mutations: More commonly seen in extrahepatic CCA and gallbladder cancer. Early-phase trials, including those evaluating zanidatumab, trastuzumab-deruxtecan, and combinations with pertuzumab, show encouraging signals. The HERIZON-BTC-01 study is currently investigating zanidatumab in HER2-amplified CCA.

3.1 IDH1 mutations

Mutations in IDH1, typically R132C/H, are detected in approximately 13% of iCCA cases. These mutations result in oncometabolite 2-HG production, leading to epigenetic dysregulation and impaired cell differentiation. The pivotal ClarIDHy trial demonstrated that ivosidenib, a first-in-class IDH1 inhibitor, significantly prolonged progression-free survival compared to placebo in previously treated IDH1-mutant CCA [21]. Though response rates are modest, disease control is frequently achieved. Ivosidenib has now been integrated into international guidelines and represents a landmark in biomarker-guided therapy for CCA.

Resistance mechanisms, including co-occurring mutations and clonal evolution, are under active investigation. Sequential sequencing and repeat biopsies are advised when progression occurs to identify emergent resistance and guide subsequent therapy selection.

3.2 MET alterations and NTRK fusions

For cholangiocarcinoma, long-tail biomarkers lie at the core of modern therapy selection. MET dysregulations and NTRK fusions occur in <2% of cases yet confer objective response rates of ≈40–60% to matched tyrosine-kinase inhibitors. Routine genomic profiling has made these rare oncogenic lesions firmly actionable in cholangiocarcinoma. In the UK, the NHS National Genomic Test Directory now requires that every advanced biliary-tract cancer sample undergo sequencing capable of detecting MET exon 14 skipping, amplification, fusion, and NTRK 1/2/3 rearrangements [23]. When a MET alteration is identified, patients may enrol in the umbrella precision-oncology trial DETERMINE (arm 06) and receive the MET inhibitor capmatinib [24]. By contrast, NTRK-positive tumours gain immediate funded access to the pan-TRK inhibitors larotrectinib (NICE TA630) or entrectinib (NICE TA644); the next-generation agent repotrectinib, approved by the US FDA in 2024, is anticipated to follow the same reimbursement route in the UK [25].

More broadly, health systems increasingly mandate comprehensive testing rather than single-gene assays: the NHS National Genomic Test Directory requires that all advanced biliary cancers undergo panel sequencing or whole-genome sequencing that captures these long-tail events, and the policy explicitly ties a positive result to funded access to MET or NTRK inhibitors. Similar coverage decisions—from Medicare's NCD 90.2 in the United States, Japan's National Health Insurance and France's Plan France Médecine Génomique have normalised reimbursement for broad NGS precisely to ensure that patients with these low-frequency, high-impact biomarkers are not missed.

3.3 HER2 amplification and overexpression

HER2 (ERBB2) amplification and overexpression are more frequently observed in extrahepatic CCA and gallbladder cancer. HER2-driven tumours represent an actionable subset, with investigational and compassionate-access agents showing promising efficacy. Zanidatumab, a HER2-targeted bispecific antibody, has demonstrated encouraging activity in HER2-positive biliary cancers in early-phase trials [26]. Other HER2-directed therapies being explored include trastuzumab-deruxtecan (T-DXd) and combinations with pertuzumab or tyrosine kinase inhibitors. Although HER2 positivity is detected in fewer than 10% of patients overall, targeted inhibition may offer a potent, biomarker-driven treatment option.

3.4 DNA repair pathways

Other alterations of interest include mutations in BRCA1/2, ATM, and mismatch repair (MMR) genes, which may confer susceptibility to PARP inhibitors or immunotherapy. BRCA1/2 mutations occur in approximately 1–7% of cholangiocarcinoma cases—varying by tumour subtype (extrahepatic ~4.8%, intrahepatic ~3.1%) [27]. The evidence for PARP inhibitor susceptibility in patients with BRCA1/2 or ATM-mutated CCA remains limited, and is similarly limited to single case reports [28], retrospective analyses, and preclinical studies that further suggest BRCA2-mutant and DDR-altered CCA respond favourably to PARP inhibitors [29] with potential synergy when combined with gemcitabine [30]. Large-scale prospective trial evidence is lacking, however early-phase and basket studies are underway to evaluate PARP inhibitor therapy in BRCA-mutated cholangiocarcinoma.

4. Immunotherapy in bile duct cancer

Immunotherapy has emerged as a promising modality in CCA, though the assessment of its efficacy in relation to specific molecular and immune contexts is ongoing. The immune landscape of CCA is typically considered "cold," due to low mutational burden, poor T-cell infiltration, and an immunosuppressive tumour microenvironment dominated by myeloid-derived suppressor cells [31] indicating a presumed lack of response to immune-directed therapies. Nevertheless, a growing number of CCA patients seemingly challenge this dogma and derive significant benefit from immunotherapy.

Immune checkpoint inhibitors such as pembrolizumab and nivolumab have shown efficacy in patients with microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) tumours. Although rare (<2% of cases), this subgroup exhibits high tumour mutational burden (TMB) and is particularly responsive to PD-(L)1 blockade, with the KEYNOTE-158 trial reporting an ORR of 40.9% in patients with MSI-H/dMMR CCA demonstrative of this susceptibility [32].

The TOPAZ-1 trial marked a turning point for immunotherapy in an unselected population whereb durvalumab (anti-PD-L1) was added to CisGem in the first-line setting for advanced BTC. The study demonstrated improved mOS (12.9 vs. 11.3 months; HR 0.76) and durable responses. Importantly, this benefit extended across anatomical subtypes and was observed regardless of PD-L1 status [4], and findings have been ratified in the three-year overall survival update with a doubling of 36-month survival from 6.9% to 14.6% [5].

Beyond TOPAZ-1 and KEYNOTE-158, additional trials are currently investigating ICI (immune checkpoint inhibitor) combination strategies such as KEYNOTE-966 (NCT04003636) evaluating pembrolizumab + CisGem vs. chemotherapy alone) [33], IMbrave151 investigating atezolizumab + bevacizumab + chemotherapy [34], and DURANCE (NCT05026476) testing durvalumab with lenvatinib and chemotherapy [35].

These trials aim to broaden the population benefitting from checkpoint blockade by combining ICIs with chemotherapy and anti-angiogenic agents.

Biomarkers under exploration include PD-L1 expression, TMB, tumour-infiltrating lymphocytes (TILs), and gene expression signatures, Biomarker enrichment remains essential, though none has yet proven sufficiently reliable for routine clinical

selection in CCA. MSI-H and dMMR phenotypes, while rare, are robust predictors of ICI benefit [36]. Tumour mutational burden (TMB) and PD-L1 expression, although more common, are not yet definitive predictors in this disease. Exploratory studies into the tumour immune microenvironment and transcriptomic signatures may enable future stratification.

Immunotherapy for cholangiocarcinoma (CCA) is hampered by tumour-intrinsic factors—such as low mutational burden, defective antigen presentation and oncogenic drivers like FGFR2 fusions or Wnt/β-catenin signalling. This lies in addition to a highly immunosuppressive tumour micro-environment (TME) rich in myeloid cells, dense stroma and hypoxia, all of which foster T-cell exclusion or exhaustion [37]. Adaptive escape emerges when PD-1 blockade up-regulates alternative checkpoints (LAG-3, TIM-3, TIGIT) or selects β-2-microglobulin–deficient clones, while dysbiosis of the gut–liver axis further blunts systemic IL-12 and IFN responses, compounding primary non-response [38].

4.1 Emerging modalities

Novel approaches and strategies are required to overcome de novo and acquired immune resistance, and treatment is shifting toward biomarker-guided combination strategies. First-line chemo-immunotherapy (e.g. durvalumab + gemcitabine/cisplatin) has improved overall survival, and current trials are layering dual checkpoint blockade, TGF-β or CSF1R inhibitors, and vessel-normalising radiotherapy to remodel the TME [39]. Parallel efforts pair FGFR2 or IDH1 inhibitors with ICIs to reverse oncogene-driven immune exclusion, while early-phase studies of peptide vaccines, personalised TIL products and CAR-T cells directed at biliary tract antigens aim to generate de novo immunity [40, 41]. Additionally, manipulating the gut microbiome—through dietary interventions, live-biotherapeutic products or faecal transplant—is emerging as a promising adjunct to enhance checkpoint efficacy [38].

Vaccine development for CCA is in its nascent stage, however recent bioinformatics-driven studies have identified tumour-associated antigens and immune subtypes in CCA suitable for mRNA vaccine design—such as MUC1, WT1, CD247, and FCGR1A—creating a framework for selecting neoantigen targets [42]. These findings support the feasibility of tailored mRNA vaccines capable of inducing antigen-specific $CD8^+$ T-cell responses in CCA—prospective data is awaited.

Integrated combination pharmaceuticals such as bispecific antibodies targeting PD-1 and another target, such as CTLA-4, represent a novel approach aimed at overcoming resistance to monotherapies. The GEMINI-Hepatobiliary trial (NCT05775159) is currently investigating volrustomig, a bispecific antibody engaging both PD-1 and CTLA-4, in patients with hepato-biliary cancers including CCA [43]. Other examples of bispecific antibodies include zanidatamab—targeting two distinct HER2 epitopes and recently FDA-approved based on the HERIZON-BTC-01 trial—and CTX-009, a DLL4/VEGF-A bispecific currently in phase II with Fast Track status, both showing encouraging response rates in advanced biliary tract cancers [4, 44].

Radiotherapy, especially stereotactic body radiotherapy (SBRT), may synergise with ICIs by inducing immunogenic cell death and enhancing antigen presentation, with evidence from the PACIFIC trial for non-small cell lung cancer supportive of this notion in solid tumours [45]. Further clinical trials exploring these combinations are in progress, and this phenomenon has been replicated in cholangiocarcinoma in small phase II studies and case series [46, 47].

Ultimately, the integration of immune profiling into standard diagnostic workups, coupled with rational combination regimens and improved patient selection, will define the next era of immunotherapy in bile duct cancer.

5. Pharmacogenomics: Personalising treatment further

Pharmacogenomics—the study of how genetic variation influences individual responses to drugs—is becoming increasingly relevant in the treatment of CCA. While pharmacogenomics is still becoming established in cancer pathways, the ability to integrate in the work up of patients with rarer cancers like CCA is important as systemic therapies become more tailored.

Fluoropyrimidines, such as capecitabine and 5-fluorouracil (5-FU), are commonly used in the adjuvant and palliative setting for CCA. Polymorphisms in the DPYD gene, which encodes dihydropyrimidine dehydrogenase, significantly increase the risk of severe toxicity, including mucositis, diarrhoea, neutropenia, and even death. Pre-emptive DPYD testing is now recommended in many European countries before initiating fluoropyrimidine-based chemotherapy.The UK MOLGEN study is exploring how pharmacogenomic variants can be systematically integrated into treatment decisions for patients with gastrointestinal malignancies, including biliary cancers [48]. Findings from MOLGEN will likely inform prospective clinical guidelines and support the development of a pharmacogenomic prescribing framework. This trial also aims to ensure ethnicity variants and ensure equitable outcomes across all patient groups [49]. Irinotecan, used off-label or in clinical trials for biliary tract cancers, is metabolised via the UGT1A1 enzyme. Patients with UGT1A1*28/*28 genotype are at increased risk of neutropenia and diarrhoea [50]. Similarly, variations in CYP3A4 and CYP2C9 affect the metabolism of several oral tyrosine kinase inhibitors (TKIs) and should be considered in dose adjustments [51].

Beyond genetics, real-time toxicity monitoring is being enhanced via the use of wearable technologies. One example is SENSECheQ, which measures touch and thermal thresholds in extremities and is designed to detect the early onset of chemotherapy-induced peripheral neuropathy (CIPN), particularly relevant for patients with CCA receiving platinum-based therapies. SENSECheQ employs sensory mapping and patient-reported outcomes to track functional decline, enabling clinicians to intervene before irreversible neuropathy develops. Preliminary data demonstrate good patient compliance and early identification of dose-limiting toxicity [52]. Recent studies in tumour agnostic cohorts have employed pendant-based wearables to generate a "Chemotherapy Resilience Index" (CRI) by measuring parameters such as step cadence and energy expenditure. These digital biomarkers distinguished between patients who tolerated chemotherapy well and those who experienced unplanned hospitalisation or dose reductions, sometimes as early as 6 days post-treatment initiation (CRI AUC = 0.88) [53]. As scalable platforms, these innovations hold promise in enhancing quality of life and informing treatment modifications in real time.

Routine capture of patient-reported outcome measures (PROMs) with cholangiocarcinoma-specific tools such as the validated EORTC QLQ-BIL21, together with smartphone companion apps like MYSUNRISE (and the NHS App), allow patients to log symptoms in real time, receive tailored advice, and escalate red-flag toxicities directly to their team, adding a digital pillar that complements pharmacogenomic testing and e-toxicity monitoring to cut adverse events and individualise dose intensity [54, 55].

A growing focus within pharmacogenomics is understanding and overcoming acquired resistance to targeted therapies. One of the clearest examples in cholangiocarcinoma is seen with FGFR2 inhibitors. Although agents such as pemigatinib and futibatinib have demonstrated efficacy in FGFR2 fusion-positive intrahepatic cholangiocarcinoma [56], resistance inevitably develops. Recent analyses have identified multiple resistance mechanisms. These include secondary mutations in the FGFR2 kinase domain (e.g., gatekeeper mutations such as V564F or N550H), which reduce inhibitor binding. Other mechanisms involve pathway reactivation via bypass signalling, such as through EGFR or MET amplification [57].

Liquid biopsy and serial ctDNA analysis are being employed to monitor clonal evolution and detect emerging resistance mutations in real time. These tools not only guide re-biopsy but also inform next-line therapy decisions. Rotation of FGFR inhibitors is an emerging strategy to overcome acquired resistance in FGFR2 fusion-positive intrahepatic cholangiocarcinoma. Resistance to first-line reversible ATP-competitive FGFR inhibitors, such as pemigatinib or infigratinib, often arises due to secondary FGFR2 kinase domain mutations and switching to irreversible FGFR inhibitors (e.g., RLY-4008, TAS-102) or combinatorial approaches targeting both FGFR and bypass pathways has shown promise in small retrospective studies and case series [51, 58].

Dual blockade strategies in FGFR2-altered cholangiocarcinoma aim to overcome both on-target resistance (e.g., secondary FGFR2 mutations) and off-target bypass mechanisms (e.g., PI3K/mTOR or MAPK pathway activation). Preclinical and early clinical studies have shown that combining FGFR inhibitors with agents targeting parallel or downstream pathways—such as mTOR inhibitors (e.g., everolimus) or MEK inhibitors (e.g., trametinib)—can restore sensitivity and prolong response duration in resistant tumours [51, 58]. The incorporation of resistance profiling into standard practice may help prolong response durations and expand the benefit of targeted therapy.

6. Future treatment algorithms: A framework for therapy selection

The integration of molecular, immunologic, and pharmacogenomic data is redefining therapeutic decision-making in CCA. Moving forward, patient stratification based on biomarkers, tumour subtype, co-morbidities, previous toxicities and pharmacogenomics will be key to optimising treatment outcomes.

1. Initial evaluation

   - Confirm histological subtype and stage
   - Perform comprehensive NGS using validated platforms (e.g., FoundationOne, Guardant360)
   - Assess MSI/dMMR status, PD-L1 expression, and TMB
   - Screen for actionable mutations (e.g., FGFR2, IDH1, BRAF, HER2, BRCA)
   - Evaluate *DPYD* and UGT1A1 variants if fluoropyrimidines or irinotecan are planned

2. First-line therapy
    - *MSI-H/dMMR*: Pembrolizumab
    - *FGFR2 fusion*: Pemigatinib or futibatinib (if available via early access or trial)
    - *IDH1 mutation*: CisGem ± durvalumab followed by ivosidenib at progression
    - *HER2 amplified*: Investigational HER2-directed therapy (e.g., zanidatumab, T-DXd)
    - *No actionable target*: CisGem + durvalumab (standard of care)
3. Second-line and beyond
    - *Re-biopsy or liquid biopsy* to assess acquired resistance or new targets
    - *FOLFOX or FOLFIRI* depending on prior toxicity and tolerability
    - *Clinical trials* involving FGFRi (RLY-4008), immunotherapy combinations, or novel agents
4. Supportive and palliative care
    - Early palliative care integration
    - SENSECheQ and similar platforms for toxicity monitoring
    - Multidisciplinary input including hepatobiliary surgery, gastroenterology, oncology, genomics, and palliative specialists.

Precision oncology initiatives such as the UK DETERMINE study and the TARGET molecular tumour board exemplify how national-scale platforms are operationalising personalised medicine for rare cancers such as cholangiocarcinoma. These initiatives support implementation by offering centralised molecular annotation, multidisciplinary case review, and clinical trial matching services to ensure patients are aligned with the most appropriate and innovative treatment pathways [24].

The DETERMINE trial is focused on using next-generation sequencing to identify actionable mutations across multiple tumour types, matching patients to a portfolio of early-phase targeted agents. By embedding genomic annotation into standard oncology workflows, these initiatives accelerate the bench-to-bedside translation of molecular discoveries.

The TARGET programme complements this by delivering a national molecular tumour board infrastructure, enabling discussion of complex cases across academic and regional centres [59]. This supports equitable access to expertise and trial opportunities regardless of geographical location. These precision oncology models are especially valuable in rare cancers like CCA, where mutation-driven treatment options are emerging rapidly but patient numbers per site remain low.

Ethical considerations in precision oncology are increasingly central to health policy. Equitable access to molecular diagnostics and novel therapies—especially for

under-represented and socioeconomically disadvantaged populations—remains a critical concern [60]. Cost-effectiveness frameworks must evolve to accommodate the high upfront costs of genomic testing and targeted drugs while recognising the long-term benefits of avoiding ineffective treatments and reducing toxicity. Health systems must balance cost-effectiveness with innovation, ensuring that advances reach all eligible patients.

Emerging policy models suggest that population-scale genomic screening, including National Test Directory, funded as a component of national cancer control strategies, may help address disparities. Moreover, public–private partnerships and academic–industry collaborations are increasingly facilitating access to targeted agents through expanded access protocols and genomic-driven trials.

Research is also intensifying into early detection and prevention. In primary sclerosing cholangitis (PSC), a major risk factor for cholangiocarcinoma, longitudinal surveillance with imaging and serum biomarkers is being refined to improve pre-symptomatic diagnosis. Trials exploring novel biomarkers such as circulating tumour DNA (ctDNA), bile-based proteomics, microbiome signatures and overcoming resistance mechanisms are underway.

Ultimately, combining genomic profiling, molecular tumour boards, and real-time trial matching will be crucial to delivering precision medicine equitably. At the same time, investment in early detection, premalignant lesion characterisation, and preventive care may enable interception of CCA at its earliest stages—potentially shifting the disease trajectory toward curative outcomes.

For patients with familial clustering or Lynch syndrome, studies are assessing the utility of periodic screening in identifying premalignant changes before progression. These efforts mirror initiatives in pancreatic cancer (e.g., EUROPAC), where high-risk cohorts are enrolled in surveillance protocols.

As research continues into early detection, premalignant lesion monitoring, and prevention strategies (e.g., surveillance of PSC or familial CCA), the goal of intercepting CCA before metastasis or advancement beyond resectability may become a future reality.

7. Conclusion and future perspectives

The management of cholangiocarcinoma is undergoing a transformation, moving from a one-size-fits-all approach to one driven by molecular and immunological insights. The last decade has seen incremental but meaningful progress in survival outcomes due to better systemic therapies and the emergence of immunotherapy. The prognosis for most patients however remains poor, necessitating further innovation including facilitating access to clinical trials and understanding resistance mechanisms.

Molecular profiling has uncovered numerous druggable targets, with FGFR2 fusions, IDH1 mutations, and HER2 amplifications now established as clinically relevant alterations. The integration of next-generation sequencing into routine care—both tissue and liquid-based—has enabled broader access to targeted therapies and improved understanding of resistance mechanisms. Immunotherapy, once considered ineffective for CCA, has now carved out a role for both biomarker-enriched and unselected populations, most notably through the success of the TOPAZ-1 trial.

Pharmacogenomics and wearable technologies like SENSECheQ promise to refine supportive care, allowing personalised dose adjustments and real-time monitoring

of toxicity. As these tools become more mainstream, the risks of overtreatment and adverse events may be significantly reduced.

Looking forward, multidisciplinary precision oncology models must be expanded to ensure that the benefits of new treatments are equitably distributed. Clinical trials remain essential, and all patients with advanced CCA should have access to genomic screening and clinical trial opportunities. New approaches to early detection, such as screening in high-risk groups or surveillance of premalignant lesions, offer hope for intercepting disease before it becomes incurable.

Ultimately, the future of bile duct cancer care lies in comprehensive integration of tumour biology, immune status, pharmacogenomics, translational advances including cancer vaccines and effector T-cell therapies, and patient values—ensuring that personalised medicine becomes the standard, not the exception.

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Bile Duct Cancer is a common type of malignant tumor of the biliary tract, starting from Cholangiocarcinoma (CAA) of the hilar region, characterized by an insidious onset, and is a prevalent malignant tumor in the biliary tract. Its diagnosis and treatment encompass multiple aspects, including epidemiology, clinical evaluation, imaging studies, pathological classification, staging, resectability assessment, perioperative management, surgical decision-making, systemic therapy, and follow-up monitoring. The contents of the book include the following chapters:

1. *Bile Duct Cancer: From Bench to Bedside – Latest Developments* offers a discussion on the latest treatment strategies and research developments for this challenging biliary tract cancer.
2. *Cholangiocarcinoma – A Comprehensive Review of Diagnostic Challenges and Novel Therapeutic Approaches* offers an exploration of bile duct cancer research, blending scientific rigor with accessibility.
3. *Diagnosis, Imaging, and Prognostic Evaluation in Cholangiocarcinoma* provides a comprehensive exploration of CCA.
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5. *Optimising Therapeutic Options for Bile Duct Cancer in the Era of Molecular Profiling, Immunotherapy and Pharmacogenomics* engages readers by exploring the latest strategies to enhance cholangiocarcinoma treatment.

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