REVIEW

10.2478/AMB-2026-0031

INTEGRATION OF GENOMIC FEATURES, IMMUNOTHERAPY AND TARGETED AGENTS IN THE TREATMENT OF GALLBLADDER AND BILE DUCT CANCER – A REVIEW

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Abstract. *Cancers of the gallbladder and bile ducts, although relatively rare, are highly lethal malignant diseases due to their aggressive biology, complex hilar and retroperitoneal anatomy, and frequently delayed diagnosis.* ***Objective:*** *To analyze the latest developments in the integration of immunotherapy and targeted agents in the treatment of gallbladder and bile duct cancers.* ***Materials and Methods:*** *Review of scientific publications using documentary analysis and content analysis.* ***Results and Discussion:*** *Gallbladder and bile duct cancer are aggressive malignant diseases with increasing incidence, especially for intrahepatic cholangiocarcinoma. Although surgery remains the primary treatment method, the integration of immunotherapy and targeted agents based on molecular profiling is transforming the therapeutic landscape and offering new hope for patients with advanced disease.*

Key words: *gallbladder cancer, bile duct cancer, surgical treatment, immunotherapy, targeted therapies*

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Received: *13 January 2026;* ***Accepted:*** *22 January 2026*

INTRODUCTION

Gallbladder and bile duct cancers, although relatively rare, are highly lethal malignancies due to their aggressive biology, complex hilar and retroperitoneal anatomy, and often delayed diagnosis. Biliary tract tumors (BTT) are a heterogeneous group of adenocarcinomas. Gallbladder cancer (GBC) is defined as a malignant neoplasm originating in the gallbladder or cystic duct. Cholangiocarcinoma (CC) is defined as cancer originating from the cholangiocytes that line the biliary tree. The anatomical classification of cholangiocarcinoma includes several subtypes that differ from each other based on their location: intrahepatic cholangiocarcinoma (ICC), originating in the bile ducts within the liver; perihilar cholangiocarcinoma (PCC), which originates in the bile ducts outside the liver at the biliary bifurcation (also known as Klatskin tumor); distal cholangiocarcinoma (DCC) in the part of the bile duct closest to the small intestine; and extrahepatic cholangiocarcinoma (ECC), as a collective term for both perihilar and distal CC. Adenocarcinoma is the most common histopathological type for both GBC and CC. According to the World Health Organization (WHO) classification, there are other, rarer histological types, such as neuroendocrine, squamous/adenosquamous, mucinous, and papillary variants. These diverse tumors show significantly different epidemiological patterns, reflecting the global burden of these diseases [1, 5]. In the International Classification of Diseases, 11th Revision (ICD-11) – the WHO's current global diagnostic coding system – gallbladder and bile duct cancers are classified with new codes that reflect more detailed anatomic and pathological distinctions compared with ICD-10 [6].

According to Globacon data for 2020, gallbladder cancer has led to approximately 116,000 new cases and 84,700 deaths worldwide. GBC is the most common cancer of the biliary system and the sixth most common cancer of the gastrointestinal tract [1, 8]. In contrast, data from the SEER registry in the US (2001-2017) show that the overall incidence of CC has increased by 43.8%, mainly due to a significant increase in ICC by 148.8%, while the incidence of ECC has increased by only 7.5%. During the same period, the incidence of cancer of unknown primary (CUP) decreased by 54.4%. This divergent trend suggests that the increase in ICC is real and cannot be attributed entirely to an artifact of CUP reclassification, which has significant implications for understanding its etiology. There is considerable regional heterogeneity in GBC, with high incidence in South American countries (Chile, Bolivia), northern India, Pakistan, Japan, and Poland. The peak incidence of CC is in northeastern Thailand. Demographically, GBC is two to six times more common in women than in men and usually affects older people, with the highest incidence in the seventh decade of life. In contrast, the largest increase in the incidence of CC is observed in younger patients (aged 18-44 years). These epidemiological changes, particularly the increase in intrahepatic disease in Western nations and among younger demographic groups, necessitate a detailed analysis of the various etiological factors and risk profiles underlying each subtype of biliary tract cancer [1, 5].

Although many cases of BTT are sporadic, a number of key risk factors have been identified, most of which involve chronic inflammation and damage to the biliary epithelium.

Table 1. Risk factors

Risk factor category	Specific factor	Associated cancer	Key data/Comments
Inflammatory and stone-related	Gallstones (cholelithiasis)	GBC	The most significant risk factor. Up to 85% of patients with GBC have gallstones. Stones >3 cm carry a 10-fold increased risk.
Primary sclerosing cholangitis (PSC)	CC (especially ICC)	The strongest risk factor in Western countries. Relative odds (OR) of 20-25 for ICC.	
Hepatolithiasis	ICC	Major risk factor; 5-10% of patients with hepatolithiasis develop ICC. OR 5-50.	
Infectious agents	Liver flukes (Opisthorchis viverrini, Clonorchis sinensis)	CC	Strong risk factor in endemic areas such as Korea, China, and Thailand.
Chronic viral hepatitis (HBV, HCV)	ICC	Significant risk factors for ICC.	
Chronic infection with Salmonella	GBC	Common risk factor in regions with high incidence of GBC.	
Congenital and structural anomalies	Biliary cysts/Caroli's disease	ICC	Associated with a significant increase in the incidence of ICC (relative risk 26.7).

Risk factor category	Specific factor	Associated cancer	Key data/Comments
Metabolic and lifestyle factors	Liver cirrhosis (any etiology)	ICC	Significant risk factor with OR 9-25.
Obesity and diabetes	ICC, GBC	Associated with an increased risk of ICC.	
Alcohol consumption	GBC, ICC	Excessive consumption increases the risk.	
Carcinogenic exposure	Industrial chemicals and radon	GBC	Increased risk for workers in the oil, paper, chemical, footwear, and textile industries, as well as for miners.
Tobacco smoking	GBC	Known risk factor.	
Hereditary factors	Family history	Lung cancer	A family history of GBC increases the risk approximately 5-fold.

In recent years, there has been a critical shift towards molecular pathology in the treatment of BTT. Understanding the different genomic characteristics of GBC, ICC, and ECC is essential for prognosis and the application of targeted therapies. The most commonly mutated genes include *TP53, KRAS, CDKN2A/B, ARID1A*, and *BAP1*. These clinically significant biomarkers are a defining characteristic of intrahepatic cholangiocarcinoma, being found much less frequently in other subtypes of BTT, and their identification by next-generation sequencing (NGS) is now standard practice for all patients with advanced disease (Table 2).

Table 2. Clinically significant biomarkers

FGFR2 **fusions/rearrangements:** Found in 15-30% of ICC cases.
IDH1/2 **mutations:** Found in 10-20% of ICC cases.
BRAF V600E **mutations:** Found in 3-6% of cases.
HER2 (ERBB2) **amplifications:** Found in up to 20% of cases.
MSI-H (mismatch repair deficiency): Found in 1-2% of cases.
***NTRK* fusions:** Found in <1% of cases.

Perineural and lymphovascular invasion are key microscopic features associated with poor prognosis. These molecular and pathological features are covered and quantified within modern cancer staging systems [2, 3]. Accurate anatomical and pathological staging is crucial. The TNM system provides the critical framework for determining prognosis, guiding treatment decisions, and determining eligibility for clinical trials. Common macroscopic growth patterns in ICC include mass-forming, periductal-infiltrating, and intraductal-growing types. Key microscopic prognostic factors are histologic grade, lymphovascular invasion, perineural invasion, and the status of surgical resection margins (R0 for negative, R1 for microscopically positive) and lymph nodes.

Summary of current TNM staging systems according to AJCC, 8th edition:

Gallbladder cancer (GBC): This is a system based on the layers of the wall. T2 tumors are divided into T2a (peritoneal side) and T2b (hepatic side), with T2b having a worse prognosis. N staging is based on the number of positive nodes (N1: 1-3; N2: ≥4), with a minimum of 6 nodes recommended for examination. Crucially, the 8th edition reclassifies metastases in the celiac, superior mesenteric, and peripancreatic lymph nodes – previously considered N2 regional disease – as distant metastases (M1);

Intrahepatic cholangiocarcinoma (ICC): T staging is based on the number of tumors, vascular invasion, and size, with the key size cutoff being 5 cm, separating T1a from T1b. Periductal invasion has been removed from T4 in the 8th edition;

Extrahepatic cholangiocarcinoma (PCC and DCC): a harmonized, N-based staging has been introduced (N1: 1-3; N2: ≥ 4). For distal (DCC) CC, T staging is based on the depth of invasion (T1: < 5 mm; T2: 5-12 mm; T3: > 12 mm) [7].

The clinical stages of disease spread determine the therapeutic approach. Resectable disease is confined and does not involve major vascular structures, allowing for curative surgery. Borderline resectable disease involves a tumor that is adjacent to critical vessels and may become resectable after neoadjuvant therapy. Locally advanced disease involves extensive vascular invasion, which precludes surgery. Metastatic disease refers to spread to distant organs and is treated primarily with systemic therapy. These pathological stages often correlate with clinical presentation.

Early diagnosis of BTT remains challenging. Initial signs and symptoms are often insidious and nonspecific, leading to frequent presentation at an advanced stage: **obstructive jaundice:** yellowing of the skin and eyes, often accompanied by severe itching of the skin (pruritus), dark urine, and white or greasy stools; **abdominal pain:** usually located in the upper right quadrant, just below the ribs; **constitutional symptoms:** unexplained weight loss, loss of appetite, constant fatigue, and fever **nausea and vomiting:** a common symptom, especially in GBC **palpable**

mass: a painless, palpable gallbladder in a patient with jaundice (Curvoisie's sign) may suggest distal biliary obstruction. Alternatively, a hard, irregular mass may be palpable if the cancer has grown to a large size or has directly invaded the liver.

GBC is often discovered incidentally during or after cholecystectomy performed for suspected benign gallstone disease. The incidence is 0.3% to 3.0% in such procedures. If detected at an early stage, the 5-year survival rate can exceed 80%. Clinical suspicion arising from these symptoms requires diagnostic evaluation for confirmation and staging.

The definitive diagnosis of BTT requires a multimodal approach. The main goals of diagnosis are to obtain a histological diagnosis, accurately determine the anatomical extent of the disease, and assess the patient's suitability for treatment.

Imaging methods include: 1. Cross-sectional imaging studies: ultrasound, contrast-enhanced computed tomography (CT), and magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) play a major role in characterizing the primary tumor and assessing local extent, vascular involvement, and distant metastases. **2.** [18F] FDG PET/CT: this method is **not** recommended for initial diagnosis due to low specificity (false positive results in inflammatory conditions), but it is valuable for preoperative staging to detect occult nodal or distant metastases that would alter the therapeutic approach, as well as for identifying disease recurrence. **3.** Invasive diagnostic and staging procedures: endoscopic ultrasound (EUS), endoscopic retrograde cholangiopancreatography (ERCP) with brush material or biopsy, and percutaneous transhepatic cholangiography (PTC) are used to obtain tissue samples and visualize the biliary tree. Staging laparoscopy is used to definitively rule out peritoneal metastases before major surgery. **4.** Laboratory markers: Serum tumor markers such as carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA), along with liver function tests, are used for diagnosis and monitoring. Once the diagnosis and stage are fully established, treatment planning proceeds [6].

Despite advances in systemic therapy, surgical resection with negative oncological resection margins (R0) remains the cornerstone of radical treatment for localized biliary tract cancers. These procedures are among the most technically challenging in surgical oncology, often requiring major hepatectomy and complex biliary reconstruction, and should only be performed in centers with high-volume and specialized hepatobiliary expertise. For gallbladder cancer, an extended cholecystectomy is performed, which includes en bloc liver resection (e.g., segments IVb/V) and regional lymphadenectomy. In incidentally detected GBC, revision radical surgery is justified for tumors in stage T2 and higher. Intrahepatic cholangiocarcinoma requires major hepatectomy with lymphadenectomy to achieve R0 resection. In extrahepatic cholangiocarcinoma, resection involves hepatectomy (for PCC) and/or resection of the bile ducts (for PCC and DCC), often requiring complex vascular and biliary reconstruction (hepaticojejunostomy).

There are various strategies for expanding resectability and managing risk. Portal vein embolization (PVE) is a preoperative technique used to induce hypertrophy of the future liver remnant (FLR), thereby reducing the risk of postoperative liver failure and expanding surgical applicability for patients who would otherwise have insufficient FLR. 70-80% of patients proceed to resection after PVE. Risk-stratified surgery is used in patients with ICC with positive lymph nodes (N1), where achieving R0 resection does not provide a significant survival advantage over R1 resection. This suggests that a less aggressive approach may be justified to reduce morbidity and facilitate adjuvant therapy. Liver transplantation is a highly selective treatment option for certain types of cholangiocarcinoma. Beyond curative surgery, systemic therapies play a crucial role in both the adjuvant and advanced stages of the disease.

The paradigm in the treatment of BTT has shifted toward a multimodal approach. Systemic therapy is now an integral part of the entire spectrum of the disease, from improving postoperative outcomes to controlling advanced disease and utilizing molecular targets.

Recommendations for adjuvant therapy in resected disease: based on the BILCAP study, adjuvant capecitabine for 6 months is the standard of care for resected BTT [1]. Chemoradiotherapy may be offered to patients with extrahepatic cholangiocarcinoma or GBC with microscopically positive resection margins (R1 resection); however, this approach is not recommended for intrahepatic cholangiocarcinoma due to the high competing risk of distant recurrence and the lack of a clear resection margin to target with postoperative radiation [4].

First-line standard of care for advanced or unresectable disease: the combination of gemcitabine and cisplatin (GemCis) is the historical chemotherapy backbone. The current standard, based on the TOPAZ-1 study, is GemCis plus the immune checkpoint inhibitor durvalumab. Results show improved median overall survival (OS) of 12.9 months (vs. 11.3 for che-

motherapy alone) and three-year OS of 14.6% (vs. 6.9%) [2]. The combination of pembrolizumab plus GemCis is another approved option [3]. Building on the molecular changes discussed earlier, a new paradigm of precision oncology has emerged, targeting specific genomic drivers found predominantly in ICC, offering new therapeutic opportunities for patients with advanced disease.

Table 3. New targeted therapies

Molecular target	Approved/recommended target agent(s)
FGFR2 **fusion/rearrangement**	Pemigatinib, Futibatinib
***IDH1* mutation**	Ivosidenib
BRAF V600E **mutation**	Dabrafenib and Trametinib
MSI-H/dMMR	Pembrolizumab
HER2 (ERBB2) **amplification**	Trastuzumab and Pertuzumab
***NTRK* fusion**	Larotrectinib, Entrectinib

In patients who are not candidates for curative or life-prolonging systemic therapy, the focus is on symptom control.

Palliative care is a critical component of treatment for most patients with BTT, aimed at maximizing quality of life through comprehensive symptom control. The main methods of biliary drainage to relieve jaundice and itching are: endoscopic stenting: via ERCP; percutaneous stenting: via PTC; surgical bypass: such as hepaticojejunostomy, used more selectively.

Other key components of palliative and supportive care: systemic palliative chemotherapy/targeted therapy; palliative radiotherapy: for localized symptom control (e.g., pain from bone metastasis); pain and symptom control: pharmacological management of pain and other cancer-related symptoms; nutritional support: management of weight loss and malnutrition; psychosocial support: provision of psychological and social support to patients and their families; specialized palliative support: early involvement of an integrated care team [9].

The prognosis for BTT remains poor overall, but is highly dependent on a combination of clinical, pathological, and molecular factors. Follow-up after treatment aims to detect recurrence early and manage treatment-related complications. **The main prognostic factors are: anatomical and pathological:** TNM stage (T stage is a critical factor for GBC), lymph node status (the most significant negative prognostic marker for resected ICC), and surgical resection status (R0 vs. R1); **histological**: tumor grade and presence of lymphovascular or perineural invasion; **clinical**: functional status of the patient and presence of symptoms such as jaundice at diagnosis; **molecular**: presence of certain molecular characteristics.

GBC has a 5-year survival rate of approximately 5%. Early-detected, incident GBC has a 5-year survival rate > 80%. The median overall survival for resected ICC is 26 months, with a 5-year overall survival rate of 26%. The median overall survival for advanced BTT treated with GemCis plus durvalumab is 12.9 months. The median overall survival from diagnosis is 6 months for ICC and 9 months for ECC. The principles of follow-up after curative treatment include clinical examination, periodic imaging (CT/MRI), and laboratory markers (e.g., CA 19-9). These factors together form the overall picture of this complex disease.

DISCUSSION

Gallbladder and bile duct cancers are aggressive malignancies with increasing incidence, especially for intrahepatic cholangiocarcinoma. Epidemiological trends, different risk profiles, and the unique molecular biology of these tumors underpin the current paradigm of multimodal treatment. Although surgery remains the only curative treatment, the integration of immunotherapy and targeted agents based on molecular profiling has been transforming therapeutic approaches and offering new opportunities for patients with advanced disease.

Ethical statements: *The authors declare that no clinical trials were used in the present study.*

The authors declare that no experiments on humans or human tissues were performed for the present study.

The authors declare that no informed consent was obtained from humans, the donors or donors' representatives participating in the study.

The authors declare that no experiments on animals were performed for the present study.

The authors declare that no commercially available immortalized human and animal cell lines were used in the present study.

Use of AI: *AI-based tools, including Google Gemini, were used solely for technical assistance with language editing and text formatting in the preparation of the manuscript. These tools did not contribute to the creation of the scientific content, data analysis, or interpretation.*

Author contributions: *All authors have read and agreed to the published version of the manuscript.*

Data availability: *All of the data that support the findings of this study are available in the main text.*

Funding: *The authors did not receive any financial support from any organization for this research work.*

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