Cancer Management and Research

Elevate Series

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REVIEW

Circulating Tumor DNA in Cholangiocarcinoma: A Precision Oncology Roadmap

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Abstract: Cholangiocarcinoma (CCA) is a rare but aggressive malignancy with a rising global incidence and few therapeutic options for advanced disease. In recent decades, precision oncology for CCA has advanced rapidly, particularly through the development of targeted therapies for patients with actionable genetic alterations. These therapies have markedly prolonged survival and improved other clinical outcomes among patients with unresectable, advanced CCA. The implementation of precision oncology largely depends on detecting genetic mutations to guide patient selection and treatment, using tumor tissue biopsies or liquid biopsies, including circulating tumor DNA (ctDNA) from blood or bile. As a minimally invasive biomarker, ctDNA shows great promise for transforming the clinical management of CCA. This review provides a comprehensive overview of the roles of ctDNA in CCA, including early detection, prognostic stratification, minimal residual disease assessment, recurrence monitoring, therapeutic target identification, and treatment response evaluation. A synthesis of existing studies indicates that bile-derived ctDNA shows superior sensitivity compared with blood-based ctDNA in capturing the genetic profiles and heterogeneity of CCA. We also propose an integrative framework that illustrates how ctDNA profiling can inform diagnosis, treatment, and surveillance across the disease continuum. Because research on ctDNA in CCA remains in its infancy, we discuss current challenges and outline future directions for translating these findings into clinical practice. Collectively, the evidence positions ctDNA—particularly bile-derived ctDNA—as a dynamic tool for real-time genomic profiling, sensitive residual disease detection, and therapy monitoring. This integrative framework provides a roadmap for translating these capabilities into clinical practice, with the potential to enable earlier, more personalized interventions and improve outcomes for patients with CCA.

Keywords: cholangiocarcinoma, circulating tumor DNA, liquid biopsy, Bile ctDNA, targeted therapy

Introduction

Cholangiocarcinoma (CCA) is a heterogeneous malignancy originating from the biliary epithelium and is classified into three anatomical subtypes: intrahepatic (iCCA), perihilar (pCCA), and distal CCA (dCCA)[1]. The global incidence of CCA has been steadily rising, exhibiting geographic variations. For instance, in regions affected by liver fluke infections, such as Southeast Asia, incidence rates can be up to 40 times higher than the global average. Other risk factors beyond liver flukes include primary sclerosing cholangitis (PSC), hepatolithiasis, viral hepatitis, cirrhosis, diabetes, and obesity.[2–4] Despite its relative rarity, accounting for approximately 3% of all gastrointestinal cancers, CCA is known for its highly aggressive nature and poor prognosis, with a median survival of less than 24 months and 5-year survival rates after resection ranging from 11% to 40%, primarily due to high recurrence rates.[5,6] Surgical resection, the only potentially curative option, is feasible in only 20–30% of iCCA patients, and adjuvant chemotherapy offers limited survival benefit.[7] For patients with unresectable CCA, treatment options have expanded beyond the traditional cisplatin-gemcitabine regimen. However, early diagnosis is challenging, mainly because the disease is often asymptomatic in its initial stages. Current diagnostic approaches rely on a combination of laboratory tests (eg, ALT, AST, γ-GT, ALP, serum bilirubin, CEA, CA19-9), imaging (abdominal ultrasound, CT, MRI-MRCP), endoscopy, and histopathology. Nevertheless, these methods collectively achieve a sensitivity of less than 60% for early detection, while specificity remains suboptimal, particularly for serum biomarkers such as CA19-9 and CEA, which are frequently elevated in benign biliary obstruction, cholangitis, and other

https://doi.org/10.2147/CMAR.S574678
Received: 16 October 2025
Accepted: 27 January 2026
Published: 6 February 2026

inflammatory hepatobiliary conditions. Imaging modalities, although indispensable for anatomical assessment and staging, often struggle to distinguish early-stage CCA from benign biliary strictures or inflammatory changes, further limiting diagnostic accuracy.[8] Moreover, tumor tissue biopsies, particularly for hilar lesions, are invasive procedures associated with risks such as bleeding, infection, bile leakage, and pancreatitis, carrying a notable complication rate of 15–20%.[9,10] As such, there is an urgent need for precise biomarkers that can improve diagnostic accuracy while minimizing patient risk.

Liquid biopsy has emerged as a transformative tool in oncology, enabling the minimally or even noninvasive detection of tumor-derived biomarkers—such as cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), RNA, extracellular vesicles, and circulating tumor cells (CTCs)—from blood, urine, or bile.[11] cfDNA comprises DNA fragments released into the bloodstream from both normal and tumor cells. ctDNA, a specific subset of cfDNA, originates from tumor cells[12] and harbors tumor-specific genetic and epigenetic alterations, including point mutations, copy number variations, chromosomal rearrangements, and DNA methylation patterns.[13,14] These alterations provide critical insights for cancer diagnosis, treatment selection, and monitoring. We note that some early or methodological studies used the term "cfDNA" due to the lack of tumor-specific molecular validation. We selectively included certain cfDNA studies, as their findings provide valuable insights into the technical feasibility and clinical translation of ctDNA analysis. A particular challenge in CCA is that its location within the biliary tree results in less ctDNA shedding into the bloodstream compared with tumors in more vascularized organs. Nevertheless, recent technical advances have substantially enhanced the sensitivity of ctDNA detection.[11] Furthermore, the proximity of CCA to the biliary system presents a unique opportunity, as ctDNA can be enriched in bile, potentially offering a more sensitive analytical approach. Several recent reviews have summarized the clinical applications of ctDNA in biliary tract cancers, largely emphasizing plasma-based assays. While bile-derived ctDNA has been mentioned in these reviews, it is often discussed as a supplementary source of tumor DNA, with limited attention to its role in specific clinical contexts.[15–18] In this review, we conducted by synthesizing existing studies on three transformative dimensions of ctDNA in the management of CCA: (1) Bile-derived ctDNA overcomes the spatial bias of plasma sampling and exhibits higher sensitivity than plasma ctDNA in detecting mutations found in matched tumors; (2) Postoperative ctDNA clearance serves as a dynamic predictor of minimal residual disease (MRD), outperforming radiographic detection of relapse by four months; and (3) Real-time monitoring of resistance during targeted therapy using ctDNA-guided adaptive sequencing to inform therapy selection. We emphasize the potential for bile-derived ctDNA to offer additional insights in specific clinical scenarios, particularly where plasma-based assays face inherent limitations. We further propose an integrative framework that synthesizes the emerging roles of ctDNA in CCA. Bridging the gap between scientific evidence and clinical practice may facilitate the transition of ctDNA from an investigational biomarker to a routine component of precision oncology for CCA.[19,20]

Roles of ctDNA in the Clinical Management of Patients with CCA

The integration of ctDNA testing into the management of patients with CCA has ushered in transformative possibilities for precision oncology. The variant allele fraction of baseline mutations can indicate tumor burden, whereas the emergence of new mutations signifies clonal evolution during disease progression and treatment.[21] Its clinical applications in early diagnosis, prognosis, and therapeutic monitoring—all of which enhance precision oncology for CCA—are discussed below.

Diagnosis

Previous studies have demonstrated that ctDNA levels in patients with solid tumors are generally higher than those in healthy controls, even in stage Ia cancer, for which ctDNA has garnered increased interest as a tool for cancer detection and monitoring.[22] For instance, a study comparing plasma cfDNA in patients with CCA and benign liver abscesses revealed that mean concentrations were significantly higher in patients with CCA both preoperatively (4.54 ng/μL) and postoperatively (3.43 ng/μL) than in benign cases (0.60 ng/μL), effectively distinguishing CCA from benign diseases.[23] The diagnostic value of ctDNA was further validated in a large-scale Asian cohort study involving 102 patients with advanced biliary tract cancer (BTC), which included iCCA (49.0%), eCCA (26.5%), and gallbladder cancer (24.5%).[24] This study demonstrated a high concordance between mutations detected in ctDNA via liquid biopsy and matched tumor tissues, with a sensitivity of 84.8% and a positive predictive value of 79.4%.[24] Ettrich et al reported an overall concordance of 74%, reaching 92% in iCCA cases.[21] Another study involving 121 patients (above 85% diagnosed with CCA) found that the concordance of mutations detected in ctDNA was greater when compared with metastatic tissue than with primary tumors, suggesting that ctDNA may

better reflect the molecular profile of advanced disease.[25] However, most of these studies were conducted in patients with late-stage disease, leaving the utility of ctDNA for early CCA detection uncertain.

Beyond mutation profiling, ctDNA methylation signatures are emerging as promising biomarkers for early cancer detection. A retrospective study identified nine methylated DNA markers by comparing methylome differences between frozen CCA samples and adjacent benign biliary epithelium and liver parenchyma, achieving a sensitivity of 76% and a specificity of 94% for CCA detection.[26] Wasenang et al further demonstrated that ctDNA methylation of OPCML and HOXD9 distinguished CCA from benign biliary diseases, with AUC values of 0.850 and 0.789, respectively.[27] JAM3 methylation was also linked to CCA diagnosis (specificity, 96.6%), tumor differentiation, and metastasis.[28] However, some studies, including a recent analysis from The Cancer Genome Atlas, found that certain methylation markers (ie, *BCAT1, IKZF1*, and *SEPT9*) did not effectively differentiate CCA from normal bile duct tissue.[29] These inconsistent findings across studies highlight the need for prospective validation in larger cohorts. Research efforts, such as the Circulating Cell-free Genome Atlas study (ClinicalTrials.gov Identifier: NCT02889978), are ongoing to develop and validate a multi-cancer early detection test using whole genome bisulfite sequencing. In a subset of 46 bile duct cancer cases, this test achieved a high sensitivity of 93.5%, demonstrating potential for early diagnosis and integration into future screening paradigms.[30]

Despite advancements, challenges remain in detecting mutations in cancers with low tumor burden or at initial stages. One study reported only a 50% concordance between ctDNA and primary tumor mutations in stage I–II iCCA patients, much lower than the concordance observed in cases with advanced disease (stage III–IV).[31] Furthermore, elevated ctDNA levels can occur in nonmalignant conditions such as autoimmune disorders, myocardial infarction, or pulmonary thromboembolism, indicating that blood-based ctDNA elevation is not disease-specific.[32] Benign diseases may share mechanisms with cancer, namely, increased cell proliferation that affects the release of cfDNA.[33] Therefore, the integration of tissue and ctDNA sequencing in the clinical evaluation of cholangiocarcinoma should be contextualized. Tissue sequencing remains the gold standard for comprehensive molecular profiling when feasible. However, in clinical scenarios where tissue is insufficient or inaccessible, or where dynamic disease monitoring is required, ctDNA analysis—particularly from bile—may serve as a valuable complementary or alternative approach. In these settings, the potential to obtain actionable molecular information through minimally invasive means may justify the cost of sequencing. To address concerns regarding background signals from non-malignant pathologies, approaches such as prioritizing bile-derived ctDNA, which enriches tumor-specific signals, and focusing on high-specificity biomarkers (eg, tumor-informed mutations or cholangiocarcinoma-specific methylation signatures) may help enhance interpretability and clinical relevance.[34]

Prognosis and Disease Progression

Accumulating evidence supports ctDNA dynamics as a surrogate biomarker for survival outcomes across various solid tumors.[35] For instance, a recent analysis from the IMvigor010 trial indicated that patients with urothelial carcinoma treated with adjuvant atezolizumab who cleared ctDNA had significantly better survival outcomes than those who remained ctDNA positive.[36] Similar trends have been observed in other cancers, where ctDNA clearance has been associated with improved radiologic response and survival, whereas persistent ctDNA positivity correlates with poorer outcomes.[37,38] An extensive cohort study involving patients with advanced BTC, comprising iCCA (49.0%), eCCA (26.5%), and gallbladder cancer (24.5%), showed that the max VAF of ctDNA can serve as a prognostic marker for patients receiving first-line systemic chemotherapy. Elevated max VAF was linked to poorer overall survival and progression-free survival(PFS) in those treated with gemcitabine/cisplatin regimens.[24] Furthermore, Ettrich et al reported that VAF levels reflected tumor burden and predicted PFS in iCCA patients. Notably, 63% of treatment-naïve patients exhibited changes in their mutational profiles during chemotherapy, further supporting the potential of ctDNA in monitoring disease progression.[21] Longitudinal plasma analyses during *FGFR* inhibitor therapy in *FGFR2* fusion–positive iCCA revealed that declining ctDNA levels aligned with clinical benefit and prolonged survival, whereas higher baseline cfDNA was associated with unfavorable outcomes.[39] Moreover, mutations in ARID1A, BAP1, PBRM1, and TP53 have been linked to poor prognosis in various cancers, including CCA. Specifically, ARID1A and PBRM1 mutations, which are involved in the SWI/SNF chromatin remodeling complex and are frequently found in CCA are associated with tumor progression,[40–42] and the presence of PBRM1 mutations in plasma ctDNA has also been correlated with adverse outcomes in CCA patients.[43] While these findings are promising, larger prospective studies are needed to validate these results in CCA.

Notably, most existing studies have focused on the associations between ctDNA concentration, VAF, specific gene mutations, or copy number variations and CCA prognosis. The clinical utility of ctDNA is not yet fully realized. Unlike established prognostic markers such as CA19-9 (cutoff: 176.3 U/mL) and CEA (cutoff: 9.6 ng/mL), the optimal cutoff for ctDNA remains undefined.[44] The heterogeneity of CCA, characterized by variations in tumor biology, molecular profiles, and treatment responses, further complicates the establishment of a universal ctDNA cutoff that accurately reflects prognosis. Therefore, large-scale clinical validation studies are essential to determine optimal ctDNA thresholds and solidify its role as a reliable prognostic biomarker in clinical practice.

MRD and Recurrence Monitoring

Patients with CCA have been reported to experience a notably high recurrence rate, reaching 60–70% even among those undergoing radical surgery, primarily attributed to MRD.[45] Accurately predicting recurrence risk and optimizing surveillance strategies are critical areas of research in CCA. A recent extensive real-world study found that patients with postoperative ctDNA-positive BTC had a median recurrence-free survival (RFS) of only 6.6 months, whereas ctDNA-negative patients did not reach a median RFS (HR=26, $P<0.0001$). Among patients with confirmed recurrence, ctDNA detected molecular relapse an average of 3.7 months earlier than radiographic imaging (range: 0.5–10.1 months), with 68.8% of cases showing ctDNA positivity while imaging remained negative. This lead time offers a critical window for early therapeutic intervention.[46] Further insights emerged from a sub-analysis of the Phase II STAMP trial, which assessed the predictive value of ctDNA in CCA patients receiving adjuvant chemotherapy.[47] Using the personalized Signatera assay, ctDNA was measured at multiple time points. Patients who tested positive for ctDNA prior to adjuvant chemotherapy tended to have shorter RFS (HR=1.7, 95% CI: 0.98–2.8, $P=0.069$) than ctDNA-negative patients. Notably, all patients who remained ctDNA-positive during adjuvant treatment experienced clinical recurrence with significantly shortened RFS, although the limited sample size may have reduced statistical power.[47] Building on this, subsequent research using a personalized 16-plex PCR-NGS assay confirmed the prognostic value of longitudinal ctDNA monitoring. Patients with ctDNA positivity before or during adjuvant chemotherapy (at 12 and 24 weeks) showed significantly worse DFS. Conversely, serial ctDNA negativity correlated with prolonged DFS, establishing ctDNA dynamics as a reliable biomarker for recurrence risk and a potential tool to guide postoperative clinical decisions.[48] A case study by Yu et al demonstrated the utility of ctDNA for detecting molecular recurrence in a patient with stage 3A iCCA before any radiographic or serologic changes. Based on rising ctDNA levels and the tumor's microsatellite instability–high and high tumor mutational burden profiles, the patient was treated with pembrolizumab, resulting in ctDNA clearance and sustained remission for more than two years. This case highlights the potential of ctDNA to inform timely intervention and individualized therapy in CCA management.[49]

With the growing recognition of ctDNA-based liquid biopsy as a dynamic biomarker for monitoring recurrence, several clinical studies have been initiated. Notably, an ongoing clinical trial (ClinicalTrials.gov Identifier: NCT06474091) at the Mayo Clinic in the United States is investigating a novel blood-based assay for detecting measurable residual disease or early recurrence/progression in patients with CCA.

Therapeutic Target Identification and Response Evaluation

The identification of actionable mutations in CCA is critical for selecting patients who may benefit from targeted therapies. Molecular profiling of BTC through NGS has uncovered potentially targetable genetic alterations in more than 40% of cases,[50,51] including *FGFR2* and NTRK fusions, IDH1 and BRAF mutations, HER2 amplification, and MSI.[52,53] Of these, *FGFR2* fusions are particularly relevant to iCCA, occurring in approximately 10–15% of patients.[54] *FGFR* inhibitors, such as pemigatinib and futibatinib, have shown promising clinical efficacy and received accelerated approval from the US Food and Drug Administration (FDA) for advanced CCA.[55,56] ctDNA testing offers a minimally invasive approach to identify these genetic alterations, facilitating the initiation of precision therapy and the evaluation of treatment response in patients with CCA.

Studies indicate that ctDNA testing is a feasible and valuable tool for iCCA patients, offering insights into clonal evolution and resistance mechanisms. A prior study suggested that strategic sequencing of *FGFR* inhibitors, guided by serial biopsies and ctDNA analysis, may prolong the duration of benefit from *FGFR* inhibition in patients with *FGFR2* fusion–positive iCCA.[57] However, ctDNA-based detection of *FGFR2* fusions remains technically challenging, with a sensitivity as low as 18%.[58] Notably, recent research has demonstrated that ctDNA can identify novel *FGFR2* fusion

partners—such as *FGFR2*-TNS1—that are not captured by conventional tissue-based NGS panels.[24] Moreover, ctDNA enables real-time assessment of treatment response and early identification of resistance mutations. For instance, a retrospective study identified *FGFR*2 fusions in the plasma of 88.9% of iCCA patients and found that 82% of those receiving *FGFR* inhibitors developed polyclonal resistance, most commonly involving the N549H mutation. Notably, resistant mutants were detectable in the plasma up to seven months before disease progression. To address resistance, next-generation *FGFR* inhibitors such as derazantinib are under clinical development.[39]

IDH1/2 mutations are present in approximately 30% of CCA patients and represent potential molecular targets for therapeutic intervention.[59] Several studies have confirmed that *IDH* mutation status identified by ctDNA is highly concordant with findings from tumor tissue and that lower ctDNA levels correlate with a longer time to treatment failure, supporting its role in monitoring therapeutic response.[60,61] These and other clinical findings have led to the development and approval of the *IDH1* inhibitor ivosidenib for previously treated, *IDH1*-mutated advanced CCA.[62] MSI–H or deficient mismatch repair (dMMR) status—detectable via ctDNA profiling—is recognized as a tumor-agnostic predictor of response to immune checkpoint inhibitors (ICIs). In two advanced CCA cases treated with pembrolizumab for MSI–H status, both patients showed persistent increases in ctDNA levels despite chemotherapy, indicating tumor progression.

However, after confirming MSI-H status via both tissue and liquid biopsy, the patients were switched to pembrolizumab, resulting in a significant reduction in ctDNA levels, suggesting effective tumor control or regression.[49,63] Currently, pembrolizumab has received tumor-agnostic approval for advanced or recurrent MSI-H/dMMR solid tumors that have progressed following standard chemotherapy.[64] MSI testing is now part of the National Comprehensive Cancer Network (NCCN) guidelines for the diagnosis and treatment of various malignancies, including CCA.[43,65] Tumors with MSI-H status often have a high tumor mutational burden (TMB), a molecular feature associated with better responses to ICIs.[66] However, MSI-H is rare in BTC, occurring in approximately 2% of cases. Most BTCs lack MSI-H or dMMR status. Moreover, not all MSI-H BTC patients benefit from ICI therapy, and iCCA is typically characterized by low TMB. A genomic profiling study of 6,130 patients with iCCA found a median TMB of 1.7 mutations per megabase (mut/Mb), with only 3.7% of patients having TMB ≥10 mut/Mb, classified as TMB-high.[44] Despite the rarity of high TMB in iCCA, assessing TMB may still provide valuable insights for predicting the potential benefit of immunotherapy. A pooled literature analysis revealed a significant correlation between the objective response rate (ORR) to PD-1/PD-L1 inhibitor monotherapy and median TMB across various tumor types, including CCA (P=0.0019).[67] For patients who are not MSI-high, current NCCN guidelines recommend nivolumab as a second-line treatment for unresectable or metastatic BTC that progresses despite standard therapies, provided that the tumor is neither MSI-high nor has high PD-L1 expression (Class 2B recommendation).[68]

Recent advances in molecular profiling and ctDNA biomarker detection have enabled numerous clinical trials investigating targeted therapies in CCA. These trials not only validate therapeutic targets but also provide critical data for evaluating treatment responses. Several important studies have demonstrated the clinical efficacy of agents targeting *FGFR2, IDH1, BRAF*, and *HER2* alterations. For instance, the FIGHT-202 phase II trial showed that pemigatinib achieved an ORR of 35.5% in patients with *FGFR2* fusion–positive iCCA, leading to its accelerated FDA approval.[55] Similarly, the Phase III ClarIDHy trial demonstrated a significant PFS benefit for ivosidenib compared with placebo in patients with advanced *IDH1*-mutated CCA.[69] Additionally, early-phase trials have reported promising results for *HER2*-directed therapies and *BRAF/MEK* inhibitors in patients with HER2 amplification or $BRAF^{V600E}$ mutations.[70] These findings highlight the importance of molecular profiling and ctDNA-based biomarker detection in guiding treatment decisions.

Enhanced Sensitivity of Bile-Based ctDNA: Addressing Spatial Bias in Blood-Based Liquid Biopsy for CCA

Bile has emerged as a promising alternative source of ctDNA for CCA, outperforming blood-based ctDNA, which is often low in abundance and highly fragmented, thereby reducing detection sensitivity and accuracy. In contrast, bile-derived ctDNA consists predominantly of longer DNA fragments with superior structural integrity compared with highly fragmented plasma ctDNA.[71–74] Moreover, both intratumoral and intertumoral heterogeneity in CCA exacerbate the spatial sampling bias inherent to plasma ctDNA analysis. For example, a study involving 1,671 patients with advanced CCA found only 18% concordance between plasma ctDNA and tissue samples for *FGFR2* fusion detection.[58] Subsequent studies have consistently shown that bile ctDNA

exhibits markedly higher sensitivity for detecting tumor-matched mutations compared with plasma. In a cohort of 42 CCA patients, the concordance of *KRAS* mutations between bile ctDNA and matched tissue was 80%, considerably greater than the 42.9% concordance between plasma ctDNA and matched tissue.[75] Gou et al performed comprehensive mutation profiling of tissue DNA, bile cfDNA, and plasma cfDNA from 28 CCA patients and demonstrated significantly higher mutation concordance in bile than in plasma (99.1% vs 78.3%, $P<0.0001$).[76] Beyond its potential for early diagnosis, bile ctDNA also holds prognostic value. Monitoring the *KRAS* mutation burden in bile ctDNA correlated significantly with patient survival ($P=0.018$), highlighting its clinical relevance.[75] Table 1 summarizes recent advances in bile ctDNA research for CCA, findings that have led the British Society of Gastroenterology to emphasize the promise of bile cfDNA testing for CCA diagnosis and management.[77,78] Despite its potential, the clinical translation of bile-derived ctDNA faces several challenges, including technical hurdles in sample processing and practical considerations related to bile sampling itself. Current extraction methods are constrained by limited sample volume and variability in DNA purity and quality.[79] Concurrently, the clinical feasibility of bile collection must also be considered. Bile sampling is an invasive procedure, typically performed during endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic biliary drainage (PTBD), interventions primarily indicated for diagnosing or alleviating biliary obstruction. Therefore, its application is considered more justified and practical in patients already scheduled for such procedures, as it can provide molecular insights without introducing additional invasive risks.[80] Key limitations include its unsuitability for screening or cases with low clinical suspicion, variability in sample quality, and the difficulty of obtaining samples in early-stage disease without biliary dilation. Collectively, these factors hinder its broad clinical adoption. To address these limitations, related technologies continue to be refined. For example, recent methodological improvements include the development by Shen et al of an efficient silica membrane-based technique for bile cfDNA extraction, which offers strong technical support for implementing bile cfDNA as a liquid biopsy biomarker in CCA diagnosis.[81]

The advantages of bile-derived ctDNA are likely attributable to its anatomical proximity to tumors, localized DNA release, and enhanced biological stability within the biliary environment. Tumor DNA is shed directly into bile via apoptosis or active

Table 1 Recent Studies of Bile-Based cfDNA/ctDNA in CCA

First Author, Year [Ref]	Study Design	Testing Method	Sample Size (n)	Detection Rate in Bile (%)	Tissue-Bile Concordance (%)	Key Findings (Bile cfDNA/ctDNA vs Plasma)
Ito et al, 2024[75]	Prospective	NGS (multigene panel) and ddPCR	BTC: 24 (CCA: 17)	58.8	NA	Positive driver mutations detected in 13/24 (54%) vs 4/24 (17%) in plasma.
Yin et al, 2024[76]	Prospective	WGS	CCA: 40	*TP53*: 27.3%; *KRAS*: 9.1%	NA	NA
Ohyama et al, 2023[78]	Retrospective	NGS (60-gene panel)	Pancreatobiliary: 87 (CCA: 59)	55	NA	Oncogenic mutations detected in 21/38 (55%) vs 9/38 (24%) in plasma ($P=0.005$).
Li et al, 2022[77]	Retrospective	NGS (425-gene panel)	BTC: 13 (CCA: 6)	84.6	90	Median cfDNA concentration: 1918 ng/mL vs 63.1 ng/mL in plasma.
Hans et al, 2021[70]	Prospective	ddPCR (*KRAS*)	BTC: 42 (CCA: 41)	48	80	*KRAS* mutation concordance with tissue: 80.0% vs 42.9% in plasma.
Guo et al, 2021[71]	Prospective	NGS (520-gene panel)	BTC: 28 (CCA: 16)	71.4	99.1	Overall SNV/indel concordance with tissue: 99.1% vs 78.3% for plasma ($P < 0.0001$).
Driescher et al, 2020[68]	Prospective	NGS (qPCR for *KRAS*)	PDAC: 58 (CCA: 12)	NA	96.2	Detection of tissue-matched mutations: 96.2% vs 31.6% in plasma.
Shen et al, 2019[79]	Prospective	Targeted deep sequencing (150-gene panel)	BTC: 10 (CCA: 6)	SNV: 48%; CNV: 43%; Indel: 9%	Sensitivity for CNV: 75%; Specificity:98.9%	NA

Abbreviations: cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; BTC, biliary tract cancer; CCA, cholangiocarcinoma; WGS, whole-genome sequencing; CNV, copy number variation; SNV, single-nucleotide variation; ddPCR, digital droplet polymerase chain reaction; qPCR, quantitative polymerase chain reaction; KRAS, Kirsten rat sarcoma viral oncogene; PDAC, pancreatic ductal adenocarcinoma; NA, not available.

secretion in localized CCA cells, allowing enrichment of long DNA fragments and avoiding systemic degradation. In contrast, blood-based ctDNA is rapidly cleared by the liver, with a half-life of minutes to 1–2 h, and undergoes enzymatic fragmentation, which can dilute tumor-specific signals below detectable levels in early-stage disease.[82] Moreover, anatomical barriers within the biliary tract prevent hepatic-renal clearance, whereas mucin hydrogels (eg, MUC5AC/MUC4) and bile acid-mediated S1PR2/ERK1/2/AKT signaling further stabilize bile-derived ctDNA, enriching its concentration and diagnostic utility.[83–85] Collectively, these factors position bile ctDNA as a highly sensitive biomarker for CCA, particularly for early detection and for monitoring localized malignancies. Figure 1 schematically summarizes the distinct release routes, molecular forms, and clearance characteristics of bile- versus plasma-derived ctDNA in cholangiocarcinoma.

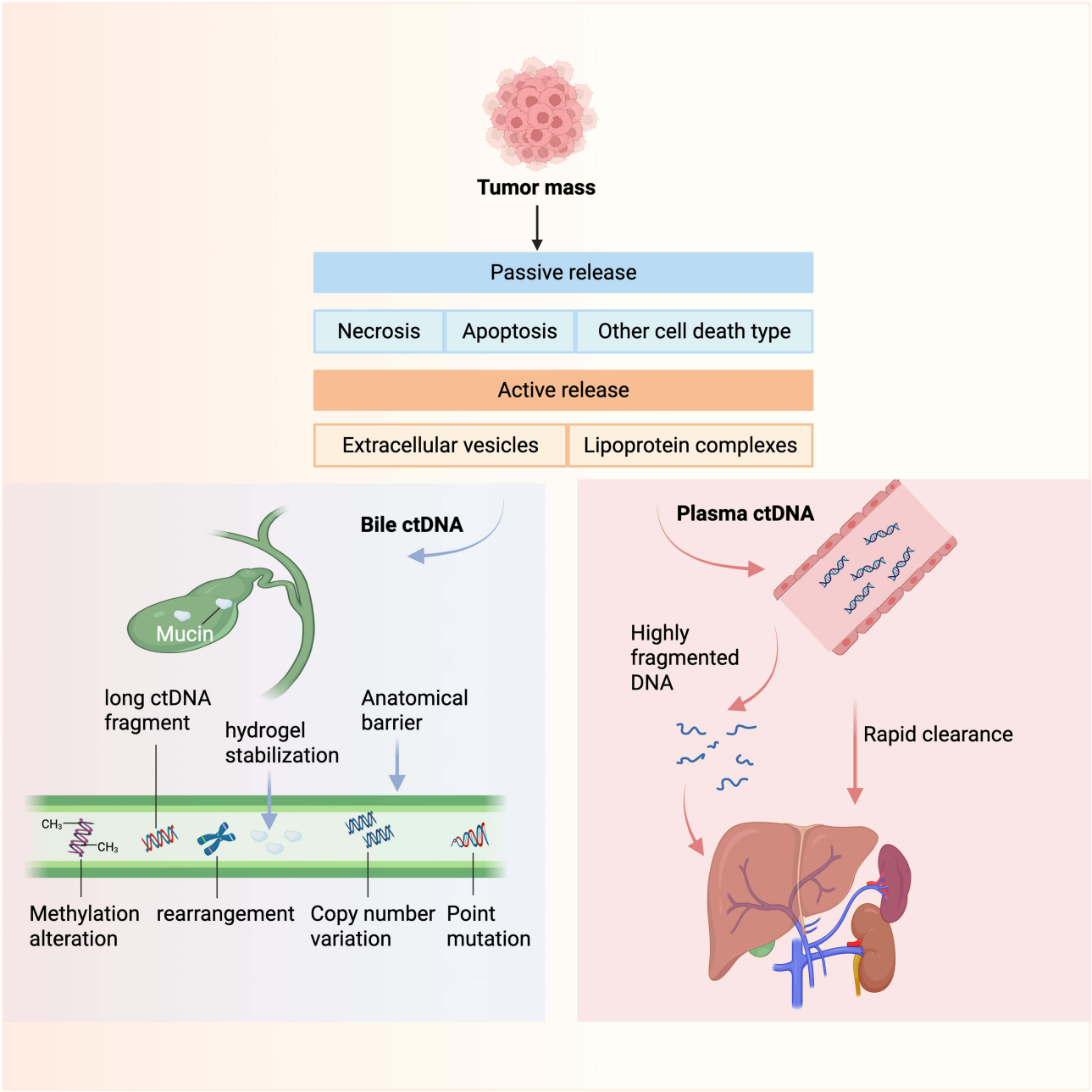

Figure 1 Differential release and clearance characteristics of bile- versus plasma-derived ctDNA in CCA. Created in https://BioRender.com. This figure provides a schematic overview of the sources, molecular characteristics, and clearance behaviors of bile- and plasma-derived ctDNA. Tumor cells release ctDNA through passive (eg, apoptosis or necrosis) or active mechanisms. In bile, ctDNA is enriched in longer and more intact DNA fragments, and its stability is enhanced by anatomical compartmentalization and biliary components such as mucins. In contrast, plasma-derived ctDNA is highly fragmented and undergoes rapid systemic clearance via hepatic, renal, and splenic pathways as well as circulating nucleases, resulting in reduced stability and lower detection sensitivity, particularly in early or localized disease.
Abbreviations: CCA, cholangiocarcinoma; ctDNA, circulating tumor DNA.

https://doi.org/10.2147/CMAR.S574678

In summary, existing evidence reveals the multifaceted potential of ctDNA in the diagnosis and treatment of CCA. Figure 2 provides a comprehensive overview, illustrating how ctDNA-based liquid biopsy can be embedded across the entire CCA management continuum. Given the unique anatomical location of CCA, adjacent bile serves as an ideal source for enriching ctDNA, providing a key solution to overcome the limited sensitivity of plasma ctDNA. To translate these scientific findings into a clear logic for clinical practice, we propose an integrative framework (Figure 3). This framework serves as a visual roadmap, organizing the existing evidence and provides a structure to navigate the potential clinical applications of ctDNA across the disease continuum.

The clinical value of this framework lies in its use of minimally invasive liquid biopsy, with bile prioritized because of its higher sensitivity, to obtain critical molecular information, thereby reducing the number of patients who require an initial invasive tissue biopsy solely for genomic profiling. The specific logical pathway is illustrated in Figure 2B. It begins with an assessment of tissue sufficiency based on tumor cellularity and DNA quality. If tissue is sufficient, tissue-based molecular testing is performed to guide targeted therapy. For cases of tissue insufficiency—resulting from an initial biopsy attempt failing for technical reasons (eg, suboptimal puncture site, scant sample volume) or where the obtained sample has tumor cellularity/DNA quality that does not meet testing standards—the next recommended step is liquid biopsy.[86] Indeed, liquid biopsy is also suitable for the following clinical scenarios: direct biopsy is difficult or poses a high risk; the patient has insufficient, inadequate, or exhausted solid tissue; complementary information to prior or future tissue testing is needed; disease progression or resistance mutations are suspected.[87] A positive liquid biopsy result can directly inform the initiation of genotype-matched therapy (eg, FGFR2 or IDH1 inhibitors). For a negative liquid biopsy result, comprehensive clinical assessment is required. If clinical suspicion for CCA remains high based on a composite assessment, a repeat biopsy using an alternative technique or site can be considered. This may include procedures such as endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) or brushing via a PTBD catheter, ideally performed by a more experienced operator or targeting a new radiographic lesion. This indicates that a "rebiopsy" is not a default step but a context-dependent option, pursued only when the minimally invasive pathway fails to provide answers and the diagnostic imperative persists. Conversely, if clinical suspicion is low or biopsy is not feasible, alternative strategies such as empiric therapy or supportive care are considered. Importantly, the framework encompasses dynamic monitoring and adaptive treatment. After therapy initiation, patients enter a phase of ctDNA-based recurrence monitoring, with bile remaining the preferred source when available. This enables the early detection of molecular relapse, allowing for treatment adjustment prior to radiographic progression. In cases of confirmed progression—such as ctDNA conversion to positivity or persistently rising levels—the pathway cycles back.

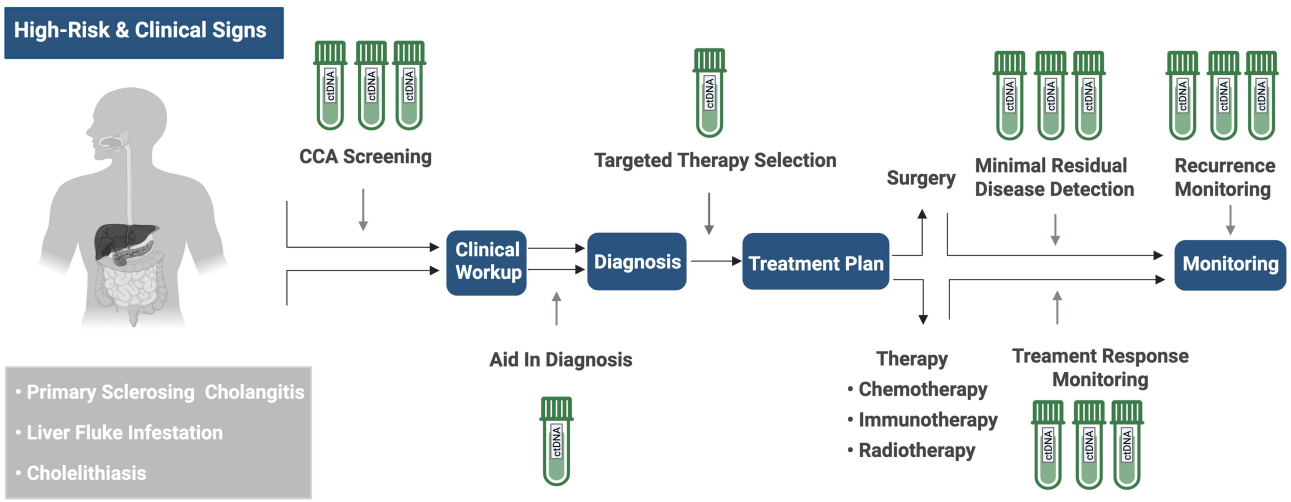

Figure 2 Clinical overview of ctDNA-based liquid biopsy applications in CCA. Liquid biopsy is utilized for: (1) routine CCA screening in high-risk populations; (2) diagnostic support in patients with clinical signs suggestive of CCA; (3) selection of targeted therapies based on tumor-specific mutational profiles; (4) minimal residual disease detection following curative-intent surgery; (5) longitudinal monitoring of treatment response during systemic therapy; and (6) surveillance for disease recurrence after complete remission or presumed cure. Single-test liquid biopsy is mainly applied for diagnostic support and targeted therapy selection, whereas serial liquid biopsies are used for screening, treatment response monitoring, minimal residual disease detection, and recurrence surveillance.
Abbreviations: CCA, cholangiocarcinoma; ctDNA, circulating tumor DNA.

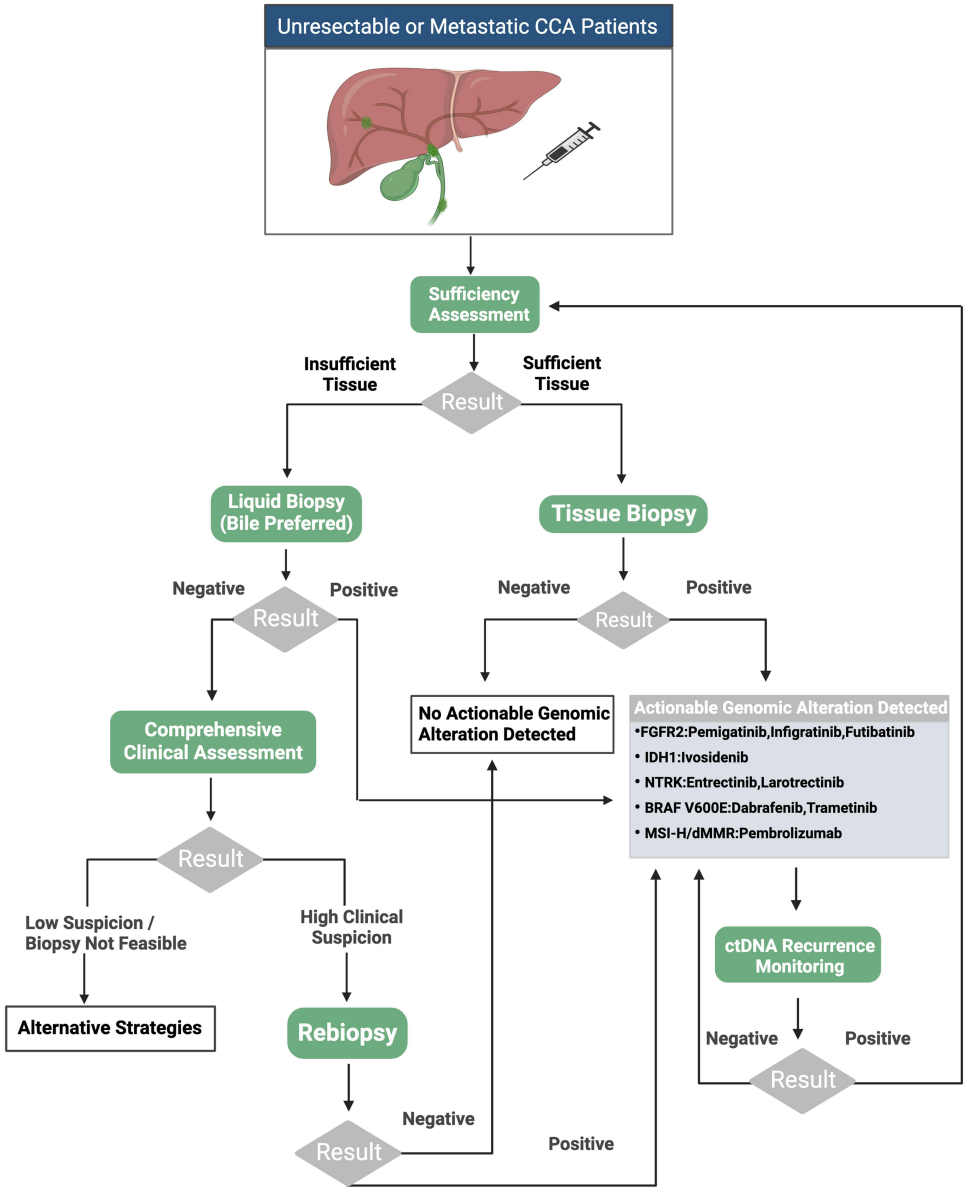

Figure 3 The Integrative Framework for ctDNA in CCA Management: This decision pathway begins with an assessment of tissue sufficiency. If tissue is adequate, tissue-based profiling guides therapy. For cases of tissue insufficiency or when tissue is inaccessible, liquid biopsy (prioritizing bile) is recommended. A positive result directly informs targeted therapy. A negative liquid biopsy triggers a comprehensive clinical reassessment. If suspicion remains high, an optimized repeat biopsy (eg, EUS-FNA) may be considered; if low, alternative strategies are pursued. Post-treatment, patients enter a ctDNA surveillance phase for early relapse detection and therapy adaptation, with confirmed progression leading back to reassessment.
Abbreviations: CCA, cholangiocarcinoma; ctDNA, circulating tumor DNA.

This can involve rebiopsy (tissue or liquid) to identify resistance mechanisms and re-engage the molecularly-guided treatment cycle, or consideration of early treatment adjustment.

In conclusion, this framework elucidates how ctDNA can be pragmatically integrated into CCA management to enable a more personalized, responsive, and less invasive paradigm.

Current Challenges and Future Perspectives

Despite the promising clinical potential of ctDNA in CCA, several critical challenges must be addressed to enable its routine clinical application. A major obstacle is the inherently low abundance of ctDNA in CCA, particularly in early-stage disease or small metastatic lesions, where tumor shedding is minimal. This low abundance leads to suboptimal

https://doi.org/10.2147/CMAR.S574678

sensitivity and increases the risk of false negatives. Additionally, ctDNA detectability varies widely among patients due to tumor heterogeneity and poorly understood biological mechanisms governing DNA release, further complicating interpretation. Another key challenge is the absence of universally applicable biomarkers. Although mutations in genes such as *IDH1, KRAS*, and *TP53* are frequently observed, their prevalence and patterns differ across CCA subtypes, so ctDNA may not comprehensively capture the full mutational landscape of the tumor. Identifying robust, subtype-specific, and dynamically responsive ctDNA markers is urgently needed to enhance diagnostic precision and therapeutic guidance. From a technical standpoint, although advancements in digital PCR and NGS have improved ctDNA analysis, issues related to sample quality, pre-analytical handling, and bioinformatic complexity continue to constrain overall reliability. Eliminating background noise and improving quantification accuracy remain key obstacles, particularly for low-frequency variants.

Looking ahead, the integration of novel technologies—such as methylation profiling, fragmentomics, and bile-derived ctDNA—has the potential to overcome current sensitivity limitations and address spatial sampling bias. Furthermore, longitudinal ctDNA monitoring, coupled with machine-learning algorithms, may facilitate real-time treatment adjustments and the early prediction of recurrence. Ultimately, unlocking the full potential of ctDNA in precision oncology for CCA will require continued innovation in assay design, biomarker discovery, and clinical validation through large-scale, prospective studies.

Conclusion

Current evidence supports ctDNA as a promising biomarker with the potential to enhance precision oncology for CCA. This review summarizes existing evidence on the roles of ctDNA in CCA, highlighting its utility as a minimally invasive or noninvasive diagnostic and monitoring tool. Notably, bile-derived ctDNA may offer higher sensitivity than blood-based ctDNA in capturing the genetic profiles and heterogeneity of CCA. The proposed integrative framework illustrates how ctDNA profiling may be considered across key stages of CCA management, including diagnosis, treatment selection, and longitudinal monitoring. Future research and well-designed clinical trials are needed to validate the clinical utility and integration of ctDNA for patients diagnosed with or at high risk of this devastating disease.

Disclosure

The authors report no conflicts of interest in this work.

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