

REVIEW

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Circulating tumor DNA–guided early detection and minimal residual disease monitoring in pancreatic and biliary cancers: evidence, barriers, and opportunities

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) and biliary tract cancers (BTCs) are characterized by dismal prognoses, largely because effective strategies for early detection are lacking and post-surgical relapse is common. Liquid biopsy—particularly circulating tumor DNA (ctDNA) analysis—offers a minimally invasive and highly specific approach to reshape the clinical management of these malignancies. In this review, we synthesize recent advances in ctDNA applications across two ctDNA-guided scenarios: early diagnosis/screening and postoperative minimal residual disease (MRD) monitoring. We trace the evolution of ctDNA detection technologies from digital PCR (ddPCR) to next-generation sequencing (NGS)–based tumor-informed and tumor-agnostic assays, and summarize key prospective and retrospective studies in pancreatobiliary tumors. A central feature of our article is the side-by-side comparison of PDAC and BTCs, highlighting shared technical barriers—low ctDNA yield, stromal desmoplasia, clonal hematopoiesis—and their distinct genomic landscapes (PDAC: *KRAS*, *TP53*, *CDKN2A*, *SMAD4*; BTCs: *IDH1/IDH2* mutations, *FGFR2* fusions, *ERBB2* or *BRAF* alterations), which together influence assay design and clinical interpretability. Finally, we outline clinical opportunities, including serial ctDNA-based risk stratification, ctDNA-informed adjuvant escalation/de-escalation, and the integration of multi-omics data, standardized reporting, and artificial intelligence (AI)–assisted signal analysis to improve sensitivity in low-shedding tumors. This framework aligns ctDNA technology with real-world clinical decision points in pancreatic and biliary cancers.

Keywords CtDNA, Pancreatic cancer, Cholangiocarcinoma, Early diagnosis, Minimal residual disease

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Introduction

The grim clinical landscape of pancreatic and biliary cancers

Pancreatic cancer and BTCs are among the most aggressive malignancies of the digestive system, posing a formidable challenge to global public health [1–3]. According to the latest GLOBOCAN 2022 data, pancreatic cancer ranks 12th in global cancer incidence but is the 6th leading cause of cancer-related mortality, with over 510,000 new cases and 467,000 deaths in 2022. Its nearly 1:1 incidence-to-mortality ratio underscores its extreme lethality [1]. Projections indicate that pancreatic cancer will become the second leading cause of cancer-related death by 2030 [4]. BTCs, though relatively rare with approximately 8,000 new cases annually in the United States, are similarly associated with a poor prognosis, with 5-year survival rates ranging from only 5% to 15% depending on the stage [3, 5]. BTCs represent a heterogeneous group of diseases, including intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), and gallbladder cancer (GBC), each with unique epidemiological features and risk factors [3, 6].

The dismal clinical outcomes for both malignancies are fundamentally rooted in their diagnosis at advanced stages and extremely high rates of postoperative recurrence. Only about 10–20% of PDAC patients are diagnosed at an early, surgically resectable stage [7]. However, even after curative-intent surgery, over 80–90% of PDAC patients and 60–70% of cholangiocarcinoma (CCA) patients will experience tumor recurrence, often due to micrometastases that are undetectable by conventional imaging at the time of surgery [8–11]. This stark clinical reality underscores the urgent need for more effective tools for early diagnosis and postoperative surveillance to improve patient survival.

Limitations of current diagnostic and monitoring tools

The standard clinical tools for the diagnosis and monitoring of PDAC and BTCs have significant limitations, which contribute to the aforementioned “diagnostic blind spot” [12, 13].

Serum biomarkers (CA19-9)

Carbohydrate antigen 19 – 9 (CA19-9) is currently the only routinely used serum biomarker, but its clinical utility is constrained by several factors [12, 14]. First, its diagnostic sensitivity (79–81%) and specificity (82–90%) in symptomatic patients are suboptimal [12, 15]. More importantly, its extremely low positive predictive value (0.5–0.9%) in the general population renders it unsuitable for screening asymptomatic individuals [12]. Second, its effectiveness is severely hampered by biological factors. Approximately 5–10% of the population has a Lewis antigen-negative genotype and cannot synthesize CA19-9,

leading to false-negative results [12, 16, 17]. Furthermore, benign conditions causing biliary obstruction, such as cholangitis or choledocholithiasis (gallstones in the bile duct), are common in patients with suspected PDAC and BTCs and can cause significant elevations in CA19-9 levels, with false-positive rates as high as 10–60% [12, 18].

Imaging (CT/MRI)

Multiphase contrast-enhanced CT and MRI are the gold standards for diagnosis and staging but have limited ability to detect early-stage lesions [13]. Small pancreatic tumors are conventionally defined in the literature as lesions with a maximum diameter ≤ 2 cm, corresponding to early-stage pancreatic ductal adenocarcinoma. Owing to their iso-dense or iso-intense appearance relative to normal pancreatic tissue and subtle imaging features, such tumors may be missed on conventional imaging in up to 40% of early-stage cases [13, 19]. Imaging also faces challenges in assessing the response to neoadjuvant therapy, as post-treatment fibrotic scar tissue is difficult to distinguish from residual tumor, often leading to an overestimation of residual disease and affecting surgical decision-making [13]. Additionally, imaging techniques can sometimes struggle to differentiate malignant tumors from benign inflammatory conditions such as focal pancreatitis or autoimmune pancreatitis [20, 21].

These limitations collectively create a dangerous “diagnostic blind spot.” An early, iso-dense pancreatic tumor in a Lewis antigen-negative patient could evade detection by both CA19-9 and CT until the disease has progressed to an advanced stage. This is precisely the gap that molecular detection technologies based on core tumor biology, such as ctDNA analysis, are poised to fill.

Liquid biopsy and ctDNA: a paradigm shift in oncology

Liquid biopsy is a revolutionary technique that enables the non-invasive acquisition of tumor information through the analysis of tumor-derived materials in bodily fluids, primarily blood [22]. Its analytes include circulating tumor cells (CTCs), exosomes, and the focus of this review—ctDNA [23, 24].

CtDNA consists of DNA fragments released into the circulatory system by tumor cells through apoptosis, necrosis, or active secretion [13, 22, 25]. These fragments are typically short (approximately 140–160 base pairs) and carry the same genetic mutations and epigenetic modifications as the primary and metastatic tumors [22, 25, 26]. The half-life of ctDNA in the blood is extremely short (often less than 2 h), making it a highly dynamic biomarker that can reflect real-time changes in the tumor genome—an advantage unmatched by traditional tissue biopsies [22, 25]. This dynamic nature gives ctDNA immense potential for monitoring treatment response,

predicting recurrence, and uncovering mechanisms of drug resistance [27].

Scope and aims of this review

Given the unprecedented pace at which research evidence on ctDNA in PDAC and BTCs is accumulating, this review aims to systematically synthesize and evaluate the latest advances from the past 2–3 years [28]. Its primary objectives are to:

1. Summarize the cutting-edge technologies and principles for ctDNA detection [29].
2. Critically appraise the clinical evidence for ctDNA in early diagnosis and MRD monitoring [29, 30].
3. Provide the first side-by-side comparative analysis of ctDNA applications in PDAC and BTCs, exploring their shared challenges (e.g., low tumor burden) and unique opportunities (e.g., different driver gene landscapes) [31].
4. Identify the current barriers to clinical translation and offer a forward-looking perspective on future research directions [32].

Methodologies for ctDNA detection and analysis

The extremely low abundance of ctDNA in the blood, often constituting less than 0.01% to 10% of total circulating cell-free DNA (cfDNA), places stringent demands on the sensitivity and specificity of detection technologies. The choice of the most appropriate technique is dictated by the clinical question at hand (e.g., tracking a known mutation versus discovering an unknown signal).

PCR-Based methods: from DdPCR to beaming

Droplet ddPCR

ddPCR technology achieves absolute quantification of target DNA molecules without a standard curve by partitioning a single sample into tens of thousands of independent, nanoliter-sized droplet reactions [33]. Its key advantages include exceptional sensitivity (detecting variant allele frequencies, VAFs, as low as 0.01%), relatively low cost, and rapid turnaround time, making it an ideal tool for tracking known, high-frequency hotspot mutations, particularly in the context of MRD monitoring [34]. In PDAC research, PCR-based approaches including digital droplet PCR (ddPCR) and competitive allele-specific PCR (castPCR) have been successfully applied to detect common driver mutations such as *KRAS*, *TP53*, *SMAD4*, and *CDKN2A* in tumor tissue and plasma [35, 36].

BEAMing (Beads, Emulsions, Amplification, and Magnetics)

BEAMing is another ddPCR technique that combines emulsion PCR with flow cytometry [37]. This technology also offers high sensitivity (VAF detection limit of

0.01–0.02%) and was one of the first liquid biopsy technologies to be clinically validated, especially for detecting mutations in colorectal cancer [38]. BEAMing allows for the precise quantification of mutant allele fractions, enabling the dynamic monitoring of tumor burden and treatment response [38].

NGS-Based methods: Targeted, Exome, and Whole-Genome (WGS) sequencing

Targeted gene panel sequencing

These NGS methods focus on a predefined set of genes associated with specific cancers. By concentrating sequencing depth on these regions of interest, targeted sequencing strikes a balance between breadth and depth, achieving high sensitivity. It is more cost-effective and has a faster turnaround time compared to whole-exome(WES) or WGS sequencing [39, 40]. Panels designed for PDAC and BTCs typically cover key driver genes such as *KRAS*, *TP53*, and *SMAD4* [41], as well as BTC-specific alterations like *IDH1* mutations and *FGFR2* fusions [3, 42].

WES and WGS sequencing

WES and WGS sequence all protein-coding regions and the entire genome, respectively. They are powerful research tools for identifying novel mutations and providing a panoramic view of the tumor genome [43]. However, their clinical application in ctDNA analysis is limited by high costs, complex data analysis, and, most critically, insufficient sequencing depth to reliably detect low-VAF mutations unless ctDNA concentrations are very high [44].

Tumor-informed vs. tumor-agnostic strategies

This is a central distinction in the application of NGS [45]. A “tumor-agnostic” (or plasma-first) approach uses a standardized gene panel to screen plasma samples directly, without prior knowledge of the tumor’s genetic makeup. In contrast, a “tumor-informed” strategy first involves sequencing the patient’s primary tumor tissue (often with WES) to identify a unique “fingerprint” of clonal genetic mutations. A personalized, highly sensitive multiplex PCR or capture probe assay is then designed based on this fingerprint to precisely track these specific mutations in the plasma [46]. This customized approach dramatically increases the sensitivity of MRD detection and has shown superior performance in recent studies of PDAC [47] and BTCs [48].

Beyond sequence: the role of epigenetic markers and fragmentomics

Methylation profiling

Aberrant DNA methylation is a universal feature of cancer and a powerful biomarker [49]. Because methylation

patterns are highly tissue-specific, analyzing the methylation profile of cfDNA can not only indicate the presence of cancer but also help infer its tissue of origin [50]. In studies of PDAC and BTCs, specific methylation signatures in plasma ctDNA have been shown to distinguish cancer patients from healthy controls with high accuracy [51–53].

Fragmentomics

This emerging field analyzes the genome-wide patterns of cfDNA fragment sizes, end-sequence motifs, and positional information to detect cancer [54]. Studies have found that ctDNA fragments are generally shorter than cfDNA from healthy cells, and their breakage patterns are non-random, reflecting the nucleosome positioning and open chromatin states of the originating tumor cells [55]. These unique “fragmentomic” features provide a cancer signal that is independent of specific genetic mutations, offering a new avenue for early detection, especially for tumors with low mutation burdens or low ctDNA shedding rates [55, 56].

The choice of methodology reflects a dichotomous strategy driven by the clinical objective. For MRD monitoring, the goal is to “hunt” a known enemy. Tumor tissue sequencing has already provided the enemy’s “fingerprint” (i.e., clonal mutations), making highly targeted and sensitive “pursuit” technologies like ddPCR or tumor-informed NGS the most efficient tools. Conversely, for early screening in an asymptomatic population, the goal is to “scout” for any unknown cancer signal. This requires a broader, more universal signal. Mutation-based panels are one approach, but technologies that capture common features of cancer, such as aberrant methylation [50] or fragmentomics reflecting altered chromatin structure [55], may have greater potential because they do not rely on finding a specific, rare mutation in a vast background

of normal DNA. This explains why no single technology is a “one-size-fits-all” solution; the optimal method is entirely dependent on the clinical application. The characteristics, advantages, and limitations of the major ctDNA detection platforms are summarized in Table 1.

Application I: early diagnosis and screening

Because the prognosis of PDAC and BTCs is directly linked to the stage at diagnosis, achieving early detection via ctDNA is critical to improving patient survival.

CtDNA for the early detection of pancreatic cancer (PDAC)

Currently, pancreatic cancer screening is limited to selected high-risk populations, including individuals with a strong family history, hereditary cancer syndromes, or germline mutations (e.g., *BRCA1/2*, *CDKN2A*, *PALB2*, and *STK11*).

Landmark studies

CancerSEEK was a pioneering multi-cancer early detection (MCED) study that integrated the analysis of ctDNA mutations in 16 genes with the detection of 8 protein biomarkers [57]. In a study involving over 1,000 patients, CancerSEEK achieved a sensitivity of 72% for stage I-III pancreatic cancer while maintaining a specificity of over 99%, demonstrating the immense potential of multi-omic integration [57].

Combined use of ctDNA and protein biomarkers

The sensitivity of relying solely on ctDNA mutations for early detection is limited, especially at stages with very low tumor burden [58]. Therefore, combining ctDNA with other types of biomarkers is the current mainstream strategy. One pivotal study showed that detecting mutations alone identified only 30% of resectable PDACs, but when combined with protein biomarkers (e.g., CA19-9,

Table 1 Comparison of major ctDNA detection platforms

Technology Platform	Principle	Throughput	Cost	Key Advantages	Key Limitations	Optimal Clinical Application
ddPCR	Droplet partitioning, absolute quantification	Low (single/few targets)	Low	Rapid, high sensitivity, absolute quantification	Limited number of targets	MRD monitoring, tracking of known mutations
BEAMing	Emulsion PCR + flow cytometry	Medium	Medium	High sensitivity, precise quantification of MAF	Technically complex, limited throughput	Monitoring treatment response
Targeted NGS Panel	Hybrid-capture/amplicon sequencing	High (multi-gene)	Medium	Balances breadth and depth, cost-effective	Cannot detect mutations outside the panel	Guiding targeted therapy, MRD monitoring
WES	Capture and sequencing of exons	High (all exons)	High	Discovers novel mutations, assesses TMB	Insufficient sensitivity for low-frequency ctDNA	Scientific discovery, tumor-informed approaches
Methylation Sequencing	Bisulfite conversion + NGS	High	High	Can determine tissue of origin, mutation-independent	Technically complex, high bioinformatics demand	Early screening, tissue-of-origin analysis
Fragmentomics	WGS analysis of fragment features	High	High	Mutation-independent, useful for low-mutation-burden tumors	Biological mechanisms still under investigation, lacks standardization	Early screening

TIMP1, and LRG1) and cfDNA/ctDNA-based markers (e.g., pancreas-specific methylation panels) [51], the detection sensitivity increased to 64% with a specificity of 99.5% [58]. More recently, a study combining CA19-9, TIMP1, and a 9-locus cfDNA methylation panel confirmed that the combined test had superior diagnostic performance to any single marker [51]. This multi-analyte, multi-omic integration has become the core strategy for improving early detection performance [28].

Prospective cohort studies

To validate the clinical utility of these methods, several prospective studies are underway. For example, the NCT02934984 study is evaluating the role of ctDNA in early screening and recurrence monitoring, while the LIQUIPANC study (NCT06391892) is exploring the use of ctDNA to guide neoadjuvant therapy decisions. These studies will provide higher-level evidence for the clinical application of ctDNA.

CtDNA for the early detection of BTCs

Progress and challenges

The application of ctDNA for the early detection of BTCs is at an earlier stage compared to PDAC but is rapidly evolving [48, 59]. The main challenge is the lower incidence of BTCs, which makes screening the general population not cost-effective. Importantly, this limitation is shared with pancreatic ductal adenocarcinoma, where cost-effectiveness studies have similarly demonstrated that population-wide screening is not feasible and that ctDNA-based approaches are primarily applicable to narrowly defined high-risk groups. Consequently, research has focused on monitoring high-risk populations [60]. Beyond plasma, emerging studies suggest that bile-derived ctDNA may offer higher tumor DNA content in biliary tract cancers, highlighting a promising complementary liquid biopsy source.

Screening high-risk populations

Patients with primary sclerosing cholangitis (PSC) have a significantly increased risk of developing CCA and are an ideal target population for screening [60, 61]. Current surveillance for PSC patients relies mainly on imaging and invasive procedures, which have low diagnostic accuracy [60, 62]. Liquid biopsy offers a highly attractive, non-invasive surveillance strategy for this population [59, 63]. Preliminary studies have shown that ctDNA analysis in PSC patients is feasible and can detect key genetic alterations associated with BTCs (e.g. *IDH1/IDH2* mutations; *FGFR2* fusions; *ERBB2* amplifications) [64, 65]. Notably, structural alterations such as *FGFR2* fusions are more challenging to detect in plasma compared with point mutations, with lower concordance rates reported. Prospective clinical trials such as NCT02809898 are

exploring the value of ctDNA as a diagnostic and prognostic biomarker for BTCs.

Comparative challenges in early detection: PDAC vs. BTCs

Tumor DNA shedding rates and ctDNA abundance

Both PDAC and BTCs are considered “low shedders” of ctDNA in their early, localized stages, which is a major obstacle to achieving high-sensitivity detection [26, 66]. The dense fibrotic stroma characteristic of PDAC may physically impede the release of DNA fragments into the bloodstream [67, 68]. The factors influencing ctDNA release are complex and include tumor volume, location (liver metastases are often associated with higher shedding rates), and the tumor’s intrinsic biological properties [69]. ctDNA shedding may also differ between intrahepatic and extrahepatic cholangiocarcinomas, potentially contributing to variability in plasma detectability. The extremely low abundance of ctDNA requires detection methods with ultra-high sensitivity and often necessitates multi-analyte strategies to amplify the signal [70, 71].

Genomic landscapes and interference from clonal hematopoiesis

The genomic profiles of PDAC and BTCs are markedly different. PDAC is dominated by *KRAS* mutations (> 90%), which provides a universal detection target but also poses a specificity challenge, as mutations are also present in precancerous lesions [72–74]. The genomic landscape of BTCs is more heterogeneous, with common targetable mutations including *IDH1*, *FGFR2* fusions, *BRAF*, and *ERBB2* [75, 76].⁶ A common and major confounding factor for both is clonal hematopoiesis of indeterminate potential (CHIP). With age, hematopoietic stem cells spontaneously acquire somatic mutations (e.g., in *DNMT3A*), and the DNA carrying these mutations is released into the plasma, where it can be mistaken for tumor-derived ctDNA, leading to false positives [77, 78]. Distinguishing true ctDNA signals from CHIP noise requires sophisticated bioinformatic algorithms, with the most reliable method being concurrent sequencing of the patient’s peripheral blood leukocytes as a control [74, 78].

The distinct genomic features of these two cancers dictate fundamentally different strategies for their early detection. For PDAC, the core challenge is specificity—how to distinguish a malignant signal from one originating from a benign lesion. Therefore, the strategy must involve detecting *KRAS* mutations in conjunction with multi-dimensional information from proteins and methylation to confirm malignant origin [74, 79]. For BTCs, the core challenge is sensitivity. Due to the lack of a single, dominant driver gene, a broad-spectrum NGS panel covering multiple genes is necessary to capture the diverse and lower-frequency driver mutations

for effective detection [75, 80]. This reveals that a “one-size-fits-all” ctDNA screening strategy is not viable; the design of the detection assay must be closely tailored to the molecular biology of the target cancer [70]. Several landmark studies that have applied these principles to the early detection of PDAC and BTCs are summarized in Table 2.

Application II: MRD monitoring and recurrence prediction

Compared to the formidable challenges of early screening, the application of ctDNA for postoperative MRD monitoring and recurrence prediction has demonstrated more mature and direct clinical value [47].

Postoperative ctdna: a powerful predictor of prognosis in pancreatic cancer

Predicting recurrence

A large body of evidence consistently demonstrates that the detection ofCtDNA after curative-intent resection of PDAC is a strong independent risk factor for tumor recurrence and poor survival [81]. Consistent prognostic associations between postoperative ctDNA detection and recurrence risk have also been reported across other solid tumors, including early breast cancer [82]. A meta-analysis showed that postoperative ctDNA positivity was associated with significantly shorter overall survival (Hazard Ratio, HR = 3.66) [83]. This finding has been validated in multiple independent cohorts, indicating that a positive postoperative ctDNA status is equivalent to the presence of MRD [84, 85]. For instance, one tumor-informed NGS study involving 35 PDAC patients confirmed that postoperative ctDNA positivity was a robust predictor

for both decreased disease-free survival (DFS HR = 3.955) and overall survival (OS HR = 5.599), detecting this molecular recurrence with a median lead time of 3.6 months before radiological evidence of disease [86]. Similarly, another study highlighted the impact of very early detection, demonstrating that in a cohort of 20 patients, a positive ctDNA status just 7 days after surgery was associated with an exceptionally high risk of recurrence (RFS HR = 16.87) [87]. However, the optimal timing for post-operative ctDNA sampling remains unclear, with studies variably assessing ctDNA at early (7–14 days) or delayed (≈ 30 days) time points, underscoring the need for standardized protocols [88, 89].

Lead time advantage over imaging

A decisive advantage of ctDNA for MRD monitoring is its ability to detect recurrence signals much earlier than conventional imaging, with a median lead time of 3 to 9 months [47, 90]. For instance, one study reported a median lead time of 3.6 months [86]. This “window of opportunity” provides a valuable chance for clinical intervention, potentially altering the disease course through earlier treatment [47]. Despite the lead-time advantage of ctDNA over imaging, there is currently no consensus or standardized therapeutic strategy initiated solely on the basis of MRD positivity in pancreatic or biliary cancers, underscoring an important unmet need for prospective interventional studies.

Guiding adjuvant therapy decisions

Postoperative ctDNA status can stratify patients for risk more accurately than traditional clinicopathological features in pancreatic and biliary cancers [91]. Patients who

Table 2 Landmark studies in early detection of PDAC and BTCs with ctdna

Study/First Author	Cancer Type	Cohort Size (Cases/Controls)	Detection Method	Key Analytes	Sensitivity (%)	Specificity (%)	Core Conclusion
Cohen JD, et al. (2018) [57]	8 Cancers (incl. PDAC)	1005 / 812	ctDNA mutations + proteins	16 genes, 8 proteins	72(for stage I-III PDAC)	> 99	Multi-omic combination enables high-specificity screening for multiple cancers.
Cohen JD, et al. (2017) [58]	PDAC	221 / 182	ctDNA mutations + proteins	, 4 proteins	64	99.5	Combining with protein biomarkers significantly improves detection sensitivity for resectable PDAC.
Ben-Ami R, et al. (2024) [51]	PDAC	86 / 86 (test set)	Proteins + ctDNA methylation	CA19-9, TIMP1, LRG1, 9 methylation loci	N/A (AUC 0.94)	N/A (AUC 0.94)	A combined protein and methylation panel outperforms CA19-9 alone.
Yang JD, et al. (2021)[52]	CCA	59 / 117	ctDNA methylation	Plasma 9 methylated DNA markers (MDMs); RRBS-discovered; tree model	AUC 0.88 (95% CI 0.81–0.95) — de novo eCCA	N/A	Accurate plasma MDM panel for de novo extra-hepatic CCA; PSC-associated eCCA shows lower performance

are ctDNA-positive (MRD-positive) postoperatively are consistently associated with a markedly increased risk of recurrence, indicating a subgroup with particularly aggressive disease biology and poor prognosis. Emerging evidence across multiple solid tumors suggests that such high-risk MRD-positive patients may derive limited benefit from standard adjuvant therapy, thereby providing a rationale for ctDNA-guided interventional trials [82]. This has led to the design of ctDNA-guided interventional trials; for example, the Adjuvant Pancreatic Therapy Guided by MRD (ctDNA) in Resected PDAC study (NCT06966440) is prospectively evaluating whether intensifying treatment in this high-risk population can improve outcomes. Conversely, MRD-negative patients have a significantly better prognosis, providing a rationale for exploring treatment de-escalation to reduce toxicity [47]. Furthermore, the clearance of ctDNA during adjuvant therapy has been shown to be a marker of treatment efficacy, correlating with better outcomes [92–94].

Postoperative ctDNA dynamics in BTCs

Prognostic value

In resectable BTCs, postoperative ctDNA positivity robustly stratifies prognosis. In a cohort of patients with resected eCCA ($n = 113$), postoperative ctDNA positivity was associated with a markedly higher risk of recurrence (RFS HR = 11.55) and anticipated radiologic relapse by a median of 4.1 months [95]. Consistently, in a prospective cohort of stage I–III BTCs ($n = 56$), ctDNA detected within the postoperative molecular residual disease window predicted an approximately 26-fold increase in recurrence risk (RFS HR = 26) and identified relapse a mean of 3.7 months earlier than imaging [96]. This stark prognostic difference is further highlighted by data showing the 1-year recurrence-free survival rate for ctDNA-positive patients was only 47.6%, compared to 85% for ctDNA-negative patients [97]. Together, these data support incorporating tumor-informed ctDNA surveillance into postoperative risk stratification in BTCs.

Monitoring treatment response

Dynamic monitoring of ctDNA levels can effectively assess the efficacy of adjuvant and even advanced-stage therapies [95, 96]. A decrease in ctDNA levels during treatment is associated with clinical benefit, while an increase often precedes radiological progression [64, 98]. This has been demonstrated in patients with FGFR2-fusion-mutant cholangiocarcinoma receiving targeted therapy, where ctDNA dynamics closely mirrored the clinical course [64].

Comparative clinical utility of MRD: guiding personalized therapy

Comparison of lead time

In both PDAC and BTCs, the lead time of ctDNA detection over imaging is consistently several months, providing a similar window of opportunity for clinical intervention [47, 96]. A study in BTCs reported a mean lead time of 3.7 months [96].

Potential for treatment escalation/de-escalation

Although the specific treatment regimens differ, the clinical framework for adjusting therapy based on MRD status is common to both diseases [81, 96]. Postoperative MRD positivity identifies a group of ultra-high-risk patients who are almost certain to relapse, making them the best candidates for clinical trials evaluating new drugs or intensified treatment regimens [90, 96]. Conversely, MRD negativity defines a low-risk group that may benefit from treatment de-escalation to avoid unnecessary toxicity [47, 96]. The DYNAMIC study in colorectal cancer has already set a successful precedent for this ctDNA-guided treatment model [94, 99].

Unique therapeutic targets

Despite the similar clinical framework, the specific therapeutic implications differ due to their distinct genomic backgrounds. In PDAC, MRD positivity might guide the use of more intensive conventional chemotherapy regimens. In BTCs, however, the presence of well-defined targets like *FGFR2* fusions and *IDH1* mutations means that detecting ctDNA with these alterations during surveillance could prompt clinicians to initiate corresponding targeted therapies before macroscopic radiological recurrence occurs, enabling early intervention at the “molecular recurrence” level [100–102]. It is also important to note that the therapeutic landscape of PDAC is evolving rapidly, with increasing incorporation of molecular stratification, including alterations associated with platinum sensitivity and the emergence of *RAS*-targeted agents. In this context, surveillance strategies integrating ctDNA and molecularly defined targets are becoming increasingly relevant in PDAC, paralleling developments previously more characteristic of biliary tract cancers.

In summary, the most mature and clinically translatable application of ctDNA in pancreatic and biliary cancers is not early screening, but rather postoperative risk stratification and MRD monitoring. Its powerful prognostic ability (extremely high HR values) and significant lead time advantage are more reliable and clinically actionable than the still-developing screening technologies [47, 81, 96]. While screening is the ultimate goal, MRD monitoring represents the “low-hanging fruit” where ctDNA technology is poised to first change clinical practice. Key

Table 3 Key studies on MRD assessment with ctDNA in PDAC and BTCs

Study/First Author	Cancer Type	Cohort Size	Detection Method	Key Time Point	Median Lead Time (vs. Imaging)	Key Prognostic Finding (HR for RFS/OS)
Du J, et al. (2024)[86]	PDAC	35	Tumor-informed NGS	Post-op/Longitudinal	3.6 months	Post-op ctDNA+: DFS HR=3.955; OS HR=5.599
Groot VP, et al. (2019) [47]	PDAC	51	Tumor-informed NGS	Post-op/Longitudinal	6.5 months	Post-op ctDNA+ associated with significantly shorter RFS and OS
Wang X, et al(2023) [87]	PDAC	20	Tumor-informed NGS	7 days post-op	N/A	7 days post-op ctDNA+: RFS HR= 16.87
Yoo C, et al. (2025)[95]	BTCs	113	Tumor-informed NGS	Post-op/Longitudinal	4.1 months	Post-op ctDNA+: RFS HR= 11.55
Yu J, et al. (2023)[96]	BTCs	56	Tumor-informed NGS	Post-op/Longitudinal	3.7 months	Post-op ctDNA+: RFS HR=26

studies demonstrating the utility of ctDNA for MRD assessment in PDAC and BTCs are detailed in Table 3.

Current challenges and future directions

Despite the promising outlook for ctDNA technology, a series of technical, clinical, and economic challenges must be overcome before its widespread adoption into clinical practice [103].

Technical hurdles: from analytical standardization to bioinformatic optimization

Standardization of analytical workflows

The lack of uniform standards is a major barrier to the clinical application of ctDNA technology. Variability in every step—from sample collection (type of blood tube, processing time), ctDNA extraction methods, to analytical platforms and result reporting—makes it difficult to compare results across different studies and laboratories [103, 104]. Although organizations like BloodPAC are promoting the establishment of consensus guidelines, they have not yet been universally adopted.

Analytical validation and error correction

Achieving the ultra-high sensitivity required for ctDNA detection depends on reliable methods to distinguish true low-frequency mutations from background errors introduced during the sequencing process. Technologies such as unique molecular identifiers (UMIs) or Duplex Sequencing are crucial for error correction but also increase the complexity and cost of the experiments [105, 106].

Bioinformatic filtering of CHIP mutations

As previously mentioned, separating the ctDNA signal from CHIP interference is a key bioinformatic challenge. While paired sequencing of peripheral blood leukocytes is the gold standard, it adds to the cost [78, 107]. Therefore, developing advanced algorithms that can efficiently identify and filter out CHIP variants based on plasma-only data is a current research hotspot [108, 109].

Clinical hurdles: biological heterogeneity and economic realities

Inter-patient heterogeneity

Even among patients with the same cancer type and stage, there is significant variability in the amount of ctDNA they release [103, 110]. The biological mechanisms behind this phenomenon of “high shedders” and “low shedders” are not yet fully understood but pose a major challenge for clinical interpretation. A negative ctDNA result in a known “low shedder” may need to be interpreted with greater caution

Cost-effectiveness

Although sequencing costs continue to decline, ctDNA testing, especially personalized tumor-informed NGS panels, remains expensive. Rigorous health economic analyses are urgently needed to demonstrate its cost-effectiveness relative to standard surveillance protocols [103]. Current cost-effectiveness studies for screening high-risk PDAC populations have focused on imaging, concluding that it is only cost-effective for the highest genetic risk groups [111]. Similar economic evaluations for ctDNA-guided treatment strategies are eagerly awaited.

Future outlook: the synergy of multi-omics and artificial Intelligence(AI)

Multi-omic integration

The future of liquid biopsy lies in integrating multiple analytes to synergistically enhance detection sensitivity and specificity [112, 113]. Combining mutational, methylation, and fragmentomic information from ctDNA with proteomics, exosome, or CTC analysis can provide a more comprehensive and robust signal of malignancy.

Application of AI in data interpretation

The massive, high-dimensional datasets generated by multi-omic liquid biopsies provide an ideal application scenario for AI and machine learning (ML) algorithms. AI can identify subtle, cancer-specific patterns—such as altered cfDNA fragment lengths at specific genomic locations or complex methylation signatures—that are

imperceptible to human analysis. By integrating these multi-omic data types, ML algorithms can build more accurate models to generate a single, robust probability score for cancer presence or predict a patient's risk of recurrence [50, 114].

Design of ctDNA-guided clinical trials

The ultimate validation of ctDNA's clinical value will come from prospective, interventional clinical trials. These trials will use ctDNA results to guide treatment decisions (e.g., escalating or de-escalating adjuvant therapy based on MRD status) and ultimately demonstrate that this strategy can improve hard endpoints such as overall survival [94].

The entire field of ctDNA is undergoing a paradigm shift from “single-analyte, manual interpretation” to “multi-analyte, AI-driven interpretation.” The liquid biopsy of the future will not be a simple “ctDNA test” but rather a comprehensive diagnostic algorithm that integrates multi-dimensional molecular information and is powered by AI. This evolution began with a focus on single mutations (like *KRAS* in PDAC, and *IDH1* mutations or *FGFR2* fusions in cholangiocarcinoma) [72, 74, 100, 115], incorporated protein biomarkers due to insufficient sensitivity, and now includes methylation and fragmentomics, creating complex, multi-layered molecular datasets [55, 112]. The complexity of handling such heterogeneous data surpasses the capabilities of traditional statistics, necessitating the intervention of AI/ML to find the optimal predictive models within the vast data landscape.⁹⁶ Thus, the technological development itself is driving the innovation of analytical methods, leading the entire field toward a more comprehensive and intelligent diagnostic future.

Conclusion

CtDNA, as a revolutionary liquid biopsy technology, offers unprecedented hope for overcoming the challenges posed by pancreatic and biliary cancers, two of the most lethal malignancies. Importantly, despite growing interest in early detection, the most mature and clinically translatable application of ctDNA in pancreatic and biliary cancers currently lies not in population-level screening, but in postoperative risk stratification and minimal residual disease monitoring. This review has systematically summarized the latest advances in ctDNA across the two key domains of early diagnosis and MRD monitoring. In early diagnosis, multi-omic strategies that integrate ctDNA mutations, methylation, fragmentomics, and protein biomarkers have significantly improved detection sensitivity and specificity, providing a viable path for screening high-risk populations. In MRD monitoring, ctDNA has demonstrated powerful prognostic capabilities, detecting molecular residual disease months before

radiological recurrence and providing a robust tool for guiding individualized adjuvant therapy and enabling precise risk stratification.

Despite substantial progress, the routine clinical implementation of ctDNA still faces numerous challenges. On the technical front, standardization of analytical workflows, further improvements in detection sensitivity, and optimization of bioinformatic algorithms (particularly for effective filtering of CHIP) are pressing issues. On the clinical front, the biological heterogeneity of tumor DNA shedding, the cost-effectiveness of testing, and the translation of ctDNA results into clinical decisions that improve patient survival require extensive further research.

The path forward is clear: to validate ctDNA-guided treatment strategies through the design and execution of prospective, interventional clinical trials that can demonstrate real improvements in patient outcomes. Concurrently, the deep integration of multi-omic technologies with AI will be crucial for developing more powerful and robust diagnostic models. Only through the collaborative efforts of academia, industry, and clinicians can the existing barriers be overcome, fully translating the immense potential of ctDNA into a clinical reality that improves the survival of patients with pancreatic and biliary cancers.

Supplementary Information

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Supplementary Material 1.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of The First Hospital of Jilin University.

Authors' contributions

Conceptualization, RL and YY; methodology, RL and ZH; investigation, RL, YY and ZH; resources, YF; data curation, RL and ZH; writing—original draft preparation, RL; writing—review and editing, YY, ZH and YF; supervision, YF; project administration, YF. All authors have read and agreed to the published version of the manuscript.

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Declarations

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