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Review

Circulating Tumor DNA in Primary and Secondary Liver Cancers: A Comprehensive Review

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Abstract

Similar to many other cancer types, liver malignancies pose the common challenges of late detection of primary tumors and recurrences. Liquid biopsies, which assess the presence of circulating tumor DNA, have emerged as a novel, non-invasive clinical tool for diagnostic and surveillance purposes. This review represents an introductory and comprehensive overview of the current circulating tumor DNA (ctDNA) literature relevant to primary and secondary liver malignancies. Herein, we highlight key findings, landmark discoveries, challenges, and future directions.

Keywords: circulating tumor DNA; ctDNA; liquid biopsy; liver cancer; liver malignancy

1. Introduction

Liver-related malignancies comprise the sixth most common cause of cancer and third leading cause of cancer death [1]. Primary liver cancers include hepatocellular carcinoma (HCC) (85–90% of cases) and cholangiocarcinoma (CCA) (10–15% of cases) [1]. Secondary liver malignancies include metastases arising from the lung, colorectum, pancreas, stomach, breast, and cecum [2]. Approximately 5% of cancer patients present with synchronous liver metastases, with the most common primary site being breast cancer for young females and colorectal cancer for young males [3]. For older patients, lung, pancreatic, and colorectal cancers are the most common primary sites, although a greater percentage of primary esophageal, stomach, small intestine, melanoma, and bladder cancers start to emerge with age [3].

Common to all liver malignancies is a worse prognosis associated with late stage of disease. The one-year survival of patients with liver metastases from any primary cancer is lower (15.1%) than those with non-hepatic metastases (24%) [3]. Advanced disease is multi-factorial due to limited sensitivity and specificity of biomarkers, more aggressive tumor biology, and lack of early, specific symptoms [4]. Current methods of liver cancer detection include radiologic imaging, tissue sampling, and traditional serum biomarkers [5]. However, radiologic imaging is limited in patients with small nodules and early microscopic lesions [6]. Tissue sampling requires invasive biopsy procedures and may yield insufficient tissue for diagnosis and high false negative rates [7,8]. Traditional serum biomarkers, such as alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen (CEA) are limited in their sensitivity and specificity [9,10].

To address these limitations, circulating tumor DNA (ctDNA) has become of interest due to its non-invasive nature through liquid biopsy and its ability to provide insight into the nature of individual patient tumor biology [11]. One meta-analysis revealed a high overall sensitivity (72.2%) and specificity (82.3%) along with diagnostic odds ratio (18.53) and area under the curve (AUC) (0.88) for ctDNA in HCC, displaying its potential as a diagnostic marker [12]. This tool, now FDA-approved for monitoring minimal residual disease for colorectal liver metastasis, holds significant potential as a surveillance and prognostic tool for liver malignancies. In terms of prognosis, patients with detectable ctDNA following curative-intent local therapy for colorectal cancer with liver metastasis have been shown to have significantly higher potential for recurrence and shorter overall survival (OS) compared to those without detectable ctDNA [13].

This comprehensive narrative review serves as an introduction to ctDNA and the landscape of ctDNA literature

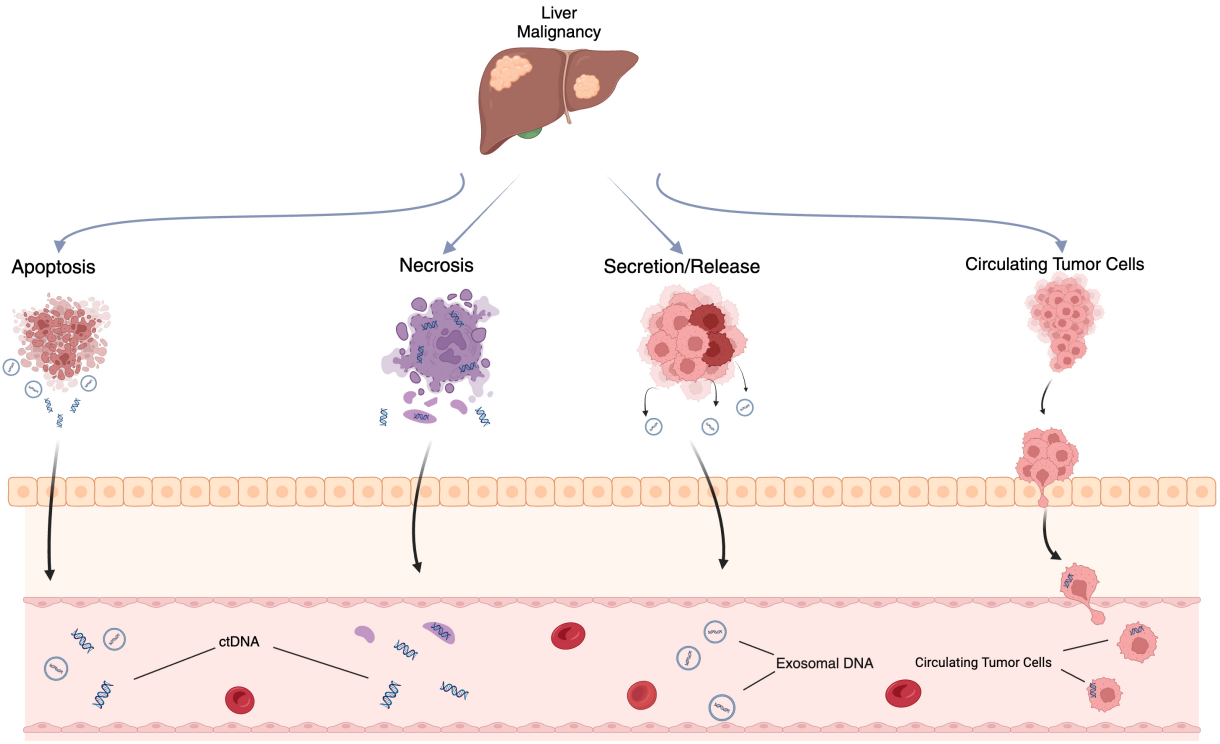

Fig. 1. Sources and origin for circulating tumor DNA (ctDNA). Created using BioRender.com.

within primary and secondary liver cancers. First, the concept of ctDNA as a biomarker is introduced, followed by summaries of key studies on clinical utility and mutational profiles by cancer subtype. Lastly, insight into challenges and limitations of current studies involving ctDNA is provided, along with commentary on future directions for research and clinical applications. Detailed descriptions of the methodologies are not included in the present review due to heterogeneity in ctDNA detection methods; however, readers are encouraged to refer to cited materials for further information.

2. Circulating Tumor DNA (ctDNA)

ctDNA refers to genomic material released by tumor cells into the patient's systemic circulation. An early study in 1977 showed the presence of cell-free DNA from peripheral blood of cancer patients, but further characterization was limited by technology at the time [14]. Recent advancements in genetic amplification technology have allowed deeper investigation into this genomic material, identifying single-nucleotide changes [15], methylation patterns [16–18], and viral sequences [19] derived from or reflective of the original tumor.

ctDNA is thought to originate from several sources, including apoptotic or necrotic tumor cells, live tumor cells, and circulating tumor cells (Fig. 1) [20,21]. Due to its relatively short half-life of up to two hours, ctDNA reflects the tumor biology in a dynamic fashion [22]. Methods of detection include droplet digital PCR (ddPCR), personalized amplicon-based NGS, personalized hybridization-based next-generation sequencing (NGS), whole-genome sequencing, exome sequencing, or array comparative genomic hybridization (Figs. 2,3) [23,24]. Detection and analytic methods can be performed in tumor-informed, which decreases risk of false positive results, or tumor-uninformed fashions, which can allow for detection of clonal evolution and tumor resistance.

3. Primary Liver Malignancies

3.1 Hepatocellular Carcinoma (HCC)

HCC is the sixth most common cancer worldwide and third leading cause of cancer-related deaths [1]. The standard diagnostic method consists of radiologic imaging (e.g., computer tomography (CT), magnetic resonance imaging (MRI)) and detection of elevated alpha-fetal protein (AFP) levels. Despite a high specificity for early HCC, AFP has limited and variable sensitivity [25,26]. In contrast, ctDNA has a reported sensitivity of 100% (42/42) and specificity of 97.4% (75/77) for detecting recurrence in patients with HCC following surgery and adjuvant therapy [27]. Recently published clinical trial results also show the ability of a liquid biopsy-based DNA methylation signature to detect HCC with 84.5% sensitivity, 95% specificity, and 0.94 AUC [18]. Further information regarding this trial, along with other completed and recruiting clinical trials for primary and secondary liver malignancies, is summarized in Table 1 (Ref. [18,28–31]) and Table 2 respectively.

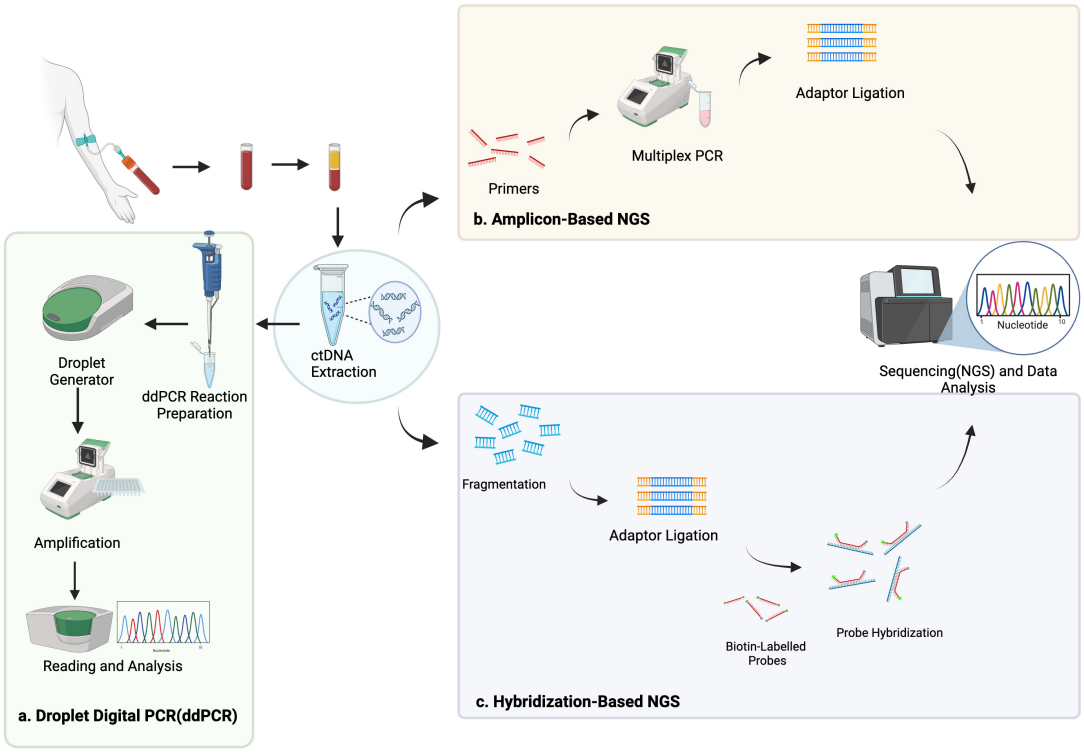

Fig. 2. Methods of ctDNA detection including droplet digital polymerase chain reaction (a), amplicon-based next-generation sequencing (NGS) (b), and hybridization-based NGS (c). Created using BioRender.com.

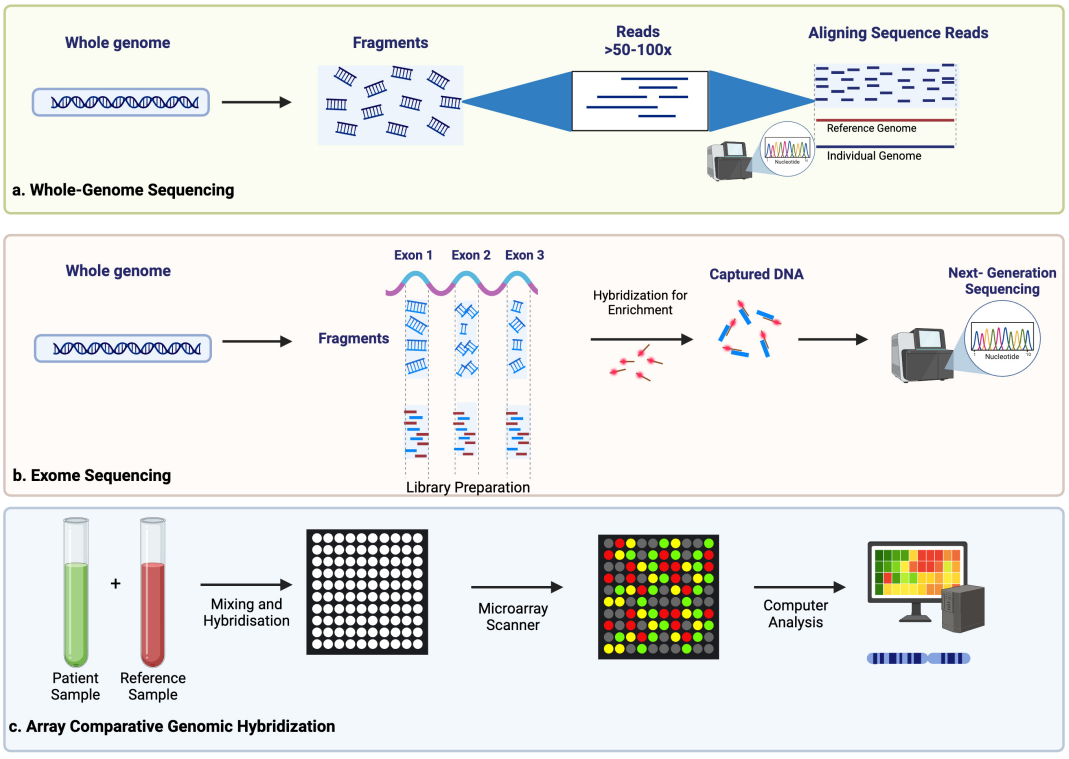

Fig. 3. Additional methods of ctDNA detection including whole-genome sequencing (a), exome sequencing (b), and array comparative genome hybridization (c). Created using BioRender.com.

Table 1. Summary of completed clinical trials for ctDNA in primary and secondary liver malignancies.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Population	Study Dates	Outcome Measures	Sponsor	Associated Publication
NCT03483922	HCC Screening Using DNA Methylation Changes in ctDNA	Observational (Case-control)	HCC	Diagnostic Test: ctDNA methylation in and it's Correlation with Development and prediction of HCC	402 participants from Dhaka area-49 healthy controls, 51 chronic HBV patients, 302 HCC patients	August 20, 2018 –June 1, 2020	Primary: Normalized median methylation values for HCC detection and specificity Results: Using 4 CpG sites validated in TCGA HCC data, HCC detection sensitivity was 84.5% and specificity was 95% with an AUC of 0.94.	HKGepitherapeutics	[18]
NCT02973204	Circulating Tumor Cells and Tumor DNA in HCC and NET	Observational	HCC	Drug: Sorafeniib, everolimus, lanreotide Procedure: Radiofrequency ablation (RFA)	Planned: 40 patients with newly diagnosed NET (of unknown primary), 30 pancreatic NET treated for known residual disease, 30 HCC patients treated with RFA or liver resection Actual enrollment: 167	November 2016– January 2020	Primary: Concordance between biopsy and plasma ctDNA mutations (ddPCR). Secondary: Detection and quantification of CTC, correlation between ctDNA and CTCs in terms of mutations, treatment response, survival.	University of Aarhus	N/A
NCT05823584	Cell-free DNA From Junction of Hepatitis B Virus Integration in HCC Patients for Monitoring Post-resection Recurrence	Observational	HCC	Surgery: Resection or liver transplant	207	December 22, 2019– December 31, 2023	Primary: Pre-operative sensitivity of vh-DNA with AFP as biomarker for HCC recurrence. Secondary: Sensitivity and specificity of vh-DNA with AFP-L3/PIVKA-II/TERTp C228T as biomarker for recurrence, clonality of recurrent HCC	TCM Biotech International Corp.	N/A

Table 1. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Population	Study Dates	Outcome Measures	Sponsor	Associated Publication
NCT05540925	Vascular Invasion Signatures in cfDNA Support Re-staging of Liver Cancer	Observational	HCC (early-stage)	Procedure: Liver resection	286	June 2016–December 2017	Primary: Recurrence free survival, overall survival stratified by risk for MVI as determined from nomogram derived from sequencing data of cfDNA (high risk >90, low-risk ≤90). Secondary: Local recurrence.	Eastern Hepatobiliary Surgery Hospital	N/A
NCT03071458	Mutational Landscape in Hepatocellular Carcinoma	Observational	HCC	Procedure: Liver transplant, radiofrequency ablation, resection	808 Total: 224 tumor, 224 non-tumor from LT 129 HCC, 129 non-tumor from RFA 342 HCC, 342 non-tumor from liver resection 40 HCC, 35 non-tumor from biopsies of advanced HCC	January 2008–May 2015	Primary: Identification of the main genetic driver and transcriptomic subgroups among a large panel of HCC. Secondary: Detection of ctDNA in patients with early and advanced HCC, review of genetic drivers and oncognic pathways with IHC, validation of tumor analyses using clinical data, pathological and IHC features, molecular classification and genetic alterations.	Institut National de la Santé Et de la Recherche Médicale, France	N/A
NCT03893695	Combination of GT90001 and Nivolumab in Patients With Metastatic Hepatocellular Carcinoma (HCC)	Interventional	HCC (Advanced or metastatic, failed first-line and/or second-line systemic therapy)	Drug: GT90001 (anti-ALK1 mAb) and Nivolumab	20	May 25 2019–September 27 2022	Primary outcome: Dose-limiting toxicity. Secondary outcomes: Overall response rate, duration of response, disease control rate, time to response, progression-free survival, pharmacokinetics, ctDNA.	Suzhou Kintor Pharmaceutical Inc	[30]

Table 1. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Population	Study Dates	Outcome Measures	Sponsor	Associated Publication
NCT06404593	Dynamic ctDNA Detection for Guiding Adjuvant Therapy and Recurrence Monitoring After Curative Resection of Colorectal Cancer Liver Metastases: A Prospective Study	Observational	CRLM	Diagnostic test: Blood sampling pre-operatively and post-operatively (serial) for ctDNA	270	June 18, 2019–December 31, 2023	Primary outcomes: Progression-free survival, overall survival.	Peking University Cancer Hospital & Institute	N/A
NCT01749332	A Pilot Study of Perihepatic Phlebotomy During Hepatic Resections	Observational	CRLM	Other: Perihepatic Phlebotomy-Perioperative and postoperative draw from peripheral, portal, and hepatic veins	117	December 2012–2019	Primary: ctDNA differences between perihepatic and peripheral ctDNA Secondary: Correlation of peripheatpic and peripheral ctDNA mutation with recurrence and survival patterns. Results: Detection of peripheral ctDNA mutant TP63 was associated with worse 2-year DSS (mt+ 79% vs. mt− 90%, $p = 0.024$). Most commonly mutated genes were *TP53* (mt*TP53*, 47.5%) and *APC* (mt*APC*, 50.8%). Substantial to almost-perfect agreement was seen between ctDNA from PERIPH and PV (mt*TP53*: 89.8%, $\kappa = 0.73$, 95% CI: 0.53–0.93; mt*APC*: 94.9%, $\kappa = 0.83$, 95% CI: 0.64–1.00) as well as (mt*TP53*: 91.5%, $\kappa = 0.78$, 95% CI: 0.60–0.96; mt*APC*: 91.5%, $\kappa = 0.73$, 95% CI: 0.51–0.95). Tumor mutations and PERIPH ctDNA had fair-to-moderate agreement (mt*TP53*: 72.9%, $\kappa = 0.44$, 95% CI: 0.23–0.66; mt*APC*: 61.0%, $\kappa = 0.23$, 95% CI: 0.04–0.42).	Memorial Sloan Kettering Cancer Center	[29]

Table 1. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Population	Study Dates	Outcome Measures	Sponsor	Associated Publication
ACTRN12615000381583	Circulating Tumour DNA Analysis Informing Adjuvant Chemotherapy in Stage II Colon Cancer (DYNAMIC)	Interventional	CRLM (stage II)	ctDNA-guided management vs. Standard clinicopathologic management of adjuvant therapy	455 randomized, 302 ctDNA-guided management, 153 standard management	August 10 2015 –July 25 2019	Results: ctDNA-guided management was noninferior to standard management for 2-year recurrence free survival (93.5% vs. 92.4% respectively; absolute difference, 1.1 percentage points; 95% CI, –4.1 to 6.2 [noninferiority margin, –8.5 percentage points]).	National Health and Medical Research Council (Australia)	[28]
NCT03415126	A Study of ASN007 in Patients With Advanced Solid Tumors	Interventional	Metastatic BRAF mutated melanoma, metastatic NRAS and HRAS mutated solid tumors, metastatic KRAS mutated CRC, metastasis KRAS mutated NSCLC, metastatic PDAC	Drug: ASN007 (ERK1/2 inhibitor)	49	January 19, 2018 –June 30, 2020	Primary: maximum tolerable dose, overall response rate. Secondary: Pharmacokinetic AUC, maximum plasma concentration, terminal elimination rate, change in baseline phosphorylated ribosomal S6 kinase in tumor biopsies, change in amount of ctDNA.	Asana BioSciences	[31]

HCC, hepatocellular carcinoma; CRLM, colorectal liver metastases; CI, confidence interval; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; ERK1/2, extracellular signal-related kinase1/2; NCT, National Clinical Trial; HBV, hepatitis B virus; TCGA, the cancer genome atlas; AUC, area under the curve; NET, neuroendocrine tumor; CTC, circulating tumor cell; cfDNA, cell-free DNA; vh-DNA, virus-host chimera DNA; AFP-L3/PIVKA-II/TERTp, alpha-fetoprotein-L3/protein induced by vitamin K absence or antagonist-II/telomerase reverse transcriptase promoter; MVI, microvascular invasion; IHC, immunohistochemistry; TP53, tumor protein 53; APC, adenomatous polyposis coli; PERIPH, peripheral; PV, portal vein; HV, hepatic vein; NRAS, neuroblastoma ras viral oncogene homolog; HRAS, Harvey rat sarcoma virus; KRAS, Kirsten rat sarcoma virus.

Table 2. Recruiting clinical trials investigating ctDNA in primary liver malignancies.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT06178809	Clinical Research on Dynamic Monitoring MRD Via Plasma ctDNA After Systemic Therapy of Hepatocellular Carcinoma	Observational	HCC	Surgery and systemic treatment	475	December 25, 2023 –December 2025	Primary: Accuracy of detection of plasma ctDNA mutation and methylation in predicting disease-free survival (DFS) or progression-free survival (PFS) in patients with primary hepatocellular carcinoma after treatment Secondary: Advance time of ctDNA dynamic detection compared with AFP+ imaging in monitoring of primary hepatocellular carcinoma recurrence or progression	Singlera Genomics Inc.
NCT06157060	Prediction of Hepatocellular Carcinoma Recurrence After Curative Treatment by Longitudinal Monitoring MRD Based on ctDNA	Observational	HCC	Surgical resection	255	November 20, 2023 –December 30, 2026	Primary: 2-year recurrence-free survival rate Secondary: Correlation between ctDNA-MRD status dynamic changes and relapsADe	Zhujiang Hospital
NCT05981066	A Clinical Study of mRNA Vaccine (ABOR2014/ IPM511) in Patients With Advanced Hepatocellular Carcinoma	Interventional	HCC	ABOR2014/IPM511 mRNA vaccine	48	July 10, 2023– December 31, 2025	Primary: Incidence and severity of adverse events, Clinically significant abnormal changes in vital signs or laboratory tests Secondary: Maximum Plasma Concentration [Cmax] and Half-time of Plasma Concentration [T1/2] of IPM511, Antigen-specific T-cell responses in peripheral blood, Change of Circulating tumor DNA (ctDNA) status, ORR, DoR, PFS, OS	Peking Union Medical College Hospital
NCT05669339	AD HOC Trial: Artificial Intelligence-Based Drug Dosing In Hepatocellular Carcinoma	Interventional	HCC	Drugs: Irinotecan, Sonidegib, and Sorafenib	12	September 2024– April 2026	Primary: Maximally tolerated dose Secondary: ORR, Change in AFP, AFP-L3, DGC, and TGF-B Drug efficacy will be measured by changes in ctDNA.	University of Florida

Table 2. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT05626985	Refinement and Validation of a Diagnostic Model (GAMAD) for Early Detection of Hepatocellular Carcinoma	Observational	HCC	Treatment per NCCN guidelines	2000	October 19, 2022–December 2024	Primary: GAMAD calculator model Secondary: GALAD calculator score, Circulating tumor DNA methylation	Singlera Genomics Inc
NCT05390112	Cohort Study of Patients With Hepatocellular Carcinoma and Circulating Tumor DNA Monitoring of Chemoembolization (Mona-Lisa)	Observational	HCC	TACE	167	May 20, 2021–December 31, 2024	Primary: Radiological response at 1 month according to mRECIST and ctDNA detection Secondary: PFS, OS	University Hospital, Rouen
NCT04134559	Checkpoint Inhibition In Pediatric Hepatocellular Carcinoma	Interventional	HCC	Pembrolizumab	18	November 1, 2020–January 1, 2025	Primary: Immune-related best overall response (irBOR) Secondary: Expression levels of infiltrating immune cells and markers of checkpoint inhibition on pre-treatment specimens, PFS, Percent change immune cell phenotype, cytokines, and circulating tumor DNA, Number of Participants with DLT, DNA sequencing of specimens	Dana-Farber Cancer Institute
NCT03839706	Relationship Between 18FDG PET/MRI Patterns and ctDNA to Predict HCC Recurrence After Liver Transplantation (PETMRIinHCC)	Interventional	HCC	Liver transplantation	20	August 22, 2018–September 2024	Primary: 18F-FDG PET/MRI results to identify aggressive HCC behavior and recurrence post transplant Secondary: 18F-FDG PET/MRI to predict HCC's poor tumoral differentiation, 18F-FDG PET/MRI are relation to circulating tumor DNA in plasma	University Health Network, Toronto

Table 2. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT06028724	A Study on the Prevalence of Clinically Useful Mutations in Solid Tumor Characterized by Next Generation Sequencing Methods on Liquid Biopsy Analysis (POPCORN)	Observational	Advanced HCC or cholangiocarcinoma	Not specified	782	May 26, 2023–May 31, 2030	Primary: Real world prevalence of clinically useful mutations in solid tumors Secondary: To identify emerging gene alterations associated with PFS or OS, To describe changes in ctDNA associated biomarkers during treatment, To evaluate the association between somatic genetic alterations and pattern of metastasis, To evaluate the association between somatic genetic alterations and the histopathological features of the tumor, To evaluate the association between somatic genetic alterations and pattern of metastasis, To evaluate the association between somatic genetic alterations and the clinical characteristic of the enrolled patients	Centro di Riferimento Oncologico - Aviano
NCT06541652	A French Multicenter Observational Retrospective Study of Rare Primary Liver Cancers (FFCD-2205)	Observational	Hepatocholangiocarcinoma, fibrolamellar hepatocellular carcinoma, epithelioid hemangioendothelioma or hepatic angiosarcoma	Diagnostic: Blood sample	150	March 26, 2024–February 2031	Primary: Description of the clinical, histological and radiological characteristics of various rare primary liver cancers (ctDNA included) Secondary: Recurrence-free survival, Progression-free survival, Overall survival	Federation Francophone de Cancerologie Digestive
NCT06391749	Clinical Validation of an MCED Test in Symptomatic Populations (K-ACCELERATE)	Observational	Benign primary liver cancer	Not specified	1000	May 2024–November 2025	Primary: Evaluate the performance of the SPOT-MAS test to detect cancer in symptomatic individuals Secondary: Feasibility of using SPOT-MAS as a triage test to assist in decision-making for follow-up high-resolution imaging or tissue biopsy procedures	Gene Solutions
NCT02838836	Tumor Cell and DNA Detection in the Blood, Urine and Bone Marrow of Patients With Solid Cancers	Observational	Primary liver cancer	Surgical resection	620	July 1, 2016–December 1, 2026	Primary: CTC/DTC numbers measured in blood, urine and bone marrow samples will be correlated with patient outcome Secondary: CTC/DTC numbers measured in blood, urine and bone marrow samples will be correlated with patient outcome	University of Missouri-Columbia

Table 2. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT05633342	Project CADENCE (C-Ancer Detected Early caN be CurEd)	Observational	Any liver cancer	Prior chemotherapy or radiotherapy	15,000	July 7, 2022–May 2025	Primary: To discover novel intracellular RNA and methylated DNA cancer biomarkers in fresh frozen tumor tissues Secondary: To select the best-performing multi-omic single-cancer, biomarker panels for each of the cancer types, and develop the corresponding Single-Cancer Early detection Algorithms (SCEAs), To discover and validate novel cell-free RNA and methylated cell-free DNA cancer biomarkers in the peripheral blood of cancer patients, To develop the best-performing multi-omic multi-cancer biomarker panel by integration and/or optimization of single-cancer panels and develop the corresponding Multi-Cancer Early detection Algorithm (MCEA), To develop *in vitro* diagnostic assay(s) for the Multi-Cancer Screening Test (MCST) and if appropriate Single-Cancer Screening Tests (SCSTs), To evaluate the clinical performance (AUC, sensitivity, specificity, tissue of origin) of the MCST and if appropriate SCSTs to discriminate cancer cases from control groups	MiRXES Pte Ltd.

ORR, objective response rate; PFS, progression-free survival; OS, overall survival; AFP, alpha-fetoprotein; DGC, des-gamma-carboxyprothrombin; TGF-B, transforming growth factor beta; NCCN, National Comprehensive Cancer Network; GALAD, (Gener+age+AFP-L3+AFP+DCP); TACE, transarterial chemoembolization; DLT, dose-limiting toxicity; 18F-FDG PET/MRI, 18F-fluorodexoyglucose positron emission tomography magnetic resonance imaging; SPOT-MAS, screening for the presence of tumor by methylation and size; DTC, disseminated tumor cell; MRD, molecular residual disease; DoR, duration of response.

Several studies have explored the clinical utility of ctDNA in HCC. In 2020, Wang *et al.* [32] found a higher cancer detection rate using pre-operative plasma ctDNA (70.4%, n = 57/81) with a panel of 4 mutations (*TP53* (c.747G>T), *TRET* (c.1-124C>T), *CTNNB1* (c.121A>G) and *CTNNB1* (c.133T>C)) compared to a detection rate of 56.8% (n = 46/81) with AFP. In addition, pre-operative detection of plasma ctDNA was associated with larger tumor size, multiple tumor lesions, microvascular invasion, and advanced Barcelona Clinic Liver Cancer (BCLC) stage, which are also associated with worse clinical prognosis [32]. Pre-operative ctDNA was also independently associated with disease-free survival (DFS) and overall survival (OS) on multivariate analysis. Another study featuring 26 operable HCC cases found that patients with at least one detectable mutation on postoperative plasma ctDNA had worse DFS than those without (17.5 months vs. 6.7 months, hazard ratio (HR) = 7.655, $p < 0.0001$), and post-operative ctDNA status was an independent risk factor for recurrence (HR = 10.293, $p < 0.0001$) [33]. Additionally, *TERT* promoter mutations on plasma ctDNA were identified as a factor for poor overall survival among 130 patients undergoing systemic chemotherapy or transcatheter arterial chemoembolization (HR = 1.94; 95% confidence interval [CI], 1.18–3.24; $p < 0.01$) [34]. High serum cfDNA levels are also an independent prognostic factor for worse OS (HR: 3.4, 95% CI: 1.5–7.6, $p = 0.004$) and increased recurrence in distant organs (HR: 4.5, 95% CI: 1.3–14.9, $p = 0.014$) for patients with hepatitis C virus-related HCC who underwent curative intent hepatectomy [35]. Additionally, serum cfDNA levels were significantly different in patients who had HCV-related HCC compared to patients with HCV only (115.9 $\pm$ 98.3 *vs* 34.4 $\pm$ 40.4 ng mL^{-1} respectively, $p < 0.0001$) [35].

Characteristics provided by ctDNA testing, such as extent of tumor mutational burden (TMB), have also been shown to correlate with survival. Wehrle *et al.* (2024) [36] demonstrated an association between TMB on post-operative ctDNA and shorter recurrence-free survival (RFS) in 48 patients with HCC following surgical resection. With immunotherapy emerging as a treatment option for HCC as suggested by the Imbrave50 trial results, this may suggest a role for adjuvant immunotherapy in this patient population. Similarly, preliminary data from Marron *et al.* (2023) [37] shows the ability of post-operative ctDNA in detecting disease relapse and shorter recurrence-free survival for patients undergoing neoadjuvant and adjuvant cemiplimab (anti-PD-1) and surgical resection.

For patients with unresectable HCC, ctDNA also plays a role in predicting treatment response. Preliminary results from a personalized, tumor-informed assay show a prolonged PFS in patients whose ctDNA became undetectable following treatment [38]. In another study, 3/4 (75%) patients undergoing immunotherapy and locoregional therapy changed from detectable to non-detectable TMB on their ctDNA following curative-intent hepatectomy [39]. CtDNA can also provide insight into the mutational landscape driving resistance to systemic therapies in advanced HCC. For example, von Felden *et al.* (2021) [40] conducted targeted sequencing of 25 genes and ddPCR of the *TERT* promoter on plasma ctDNA, and found that patients with mutations in the PI3K/MTOR pathway exhibited significantly shorter PFS (2.1 vs. 3.7 months, $p < 0.0001$) following treatment with tyrosine kinase inhibitors compared to those without such pathway-specific mutations. For these 77 HCC, the most frequently mutated genes on ctDNA were *TERT* promoter (51%), *TP53* (32%), *CTNNB1* (17%), *PTEN* (8%), *AXIN1*, *ARID2*, *KMT2D*, and *TSC2* (6% each) [40].

Other studies have used ctDNA to investigate mutational pathways driving HCC tumorigenesis. For example, Ikeda *et al.* (2018) [41] performed ctDNA and tissue NGS testing on 26 patients with HCC and identified common mutations of *TP53* (50%), *CTNNB1* (100%), and *ARID1A* (90%). An *et al.* (2019) [33] assessed the presence of nine presumptive driver genes for HCC (*TP53, AXIN1, CTNNB1, CDKN2A, ARIN1A, ARID2, SMARCA4, KEAP1* and *NFE2L2*) and found 37 driver events in 88.5% cases (23/26). On an epigenetic level, aberrant promoter methylation of tumor suppressor genes *p16*, *GSTP1*, and *RASSF1A* have been noted in both the plasma and tumor tissue of patients with HCC [42–46]. More specifically, *p16* methylation was noted in 73% (16/22) HCC tissues and 81% (13/16) HCC plasma/serum samples, while being absent in the plasma/serum of healthy patients and patients with chronic hepatitis/cirrhosis [43]. Hypermethylation of *GSTP1*, which encodes glutathione S-transferase, was observed in 88.5% (23/26) of HCC tumor tissue and 50% (16/32) patients with HCC, while none of the normal peripheral blood mononuclear cell samples from healthy patients (n = 12) had aberrant *GSTP1* methylation [45]. For *RASSF1A*, aberrant promoter methylation was detected in 92.5% (37/40) of HCC tissues and 42.5% (17/40) of paired plasma, while being associated with HCC tumor size of at least 4 centimeters ($p = 0.035$) [46]. Hypomethylation of serum *LINE-1* has also been shown to be a significant and independent prognostic marker of overall survival for patients with HCC [47]. Additionally, the average level of serum LINE-1 hypomethylation differed significantly among patients who were healthy and those with HCC, cirrhosis, or hepatitis B virus [47]. Thus, specific genetic and epigenetic changes within HCC tumors may be detectable peripherally from patient plasma/serum, establishing another method of clinical diagnosis.

Finally, we hypothesize potential utility of ctDNA in transplant selection criteria. Studies have demonstrated generally equivalent outcomes across the many currently described selection criteria, indicating the current spectrum of biomarkers are ineffective at advancing our discriminatory capability [48–50]. In contrast, ctDNA status has been

shown to be associated with shorter RFS and higher recurrence rate based on serial ctDNA testing [51]. In a study by Huang *et al*. (2024) [51] featuring 74 patients undergoing liver transplant for cancer, patients with plasma ctDNA detected postoperatively had a shorter RFS of 17.2 months (vs. 19.2 months, $p = 0.010$) and higher recurrence rate (46.2% vs. 21.3%, $p < 0.0001$) compared to those without detectable ctDNA postoperatively. Significantly, dynamic changes in ctDNA could also predict disease progression prior to changes in levels of traditional tumor biomarkers. In the same study, increase in ctDNA levels occurred prior to changes in AFP and DCP (des-gamma-carboxy prothrombin) for one patient, in whom recurrent liver tumor lesions were subsequently found on MRI [51]. Initial data are promising in this sense, and we hypothesize that addition of ctDNA to morphologic characteristics may improve our ability to stratify pre-transplant oncologic risk.

3.2 Cholangiocarcinoma (CCA)

CCA accounts for 10–15% of primary liver cancers. In the U.S., Australia, and Europe, CCA affects 0.3–3.5 individuals per 100,000 people, but can also reach incidences of 85 cases per 100,000 people in areas such as northeastern Thailand, where liver fluke infection is more common [52]. 5-year overall survival rates for CCA are only about 15–20%, even after curative-intent surgery and adjuvant therapy [53]. Timely diagnosis of CCA is challenging due to limited samples obtained during biopsy, equivocal results of diagnostic testing and radiologic imaging, and lack of specific tumor markers [54]. Therefore, many patients have advanced or systemic disease at time of diagnosis, precluding surgical resection, which is considered the gold-standard therapy [55]. CA19-9 is a traditional serum biomarker used in diagnosing and monitoring CCA. However, its poor sensitivity and specificity, high false positive rate, and unreliable nature in patients with benign conditions including primary sclerosing cholangitis render it a suboptimal biomarker [56,57].

In terms of clinical utility, ctDNA has been shown to predict survival in patients with CCA. A study by Uson Junior *et al*. (2022) [58] featured patients with metastatic intrahepatic CCA, extrahepatic CCA, and gallbladder cancer who underwent ctDNA testing prior to initiation of first-line treatment with platinum-based chemotherapy. After adjusting for cancer subtype, metastatic site, largest tumor size, age, sex, and CA19-9 levels, each 1% increase in ctDNA level was associated with HR of 13.1 in OS [58]. Additionally, when stratifying dominant clonal allele frequency (DCAF) from ctDNA by quartile (ctDNA $\leq$0.6%, 0.6%–3%, 3%–10%, and $\geq$10%), there was a significant association with PFS ($p = 0.014$) and OS (0.001) [58]. Wintachai *et al*. (2021) [59] have also shown the diagnostic value of ctDNA in CCA. In particular, they identified cut-off values of ctDNA of 0.2175 and 0.3388 ng/uL to distinguish CCA from healthy patients and those with benign biliary diseases at high sensitivity (88.7, 82.3%) and specificity (96.7%, 57.6%). Plasma cfDNA had the highest AUC in discriminating CCA from patients with benign biliary disease (AUC: 0.72, 95% CI: 0.61–0.83) compared to CA19-9 (AUC: 0.59, 95% CI: 0.46–0.71) and CEA (AUC: 0.50, 95% CI: 0.36–0.65), showing superior diagnostic efficacy of ctDNA [59].

More recently, preliminary results from the STAMP (adjuvant gemcitabine plus cisplatin (GemCis) versus capecitabine (CAP) in node-positive extrahepatic cholangiocarcinoma (CCA)) trial show feasibility of ctDNA monitoring prior to and during adjuvant chemotherapy, with improved RFS in patients who remained ctDNA negative on adjuvant chemotherapy relative to patients who were ctDNA positive [60]. The utility of tumor-informed ctDNA testing-based minimal residual disease detection in CCA was also recently exemplified in a case study published by Yu *et al*. [61]. In this case, tumor-informed ctDNA testing identified high levels of microsatellite instability and tumor mutational burden, leading to early treatment with pembrolizumab and a DFS within the study follow up period of two years.

Given the wide genetic heterogeneity of CCA, investigation of tumor mutational profiles with ctDNA is valuable. One multi-institutional study of 1671 patients with advanced biliary tract cancer showed fibroblast growth factor receptor 2 (*FGFR2*) fusions, isocitrate dehydrogenase 1 (*IDH1*) mutations, and *BRAF* V600E mutations to be clonal alterations, likely representing early oncogenic drivers [62]. A smaller scale study of 71 patients with CCA showed alterations in *TP53* (38%), *KRAS* (28%), and *PIK3CA* (14%) as the most common [63]. Another study surveying the mutational landscape of biliary tract cancers using ctDNA among 124 patients identified *TP53* and *KRAS* as the most common alterations in all subtypes of disease, followed by *FGFR2* for the intrahepatic subtype, *ARID1A* for the extrahepatic subtype, and *CDK6*, *APC*, and *SMAD4* for the gallbladder subtype [64]. Interestingly, the spectrum of detectable alterations on ctDNA can vary based on age, as patients with early-onset biliary tract cancer (or less than 50 years of age) were shown to have higher rates of *FGFR2* fusions or single-nucleotide variations (21%) compared to those greater than 50 years of age (2%, $p = 0.2$). Conversely, older patients had higher rates of *TP53* mutations (67%) compared to early-onset cancer patients (35%, $p = 0.6$) [64].

Matching systemic therapy regimens based on ctDNA molecular testing may lead to improved treatment outcomes. For example, a study featuring 80 patients who underwent systemic treatment for biliary tract cancers showed significantly prolonged PFS (HR = 0.60 [0.37–0.99], $p = 0.047$) and higher rates of disease control (61% vs. 35%, $p = 0.04$) for patients whose therapy were molecularly matched to ctDNA and/or tissue-DNA genomic profiling compared to those with unmatched regimens [63]. Following targeted

inhibitor treatment, serial ctDNA measurements can also provide insight into mechanisms of acquired resistance. For example, Varghese *et al*. (2021) [65] and Goyal *et al*. (2017) [66] have shown acquisition of new mutations for patients with metastatic iCCA on FGFR-targeting treatments, while Cleary *et al*. (2022) [67] has shown identification of secondary *IDH1* and acquired *IDH2* mutations following treatment with ivosidenib. Given that resistance may occur due to clonal evolution as well, single site biopsy results may not be reliable in capturing polyclonal states as ctDNA.

4. Secondary Liver Malignancies

Colorectal Liver Metastases (CRLM)

Colorectal cancer (CRC) is the third most lethal cancer worldwide and the most common cause of liver metastasis for young males [1,3]. About 30–50% of patients with CRC experience liver metastasis, with a 10-year survival of only 5% [68]. Traditional biomarkers, such as carcinoembryonic antigen (CEA), are limited by low sensitivity and specificity in detecting CRLM [69]. In contrast, ctDNA has been shown to have high sensitivity rates across all stages of CRC [70].

Recently, the landmark DYNAMIC trial showed that ctDNA-guided management was noninferior to standard management for adjuvant therapy following curative-intent surgery for stage II CRC with respect to two and five-year RFS (Table 1) [28,71,72]. For example, five-year RFS was 88% for the ctDNA-guided group, which was similar to 87% in the standard management group (difference 1.1%, 95% CI: –5.8%–8%) [72]. Five-year OS was also similar between the two groups (93.8% vs. 93.3% for ctDNA vs. standard, HR: 1.05; 95% CI: 0.47–2.37, $p = 0.887$) [72]. Long-term follow-up of these trials showed the significance of ctDNA clearance at time of adjuvant therapy completion, as patients with ctDNA clearance had a much higher RFS (85.2%) compared to those with ctDNA persistence (20%) (HR: 15.4, 95% CI: 3.91–61.0, $p < 0.001$) [73].

Similarly, a study featuring 48 patients with CRLM with paired pre- and post-hepatectomy ctDNA showed that negativity of ctDNA following hepatectomy, whether ctDNA+/– or ctDNA–/–, is associated with improved RFS compared to ctDNA+/+, after adjusting for prehepatectomy chemotherapy, synchronous disease, and presence of 2+ CRLM (ctDNA+/–: HR = 0.21, 95% CI 0.08–0.53; ctDNA–/–: HR = 0.21, 95% CI 0.08–0.56) [74]. These findings have led to a single-institution, risk-stratified, prospective trial evaluating ctDNA-directed chemotherapy for patients with following hepatectomy for CRLM (NCT05062317) (Table 3). Likewise, another study by Wehrle *et al*. (2023) [75] showed an association with positive postoperative ctDNA and increased likelihood of disease recurrence ($p = 0.090$). A study by Bolhuis *et al*. (2021) [76] showed early evidence for this relationship—detectable postoperative ctDNA was associated with shorter median RFS (4.8 vs. 12.1 months) and lack of response on pathology. Overall, a recent systematic review and meta-analysis by Wullaert *et al*. (2023) [13] summarized supporting evidence for ctDNA as a prognostic marker, as the presence of ctDNA following surgery had a hazard ratio of 3.12 (2.27–4.28, 95% CI) for recurrence and 5.04 (2.53–10.04, 95% CI) for overall survival.

There are ongoing studies assessing selection criteria for liver transplant for colorectal liver metastasis based on novel pre-transplant protocols, including suggestions that ctDNA may help assess disease burden prior to transplantation [77–79]. This suggestion has not yet been validated but is of interest in guiding patient selection in this relatively novel disease approach.

In terms of mutational landscape, ctDNA can also provide insight into mutations or genetic alterations driving CRLM. In the same study of 51 CRLM patients by Wehrle *et al*. (2023) [75], the most common mutations detected on ctDNA in the were *TP53* (57%), *APC* (53%), *KRAS*, (37%) and *EGFR* (24%). Another study by Shi *et al*. (2022) [80] similarly found *KRAS*, *APC*, and *TP53* to be the most commonly altered genes among 41 patients with metastatic CRC. Such patterns are in line with mutational profile analyses conducted on tissue for CRLM [81,82]. Additionally, alterations on ctDNA have been shown to be associated with therapeutic response, as patients with low-*KRAS* mutational burden had improved response rates, PFS, and OS within the Shi *et al*. (2022) study [80]. On an epigenetic level, methylation status of certain gene promoters have also been used for diagnostic, prognostic, and monitoring purposes. A few examples of commercially available tests that detect altered promoter methylation patterns include the Epi proColon (*SEPT9*) [83], ColoDefense (*SEPT9, SDC2*) [84], SpecColon (*SFRP2, SDC2*) [85], and TriMeth (*C9orf50, KCNQ5, CLIP4*) [86]. Compared to standard diagnostic tumor biomarkers (e.g., CEA, CA19-9), methylation markers *SEPT9, DCC, BOLL, and SFRP2* were shown to have a stronger correlation with tumor volume and operability [87].

5. Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive solid tumor with poor prognosis and high recurrence rates. It is the sixth leading cause of cancer mortality in both sexes, accounting for 5% of all cancer deaths globally [1]. A significant challenge in improving outcomes in PDAC is early detection of primary tumors and metastases. One study investigating ctDNA via ddPCR found higher rates of ctDNA detection in patients with occult metastases (41% vs. 14.6%, $p = 0.001$) compared to patients without occult metastases [88]. In fact, ctDNA was determined to be an independent predictor of occult metastases (OR: 3.113, $p = 0.039$) with a sensitivity of 66.7% and specificity of 81.6% [88].

Table 3. Recruiting clinical trials investigating ctDNA in secondary liver malignancies.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT06300463	Platform Study of Immunotherapy Combinations in Colorectal Cancer Liver Metastases	Interventional	CRLM	Three arms: (1) Botensilimab + Balstilimab (2) Botensilimab + Balstilimab + AGEN1423 (3) Botensilimab + Balstilimab + Radiation	24	March 26, 2024 –March 2027	Primary: Mean CD8: Treg ratio, as determined by flow cytometry of tumor tissue, at time of surgical resection in each treatment arm Secondary: Number of Treatment-Related Adverse Events (TRAEs) as assessed by CTCAE v5.0 per treatment arm, Pathological Response Rate Per Arm, Radiographic Response Rate Per Arm, Number of Participants Per Arm with ctDNA Clearance	Weill Medical College of Cornell University
NCT06225843	Sotevtamab (AB-16B5) Combined With FOLFOX as Neoadjuvant Treatment Prior to Resection of Colorectal Cancer Liver Metastasis (EGIA-003)	Interventional	CRLM	Drugs: Sotevtamab and FOLFOX	17	February 15, 2024 –June 2025	Primary: Rubbia-Brandt score at surgery, Treatment-Emergent Adverse Events Secondary: Objective Response Rate (ORR), Quantity of circulating tumor DNA (ctDNA), Sotevtamab concentrations in plasma, Presence of ADA (anti-sotevtamab antibodies)	Alethia Biotherapeutics
NCT06199232	Targeted Treatment Plus Tislelizumab and HAIC for Advanced CRCLM Failed From Standard Systemic Treatment	Interventional	CRLM (who underwent ctDNA genotyping)	Drugs: HAIC+ Targeted therapy + PD-1 inhibitor	47	January 23, 2024– January 23, 2027	Primary: PFS rate at 6 months Secondary: PFS, OS, Intrahepatic PFS, ORR, DCR, Number of patients with treatment-related adverse events	Peking University
NCT06111105	GUIDE.MRD-01-CRC: Clinical Validation and Benchmarking of Top Performing ctDNA Diagnostics - Colorectal Cancer	Observational	CRLM	Curative-intent resection and candidate for adjuvant chemotherapy	590	August 1, 2023 –July 31, 2030	Primary: Collection of clinical plasma samples at relevant time points for ctDNA diagnostics Secondary: 3-year recurrence-free survival, Lead time between ctDNA detection and clinical recurrence, Prognostic value of ctDNA analysis at relevant time points	Claus Lindbjerg Andersen
NCT05815082	ctDNA-guided Adjuvant Chemotherapy in Liver Metastasis of Colorectal Cancer	Interventional	CRLM (patients with post-operative ctDNA negative only)	Two arms: (1) Surveillance (2) FOLFOX chemotherapy regimen, single-agent 5-FU/LV, capecitabine, or combination with targeted therapy	490	March 20, 2023– February 20, 2033	Primary: 3-year progression-free survival, 5-year progression-free survival Secondary: 3-year overall survival, Complications	Sixth Affiliated Hospital, Sun Yat-sen University

Table 3. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT05797077	Postoperation Maintenance Therapy for Resectable Liver Metastases of Colorectal Cancer Guided by ctDNA	Interventional	CRLM (patients with post-operative ctDNA positive only)	Two arms: (1) Colorectal resection surgery + FOLFOX chemotherapy regimen + Capecitabine maintenance (2) Colorectal resection surgery + FOLFOX chemotherapy regimen	346	February 20, 2023 –February 20, 2031	Primary: 3-years Progression Free Survival, 5-years Progression Free Survival Secondary: 3-years overall survival, 5-years overall survival, Complications	Sixth Affiliated Hospital, Sun Yat-sen University
NCT05787197	ctDNA in CRC Patients Undergoing Curative-intent Surgery for Liver Metastases (CLIMES)	Observational	CRLM	Curative-intent surgical resection + chemotherapy	232	January 9, 2024–June 30, 2027	Primary: Disease-free survival (DFS) Secondary: Number of event-free survival (EFS) in patients who undergo curative-intent resection of CRLM, Overall survival (OS) n patients who undergo curative-intent resection of CRLM, Time to surgical failure (TSF) in patients who undergo curative-intent resection of CRLM, Prognostic value of ctDNA, Prognostic factor(s) for disease recurrence and survival, Association between ctDNA and clinical features	GERCOR - Multidisciplinary Oncology Cooperative Group
NCT05755672	On-treatment Biomarkers in Metastatic Colorectal Cancer for Life (On-CALL)	Observational	CRLM	Treatment with curative intent: Chemotherapy and/or resection	100	March 1, 2023–March 2033	Primary: Follow-up examination of tumor remission, progression or recurrence from histological samples (tumor tissue targeted deep sequencing) and ctDNA analysis Secondary: Quality of life changes (EORTC-QLQ-C30 and EORTC-QLQ-CR29) prior to and after neoadjuvant and/or adjuvant treatment.	Region Skane

Table 3. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT05677113	A Study of QBECO Versus Placebo in the Treatment of Colorectal Cancer That Has Spread to the Liver (PERIOP-06)	Interventional	CRLM	Two arms: (1) QBECO (2) Placebo	115	August 30, 2023–February 1, 2030	Primary: 2-year Progression-Free Survival (PFS) rate Secondary: Clearance of ctDNA, Side-effect profile of QBECO, Quality of recovery, Five-year overall survival	Sunnybrook Health Sciences Centre
NCT05579340	Postoperative Exercise Training and Colorectal Cancer Liver Metastasis (mCRC-POET)	Interventional	CRLM	Surgical resection, adjuvant chemotherapy/radiotherapy, and exercise training	66	April 2023–April 2025	Primary: Change in peak oxygen consumption (VO2peak) Secondary: 3-years recurrence-free survival, 3-years overall survival, Changes in Aerobic Capacity, Changes in Muscle strength, Changes in Functional performance, Changes in Body composition and anthropometrics, Changes in Systolic/Diastolic Blood pressure, Changes in Heart rate, Changes in Blood biochemistry, Changes in Cytokine levels in blood, Changes in Immune cells in blood, Changes in Osteonectin, Changes in Patient-reported symptomatic adverse events, Changes in Health-related quality of life, Changes in Depression, Changes in Anxiety, Changes in Physical activity, Changes in Circulating tumor DNA, Changes in DNA methylation, Changes in treatment tolerance, Postoperative hospital admissions, Postoperative complications	Rigshospitalet, Denmark
NCT05398380	Liver Transplantation for Non-resectable Colorectal Liver Metastases: Translational Research	Interventional	CRLM	Liver transplantation	35	January 1, 2022–December 31, 2026	Primary: Five years overall survival Secondary: 1 and 3 year overall survival, 1, 3, and 5 year recurrence free survival, Number of patients that drop-out of the study prior to receive intervention, Patterns of cancer recurrence after liver transplantation, Changes in quality of life assessed by EORTC QLQ-C30 questionnaire Other: Percentage of intratumoral genetic heterogeneity of metastatic liver via scRNA-sequencing, percentage of patients with ctDNA (pre-chemotherapy, pre-transplantation, every 3 months after transplantation)	Hospital Vall d'Hebron

Table 3. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
NCT05240950	Anti-CEA CAR-T Cells to Treat Colorectal Liver Metastases	Interventional	CRLM	Anti-CEA CAR-T Cells	18	August 25, 2022–December 25, 2026	Primary: Incidence and severity of adverse events, recurrence by ctDNA MRD detection or imaging diagnosis, 2-year RFS rate based on imaging Secondary: Pharmacokinetics (PK) indicator (Cmax or AUC)	Changhai Hospital
NCT05068531	Early Detection of Treatment Failure in Metastatic Colorectal Cancer Patients (eDetect-mCRC)	Observational	CRLM	Resection + FOLFOX-based preoperative neoadjuvant systemic chemotherapy	100	September 1, 2022–October 2026	Primary: Radiological response to pre-operative chemotherapy, Biochemical response to pre-operative chemotherapy, Pathological response to pre-operative chemotherapy, Tumor response to pre-operative chemotherapy, Histopathologic growth pattern, Post-operative minimal residual disease, Time to radiological recurrence, Time to biochemical recurrence, Time to tumor recurrence as assessed by detection or change in level of circulating tumor DNA Secondary: Incidence and grade of FOLFOX-induced neuropathy, Incidence of allergic reaction to oxaliplatin, Incidence of hospitalization for febrile neutropenia, Ninety-day post-surgical complications, Disease-specific survival	Centre hospitalier de l'Université de Montréal
NCT05062317	ctDNA-Directed Post-Hepatectomy Chemotherapy for Patients With Resectable Colorectal Liver Metastases	Interventional	CRLM	Two arms: (1) Capecitabine or 5-fluorouracil (2) FOLFOX (5-fluorouracil, leucovorin and oxaliplatin) or FOLFIRI (5-fluorouracil, leucovorin and irinotecan) with or without bevacizumab	120	April 26, 2022–February 28, 2026	Primary: 1-year RFS rate following liver resection of CRLM with curative intent among ctDNA negative patients who receive risk-stratified post-operative chemotherapy. Secondary: RFS following liver resection of CRLM in ctDNA positive patients, OS following liver resection among ctDNA negative and positive patients, proportion of ctDNA negative at 1-year post-resection, survival of ctDNA negative patients undergoing ctDNA-guided postoperative chemotherapy to historical controls, proportion of patients in each arm who change chemotherapy in response to ctDNA measurement, delineation of pattern of disease recurrence, ctDNA sensitivity and specifcity for predicting disease recurrence,	M.D. Anderson Cancer Center

Table 3. Continued.

NCT	Study Title	Study Type	Liver Cancer Type	Intervention	Sample Size	Study Dates	Outcome Measures	Sponsor
							evaluation of MD Anderson Symptom Inventory (MDASI-GI) during course of postoperative therapy, evaluation and correlation of patient molecular subtypes and characterization of tumor biologic factors associated with ctDNA detection, surgery-related adverse events up to 90 days post-operatively, chemo-related adverse events up to 30 days following last dose of chemotherapy.	
NCT03223779	Study of TAS-102 Plus Radiation Therapy for the Treatment of the Liver in Patients With Hepatic Metastases From Colorectal Cancer	Interventional	CRLM	TAS-102 + Radiation	56	October 13, 2017–January 2025	Primary: Maximum Tolerated Dose (MTD), Duration of Local Control Secondary: Toxicity associated with TAS-102 combined with SBRT, PFS, OS, Association between KRAS or BRAF mutation status with local control, Serial ctDNA measurements	Massachusetts General Hospital
NCT06227728	Analysis of PD-L1, TMB, MSI and ctDNA Dynamics to Predict and Monitor Response to Immunotherapy in Metastatic Cancer	Observational	Stage IV cancer with known metastases (lung, colorectal, breast, gastric, etc.)	Drugs: Immune checkpoint inhibitors	50	March 22, 2024–December 31, 2026	Relationship between ctDNA dynamics and clinical response to immune checkpoint inhibitors (ICIs), Compare and combine ctDNA dynamics and RECIST1.1 to predict clinical response in case of pseudoprogression, Investigate the prognostic value of ctDNA clearance with PFS and OS, Compare the prognostic values of PD-L1, TMB and MSI in predicting clinical response to ICI, best indicator(s) for ICI response	Gene Solutions

TMB, tumor mutational burden; CTCAE, Common Terminology Criteria for Adverse Events; FOLFOX, folinic acid, fluorouracil, oxaliplatin; HAIC, hepatic artery infusion pump; PD-1, programmed cell death protein 1; scRNA, small conditional RNA; FOLFIRI, fluorouracil, leucovorin, fluorouracil, irinotecan; TAS, trifluridine/tipiracil; SBRT, sterotactic body radiation therapy; MSI, microsatellite instability; ICI, immune checkpoint inhibitor.

In terms of survival, ctDNA has been found to be independently associated with worse OS and PFS on multivariable analysis of 104 patients with advanced pancreatic cancer and liver metastasis (HR = 3.1, 95% CI = 1.9–5.0, $p < 0.0001$; HR 2.6, 95% CI = 1.7–4.0, $p < 0.0001$, respectively) [89]. Additionally, detection of ctDNA correlates with increased number of liver lesions, presence of lung and/or peritoneal metastases, tumor burden, and CA19-9 levels [89]. PDAC with liver metastasis has been associated with increased rates of *KRAS* mutations (78%) and higher median maximum variant allele frequency (VAF) (1.9%) compared to other metastatic sites, indicating a more aggressive tumor biology [90]. In addition, somatic copy number alterations of ctDNA seems to be unique to PDAC patients with liver metastasis [91]. Overall, for pancreatic cancer generally, detection of *KRAS*-mutated ctDNA has been shown to feasibly predict early progression of pancreatic cancer [92], while somatic mutation burden on ctDNA can also predict treatment response to first-line chemotherapy [93].

For clinical management, ctDNA-guided treatment is now being explored following upfront resection of PDAC in the AGITG DYNAMIC-Pancreas trial. Preliminary results show association of ctDNA with earlier recurrence, as patients with positive ctDNA 5 weeks following tumor resection had a lower median RFS compared to ctDNA negative patients (13 vs. 22 months, HR: 0.52, $p = 0.003$) [94].

Regarding mutational landscape, ctDNA whole exome sequencing has been used to identify unique molecular profiles in patients with aggressive pancreatic cancer and those with liver metastasis. In particular, enrichment of somatic mutations in *KRAS*, *LAMA1*, *FGFR1*, and *IFF01* in tumor cells and mutations pertaining to the adaptive immune response (*HLA-H, HLA-DRB1, TRBV6-7*) have been noted on ctDNA for pancreatic cancer with liver metastasis [95]. Concurrent *KRAS* copy number gains and somatic mutations on ctDNA have also been associated with extremely poor overall survival for patient with PDAC and metastatic liver lesions [96].

6. Lung Cancer

Lung cancer is the most frequently diagnosed cancer and primary cause of cancer-related deaths worldwide (18.7%) [1]. Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer cases [97]. Approximately 15% of patients with NSCLC have metastasis to the liver, which is associated with the worst prognosis and resistance to targeted therapy against epidermal growth factor receptor (EGFR) [98,99]. According to the National Cancer Comprehensive Network (NCCN) guidelines, ctDNA testing is warranted for patients with advanced NSCLC who are deemed medically unfit for invasive tissue sampling, have insufficient tissue sample for analysis, or uncertain timing of tissue acquisition. Sensitivity and specificity of ctDNA for NSCLC are 70–94% and 90% respectively [100–102].

Noninvasive versus Invasive Lung Evaluation (NSCLC) features the most extensive collection of ctDNA related studies. In the NILE trial, ctDNA was shown to have a 100% positive predictive value and greater than 98.2% concordance with tissue for FDA-approved targets (e.g., *EGFR*, *ALK*, *ROS1*, *BRAF*) in 34 patients with ctDNA testing prior to treatment of metastatic NSCLC [103]. Additionally, ctDNA was shown to be noninferior compared to standard-of-care tissue genotyping for identifying guideline-recommended biomarkers while decreasing median turnaround time (9 vs. 15 days, $p < 0.0001$) compared to tissue [103]. The ACCELERATE trial further underscored the ability of ctDNA to decrease result turn-around time, as patients with advanced NSCLC and ctDNA testing had a lower median time from referral to treatment initiation compared to those undergoing tissue genotyping (39 days (interquartile range (IQR), 27–52) vs. 62 (IQR, 44–82, $p < 0.001$) [104]. When assessed in a larger multicohort study through the Blood First Assay Screening Trial (BFAST), ctDNA guided treatment of patients with *ALK* mutations had a high objective response rate (87.4% [78.5–93.5] by investigator, 92% [84.1–96.7] by independent facility) [105]. One recent nonrandomized controlled trial demonstrated feasibility for using ctDNA in de-escalating tyrosine kinase inhibitor treatment following local consolidative therapy in advanced NSCLC patients while attaining full remission, thereby minimizing unnecessary treatment toxicity [106].

A few studies have investigated the use of ctDNA specifically in the context of liver metastasis for lung cancer. One study noted that concordance of therapeutically targetable mutations on ctDNA with tissue-based genotyping results was highest for patients with liver metastases (100%, 13/13) compared to patients with metastatic disease to other sites (46.2% concordance), indicating promising potential for the clinical applicability of ctDNA-guided treatment for this patient population [107]. When surveying the mutational landscape of 115 patients with NSCLC and liver metastasis, Zhao *et al.* (2024) [108] identified *TP53* and *EGFR* as the most frequently altered genes on ctDNA. This is consistent with findings from Jiang *et al.* (2021) [109], who found *TP53* and *EGFR* to be the most commonly mutated genes for lung adenocarcinoma with liver metastasis. Interestingly, this group also noted higher similarity in mutational and copy number between paired primary lesions and metastases in patients with liver metastases compared to those with brain metastases, indicating a more linear progression model for hepatic metastatic lesion development [109]. Another study by Lam *et al.* (2021) [110] found that visceral metastasis (hepatic, adrenal, renal, or splenic) was associated with increased ctDNA VAF and greater tumor burden. Their discussion highlights a hypothesis regarding decreased clearance of ctDNA in hepatic metastasis specifically, potentially leading to high ctDNA VAF [110].

7. Breast Cancer

Breast cancer is the most common malignancy in female patients globally, with the liver being third most common site of metastasis [1,111]. Several studies have explored the utility of ctDNA in the context of breast cancer at various stages of treatment. For example, a study following 283 patients with a tumor-informed approach found that ctDNA positivity was a notable adverse prognostic factor for distant RFS for both triple negative (TNBC) and hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative cancer subtypes [112]. Interestingly, ctDNA concentration was found to be strongly associated with pathologic complete response and residual cancer burden in TNBC specifically [112]. The I-SPY2 trial results also support the use of ctDNA as a tool for guiding de-escalation of therapy, as early ctDNA clearance at 3 weeks following treatment initiation predicted good outcomes [113]. Another multicenter trial (plasmaMATCH) showed a high sensitivity of digital PCR ctDNA testing (93%) and high concordance rate with targeted sequencing (96–99%) [114]. Among 1044 patients, 533 (51.1%) were identified to have potentially targetable mutations (e.g., *PIK3CA, ESR1, HER2, AKT1, PTEN*). Additionally, patients who underwent ctDNA guided treatment (neratininb for *HER2* mutant and capivasertib for *AKT1* mutation) were found to have comparable durable responses to those who received treatments based on tissue testing [114].

For liver metastasis specifically, one multicenter study featuring 223 metastatic breast cancer patients (NCT05079074), of which 30.9% (72/233) had liver metastasis, demonstrated prolonged PFS for patients with no alterations detected on pre-treatment ctDNA compared to patients with at least 1–2 or 3–4 alterations on ctDNA (6.63 vs. 5.70 vs. 4.90 months respectively, $p < 0.05$) [115]. Another study including 58 patients with metastatic breast cancer showed that copy number changes of ctDNA was significantly correlated with the presence of hepatic metastases ($p = 0.002$), suggesting a high rate of variant mutant DNA in the circulation of patients with metastatic disease [116].

Evaluation of ctDNA has also been used to study tumoral genetics and resistance patterns in breast cancer. One study surveying the ctDNA mutational landscape in Chinese women with breast cancer found the most prevalent mutated genes to be *PIK3CA* (24%), *TP53* (43%), and *ERBB2* (14%) [117]. They also identified significant associations between cfDNA yield and cancer stage ($p = 0.033$, $r = 0.9$) [117]. A proof-of-concept study featuring a ER+/HER2+ patient with mixed invasive ductal-lobular carcinoma showed the ability of ctDNA to capture mutations present in the primary tumor and/or liver metastasis, while primary tumor biopsy sites failed to reliably identify all mutations in the metastasis [118]. Another study evaluating paired plasma and tissue samples from 40 HR+ early-stage breast cancer patients found a broader mutation spectrum with ctDNA compared to tissue DNA, while maintaining dependable assessments of microsatellite instability, tumor mutational burden, loss of heterogeneity, and homologous recombination deficiency [119]. Significantly, ctDNA was able to detect mutations in *ESR1* early—potentially identifying patients who are at risk for resistance to endocrine therapy—along with mutations in DNA damage response and proliferative signaling pathways [118]. For example, one patient who had tumor recurrence and liver metastasis had mutations in *PIK3CA*, *ESR1*, and *TP53* [119]. A separate study with whole genome sequencing from ctDNA in two HR+/HER2- breast cancer patients with liver metastasis identified similar driver mutations (e.g., *PIK3CA, ESR1*) and convergent evolution for drug resistant mutants following endocrine therapy [120].

8. Challenges & Future Directions

Several challenges remain for the use of ctDNA in clinical practice. Firstly, concordance between ctDNA and tissue-based DNA have shown varying rates based on cancer and platform type [29]. When possible, exploration of paired tissue sequencing may provide insight into the presence of tumor-based mutations, while also elucidating ctDNA specific mutations. Additionally, the differences in sequencing methods among commercial and research platforms for ctDNA detection and sequencing may impede comprehensive interpretation of results. Furthermore, certain somatic alterations, such as detection of fusions, are still not as accurately captured on ctDNA compared to primary tissue biopsy sequencing [121]. In the clinic, timing and administration of ctDNA testing may be logistically difficult to obtain both pre- and post-operative testing results for each patient. Furthermore, managing cost of the commercially available assays is necessary to ensure equitable access to cancer care. Given the expanding nature of this field, initial studies have featured smaller sample sizes for feasibility testing and retrospective experimental designs, leading to limitations in drawing reliable conclusions. Thus, increased recruitment, prospective studies, and incorporation into clinical trials may improve our knowledge and understanding of ctDNA and its results. Additionally, although advantages of ctDNA testing include its non-invasive nature, rapid result times, and potential for treatment de-escalation, the impact of liquid biopsy with respect to patient quality-of-life remains to be explored and will likely require investigation based on specific cancer type.

Despite these challenges, the future clinical applications of ctDNA are promising. For diagnostic purposes, ctDNA may be helpful in cancer types for which adequate tissue sampling is difficult to obtain. As prospective studies evolve for primary liver malignancies, ctDNA can be used to select neoadjuvant therapy regimens for high-risk patients and guide de-escalation of therapy by monitoring minimal residual disease. Such changes can significantly impact patient care by decreasing time to treatment, min-

imizing unnecessary toxicity, and tailoring targeted therapy. ctDNA-guided de-escalation of adjuvant therapy or post-treatment surveillance can also reduce overall cost of care [122,123]. Additionally, ctDNA may identify unique, novel mutations for targeted therapy development and allow for serial monitoring of clonal evolution of tumors and development of resistance mechanisms in a dynamic manner [66,124]. Particularly for immunotherapy, several parameters (e.g., microsatellite instability, high tumor mutational burden) are identifiable from ctDNA and can provide personalized predictive value for the benefit of immune checkpoint inhibitors, as shown in the KEYNOTE 158, MYSTIC phase III, and OAK (atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer) clinical trials [125–128]. On a larger scale, with a refined understanding of ctDNA results, liquid biopsy may even be considered for screening purposes, although associated ethical and cost-related challenges may arise.

For research, several avenues remain to be explored for the use of ctDNA within liver cancer. In addition to delineating mutational profiles on ctDNA, epigenomic analysis of tumor-specific methylation patterns has been preliminarily explored and warrants further investigation [129]. As highlighted above, the utility of ctDNA within patients receiving liver transplant for liver cancer also requires further investigation to ensure unwanted sources of cfDNA are not introduced by the donor organ during data acquisition and interpretation. Combining the use of ctDNA with established serum tumor biomarkers and other liver-specific factors is also an open area for investigation for clinical research for patients with cancer and those undergoing liver transplant. On a mechanistic level, research into the exact origins and biologic basis of circulating tumor cells and ctDNA may be helpful in elucidating the mismatch between ctDNA mutational profiles and tumor tissue profiles. The sensitivity and specificity of ctDNA in early-stage malignancies, including primary liver cancers, remains limited due to smaller amounts of ctDNA shedding [22,130]. For example, large-scale ctDNA profiling of 236 HCC patients with staging data revealed a lower sensitivity for ctDNA detection for stage I-III cancers (68%, 95% CI: 62.6%–73.4%) compared to stage IV cancers (86.3%, 95% CI: 83.6%–89%) [130]. Although strides are being made to detect alterations in ctDNA for early stage cancers [22,101,131], further research is needed to optimize detection of localized cancers at such timepoints for diagnostic purposes. Lastly, for studies evaluating ctDNA in other primary cancer types (e.g., colorectal, pancreas, lung, breast), including subgroup analyses based on metastatic site is crucial to broadening our knowledge of the mutational landscape and tumor biology in secondary liver malignancies.

9. Conclusion

Circulating tumor DNA has several advantages for liver malignancies in the modern era, such as its non-invasive nature, quick turnaround time with results, detection of actionable mutations, and monitoring of minimal residual disease. Information generated from this tool can guide treatment decisions, such as de-escalation of therapy, initiation of targeted therapy, or treatment switches, thereby facilitating precision oncology. Barriers and challenges to consider when implementing ctDNA testing in the clinical setting include evaluating concordance with patient tumor tissue, deciding on the use of tumor-informed versus uninformed approaches, and timing of pre- and post-treatment ctDNA. As liquid biopsy platforms evolve, ctDNA will become a clinically significant tool in guiding neoadjuvant and adjuvant therapy agents and timelines, while shifting diagnostic and surveillance guidelines. Future studies involving liver cancers should include larger scale studies with paired tissue sampling, while prospective clinical trials can consider integrating serial ctDNA measurements into treatment arms and subgroup analyses based on metastatic site to evaluate its full utility.

Author Contributions

The study was conceptualized and conducted under the direction of FA and DCHK. Literature review and analysis was performed by HH and CJW. Manuscript drafting was performed by HH and CJW. NT, KS, CJ, SS, RP, JME, MWL, KH, AS, and CM contributed substantially to the acquisition of references for the work. Figures were created by KS, CJ, SS, HH, CJW, and OFK. Tables were created by HH, CJW, and PK. Critical manuscript review was performed by all authors. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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