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Review

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Circulating tumor DNA to monitor treatment response in solid tumors and advance precision oncology

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Circulating tumor DNA (ctDNA) has emerged as a dynamic biomarker in cancer, as evidenced by its increasing integration into clinical practice. Carrying tumor specific characteristics, ctDNA can be used to inform treatment selection, monitor response, and identify drug resistance. In this review, we provide a comprehensive, up-to-date summary of ctDNA in monitoring treatment response with a focus on lung, colorectal, and breast cancers, and discuss current challenges and future directions.

Background

The therapeutic landscape for cancer has transformed significantly over the past two decades with the advent of precision oncology. This paradigm shift underscores the critical importance of aligning patients with the appropriate molecular therapies at the right time, aiming to improve clinical outcomes while minimizing the use of ineffective and potentially toxic treatments. However, the methodologies for monitoring treatment response have not evolved at the same pace, still relying heavily on imaging and biomarkers that often lack specificity. These approaches, while useful, have significant limitations, including a lack of sensitivity and an inability to provide detailed molecular insights that are crucial for clinical decision-making in the era of targeted therapies.

One of the critical challenges in oncology is the dynamic stratification of patients for appropriate treatments. Real-time stratification is essential to ensure that therapeutic interventions are timely and effective. Traditional tissue biopsies, although informative, are often not feasible for repeated use due to their invasive nature. They also fail to capture the full extent of tumor heterogeneity and dissemination, particularly in metastatic settings[1].

Imaging techniques, such as those adhering to the Response Evaluation Criteria in Solid Tumors (RECIST), remain the gold standard for monitoring treatment response[2]. The RECIST are a set of standardized criteria developed to provide a consistent method for assessing tumor response to treatment. These criteria are commonly used in clinical practice and in clinical trials to evaluate the efficacy of chemotherapy and targeted therapy and are recognized by regulatory bodies like the Food and Drug Administration (FDA) and European Medicines Agency (EMA). An updated version was recently introduced for evaluating responses in patients treated with immunotherapy, called iRECIST[3]. RECIST and iRECIST primarily rely on imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), to measure changes in tumor size. However, the focus on macroscopic anatomical changes in tumor size do not always correlate with molecular or cellular changes that are critical for understanding the effectiveness of targeted therapies and immunotherapies, and in particular, does not detect microscopic disease such as in minimal residual disease (MRD), which may lead to relapse. There is also a lack in sensitivity in detecting early treatment responses, particularly in cases where tumors do not shrink significantly but undergo necrosis or other changes.

Liquid biopsy has emerged as a pivotal modality for cancer surveillance through the analysis of circulating biomarkers in biofluids such as blood, urine or saliva[4]. Unlike conventional tissue biopsies that require surgical procedures, liquid biopsy is a minimally invasive approach for real-time analysis of cancer burden, disease progression, and response to treatment[5]. The procedural ease, low cost, and diminished invasiveness of liquid biopsy confer substantial promise for integration into routine clinical practice, providing a dynamic platform for personalized therapeutic interventions and quick adaptations to evolving disease states[4,5].

Several analytes can be studied through liquid biopsy, including circulating tumor cells (CTCs), extracellular vesicles (EVs), and circulating tumor DNA (ctDNA). CTCs are tumor cells that have shed from the primary tumor and circulate in the bloodstream[6,7], while EVs include a diverse group of nano-sized particles encased in a lipid bilayer membrane released by various cell types[8,9]. Both EVs and CTCs have been shown to play central roles in tumor invasion and metastasis[8,10]. On the other hand, circulating tumor DNA (ctDNA) refers to small fragments of DNA that are released by tumor cells into the bloodstream. Nowadays, studies are exploring a multi-omic approach to liquid biopsy research, in which multiple analytes are analyzed within the same liquid biopsy sample[11].

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ctDNA arguably holds the most clinical potential as a biomarker, as demonstrated by its clinical adoption in several applications, and as such will be the focus of the present review. Since blood, irrespective of health state, contains cell-free DNA (cfDNA) derived mainly from the physiologic apoptosis of hematopoietic and other normal cells[12,13], evaluating ctDNA involves detecting a tumor-specific characteristic (e.g., somatic mutation, methylation profile or viral sequence) that distinguishes it from cfDNA of non-tumor origin. The quantity of ctDNA found in the blood has been correlated to tumor burden and cell turnover[14], ranging from below 1% of total cfDNA in early-stage cancer to upwards of 90% in late-stage disease[15]. The half-life of cfDNA in circulation, estimated between 16 min and several hours[16,17], enables real-time monitoring of tumor heterogeneity and subclonal changes[18–20]. Because cfDNA is thought to be released largely as a result of cell death[21], it can provide information on treatment response, with recent data suggesting that early ctDNA release may reflect outcomes across various tumor types[22–26]. Moreover, mutations found in cfDNA from patient plasma likely encompass both primary tumor and metastatic sites, making it representative of systemic disease[27]. Finally, recent studies have revealed that fragmentation patterns can also be used to differentiate ctDNA from normal cfDNA[28]. Indeed, cfDNA fragmentation and end motifs have been shown to inform on pathological states[29–31]. Furthermore, cfDNA fragment size may reflect the emission process[32,33], adding another layer of insight into the dynamics of ctDNA.

While ctDNA has been investigated for cancer diagnostics and prognostication, arguably its most immediate clinical application is for the assessment of treatment response and MRD, as emphasized by the nature of the several ctDNA assays already integrated into clinical practice[34–36]. ctDNA offers advantages in providing a simple approach to detect minimal levels of disease specifically and non-invasively, allowing assessment of response to treatment, presence of residual disease, and emergence of resistance[37,38] (Fig. 1).

Despite its promise, the clinical adoption of ctDNA faces several challenges. Low ctDNA abundance and the lack of technical standardization are significant hurdles. Addressing these challenges requires refining detection methods, establishing standardized protocols, and conducting large-scale clinical trials to validate the clinical utility of ctDNA across diverse cancer populations. While ctDNA is presently being explored for multiple purposes, including early cancer diagnostics and prognostication, we believe that the most immediate clinical application of ctDNA is in the monitoring of treatment response[39,40]. This review aims to provide a comprehensive overview of the current state of ctDNA research, including the novel methodologies and biofluids used for analysis, focusing specifically on the application of ctDNA in assessing treatment response in the most common solid tumors. We highlight clinical applications, ongoing trials, and emerging research. Additionally, we describe the analytical challenges and hurdles to its clinical adoption and discuss future directions.

Approaches to ctDNA monitoring: advances and challenges

Given the low abundance of ctDNA compared to non-cancer (normal) cfDNA, highly sensitive techniques are essential for effectively detecting tumor-specific DNA in the circulation. Numerous methods have been applied and developed for ctDNA detection and analysis, predominantly focusing on identifying tumor-specific somatic mutations or other genomic alterations (previously reviewed in Siravegna et al.[41] and Pessoa et al.[42]). Driver mutations detected in ctDNA are frequently utilized as an indicator of disease burden, under the assumption that these somatic events will be represented in a large proportion of the cancer cells[43]. Levels of ctDNA through the detection of such mutations can be tracked longitudinally to assess disease burden[44,45], MRD[46–48], and treatment response[49,50]. Assessing molecular response using ctDNA involves evaluating ctDNA clearance after treatment, percent change from baseline, and other quantitative measures. Additionally, emerging alterations such as acquired resistance mutations to targeted therapies or chemotherapy are invaluable as biomarkers to gauge treatment response and to select/modify treatment approaches[51,52].

Targeted approaches, such as polymerase chain reaction (PCR) methods, can detect mutations with high sensitivity and rapid turnaround times. Techniques like quantitative PCR (qPCR), digital (d)PCR and BEAMing (beads, emulsion, amplification, and magnetics) are often employed, and have been thoroughly reviewed in Wang et al.[53] and Kim et al.[54]. These methods are particularly useful for tumor-informed analyses, where sequencing conducted on primary tumor tissue is used to identify mutations or somatic alterations of interest, which can then be targeted using these assays. In contrast, commonly mutated genes in specific cancers, such as *BRAF* in melanoma[55], *KRAS* in lung and colorectal cancer (CRC)[56,57], *ESR1* and *PIK3CA* in breast cancer[58,59], androgen receptor (*AR*) in prostate cancer[60], *IDH* in gliomas[61], and *GNAQ/11* in uveal melanoma[62], can serve as targets even without tumor tissue analysis. These targeted approaches facilitate rapid and cost-effective analyses but are limited to a small number of mutations or alterations that can be monitored per assay.

In recent years, next-generation sequencing (NGS) methodologies have advanced significantly, offering broader ranges of genomic alterations within patient samples without necessitating a tumor-informed approach[63–65]. NGS technologies, including whole-exome sequencing (WES) and whole-genome sequencing (WGS), as well as targeted approaches such as tagged-amplicon deep sequencing (TAm-Seq)[66], Safe-Sequencing System (Safe-SeqS)[67], CAncer Personalized Profiling by deep Sequencing (CAPP-Seq)[63], and targeted error correction sequencing (TEC-Seq)[68] allow for comprehensive assessments of numerous patient-specific genomic changes, providing a more detailed understanding of the disease. These methods are particularly relevant for heterogeneous cancers with high genomic instability. However, a concern with NGS is that the PCR step present in most NGS workflows introduces low-frequency errors which can

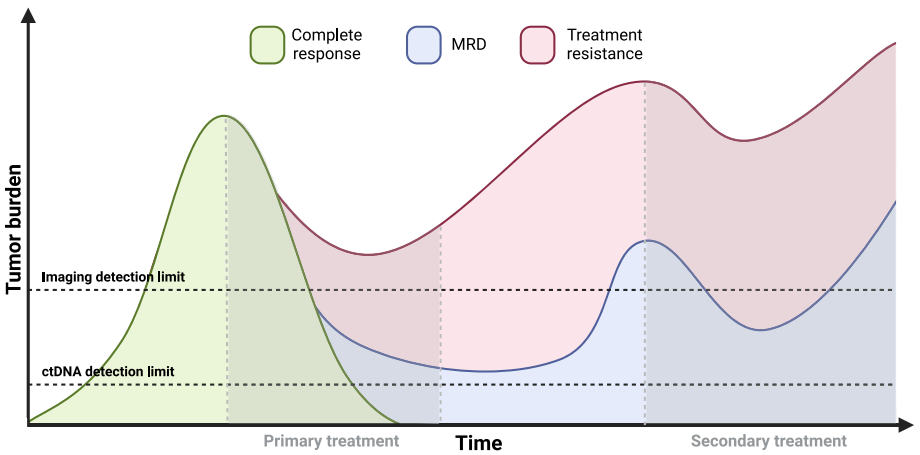

Fig. 1 | ctDNA levels to monitor treatment response. ctDNA can offer a more sensitive and personalized approach to monitoring disease compared to imaging or other conventional methods. This sensitivity can be explored in several ways: to assess response, MRD, and emergence of resistance.

be misidentified as low-frequency variants present in the ctDNA. As such, many methods rely on unique molecular identifiers (UMIs), which are molecular barcodes tagged onto DNA fragments before PCR amplification, which can help to filter out true mutations from sequencing artefacts[69]. In 2012, Schmitt et al. introduced Duplex Sequencing, which became the gold-standard of high-accuracy sequencing[69]. This method tags and sequences each of the two strands of a DNA duplex, relying on the fact that true mutations would be found in the same position on both strands, thus further improving error correction by UMIs[69]. However, generating a duplex consensus is highly inefficient, and thus several methods have been developed to address this shortcoming, including SaferSeqS[70], NanoSeq[71], and Singleton Correction[72]. Notably, most recently in 2023, Bae et al. developed Concatenating Original Duplex for Error Correction (CODEC), which allows for 1000-fold higher accuracy than NGS and uses up to 100-fold fewer reads than duplex sequencing, by reading both strands of each DNA duplex with single NGS read pairs[73]. NGS methodologies used for ctDNA analysis have also been thoroughly reviewed in Ferreira da Silva, et al.[74].

However, despite the continuing development of more sensitive NGS methodologies, the limitation of low input ctDNA amounts in early-stage cancers and low-shedding tumors still exists[63,75–77]. Interestingly, in 2024 Martin-Alonso et al. proposed the use of priming agents to transiently reduce cfDNA clearance in vivo to address the low levels of ctDNA in circulation, presenting a fascinating future direction for the ctDNA field[78]. Nevertheless, the presence of clonal hematopoiesis of indeterminate potential (CHIP) variants in the cfDNA pool can lead to false positives when sequencing[79,80]. Moreover, sequencing methods face challenges related to cost and longer analysis times, constraining their use in real-time decision-making[51,81]. Finally, the low input volumes of cfDNA require careful trade-offs between the breadth and depth of analysis to detect low copy alterations.

Beyond mutational analysis, non-mutation-based approaches have been explored for ctDNA monitoring. For example, viral cfDNA has been investigated for cancer types with viral etiologies, such as those associated with human papillomaviruses (HPV) (oropharyngeal and cervical cancers)[82,83], hepatitis B virus (hepatocellular carcinoma)[84], and herpesviruses (certain lymphomas, nasopharyngeal cancer, Kaposi sarcoma)[85–87]. Otherwise, DNA methylation or fragmentome analysis have also been used for ctDNA analyses[32,33,88]. Such approaches can help overcome traditional challenges associated with genomic ctDNA studies, such as confounding data from CHIP variants[80].

DNA methylation analysis can, in many ways, provide information that is similar to other genetic analyses. As with mutational analysis, promoter hypermethylation can be used for longitudinal disease monitoring at specific sites[89–91]. This generally requires information obtained from tumor tissue, often done using arrays[92]. Traditionally, DNA methylation analysis has relied on bisulfite conversion, with analytical methods such as whole genome bisulfite sequencing (WGBS) and targeted bisulfite sequencing. These methods have shown to be effective in longitudinal monitoring of cancer patients in both plasma[93] and urine[92,94]. Single gene hypermethylation, particularly of tumor suppressor genes such as *p16* in hepatocellular carcinoma, *septin 9* in CRC, and *MGMT* in gliomas[89–91], has also been an effective tool to detect cancer in liquid biopsies. More recently, bisulfite-free methods have been incorporated to reduce the challenges associated with DNA degradation caused by bisulfite conversion[95]. Importantly, methods such as chromatin immunoprecipitation sequencing (ChIP-Seq) and other immunoprecipitation-based methods such as methylated DNA immunoprecipitation sequencing (MeDIP-Seq) are gaining popularity in cfDNA analysis, with especially useful applications in determining the cells of origin of cfDNA[96–98].

cfDNA fragmentomics is an emerging field in liquid biopsy, referring to the study of cfDNA fragmentation patterns, fragment sizes, and end characteristics[99–101]. Studies have demonstrated that cancer patients often exhibit more diverse fragmentation patterns, leading to an overall smaller average fragment size[102], which can be used to distinguish cancer from non-cancer derived cfDNA. In fact, qPCR was used by Diehl et al. to first suggest that cfDNA fragments containing mutant sequences are generally shorter than non-mutant cfDNA fragments[103]. Moreover, using an automated electrophoresis system, our group has shown significant increases in cfDNA fragment sizes in the plasma of HPV-positive head and neck cancer patients after treatment, suggesting a potential additional biomarker for monitoring treatment response[104]. Recently, the development of novel bioinformatics methodologies has allowed for the acquisition of larger, higher-throughput, and more detailed fragmentomic data[28,105–109]. For instance, in 2019, Cristiano et al. developed their method for the genome-wide analysis of cfDNA fragmentation patterns using low-coverage WGS called DELFI (DNA evaluation of fragments for early interception)[28]. Combining this machine learning model that incorporates genome-wide fragmentation profiles with mutation-based cfDNA analyses, the sensitivity of cancer detection was 91%. Other novel methods for fragmentomic analysis that have been developed in recent years include orientation-aware cfDNA fragmentation (OCF)[110], epigenetic expression inference from cfDNA-sequencing (EPIC-seq)[111], motif diversity score (MDS)[112], windowed protection score (WPS)[105], assessing transcription factor-binding site accessibility (TFBS)[113], LIQUORICE[114], and Griffin[115] which have all been thoroughly reviewed in Liu[100] and Thierry et al.[101].

Recently, multimodal approaches combining various types of analyses have become more common in the liquid biopsy field. Studies now often incorporate combinations of multiple types of analyses, such as copy number alterations (CNA), genomic, epigenetic, and fragmentomic analyses on cfDNA samples[77,98,116,117]. For example, Parikh et al. showed that integrating epigenomic signatures increased sensitivity for recurrence by 25–36% *vs.* genomic alterations alone[118]. Moreover, the spectrum of biofluids used for ctDNA analysis has also expanded beyond plasma to include urine[26,119–122], saliva[104,123–125], and cerebrospinal fluid (CSF)[126–129], among others, for monitoring treatment response and disease progression. Figure 2 presents an overview of the current and emerging range of biofluids and methodologies that can be used for ctDNA analysis.

Although most ctDNA studies to date have been observational in nature, the potential role of ctDNA monitoring to help guide clinical decision-making cannot be overlooked. In fact, in recent years, several clinical trials have been initiated to evaluate the use of ctDNA monitoring in the clinical setting. Specifically, the potential for ctDNA use in helping clinicians with initial treatment selection or treatment modification based on ctDNA response have been explored. In the following sections, we will focus on the use of ctDNA to monitor treatment response, MRD, and resistance in common solid cancers, and we will highlight current clinical trials using ctDNA.

Lung cancer

As the leading cause of cancer-related deaths worldwide, lung cancer has been extensively studied in the liquid biopsy field[130]. The approval of the first diagnostic liquid biopsy assay by the FDA in 2016 for use in non-small cell lung cancer (NSCLC)[34] marked a significant milestone in the use of liquid biopsies. The Cobas EGFR Mutation Test v2 (Roche Molecular Systems, Inc) is a real-time PCR assay that can detect 42 mutations in exons 18, 19, 20 and 21 of the *EGFR* gene, and which can be used in the clinic for the prescription of EGFR inhibitors in NSCLC patients when biopsied tumor tissue is not available[34]. Since then, two other liquid biopsy-based tests were approved by the FDA for use in treatment selection in NSCLC patients, the Guardant 360 CDx and the FoundationOne Liquid CDx tests, which are both NGS panel-based assays[35,36]. Currently, there exists a growing body of research focusing on the dynamic monitoring of ctDNA in lung cancer patients in order to assess treatment response, which will be reviewed below. Although the majority of these studies have centered on NSCLC, which makes up 85% of lung cancer cases[130], there has also been exploration into ctDNA monitoring in small cell lung cancer (SCLC) patients in recent years.

Early studies evaluating ctDNA in NSCLC predominately used PCR-based methods to detect specific point mutations in cfDNA, targeting genes such as *KRAS* or *EGFR*[56,131]. With the advent of NGS, sequencing-based methods for ctDNA analysis in NSCLC gained traction. Notably, in 2014, Newman et al. introduced CAPP-Seq and demonstrated its utility in

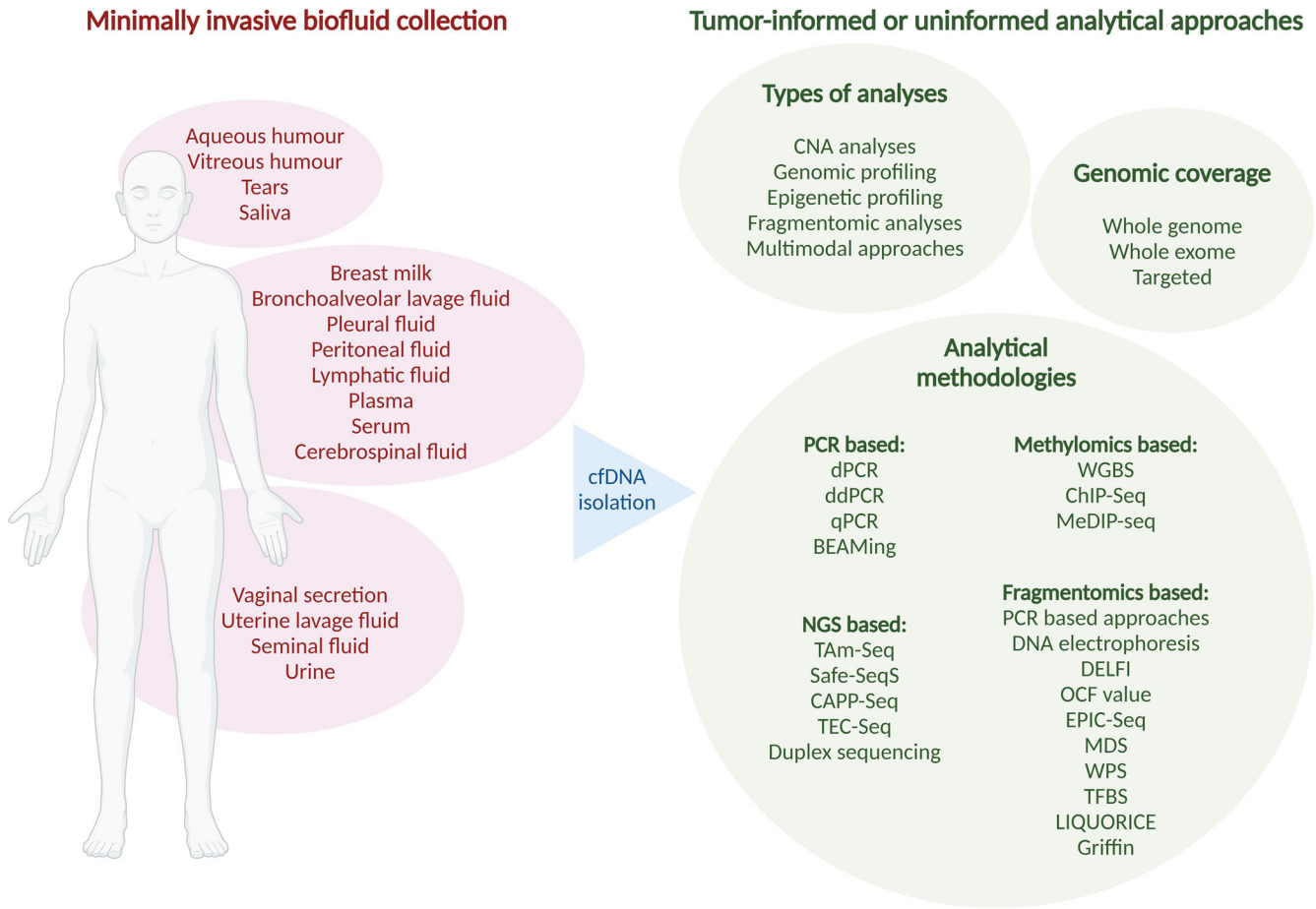

Fig. 2 | Types of biofluids, analyses, genomic regions, and methodologies that can be used for ctDNA monitoring in cancer patients. A vast range of biofluids can be collected from cancer patients for cfDNA isolation. Following this, tumor-informed or uninformed, as well as mutation or non-mutation based analytical approaches can be used for ctDNA analysis, with multimodal studies becoming more common in the field.

detecting ctDNA in NSCLC patients[63]. In their study, ctDNA was detectable in 100% of stage II-IV ($n = 9$) and 50% of stage I ($n = 4$) NSCLC patients, and ctDNA levels significantly correlated with tumor volume[63]. This method was later was used by Chaudhuri et al. for longitudinal monitoring of ctDNA in lung cancer patients with localized disease (stages I-III) undergoing treatment with curative intent and was found to be useful in detecting post-treatment MRD[132]. Further, in 2020, Moding et al. used CAPP-Seq to analyze ctDNA in 218 samples from 65 NSCLC patients with locally advanced disease and found that ctDNA dynamics early during consolidation immune checkpoint inhibition could be used to identify patients responding to the treatment[50]. In a cohort of 100 NSCLC stage I-III patients from the TRACERx (TRAcking non-small-cell lung Cancer Evolution Through Therapy (Rx)) study, phylogenetic ctDNA analysis was used to track the development of subclonal mutations and metastases[133]. Additionally, many studies have highlighted the prognostic value and clinical utility of evaluating ctDNA dynamics in NSCLC through changes observed in pre- and post-surgery samples[119,134,135]. Moreover, other studies have highlighted the usefulness of using ctDNA to detect MRD in the post-operative period in NSCLC[46,136,137].

With the advent of immunotherapy and targeted therapies, there has been a paradigm shift in the treatment landscape of NSCLC, particularly for patients with late-stage and metastatic disease. Longitudinal monitoring of ctDNA during personalized NSCLC treatments has become increasingly relevant, aiding in the detection of acquired resistance mutations and predicting patient response to therapies[138–144]. For instance, studies have used NGS and/or dPCR to monitor ctDNA during treatment with EGFR-tyrosine kinase inhibitors (TKIs) such as afatinib[138] or osimertinib[140]. In the latter study, it was found that plasma ctDNA response to treatment occurs early after treatment, suggesting that optimal ctDNA assessment may be within the first cycle of therapy (before standard imaging timepoints). Furthermore, longitudinal ctDNA monitoring has been especially useful in detecting mutations that confer acquired resistance to targeted therapies, such as *EGFR* T790M resistance mutations during EGFR-TKI treatment[145,146] as well as response to ALK TKIs[143] and BRAF inhibitors[144]. This presents a potential means to monitor ctDNA for resistance mutations even before the development of disease progression. Moreover, recent studies have explored longitudinal disease monitoring with ctDNA to predict patient response to treatments and allow for earlier clinical decision-making and therapy intervention[147–151]. For instance, the European Organisation for Research and Treatment of Cancer (EORTC) Lung Cancer Group 1613 APPLE phase II clinical trial was designed to evaluate the feasibility of using longitudinal plasma *EGFR* T790M monitoring by Cobas EGFR test v2 in determining the best treatment administration of gefitinib and osimertinib[150]. Overall, they found that serial monitoring of ctDNA allowed for identification of molecular progression before RECIST progression and thus, allowing for an earlier switch from gefitinib to osimertinib in 17% of patients, resulting in satisfactory progression-free-survival (PFS) and overall survival (OS). A summary of the completed clinical trials assessing the use of ctDNA for clinical interventional purposes with published results in lung cancer can be found in Table 1. Also, there are many clinical trials that are ongoing to assess the use of ctDNA for interventional purposes. For instance, stage 1 of the BR.36 study was recently published, which is a phase 2 clinical trial of ctDNA molecular response-adaptive immuno-chemotherapy for treatment-naïve NSCLC[151]. This first

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Table 1 | Completed clinical trials with published results that used ctDNA for clinical decision making and treatment intervention in lung, colorectal, and breast cancers[a]

Cancer Type	Study Name/ Clinical Trial Identifier/ Publications	Number of Patients	Study Goal	ctDNA Methodology	Key Findings
NSCLC	LiquidLung-O NCT02769286[290,291]	119	To determine treatment efficacy of osimertinib in patients with NSCLC with activating *EGFR* mutations (cohort 1) or T790M *EGFR* mutations (cohort 2) which were detected from ctDNA.	PCR (PANA Mutyper R EGFR assay and Cobas EGFR Mutation Test v2)	Cohort 1: Osimertinib had favorable outcomes in the first-line treatment of metastatic NSCLC with activating *EGFR* mutations in ctDNA as well as tumor DNA. Cohort 2: Osimertinib had favorable outcomes in patients with NSCLC with T790M detected from ctDNA, with unknown tumor mutation status.
NSCLC	Liquid-Lung-A NCT02629523[292]	331	To determine the efficiency of afatinib in treatment-naïve patients with lung cancer harboring *EGFR* exon 19 deletions or exon 21 point mutations detected in ctDNA.	PCR (PANA Mutyper R EGFR assay)	Afatinib showed similar objective response rate and PFS in patients with lung cancer harboring *EGFR* mutations in their ctDNA regardless of tumor *EGFR* mutation results. Thus, the survival benefit of afatinib treatment can be achieved by using noninvasive (ctDNA) assays.
NSCLC	BFAST NCT03178552[293–295]	2219	To evaluate the relationship between blood-based NGS detection of actionable genetic alterations and activity of targeted therapies or immunotherapy in treatment-naive advanced or metastatic NSCLC.	NGS (FoundationACT and a blood-based tumor mutational burden assay)	Results from cohorts A, B, C published. Overall, the trial results reveal the clinical application of blood-based NGS as a method to inform clinical decision-making.
NSCLC	APPLE NCT02856893[150]	103	To evaluate the feasibility of using longitudinal plasma *EGFR* T790M monitoring in determining the best treatment administration of gefitinib and osimertinib.	PCR (Cobas EGFR mutation test v2)	Results from arms B and C published. The serial monitoring of ctDNA T790M status was not only feasible but lead to the identification of molecular progression before RECIST progression and an earlier switch to osimertinib in 17% of patients with satisfactory PFS and OS outcomes.
NSCLC	ACCELERATE NCT04863924[296]	150	To determine the association between ctDNA genotyping before tissue diagnosis and time to treatment.	NGS (InVisionFirst-Lung)	The median time to treatment was 39 days for the ACCELERATE cohort vs 62 days for the reference cohort. Thus, the use of plasma ctDNA genotyping before tissue diagnosis was associated with accelerated time to treatment.
NSCLC	LOCAL NCT03046316[297]	60	To assess the feasibility of de-escalation of TKI treatment guided by ctDNA for achieving complete remission after local consolidative therapy.	NGS (oncoMRD-B panel of 338 genes (GenePlus))	Overall, a ctDNA-guided adaptive de-escalation TKI treatment strategy is feasible for patients with advanced NSCLC.
CRC	TRIUMPH UMIN000027887[200]	30	To evaluate the efficacy of pertuzumab plus trastuzumab for mCRC with *HER2* amplification confirmed by tumor tissue or ctDNA analysis.	NGS (Guardant360)	ctDNA genotyping can identify patients who benefit from dual-HER2 blockade and monitor treatment response.
CRC	CHRONOS NCT03227926[196]	52	To identify *RAS*/*BRAF*/*EGFR* mutations in ctDNA to tailor a chemotherapy-free anti-EGFR rechallenge with panitumumab.	NGS and ddPCR	ctDNA analysis is an effective, safe, and timely method to guide anti-EGFR rechallenge therapy with panitumumab in patients with mCRC.
CRC	DYNAMIC ACTRN12615000381583[49]	455	To assess whether a ctDNA-guided approach could reduce the use of ACT without compromising recurrence risk.	NGS (Safe-SeqS)	A ctDNA-guided approach reduced ACT use without compromising recurrence-free survival.
CRC	COBRA NCT04068103[298]	635	To evaluate if positive ctDNA after resection can identify patients who will benefit from ACT.	NGS (Guardant LUNAR assay)	Preliminary results in 635 patients showed no improvement in ctDNA clearance after 6 months of chemotherapy for patients with ctDNA detected following resection of stage IIA colon cancer. The phase II endpoint was not met and further enrollment has been stopped.
Breast	PlasmaMATCH NCT03182634[299]	1034	To determine the ability of ctDNA testing to select patients for mutation-directed therapy.	ddPCR and NGS (Guardant360)	The analysis of ctDNA allows for accurate genotyping, facilitating the identification of mutation-specific treatments for breast cancer. Results highlighted the effectiveness of targeted therapies for uncommon *HER2* and *AKT1* mutations, confirming their potential as actionable targets for treatment.

Table 1 (continued) | Completed clinical trials with published results that used ctDNA for clinical decision making and treatment intervention in lung, colorectal, and breast cancers[a]

Cancer Type	Study Name/ Clinical Trial Identifier/ Publications	Number of Patients	Study Goal	ctDNA Methodology	Key Findings
Breast	PADA-1 NCT03079011[223]	1017	To demonstrate the effectiveness of early therapy change based on increasing *ESR1* mutation in blood, while evaluating the overall safety of combining fulvestrant and palbociclib.	multiplex ddPCR	The early therapeutic targeting of blood *ESR1* mutation in ER+/HER2- advanced breast cancer resulted in significant clinical benefit.
Breast	ACTDNA NCT05079074[300,301]	223	To evaluate the efficacy of re-subtyping and determining treatment strategy based on ctDNA alterations.	NGS	Patients with druggable ctDNA alterations showed significant improvements in PFS and disease control rate when receiving guided therapy, compared to those receiving standard treatment.
Breast	c-TRAK-TN NCT03145961[232]	208	To assess the utility of ctDNA in detecting residual disease following patients' standard primary treatment for TNBC.	ddPCR	Patients had a high rate of metastatic disease on ctDNA detection. Implementation of MRD detection with personalized ctDNA assays was clinically achievable.

ACT adjuvant chemotherapy, *ctDNA* Circulating tumor DNA, *ddPCR* droplet digital PCR, *ER* Estrogen receptor, *MBC* Metastatic breast cancer, *mCRC* metastatic colorectal cancer, *MRD* Minimal residual disease, *NGS* Next-generation sequencing, *NSCLC* Non-small cell lung cancer, *OS* Overall survival, *PCR* Polymerase chain reaction, *PFS* Progression-free survival, Safe-SeqS: Safe-Sequencing System, *TNBC* Triple negative breast cancer, *TKI* Tyrosine kinase inhibitor, *VAF* variant allele frequency.
[a]Table 1 includes interventional, ctDNA lead, completed or terminated clinical trials with published results.

observational stage of the clinical trial showed that ctDNA molecular response could identify patients with metastatic NSCLC less likely to achieve favorable clinical outcomes with single-agent PD-1 therapy. In stage 2 of the BR.36 trial, which is ongoing, patients at risk of progression as determined by ctDNA analysis, will be randomized into either treatment intensification or continuation of therapy study arms. Additionally, there are several ongoing clinical trials to determine if it is beneficial to provide additional treatment to NSCLC patients who have detectable ctDNA (MRD) after their initial treatments or surgeries, including the ADAPT-E[152], ADAPT-C[153], and ctDNA Lung RCT trials[154]. Figure 3 below exemplifies the current applications of ctDNA in precision oncology for NSCLC.

Despite the extensive studies on ctDNA kinetics during cancer treatment in NSCLC, there is limited research focused on ctDNA monitoring in patients with SCLC, an aggressive disease comprising 15% of lung cancer cases[130]. As SCLC is characterized by nearly consistent inactivation of *TP53*, early ctDNA studies used PCR-based methods for the detection of *TP53* mutations in cfDNA of SCLC patients[155,156]. Subsequent studies have employed NGS for longitudinal ctDNA analysis in SCLC and demonstrated its ability to monitor disease[157–163]. Notably, Lovly's group, using a targeted sequencing panel of 14 frequently mutated genes in SCLC, demonstrated the potential of ctDNA analysis in providing evidence of disease relapse before conventional imaging[157], as well as serving as a prognostic indicator post-treatment[160]. Likewise, Nong et al. used targeted deep sequencing of 430 genes on pre and post-treatment plasma samples in 22 SCLC patients, and found that mutations in DNA repair and NOTCH signaling pathways were enriched post-treatment, exemplifying that NGS can allow for the analysis of the genomic evolution of SCLC[158]. More recently, WGS was used for the detection of CNA in parallel with targeted sequencing of 110 SCLC-associated genes to analyze cfDNA from 69 SCLC patients, of which longitudinal samples were obtained from 6 patients[161]. Moreover, a recent study from Sivapalan et al. used targeted error-correction sequencing and chromosomal arm-level structural alterations analysis on serial plasma ctDNA and matched white blood cell DNA from 33 metastatic SCLC patients receiving chemotherapy or immunotherapy regimens[162]. Overall, both these studies highlight the potential of using novel methodologies combined with dynamic monitoring of ctDNA in order to assess molecular response to treatment in SCLC patients.

Colorectal cancer

CRC is one of the most common cancers worldwide and has demonstrated high shedding of ctDNA into the bloodstream, making liquid biopsy a useful tool in assessing treatment response and progression in this disease[164,165]. An early and pivotal study by Diehl et al. demonstrated that ctDNA measurements could be used to reliably monitor tumor dynamics in CRC patients undergoing surgery[16]. Specifically, they showed that median ctDNA decreased by 96.7% in less than a day and by 99% within 10 days, with detectable ctDNA after surgery associated with relapse. Since then, numerous other studies have demonstrated that the presence of ctDNA post-treatment (MRD) was associated with a higher likelihood of relapse[47,118,166–170]. Notably, Henriksen et al. conducted a nationwide Danish cohort study in 851 stage II-III CRC patients treated with curative intent[171]. They found that ctDNA detection, both post-operatively and serially, was associated with recurrence.

MRD detection is particularly important in high-risk stage II CRC patients. While the management of patients with stage I, III, and IV CRC is quite standardized, therapeutic strategies for stage II CRC are not straightforward, as a one-size-fits-all treatment is not suitable in this setting[172,173]. Current adjuvant management in resected stage II colon cancers is based on risk stratification using clinical and pathologic prognostic factors. ctDNA is thus being investigated for its ability to assess the need for adjuvant chemotherapy in stage II patients[174]. Large and prospective studies have shown that ctDNA is predictive of recurrence in patients with resected stage II colon cancer[47,175–177]. Notably, in a pioneering study by Tie et al. in 230 patients with stage II colon cancer, post-operative ctDNA levels were

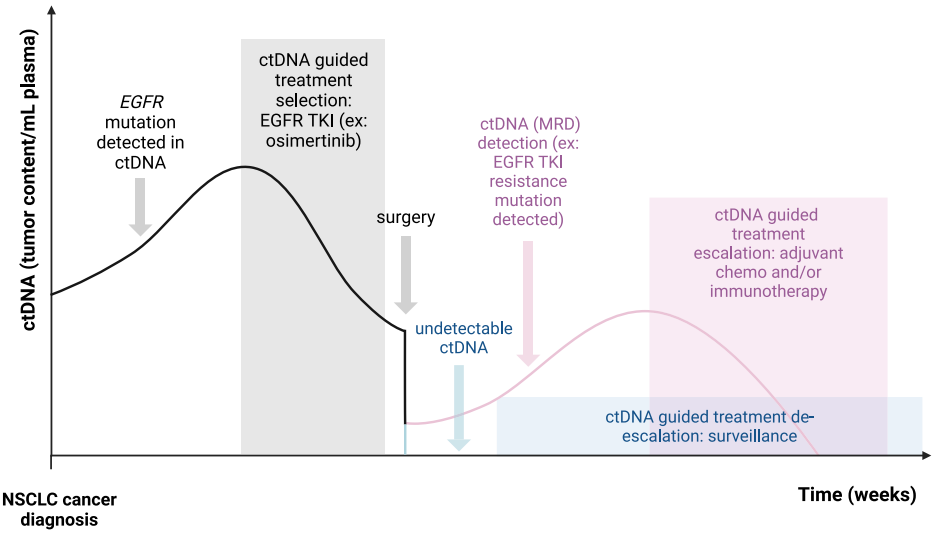

Fig. 3 | Current ctDNA precision oncology applications in NSCLC. ctDNA can be used for first- and second-line therapy selection, acquired resistance detection, and MRD detection in NSCLC.

prognostic, with a negative result associated with a significantly increased recurrence-free survival[47].

In contrast to stage II disease, adjuvant chemotherapy has been the standard of care for stage III CRC patients since the 90s[178]. However, in more recent years, studies have demonstrated that many stage III CRC patients can achieve 5-year disease-free survival even without adjuvant chemotherapy[179]. Thus, ctDNA monitoring has emerged as a powerful tool to potentially select stage III CRC patients for adjuvant therapy. Two prospective studies involving only stage III patients aimed to determine the clinical utility of using ctDNA to guide treatment[166,180]. The first study of 96 colon cancer patients treated with surgery and adjuvant chemotherapy showed significantly lower 3-year recurrence-free interval in patients with detectable *vs.* undetectable ctDNA both after surgery and after chemotherapy treatments[166]. In the second study, 168 CRC patients with stage III disease were treated with curative intent[180]. Of these, 80% of postoperative ctDNA-positive patients treated with adjuvant chemotherapy relapsed, while only patients who cleared ctDNA permanently during adjuvant chemotherapy remained relapse-free. Interestingly, the researchers also observed that the rate at which ctDNA increased following treatment was significantly related to patient survival. Additionally, a *post-hoc* analysis of the PRODIGE-GERCOR IDEA-France trial analyzed ctDNA in 1017 patients collected at both post-surgery and pre-chemotherapy timepoints, who were randomly assigned to receive either 3-month or 6-month oxaliplatin-based adjuvant chemotherapy. Only 13.8% of the patients were ctDNA-positive after surgery, and these patients had a lower 3-year disease-free survival rate compared to those who were ctDNA-negative[181]. Overall, these studies emphasize the potential of using ctDNA monitoring for therapy selection in stage III CRC patients, which could potentially reduce the number of patients exposed to toxic anti-cancer treatments.

ctDNA has been most extensively studied in the context of stage IV metastatic (m)CRC, because of the high systemic cancer burden and corresponding elevated levels of ctDNA shed, providing a rich source for analysis. Given the numerous studies in mCRC, meta-analyses have been conducted to analyze the collective data and have shown that the presence and levels of ctDNA are strongly associated with OS and risk of recurrence[182,183]. Jones et al. identified 28 studies, reporting on 2823 patients, where the measurement of ctDNA in stage IV CRC was correlated with clinical outcome[182]. They determined that ctDNA was positive in 80–90% of patients prior to treatment with a strong correlation between detectable ctDNA after treatment (surgery or chemotherapy) and OS as well as PFS. Of note, the meta-analysis revealed that ctDNA consistently serves as an early indicator of long-term prognosis in irresectable disease, with changes after just one cycle of systemic therapy proving to have prognostic value. Similarly, Wullaert et al. conducted a meta-analysis investigating the association between ctDNA in patients undergoing curative-intent local therapy for CRC liver metastasis[183]. Their findings revealed that following surgery, ctDNA-positive patients had a significantly higher risk of recurrence and shorter OS compared to those who were ctDNA-negative. A similar association was observed in patients who remained ctDNA-positive after completing adjuvant therapy.

Overall, the studies investigating ctDNA as a biomarker to monitor and predict response to treatment in CRC can be summarized by a large meta-analysis by Reece et al. on 92 studies[184]. The studies collectively showed that ctDNA is a reliable measure of tumor burden and useful in assessing the adequacy of surgical tumor clearance, with changes in ctDNA levels reflecting the response to systemic treatments as well as emergence of new mutations, allowing more sensitive monitoring than currently used clinical tools. As such, numerous clinical trials are currently underway to evaluate the effectiveness of ctDNA-based treatment interventions in CRC. Notably, CIRCULATE-US[185], TRACC Part C[186], IMPROVE-IT2[187], PEGASUS[188], BESPOKE[189], and AGITG DYNAMIC-Rectal[190] are all large ongoing clinical trials evaluating the use of ctDNA (MRD) detection to guide adjuvant treatment decisions. Additionally, recent reviews by Conca et al.,[191] and Roazzi et al.,[192] address the current state of ongoing clinical trials of ctDNA-guided treatment in CRC. Below we present a summary of the completed clinical trials designed to assess the use of ctDNA for interventional purposes in CRC (Table 1).

ctDNA also holds promise in elucidating the intricate landscape of resistance mechanisms that emerge during treatment in CRC, which can help guide clinical decision-making and therapeutic strategies. Initially, the application of ctDNA in mCRC predominantly focused on detecting the mutational status of key genes such as *RAS* and *BRAF*, pivotal for guiding patient selection for anti-EGFR treatment alongside chemotherapy in the first-line setting. For example, studies have shown that mCRC patients with *RAS* mutations on ctDNA have significantly worse response rates and survival after rechallenge with anti-EGFR agents compared to *RAS* wildtype patients[52,193,194]. In a two phase-trial composed of REMARRY (monitoring phase) and PURSUIT (trial phase), the authors reported that patients with RAS-negative ctDNA benefited from anti-EGFR rechallenge therapy[195]. This was confirmed by the landmark CHRONOS trial that used ctDNA to select patients for chemotherapy-free anti-EGFR rechallenge with panitumumab[196]. Similarly, in the VELO randomized clinical trial, mCRC patients with pretreatment *RAS/BRAF* wildtype ctDNA experienced prolonged clinical benefit when treated with panitumumab plus trifluridine-tipiracil, compared to those treated with trifluridine-tipiracil alone[197]. This suggests that ctDNA could be useful to select patients who may benefit from of anti-EGFR rechallenge. Expanding beyond EGFR-directed therapies, ctDNA analysis presents an avenue for identifying candidates for alternative

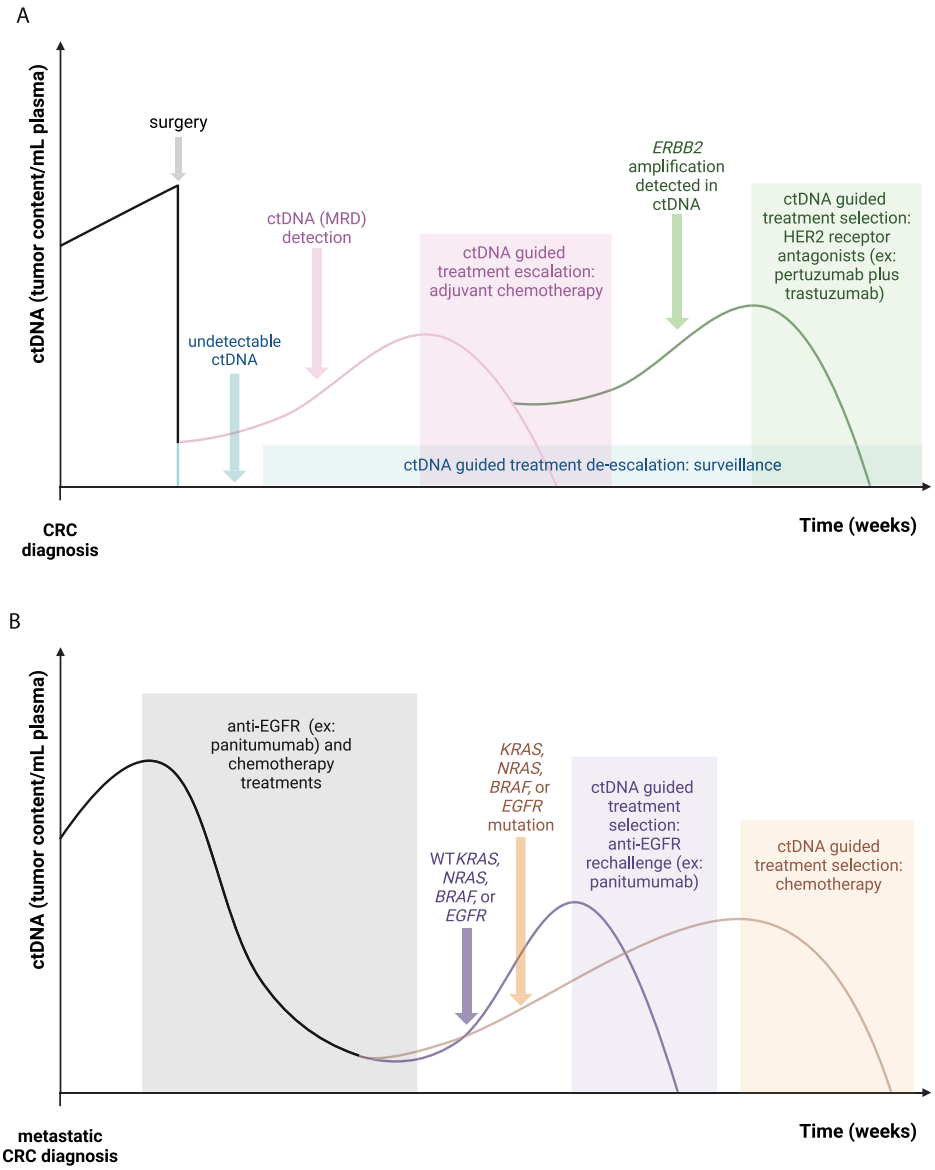

Fig. 4 | Current ctDNA precision oncology applications in CRC. A ctDNA is being explored for the detection of MRD and treatment escalation/de-escalation in the adjuvant setting in CRC. The detection of specific alterations in ctDNA such as HER2 amplifications or **B** *KRAS, NRAS, BRAF*, or *EGFR* mutations can be used to guide personalized treatment decisions.

treatment modalities, including anti-HER2 regimens, in mCRC[198–200]. Moreover, comprehensive ctDNA profiling has unveiled a spectrum of additional genetic alterations implicated in acquired resistance[196,201–205]. Figure 4 outlines the current applications of ctDNA for personalized treatment monitoring in CRC.

Breast cancer

Breast cancer is a highly diverse disease with various tumor subtypes, each demanding distinct treatment strategies based on molecular and genomic profiling of the tumor tissue. For these reasons, molecular biomarkers to monitor treatment response and effectiveness are vital to precision oncology strategies in this disease context. ctDNA has been extensively linked to outcome in breast cancer (reviewed in Xu et al.[206]). Notably, our recent meta-analysis reporting data from 4264 patients in 37 studies on metastatic breast cancer demonstrated that detection of ctDNA was associated with worse OS, PFS, and disease-free survival[207].

Several studies have shown the potential of ctDNA in guiding treatment and improving patient outcomes in breast cancer. Riva F et al.[208] reported that tailored ctDNA detection using droplet (d)dPCR yielded a 75% detection rate at baseline during neoadjuvant chemotherapy in triple negative breast cancer (TNBC)[208]. They found that neoadjuvant chemotherapy led to a rapid reduction in ctDNA levels and the absence of MRD post-surgery. A gradual decline in ctDNA during neoadjuvant chemotherapy was significantly associated with a shorter survival period in TNBC. Aguilar-Mahecha et al.[209] demonstrated that early on-treatment levels of genomic instability in ctDNA can predict treatment response and outcomes in MBC[209]. They found that patients classified as 'responders', showed decreased levels of genomic instability 3 months post-treatment compared to the 'non-responder' group. Lin et al.[210] have also demonstrated its utility as a robust predictor for relapse in patients with stage II to III breast cancer undergoing neoadjuvant therapy, with ctDNA-positive patients post-neoadjuvant therapy having worse recurrence-free survival than ctDNA-negative patients[210]. Interestingly, of the 13 study participants who achieved a pathologic complete response, only 2 had detectable ctDNA after neoadjuvant therapy, and both of these individuals went on to develop metastasis. In contrast, the remaining 11 subjects did not experience recurrence or metastasis. Ortolan et al.[211] demonstrated that ctDNA can effectively predict the prognosis of TNBC patients undergoing neoadjuvant chemotherapy[211]. They found that ctDNA was detected in 77% of evaluable cases before the start of neoadjuvant chemotherapy, whereas it was present in only 43% of cases after the treatment. In the metastatic setting, ctDNA has been used for treatment tailoring, tracking mechanisms of drug resistance,

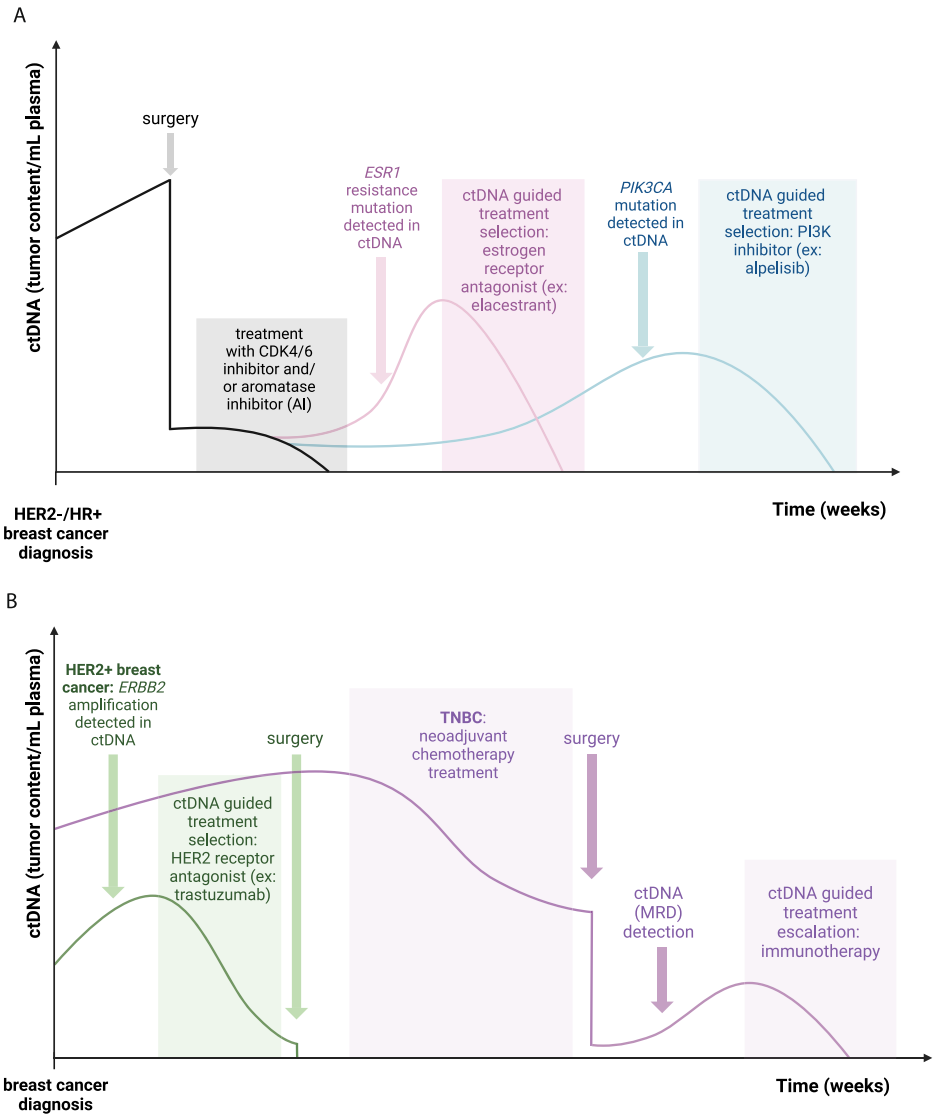

Fig. 5 | Current ctDNA precision oncology applications in breast cancer. A In HR+ breast cancer, ctDNA can be used to detect the emergence of *ESR1* resistance mutations and second-line therapy selection. ctDNA can also be used for *PIK3CA* mutation detection and personalized treatment recommendation of PI3K inhibitors. **B** ctDNA can be used for MRD detection and treatment escalation. In HER2+ breast cancer, ctDNA can be used for detection of HER2 amplifications and therapy selection.

and for predicting disease response before imaging (reviewed in Amato et al.[212]). These findings highlight the value of ctDNA in evaluating and guiding treatment strategies in breast cancer.

The incorporation of ctDNA analysis into breast cancer clinical trials has increasingly become a crucial component in personalizing treatment approaches. The SOLAR-1 trial, a phase 3 study, assessed the safety and effectiveness of the PIK3CA inhibitor alpelisib when combined with fulvestrant in patients with *PIK3CA*-mutated, hormone receptor-positive (HR+) / HER2- breast cancer[213,214]. Plasma ctDNA analysis revealed worse OS for *PIK3CA*-mutated patients treated with fulvestrant alone *vs.* in combination with alpelisib. Consequently, the American Society of Clinical Oncology (ASCO) updated its guidelines in 2022 to recommend ctDNA testing for HR+/HER2- MBC to identify patients eligible for alpelisib plus fulvestrant treatment[215]. Also, CAPTURE is an ongoing phase II multicentre clinical trial in Australia studying the use of alpelisib plus fulvestrant in ER+/HER2- advanced breast cancer patients with detectable PIK3CA mutations in ctDNA who had progressed during or after treatments with CDK4/6 inhibitors and aromatase inhibitors[216]. ctDNA analysis of patients enrolled in the phase Ib MONALEESASIA trials identified several genomic alterations associated with treatment response to the CDK4/6 inhibitor ribociclib[217]. Subsequently, in phase III of the MONALEESA trial, Andre et al.[218] found that patients with *ERBB2, FAT3, FRS2, MDM2, SFRP1*, and *ZNF217* mutations had better PFS with ribociclib compared to placebo, while mutations in *ANO1, CDKN2A/2B/2C*, and *RB1* reduced ribociclib sensitivity[218]. Table 1 offers a summary of various published clinical trials that evaluated the application of ctDNA monitoring for clinical interventions in breast cancer.

The identification of treatment resistance in breast cancer can also be greatly aided by the use of ctDNA. *ESR1* mutations are well established to drive resistance to endocrine therapy in *HR+* breast cancer. In 2016, Fribbens et al. assessed the impact of *ESR1* mutations on the sensitivity to standard therapies from two phase III randomized trials: the SoFEA Trial[219] and the PALOMA3 trial[220] indicating that plasma *ESR1* mutations after aromatase inhibitor therapy progression could guide subsequent endocrine therapy[221]. The ALERT study further supported this by linking dynamic ctDNA fluctuations during treatment cycles with clinical benefits and resistance in *ER+*MBC patients[222]. In the PADA-1 trial, ER+/HER2- patients with rising plasma *ESR1* ctDNA levels during first-line aromatase inhibitor and palbociclib therapy were randomized to either continue their current treatment or switch to fulvestrant and palbociclib[223]. Patients in the fulvestrant group had improved PFS compared to the aromatase inhibitor group. Similarly, in the EMERALD trial, patients with *ESR1* mutations treated with elacestrant showed improved survival compared to those receiving standard care[224]. As a result, ASCO now recommends ctDNA testing to detect *ESR1* mutations in advanced ER+/HER2- breast cancer patients who have progressed after endocrine therapy, to identify those who

might benefit from treatment with elacestrant[225]. This recommendation highlights the importance of ctDNA biomarker studies and demonstrates the rapid integration of ctDNA biomarker studies into clinical practice.

The utility of ctDNA extends to the detection of MRD through post-treatment monitoring, which is pivotal in predicting relapse in breast cancer patients[226]. A landmark study conducted by Garcia-Murillas et al. in 2015[227] showed that ctDNA positivity after curative-intent surgery was a strong predictor of relapse, with a median lead time of 7.9 months before clinical recurrence[227]. They demonstrated that ctDNA analysis could detect MRD with a sensitivity of 93% and specificity of 100%. Similarly, Olsson et al. demonstrated that ctDNA could detect metastasis an average of 11 months before clinical detection, showing high accuracy in distinguishing patients with and without clinically detected recurrence after surgery[228]. Further supporting these findings, Magbanua et al.[229] isolated cfDNA from 84 high-risk early breast cancer patients in the neoadjuvant I-SPY 2 trial[229]. They found that the absence of ctDNA clearance was a strong predictor of poor response and metastatic recurrence, whereas clearance was linked to improved survival, even in patients who did not achieve a pathologic complete response. Zhou et al.[230] also showed that detection of ctDNA in post-treatment samples was linked to a substantial likelihood of future recurrence and an unfavorable response to neoadjuvant treatment[230]. Coombes et al.[220] also found that ctDNA detected pre-clinically or radiologically in 16 of 18 patients (89% sensitivity) predicted metastatic relapse up to 2 years in advance, with a median lead time of 8.9 months and 100% specificity[220]. However, Coakley et al.[231] noted that different detection methodologies, (NGS *vs.* dPCR) affect lead times from ctDNA detection to clinical relapse[231]. In a cohort of 22 early-stage breast cancer patients, NGS provided a lead time of 6.1 months compared to 3.9 months with dPCR. Despite the small sample size, these findings highlight the potential considerations when using ctDNA for MRD detection. The c-TRAK TN trial, a phase II clinical trial, prospectively evaluated the effectiveness of ctDNA in detecting MRD and guiding therapy in early-stage TNBC[232]. In the trial, ctDNA-positive patients were assigned to either receive pembrolizumab or to be placed in an observation group. Interestingly, none of the participants in the intervention arm achieved ctDNA clearance. However, the assessment was limited by the small sample size of patients receiving therapy, which resulted from a higher-than-anticipated rate of metastasis among study participants. Overall, these studies support the potential of ctDNA for use as a valuable tool in early detection of MRD and metastasis. As such, a large scale multicenter ongoing clinical trial, MiRaDoR, is using ctDNA surveillance on HR-positive/HER2-negative early-stage BC patients to detect MRD and provide treatment at the event of ctDNA positivity[233].

The FDA has approved certain ctDNA tests as companion diagnostics for guiding targeted therapies in breast cancer. For instance, the Guardant360 CDx NGS panel detects *ESR1* mutations in estrogen receptor-positive (ER+)/HER2- metastatic breast cancer (MBC), identifying candidates for elacestrant[234]. Similarly, the Qiagen therascreen *PIK3CA* RGQ PCR Kit can identify *PIK3CA* mutations in ctDNA from plasma samples for breast cancer patients who may be eligible for treatment with alpelisib[235]. FoundationOne CDx identifies patients eligible for targeted therapies by detecting *ERBB2* (HER2) amplifications for treatment with trastuzumab, ado-trastuzumab emtansine, and pertuzumab. It also detects *AKT1* and *PTEN* alterations that can benefit from AKT inhibitors like capivasertib and ipatasertib[35,236,237]. These FDA-approved ctDNA tests and genomic profiling assays are crucial in guiding the selection of targeted therapies for breast cancer patients, potentially improving outcomes.

The landscape of ctDNA research in breast cancer is evolving, with several novel trials underway. Also, recently developed predictive models for neoadjuvant chemotherapy response in breast cancer patients, incorporating genomic features and clinical factors, demonstrate effective discrimination between pathologic complete response and non-complete response with ctDNA status-enhancing predictive accuracy[238]. The MAGNETIC 1 trial evaluates ctDNA's diagnostic potential in monitoring HR + MBC during first-line endocrine therapy[239]. The CIPHER study explores ctDNA's role in triple-negative and HER2+ early-stage breast cancer, enabling tailored interventions for these aggressive subtypes[240]. These studies, among others, are paving the way for the integration of ctDNA into clinical practice, allowing earlier detection of MRD and treatment resistance, and ultimately improving long-term survival rates and quality of life for breast cancer patients. Figure 5 summarizes the current applications of ctDNA monitoring in the various breast cancer subtypes.

Other cancers

In the following section we will outline other notable advances and applications of ctDNA monitoring in other solid tumor types. To begin, ctDNA has emerged as a promising marker for monitoring disease progression and post-treatment relapses in melanoma[241,242]. In recent years, the treatment landscape for melanoma has shifted tremendously with the emergence of new targeted therapies as well as the remarkable promise of immunotherapies. For instance, BRAF and MEK inhibitors[243] as well as immunotherapies such as anti-PD1 treatment[244,245] have been shown to increase patient survival[246,247]. However, selection of patients is essential and the response to such treatments is not always long-lasting, with secondary resistances often occurring. A study by Haselmann et al. in 634 stage I to IV melanoma patients showed that detection of *BRAF* mutant ctDNA preceded relapse as assessed by RECIST and was more specific than serum S100 and lactate dehydrogenase[248]. Additionally, Varaljai et al. assessed the presence of *BRAF, NRAS*, and *TERT* mutations in ctDNA in 96 patients with advanced-stage melanoma, observing that changes in ctDNA correlated with treatment response and that increasing ctDNA levels predicted disease progression significantly earlier than did routine radiologic scans, with a mean lead time of 3.5 months[249]. Moreover, ctDNA has been utilized to assess response to immunotherapies in melanoma. One study found that longitudinal assessment of ctDNA in patients receiving PD1 inhibitors was an accurate predictor of tumor response as well as survival[250]. Another study demonstrated that positive ctDNA during treatment (week 2 or 4) was an early predictor of a complete lack of clinical benefit under anti-PD1[251]. Importantly given the common resistance to immunotherapies, a previous study demonstrated emergence of dynamic complexity in mutational profile of ctDNA during treatment with pembrolizumab or nivolumab treatment, including multiple *BRAF* mutations in the same patient, clinically relevant *BRAF* mutations emerging through therapy and co-occurring subclonal *BRAF* and *NRAS* mutations[246].

Interestingly, ctDNA has also been investigated in uveal melanoma, a rare form of melanoma that develops in melanocytes of the uveal track. While uveal melanoma represents only 5% of melanomas, it is the most common intraocular tumor in adults and is associated with high mortality rates[252]. Liquid biopsy is particularly useful in this context, where the diagnosis of uveal melanoma is made through imaging, with highly invasive intraocular biopsies usually performed only for prognostication[253]. Blood, as well as aqueous humor and vitreous humor, have been investigated as sources of ctDNA, and data has shown correlations between ctDNA and disease severity, progression, metastasis and outcome[62,254–257]. The clinical utility of ctDNA in monitoring treatment response has also been tested, including in the context of novel targeted therapies such as immunotherapy[65,258,259] and protein kinase C inhibition[260]. In a phase II trial of tebentafusp, a soluble T cell receptor bispecific, in 127 patients with treatment-refractory metastatic uveal melanoma (NCT02570308), early on-treatment reduction in ctDNA was strongly associated with OS, even in patients with radiographic progression[261].

In the realm of prostate cancer, several studies have highlighted the potential use of ctDNA as a biomarker for various clinical applications, such as predicting postoperative recurrence[262], monitoring drug resistance[262], and assessing treatment response[81]. In particular, many studies have explored the feasibility of detecting *AR* mutations and splice variants in ctDNA[263–267]. For instance, in a study using a 3,334 patient cohort of metastatic castration-resistant prostate cancer, it was demonstrated that ctDNA contained additional alterations not found in tumor tissue, including a broad spectrum of *AR* resistance alterations and somatic *BRCA1/2* mutations and reversions[268]. Also, a study by Romanel et al. investigated 274 plasma

samples from 97 castration-resistant prostate cancer treated with abiraterone and found that ctDNA was useful in studying the dynamic evolution of *AR* alterations throughout treatment[265]. Specifically, this study provided evidence that resistant *AR* subclones can be detected in ctDNA even before clinical evidence of disease progression. In a more recent study, a shift in the ctDNA population towards *AR* augmentation after treatment with AR signaling inhibitors was observed[81]. Recent research also suggests that metastatic castration resistant prostate cancer patients with *AR* alterations on ctDNA had inferior OS after disease progression on the first AR signaling inhibitor compared to those without *AR* ctDNA mutants[269].

The dire prognosis of central nervous system tumors has prompted new avenues for biomarker discovery to monitor patient response to treatment. However, blood-based liquid biopsy analysis is challenged by the highly selective nature of the blood brain barrier[270]. As such, many studies have turned towards the use of CSF as a source of ctDNA[127,271]. Indeed, several studies have reported ctDNA concentrations in several orders of magnitude higher in CSF than plasma[127,272] or urine[273], where typical plasma variant allele frequencies are <1%[271,274]. Notably, serial monitoring of CSF has been used to detect MRD and treatment response in brain cancers[48,271,275]. In fact, a recent clinical trial showed that analyzing H3 K27M-mutant diffuse midline gliomas from CSF ctDNA was a reliable approach to confirm treatment response and identify subsequent tumor progression[275]. Similar studies have also been explored in the context of brain metastases. For instance, using CSF cfDNA, Pentsova et al.[129] detected high-confidence somatic alterations in 63% of patients with central nervous system metastases of solid tumors, 50% of patients with primary brain tumors, and 0% of patients without central nervous system involvement[129]. While these studies advocate for the use of CSF in primary brain tumors and brain metastases, lumbar punctures remain significantly more invasive than blood or urine collection[276].

In recent years, viral ctDNA in virus-driven malignancies, which account for ~10% of the worldwide cancer burden[277], has also been investigated as a tool to track disease progression and recurrence. This is especially prominent in cancers driven by HPV such as oropharyngeal and cervical cancers[278]. However, examining viral DNA as opposed to mutations poses different challenges, since viral sequences diverge over time and across populations[279]. This has led to a wide array of experimental techniques for detecting different portions of the viral genome. Generally, HPV-ctDNA has been examined using ddPCR or HPV-specific sequencing techniques, and has been shown to be a tool for monitoring treatment response in bodily fluids, including plasma and saliva[83,104]. While multiple studies have shown significant relationships between viral ctDNA levels and treatment response[83,280–282], certain challenges still exist in these analyses, especially the variable copy number caused by differences in the degree of integration of viral DNA into the host genome[280,281,283]. Globally, there has been a high degree of sensitivity in studies monitoring plasma ctDNA in HPV-related head and neck cancers and in more advanced cervical cancers, though certain studies have demonstrated lower sensitivity for lower-grade cervical cancer[281,284].

Limitations and future directions

While ctDNA is emerging as a powerful tool for monitoring treatment response, certain limitations still exist that hinder its widespread implementation into clinics. Particularly, there is a lack of guidelines for optimal liquid biopsy sampling timepoints, such as the ideal time after treatment to detect MRD and predict patient relapse. Furthermore, there is also a lack of standardization of pre-analytical steps, including the type of collection tube or the speed and number of centrifugation steps needed for processing samples[285,286]. There is also a lack of standardization of downstream analytical assays used, such as the use of tumor-informed or uninformed approaches, which ultimately leads to variability in clinical trial results[287]. In addition, ctDNA analysis can be limited by constraints such as low levels of input DNA, rapid degradation of DNA fragments, and potential confounding data from other diseases cancer patients might have simultaneously[288]. Future research should focus on refining detection methods and establishing standardized protocols across diverse cancer populations[285,288,289]. Overall, liquid biopsy holds tremendous potential, both as a source of non-invasive biomarkers and as a methodology for studying cancer. However, we lack a solid understanding of the fundamental origin of tumor-derived molecules. Addressing these gaps is the first step to standardizing ctDNA analysis and interpretation and determining its clinical utility in different cancer contexts. By tracking disseminated molecules and the evolving dynamics of cancer, we will identify molecular alterations that could be used for non-invasive diagnosis, prognosis, and detection of treatment resistance, finding new opportunities for personalized strategies to prevent and manage disease.

Data availability

No datasets were generated or analysed during the current study.

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Author contributions

J.V.B. was responsible for conceptualization. A.B., M.N., T.F., K.D., A.N., N.K., A.C., and J.V.B. were responsible for writing the first draft of the manuscript. All authors contributed in editing and approving the final manuscript.

Competing interests

The authors declare no competing interests.

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