The Journal of Liquid Biopsy 4 (2024) 100146

Contents lists available at ScienceDirect

The Journal of Liquid Biopsy

journal homepage: www.sciencedirect.com/journal/the-journal-of-liquid-biopsy

# Liquid biopsy into the clinics: Current evidence and future perspectives

Myrto Boukovala [a], C. Benedikt Westphalen [b], Victoria Probst [b,*]

[a] Servier Deutschland GmbH, Germany
[b] LMU Munich, Germany

ARTICLE INFO

*Keywords:*
Liquid biopsy
ctDNA
Minimal residual disease
Clinical application

ABSTRACT

As precision oncology has become a major part of the treatment landscape in oncology, liquid biopsies have developed as a particularly powerful tool as it surmounts several limitations of traditional tissue biopsies. These biopsies involve most commonly the isolation of circulating extracellular nucleic acids, including cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA), as well as circulating tumor cells (CTCs), typically from blood.

The clinical applications of liquid biopsies are diverse, encompassing the initial diagnosis and cancer detection, the application as a tool for prognostication in early and advanced tumor settings, the identification of potentially actionable alterations, the monitoring of response and resistance under systemic therapy and the detection of resistance mechanisms, the differentiation of distinct immune checkpoint blockade response patterns through serial samples, the prediction of immune checkpoint blockade responses based on initial liquid biopsy characteristics and the assessment of tumor heterogeneity. Moreover, molecular relapse monitoring in early-stage cancers and the personalization of adjuvant or additive therapy via MRD have become a major field of research in recent years.

Compared to tissue biopsies, liquid biopsies are less invasive and can be collected serially, offering real-time molecular insights. Furthermore, liquid biopsies may allow for a more holistic evaluation of a patient's disease, as they assess material from all tumor sites and can theoretically reflect tumor heterogeneity. Furthermore, quicker turnaround-time also constitutes an advantage of liquid biopsies. Disadvantages or hurdles include the challenge of detecting low amounts of tumor deposits in peripheral blood or other fluids and the potential of different amounts tumor-shedding from different metastatic sites, as well as potentially false-positive from clonal hematopoietic mutations of indeterminate potential (CHIP) mutations. The clinical utility of liquid biopsies still must be validated in most settings and further research has to be done. Clinal trials including alternate bodily fluids and leveraging AI-technology are expected to revolutionize the field of liquid biopsies.

## 1. Introduction

In the era of precision oncology, molecular diagnostics have had an immense impact on patient diagnosis and treatment, thus leading to improved patient outcomes [1]. Liquid biopsy has emerged as a particularly powerful tool as it could potentially surmount several limitations of traditional tissue biopsies. Liquid biopsies are minimally to non-invasive and offer an easily-accessible alternative for molecular profiling, especially when tumor tissue is not available. Furthermore, they assess the tumor-derived components across tumor sites, thus having the theoretical potential to serve as a real-time, spatial and temporal cancer monitoring approach [2]. Although there is promising data for the clinical validity of liquid biopsies in various settings including cancer detection and disease monitoring, clinical utility remains to be proven in most settings.

The goal of this review is to provide an overview of the main potential clinical applications of liquid biopsies, including current evidence and recommendations, ongoing research and future perspectives, with a main focus on circulating tumor deoxyribonucleic acid (ctDNA) and circulating tumor cells (CTCs) in solid tumors.

## 2. Background

The term ‘liquid biopsy’ refers to the examination of biological fluids through cellular and molecular techniques in order to derive information about cancer biology and dynamics. The most widely examined and most commonly used analyte is definitely blood. However, liquid biopsies have been also explored and delivered promising results in a wide range of alternative –more or less easily accessible-bodily fluids including urine

* Corresponding author.
*E-mail address:* victoria.probst@med.uni-muenchen.de (V. Probst).

https://doi.org/10.1016/j.jlb.2024.100146
Received 10 January 2024; Received in revised form 5 February 2024; Accepted 6 February 2024

[3–5], pleural effusion [6–8], ascites effusion [7,9], cerebrospinal fluid [7,10–12] and saliva [13,14] (Fig. 1).

There are several cellular and molecular techniques that have been studied in the assessment of liquid biopsies, offering different types of information (Fig. 1). The most commonly employed approaches involve the analysis of cell-free (cf) and ctDNA, which is the subset of cfDNA directly derived from tumor.

Two primary methodologies have been explored for analyzing ctDNA/cfDNA: targeted and untargeted strategies. The former concentrate on specific gene rearrangements or mutations, whereas the latter provide a more comprehensive examination and surveillance of the tumor genome, irrespective of prior information about the tumor [16]. Among the techniques applied to assess ctDNA/cfDNA on a genomic level are digital droplet polymerase chain reaction (ddPCR) and BEAMing (beads, emulsions, amplification, and magnetics) as well as sequencing techniques like tagged-amplicon deep sequencing (TAm-Seq), cancer personalized profiling by deep sequencing (CAPP-Seq), whole exome sequencing (WES), and whole genome sequencing (WGS) [15,16]. These approaches range from single-gene testing to large testing panels and can be tumor-informed, i.e. assess specific alterations based on prior molecular profiling of tumor tissue, or tumor-agnostic, which usually involves a wider, less specific panel. Currently used methodologies of cfDNA/ctDNA analysis are facing the challenge of low-shedding cancers, especially in early-stage disease, as well as the potential of confounding by clonal hematopoietic mutations of indeterminate potential (CHIP) and germline mutations. CtDNA assays also tend to have reduced sensitivity for the detection of gene fusions, as compared to tissue RNA testing [2]. These are potential limitations that need to be taken into consideration when interpreting ctDNA results [2]. On an epigenetic level, DNA methylation analyses mainly through bisulfite sequencing have also been explored [15,16]. CfDNA fragmentation analysis is an emerging technology that has already delivered promising results. Another emerging analysis could be the evaluation of nucleosome occupancy of cfDNA by deep sequencing methods [15,16].

Circulating tumor cells (CTCs), which are cells shed by the tumor into the bloodstream, were one of the first widely used liquid biopsy biomarkers. While they facilitate a wide range of analyses including genomic, transcriptomic, proteomic, methylation, functional and single-cell analyses, their amount in blood in usually low and they are more difficult to isolate [15,16]. Several other tumor-derived components that have also been examined, however less widely than ctDNA and CTCs, include cell-free ribonucleic acid (cfRNA), proteins, extracellular vesicles as well as tumor educated platelets (TEP) and particularly tumor educated platelets ribonucleic acidTEP-RNA [15–17]. The detailed description of liquid biopsy techniques is beyond the scope of this review. Excellent review papers focusing on this topic have been published in several journals [15–17].

Probably the biggest advantage of liquid biopsies is that they are minimally-to non-invasive and much easier to perform in contrast to traditional tissue diagnostics that require a tumor biopsy and place the patient at a significantly higher risk for procedure-related complications. As a result, multiple liquid biopsies can be performed over time, enabling real-time monitoring of cancer evolution. Furthermore, liquid biopsies may allow for a more holistic evaluation of a patient's disease, as they assess material from all tumor sites and can theoretically reflect tumor heterogeneity. Finally, liquid biopsies tend to be less expensive and have generally quicker turnaround times than tissue molecular diagnostics. One major disadvantage of liquid biopsies is that tumors may shed low amounts of components, making detection in peripheral blood more challenging. Furthermore, shedding of tumor-derived components may differ across various tumor sites and thus hinder the evaluation of tumor heterogeneity. Although there is abundant evidence on the analytical and clinical validity of numerous assays in a wide-range of tumor entities and indications, their clinical utility in most settings has not been validated yet [2,15,17]. (Table 1).

## 3. Potential clinical applications of liquid biopsies in oncological practice

Liquid biopsies offer a wide range of potential clinical applications, as summarized in Fig. 2. Their common objective is to complement standard clinical, imaging and tissue assessments in order to optimize patient management.

**Table 1**
Advantages and disadvantages of liquid biopsy.

| Advantages | Disadvantages |
|---|---|
| ✓Minimally- to non-invasive and<br>✓Low organizational effort required<br>✓ Multiple assessments over time possible<br>✓ Assessment of tumor heterogeneity<br>✓ Lower cost that tissue diagnostics (generally)<br>✓ Shorter turnaroundtimes | – Low shedding of tumor-derived materials may hinder detection<br>– Tumor-shedding may differ across tumor sites hindering heterogeneity assessment<br>– Clinical utility remains to be proven (in most settings) |

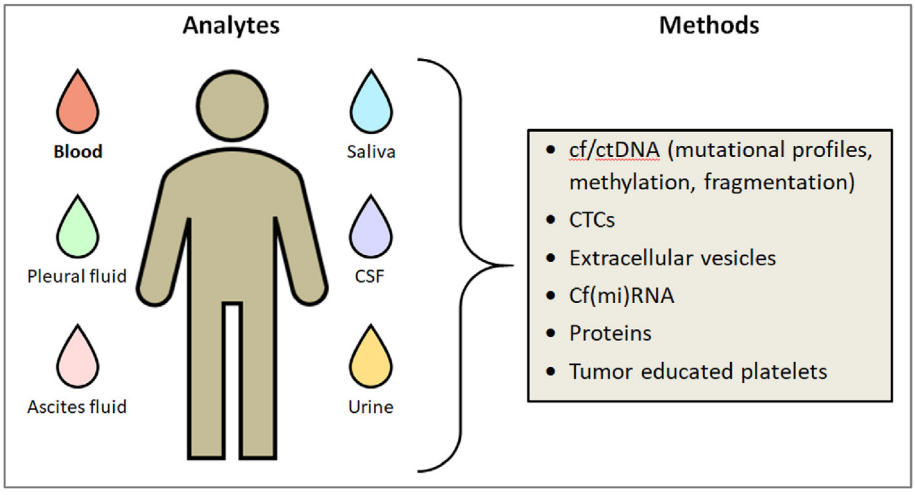


**Fig. 1.** cfDNA = cell free DNA, CSF = cerebrospinal fluid, CTCs = circulating tumor cells, ctDNA = circulating tumor DNA.

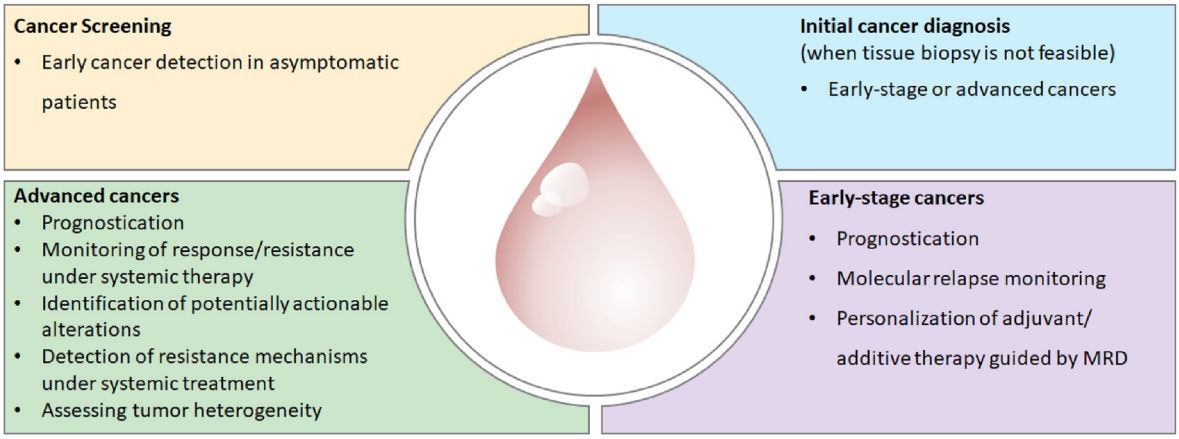


**Fig. 2.** Overview of clinical applications of liquid biopsies.

### *3.1. Cancer screening and early cancer detection*

One of the potential liquid biopsy applications of highest interest is cancer screening and early cancer detection in asymptomatic populations. Detecting cancer at an early stage, when it is still amendable to curative therapies, may have tremendous impact on patient outcomes and help reduce cancer mortality. As outlined by the European Society for Medical Oncology (ESMO) Precision Medicine Working Group, a reliable liquid biopsy screening tool would require high specificity combined with clinically meaningful sensitivity and would ideally classify the tissue of origin [2]. This is particularly challenging with cf/ctDNA techniques as especially early-stage tumors shed little ctDNA [2]. Several methodological approaches yielding promising results have been explored for this purpose.

Initial approaches for cancer detection through liquid biopsies were cfDNA based. Phallen et al. utilized targeted error correction sequencing to examine cfDNA and were able to detect somatic mutations in the plasma of solid tumor patients with early-stage disease at a rate ranging from 59 to 71% [18]. Analysis of cfDNA in pregnant women who submitted blood for noninvasive prenatal testing showed an abnormal genomic profile not consistent with fetal abnormalities in about 10 of 100,000 cases. In 18 of 43 of these cases, the mother was eventually diagnosed with a malignancy, suggesting that cfDNA can be an early biomarker for cancer detection [19]. Assessment of mutations in cfDNA combined with circulating proteins as performed in CancerSEEK testing has been able to detect non-metastatic, clinically detected solid malignancies with fairly high sensitivity of 69–98% for cancers of the ovary, liver, stomach, pancreas, and esophagus, and specificity greater than 99% [20].

DNA methylation-based approaches have also been extensively explored in this setting. Epi proColon®, which is an FDA approved blood-based test for colorectal cancer (CRC) screening, utilizes real time PCR to detect methylated septin 9 -a biomarker for CRC- in cfDNA. In the prospective PRESEPT Study (NCT00855348), Epi proColon® demonstrated a standardised sensitivity of 48.2% (35%, 63% and 46% for Stage I, II, and III, respectively) with a specificity of 91.5% [21]. The test is currently intended for persons aged 50 and older who are unwilling or unable to be screened by standard methods [22]. Methylation analyses of cfDNA using different methods including targeted and genome-wide bisulfite sequencing have also been employed by different research groups for cancer detection across several malignancies, exhibiting robust performance with high specificity and encouraging sensitivity [23–28]. Methylation analysis has also been shown to be a useful tool in tumor classification through liquid biopsies [25,26,28]. DNA fragmentation analyses have also been employed to detect cancer, including the evaluation of genome-wide fragmentation patterns of cfDNA [29] as well as fragmentation size [30].

Non-ctDNA-based approaches that have been explored for cancer screening include extracellular vesicle analyses. In a case-control study including cancer cases of the pancreas, ovary and bladder, Hinestrosa et al. used an extracellular vesicle protein measurement approach that yielded a specificity of 99.5% with sensitivity of 71.2%. Detection rates for Stage I cancer ranged from 43.8% in bladder cancer to 95.5% in pancreatic cancer [31]. Tumor educated platelet RNA analyses have also been explored in this setting yielding promising results regarding cancer detection and classification [32–34].

This encouraging data of different liquid biopsy approaches as cancer screening tools needs to be further evaluated in large-scale screening studies to assess not only their reliability as screening tools but also their clinical utility and impact on clinical outcome. Currently, the use of multi-cancer liquid biopsy screening tools is not considered part of standard clinical practice by the ESMO Precision Medicine Working Group [2].

### *3.2. Initial cancer diagnosis and cancer genotyping (when tissue biopsy is not feasible), early stage or advanced cancers*

In most cases, a suspected solid cancer diagnosis on imaging is confirmed by tissue biopsy. Nevertheless, identification of pathogenomic mutations in ctDNA can assist in the initial cancer diagnosis and cancer genotyping in patients with tumors that are difficult to biopsy or patients with contraindications for tissue sampling [15,35].

Jensen et al. [36] conducted a study involving patients with tumors deemed arduous to biopsy. They successfully isolated cfDNA and employed low-coverage genome-wide sequencing techniques. Findings from this study, as well as others, underscore the potential of liquid biopsy to offer molecular insights in cases where the acquisition of sufficient tissue for molecular profiling is challenging. The ESMO Precision Medicine Working Group has recommended ctDNA analysis for advanced cancer genotyping in several entities, including breast, gastric, hepatocellular, and non-small cell lung cancer (NSCLC), in cases where tissue analysis is not possible [2].

However, in this context the differentiation of pathogenomic and CHIP mutations is of utmost importance, requiring a high level of expertise in the use and interpretation of the applied genomic test. In case of negative liquid biopsy results, the potential of false negatives needs to be taken into consideration and guide further management of the patient on an individualized basis [2]. Also, the use of alternate bodily fluids apart from blood like urine, pleural, ascites and cerebrospinal fluid, needs to be further explored in this setting [15].

### *3.3. Clinical applications in early-stage cancer*

Blood ctDNA is the most widely studied liquid biopsy approach for

the assessment of early-stage cancer. As early-stage cancers are known to shed lower levels of ctDNA compared to metastatic disease, specialized assays with high sensitivity and the ability to detect very low ctDNA levels need to be employed in this setting [2].

### 3.3.1. Prognostication

Detection of ctDNA by various methodologies including tumor-informed and -agnostic techniques prior or after curative-intent therapy has been consistently shown to have a negative prognostic value and has been linked to high risk of recurrence/relapse in patients with early-stage cancer, with clinical validity shown in many studies [2,37]. Recent meta-analyses demonstrated a fairly high specificity (>90%) but moderate sensitivity in this setting [38–40]. Current evidence suggests that serial ctDNA testing may improve sensitivity in the (post-)adjuvant setting [41–44]. Tumor-informed personalized ctDNA assays like Signatera™ or TARDIS that assess multiple known mutations have been developed in this setting, showing robust testing performance [44–46].

In patients with resectable colon cancer (localized or oligometastatic), several studies as well as a series of recent meta-analyses indicate that detectable ctDNA after surgery as well as after adjuvant chemotherapy are associated with a higher risk of recurrence [47–56]. In early-stage breast cancer, ctDNA detection at baseline and after curative treatment has also been linked to relapse [57–59]. Studies in other solid tumors including pancreatic, bladder, lung, and esophageal cancer as well as melanoma have yielded similar results, indicating the association of ctDNA detection directly after curative-intent therapy or during surveillance with high risk of recurrence across tumor types and ctDNA assays [37,42,43,60–65]. Further observational studies investigating the prognostic role of ctDNA in early-stage cancer are underway in several entities, including NSCLC (ex. NCT05382052, NCT05167604), breast (ex. NCT04353557), gastric (ex. NCT04943406), endometrial (ex. NCT05955079), and pancreatic cancer (ex. NCT04246203, NCT05400681, NCT05853198).

### 3.3.2. Personalization of adjuvant/additive therapy guided by minimal residual disease (MRD)

Regarding clinical utility, ctDNA positivity directly after curative-intent therapy -also referred to as minimal residual disease (MRD)- is of special interest, as it may have the potential to personalize adjuvant patient management. In a subset analysis of the IMvigor010-trial (NCT02450331), a randomized phase III trial comparing atezolizumab to observation after surgical resection for operable urothelial cancer, clinical benefit of immunotherapy in terms of disease-free survival was shown to be restricted to patients with detectable ctDNA after surgery [66]. Several studies and a meta-analysis in early-stage NSCLC have demonstrated that patients with detectable MRD benefited from adjuvant therapy including chemotherapy, targeted therapy, and immunotherapy in terms of recurrence-free survival while MRD-negative patients did not confer relevant benefit [41,62,65,67,68]. At the same time, MRD negativity could potentially guide de-intensification of adjuvant/additive treatment in a subset of patients, thus avoiding overtreatment and sparing patients of unnecessary toxicity. In the randomized DYNAMIC trial in stage II colon cancer comparing adjuvant treatment decisions guided by ctDNA MRD results vs. standard clinicopathological features, the use of adjuvant therapy was reduced in the MRD-guided group without compromising recurrence-free survival [69]. Although this data is highly suggestive of the potential of MRD-status to refine adjuvant management both through intensification and de-intensification of treatment, further evidence from ideally randomized clinical trials is required to thoroughly evaluate this notion. Currently, several ongoing clinical trials aim to examine MRD-driven adjuvant/additive therapy in different tumor entities (Table 2).

### 3.3.3. Molecular relapse monitoring

Another clinical application of particular interest in early-stage cancer is ctDNA-based monitoring during surveillance in patients with

**Table 2**
ctDNA-MRD in early-stage cancer – Ongoing trials.

| Setting | Disease | Examples of ongoing trials |
|---|---|---|
| MRD-guided adjuvant treatment | Bladder cancer | IMvigor011 (NCT04660344) |
| | Breast cancer | LEADER (NCT03285412) |
| | Colorectal cancer | MEDOCC-CrEATE (NL6281/NTR6455)<br>DYNAMIC-Rectal (ACTRN12617001560381)<br>CIRCULATE (NCT04089631)<br>CIRCULATE- PRODIGE 70 (NCT04120701)<br>CIRCULATE JAPAN – VEGA (jRCT1031200006)<br>CIRCULATE-US (NCT05174169)<br>COBRA (NCT04068103)<br>CIRCULATE-Spain (EudraCT 2021-000507-20)<br>DYNAMIC-III (ACTRN-12617001566325)<br>TRACC (Part C) (NCT04050345)<br>SYNCOPE (NCT04842006)<br>BNT122-01 (NCT04486378)<br>PEGASUS (NCT04259944)[a]<br>NCT03803553[a] |
| | NSCLC | MERMAID-1 (NCT04385368)<br>LUN0115 (NCT04585477)<br>LUN0114 (NCT04585490) |
| | Solid tumors, microsatellite instability-high (MSI-H) | NCT03832569[a] |
| Molecular relapse monitoring | Breast cancer | DARE (NCT04567420)<br>ZEST (NCT04915755)<br>TRAK-ER (NCT04985266) c-TRAK-TN (NCT03145961) |
| | Colorectal cancer | CIRCULATE JAPAN – ALTAIR (NCT04457297)<br>IMPROVE-IT2 (NCT04084249)<br>NCT04752930 |
| | NSCLC | MERMAID-2 (NCT04642469) |

[a] Intervention (also) guided by ctDNA assessment after adjuvant therapy.

**Table 3**
Monitoring of treatment response and guiding therapy decisions – Ongoing trials.

| Setting | Disease | Examples of ongoing trials |
|---|---|---|
| Monitoring of treatment response | Breast cancer | MONDRIAN (NCT04720729)<br>NCT03947736<br>NCT05816642 |
| | Colorectal cancer | NCT04555369 |
| | NSCLC | ELUCID (NCT03926260)<br>PRELUCA (NCT05889247)<br>LIBERTYLUNG (NCT04790682)<br>NCT05415358 |
| | Renal Cell Carcinoma | NCT05816642 |
| | Castrate Resistant Prostate Cancer (CRPC) | NCT05116579 |
| | Solid tumors | FLUIDO (NCT04793061) |
| Monitoring of treatment response and guiding therapy decisions | Breast cancer | NCT05770531<br>NCT05770531 |
| | Colorectal cancer | RAPID-1 (NCT04786600)<br>OPTIMIZE (NCT04680260)<br>TACT-D (NCT03844620) |
| | NSCLC | NCT05816642 |
| | CRPC | PROTRACT (NCT04015622) |

macroscopically no evidence of disease after curative-intent treatment. The prognostic value of ctDNA detection during surveillance has been shown in several studies and has been extensively discussed above. However, how "ctDNA relapse" could meaningfully affect patient management remains unclear. For instance, ctDNA relapse could potentially trigger closer monitoring through clinical and imaging evaluations in order to detect radiographic relapse earlier when the tumor might still be amendable to curative-intent treatment. Furthermore, earlier systemic treatment targeting MRD might potentially delay treatment progression in analogy to the approved hormonal therapies that are routinely applied in biochemically-recurrent prostate cancer. Currently, there is very limited evidence in this field. In the recently published c-TRAK TN study, patients with moderate- and high-risk early-stage triple-negative breast cancer underwent ctDNA surveillance via tumor-informed ddPCR after curative-intent therapy and ctDNA positive patients were randomized 2:1 to pembrolizumab vs. observation. Interestingly, the authors reported a very high rate of metastases in imaging at the time of ctDNA detection (23/32, 72%), highlighting the need for more sensitive testing [70]. Further studies evaluating how molecular relapse monitoring may affect patient management are underway (Table 2).

Due to the absence of robust evidence from prospective clinical trials regarding the clinical utility of MRD-guided decisions after curative-intent treatment and during surveillance, the ESMO Precision Medicine Working Group does not currently recommend the adoption of MRD-testing in the routine clinical practice of early-stage cancer [2].

#### 3.3.4. CTCs

Apart from ctDNA-based approaches, CTC-based approaches have also been studied in the early-stage cancer setting, mainly focusing on prognostication. Most evidence comes from early-breast cancer studies, with many of them using the CellSearch assay, and suggests that high CTC levels and/or a CTC level increase prior, during or directly after curative-intent treatment have a negative prognostic value in terms of relapse-free and/or overall survival [71–77]. Baseline CTCs and maximal CTCs were also shown to be associated with relapse and shorter overall survival in patients with oral and oropharygeal squamous cell cancer undergoing induction chemotherapy followed by curative surgery combined with postoperative radiotherapy [78].

### 3.4. Clinical applications in advanced cancer

#### 3.4.1. Evaluation of targetable alterations

Although standard-of-care genomic profiling to detect targetable alterations still includes tissue-based testing, numerous prospective and retrospective studies have proven a high specificity and positive predictive values, ranging from 95% to 99%, when comparing ctDNA liquid biopsy results with those obtained through tissue-based testing in advanced tumors [79–83]. Therefore, its application in this clinical setting has been considered valuable and has led to the approval of several single- and multigene tests by the FDA.

The first Food and Drug Administration (FDA) -approved liquid biopsy assay, known as the cobas epidermal growth factor receptor (*EGFR*) mutation test v2, utilizes PCR-based technology and was approved as a companion diagnostic test for *EGFR* mutations present in plasma cfDNA from patients with advanced-stage NSCLC who could potentially benefit from the *EGFR* tyrosine kinase inhibitor (TKI) erlotinib [46]. The second PCR-based assay to be approved as a companion diagnostic test was the therascreen phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) RGQ PCR kit. The underlying data for approval was retrieved from the phase III SOLAR-1 trial. The test is designed to assess the eligibility for treatment with the *PIK3CA* inhibitor alpelisib in combination with fulvestrant in hormone receptor–positive advanced breast cancer [84]. For both PCR-tests, reflex testing of tumor tissue is advised in case of negative test results [46,84].

Concerning next-generation sequencing-(NGS)-based multigene liquid biopsy tests, Guardant360® as well as FoundationOne® Liquid CDx have been approved as companion diagnostics for general molecular profiling and for several targeted therapies in recent years [85,86]. Guardant360® was primarily intended for the detection of *EGFR* mutations for eligibility assessment for Osimertinib [87]. FoundationOne® Liquid CDx was first approved for patients with advanced prostate cancer and NSCLC. The approval was extended to breast and ovarial cancer [88].

Apart from the approved tests and indications, the detection of actionable alterations has been studied in several other entities, such as cancer of unknown primary (CUP), gastroesophageal and colorectal cancer as well as gynecological tumors, with a percentage of at least 1 pathogenetic alteration detected ranging from 68 to 82% [89–94]. These high detection rates support the assessment of alterations using liquid biopsy testing.

The ESMO Precision Medicine Working Group has stated that the degree of supporting evidence regarding the clinical validity of ctDNA assays is substantial enough that validated and suitably sensitive ctDNA testing can be incorporated into standard clinical practice for the purpose of genotyping in advanced disease [2].

#### 3.4.2. Prognostication

As for early-stage cancers, liquid biopsies have been assessed for potential prediction of outcome in various tumor entities [90–95]. Predictors of poorer outcome were shown to include the following: concordance between tissue and liquid ctDNA alterations, elevated levels of ctDNA (in %) and higher numbers of ctDNA alterations [96–98].

#### 3.4.3. Assessment of baseline tumor mutational burden (TMB) and microsatellite status to predict checkpoint inhibitor (CPI) response

Response to CPI therapy has been shown to be associated with TMB and high microsatellite instability (MSI) detected by tumor tissue-based tests [99–101]. Several studies have been performed to assess this association also for blood-derived TMB and MSI status. Georgiadis et al. as well as Gandara et al. both detected a positive correlation [102,103]. For the validation of MSI detection using the cfDNA-based Guardant360® test, Willis et al. performed a study with 1145 samples of cfDNA that were correlated with tissue samples. The overall accuracy found was 98.4 % [104].

For TMB detection via liquid biopsy, the ESMO acknowledges its importance as a highly relevant field of research, but does not currently recommend the selection of patients for CPIs based solely on its result [2].

#### 3.4.4. Assessment of response and prediction outcome of CPI-based therapy

Early assessments under CPI are needed in the clinical routine in order to better differentiate tumor response. Especially pseudo-progression, the initial increase in tumor size in radiographic images probably fueled by immune inflammation, can be frequently detected at the beginning of CPI treatment, in spite of overall clinical response. Cf/ctDNA have therefore been examined as a potential tool for assessing therapy response under CPI in several trials.

In a study performed by Kato et al. [90] that employed serial cfDNA-assessments for patients undergoing CPI-treatment in various tumor entities, patients were divided into a cohort with high or low cfDNA-derived average adjusted changes in variant allele frequency (VAF). The factor served as an independent predictor of clinical benefit rate, PFS and OS. Zhang et al. assessed ctDNA-levels in patients receiving durvalumab ± tremelimumab and could detect a correlation of on-treatment reductions of VAF with longer PFS, OS and objective response rate [105]. Ricciuti et al. assessed the % change in ctDNA in patients treated with pembrolizumab ± platinum/pemetrexed. A reduction of detectable ctDNA correlated with higher response rates, progression-free survival (PFS) and median overall survival (mOS) [106].

Together with imaging tools, the usage of quantitative ctDNA and VAF assessment could hence offer additional information especially for early response assessment and prediction of outcome.

*3.4.5. Serial sampling for monitoring treatment response for targeted therapy and chemotherapy*

Apart from CPI-treatment, additional tools for the assessment of treatment response in patients receiving targeted therapy or chemotherapy are also in demand. Several studies have therefore analysed the use of ctDNA or CTCs in this setting. In a study by Tie et al., 53 metastatic colorectal cancer (mCRC) patients receiving standard first-line chemotherapy were included. (25851626) ctDNA was assessed before treatment initiation and before the second cycle. Significant ctDNA reduction was found to be associated with responses in the staging imaging examination after 8–10 weeks. Cao et al. evaluated mutational changes in ctDNA in patients with advanced CRC and could detect that dynamic changes could predict disease progression [107]. Concerning CTCs, a phase II trial performed by Punnoose et al. showed that a reduction of CTCs was in accordance with radiographic response and longer PFS [108].

Although traditional tumor markers find widespread application for treatment monitoring in current clinical practice, ctDNA has been shown to be superior in this setting in different tumor entities. Dawson et al. evaluated the correlation of imaging results with cancer antigen 15-4 (CA15-4) compared to ctDNA results in patients with breast cancer. ctDNA was found to more precisely correspond to tumor burden and to establish a greater dynamic range [15,109] Parikh et al. showed that a decrease in ctDNA after 4 weeks of chemotherapy more accurately predicted partial response and clinical benefit than carcinoembryonic antigen (CEA) [110]. It was furthermore evaluated by Parkinson et al. that the decrease of ctDNA more precisely predict time to progression than cancer antigen 125 (CA-125) levels in patients with ovarian cancer [111] (Table 3).

*3.4.6. Detection of resistance mechanisms under systemic treatment*

Another important field of interest in the application of ctDNA is the potential detection of resistance mechanism that evolve under treatment. Plasma ctDNA analysis allows for the monitoring of various resistance mechanisms to targeted therapy [46]. These resistance mechanisms include co-mutations that can influence treatment decisions across multiple cancer types, with particular significance observed in patients with NSCLC and colorectal cancer (CRC) [112,113]. The appearance of kirsten rat sarcoma virus (*KRAS)* mutations has proven to be of particular interest in this regard, serving as a resistance mechanism to *EGFR*-targeted therapy in patients with CRC. This mutation can be detected via plasma cfDNA analysis [114,115]. In a recent trial, Topham et al. compared the acquisition of different alterations between patients with refractory mCRC with or without prior anti-*EGFR*-based therapy [116]. The result showed a higher significant mutation frequency among the anti-*EGFR*-cohort than the control-group (12 vs. 2). *KRAS* and *EGFR* mutations were detected in 56% and 58% of patients having received anti-*EGFR*-therapy. Similarly, cfDNA analysis can also identify *EGFR* resistance mutations in patients with *EGFR*-mutant NSCLC, aiding in the selection of the most appropriate subsequent treatment option [117].

It has been shown in prior studies that acquired *RAS* and *EGFR* mutations are not statically detectable but may decay after the withdrawal of anti-*EGFR*-therapy [116,118]. Therefore, ctDNA may prove to be an important tool when considering a re-challenge with anti-EGFR-treatment. Several trials have been performed to assess this issue, among them the CHRONOS trial that could detect clinical benefit in cases of a re-challenge without ctDNA-based detection of rat sarcoma (*RAS),* V-Raf Murine Sarcoma Viral Oncogene Homolog B *(BRAF)* or EGFR-extracellular domain (*ECD*) mutations [118–121]. The ESMO has acknowledged ctDNA-guided anti-*EGFR* rechallenge in mCRC [2].

In a phase I/II trial of the PI3K-alpha inhibitor alpelisib, administered alongside an aromatase inhibitor for patients with hormone receptor-positive breast cancer, Razavi et al. [122] used ctDNA analysis and identified that the development of resistance was associated with the presence of loss-of-function Phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) mutations and activating mutations in estrogen receptor 1 (*ESR1)*. Serial ctDNA assessment in patients with human epidermal growth factor receptor 2 (*HER2*) -positive metastatic breast cancer treated with oral anti-*HER1/HER2* tyrosine kinase inhibitors showed a correlation of *HER2*-amplification and disease progression [123]. Other clinical trials have further highlighted the potential of serial ctDNA evaluation for detection of progression [109,111].

Next to assessing known resistance mechanisms, detecting unknown mechanisms through cfDNA has been studied in several trials, either using large panels or a targeted approach in cases where some probable association with resistance to a specific treatment is known [124,125]. However, CHIP mutations may limit tumor agnostic-strategies [2].

CTC-based assessments have also been applied for identifying mechanisms of resistance during therapy. An important variant to highlight in this context is the androgen receptor splice variant 7 (AR-V7). Detection of this variant in CTCs has been associated with worse prognosis in patients with metastatic castration-resistant prostate cancer, as demonstrated by Antonarakis et al. [126] and Scher et al. [127]. The PROPHECY study, a prospective, multicenter trial, further showed it to be linked to lower PFS and OS [46,128].

*3.4.7. Assessment of ctDNA/CTCs originating from multiple metastatic sites and tumor heterogeneity*

Heterogeneity of tumor cells, especially in the metastatic setting, may play an important role in treatment approaches. Tissue-derived tumor samples can only provide the assessment of a limited amount of tumor material at a specific timepoint. Liquid biopsies could therefore potentially act as an additional tool for complementing the genetic landscape of the tumor, as ctDNA/CTCs can originate from the shedding of several tumor sites and can be evaluated serially [15,96]. However, different tumor sites may shed variable amounts of tumor-derived components, which may be a limiting factor [2].

## 4. Future perspectives

Although several liquid biopsy assays have demonstrated clinical validity in various settings, clinical utility remains to be proven. In order to generate robust evidence of clinical utility, a prospective randomized design or in some cases a prospective-retrospective design, using archival blood samples from a previously conducted prospective trial, needs to be applied [2]. In early-stage cancer, numerous randomized clinical trials are currently investigating the clinical utility of MRD-guided adjuvant treatment and molecular relapse monitoring, while in the advanced disease setting, the focus of ongoing trials is serial liquid biopsy testing to monitor disease response and resistance. Emerging evidence of these studies is expected to address the question how liquid biopsies can inform treatment decisions in a meaningful way and thus improve patient outcome.

The studies on liquid biopsy testing in alternative analytes other than blood such as urine, pleural/ascites effusion or saliva have provided preliminary yet very promising evidence with regard to cancer diagnosis [5,129–131], monitoring of treatment response and relapse [3,132], as well as molecular profiling [6,9]. Ongoing trials in this field are expected to deepen our understanding on the potential of these analytes. Some examples of ongoing trials include NCT05453591 (urine, breast cancer), NCT05141383 and NCT04079699 (urine, prostate cancer), NCT05122507 (saliva, head and neack cancer), and NCT05461430 (ascites and pleural fluid, tumoragnostic).

Artificial intelligence (AI) has many clinical applications and is expected to play an integral role in medical scientific development, including biomolecular research. AI has been already widely employed in liquid biopsy assay development and has thus contributed substantially to the evolution of this field. For instance, machine learning has been used to improve the performance for CTC assessment [133] as well as for early cancer detection and differentiation of cancer origin through several techniques including cf/ctDNA, methylation, RNA, extracellular vesicle and protein analyses [28,134,135]. The Lung- CLiP algorithm is

an example of a machine-learning approach that integrates targeted sequencing of plasma cfDNA and matched leukocyte DNA to robustly discriminate early-stage lung cancer patients from risk-matched controls [136]. Furthermore, machine learning has also been applied to incorporate molecular liquid biopsy with clinical and radiological patient data in order to assist clinical decisions [137]. Several machine-learning platforms dedicated to analyze liquid biospy data have been developed, with the potential to further improve their performance as they receive additional information input [138,139]. Overall, AI is expected to be an integral part of liquid biopsy analytics and facilitate integration into clinical decision making.

## 5. Conclusion

Liquid biopsies constitute a modern and promising technology with increasing usage that can potentially further facilitate the integration of precision medicine into clinical routine. Both ctDNA/cfDNA and CTCs allow for serial collection and provide real-time molecular and response data, especially for patients in which tissue biopsies are not recommended. Numerous trials have shown promising results in various fields, such as for predicting CPI response, monitoring response and detecting resistance under chemotherapy or targeted therapy as well as MRD-detection and monitoring, suggesting a valuable prognostic and potentially predictive tool in early and advance cancer settings. In numerous cases, liquid biopsies could therefore be used as a complementary tool to standard tissue biopsies during the course of the disease or for more detailed analysis. Moreover, in patients for whom tissue biopsies are not feasible, liquid biopsies already play an important role for genotyping and guiding therapy decisions.

Several FDA-approved liquid biopsy companion diagnostics are already available, such as those for *EGFR* and *PIK3CA* alterations as well as multigene panels. These authorizations signify a turning point in the extensive adoption of liquid biopsy within clinical settings, particularly among patients with advanced-stage cancer. The subsequent horizon for employing liquid biopsy in clinical practice is anticipated to focus on the systemic treatment of individuals with MRD. Further trials are needed to gain approval by health technology authorities and be granted reimbursement.

However, several potential limitations, especially false-positive results from ctDNA assessments due to CHIP mutations as well as low shedding of tumor-derived materials that potentially hinder detection of ctDNA/CTCs, still have to be taken into account. Challenges for the integration of liquid biopsies into clinical practice include standardization, analyte validation and demonstration of clinical utility as well as regulatory considerations. Emerging data from ongoing clinical trials in various setting, also including alternate bodily fluids than blood and leveraging AI-technology, are expected to revolutionize the liquid biopsy field in the near future.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Victoria Probst reports a relationship with Nordic Pharma UK that includes: travel reimbursement. C. Benedikt Westphalen reports a relationship with Roche that includes: board membership, funding grants, speaking and lecture fees, and travel reimbursement. C. Benedikt Westphalen reports a relationship with Amgen Inc that includes: board membership and speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Bayer AG that includes: board membership, speaking and lecture fees, and travel reimbursement. C. Benedikt Westphalen reports a relationship with Bristol-Myers Squibb Co that includes: board membership and speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Chugai Pharmaceutical Co Ltd that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Celgene GmbH that includes: board membership, speaking and lecture fees, and travel reimbursement. C. Benedikt Westphalen reports a relationship with Dr. Falk Pharma AG that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with GSK that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Merck Sharp & Dohme Corp that includes: board membership and speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Merck & Co Inc that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Janssen Pharmaceuticals Inc that includes: board membership, speaking and lecture fees, and travel reimbursement. C. Benedikt Westphalen reports a relationship with Ipsen that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Servier Monde that includes: board membership, speaking and lecture fees, and travel reimbursement. C. Benedikt Westphalen reports a relationship with Sirtex Medical Inc that includes: speaking and lecture fees. C. Benedikt Westphalen reports a relationship with Taiho Oncology Inc that includes: speaking and lecture fees and travel reimbursement. C. Benedikt Westphalen reports a relationship with Incyte Corporation that includes: board membership. C. Benedikt Westphalen reports a relationship with Baxalta that includes: board membership. C. Benedikt Westphalen reports a relationship with Rafael Pharmaceuticals Inc that includes: board membership. C. Benedikt Westphalen reports a relationship with RedHill Biopharma Ltd that includes: board membership and travel reimbursement. C. Benedikt Westphalen reports a relationship with ESMO that includes: board membership. C. Benedikt Westphalen reports a relationship with German Cancer Aid that includes: board membership. C. Benedikt Westphalen reports a relationship with German Cancer Society that includes: board membership. C. Benedikt Westphalen reports a relationship with European Commission that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Myrto Boukovala reports a relationship with Servier Deutschland GmbH that includes: current employment at the company.

## References

[1] Normanno N, et al. Cancer Biomarkers in the era of precision oncology: addressing the needs of patients and health systems. Semin Cancer Biol 2022;84:293–301.
[2] Pascual J, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022;33(8):750–68.
[3] Husain H, et al. Monitoring daily dynamics of early tumor response to targeted therapy by detecting circulating tumor DNA in urine. Clin Cancer Res 2017; 23(16):4716–23.
[4] Zhang J, Zhang X, Shen S. Treatment and relapse in breast cancer show significant correlations to noninvasive testing using urinary and plasma DNA. Future Oncol 2020;16(13):849–58.
[5] Oshi M, et al. Urine as a source of liquid biopsy for cancer. Cancers 2021;13(11).
[6] Tong L, et al. Tumor-derived DNA from pleural effusion supernatant as a promising alternative to tumor tissue in genomic profiling of advanced lung cancer. Theranostics 2019;9(19):5532–41.
[7] Villatoro S, et al. Prospective detection of mutations in cerebrospinal fluid, pleural effusion, and ascites of advanced cancer patients to guide treatment decisions. Mol Oncol 2019;13(12):2633–45.
[8] Sorolla MA, et al. Diving into the pleural fluid: liquid biopsy for metastatic malignant pleural effusions. Cancers 2021;13(11).
[9] Han MR, et al. Clinical implications of circulating tumor DNA from ascites and serial plasma in ovarian cancer. Cancer Res Treat 2020;52(3):779–88.
[10] Pentsova EI, et al. Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid. J Clin Oncol 2016;34(20):2404–15.
[11] Miller AM, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature 2019;565(7741):654–8.
[12] Bobillo S, et al. Cell free circulating tumor DNA in cerebrospinal fluid detects and monitors central nervous system involvement of B-cell lymphomas. Haematologica 2021;106(2):513–21.
[13] Cheng J, Nonaka T, Wong DTW. Salivary exosomes as nanocarriers for cancer biomarker delivery. Materials 2019;12(4).
[14] Nonaka T, Wong DTW. Saliva diagnostics: salivaomics, saliva exosomics, and saliva liquid biopsy. J Am Dent Assoc 2023;154(8):696–704.
[15] Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. J Hematol Oncol 2022;15(1):131.
[16] Lone SN, et al. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Mol Cancer 2022;21(1):79.

[17] Poulet G, Massias J, Taly V. Liquid biopsy: general concepts. Acta Cytol 2019; 63(6):449–55.
[18] Phallen J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017;9(403).
[19] Dharajiya NG, et al. Incidental detection of maternal neoplasia in noninvasive prenatal testing. Clin Chem 2018;64(2):329–35.
[20] Cohen JD, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359(6378):926–30.
[21] Church TR, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut 2014;63(2):317–25.
[22] Epigenomics. Epi proColon® 2.0 CE. 2019 November 2023]; Available from: https://www.epiprocolon.com/wp-content/uploads/sites/3/2019/08/MKT_0049DE_Epi_proColon_20CE_Medical_Professionals_rev3_singles.pdf.
[23] Xu RH, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater 2017;16(11):1155–61.
[24] Liu L, et al. Targeted methylation sequencing of plasma cell-free DNA for cancer detection and classification. Ann Oncol 2018;29(6):1445–53.
[25] Liu MC, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol 2020;31(6):745–59.
[26] Shen SY, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature 2018;563(7732):579–83.
[27] Chan KC, et al. Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing. Proc Natl Acad Sci U S A 2013;110(47):18761–8.
[28] Klein EA, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol 2021;32(9): 1167–77.
[29] Cristiano S, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature 2019;570(7761):385–9.
[30] Mouliere F, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med 2018;10(466).
[31] Hinestrosa JP, et al. Early-stage multi-cancer detection using an extracellular vesicle protein-based blood test. Commun Med 2022;2:29.
[32] Best MG, et al. RNA-seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. Cancer Cell 2015; 28(5):666–76.
[33] Best MG, et al. Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets. Cancer Cell 2017;32(2):238–252 e9.
[34] Campolo F, et al. Platelet-derived circRNAs signature in patients with gastroenteropancreatic neuroendocrine tumors. J Transl Med 2023;21(1):548.
[35] Lebofsky R, et al. Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Mol Oncol 2015;9(4):783–90.
[36] Jensen TJ, et al. Genome-wide sequencing of cell-free DNA enables detection of copy-number alterations in patients with cancer where tissue biopsy is not feasible. Mol Cancer Therapeut 2021;20(11):2274–9.
[37] Moding EJ, et al. Detecting liquid remnants of solid tumors: circulating tumor DNA minimal residual disease. Cancer Discov 2021;11(12):2968–86.
[38] Mittal A, et al. Utility of ctDNA in predicting relapse in solid tumors after curative therapy: a meta-analysis. JNCI Cancer Spectr 2023;7(4).
[39] Zhong R, et al. Accuracy of minimal residual disease detection by circulating tumor DNA profiling in lung cancer: a meta-analysis. BMC Med 2023;21(1):180.
[40] Guo RQ, et al. Clinical significance of circulating tumor DNA in localized non-small cell lung cancer: a systematic review and meta-analysis. Clin Exp Med 2023; 23(5):1621–31.
[41] Moding EJ, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. Nat Cancer 2020;1(2):176–83.
[42] Chaudhuri AA, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 2017;7(12):1394–403.
[43] Christensen E, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. J Clin Oncol 2019;37(18):1547–57.
[44] Coombes RC, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res 2019;25(14):4255–63.
[45] McDonald BR, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med 2019; 11(504).
[46] Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic - implementation issues and future challenges. Nat Rev Clin Oncol 2021;18(5): 297–312.
[47] Tie J, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016; 8(346):346ra92.
[48] Tarazona N, et al. Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. Ann Oncol 2019; 30(11):1804–12.
[49] Tie J, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 2019;5(12): 1710–7.
[50] Parikh AR, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. Clin Cancer Res 2021;27(20):5586–94.
[51] Taieb J, et al. Prognostic value and relation with adjuvant treatment duration of ctDNA in stage III colon cancer: a post hoc analysis of the PRODIGE-GERCOR IDEA-France trial. Clin Cancer Res 2021;27(20):5638–46.
[52] Chidharla A, et al. Circulating tumor DNA as a minimal residual disease assessment and recurrence risk in patients undergoing curative-intent resection with or without adjuvant chemotherapy in colorectal cancer: a systematic review and meta-analysis. Int J Mol Sci 2023;24(12).
[53] Yekeduz E, et al. ctDNA as a prognostic factor in operable colon cancer patients: a systematic review and meta-analysis. Future Oncol 2021;17(3):349–57.
[54] Fan X, Zhang J, Lu D. CtDNA's prognostic value in patients with early-stage colorectal cancer after surgery: a meta-analysis and systematic review. Medicine (Baltim) 2023;102(6):e32939.
[55] Mi J, et al. Circulation tumour DNA in predicting recurrence and prognosis in operable colorectal cancer patients: a meta-analysis. Eur J Clin Invest 2022; 52(12):e13842.
[56] Faulkner LG, et al. The utility of ctDNA in detecting minimal residual disease following curative surgery in colorectal cancer: a systematic review and meta-analysis. Br J Cancer 2023;128(2):297–309.
[57] Garcia-Murillas I, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7(302). 302ra133.
[58] Olsson E, et al. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med 2015;7(8):1034–47.
[59] Garcia-Murillas I, et al. Assessment of molecular relapse detection in early-stage breast cancer. JAMA Oncol 2019;5(10):1473–8.
[60] Sausen M, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun 2015;6:7686.
[61] Jiang J, et al. Circulating tumor DNA as a potential marker to detect minimal residual disease and predict recurrence in pancreatic cancer. Front Oncol 2020;10: 1220.
[62] Xia L, et al. Perioperative ctDNA-based molecular residual disease detection for non-small cell lung cancer: a prospective multicenter cohort study (LUNGCA-1). Clin Cancer Res 2022;28(15):3308–17.
[63] Azad TD, et al. Circulating tumor DNA analysis for detection of minimal residual disease after chemoradiotherapy for localized esophageal cancer. Gastroenterology 2020;158(3):494–505 e6.
[64] Tan L, et al. Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA. Ann Oncol 2019;30(5):804–14.
[65] Shen H, et al. Potential clinical utility of liquid biopsy in early-stage non-small cell lung cancer. BMC Med 2022;20(1):480.
[66] Powles T, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nature 2021;595(7867):432–7.
[67] Qiu B, et al. Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC. Nat Commun 2021;12(1):6770.
[68] Zhang JT, et al. Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer. Cancer Discov 2022;12(7):1690–701.
[69] Tie J, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022;386(24):2261–72.
[70] Turner NC, et al. Results of the c-TRAK TN trial: a clinical trial utilising ctDNA mutation tracking to detect molecular residual disease and trigger intervention in patients with moderate- and high-risk early-stage triple-negative breast cancer. Ann Oncol 2023;34(2):200–11.
[71] Pachmann K, et al. Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. J Clin Oncol 2008;26(8):1208–15.
[72] Ma G, et al. Heterogeneous circulating tumor cells correlate with responses to neoadjuvant chemotherapy and prognosis in patients with locally advanced breast cancer. Breast Cancer Res Treat 2023;201(1):27–41.
[73] Maurer M, et al. Increased circulating epithelial tumor cells (CETC/CTC) over the course of adjuvant radiotherapy is a predictor of less favorable outcome in patients with early-stage breast cancer. Curr Oncol 2022;30(1):261–73.
[74] Rack B, et al. Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. J Natl Cancer Inst 2014;106(5).
[75] Riethdorf S, et al. Prognostic impact of circulating tumor cells for breast cancer patients treated in the neoadjuvant "geparquattro" trial. Clin Cancer Res 2017; 23(18):5384–93.
[76] Bidard FC, et al. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis. J Natl Cancer Inst 2018;110(6): 560–7.
[77] Sparano J, et al. Association of circulating tumor cells with late recurrence of estrogen receptor-positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol 2018;4(12):1700–6.
[78] Inhestern J, et al. Prognostic role of circulating tumor cells during induction chemotherapy followed by curative surgery combined with postoperative radiotherapy in patients with locally advanced oral and oropharyngeal squamous cell cancer. PLoS One 2015;10(7):e0132901.
[79] Leighl NB, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 2019;25(15):4691–700.
[80] Aggarwal C, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncol 2019;5(2):173–80.
[81] Maron SB, et al. Circulating tumor DNA sequencing analysis of gastroesophageal adenocarcinoma. Clin Cancer Res 2019;25(23):7098–112.
[82] Mack PC, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non-small cell lung cancer: analysis of over 8000 cases. Cancer 2020;126(14):3219–28.

[83] Oxnard GR, et al. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 2014;20(6):1698–705.
[84] Andre F, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019;380(20):1929–40.
[85] FoundationOne®Liquid CDx. [cited 2023 08 October]; https://www.foundationmedicine.de/de/our-services/liquid.html].
[86] Guardant360® [cited 2023 08 October]; https://guardanthealth.com/products/tests-for-patients-with-advanced-cancer/].
[87] Administration, U.S.F.a.D. Guardant360 CDx – P200010/S008 [cited 2023 08 October]; https://www.fda.gov/medical-devices/recently-approved-devices/guardant360-cdx-p200010s008 ].
[88] Administration, U.S.F.a.D. FDA approves liquid biopsy NGS companion diagnostic test for multiple cancers and biomarkers. [cited 2023 08 October] https://www.fda.gov/drugs/fda-approves-liquid-biopsy-ngs-companion-diagnostic-test-multiple-cancers-and-biomarkers].
[89] Kato S, et al. Utility of genomic analysis in circulating tumor DNA from patients with carcinoma of unknown primary. Cancer Res 2017;77(16):4238–46.
[90] Kato S, et al. Analysis of circulating tumor DNA and clinical correlates in patients with esophageal, gastroesophageal junction, and gastric adenocarcinoma. Clin Cancer Res 2018;24(24):6248–56.
[91] Shatsky R, et al. Next-generation sequencing of tissue and circulating tumor DNA: the UC San Diego Moores Center for personalized cancer therapy experience with breast malignancies. Mol Cancer Therapeut 2019;18(5):1001–11.
[92] Schwaederle MC, et al. Utility of genomic assessment of blood-derived circulating tumor DNA (ctDNA) in patients with advanced lung adenocarcinoma. Clin Cancer Res 2017;23(17):5101–11.
[93] Choi IS, et al. Genomic profiling of blood-derived circulating tumor DNA from patients with colorectal cancer: implications for response and resistance to targeted therapeutics. Mol Cancer Therapeut 2019;18(10):1852–62.
[94] Charo LM, et al. Clinical implications of plasma circulating tumor DNA in gynecologic cancer patients. Mol Oncol 2021;15(1):67–79.
[95] Okamura R, et al. Comprehensive genomic landscape and precision therapeutic approach in biliary tract cancers. Int J Cancer 2021;148(3):702–12.
[96] Adashek JJ, Janku F, Kurzrock R. Signed in blood: circulating tumor DNA in cancer diagnosis, treatment and screening. Cancers 2021;13(14).
[97] Mardinian K, et al. Temporal and spatial effects and survival outcomes associated with concordance between tissue and blood KRAS alterations in the pan-cancer setting. Int J Cancer 2020;146(2):566–76.
[98] Rosenberg S, et al. Survival implications of the relationship between tissue versus circulating tumor DNA TP53 mutations-A perspective from a real-world precision medicine cohort. Mol Cancer Therapeut 2020;19(12):2612–20.
[99] Petrelli F, et al. Outcomes following immune checkpoint inhibitor treatment of patients with microsatellite instability-high cancers: a systematic review and meta-analysis. JAMA Oncol 2020;6(7):1068–71.
[100] Sun Z, et al. Identification of microsatellite instability and immune-related prognostic biomarkers in colon adenocarcinoma. Front Immunol 2022;13:988303.
[101] Goodman AM, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Therapeut 2017;16(11):2598–608.
[102] Georgiadis A, et al. Noninvasive detection of microsatellite instability and high tumor mutation burden in cancer patients treated with PD-1 blockade. Clin Cancer Res 2019;25(23):7024–34.
[103] Gandara DR, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24(9):1441–8.
[104] Willis J, et al. Validation of microsatellite instability detection using a comprehensive plasma-based genotyping panel. Clin Cancer Res 2019;25(23):7035–45.
[105] Zhang Q, et al. Prognostic and predictive impact of circulating tumor DNA in patients with advanced cancers treated with immune checkpoint blockade. Cancer Discov 2020;10(12):1842–53.
[106] Ricciuti B, et al. Early plasma circulating tumor DNA (ctDNA) changes predict response to first-line pembrolizumab-based therapy in non-small cell lung cancer (NSCLC). J Immunother Cancer 2021;9(3).
[107] Cao H, et al. Circulating tumor DNA is capable of monitoring the therapeutic response and resistance in advanced colorectal cancer patients undergoing combined target and chemotherapy. Front Oncol 2020;10:466.
[108] Punnoose EA, et al. Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. Clin Cancer Res 2012;18(8):2391–401.
[109] Dawson SJ, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368(13):1199–209.
[110] Parikh AR, et al. Serial ctDNA monitoring to predict response to systemic therapy in metastatic gastrointestinal cancers. Clin Cancer Res 2020;26(8):1877–85.
[111] Parkinson CA, et al. Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: a retrospective study. PLoS Med 2016;13(12):e1002198.
[112] Li S, et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. Br J Cancer 2014;110(11):2812–20.
[113] De Roock W, et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 2011;12(6):594–603.
[114] Misale S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012;486(7404):532–6.
[115] Diaz Jr LA, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012;486(7404):537–40.
[116] Topham JT, et al. Circulating tumor DNA identifies diverse landscape of acquired resistance to anti-epidermal growth factor receptor therapy in metastatic colorectal cancer. J Clin Oncol 2023;41(3):485–96.
[117] Mok TS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376(7):629–40.
[118] Parseghian CM, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. Ann Oncol 2019;30(2):243–9.
[119] Montagut C, et al. Efficacy of Sym004 in patients with metastatic colorectal cancer with acquired resistance to anti-EGFR therapy and molecularly selected by circulating tumor DNA analyses: a phase 2 randomized clinical trial. JAMA Oncol 2018;4(4):e175245.
[120] Van Emburgh BO, et al. Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. Nat Commun 2016;7:13665.
[121] Siravegna G, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 2015;21(7):795–801.
[122] Razavi P, et al. Alterations in PTEN and ESR1 promote clinical resistance to alpelisib plus aromatase inhibitors. Nat Cancer 2020;1(4):382–93.
[123] Ma F, et al. ctDNA dynamics: a novel indicator to track resistance in metastatic breast cancer treated with anti-HER2 therapy. Oncotarget 2016;7(40):66020–31.
[124] O'Leary B, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018;8(11):1390–403.
[125] Murtaza M, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013;497(7447):108–12.
[126] Antonarakis ES, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371(11):1028–38.
[127] Scher HI, et al. Assessment of the validity of nuclear-localized androgen receptor splice variant 7 in circulating tumor cells as a predictive biomarker for castration-resistant prostate cancer. JAMA Oncol 2018;4(9):1179–86.
[128] Armstrong AJ, et al. Prospective multicenter validation of androgen receptor splice variant 7 and hormone therapy resistance in high-risk castration-resistant prostate cancer: the PROPHECY study. J Clin Oncol 2019;37(13):1120–9.
[129] Yuvaraj M, et al. Fluorescence spectroscopic characterization of salivary metabolites of oral cancer patients. J Photochem Photobiol, B 2014;130:153–60.
[130] Iliev R, et al. Expression levels of PIWI-interacting RNA, piR-823, are deregulated in tumor tissue, blood serum and urine of patients with renal cell carcinoma. Anticancer Res 2016;36(12):6419–23.
[131] Liu B, et al. Detection of promoter DNA methylation in urine and plasma aids the detection of non-small cell lung cancer. Clin Cancer Res 2020;26(16):4339–48.
[132] Hann HW, et al. Detection of urine DNA markers for monitoring recurrent hepatocellular carcinoma. Hepatoma Res 2017;3:105–11.
[133] Iyer A, et al. Integrative analysis and machine learning based characterization of single circulating tumor cells. J Clin Med 2020;9(4).
[134] Liu L, et al. Machine learning protocols in early cancer detection based on liquid biopsy: a survey. Life 2021;11(7).
[135] Nakamura K, et al. An exosome-based transcriptomic signature for noninvasive, early detection of patients with pancreatic ductal adenocarcinoma: a multicenter cohort study. Gastroenterology 2022;163(5):1252–1266 e2.
[136] Chabon JJ, et al. Integrating genomic features for non-invasive early lung cancer detection. Nature 2020;580(7802):245–51.
[137] Ye M, et al. A classifier for improving early lung cancer diagnosis incorporating artificial intelligence and liquid biopsy. Front Oncol 2022;12:853801.
[138] Shen H, et al. A web-based automated machine learning platform to analyze liquid biopsy data. Lab Chip 2020;20(12):2166–74.
[139] Halner A, et al. DEcancer: machine learning framework tailored to liquid biopsy based cancer detection and biomarker signature selection. iScience 2023;26(5):106610.