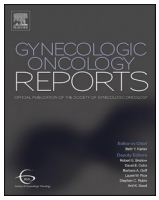
Gynecologic Oncology Reports 54 (2024) 101431

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor



Circulating cell-free (cf)DNA analysis: Current technologies and applications in gynecologic cancer

Sarah H Kim [a], Britta Weigelt [b,*]

[a] Gynecology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[b] Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

A B S T R A C T

Cell-free DNA (cfDNA) analysis has several promising clinical applications in the management of cancer patients, with clinical validity established in different types of solid tumors (e.g., lung, breast, and colon cancer). Cancers harbor unique genetic alterations that can be detected in the plasma and other bodily fluids of cancer patients, constituting an alternate source of tumor-derived DNA. Technologic advances and wide-spread availability of next-generation sequencing (NGS) have made sequencing analysis of circulating tumor DNA (ctDNA) possible, employing both off-the-shelf and personalized tumor-informed panels. Tumor size, disease burden and high-grade histologic types have been shown to correlate with ctDNA levels across multiple solid cancer types. Detection of tumor-derived genetic alterations in plasma-derived cfDNA can facilitate diagnosis, guide treatment selection, and serve as a biomarker for treatment response and prognostication. Molecular residual disease (MRD) is at the forefront of cfDNA analysis, with implications in treatment de-escalation/ escalation in the neoadjuvant and adjuvant settings. The development of cfDNA analysis in early detection of cancers is under active investigation. Proof-of-principles studies in gynecologic cancers have demonstrated feasibility and potential for innovation in cancers lacking specific biomarkers, including the tracking of human papillomavirus (HPV) cfDNA in patients with cervical cancer. In this review, we outline the assays currently available for cfDNA sequencing/ ctDNA detection, the role of cfDNA analysis in clinical decision-making and the current status and potential clinical uses of cfDNA research in gynecologic cancers.

1. Background circulating cell-free (cf)DNA

Over the past decade, circulating cell-free (cf)DNA analysis has received great attention in oncology research with a wide spectrum of promising tumor biomarker-related applications ranging from surrogates for traditional biopsies (i.e., “liquid biopsy”) and prognosis to minimally invasive serial monitoring of treatment response and detection of minimal residual disease and therapy resistance mechanisms (Alix-Panabieres and Pantel, 2021). While cfDNA analysis has been validated as a clinical biomarker in several different solid malignancies, the analysis of cfDNA remains relatively new to the field of gynecologic oncology and optimal clinical applications of the emerging technology remain unclear.

Cancers harbor unique sets of genetic alterations acquired throughout tumorigenesis, and next-generation sequencing (NGS)-based testing has become more broadly available as a method to identify these genetic alterations in tissue-derived tumor DNA for diagnosis and therapy decision-making (Berger and Mardis, 2018). Circulating tumor (ct) DNA found in the plasma of cancer patients, which is the cfDNA of tumor origin (see next paragraph), has been shown to constitute an alternative source of tumor-derived DNA. While the detection of these cancer-associated mutations in blood have initially proved technically challenging, sequencing technologies and bioinformatics analyses have advanced over the recent years and both genetic and epigenetic tumor-derived alterations can be detected with high specificity and sensitivity.

Tissues/ cells release non-encapsulated fragmented cfDNA into the blood stream and other body fluids through various mechanisms, including necrosis and apoptosis (Crowley et al., 2013). Within this cfDNA, the DNA fraction that is derived from cancer cells is referred to as ctDNA (Fig. 1). It is important to note that cfDNA is not cancer-specific, and it is thought that in healthy individuals most cfDNA in plasma originates from hematopoietic cells (Lui et al., 2002). In fact, in healthy individuals, cfDNA can be detected at low levels in plasma, and are increased in those with inflammatory disease or after stroke or surgery (Gaitsch et al., 2023; Underhill, 2021). In general, higher levels of cfDNA have been found in cancer patients compared to healthy controls (Meddeb et al., 2019), however, this may only be marginal in patients with early-stage/ low volume disease. Importantly, tumor size and extent of disease burden/ stage are correlated with the levels of detectable ctDNA across solid cancer types, and patients with high-stage or metastatic disease have been shown to have the highest fraction of ctDNA in plasma (Bettegowda et al., 2014). Also, the subset of cases with

* Corresponding author at: Memorial Sloan Kettering Cancer Center, Department of Pathology and Laboratory Medicine, 1275 York Avenue, New York, NY 10065.
E-mail address: weigeltb@mskcc.org (B. Weigelt).

https://doi.org/10.1016/j.gore.2024.101431
Received 23 April 2024; Received in revised form 4 June 2024; Accepted 9 June 2024
Available online 13 June 2024

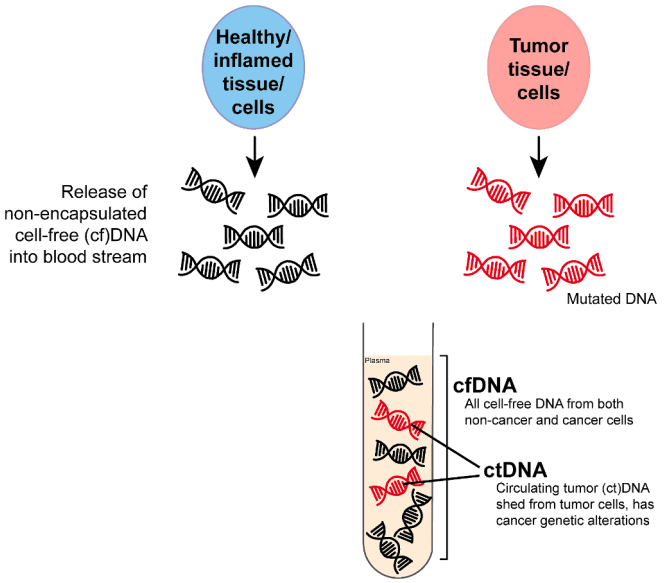


Fig. 1. Circulating cell-free (cf)DNA and circulating tumor (ct)DNA. Non-encapsulated circulating cell-free (cf)DNA is released from healthy and inflamed tissues as well as tumor tissues into the blood stream. cfDNA refers to all cell-free DNA from all sources, non-cancer and cancer cells. ctDNA refers specifically to cell-free DNA shed from tumor cells, and this DNA harbors genetic alterations that can be detected through sequencing analysis of cfDNA.

detectable ctDNA varies by tumor type, and many of the initial studies were performed in cancer types with early hematogenous spread such as breast and lung cancer (Bettegowda et al., 2014). In gynecologic cancer, work has been focusing on utilizing cfDNA analysis for disease monitoring and detection of minimal/ molecular residual disease (MRD), and for the identification of specific genetic alterations, such as *BRCA1/2* mutations in ovarian cancer or microsatellite instability (MSI) in endometrial cancer. Currently, a liquid biopsy test for *BRCA1/2* mutation detection in ovarian cancer as well as cfDNA-based MRD assessment and immunotherapy response monitoring are available for clinical use in gynecological cancers (see below; Table 1 and Fig. 3). However, their optimal use, timing of therapeutic (de-)escalation, and effect on patient outcomes are yet to be defined in prospective clinical studies.

In this review article, we outline the assays currently available for cfDNA sequencing/ ctDNA detection, the role of cfDNA analysis in clinical decision-making and in gynecologic cancers.

2. Assays for cfDNA sequencing/ ctDNA detection

For the detection of mutations in peripheral blood, cfDNA is extracted from plasma, for which different manual and automated methods are available. While there are ways to enrich for ctDNA, for example through fragment size selection as the fragment length of ctDNA is shorter (median ~ 144 bp) than that of cfDNA (median ~ 167 bp) (Underhill, 2021; Udomruk et al., 2021), analyses are generally performed on the entire isolated cfDNA. Thus, sensitive and specific assays are required to detect the ctDNA fraction within the cfDNA. For the cfDNA sequencing analysis, there are a wide range of technologies and assays available and still emerging, both in the commercial and academic settings. The choice of assay is dependent on the application and disease setting. In general, for the detection of genetic alterations, two types of approaches are utilized, the off-the-shelf sequencing panels designed to detect mutations in frequently altered cancer-related genes and the personalized or also called 'bespoke" sequencing panels (Moding et al., 2021) (Fig. 2). While for off-the-shelf panels the same panel is applied for cfDNA sequencing analysis for each patient, for personalized/ bespoke panels, first the tumor DNA is sequenced. A set of genetic alterations identified in the tissue is then selected to design a personalized panel and applied to detect the tumor/patient-specific mutations in the cfDNA from the same individual, thus every patient has a unique panel (Moding et al., 2021) (Fig. 2).

Table 1
Examples of liquid biopsy companion diagnostic indications for solid tumors.

Cancer type	Gene	Drug	Diagnostic test provider(s)
NSCLC	*KRAS*	KRAS inhibitor	Agilent Resolution ctDx FIRST assay (Resolution Bioscience, Inc.); Guardant360 CDx (Guardant Health, Inc.)
NSCLC	*EGFR*	EGFR inhibitor	cobas EGFR Mutation Test v2 (Roche Molecular Systems, Inc.); Foundation One Liquid Cdx (Foundation Medicine, Inc.); Guardant360 CDx
NSCLC	*MET*	MET inhibitor	FoundationOne Liquid CDx
NSCLC	*ROS1*	Trk A B C, ALK, and ROS1 inhibitor	FoundationOne Liquid CDx
NSCLC	*ALK*	ALK inhibitor	FoundationOne Liquid CDx
NSCLC	*ERBB2*	HER2 antibody drug conjugate	Guardant360 CDx
NSCLC, colorectal cancer	*BRAF*	BRAF inhibitor combination	FoundationOne Liquid CDx
Ovarian cancer, prostate cancer	*BRCA1* and *BRCA2*	PARP inhibitor	FoundationOne Liquid CDx
Prostate cancer	*ATM*	PARP inhibitor	FoundationOne Liquid CDx
Breast cancer	*PIK3CA*	PI3K inhibitor	FoundationOne Liquid CDx; therascreen PIK3CA RGQ PCR Kit (QIAGEN GmbH)
Breast cancer	*ESR1*	Estrogen receptor degrader (SERD)	Guardant360 CDx
Solid tumors	*NTRK1/ 2/3*	Trk A B C, ALK, and ROS1 inhibitor	FoundationOne Liquid CDx

NSCLC, non-small cell lung cancer. Obtained from US Food and Drug Administration (US Food Drug Administration, 2023), accessed April 2024.

The technical approaches for both off-the-shelf and personalized sequencing panels vary and include digital PCR for the assessment of one or more common mutations in cancer-related genes, amplicon NGS sequencing or hybrid capture-based NGS panels as well as whole-genome sequencing. Most panels focus on the detection of tumor-specific mutations in cfDNA, however copy-number alterations, structural variants, structural variant breakpoints and translocations can also be assessed. Different assays and approaches have varying levels of detection (LOD) of ctDNA in plasma, which can be increased by higher cfDNA input for the sequencing analysis, the depth of sequencing, the number of genetic alterations assessed or by capturing multiple mutations in the same DNA fragment (i.e., 'in-phase') (Moding et al., 2021; Kurtz et al., 2021).

The combination of low ctDNA quantities in the plasma with high sequencing depth may lead to the introduction of sequencing artifacts, which in turn may limit analytical sensitivity and impact specificity. Furthermore, to maintain the specificity of ctDNA detection it is critical to differentiate cancer-signals from background normal biological variation. The main source of biological noise in cfDNA analyses is clonal hematopoiesis (CH) (Hu et al., 2018). CH is a part of the normal process of aging and involves the accumulation of somatic mutations in hematopoietic stem cells and their clonal expansion in blood cells (Buttigieg and Rauh, 2023). Importantly, and as mentioned above, most non-cancerous cfDNA in plasma originates from hematopoietic cells. It has been shown through high-intensity sequencing that CH is highly present in the general population and that CH-related mutations can be detected in the plasma of the vast majority of patients with and without

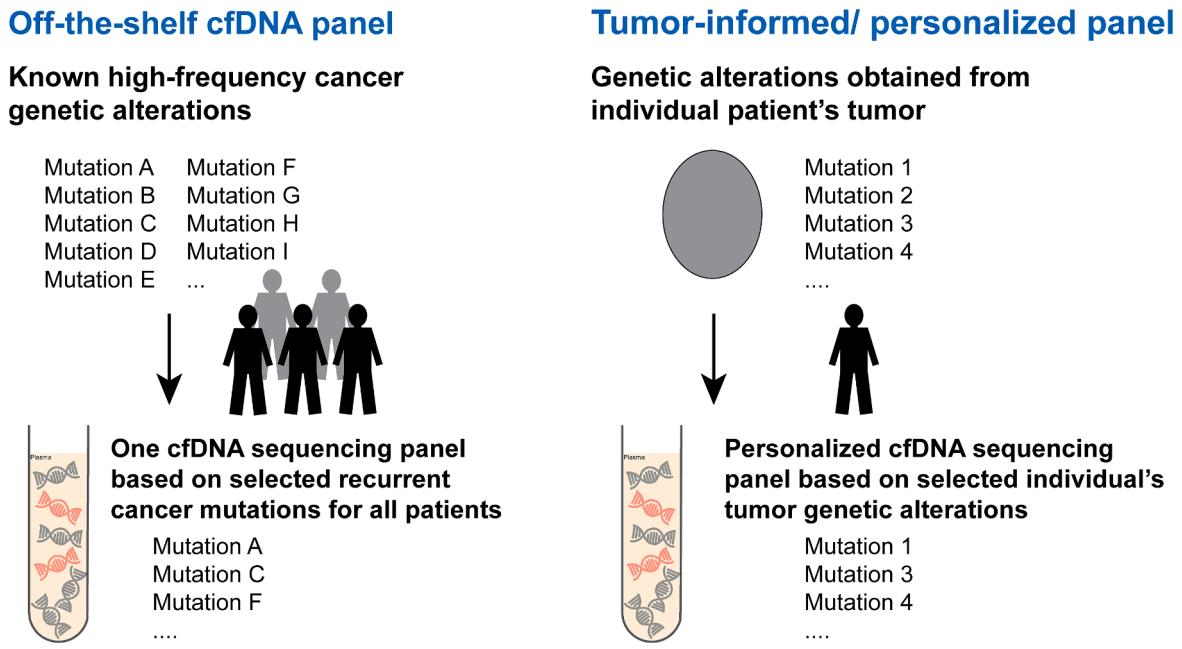


Fig. 2. Sequencing panels for the analysis of circulating cell-free (cf)DNA. Off-the-shelf cfDNA panels are designed based on known high-frequency cancer genetic alterations. Such panels focus on the analysis of commonly altered cancer-related genes, and the same cfDNA sequencing panel is used for all patients. In contrast, tumor-informed, personalized or also called bespoke cfDNA panels are designed based on an individual's tumor-specific genetic alterations. First, sequencing of a patient's tumor is performed; from the identified tumor genetic alterations a tumor-informed/ personalized cfDNA sequencing panel is designed. Thus, every patient has a unique personalized cfDNA assay.

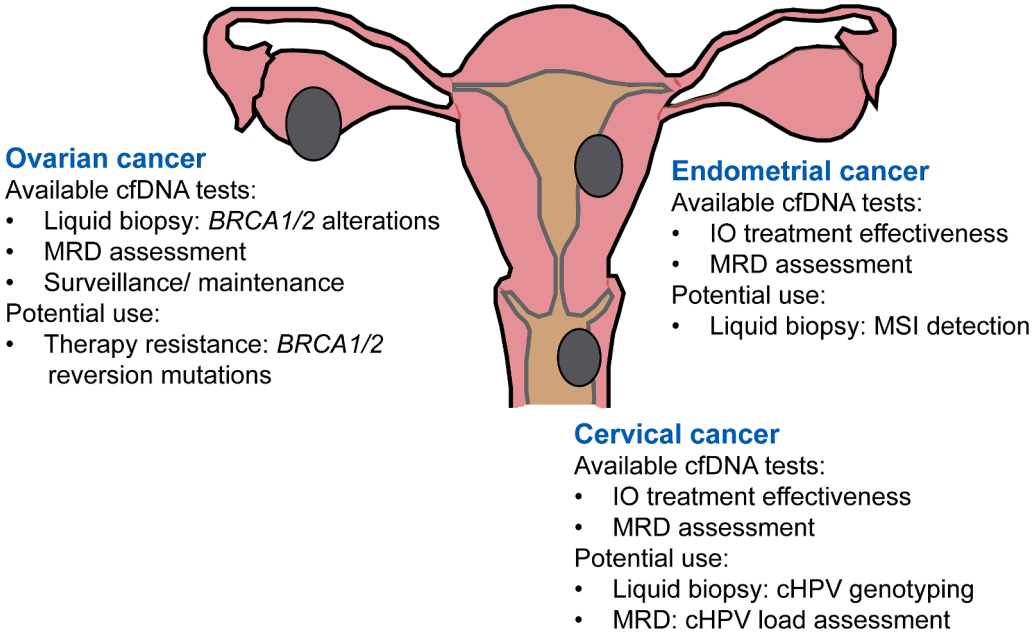


Fig. 3. Current and potential clinical applications of circulating cell-free (cf)DNA analysis in gynecologic cancers. The analysis of cfDNA remains an active area of investigation in gynecologic cancer. In ovarian cancer, cfDNA or liquid biopsies can be analyzed to detect *BRCA1/2* alterations and resistance mechanisms to systemic therapy. In addition to somatic mutations, microsatellite instability (MSI) can be detected in cfDNA of endometrial cancer patients and circulating HPV DNA (cHPV) can be measured and genotyped in the plasma of cervical cancer patients. In both endometrial and cervical cancer, immunotherapy (IO) effectiveness can be assessed in cfDNA-based assays. In ovarian, endometrial and cervical cancer, monitoring of cfDNA as a measure of minimal/ molecular residual disease (MRD) has been demonstrated and has potential implications in treatment selection, escalation and/ or de-escalation.

cancer (Razavi et al., 2019). CH mutations are similar to those found in hematologic cancers and other cancer types, including gynecologic cancers, and most commonly affect *DNMT3A*, *JAK2*, *TP53*, *TET2*, *ASXL1* and *SF3B1* (Hu et al., 2018; Razavi et al., 2019). These hematopoietic cell-derived CH-related mutations in cfDNA could disguise as tumor-derived, and their incorrect classification as tumor-associated ctDNA may have consequences for patient management. It is therefore important to restrict the mutation analysis in cfDNA to those alterations also present in the matched tumor tissue of the same patient (i.e., genotyping) and/ or to perform matched cfDNA - white blood cell sequencing for

accurate variant interpretation and discrimination between CH– and tumor-derived mutations (Hu et al., 2018). Furthermore, tagging of individual DNA molecules with unique identifiers (i.e., molecular barcoding) coupled with sophisticated computational tools can also be employed to identify and suppress recurrent background errors (Abbosh et al., 2019). More recently, multi-modal or multi-analyte cfDNA assays are being developed which combine genetic and epigenetic methylation analyses with protein tumor markers and/or viral genomes amongst others (Alix-Panabieres and Pantel, 2021), which may lead to improved sensitivity and specificity.

3. cfDNA analysis in clinical decision-making

The analysis of cfDNA is rapidly evolving in the field of oncology, with a number of already approved tests (Table 1) as well as promising clinical applications and advantages over traditional cancer screening tools and diagnostic tests. As a liquid biopsy, ctDNA levels can be quantified in plasma using a blood sample, avoiding invasive tissue biopsies. Furthermore, while tissue biopsies provide a single snapshot of the tumor, cfDNA sequencing can detect the entire repertoire of somatic genetic alterations found in primary tumors and metastatic disease in one test, thus accounting for intra- and inter-tumoral heterogeneity (De Mattos-Arruda et al., 2014), can detect multiple cancers in a single patient (Zhang et al., 2024), and can be more frequently repeated for monitoring purposes.

In oncology cfDNA research, lung, breast and colon cancer remain the most well-studied cancer types to date, and not surprisingly, are the most active in clinical trials (Zhang et al., 2024). In gynecologic cancer, published cfDNA focused research studies and clinical trials are more limited (see below). The first liquid biopsy test was approved by the Food and Drug Administration (FDA) in 2016 in non-small cell lung cancer (NSCLC) to detect epidermal growth factor receptor (*EGFR*) gene mutations as a companion diagnostic (CDx) for EGFR-specific tyrosine kinase inhibitors (Kwapisz, 2017). To date, FDA-approved liquid biopsy CDx tests are available beyond NSCLC, including for breast, ovarian cancer, prostate and colon cancer as well as tumor type agnostic tests (Table 1). These CDx tests are designed to provide cfDNA-based assessment of specific genetic alterations that serve as biomarkers for the selection of specific targeted therapies. Of importance for ovarian cancer, a CDx liquid biopsy test is available for the detection of *BRCA1* and *BRCA2* mutations, which is a companion diagnostic to identify patients who may benefit from treatment with poly ADP ribose polymerase (PARP) inhibitors (Medicine, 2021) (Table 1).

Another clinical application that has emerged is cfDNA analysis in the setting of minimal/ molecular residual disease (MRD) detection. MRD refers to tumor cells remaining in the body after surgery and/or systemic treatment. Landmark analysis is the identification of MRD through cfDNA analysis at one defined time point shortly after curative therapy and is closely associated with recurrence risk across solid tumor types (Moding et al., 2021; Christie et al., 2017). MRD detection can therefore be employed to guide surveillance strategies and adjuvant therapy decision-making for those patients at highest risk of relapse. In addition, the detection of ctDNA in plasma at the time of diagnosis has been shown to be prognostic in many disease types and proportionate to disease burden in a given patient (Bettegowda et al., 2014; Moding et al., 2021; Han et al., 2024). Furthermore, there is evidence to suggest that incorporating ctDNA detection into anatomical staging may help refine the prognostic value of initial staging, as recently shown in patients with NSCLC (Yang et al., 2018).

In addition to landmark analyses, dynamic measurements of MRD through the assessment of multiple post-treatment blood draws over time are used for disease monitoring and the early detection of disease recurrence across cancer types. Importantly, using such serial cfDNA analysis has been shown to detect recurrence of molecular relapse with occasionally significant lead times of many months over clinical or radiological detection in different cancer types (Garcia-Murillas et al., 2015; Tie et al., 2016; Chaudhuri et al., 2017; Hou et al., 2022). cfDNA-based MRD detection has been shown to be more sensitive than existing blood-based biomarkers and thus has the potential to serve as a biomarker particularly for those disease types in which blood markers are not available. In the neoadjuvant setting, the detection of ctDNA following systemic therapy is associated with decreased progression-free survival (PFS) and overall survival (OS) (Magbanua et al., 2023). Although commercial assays are offering cfDNA-based tests for MRD assessment, clinical studies are required to define at what time point of an observed change in MRD/ levels of ctDNA to escalate or de-escalate treatment. Also, it has yet to be demonstrated that early disease interception, meaning the treatment of disease following early cfDNA/cfDNA detection, has a favorable impact on prognosis.

The assessment of serial cfDNA samples during or after adjuvant treatment can also provide a means for real-time monitoring of resistance mechanisms to guide treatment decisions. During systemic treatment, cancers may acquire resistance mutations. For example, serial cfDNA analysis has been employed for the detection of *KRAS* mutations in plasma during anti-EGFR therapy in initially *KRAS* wild-type colorectal cancers, a known mechanism of acquired resistance (Diaz et al., 2012; Misale et al., 2012). Similarly, surveillance of the emergence of *ESR1* mutations in liquid biopsies of estrogen receptor-positive breast cancer patients as a mechanism of resistance to aromatase inhibitors has been reported (De Santo et al., 2019).

Effective screening modalities are currently available for only a subset of cancers. Based on the findings that the detection of tumor mutations in plasma can at times be more sensitive than existing biomarkers and can have lead time over imaging, there has been great interest in the development and clinical application of cfDNA analysis for early detection (Batool et al., 2023). The advantages of early detection are obvious and multiple, increasing the window of intervention and thereby improving clinical outcomes. The small amounts of tumor-derived DNA in plasma of pre-invasive or early-stage cancers coupled with the LODs of current technologies, however, has proven challenging. Currently, the only FDA-approved plasma-based screening test is for colon cancer, with a sensitivity of 83.1 % in the detection of colorectal cancer, however of only 13.2 % for detection of precancerous lesions (Chung et al., 2024). Efforts are ongoing for the realization of plasma-based screening for commonly occurring cancers, which has the potential to improve compliance with screening, decrease health disparities and improve access on a global level (Medina et al., 2023).

4. Role of cfDNA analysis in gynecologic cancer

Unlike in other solid malignancies, where cfDNA analysis has been implemented as a clinical biomarker, in gynecologic oncology, liquid biopsy marker studies are generally at an earlier phase of investigation (Fig. 3). Given the increased understanding of the molecular landscape of gynecologic cancers and molecular markers associated with outcome, coupled with the lack of specific biomarkers and screening modalities, cfDNA analysis has the potential to impact patient care. Results of currently ongoing clinical trials are eagerly awaited.

4.1. Ovarian cancer

Consistent with observations in other cancer types, cfDNA levels in ovarian cancer patients have been shown to correlate with stage, disease burden, CA-125 levels and computed tomography (CT) findings (Hou et al., 2022). Earlier studies demonstrated that tumor-derived *TP53* mutations could be found in matched blood samples, correlated with disease burden and time to progression following chemotherapy (Parkinson et al., 2016). Circulating DNA methylation levels of *CDH1, RASSF2A* and *BRCA1* have been studied as potential screening biomarkers in the early detection of ovarian cancer (Dvorska et al., 2019; Giannopoulou et al., 2017; Ibanez de Caceres et al., 2004), with most studies yielding relatively low sensitivities but high specificities of

around 90 %, suggesting this could be used in conjunction with other available tests, such as CA125 levels and imaging. While the practicality in the clinical setting remains up for debate, the presence of mutations in ctDNA can predict early relapse, at lower thresholds than existing biomarkers, clinical or radiographic studies (Garcia-Murillas et al., 2015).

A cfDNA analysis application of interest in high-grade serous ovarian cancer involves the identification of resistance mechanisms. In patients with germline *BRCA1* or *BRCA2* mutations with platinum-resistant/ refractory ovarian cancer, NGS of cfDNA revealed *BRCA1/2* reversion mutations, a known mechanism of resistance to platinum agents and PARP inhibitors (Christie et al., 2017; Weigelt et al., 2017). The detection of *BRCA1/2* reversion mutations in cfDNA were then shown to predict primary and acquired resistance to PARP inhibition in patients with high-grade serous ovarian cancer (Lin et al., 2019).

For patients with ovarian cancer, a liquid biopsy test to detect *BRCA1* or *BRCA2* mutations in plasma for PARP inhibitor treatment is available for clinical use (FoundationOne Liquid CDx (Medicine, 2021; US Food Drug Administration, 2023); Table 1). In addition, commercial cfDNA tests for MRD assessment to identify high-risk ovarian cancer patients benefiting from additional treatment, for surveillance/ maintenance for early recurrence detection, and/or for immunotherapy treatment effectiveness assessment are now available (e.g., from Natera, Invitae, Northstar, Foundation Medicine, and Guardant Health). It should be noted, however, that some of these tests are solid tumor type agnostic and that the time points and types of therapeutic interventions upon changes in cfDNA in the plasma of ovarian cancer patients are yet to be defined.

There are currently several active prospective clinical trials assessing cfDNA analysis in epithelial ovarian cancers (NIH National Library of Medicine, 2024), focusing primarily on early detection (e.g., NCT06249308, NCT05693987) and MRD detection and treatment response (e.g., NCT03691012, NCT06071286).

4.2. Endometrial cancer

Endometrial cancer is one of the few common cancer types with both an increase in incidence and in mortality (Siegel et al., 2024). Blood-based biomarkers such as CA-125 are of limited utility, making cfDNA analysis an attractive potential biomarker for disease monitoring and prognostication. Furthermore, while stage I disease carries a favorable prognosis, different pathologic and molecular factors are associated with variable PFS. cfDNA analysis for MRD in this setting may aide in determining which patients would benefit most from adjuvant therapy. However, unlike in ovarian cancer, distant metastatic disease is less common in patients with endometrial cancer, resulting in decreased tumor-derived genetic material shed into the blood stream, posing a challenge to ctDNA detection.

Preliminary studies demonstrated detection of cfDNA primarily in endometrial cancer patients with high-risk disease (Feng et al., 2021; Cicchillitti et al., 2017), and NGS of plasma detected hotspot mutations in 33 % of patients at the time of hysterectomy (Bolivar et al., 2019). In a recent proof of principle study, cfDNA levels correlated with stage, and serial measurements of ctDNA reflected response to treatment, disease progression and recurrence. Somatic mutations in the tumor were accurately identified in cfDNA in over 90 % of cases and the presence of ctDNA at baseline or postoperatively was found to be significantly associated with reduced PFS (Ashley et al., 2023). Furthermore, recent studies have reported on the detection of microsatellite instability (MSI) in uterine aspirates and cfDNA as a means of minimally invasive subtyping of endometrial cancers and to monitor response to immune checkpoint inhibition (Casas-Arozamena et al., 2023; Manning-Geist et al., 2022).

The current landscape of adjuvant treatment for endometrial cancer is a moving target. Different clinical, histopathological and now molecular features are incorporated in the staging system, used to determine prognosis and aide with patient selection for adjuvant therapy. A potentially meaningful clinical application of cfDNA analysis is adjuvant treatment stratification through post-surgical monitoring of cfDNA as a measure of MRD. Recio and colleagues analyzed the post-surgical ctDNA in 101 patients with uterine malignancies, and consistent with previous findings, those patients with higher risk histologic types, were more likely to have detectable ctDNA following surgery. Importantly, after adjusting for histologic type, mismatch repair (MMR) and p53 status, detectable ctDNA or MRD following surgery was the only significant risk factor for recurrence (Recio et al., 2024). Solid tumor type-agnostic and gynecologic cancer-specific cfDNA-based assays for MRD assessment and/or for immunotherapy treatment effectiveness are now available for endometrial cancer patients (e.g., from Natera, Invitae, Northstar, Foundation Medicine, and Guardant Health). Clinical trials are currently ongoing investigating cf/ctDNA analysis in endometrial cancer (NIH National Library of Medicine, 2024), however, assessing early detection (e.g., NCT06083779) and prognosis/ prediction (e.g., NCT05049538, NCT05504161).

4.3. Cervical cancer

Despite the widespread availability of cervical cancer screening with cytology and human papillomavirus (HPV) testing and the HPV vaccine, the rates of cervical cancer in the United States, while they have decreased substantially since the 1970s, have remained largely unchanged in the past decade (Siegel et al., 2024). This may be in part attributed to lack of access and/ or lack of compliance with screening, insufficient patient education regarding cervical cancer and the uptake and effectiveness of the HPV vaccine. As infection with high-risk HPV is associated with the majority of cervical cancers, HPV cfDNA in cervical cancer offers additional opportunities for the development of biomarkers for diagnosis and disease monitoring, as the presence, type and load of HPV can be inferred in cfDNA (Kang et al., 2017; Jeannot et al., 2016). Furthermore, the ability to acquire liquid biopsies to correctly identify HPV genotypes has implications for cancer-directed immunotherapy through targeting of HPV oncoproteins (Kang et al., 2017; Jeannot et al., 2016). A recent study demonstrated the independent association of persistent HPV ctDNA following chemoradiation with inferior PFS, suggesting consideration for escalation of treatment in these patients at higher risk for recurrence (Han et al., 2024). For all solid tumors, including cervical cancer, cfDNA for MRD/ disease-monitoring and monitoring of response to immune-checkpoint inhibitor therapy are commercially available (see above). Akin to endometrial cancer, there are only a few active clinical trials in cervical cancer currently ongoing (National Library of Medicine, 2024), primarily assessing cfHPV DNA as a potential biomarker (e.g., NCT05606133, NCT05950087) (Fig. 3).

5. Conclusion

Recent studies using higher sensitivity technologies and advanced bioinformatics methods have shown encouraging potential of cfDNA analysis in gynecologic cancer, including MRD detection for treatment escalation and/or de-escalation, early disease recurrence detection, and assessment of immunotherapy effectiveness. In addition, in ovarian cancer, liquid biopsies are used for the detection of *BRCA1/2* genetic alterations and of resistance mechanisms to systemic therapy. In endometrial cancer, cfDNA sequencing has been employed for somatic mutation and MSI detection, whereas in cervical cancer patients, cHPV measurement and genotyping in plasma is of great interest. While cfDNA assays are now available for clinical use in patients with gynecologic cancer, the ongoing clinical trials will provide further information on the clinical applications and effectiveness of cfDNA analyses.

CRediT authorship contribution statement

Sarah H Kim: Writing – review & editing, Writing – original draft,

Investigation, Conceptualization. **Britta Weigelt:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization.

Conflict of interest statement

B. Weigelt reports research funding from REPARE Therapeutics, and employment of an immediate family member at AstraZeneca.

Acknowledgments

This work was supported in part by a Cancer Center Support Grant of the NIH/NCI (Grant No. P30CA008748). B. Weigelt is funded in part by Breast Cancer Research Foundation and Cycle for Survival grants.

References

Abbosh, C., Swanton, C., Birkbak, N.J., 2019. Clonal haematopoiesis: a source of biological noise in cell-free DNA analyses. Ann Oncol. 30, 358–359.
Alix-Panabieres, C., Pantel, K., 2021. Liquid Biopsy: From Discovery to Clinical Application. Cancer Discov. 11, 858–873.
Ashley, C.W., Selenica, P., Patel, J., Wu, M., Nincevic, J., Lakhman, Y., et al., 2023. High-Sensitivity Mutation Analysis of Cell-Free DNA for Disease Monitoring in Endometrial Cancer. Clin Cancer Res. 29, 410–421.
Batool, S.M., Yekula, A., Khanna, P., Hsia, T., Gamblin, A.S., Ekanayake, E., et al., 2023. The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring. Cell Rep Med. 4, 101198.
Berger, M.F., Mardis, E.R., 2018. The emerging clinical relevance of genomics in cancer medicine. Nat Rev Clin Oncol. 15, 353–365.
Bettegowda, C., Sausen, M., Leary, R.J., Kinde, I., Wang, Y., Agrawal, N., et al., 2014. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 6, 224ra24.
Bolivar, A.M., Luthra, R., Mehrotra, M., Chen, W., Barkoh, B.A., Hu, P., et al., 2019. Targeted next-generation sequencing of endometrial cancer and matched circulating tumor DNA: identification of plasma-based, tumor-associated mutations in early stage patients. Mod Pathol. 32, 405–414.
Buttigieg, M.M., Rauh, M.J., 2023. Clonal Hematopoiesis: Updates and Implications at the Solid Tumor-Immune Interface. JCO Precis Oncol. 7, e2300132.
Casas-Arozamena, C., Moiola, C.P., Vilar, A., Bouso, M., Cueva, J., Cabrera, S., et al., 2023. Noninvasive detection of microsatellite instability in patients with endometrial cancer. Int J Cancer. 152, 2206–2217.
Chaudhuri, A.A., Chabon, J.J., Lovejoy, A.F., Newman, A.M., Stehr, H., Azad, T.D., et al., 2017. Early Detection of Molecular Residual Disease in Localized Lung Cancer by Circulating Tumor DNA Profiling. Cancer Discov. 7, 1394–1403.
Christie, E.L., Fereday, S., Doig, K., Pattnaik, S., Dawson, S.J., Bowtell, D.D.L., 2017. Reversion of BRCA1/2 Germline Mutations Detected in Circulating Tumor DNA From Patients With High-Grade Serous Ovarian Cancer. J Clin Oncol. 35, 1274–1280.
Chung, D.C., Gray 2nd, D.M., Singh, H., Issaka, R.B., Raymond, V.M., Eagle, C., et al., 2024. A Cell-free DNA Blood-Based Test for Colorectal Cancer Screening. N Engl J Med. 390, 973–983.
Cicchillitti, L., Corrado, G., De Angeli, M., Mancini, E., Baiocco, E., Patrizi, L., et al., 2017. Circulating cell-free DNA content as blood based biomarker in endometrial cancer. Oncotarget. 8, 115230–115243.
Crowley, E., Di Nicolantonio, F., Loupakis, F., Bardelli, A., 2013. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol. 10, 472–484.
De Mattos-Arruda, L., Weigelt, B., Cortes, J., Won, H.H., Ng, C.K., Nuciforo, P., et al., 2014. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. Ann Oncol. 25, 1729–1735.
De Santo, I., McCartney, A., Migliaccio, I., Di Leo, A., Malorni, L., 2019. The Emerging Role of ESR1 Mutations in Luminal Breast Cancer as a Prognostic and Predictive Biomarker of Response to Endocrine Therapy. Cancers (basel). 11.
Diaz Jr., L.A., Williams, R.T., Wu, J., Kinde, I., Hecht, J.R., Berlin, J., et al., 2012. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 486, 537–540.
Dvorska, D., Brany, D., Nagy, B., Grendar, M., Poka, R., Soltesz, B., et al., 2019. Aberrant Methylation Status of Tumour Suppressor Genes in Ovarian Cancer Tissue and Paired Plasma Samples. Int J Mol Sci. 20.
Feng, W., Jia, N., Jiao, H., Chen, J., Chen, Y., Zhang, Y., et al., 2021. Circulating tumor DNA as a prognostic marker in high-risk endometrial cancer. J Transl Med. 19, 51.
Gaitsch, H., Franklin, R.J.M., Reich, D.S., 2023. Cell-free DNA-based liquid biopsies in neurology. Brain. 146, 1758–1774.
Garcia-Murillas, I., Schiavon, G., Weigelt, B., Ng, C., Hrebien, S., Cutts, R.J., et al., 2015. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med. 7, 302ra133.
Giannopoulou, L., Chebouti, I., Pavlakis, K., Kasimir-Bauer, S., Lianidou, E.S., 2017. RASSF1A promoter methylation in high-grade serous ovarian cancer: A direct comparison study in primary tumors, adjacent morphologically tumor cell-free tissues and paired circulating tumor DNA. Oncotarget. 8, 21429–21443.
Han, K., Zou, J., Zhao, Z., Baskurt, Z., Zheng, Y., Barnes, E., et al., 2024. Clinical Validation of Human Papilloma Virus Circulating Tumor DNA for Early Detection of Residual Disease After Chemoradiation in Cervical Cancer. J Clin Oncol. 42, 431–440.
Hou, J.Y., Chapman, J.S., Kalashnikova, E., Pierson, W., Smith-McCune, K., Pineda, G., et al., 2022. Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer. Gynecol Oncol. 167, 334–341.
Hu, Y., Ulrich, B.C., Supplee, J., Kuang, Y., Lizotte, P.H., Feeney, N.B., et al., 2018. False-Positive Plasma Genotyping Due to Clonal Hematopoiesis. Clin Cancer Res. 24, 4437–4443.
Ibanez de Caceres, I., Battagli, C., Esteller, M., Herman, J.G., Dulaimi, E., Edelson, M.I., et al., 2004. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. Cancer Res. 64, 6476–6481.
Jeannot, E., Becette, V., Campitelli, M., Calmejane, M.A., Lappartient, E., Ruff, E., et al., 2016. Circulating human papillomavirus DNA detected using droplet digital PCR in the serum of patients diagnosed with early stage human papillomavirus-associated invasive carcinoma. The Journal of Pathology Clinical Research. 2, 201–209.
Kang, Z., Stevanovic, S., Hinrichs, C.S., Cao, L., 2017. Circulating Cell-free DNA for Metastatic Cervical Cancer Detection, Genotyping, and Monitoring. Clin Cancer Res. 23, 6856–6862.
Kurtz, D.M., Soo, J., Co Ting Keh, L., Alig, S., Chabon, J.J., Sworder, B.J., et al., 2021. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. Nat Biotechnol. 39, 1537–1547.
Kwapisz, D., 2017. The First Liquid Biopsy Test Approved. Is It a New Era of Mutation Testing for Non-Small Cell Lung Cancer? Annals of Translational Medicine. 5, 46.
Lin, K.K., Harrell, M.I., Oza, A.M., Oaknin, A., Ray-Coquard, I., Tinker, A.V., et al., 2019. BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma. Cancer Discov. 9, 210–219.
Lui, Y.Y., Chik, K.W., Chiu, R.W., Ho, C.Y., Lam, C.W., Lo, Y.M., 2002. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. Clin Chem. 48, 421–427.
Magbanua, M.J.M., Brown Swigart, L., Ahmed, Z., Sayaman, R.W., Renner, D., Kalashnikova, E., et al., 2023. Clinical significance and biology of circulating tumor DNA in high-risk early-stage HER2-negative breast cancer receiving neoadjuvant chemotherapy. Cancer Cell. 41 (1091–102), e4.
Manning-Geist BP, J.A.; Marra, A.; Da Cruz Paula, A.; Hanlon, E.J.; Abu-Rustum, N.; Shah, R.H.; Beger, M.F.; Hensley, M.L.; Zamarin, D.; Weigelt, B.; Friedman, C.F. Cell-free DNA analysis as a molecular tool to monitor response to immune checkpoint inhibition in endometrial cancer. American Society of Clinical Oncology: Journal of Clinical Oncology; 2022.
Meddeb, R., Dache, Z.A.A., Thezenas, S., Otandault, A., Tanos, R., Pastor, B., et al., 2019. Quantifying circulating cell-free DNA in humans. Sci Rep. 9, 5220.
Foundation Medicine. FoundationOne Liquid CDx. URL: https://www.foundationmedicine.com/sites/default/files/document/F1LCDx_SS.pdf. 2021.
Medina, J.E., Dracopoli, N.C., Bach, P.B., Lau, A., Scharpf, R.B., Meijer, G.A., et al., 2023. Cell-free DNA approaches for cancer early detection and interception. J Immunother Cancer. 11.
Misale, S., Yaeger, R., Hobor, S., Scala, E., Janakiraman, M., Liska, D., et al., 2012. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 486, 532–536.
Moding, E.J., Nabet, B.Y., Alizadeh, A.A., Diehn, M., 2021. Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease. Cancer Discov. 11, 2968–2986.
NIH National Library of Medicine. ClinicalTrials.gov. URL: http://clinicaltrials.gov/. 2024.
Parkinson, C.A., Gale, D., Piskorz, A.M., Biggs, H., Hodgkin, C., Addley, H., et al., 2016. 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. 13, e1002198.
Razavi, P., Li, B.T., Brown, D.N., Jung, B., Hubbell, E., Shen, R., et al., 2019. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med. 25, 1928–1937.
Recio, F., Scalise, C.B., Loar, P., Lumish, M., Berman, T., Peddada, A., et al., 2024. Post-surgical ctDNA-based molecular residual disease detection in patients with stage I uterine malignancies. Gynecol Oncol. 182, 63–69.
Siegel, R.L., Giaquinto, A.N., Jemal, A., 2024. Cancer statistics, 2024. CA Cancer J Clin. 74, 12–49.
Tie, J., Wang, Y., Tomasetti, C., Li, L., Springer, S., Kinde, I., et al., 2016. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 8, 346ra92.
Udomruk, S., Orrapin, S., Pruksakorn, D., Chaiyawat, P., 2021. Size distribution of cell-free DNA in oncology. Crit Rev Oncol Hematol. 166, 103455.
Underhill, H.R., 2021. Leveraging the Fragment Length of Circulating Tumour DNA to Improve Molecular Profiling of Solid Tumour Malignancies with Next-Generation Sequencing: A Pathway to Advanced Non-invasive Diagnostics in Precision Oncology? Mol Diagn Ther. 25, 389–408.
US Food & Drug Administration. List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools). URL: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools. 2023.
Weigelt, B., Comino-Mendez, I., de Bruijn, I., Tian, L., Meisel, J.L., Garcia-Murillas, I., et al., 2017. Diverse BRCA1 and BRCA2 Reversion Mutations in Circulating Cell-Free

DNA of Therapy-Resistant Breast or Ovarian Cancer. Clin Cancer Res. 23, 6708–6720.
Yang, M., Forbes, M.E., Bitting, R.L., O'Neill, S.S., Chou, P.C., Topaloglu, U., et al., 2018. Incorporating blood-based liquid biopsy information into cancer staging: time for a TNMB system? Ann Oncol. 29, 311–323.
Zhang, K., Fu, R., Liu, R., Su, Z., 2024. Circulating cell-free DNA-based multi-cancer early detection. Trends Cancer. 10, 161–174.