The Advent of Circulating Tumor DNA in the Management of Ovarian Cancer

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Abstract

Genomically actionable mutations are increasingly used to deliver personalized medical care for patients with ovarian cancer (OC). Liquid biopsy applications encompass the identification and study of circulating tumor DNA (ctDNA), cell-free DNA, circulating tumor cells, and sometimes circulating miRNAs. In the current practice, ctDNA is mostly utilized. The multiple clinical applications of liquid biopsy in oncology have facilitated the implementation of precision medicine in practice. Though not still ready for clinical use in OC daily practice, the use of liquid biopsy in the experimental setting has revolutionized the study of the mechanisms of carcinogenesis and treatment resistance underlying the clinical disease progression. Moreover, as a minimally invasive approach, liquid biopsy can be used to predict response to antineoplastic therapies, including standard platinum-based chemotherapy regimens and PARP inhibitors. In addition, liquid biopsy can also be used in OC to predict recurrence, inform on the prognosis and anticipate clinical progression-free survival events. In this chapter, the clinical relevance and utility of blood-based ctDNA in OC are reviewed.

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Keywords

Ovarian cancer · Liquid biopsy · Circulating tumor DNA · Outcomes

5.1 Introduction

Ovarian tumor components such as circulating tumor cells (CTCs) (Romero-Laorden et al. 2014), circulating tumor DNA (ctDNA) (Esposito et al. 2014), microRNAs (Wang et al. 2017), and cell–cell communicating exosomes, which are nano-sized vesicles containing nucleic acids and proteins (Li and Wang 2017), can be released into the bloodstream during tumor apoptosis, necrosis, and metastatic spread. Noninvasive quantitative and qualitative assessment of these tumors highly informative "*gold constituents*" may be accomplished with the advent of highly sensitive technologies such as digital polymerase chain reaction (PCR) and next-generation sequencing (NGS) platforms (Zhang et al. 2017) as well as the FDA-approved CellSearch® immuno-magnetic system for CTCs detection and characterization (Sun et al. 2018). This field of oncology is rapidly evolving and has literally experienced an *explosion* of liquid biopsy studies. Ovarian cancer (OC) is a particularly suitable and an ideal candidate for liquid biopsy: first, it sheds a higher quantity of tumor materials in the bloodstream; then, a significant proportion of women can experience a tumor recurrence after the primary treatments and/or tumor progression, after the initial systemic chemotherapy. Therefore, the clinical OC setting recalls the need to identify biomarkers of prognosis, early recurrence and prediction of treatment sensitivity. With the emerging precision oncology, ctDNA-based approaches have provided considerable and actionable data for the development of tools for (a) early detection, (b) real-time and longitudinal monitoring of therapy response, (c) detection of residual disease and recurrence, and (d) study of tumor heterogeneity (Steffensen et al. 2014; Wan et al. 2017; El Bairi et al. 2017a, b; Van Berckelaer et al. 2016) (Fig. 5.1). In this chapter, the advent of ctDNA in OC is reviewed based on several recent developments.

5.2 Circulating Tumor DNA as a Biomarker in Ovarian Cancer

Several recent human trials investigated the clinical value of ctDNA in OC (Table 5.1). Pereira et al. examined the role of ctDNA as a prognostic biomarker in 22 women with OC at the time of surgery and throughout the treatment course, using digital PCR and NGS to identify relevant mutations (Pereira et al. 2015). Notably, this study detected ctDNA in 93.8% of patients comparing it with computed tomography scan findings and CA-125 marker results. Moreover, ctDNA after 6 months of adjuvant treatment was found undetectable and associated with better PFS and OS ($p = 0.0011$ and $p = 0.0194$, respectively) suggesting its potential impact as a prognostic biomarker for disease recurrence and survival rate (Pereira et al. 2015). However, the prognostic value of ctDNA seems to be limited by

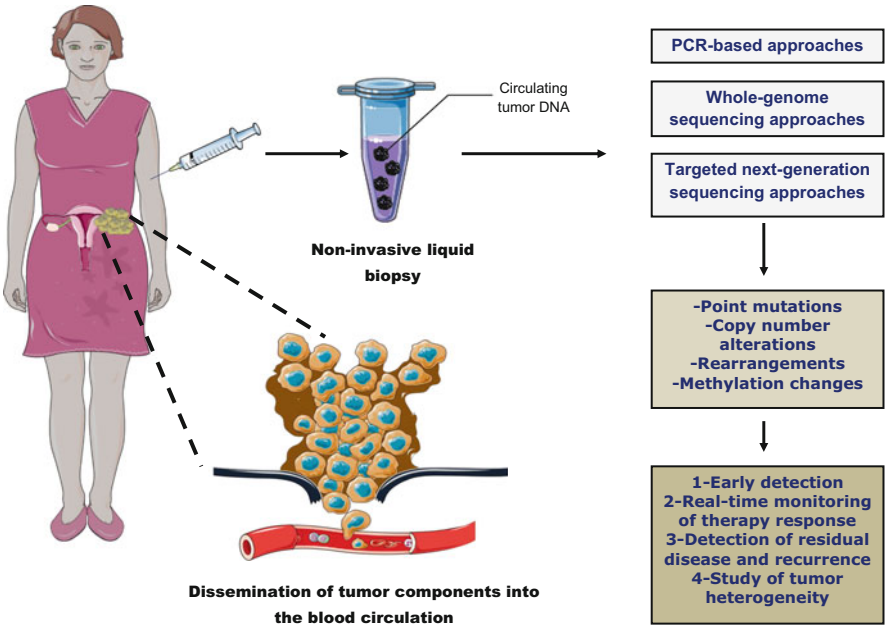

Fig. 5.1 Dissemination of tumor components into the blood circulation

the lack of sufficient data on its correlation with tumor size and stage. The ctDNA based analysis has revealed that mutated *TP53* was the most prevalent gene alteration, followed by low frequent mutations in *PTEN*, *PIK3CA*, *MET*, *KRAS*, *FBXW7*, and *BRAF* genes in patients with high-grade serous tumors (Pereira et al. 2015). To date, the rich source of data regarding initial *TP53* mutations revealed its important driver role in basal-like breast cancers and OC (reviewed elsewhere: Silwal-Pandit et al. 2017). Notably, OC patients with mutated *TP53* appear to have better survival and to be sensitive to chemotherapy (Leijen et al. 2016; Wong et al. 2013). In a retrospective analysis, Parkinson et al. found that decreased *TP53* mutant allele fraction (>6%) in ctDNA is an independent predictive biomarker for time-to-progression endpoint (TTP) (HR: 0.22, 95% CI: 0.07–0.67, $p = 0.008$) in relapsed high-grade serous OC (Parkinson et al. 2016). Furthermore, ctDNA levels were strongly correlated with the total volume of disease ($p < 0.001$) compared to the gold-standard CA-125 biomarker. Likewise, a significant correlation between mutated *TP53* in ctDNA and CA-125 ($p < 0.001$) was reported, thus suggesting its possible use as a highly specific biomarker to predict platinum-based treatment response (Parkinson et al. 2016). Recently, a large study enrolling 121 OC patients demonstrated that the NGS-based detection of somatic and germline *BRCA* mutations in ctDNA is feasible when the standard diagnostic testing is not satisfactory (Ratajska et al. 2017). *BRCA* reversion mutations (also known as *back mutations*) are a mechanism that may explain the acquired resistance to platinum

Table 5.1 Impact of circulating tumor DNA in ovarian cancer management (data from the last 5 years)

Author/year	No. of patients (histology)	Clinical impact	Genetic alteration	Study technique
Rusan et al. (2020)	32 (HGSOC[β])	Response to PARP inhibitors	*HOXA9* methylation	In-house digital droplet PCR
Noguchi et al. (2020)	51 (miscellaneous)	Prediction of progression-free survival (PFS)	Somatic mutations in *TP53, APC, KRAS, EGFR, MET, PIK3CA, NPAP1,* and *ALK*	Illumina NextSeq 500
Ogasawara et al. (2020)	255 (miscellaneous)	Prediction of recurrence	Somatic *PIK3CA* and/or *KRAS* mutations	Digital droplet PCR
Alves et al. (2020)	11 (miscellaneous)	Prediction of disease-free survival	–	Quantitative real-time PCR
Lin et al. (2018)	112 (HGSOC)	Response to PARP inhibitors	Reversion*BRCA1/2* mutations	Guardant360 assay (Illumina HiSeq)
Slavin et al. (2018)	2010	Identification of incidental germline mutations	Variants in 16[†] genes associated with hereditary cancers	Guardant360 assay (Illumina HiSeq)
Giannopoulou et al. (2018)	50 (HGSOC)[¥]	Prediction of overall survival (OS) and PFS	*ESR1* methylation	Real-time methylation-specific PCR
Christie et al. (2017)	30 (HGSOC)	Therapy response	Reversion *BRCA*1/2 mutations	Targeted sequencing (Illumina MiSeq)
Widschwendter et al. (2017)	151 (miscellaneous)	Early detection and therapy response	DNAme-Marker Panel	Bisulfite sequencing (Illumina MiSeq/HiSeq 2500)
Ratajska et al. (2017)	121 (HGSOC; 72%)	Monitoring of PARP inhibition	*BRCA*1/2	Next-generation sequencing (Illumina)
Parkinson et al. (2016)	40 (HGSOC)	Therapy response	*TP53*	Digital PCR
Harris et al. (2016)	10 (HGSOC)	Therapy response and relapse monitoring	Somatic chromosomal rearrangements	Next-generation sequencing (Illumina HiSeq 2000) and qPCR
Pereira et al. (2015)	22 (21 HGSOC and 1 mixed mesodermal tumor)	Therapy response and survival	*TP53* and other low frequent mutated genes[‡]	Next-generation sequencing (Illumina HiSeq 2500 and Ion

(continued)

Table 5.1 (continued)

Author/year	No. of patients (histology)	Clinical impact	Genetic alteration	Study technique
				Torrent PGM-Ion AmpliSeq™ Cancer Hotspot Panel v2) and digital PCR

[β]including four patients with non-serous tumors. [†]APC, ATM, BRCA1, BRCA2, CDKN2A, KIT, MLH1, NF1, PTEN, RB1, RET, SMAD4, STK11, TP53, TSC1, and VHL. [‡]PTEN, PIK3CA, MET, KRAS, FBXW7, and BRAF. Abbreviations: *ALK* anaplastic lymphoma kinase, *APC* adenomatous polyposis coli, *BRCA* breast cancer gene, *DNA* deoxyribonucleic acid, *EGFR* epidermal growth factor receptor, *ESR1* estrogen receptor 1, *HGSOC* high-grade serous ovarian cancer, *HOXA9* homebox A9, *KRAS* Kirsten rat sarcoma, *MET* mesenchymal–epithelial transition factor, *NPAP1* nuclear protein-associated protein 1, *PARP* poly-ADP ribose polymerase, *PIK3CA* phosphatidylinositol-4,5-bisphosphate 3-kinase, *TP53* tumor protein 53, *PCR* polymerase chain reaction, *qPCR* quantitative polymerase chain reaction. [¥]53 primary tumors and 50 corresponding plasma samples

and PARP inhibition-based chemotherapy in OC and leading to the restoration of wild-type functions of this gene (Sakai et al. 2008; Norquist et al. 2011). These secondary mutations can take place in germline or somatic mutated *BRCA* alleles (Carneiro et al. 2018) and usually alter the structure of the primary frameshift into an in-frame internal deletion and leads to partly functional BRCA proteins (Ganesan 2018). As demonstrated by the previous study, detection of secondary reversion *BRCA* mutations in ctDNA allows the selection of patients that can benefit from PARP inhibition therapy which has recently shown a potential clinical response in OC (Ratajska et al. 2017; Ledermann 2016). Furthermore, in another study that recruited 30 patients with recurrent high-grade serous OC with known germline *BRCA1* or *BRCA2* status, reversion mutations detected in ctDNA based on targeted NGS assay were found to drive poor response to PARP inhibition and platinum-based treatments (Christie et al. 2017). However, despite reversion *BRCA* mutations were identified in ctDNA in an unbiased manner, this approach is limited by the fact that wild-type *BRCA* alleles-based DNA may be abundantly released in the blood from normal cells which will possibly influence the sensitivity of these NSG assays (Christie et al. 2017). More recently, 10,888 unselected patients with advanced cancers (stage III/IV), including OC patients ($n = 210$, 2%), were enrolled in a large cohort to identify incidental germline mutations in 16 actionable genes based on the Guardant 360™ NGS-based assay (Slavin et al. 2018). Variants in clinically targetable genes, such as *BRCA* in ctDNA, were found to be the highest among patients with OC compared with other advanced cancers (8.13% vs. 3.46%, 3.34%, and 2.2% for prostate, pancreatic, and breast tumors respectively) (Slavin et al. 2018). Similarly, Lin et al., in a study ($n = 112$) that used Guardant-360assay, has recently shown that patients with OC, without *BRCA* reversion mutations, had longer median PFS than those with reversion mutations identified in ctDNA before PARP inhibitors-based treatments (9.0 vs. 1.8 months; HR: 0.12; $p < 0.0001$) (Lin

et al. 2018). Moreover, baseline ctDNA in OC at the time of diagnosis has also a value for predicting recurrence. In this regard, a large retrospective cohort of 255 patients with epithelial OC demonstrated that the presence of detectable ctDNA is an independent biomarker for recurrence (HR: 0.38, 95% CI: 0.18–0.79; $p = 0.01$) (Ogasawara et al. 2020). This suggests that tumor seeding can occur in localized OC. Moreover, other pilot studies confirmed the feasibility of this noninvasive approach in predicting outcomes in patients with OC (Alves et al. 2020; Noguchi et al. 2020). Taken together, these preliminary findings can establish prognostic value and efficient real-time monitoring of anticancer treatments, if validated in large cohorts using standardized assays such as companion diagnostics. Remarkably, concordance between genomic alterations in ctDNA and primary tumors was also noticed suggesting an added value of this approach as a diagnostic tool (reviewed elsewhere: Cheng et al. 2017). Moreover, the combination of cell-free DNA with CA125 and the emerging biomarker HE4 may improve the accuracy of OC detection as supported by an earlier report (Shao et al. 2015). Therefore, multimarker panels are supposed to improve the sensitivity and specificity of this liquid biopsy-based approach.

Another application of liquid biopsy is the ability to assess gene methylation and other epigenetic changes as biomarkers for early detection and prognostication purposes (El Bairi et al. 2018; Tomasetti et al. 2017). In OC, the clinical significance of methylation patterns in ctDNA has been examined by Widschwendter et al. in 151 patients with various histologies, based on a multi-marker panel (three methylated regions *COL23A1*, *C2CD4D*, and *WNT6* genes) using bisulfite sequencing; the pretreatment of DNA samples before the sequencing is one standard procedure to study the DNA methylation pattern (Widschwendter et al. 2017). This methylation panel has demonstrated to discriminate patients with OC from healthy women or patients with a benign pelvic mass, with specificity and sensitivity of 90.7% (95%, CI: 84.3–94.8%) and 41.4% (95% CI: 24.1–60.9%) respectively (Widschwendter et al. 2017). Remarkably, this panel showed superiority in predicting chemotherapy response compared with CA-125 (78% of responders and 86% of non-responders ($p = 0.04$) vs. 20% and 75% respectively) (Widschwendter et al. 2017). Correlation between changes in methylation in primary tumors and ctDNA based on real-time methylation PCR (mPCR) and its association with clinical outcomes was also reported in a recent study enrolling 50 patients with high-grade OC (Giannopoulou et al. 2018). Methylated *ESR1* in ctDNA, a gene encoding for the estrogen receptor, was found to be significantly associated with primary tumors ($p = 0.004$) (Giannopoulou et al. 2018). Importantly, methylated *ESR1* was also found to predict better overall survival ($p = 0.027$) and progression-free survival ($p = 0.041$) (Giannopoulou et al. 2018). More recently, homeobox A9 (*HOXA9*) promoter methylation in ctDNA was found to predict response to PARP inhibitors (Rusan et al. 2020). The findings of this cohort ($n = 32$) of a phase II trial that investigated veliparib for platinum-resistant OC patients with *BRCA* mutations demonstrated that detectable methylated *HOXA9* at baseline and before each treatment cycle was associated with worse outcomes. Patients that were positive for this biomarker had a reduced PFS (5.1 vs 8.3 months; $p < 0.0001$) and OS (9.5 vs

19.4 months; $p = 0.002$). This longitudinal monitoring also showed that patients that were positive at baseline and that had undetectable methylated *HOXA9* ctDNA showed improved outcomes on multivariate analysis (Rusan et al. 2020).

In addition to point mutations and DNA methylation, chromosomal rearrangements in ctDNA were also investigated based on whole-genome sequencing technology and appear to have greater tumor specificity in OC (Harris et al. 2016). Aberrant chromosomal junctions were identified in ctDNA of OC patients ($n = 8$) before cytoreductive surgery in which five subjects had undetectable post-surgical ctDNA and therefore, supporting its possible use for monitoring therapeutic interventions (Harris et al. 2016). Still, results from these proof-of-principle studies remain immature in these small populations of OC patients. Also, these studies have been conducted based on relatively small samples and different methodologies and technologies which require meta-analytic approaches to combine their data. In this perspective, only one previous meta-analysis was performed by Zhou et al. and it has pooled the results of nine studies (462 patients and 407 controls) to assess the diagnostic value of circulating cell-free DNA (cfDNA) in OC (Zhou et al. 2016). Pooled sensitivity of cfDNA (0.70; 95% CI: 0.65–0.74) was found poor but its specificity (0.90; 95% CI: 0.87–0.93) reached an acceptable value for OC diagnosis (Zhou et al. 2016). As expected, subgroup analysis indicated that studies with large sample sizes detected OC accurately compared with small sample ones. In the case of specimen types, plasma-based assays were found to have high sensitivity but low specificity (0.72 and 0.89, respectively) in comparison with serum-based tests (0.65 and 0.93, respectively) (Zhou et al. 2016). When compared with the most recent meta-analysis by Dayyani et al. that investigated the diagnostic value of the standard CA-125 showing an area under the curve (AUC) of 0.883 (95%; CI: 0.771–0.950) (Dayyani et al. 2016), the AUC of cfDNA was relatively greater [0.89 (95%; CI: 0.83–0.95)], thus demonstrating a better accuracy (Zhou et al. 2016). In this meta-analysis, significant heterogeneity (sensitivity: $I^2 = 85.2\%$ and specificity: $I^2 = 78.5\%$) was observed among enrolled studies (Zhou et al. 2016). Meta-regression was utilized to identify the source of this heterogeneity and accordingly, no covariates such as study design, sample type, location, etc. were found to influence it and therefore the source of this heterogeneity could not be detected (Zhou et al. 2016). Furthermore, potential bias and quality appraisal of methodological quality of selected studies for the meta-analysis was assessed using QUADAS-2 (Whiting et al. 2011). This tool indicated that the study design did not considerably involve the accuracy of cfDNA as a diagnostic biomarker for OC (Zhou et al. 2016). As this field is rapidly evolving, future meta-analyses will provide sizable evidence when additional studies are available.

5.3 Perspectives: Ongoing Clinical Trials Investigating ctDNA for Ovarian Cancer

Clinical trials on this topic (Table 5.2) have the potential to provide accurate findings by increasing power and providing well-designed biomarker cohorts. The design of clinical trials for several interventions across the cancer continuum embraces

Table 5.2 Summary of ongoing clinical trials assessing ctDNA as a biomarker for diagnosis, prognosis and therapy response prediction in ovarian cancer

Trial identifier	Purposes/Objectives	Study design	Enrollment[a]	Sponsor
NCT03614689	Study of correlation between ctDNA, OC recurrence, mutational status, therapy response, and characteristics of immune repertoire before and after therapy	Prospective	100	Geneplus-Beijing Co. Ltd. in collaboration with Peking Union Medical College Hospital
NCT03155451	Detection of ctDNA in plasma for OC diagnosis	Prospective case-control	43	RenJi Hospital
NCT03691012	Application of ctDNA in peripheral blood as a biomarker for recurrence of stage I-IV epithelial OC after debulking surgery or following adjuvant chemotherapy	Prospective and multicenter	100	Walter and Eliza Hall Institute of Medical in collaboration with Johns Hopkins University
NCT03302884 (CIDOC)	Exploration of ctDNA dynamics as a biomarker for early OC recurrence and treatment efficacy after front-line treatments	Prospective and multicenter	150	Institut Paoli-Calmettes in collaboration with AstraZeneca
NCT02822157 (CLIO)	Assessment of ctDNA for monitoring olaparib-based treatment in OC	Randomized phase II trial (crossover assignment)	160	Universitaire Ziekenhuizen Leuven in collaboration with AstraZeneca
NCT03622983 (PELVIMASS2)	Collection of biological samples including ctDNA and detailed clinical data for future personalized medical interventions such as prediction of treatment response in patients with pelvic cancers	Prospective	500 (pelvic neoplasms including OC)	Centre Hospitalier Intercommunal Creteil
NCT03017573 (SCANDARE)	Correlation between ctDNA levels, de novo mutations, and immune response	Prospective	500 (ovarian, breast, head, and neck cancers)	Institut Curie

NCT02489058 (OLALA)	Study of ctDNA for monitoring therapy response to olaparib	Retrospective/prospective	100	University Health Network, Toronto
NCT03783949 (EUDARIO)	Study of ctDNA as a biomarker in a 3-arm phase II trial assessing safety/efficacy of ganetespib combined with carboplatin followed by niraparib vs. ganetespib+ carboplatin followed by ganetespib and niraparib vs. carboplatin + standard chemotherapy followed by niraparib maintenance in platinum-sensitive OC (as an additional outcome measure)	Randomized multicenter phase II study (parallel assignment)	120	Universitaire Ziekenhuizen Leuven in collaboration with European Commission
NCT03277209	Study of changes in ctDNA dynamics before and after a treatment based on continuous intravenous administration of plerixafor (a CXCR4 antagonist) and its impact on immune microenvironment in pancreatic, ovarian, and colorectal carcinomas patients(as an additional outcome measure)	Interventional phase I trial	–	Weill Medical College of Cornell University in collaboration with Cambridge University Hospitals NHS Foundation Trust
NCT02644369 (INSPIRE)	Study of changes in ctDNA as a genomic biomarker for therapy response to pembrolizumab in advanced solid cancers including epithelial OC (as a secondary outcome measure)	Interventional phase II trial	100	University Health Network, Toronto in collaboration with Merck Sharp & Dohme Corp.
NCT02797977	Assessment of ctDNA as a predictive biomarker for Chk1 inhibition in advanced cancers including high-grade OC	Nonrandomized phase I/II trial	140	Sierra Oncology, Inc.

(continued)

Table 5.2 (continued)

Trial identifier	Purposes/Objectives	Study design	Enrollment[a]	Sponsor
NCT01350908	Quantification of ctDNA in blood samples of OC patients and comparison of related detection techniques (PAP pyrophosphorolysis activated polymerization), BEAMing, and NGS)	–	25	Institut Curie
NCT02811224	Determination of sensitivity of a detecting assay of ctDNA in OC	Prospective case control	50	Scripps Translational Science Institute

[a]estimated sample size

innovative technologies to boost cancer care advancements and validate the clinical utility, safety, and effectiveness. Research questions, in fact, shall find validations only in the context of controlled studies. The use of liquid biopsy in clinical trials has been initially developed to study the treatment response. However, while liquid biopsy applications have found room in the clinical care of some patients with selected tumor types, mostly as plasma-based assays for non-small cell lung cancer, several clinical trials are assessing their utility in a spectrum of diseases (Snow et al. 2019).

The presence of tumor ctDNA has been historically identified in healthy subjects (Mandel and Metais 1948), and in patients with cancer, suggesting ab initio a role for both the early diagnosis and treatment of human cancers. In OC, the identification of ctDNA in healthy subjects has prompted the applications for the screening of solid tumors, providing the high capacity of DNA shedding into the plasma of some cancers (Alharbi et al. 2018). The lack of effective screening mechanisms based on plasma markers and imaging for OC has illuminated an important unmet need, for the deadliest women's pelvic tumor (Jacobs et al. 2016). The first clinical studies of the screening of OC have provided quite variegated results: essentially, sensitivity is interestingly elevated with ctDNA but diagnostic specificity still too low for screening purposes (Vanderstichele et al. 2017). Whole-genome sequencing, targeted gene sequencing by quantitative PCR, and DNA methylation pattern studies have been utilized in clinical trials for OC screening (Pereira et al. 2015); however, the definition of an exact role and clinical position is still a matter of research. To date, no cancer screening has been successfully implemented on liquid biopsy, though highly promising (Lo and Lam 2020). Presently, one clinical trial led by Shanghai Jiao Tong University in China and based on the study of the ctDNA methylation levels by deep sequencing-Sequencing is ongoing for screening purposes (NCT03155451). The incorporation of the information from ctDNA will aid in the definition of effective early detection interventions for patients at average or increased risk of OC, alone or in the context of more complex decisional algorithms. Possibly, high-performing ctDNA-based strategies will help reduce the incidence of advanced disease, inform on the appropriate timing of prophylactic surgeries in high-risk patients and enhance the family screening, for selected pedigrees.

Levels of ctDNA are influenced also by the disease burden and affected in the quantity and quality by the carcinogenesis dynamics of clone selection-turnover and treatment responses. The concept of earlier treatment in OC, based on the use of plasma biomarkers of relapse (e.g., CA125), has never been truly supported in women receiving and completing primary treatments (Krell et al. 2017). The CA125-triggered treatment has not been demonstrated to improve the outcome in women with no macroscopic OC recurrence (Krell et al. 2017). However, CA125 is an imperfect biomarker, and susceptible to a number of non-oncogenic phenomena, including inflammatory processes (Kim et al. 2016). So far, the definition of the most meaningful prognostic determinants in OC patients is based on the clinical and radiological findings, e.g., platinum sensitivity (Krell et al. 2017). Therefore, clinical implementation of plasma-based markers that better predict the true cancer relapse

events are highly warranted, to understand if the therapeutic exposure of the initial clones driving the recurrence in the preclinical stage can improve cancer survival. Based on these assumptions, prospective clinical trials have been designed and are ongoing to identify and validate ctDNA-based biomarkers for recurrence of stage I-IV epithelial OC after debulking surgery or following adjuvant chemotherapy (NCT03691012) and explore the ctDNA dynamics (NCT03302884/CIDOC).

In addition, several trials are also exploring the opportunity to study the variations in the ctDNA during treatment or the identification of resistance-driving clones. The phase 2 clinical trial ARIEL2 enrolled patients to receive the anti-PARP rucaparib; a subset of patients performed a liquid biopsy, to understand how the quantitative changes in the ctDNA could predict treatment response. None of the patients with persistently elevated ctDNA experienced a radiological tumor response, while 80% of patients with a demonstrated reduction of ctDNA (i.e., decreased level of 50% or more after a single treatment cycle) experienced a radiological tumor response, suggesting a possible predictive role (Piskorz et al. 2016). Therefore, prospective clinical trials have been designed to understand how ctDNA quantitative dynamics can affect the prognosis and serve as clinically useful and valid predictive biomarkers (NCT03302884/CIDOC). Also, ctDNA quantitative evaluations can be useful to understand the on-target mechanisms of resistance, as discussed above for the intragenic reversion mutations of *BRCA1/2*, linked to acquired resistance to PARP inhibitors (Christie et al. 2017). The ongoing prospective clinical trials aim to confirm the clinical value of longitudinal mutational evaluations with ctDNA during treatments for PARP inhibitors (NCT02822157/CLIO, NCT02489058/OLALA) and/or other targeted agents (NCT03622983/PELVIMASS2, NCT03783949/EUDARIO, NCT02797977).

Moreover, experimental evidence has demonstrated a possible role of liquid biopsy in the monitoring of response to immunotherapeutic agents (IO). The assessment of tumor response in patients receiving IO has been sometimes challenging, especially for patients experiencing an initial tumor progression followed by a durable cancer response (i.e., pseudo-progression). Accordingly, ctDNA-based assays that correlate with the true cancer burden may be desirable. Indeed, one study confirmed the prognostic value of ctDNA reduction in patients receiving IO, including a cohort of women with high-grade serous ovarian cancer (Bratman et al. 2020). This recapitulates the findings with chemotherapy and targeted agents. Consistently, ctDNA applications in IO treatment response monitoring have been implemented in ongoing clinical studies (NCT03017573/SCANDARE, NCT03277209, NCT02644369/INSPIRE). The possibility to collect samples during routine clinical procedures for standard clinical assessments of patients with OC is a major favoring characteristic for the clinical implementation of liquid biopsy, as its noninvasive nature. While the utility, reproducibility, and value of ctDNA assays in the clinical practice are still investigational, the OC biology and the preliminary exploratory findings from small cohorts suggest a promising role in the clinical practice, across the spectrum of cancer continuum.

5.4 Feasibility, Availability, and Accessibility of Liquid Biopsy-based Methodologies for Clinical Applications: Addressing Barriers, Framing Solutions for Cancer Resilient Health Systems

The implementation of innovative medical technologies developed in resource-rich settings can often encounter barriers in different health system contextures (Lustberg et al. 2018). For the approved indications, the role of assays based on liquid biopsy is complementary, and not entirely intended to replace tissue-based diagnostics; they are used mostly to characterize predictive and prognostic biomarkers (Goodsaid 2019). As a result, a number of regulators and decision-makers have questioned the true clinical utility of ctDNA assays outside clinical trials, thus they have not supported the coverage by the national health insurance schemes (Lustberg et al. 2018). The liquid biopsy technologies are sophisticated and costly, therefore demanding elevated financial resources and skilled health personnel.

In low- and middle-income countries, the implementation of effective cancer control programs is challenged by the scarcity of resources, often weakened by non-resilient health systems, unprepared to face the rapidly increasing cancer burden (Wambalaba et al. 2019). Accordingly, the selection and prioritization of cancer interventions are critical to assure the delivery of quality cancer interventions to a large proportion of the population, pursuing for a universal health care. Nevertheless, some authors have reported possible benefits in the implementation of ctDNA techniques in low- and middle-income countries. The possibility to collect blood samples virtually anywhere, stored in local laboratories, and then analyzed in reference centers is one of the advantages (Temilola et al. 2019). For many patients, in fact, the first and most important barrier to cancer care is to have a diagnosis of the malignancy, to seek medical care, and to perform the diagnostic tissue biopsy—representing one of the most significant reasons for delays in cancer treatments and advanced cancer presentations (Brand et al. 2019; Trapani et al. 2021). However, evidence to support a complete replacement of tissue biopsy with liquid biopsy for diagnostic purposes is not entirely supported, as the role of ctDNA assays is mostly complementary, and not intended to make the diagnosis of cancer (Adeola et al. 2017). Therefore, no implementation should be endorsed in the absence of good prospective clinical data, and validations in the ethnic subgroups of interest. For example, only a minority of the patients enrolled in the clinical studies of liquid biopsy belong to African ancestry, and African-based studies are only a small number. One research showed that the majority of African-based studies were done in Egypt, with a few other studies from Northern Africa and South Africa (Temilola et al. 2019). Advocating for inclusiveness in clinical trials and evaluating the local utility of new medical technologies have emerged as health imperatives, ensuring valuable investments with measurable population health and economic gains (Dilla et al. 2015).

The implementation of innovative health interventions like liquid biopsy with no cognition of the utility, health gains, budgetary impact, and reimbursement decisions are common sources of inefficiency in the health investments. For example, three African countries (Kenya, Tunisia, and South Africa) have made available to the

public some liquid biopsy kits (Kinyua 2018); however, these interventions have soon become prerogative of only a minority of the populations, as they are de facto unaffordable to the greatest proportion of the patients. The financial barriers and the lack of consistent data on effectiveness and cost-effectiveness can prevent all the good narratives to develop implementation research of liquid biopsy in low- and middle-income countries, including serving the most remote areas and disadvantaged populations. In addition, the risk to increase the health care gap is large, including through an elevated exposure to catastrophic health expenditure.

Nowadays, it is imperative to expand the options of cancer services in low- and middle-income countries through a phased approach. The safety, feasibility, sustainability, and cost-effectiveness of new technologies must be viewed in a context-appropriate cancer planning perspective, and not as a mere race for the most innovative devices. Therefore, research investments must be oriented to boost the local capacities through international and national efforts, including regulated agreements with the private sector, and always developed in alignment with the goals of the national cancer control planning (Jamison et al. 2018).

The use of liquid biopsy could also help in the promotion of the best treatment practices, in the context of clinical trials. Whether liquid biopsy should be implemented in the clinical practice for women with OC in low- and middle-income countries nowadays is unlikely to be realistic. However, the strengthening of clinical research is the imperative of the cancer agenda, including with the use of new technologies—when intended to enforce the local evidence-based practices, scale-up the workforce, and develop training programs—resulting in a health system benefit of the cancer research, therefore translated in a population benefit with societal gains. Major advancements in cancer care will be stated only under a goal-oriented research agenda, making sure that priority investments are not distracted by more appealing but not presently useful interventions. It is necessary to work for population-based cancer care that is affordable, accessible, and designed to respond to local health needs through global health tools and technologies.

5.5 Conclusions

Liquid biopsy is a novel noninvasive approach that can provide a more accurate prognostic evaluation and prediction of therapeutic response. Moreover, its potential role in the early detection of the disease and in cancer screening needs to be further investigated. To date, OC represents the fifth cause of death from cancer in the women population and it has the worst prognosis among gynecological tumors (Giannopoulou et al. 2019). This aggressive cancer is still diagnosed at an advanced stage despite general improvements made in the management of the disease. The lack of clearly defined biomarkers for early detection plays an important role that has to be addressed. Liquid biopsy may represent a new promising tool in the management of OC, offering improvements in monitoring the disease course, treatment response, and prediction of resistance to anticancer therapies. It may be useful to develop more personalized and evidence-based therapy for this aggressive disease.

There is still much to do for an optimal management and a better therapeutic outcome for women with OC. The available data are based on pilot exploratory studies. Improved and standardized techniques, reproducibility of results, large OC patients sampling, and longer follow-up are mandatory before implementing ctDNA approach in clinical practice. Additional data and further reading are detailed in prior reviews (Box 5.1).

Box 5.1 Recommended reading of particular interest

	DOI
Keller L, et al. **Clinical relevance of blood-based ctDNA analysis: mutation detection and beyond**. Br J Cancer. 2020.	10.1038/s41416-020-01047-5
Pantel K, Alix-Panabières C. **Liquid biopsy and minimal residual disease - latest advances and implications for cure**. Nat Rev Clin Oncol. 2019;16(7):409–424.	10.1038/s41571-019-0187-3
Cescon DW et al. **Circulating tumor DNA and liquid biopsy in oncology**. Nat Cancer. 2020, 1, 276–290.	10.3390/cancers12102880
Cheng ML, et al. **Circulating tumor DNA in advanced solid tumors: Clinical relevance and future directions**. CA Cancer J Clin. 2020.	10.3322/caac.21650
Pessoa LS, et al. **ctDNA as a cancer biomarker: A broad overview**. Crit Rev Oncol Hematol. 2020;155:103109.	10.1016/j.critrevonc.2020.103109
Davidson B. **Circulating tumor cells and cell-free nucleic acids in patients with gynecological malignancies**. Virchows Arch. 2018;473(4):395–403.	10.1007/s00428-018-2447-5
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Asante DB, et al. **Liquid biopsy in ovarian cancer using circulating tumor DNA and cells: Ready for prime time?** Cancer Lett. 2020;468:59–71.	10.1016/j.canlet.2019.10.014

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