

Review Article

# New insights of liquid biopsy in ovarian cancer

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## Abstract

Through the development of new analysis technologies, many issues regarding the approach to tumoral diseases have been elucidated. With analytical assays developed in the last years, various omics technologies have evolved in such a manner that the characteristics of tumor cells and products can be evaluated (assessed) in the bloodstream of cancer patients at different times. Ovarian Cancer (OC) is one of the most difficult to diagnose tumors, with low survival rates due to the high heterogeneity of these diseases that are distinct in terms of etiology and molecular characteristics, but which simply share an anatomical appearance. Recent findings have indicated that several types of ovarian cancer classified into different histotypes are in fact derived from non-ovarian issues and share few molecular similarities. Within this context, ovarian cancer screening and diagnosis can be made through the evaluation of circulating tumor cells in peripheral blood using liquid biopsy technologies. Advances in the study of various molecules analyzed by liquid biopsy have shown that elucidation of intratumoural and intertumoural heterogeneity and spatial and temporal tumor evolution could be traced by serial blood tests rather than by histopathological analyses of tissue samples from a primary tumor. Therefore, evaluation of some molecules such as circulating tumor cells (CTC), circulating tumor DNA (ctDNA), circulating cell-free RNA (non-coding and mRNA, extracellular vesicles), tumor-educated platelets or different miRNAs using liquid biopsy could lead to improvement of patient management.

## Abbreviations

OC: Ovarian Cancer; TVUS: Transvaginal Ultrasound; CT: Computerized Tomography; PET-CT: Positron Emission Computerized Tomography; HE4: Human Epididymis Protein 4; ISLB: International Society of Liquid Biopsy; ICGC: International Cancer Genome Consortium; FDA - Food Drug Administration; TCGA: The Cancer Genome Atlas; HTAN: Human Tumor Atlas Network; ILSA: International Liquid Biopsy Standardization Alliance; FNHI: The Foundation for the National Institutes of Health; BC: Biomarkers Consortium; QCMs: Quality Control Materials; ENCODE: Encyclopedia Of DNA Elements; BEAM: Beads, Emulsion, Amplification, Magnetics; cfDNA: Circulating cell-free DNA; cfRNA: Circulating cell-free RNA; CTC: Circulating Tumor

Cells; ctDNA: Circulating tumor DNA; circRNA: Circular RNA; HE4: Human Epididymis Protein 4; LncRNA: Long non-coding RNA; TsncRNAs: Trans- noncoding RNA; miRNA: Micro RNA; mRNA: messenger RNA; NGS: Next Generation Sequencing; OC: Ovarian Cancer; PCR: Polymerase Chain Reaction; WGS: Whole-Genome Sequencing; WES: Whole-Exome Sequencing

## Introduction

The aim of this study is to provide an overview of recent findings in the field of diagnosis and evaluation of prognosis, including the possibility of detection of small residual tumors of ovarian carcinomas, in order to improve the management of particular cases. In this article, we summarized the main used biomarkers in OC diagnosis that could be analyzed through

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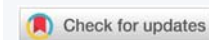
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liquid biopsy assays, which is a non-invasive technique that allows serial sampling collection and analysis, for monitoring dynamic tumor changes over time. Liquid biopsy as a tool for diagnosing cancer refers to a simple and less invasive procedure that could provide valuable information in clinical oncology just by analyzing a small number of tissues, often a single peripheral blood sample.

Ovarian cancer has the highest mortality rate of all gynecological cancers worldwide being frequently diagnosed at an advanced stage [1,2].

The classification of ovarian cancer is generally made according to the stage at the time of tumor discovery, early or advanced stage and according to the histology of the tumor, classified as epithelial or non-epithelial, of which the most frequent is the high-grade serous ovarian carcinoma (HGSOC) [2-4]. This type of cancer is characterized by an unusual dissemination mechanism: rapid growth, disruption of ovarian tumor capsules and malignant cells spread into the peritoneal cavity which usually involves the accumulation of ascites. Therefore, the development of new methods for investigating circulating cells is widely reported as a rational avenue for improving clinical efficiency and preventing the progression of disease in patients with ovarian cancer. Given the challenges of screening, diagnosis and monitoring, and the impact that the early diagnosis has on the patient's prognosis, new emerging tools are needed to increase the precision of the diagnosis and to monitor and better understand and predict the response to the treatment [4,5].

In the context of ovarian cancers, classical diagnostic and screening procedures are not efficient, leading to late-stage detection of the disease, which results in inefficient treatments and poor survival rates. Usually, tumors of the ovary are diagnosed by conventional methods such as tissue biopsy, imaging techniques (transvaginal ultrasound - TVUS), computerized tomography scan (CT, PET-CT) and evaluation of some biomarkers from circulating blood. Ovarian tissue biopsy is obtained by different invasive techniques such as surgical, needle biopsy, or imaging-guided-biopsy, in which some solid tissue fragments are removed for pathological examination. In these procedures, limitations of collecting the right pieces of the samples can restrict the information about the tumor heterogeneity and detection of possible metastasis in other sites. As for biomarkers, they can be divided into several categories, such as biomarkers specific for the diagnosis of OC (one of the commonly used combinations being the CA125, Human Epididymis Protein 4 - HE4 and mesothelin), biomarkers used as a prognosis for OC among which we could list predictive biomarkers and treatment response biomarkers, more recently used in personalized medicine in patients with OC [6,7]. Over the last decade, many studies have been made for the detection and management of OC and numerous molecules have been considered promising diagnostic and/or prognostic biomarkers, such as CTC and

ctDNA. In this context, we aim to discover the newest and relevant research in the domain of OC and we carried out a literature search in public databases such as PubMed, Google Academic, NCBI, Science Direct, etc., using keywords liquid biopsy and ovarian cancer.

### **Liquid biopsy - non-invasive molecular analysis tool used in diagnosis and therapies ajustement in OC**

Liquid biopsy is a technique that refers to the evaluation of some types of cells or sub-cellular structures from biological fluids, by diverse molecular analysis. Due to the promising potential of this non-invasive method of analysis, liquid biopsy is used nowadays in clinical practice in prenatal screening and in special in oncology for investigating circulating cells, sub-cellular structures, or other bio-molecules. These types of molecules are considered biomarkers that fulfill important roles in diagnostic, prognostic or monitoring therapy response, in order to change the treatment in a timely manner to prevent the recurrence of the disease [8,9]. Several single and multi-gene assays using different omics technologies for detecting genetic alteration of some circulating cells that carried out genetic information about molecular profiling of the primary, metastatic or recurrent tumors were approved by international organizations. International Cancer Genome Consortium (ICGC), Food Drug Administration (FDA), The Cancer Genome Atlas (TCGA), Human Tumor Atlas Network and the International Liquid Biopsy Standardization Alliance (ILSA) has made large collaborative studies, with the purpose of better understanding tumor biology and elucidating the intratumoral and intertumoral heterogeneity. Other processes intensively studied lately are represented Through the interaction between tumoral cells, metabolites that these cells release in the microenvironmental space and their interaction with the genome. Furthermore, the mechanisms by which metabolic and homeostatic processes are dysregulated and monitoring therapy response or targeted therapy have evolved. This progress was made in order to apply standardization of some biomarkers that could be used for screening to provide chances of early detection of cancers or for monitoring the response to targeted therapy.

### **Use of liquid biopsy in clinical practice**

As a result of numerous technological advances in the fields of genetics, genomics, and oncology, promising developments in precision medicine have evolved lately. Among these signs of progress, liquid biopsy ranks one of the first places in customization of individual cases, by evolution made in the approach regarding diagnostic and therapy. Today, liquid biopsy is used as an alternative to solid biopsies, due to the non-invasive characteristic of this procedure, by which genetic material necessary to evaluate the genomic variation of a tumor, is obtained. By liquid biopsy, some types of molecules are obtained in order to characterize or evaluate the prognostic and evolution of the tumor. These include CTC and ctDNA, being the most used biomolecules to customize health care

for individual patients [10-13]. In this scope, the International Society of Liquid Biopsy (ISLB), founded in 2017, deals with the regulation and standardization of recommendations in the field of diagnosis by liquid biopsy. The main purpose of this society is to coordinate the research efforts and strategies of all specialists worldwide. Few working groups have put their efforts together to introduce in clinical practice the use of many biomarkers obtained and quantified by liquid biopsy. One of them is The Foundation for the National Institutes of Health (FNIH), Biomarkers Consortium (BC), whose main the research area is represented by biomarker use, especially in new drug development and medical diagnostics in cancers patients and discovery and implementation of Quality Control Materials (QCMs) like in the ctDNA Quality Control Materials Project [10,15-17]. The importance of liquid biopsy in clinical practice it's recognized in oncology by supporting the clinical decision in order to classify and characterize cancer types, by identifying the mutation for targeted therapy, monitoring therapy response [10,15], as it is shown in Figure 1.

In ovarian cancer, although many biomarkers are available in the clinic, does not exist yet a set of specific universally accepted and used biomarkers that allow early diagnosis and monitoring disease progression. In the following chapters, we will discuss the most commonly used types of biomolecules in ovarian cancer, obtained through liquid biopsy.

### Circulating biomarkers in OC usually detected through liquid biopsy

Many molecules produced by tumoral cells or cells in the environment of a cancer type could be evaluated in body fluids, especially in peripheral blood, by qualitative or quantitative techniques. These molecules can be considered a tumor biomarkers, because they originate from various cellular sources such as cytoplasmic proteins, surface antigens or receptors, enzymes, hormones, or another specific type cells, especially oncogenes and their products [14]. Various isolation methods have been developed either based on biological or by physical properties for CTC, ctDNA, circulating

cell-free RNA (non-coding and messenger RNA, extracellular vesicles), tumor-educated platelets, or different miRNAs, which are evaluated by liquid biopsy. CTCs are detected by several methods of gene expression analysis (RT-PCR, FISH), by immunocytochemistry (ICC) or a combination of both [15].

**Circulating tumor cells (CTC):** CTCs are major components of liquid biopsies for clinical diagnosis, prognosis and real-time treatment monitoring [18,19], as their significance was first reported by Thomas Ashworth in 1869 [20]. These cancer cells preserve characteristics and the heterogeneity of the primary tumor, while they can potentially initiate subsequent metastases. Due to their significance, several technologies have been developed, targeting the enrichment, isolation, and detection of CTCs in liquid biopsies. In total, these technologies can be categorized into 3 groups: the ones that probe the biological [20,21] or physical [23,24] properties of CTCs and the functional assays [25,26], as summarized in Table 1.

The prognostic value of CTCs is focused on the ability to discriminate potentially metastatic clones, while the elucidation of the molecular and biological properties of CTCs can facilitate optimized treatment. Monitoring of transcripts in CTCs have been shown to have substantial predictive value for prostate cancer patients [32], while probing for CTCs in HR-positive breast cancer patients has been shown to have the potency to predict late recurrence [33]. Several studies have denoted the significance of molecular and biological characteristics of CTCs in the early differential diagnosis of cancer [34] and prevention of metastasis [35]. Continuous enumeration of CTCs in cancer patients has been shown to be a valuable tool for prognosis and treatment evaluation [36,37].

**Circulating tumor DNA (ctDNA):** Apart from CTCs, the thought of investigating liquid biopsies has been mainly based on ctDNA, as it is an accessible target that facilitates prognosis and treatment monitoring, while it has the potential to become an early cancer diagnosis tool. The release of ctDNA into circulation is done by passive mechanisms, such as cell death, as well as active mechanisms of cells, such as the release of

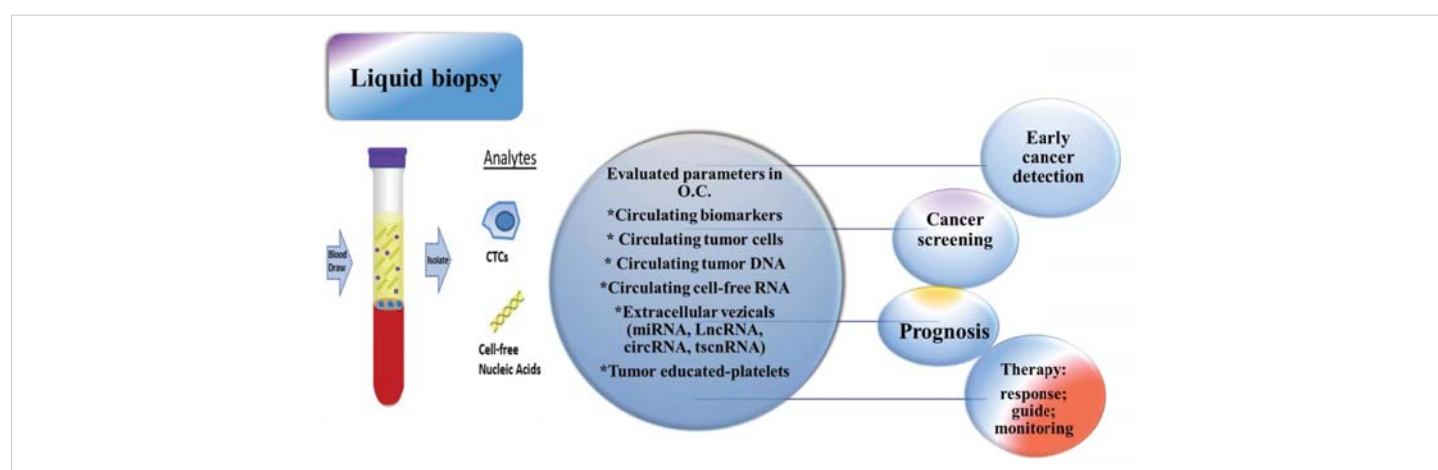


Figure 1: The importance of liquid biopsy in clinical practice.



**Table 1:** Technologies for the isolation and detection of CTCs in liquid biopsies.

Targeting method	Targets	References
Biological properties of CTCs. Positive and/or negative selection.	Positive selection: EpCAM, cytokeratins such as CK18, CK19 and CK8. Negative selection: CD45, CD66b antigens.	[27,28]
Physical properties of CTCs	size, density, electrostatic properties and the greater ability of CTCs to deform.	[29]
Functional assays	Protein secretion from CTCs, preferential binding of CTCs to	[30,31]

**Table 2:** Role of miRNAs in OC.

Structure	Role of miRNAs in OC	References
miR-21-5p	miR21 transferred from CAAs to the cancer cells confer chemoresistance by binding to its direct novel target-APAF1	[73,79]
miR-506	miR506 downregulation promotes an aggressive phenotype in OC	[100]
miR-141	Is a b-Catenin, TCF7L2, SOX17 inhibitor/inhibitor/activator Up-modulated in ovarian carcinoma	[69]
miR-126	miR126 may serve tumor suppressor roles by inducing G1 cell cycle arrest and suppressing invasion in ovarian cancer cells, by targeting VEGF expression	[101]
miR-200 family	EMT regulation (double-negative feedback loop) Up-regulated in ovarian tumors compared to normal cells and tissues	[75-78,84]

extracellular vesicles [38]. Previously identified mutations in primary tumors can be found in ctDNA to monitor the responsiveness of treatment [39], whereas novel lesions can be indicated with a non-targeted sequencing approach [40]. The fact that ctDNA is mixed with cfDNA from healthy cells renders difficult the detection of variants, however, several technologies have demonstrated effective detection of variants having frequency down to 0.01% [41]. The detection of variants can be done among others by digital PCR, NGS, or BEAMing [42]. As cancer development is characterized by epigenetic modifications as well, there is an increasing interest to identify specific aberrant methylation patterns in ctDNA, mainly in promoter regions [43]. Generally, ctDNA has been found to be increased in cancer patients compared to healthy individuals [44], while the prognostic value of ctDNA has been indicated by several studies connecting specific variants [45] or methylation markers [46] with the survival rates of the patients. The assessment of ctDNA has been found to be vital for the selection of therapy in cancer patients with difficult-to-resect tumors [47] and for monitoring treatment resistance [48]. In the study of Willis, et al. microsatellite instability assessment has been performed using ctDNA, which is a key biomarker for the administration of immunotherapies in several cancer types, including ovarian [49].

#### Circulating cell-free RNA (non-coding and mRNA):

The presence of cfRNAs in the blood is associated with the levels they have at the tissues of origin and their release rate from the cells. As in several cancer types is hard to obtain adequate cfDNA from the plasma of the patients, due to low concentrations, cfRNA could fill in the gap and facilitate early cancer type and subtype diagnosis together with localization of the primary tumor. The significance of cfRNA as

a diagnostic, prognostic, and treatment response biomarker has been mainly studied focusing on miRNAs and mRNAs (messenger RNA). Although it has been shown that changes in the expression levels of specific miRNAs could be used in cancer monitoring [50], assays targeting only miRNAs lack reproducibility and specificity as they are prone to processing biases [51]. The investigation of mRNAs with known cancer-driving mutations or fusions and the assessment of their expression levels has substantial usage in clinical practice [52]. The selection of specific mRNAs that are not found in the plasma of healthy individuals but are present in the liquid biopsies of cancer patients could supplement cfDNA assays in cancer monitoring [53]. Targeting biomarkers that are tissue-specific, such as overexpressing cfRNAs or methylation patterns on cfDNA, could facilitate clinical practice in the prediction of tumor tissues of origin (TOO).

**Extracellular vesicles:** There are three types of EVs that can be differentiated by their size and biogenesis, namely exosomes, microvesicles and apoptotic bodies [54]. The smallest ones, ranging from 30 to 150 nm in diameter, are the exosomes, which are currently considered an important component of cell-to-cell communication [55]. Their cargo contains target molecules for cancer monitoring, such as miRNA, mRNA and cfDNA, they could facilitate clinical practice by providing a reflection of the cancer cells they originate from. The stress conditions which are exerted on cancer cells can alter normal vesicular biogenesis and exocytosis together with the composition of the vesicles. Exosomes can be found in adequate concentrations in liquid biopsies, while they are very stable and tolerant to transportation [56,57]. These attributes together with the fact that the target molecules, such as sensitive cfRNA, remain protected within their lipid bilayer render them a promising source of biomarkers.

**Tumor-educated platelets (TEPs):** Among others, the functionality of platelets has been associated with thrombus formation to induce hemostasis, the communication of immune cells, the propagation of inflammation, as well as the creation of new blood vessels. It has been noted that there is an active interplay between cancer cells and TEPs which helps metastasis from intravasation to extravasation and the formation of metastatic niche, through epithelial to mesenchymal transition (EMT) induction of cancer cells, intravascular protection of cancer cells in clots and neoangiogenesis at the metastatic site [58-60]. The "education" process of platelets takes place through the direct acquisition of biomolecules from the tumor and the cells around it via extracellular vesicles, indirectly through post-transcriptional splicing of RNAs in platelets as well as through altering the transcriptional profile of megakaryocytes [61,62]. Even though platelets lack a nucleus, they are capable of protein synthesis, which gives them an active role in cancer propagation [63]. Considering that TEPs contain molecules of interest, such as RNAs from cancer cells, could be targeted by assays to contribute to early cancer diagnosis, prognosis and treatment response prediction [64].



## Use of biomarkers obtained through liquid biopsy as a prognostic tool in personalized medicine in OC patients

The conventional biomarkers used in the clinical diagnosis of ovarian cancer are serum cancer antigen 125 (CA125), Human Epididymis Protein 4 (HE4) and transvaginal ultrasonography. Using these biomarkers alone for differential diagnosis is insufficient due to the low sensitivity and specificity of these procedures, so, many studies were made and furthermore are coming up in order to explore the relation between miRNA and ovarian cancer and to improve diagnosis, prognosis and treatment methods. The development of tumors is imposed by the tumor microenvironment. Extracellular matrix molecules regulate cancer invasion and metastasis and, at the same time, downregulation of miRNAs controls tumor spreading by degrading extracellular matrix [65,66].

**miRNAs are used as a source of liquid biopsy in ovarian cancer:** One of the RNA types involved in cancer oncogenesis, prognosis, and treatment response is microRNA (miRNA), a small, non-coding RNA, containing between 19-25 nucleotides, with multiple roles in almost all cell functions, such as cell differentiation, proliferation, or death. The miRNA is endogenously synthesized in the cell nucleus, by DNA polymerase 2 and its precursor is processed by Drosha and Pasha enzymes and transported into the cytoplasm. The 70-100 nucleotides pre-miRNA are then cleaved by Dicer into a functional 22 nucleotides miRNA [67,68]. The miRNA regulates the post-transcriptional gene expression by getting incorporated in an mRNA silencing complex, by inhibiting the mRNA translation or by degrading the mRNA. This process is based on its complementarity with the target zone, although it is not always necessary, as one miRNA can sometimes regulate more than one mRNA [67,69,70]. Oncogenesis can be influenced by both the downregulation and upregulation of miRNA. By downregulating miRNA, the tumor genesis can be suppressed, whereas upregulation of miRNA can act as an enhancer, promoting abnormal cell growth dysregulating apoptosis, promoting neovascularization and an inflammatory environment [71]. Because of its multiple roles in cell biology, miRNA can also be used as a biomarker in medical practice, as it is expressed in fluids like blood and its components plasma and serum, in urine, saliva and breast milk, in both oncologic patients and healthy people. The genetic signature of circulating miRNA is identical to the tissular miRNA, thus providing more accessible tools for detecting the presence of ovarian cancer, its histological type, prognosis, and [71,72]. MiRNA can be used as a biomarker due to its stability in the bloodstream, even in extreme conditions such as variations in pH or temperature. Its degradation is prevented by binding to serum proteins or lipids or by being incorporated into small vesicles that resulted from apoptosis [73,74]. Dosing the circulating miRNA can be used as a *diagnostic tool*, as some specific miRNAs are modified in ovarian cancer patients compared to healthy individuals. In a study from 2006, Zhang, et al. found that the miR-200 family

(miR-200a, miR-200b, miR-200c, miR-141) is upregulated in ovarian cancer, while a study in 2015 showed that miR- 92, miR-15a, and miR-21 are also upregulated in patients with ovarian cancer [75,76]. Some miRNA may also be associated with certain ovarian cancer histology. The miR-200 family is found in all subtypes of ovarian cancer, but endometrioid tumors presented upregulated miR-21 and downregulated miR-222. Other miRNAs are downregulated in ovarian cancer patients, for example, miR-9, miR-31, miR-34, miR-503, miR-506 and miR-507 [ 67-69,100]. The miRNA can also be used as a *prognostic tool*. For example, as found in a study from 2016 by Nakamura, patients with lower levels of miR-200 family or with lower methylation of let 7a-3, had a lower survival rate [77]. Another study group demonstrated that miR-21 can be used as an independent prognostic factor in ovarian cancer, as its higher values are associated with more advanced disease, lower histologic differentiation, and lower survival [74]. Another role of miRNA is to predict *the response to therapy*, as indicated in a study by Kapetanakis, et al. They showed that the levels of miR-200b during chemotherapy can be correlated to the treatment response. For patients with decreasing levels of miR-200b, the progression-free survival was longer than in those with increasing levels of miR-200b, who had an increased risk of progression [78]. Oncologic treatment was developed by making use of the miRNA and its function, the treatment of cachexia associated with inflammation and high levels of miR-21 being one such example. Inhibition of microvesicles containing miR-21 and their interaction with myocytes has been a promising strategy for the treatment of severe weight and muscle loss in cancer patients [73]. By studying miRNAs in ovarian cancer patients, we have gained insights into the complexity of their roles in cellular mechanisms, in both normal and cancerous cells. Their clinical utility is yet to be determined, but recent studies have given promising perspectives on using them in daily practice as easy-to-determine biomarkers [73]. In Table 2 we have summarized the role of some miRNA associated with ovarian carcinoma.

**LncRNAs, used as a source of liquid biopsy in ovarian cancer:** Recently, a significant role in ncogenesis has been attributed to the long non-coding RNAs (lncRNAs), from an oncogenic perspective, and more scientists, are studying this subject for a better understanding of its impact in the field of cancer, especially ovarian cancer [86]. LncRNAs are a subclass of non-coding RNAs, that is not translated into protein and that contains approximately 200 nucleotides, which differs from short ncRNAs [87]. They have a big impact on the cell environment as transcriptional regulators, due to the specific interactions with other cell components, such as proteins, DNA, or RNA [88]. Mutations in the structure of lncRNA can change the basic cell functions, leading to the growth of cancer tumors. Therefore, lncRNA could be used as a useful biomarker in the development of cancer, such as ovarian cancer [86]. In ovarian cancer development, lncRNA acts as a process suppressor, as



it can induce autophagy in the cell. A remarkable example is lncRNA GAS8-AS1, which demonstrated this capability by binding to the multi-domain protein Beclin1, the main compound in the process of autophagy. Also, Meg3, a lncRNA that has a specific role in ovarian oncogenesis, suppressing tumoral transformation, has been identified by both Oliveira, et al. and Zamaraev, et al. [86,89]. Regarding the impact on cell apoptosis, it has been observed that the apoptotic process is affected by lncRNA, which acts on the tumor suppressor p53, by regulating it. Chemoresistance in patients with OC can be induced when lncRNA suppresses p53 protein or Bcl-2 family proteins (proteins with pro-apoptotic action) [87]. Several studies have shown that lncRNA is involved in fundamental signaling pathways, such as PI3K/Akt signaling, affecting the process of malignant proliferation [86]. In the immune system, the lncRNA is involved in the release of an anti-tumor immune response, which leads to OC cells being undetected by immune system cells, making lncRNA a goal in OC immunotherapy [90]. One way to find drugs for OC patients could be to use compounds that could act directly on the lncRNA. Thus, by means of lncRNA, it may be possible to regulate the apoptosis of OC cells [91]. Nevertheless, not all the actions and functions of lncRNA is yet known. Several studies in this area will help researchers in the future to lay the groundwork for ovarian cancer therapy, targeting the lncRNA directly [92].

**circRNAs in OC:** Circular RNAs are RNA molecules that belong to the lncRNA class and they range in size from hundreds of nucleotides to thousands of nucleotides [93]. Their structure is in the form of a closed, circular loop and they are not usually translated into proteins and have a non-polyadenylated structure [94]. The important characteristics of circular RNA are represented by the ubiquitous presence, the stability of their structure, their conserved structure, but also the multitude of roles that they fulfill, such as the involvement in the process of splicing and translation regulation. The circular shape of the molecules generates a much more stable structure against RNases, compared to the linear structure of other RNA molecules [95]. Over time, scientists have shown major involvement of circular RNA molecules in most cancers, including OC. The action of circular RNAs in OC is to control the process of cell proliferation. It has been observed that there is a close association between the stages of initiation and progression of ovarian cancer and the deregulation of circular RNA molecules, which leads to the use of circular RNA as biomarkers [93]. Ning, et al. found that circular RNA has basic functions in oncogenesis because it regulates the expression of certain genes. Thus, they found that 6 types of circular RNA (circ-EXOC6B, circ-BNC2, circ-FAM13B, circ-N4BP2L2, circCELSR1 and circ-RHOBTB3) are associated with certain clinical and pathological features of ovarian cancer. They also found that the circular RNA molecules, circEXOC6B and circ-N4BP2L2, can be used as biomarkers for the prognosis of ovarian cancer [96].

**tsncRNA used as a source of liquid biopsy in ovarian cancer:** TsncRNAs (trans- noncoding RNA) belong to small non-coding RNA, a relatively new described class of biomolecules that are involved in various biological processes, such as regulation of gene expression at transcriptional and posttranscriptional levels [97]. Like it was demonstrated by ENCODE project (Consortium, E.P. The ENCODE (Encyclopedia of DNA Elements), 90% of the whole human genome contains functional ncRNA [98]. In the last decade, it was well documented that EVs isolated and analyzed by liquid biopsies, are a carrier of various cargo, including tsncRNA molecules, and serve to transport genetic material between cells, with involvement in, regulating important biological processes, such as the proliferation and differentiation of cells [99]. Recent studies have shown that circulating tsncRNA molecules are present in 14 biological samples processed in patients diagnosed with epithelial ovarian cancer or other ovarian tumors, thus suggesting their potential as biomarkers for both diagnosis and prognosis.

### Main difficulties of biomarker research in ovarian carcinomas

Despite the rapid progress in the field of liquid biopsy technology, however, there is still a gap between research studies realized on cohorts of patients with ovarian cancers and clinical practice application of these findings.

Liquid biopsy is not a routinely used diagnostic test, due to the lack of uniform screening strategies between countries across the world. Although these procedures, could obtain valuable information about the quick identification of the clonal evolution of cancer cells and also monitorisation of the development of changes leading to drug resistance in the future, there are limitations in collecting, separating and analyzing the right biomarkers in the blood sample due to many altered cells involved in the carcinogenic process. Another concern raised by the application of liquid biopsy to cancer screening in the general population is made by false-positive results that could be obtained, because of the constant accumulation of genetic and epigenetic alteration in the microenvironment of the tumoral site and free circulation of diverse molecules that are evaluated through this technique [105,106].

Liquid biopsy assays are obtained by evaluation of large gene panels through whole-genome sequencing (WGS) or whole-exome sequencing (WES) data analysis of tumor tissue samples and also could be customized into personalized gene panel, but the effectiveness and potential economic impact of including this procedure into general health screening programs are not yet justified. For that, the public healthcare systems must establish collaboration with multiple stakeholders such as patient organizations, the regulatory and policymakers authorities to support economic cost and to establish the conditions of implementation and the medical and scientific community.





The development of multi-analyte panels correlates with analysis of patient history, clinical data and liquid biopsy marker profiles are essential to improve diagnostic accuracy [102-104].

## Conclusion and perspectives

Although many biomarkers have been studied over time, and some of them have been used to assess the status, progression and efficacy of the drug therapy in ovarian cancer, till now, is still no clear set of specific biomolecules which can be used as a reference standard in ovarian cancer detection or in evaluating therapy. In the last decade, liquid biopsy has evolved as a revolutionary technique by which diverse genomic and proteomic information about patients with cancers can be deciphered. The various biomolecules are separated by the technique of liquid biopsy can be both diagnostic and prognostic biomarkers in OC and can be used to predict tumorigenesis in ovarian cancers and other types of gynecological tumors. With the development of new technologies in the field of molecular biology such as NGS (next generation sequencing), biological samples obtained by liquid biopsy techniques can be processed, which provide data on specific mutations that may occur in target genes involved in cancer processes. This information is extremely useful both for the diagnosis, prognosis and screening of various types of cancer, as well as for predicting a patient's response or resistance to receiving treatments, which may allow early diagnosis of disease progression.

## Author contributions

Conceptualization, F.D., P. A.; methodology, F.D., F.A.G., D. C.; investigation, P. A., M. A.M. N., C.G.N, F. A. G., D. R. C. and. F.D.; writing—original draft preparation, P. A., F. A. G., M. A.M. N., C.G.N, D. R. C., F.D.; writing—review and editing, F. A. G., F.D., M. A.M. N., C.G.N., supervision F.D., P. A.; project administration, F. D., F. A. G., M. A.M. N.

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