Reducing Ovarian Cancer Mortality Through Early Detection: Approaches Using Circulating Biomarkers
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ABSTRACT

More than two-thirds of all women diagnosed with epithelial ovarian cancer (EOC) will die from the disease (>14,000 deaths annually), a fact that has not changed considerably in the last three decades. Although the 5-year survival rates for most other solid tumors have improved steadily, ovarian cancer remains an exception, making it the deadliest of all gynecologic cancers and five times deadlier than breast cancer. When diagnosed early, treatment is more effective, with a 5-year survival rate of up to 90%. Unfortunately, most cases are not detected until after the cancer has spread, resulting in a dismal 5-year survival rate of less than 30%. Current screening methods for ovarian cancer typically use a combination of a pelvic examination, transvaginal ultrasonography, and serum cancer antigen 125 (CA125), but these have made minimal impact on improving mortality. Thus, there is a compelling unmet need to develop new molecular tools that can be used to diagnose early-stage EOC and/or assist in the clinical management of the disease after a diagnosis, given that more than 220,000 women are living with ovarian cancer in the United States and are at risk of recurrence. Here, we discuss the state of advancing liquid-based approaches for improving the early detection of ovarian cancer.

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Introduction

Ovarian cancers are a heterogeneous group of malignancies arising from or involving the ovary and/or fallopian tubes (1). Ovarian cancers are broadly classified into non-epithelial ovarian cancer and epithelial ovarian cancer (EOC) and that the latter is attributed to the majority of ovarian cancer–related deaths (2). Overall, EOC is the fifth leading cause of cancer-related death among American women, making it the deadliest cancer of the reproductive system (3). EOC is a heterogeneous disease, comprising several histotypes (serous, ~70%; endometrioid, ~10%; clear cell, ~10%; and mucinous, ~3%–10%) with distinct epidemiologic, molecular, and clinical features. High-grade serious carcinomas (HGSOC) are estimated to be 50% to 60% of all ovarian malignancies and account for half of all EOCs.

EOCs are diagnosed predominantly at an advanced stage with widespread metastases throughout the peritoneal cavity (4–6). Manifestations of the disease are typically vague and do not become apparent until the disease is advanced and difficult to treat. Thus, ovarian cancer is caught at an early stage only in about one-fifth of all cases according to the National Ovarian Cancer Coalition. Furthermore, no reliable screening tests are currently available for ovarian cancer, and current diagnostic tools for early detection remain inadequate. Early detection of ovarian cancer at a localized stage (stages IA and IB) results in far better disease prognosis: according to the American Cancer Society (2019), the projected 5-year survival rate for these patients is about 92%. However, only 15% of all ovarian cancers are successfully diagnosed at this stage. Patients with advanced-stage HGSOC have a worst prognosis (~32% and ~15% for 5- and 10-year survival, respectively) compared with patients diagnosed with early-stage HGSOC (~71% and ~53% for 5- and 10-year survival, respectively); ref. 7. Moreover, 30 years of statistics gathered by the NIH Surveillance, Epidemiology, and End Results (SEER) Program indicate that approximately 70% of patients diagnosed with ovarian cancer will experience disease recurrence after initial benefit from chemotherapy (8). These statistics clearly emphasize the need to develop tools to detect ovarian cancer at an earlier stage.

The two most commonly used screening tests in the clinic are transvaginal ultrasound (TVUS) and blood tests for elevated cancer antigen-125 (CA125) protein levels. It is difficult to distinguish benign from malignant tumors by TVUS and, hence, the need for more invasive biopsies. On the other hand, CA125 protein levels are typically not elevated in the serum of up to 50% of patients with stage I ovarian cancer; thereby, it is not as reliable for early detection (9). Also, blood tests quantifying CA125 protein level are not specific to ovarian cancer, as CA125 elevation can be potentiated by many factors (10). Unlike breast cancer in which a biopsy can be performed for diagnosis, EOC screening requires invasive surgery to make
a definitive diagnosis (11, 12) and therefore false-positive surgeries must be limited to at most 10 per screen-detected cancer, that is, a positive predictive value (PPV) exceeding 10%, which for the patient is quite unacceptable. Thus, the low prevalence of this cancer in the general postmenopausal population (1 in 2,500) requires an effective screening strategy to have a high sensitivity for early-stage disease (>75%) and a very high combined specificity (99.6%) to achieve a PPV exceeding 10% (11–19). When CA125 is interpreted longitudinally with the risk of ovarian cancer algorithm (ROCA) referring 2% annually at highest risk to transvaginal ultrasound (TVUS), this “2-stage screening strategy” achieved a combined specificity of 99.8%, a 22% PPV—greater than the 10% lower limit, and detected an increased but still modest proportion of EOCs in early stage (11, 20, 21). In a 2017 landmark study, the United Kingdom Collaborative Trial of Ovarian Cancer Screening compared several screening approaches in more than 200,000 postmenopausal women (median follow-up of 11.1 years): (i) serum CA125 level evaluated using ROCA (50,640 women), (ii) annual TVUS screening (50,639 women), or (iii) no screening (101,359 women). Unfortunately, like other ovarian cancer screening studies primarily focused on measuring serum CA125 levels (22–27), this study did not show a significant decrease in ovarian cancer mortality (28). This clinical problem highlights the urgent need to expand the class of liquid-based biomarkers to detect a substantially greater proportion of cancers in early stage, while maintaining a 98% annual specificity for referral to imaging (e.g., TVUS).

**Liquid-Based Biopsies: Can These Developing Molecular Tools Be Used to Improve Early Detection of Cancer?**

Pathologic analysis of tumor tissue biopsies has been the gold standard in the initial diagnosis of cancer, but liquid biopsies, which analyze tumor-derived material circulating in the bloodstream and other bodily fluids, are rapidly gaining traction in the clinic. Liquid biopsy is a minimal or noninvasive technology that detects molecular biomarkers using liquid sample without the need for costly or invasive procedures. These tests have considerable potential in oncology, e.g., for early detection of cancer, treatment and recurrence monitoring, and as surrogates for traditional biopsies with the purpose of predicting response to treatments and the development of acquired resistance. A liquid biopsy can provide information about the genetic landscape of all cancerous lesions (primary and metastases) as well as offer the opportunity to systematically track genomic evolution. The liquid biopsy biomarker types are primarily segmented into circulating tumor cells (CTCs), cell-free DNA (cfDNA), and extracellular vesicles (EV; Fig. 1). The discovery of CTCs dates back to the 1860s, when cells that were morphologically identical to the tumor were identified in the blood of a patient with metastatic cancer (29). Their potential significance was not fully realized until the late 2000s, when the number of CTCs in the bloodstream were shown to have prognostic significance in various different tumor types, for example, metastatic breast, metastatic castration-resistant prostate, and metastatic colorectal cancer (30). Another way that tumors release biomarker information is through tumor cell necrosis and the release of dead cells or cell fragments. These cells are engulfed by phagocytes that process the tumor cell DNA into small fragments (160–180 base pairs in length) of nucleic acids, which is then released into the bloodstream (31, 32). Although levels of tumor DNA have been shown to mirror tumor burden, interpretation is often complicated by the presence of other cfDNA derived from non-tumor cells. Therefore, a number of highly sensitive methods have been developed to detect aberrations found in circulating tumor DNA (ctDNA), including mutation, amplification, chromosomal rearrangement, and hypermethylation. A third biomarker, which has been known for years, has reemerged and is showing great promise. This member of the EV family, known as exosomes or small EVs, are nano-sized vesicles (30–150 nm) of endocytic origin, which are produced and released by most cell types under normal physiologic and in diseased states (33, 34). Once considered little more than garbage cans whose job was to discard unwanted cellular components, recent discoveries have sparked considerable interest in exosomes as circulating biomarkers. Exosomes are informative molecules that carry cargo representative of their originating cell including nucleic acids, cytokines, membrane-bound receptors, and a wide assortment of other, biologically active lipids and proteins (35–38). This cargo remains functional upon entry or fusion with a recipient cell, thus exosomal transfer is now considered an important form of cell–cell communication in normal and pathologic states, such as cancer. Because exosomes can travel systemically throughout the body, efforts are underway to exploit them as potential biomarkers to detect and monitor disease states. In this review, we will focus on the current methods and advancements using liquid-based biopsies that have shown promising potential in the early detection of ovarian cancer.

**Circulating Tumor Cells**

First observed in 1869 by Thomas Ashworth in the blood of an individual with metastatic disease, CTCs have long been thought to be an important component of tumor dissemination (29, 39, 40). CTCs are cancer cells that have detached from the tumor and are found at extremely low levels in the bloodstream. Modern research on CTCs has focused on CTC enumeration and characterization. Some speculate that tumor cell shedding is an early event in tumorigenesis and might be useful for early detection, but few studies have shown utility in this clinical setting. As a blood-based, FDA-approved biomarker, CTCs were thought useful for determining the prognosis of patients with metastatic breast, colorectal, and prostate cancers (41–45). As the only FDA-cleared device for the enumeration of CTCs in whole blood, CellSearch (Menarini Silicon Biosystems, Inc.) was originally used for the evaluation of CTC
numbers in patients with cancer to help predict prognosis and overall survival (30). However, clinical enumeration of these circulating cells has become less useful and costly, due to the fact CTC tests are rarely reimbursed by insurance. Regardless, the interest in CTCs continues and many different platforms for CTC isolation have been developed for research as well as clinical use in recent years (46). Nevertheless, CTC isolation and characterization remain technically challenging and progress has been hampered by the difficulty in rapidly and effectively isolating pure populations of CTCs using liquid biopsy.

The three main categories of isolation techniques are immune-affinity–based methods, size-/density-based methods, and microfluidic device–based methods (ref. 47; Fig. 2). The earliest approaches utilized positive and negative selection for common CTC surface antigens or organ-specific markers to differentiate CTCs from other cell types. In these studies, researchers used antibody-coated beads to positively select CTCs from the cellular milieu or deplete the milieu of other cell types with a negative selection marker. EpCAM, a cell adhesion protein found on epithelial cells, is often used as a positive selection marker for CTCs (48). The most common negative selection marker is CD45 because it is found only on cells of hematopoietic origin. First reported in 2000, the ISET (isolation by size of epithelial tumor cells) system is based on cell size filtration and has been shown to effectively recover CTCs for diagnostic cytopathologic analysis (49). However, smaller CTCs less than 8 μm in diameter can be lost using this method, due to the selective pore size. Microfluidic devices offer several attractive advantages for CTC-based studies, such as continuous sample processing using a small volume to reduce loss and integration of downstream analyses on the chip itself. These devices can also reduce hands-on time and increase consistency of heterogeneous CTC profiles (50). Some microfluidic devices integrate existing CTC isolation techniques within their design, such as the size-dictated immunocapture chip (SDI-Chip; ref. 51). The SDI-chip is composed of two mirrored anti-EpCAM antibody-coated micropillar arrays that selectively enhance the interaction of CTCs with the selection antibodies, resulting in efficient capture of CTCs based on the principle of deterministic lateral displacement (52). The disadvantage to the EpCAM-based capture is that not all CTCs express EpCAM (53). In an effort to move away from the bias introduced by affinity-based isolation, researchers have begun to focus on label-free surface expression–independent

Figure 1.
A graphical representation of a variety of approaches ovarian cancer researchers can take to evaluate blood-based liquid biopsies for cancer-associated biomarkers to aid in early detection of malignancy.
microfluidic devices to isolate CTCs. A label-free approach is optimal because CTCs are very heterogeneous and fluid in their gene expression, making any affinity-based approach biased toward cells expressing a certain marker. Several label-free CTC separation techniques have arisen in recent years including a microfluidic flow fractionation approach, acoustic-based separation, and deterministic lateral displacement separation (hence the name) of 56 sharp turns, which results in size-based enrichment of CTCs depleted of hematopoietic cells. Improvements can be made on the purity of resultant CTCs by double Labyrinth separation. Labyrinth output can even be used with various downstream single-cell analysis tools including BioMark Real-Time PCR System (Fluidigm), DEPArray (Silicon Biosystems), and droplet digital PCR (Raindance).

CTC enumeration data can provide only limited insight into the underlying biology of malignant disease. More recent research efforts have been focused on the molecular characterization and functional analysis of CTCs. Using less than 1 mL of blood, Racila and colleagues demonstrated that 12 of 14 patients with clinically organ-confined breast cancers and 3 of 3 patients with organ-confined prostate cancers had excess epithelial cells detected in their blood (57). However, the diagnostic utility of CTCs in ovarian cancer has been decidedly underwhelming. In the first studies led by us, CTCs (as circulating endothelial cells/circulating endothelial progenitors) were found to be rare in patients with ovarian cancer and were not overly informative when evaluated along with activity of molecular targeted therapies (58, 59). In a 2018 study, CTCs were detected in only 5 out of 29 patients (17.2%) with proven primary ovarian cancer (Table 1). CTCs were shown to be more prevalent in patients with metastases to the ovary, rather than in primary ovarian cancer (43). Although CTCs may not be useful as early detection biomarkers, CTC status in ovarian cancer is associated with overall survival and progression-free survival according to a recent meta-analysis of 11 publications and 1,129 patients (60).

Circulating Tumor DNA

It was reported for the first time in 1977 by Leon and colleagues that patients with cancer have increased levels of

![Figure 2.](image)

Examples of methods to capture circulating tumor cell from whole blood.

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<th>Selection parameters</th>
<th>Case size</th>
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Note: Pairs of sensitivity and specificity in this table are not directly comparable with each other because they are calculated on the basis of different cutoffs throughout.

*Sensitivity (Se) and specificity (Sp) pair values listed correspond to a specific decision cutoff utilized in each study.
free DNA in their blood serum (61). Cell-free DNA broadly refers to all circulating DNA, whereas ctDNA is specifically tumor-derived as the name suggests. Unfortunately, typical DNA isolation methods do not distinguish DNA by its origin, so scientists have developed several strategies to increase the proportion of ctDNA isolated from serum or plasma. Enrichment for ctDNA is optimal because it increases the likelihood of uncovering biomarkers specific to the cellular heterogeneity of the tumor. Apoptotic DNA fragmentation is thought to be the main source of ctDNA due to its average size of 70 to 200 base pairs, which corresponds to the length of DNA around a nucleosome (62). The amount of ctDNA can be highly variable. ctDNA can comprise approximately 0.01% ± 90% of total circulating DNA (63–65). Even so, ctDNA has been used to detect tumor-specific mutations, loss of heterozygosity, DNA integrity, microsatellite alterations, and epigenetic alterations (62, 66). Importantly, ctDNA can be used to quantify the level of disease burden and reveal the genomic landscape of the tumor. Researchers have estimated that the half-life of ctDNA ranges from 16 minutes to 2.5 hours, based on the degradation kinetics of DNA in circulation (67–69). This makes ctDNA a temporal snapshot of tumor status. Even with this short half-life, promising results have been shown in colorectal cancer, in which >60% of early-stage tumors can be detected through sensitive mutational analysis of DNA fragments in plasma (64). Figure 3 depicts typical analysis methods used to evaluate ctDNA and its required sensitivity as well as various applications.

As we move toward the future, more and more advancements are taking place to increase the sensitivity of current DNA-based analysis methods such as real-time PCR, microfluidic digital-drop PCR, BEAMing, Safe-SeqS, CAPP-Seq, and other next-generation sequencing techniques (70). In a recent retrospective study, researchers determined that detection of mutations in TP53 using high-grade serous ovarian carcinoma patient plasma has the potential to assess clinical prognosis and response. This study also demonstrated that the amount of ctDNA correlated with tumor volume as determined by three-dimensional (3D) volume reconstruction from CT images (71). Overall, all patients with cancer evaluated in this study with a tumor volume greater than 20 cm³ had reliably detectable TP53 mutations; however, the role of ctDNA mutation screening for early detection remains unproven. Using a mathematical model, researchers postulate that tumors in the millimeter diameter range can only be detected using secreted blood biomarkers under ideal conditions of extremely high rates of biomarker secretion and essentially zero background from healthy cells (72, 73). Thus, very sensitive technology is necessary to detect ctDNA from early-stage ovarian cancer.

Epigenetic changes can also be reliably detected in ctDNA. Aberrant methylation in circulating DNA has been shown in many cancer types (74). Cancer cells use promoter methylation to deregulate gene expression and can be informative as a noninvasive biomarker. The hypermethylation of important DNA repair genes is often an early step in carcinogenesis. In 2011, Liggett and colleagues determined that the methylation of three promoters differenitiated patients with ovarian cancer from healthy controls with a sensitivity of 90% and a specificity of 86.7% (75). These methylated genes, RASSF1A, CALCA, and EP300, are associated with tumor suppression, calcium regulation, and histone acetylation, respectively. In addition, promoter methylation of RASSF1A and PGR-PROX was

Figure 3.
Sensitivity, methods, and applications for circulating tumor DNA analysis. Overview of the most common applications and techniques for ctDNA analysis. ctDNA represented by red DNA strands in blood collection tubes (adapted from ref. 102).
informative for distinguishing ovarian cancer from benign ovary disease (sensitivity 80%, specificity 73.3%; ref. 75). Methylation patterns found in ctDNA have even shown the potential to diagnose a subset of ovarian cancers up to 2 years in advance of elevated CA-125 levels (Table 1; ref. 76).

It is thought that solid malignant neoplasms shed DNA into the circulatory system most often by necrosis, rather than apoptosis as in normal tissue (77). Necrosis typically results in larger and less uniform DNA fragments than apoptosis. Because of this, DNA integrity is increased in cancer and can be analyzed using ctDNA. Using the most common mobile element in the human genome, Alu repeats, researchers can derive a DNA integrity index that can differentiate patients with cancer from healthy individuals by isolating circulating DNA from plasma. This approach was used by Wang and colleagues to establish a cutoff index value of 0.59, meaning all samples above this index are likely malignant (77). This study demonstrated inadequacies in sensitivity of the assay (62%); however, specificity was 100% (Table 1). DNA integrity analysis represents a less-expensive and simpler alternative to DNA sequencing.

**EVs in Ovarian Cancer**

Extracellular vesicles compared with CTCs and cfDNA are considered a relatively new class of cancer biomarker. EVs are secreted by cells as a form of intercellular communication. These EVs can shuttle nucleic acids, lipids, and proteins from their cell of origin to surrounding cells to regulate the function of other cells (78). EVs are classified according to size (from a few nanometers to a few micrometers) and subcellular origin (79). A subtype of EVs, termed exosomes, is endocytic in origin and includes 60- to 80-nm small exosomes (Exo-S), as well as 90- to 120-nm large exosomes (Exo-L; ref. 80). In general, the term "exosomes" is broadly used to refer to a heterogenous mixture of small EVs (sEVs) that are less than 200 nm in size; this is due to the fact that widely used purification methods (such as differential ultracentrifugation) cannot definitively isolate EV class based on subcellular origin (81).

The role of exosomes in cancer metastasis has been demonstrated in various cancer types (82–85), establishing a rationale for exploring exosome-based diagnostics. In the ovarian cancer context, our laboratory has shown that tumor-derived exosomes from CP30 and CP70, which are both platinum-resistant ovarian cancer cell lines, imparted platinum resistance to a platinum-sensitive cell line (A2780; ref. 86). In another in vitro study, EOC cells were shown to secrete exosomes that affected the phenotype of mesothelial cells of the peritoneum via transfer of CD44, a cell surface glycoprotein that plays a role in cell adhesion and migration. The mesothelial cells, in turn, increased CD44 surface and MMP-9 secretion, resulting in the degradation of the extracellular matrix and promoting ovarian cell invasion (87). Collectively, these studies suggest that exosomes represent a general mechanism by which one cell type can modulate the phenotype and characteristics of neighboring cells.

Ovarian tumor–derived EVs are not only rich in proteins but these contain miRNAs. miRNAs are small noncoding RNAs that can target messenger RNA thereby altering gene expression. miRNA profiling of EpCAM-captured exosomes from ovarian cancer patients’ serum identified miR-21, miR-141, miR200a, miR200b, miR-200c, miR-203, miR-205, and miR-214 to be elevated compared with controls. A high expression of let-7 has been correlated to ovarian cancer cell invasiveness in SKOV3 (88). It has been demonstrated that miR-1246 expression in ovarian tumor–derived exosomes can aid tumor growth by downregulating Cav1 expression, thereby increasing P-glycoprotein expression in infiltrating pro-tumorigenic immune cells (M2-type macrophages; ref. 89). This makes the tumor microenvironment favorable because this confers drug resistance to paclitaxel (89). In addition, other studies have shown that miRNAs from ovarian cancer tumor–derived EVs can confer drug resistance through other pathways (82, 90–92).

Because there are various evidence of tumor-derived EVs contributing to the progression of ovarian cancer (87, 93, 94), it is logical to assess the potential of EVs for diagnostics. Recently, several technologies have been developed to capture exosomes from minute volumes of starting material to surpass the problem of requiring larger sample volumes when using conventional methods such as ELISA and immunoblotting (Table 2). Technologies such as microfluidic chips and the nano-plasmonic exosome (nPLEX) platform can be conjugated to antibodies to allow for capture and profiling of exosomes. One study has assessed exosomes derived from ascites of patients with ovarian cancer using the nPLEX platform (95). Initially, molecular profiling of antibodies was used to coat the surface of nanoholes using selected ovarian cancer markers based from literature. Ovarian cell lines and exosomes derived from the same cell lines were then profiled. The authors found that the combination of EpCAM and CD24 can distinguish ovarian tumor–derived exosomes with a detection accuracy of 97%. Furthermore, it was found that these two markers are highly expressed in the ascites of patients with ovarian cancer (n = 20) compared with the control population (n = 10), which consisted of ascites from nonmalignant conditions (95). These findings correspond with a previous report that identified that EpCAM and CD24 are found in exosomes of cultured cell lines and malignant ascites (96). Our laboratory was the first to demonstrate the capture of exosomes directly from the plasma of patients with ovarian cancer using a microfluidic chip (97). This was followed by the development of a microfluidic chip called ExoSearch (Fig. 4) by Zhao and colleagues (2016), wherein exosomes from plasma of healthy controls and patients with ovarian cancer were captured using anti-CA-125, anti-EpCAM (Epithelial Cell Adhesion Molecule), and anti-CD24 (cluster of differentiation 24 or heat stable antigen CD24; ref. 98). In this study, it was...
observed that exosomal CA125 and EpCAM may be used to discriminate patients with ovarian cancer from the healthy controls (98). Most recently, our group together with Zhang and colleagues (2019) has reported the development of an ultrasensitive analysis of exosomes using a 3D nanostructured herringbone chip (nano-HB; ref. 99). In this small case–control study, we report that exo-folate receptor alpha as well as exo-EpCAM and exo-CD24 could be detected in small volumes of plasma samples (as little as 2 μL) and could significantly distinguish patients with ovarian cancer from cancer-free controls (area under the curve = 1; Fig. 5; ref. 99). While these types of devices have yet to be assessed under the rigor of clinical trials or in longitudinal samples from asymptotic patients who later develop cancer, these devices clearly hold promise.

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Note: Pairs of sensitivity and specificity in this table are not directly comparable with each other because they are calculated on the basis of different cutoffs throughout.

*As reported by each study.

Conclusion

Liquid-based approaches to detect cancer, although gaining traction, are not new. Complete blood cell counts, including white blood cells, red blood cells, and platelets, are used to help diagnose blood disorders, including leukemia and lymphoma. Prostate-specific antigen is used to help diagnosis prostate cancer, whereas calcitonin, α-fetoprotein, and human chorionic gonadotropin are used to diagnosis medullary thyroid cancer, liver cancer, and germ cell tumors, such as testicular cancer and ovarian cancer, respectively. Liquid-based biopsies are likely here to stay and as technologies improve so will their clinical utility. These types of assays, which are considerably less invasive when compared with the tissue biopsy procedure, will help further advance personalized targeted therapy and...
immunotherapy by offering a source of easily obtainable material for mutation analysis. Liquid biopsies also have the potential to be useful in efficacy assessment, especially if imaging cannot be used or the interpretation is problematic, and in real-time monitoring of molecular profiles and clonal evolution in patients undergoing cancer therapy to detect metastatic relapse or metastatic progression as well as mechanisms of resistance.

Figure 5.
A, Schematic of the 3D nanostructured herringbone chip (nano-HB), method of nano-HB chip fabrication (B), a nano-HB chip with magnified SEM images in the middle (C), and testing clinical samples on the nano-HB chip (D, left). CD24, EpCAM, and FRα on exosomes derived from the plasma of patients with ovarian cancer (n = 20) and control population (n = 10) were quantified. Error bars, SD (n = 3). E, Plot of the exosomal protein concentration captured on the nano-HB chip versus ovarian cancer staging, with stage I/II considered as early and stage III/IV as advanced (reprinted with permission from ref. 99). BKG, Background; CSA, colloidal self-assembly; SEM, scanning electron microscopy.
Even with the growing excitement surrounding this area of translational research, significant improvements in liquid biopsy platforms and techniques will be required to advance the promises of precision cancer medicine. At present, a liquid biopsy test in itself cannot simply replace the gold standard tissue biopsy–based test; they primarily serve as a complimentary test. As such, there are several remaining challenges impeding the wider adoption of liquid biopsy–based clinical tests. First, it is important to determine the level of sensitivity of a given liquid-based assay. It is well established that CTCs, ctDNA, and tumor-derived EVs are relatively rare compared with the number of “other molecules” found in a blood or bodily fluid sample; therefore, major hurdles remain to improve the test’s detection ability. Furthermore, it is not clear if a given test can accurately dissect the heterogeneity of a tumor and identify the “bad actors” among the other tumor subclones. Second, the majority of liquid biopsy assays lack extensive clinical validation resulting in limited reimbursements and utilization of these next-generation tests within the medical community (100). It is, therefore, essential to rigorously validate and demonstrate to both providers and payers the value of liquid biopsies in the clinical setting before they impact clinical practice and are routinely reimbursed. As we move toward the future, the technologies supporting the development of liquid biopsy assays will continue to improve and in turn the sensitivity and specificity of the test. Currently, strides are being made in improving single CTC capture approaches and platforms. With the adoption of digital drop PCR, researchers are able to increase the sensitivity of detection of low-abundance mutations in the ctDNA (101). Microfluidic technologies are also proving suitable for isolation of low-abundance cancer-associated EVs/exosomes (99).

In summary, developing effective screening tests for early detection of ovarian cancer remains one of the most significant unmet needs in the diagnosis and treatment of this disease. It is vital to identify new liquid-based tests and to develop an effective strategy to identify ovarian cancer at its earliest stages so that women stand the best chance for successful treatment and improved survival. To implement a population-wide ovarian cancer screening strategy, there must be evidence that the test is both sensitive enough to detect the cancer early and specific enough not to cause harm to healthy people. It is clear that when cancer, any cancer, is detected late, that is, after it has metastasized to other parts of the body, the outcomes are generally grim. These new liquid-based platforms represent potentially valuable clinical tools to evaluate various circulating biomarkers at a specificity and sensitivity necessary to detect ovarian cancer while it is still localized. In the next decade, liquid-based biopsies will become important clinical tools for cancer screening, diagnosis, prognosis evaluation, drug response predictions, and disease monitoring. However, the analytical and clinical utility of such tools must be rigorously established before this can be applied. Successful “bench to bedside” transition will require extensive clinical validation, which demonstrates a significant decrease in the mortality of women diagnosed with ovarian cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


29. Ashworth T. A case of cancer in which cells similar to those in the tumours were seen in the blood in the after death. Australasian Med J 1869;112:4970–5.


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