Langenbeck's Archives of Surgery (2019) 404:517–525
https://doi.org/10.1007/s00423-019-01788-9

REVIEW ARTICLE

Liquid biopsy for the detection and management of surgically resectable tumors

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Received: 25 February 2019 / Accepted: 14 April 2019 / Published online: 5 August 2019

Abstract

Background Traditional biopsies have numerous limitations in the developing era of precision medicine, with cancer treatment that relies on biomarkers to guide therapy. Tumor heterogeneity raises the potential for sampling error with the use of traditional biopsy of the primary tumor. Moreover, tumors continuously evolve as new clones arise in the natural course of the disease and under the pressure of treatment. Since traditional biopsy is invasive, it is neither feasible nor practical to perform serial biopsies to guide treatment in real time.

Purpose The current manuscript will review the most commonly used types of liquid biopsy and how these apply to surgical patients in terms of diagnosis, prediction of outcome, and guiding therapy.

Conclusions Liquid biopsy has the potential to overcome many of the limitations of traditional biopsy as a highly tailored, minimally invasive, and cost-effective method to screen and monitor response to treatment. However, many challenges still need to be overcome before liquid biopsy becomes a reliable and widely available option.

Keywords Cancer · Liquid biopsy · Traditional biopsy · Circulating tumor cells · Circulating tumor DNA · Precision Medicine

Introduction

The diagnosis and management of cancer often rely on the information provided by biopsy of a suspicious lesion. This implies removing cells or tissues from the primary or metastatic mass for analysis. In a traditional biopsy, the specimen is obtained from the primary tumor or a metastatic site by means of a biopsy needle (core needle or fine needle aspiration) or surgical procedure (incisional biopsy or excisional biopsy). This type of traditional biopsy is still the mainstay for diagnosis of invasive cancer over that of a benign non-neoplastic mass or a precursor lesion, confirmation of tumor type in cases of uncertainty, determination of cancer subtype, and source of tissue for molecular analysis. Despite the useful information provided by a tissue diagnosis, these are invasive interventions that provide limited information regarding tumor biology and expose patients to procedural risks.

The current use of traditional biopsy in the management of cancer is highly variable and depends on tumor type. For example, the diagnosis of most solid pancreatic tumors can be made with a high degree of certainty based on clinical presentation and high-quality imaging alone. In this regard, a tissue diagnosis is not required prior to surgical resection of localized cancers and a biopsy provides very little useful information in terms of biomarkers that direct management. Currently, the main use of a biopsy in patients with pancreatic cancer is for the establishment of a tissue diagnosis required for those patients being considered for neoadjuvant therapy. This is in contrast to the management of localized breast tumors in which a biopsy is necessary to differentiate benign from malignant lesions, type of precursor lesion, and the status of clinically important biomarkers such as receptor status. Molecular analysis performed through immunohistochemistry, DNA mutational analysis, and RNA expression profiling may yield important information that has the potential to impact treatment. Such is the case of HER2 neu positive lung cancers [1] or estrogen/progesterone-receptor positive breast cancers [2].

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A traditional biopsy is a "snapshot" in time, a one-time event in the course of cancer treatment. Since traditional biopsy is invasive, it is neither feasible nor practical to perform serial biopsies to guide treatment in real time. In contrast, tumors continuously evolve as new clones arise in the natural course of the disease and under the pressure of treatment. Most tumors consist of multiple cellular clones and each has the potential for a unique biological behavior. This poses a big challenge in the choice and monitoring of therapy, as an effective treatment might be introduced early with significant initial response, but failing to maintain results as resistant clones survive and multiply. Tumor heterogeneity also introduces risk of sampling error with the use of traditional biopsy. The inability to detect the aggressive clones, among all clones, that drive outcome has clinical implications in terms of guiding management in a precision approach. The development of methods for global genetic analysis, expression profiling, and protein expression has led to the identification of novel biomarkers that have the potential to direct therapy. It has also reinforced the understanding that a single disease process such as cancer or genetic diseases might find its roots in a plethora of mutations in a variety of genes that define the course of the disease and determine different responses to treatment among patients. This realization has paved the road to the emergence of so-called "precision medicine", which strives to provide treatment based on an individual's unique tumor biology. The application of targeted therapies is made possible by the identification of genomic-level aberrations that characterize a subpopulation of patients within a disease process that will benefit from them [3]. These developments in the understanding of tumor biology have changed the role and mechanism of biopsy.

Liquid biopsy refers to the analysis of any bodily fluid for cellular or molecular markers of cancer. It has the ability to overcome limitations of traditional biopsy and further advance the understanding of tumor biology. It provides the opportunity to gain access to tumor cells through a minimally invasive method such a blood draw, with little discomfort and virtually no risks. This advantage goes beyond patient comfort and safety; it enables tumor characterization in patients in whom biopsy might have been impossible previously: frail patients, technically challenging, or inaccessible location. It may help prevent delays in diagnosis and treatment in these patients. Moreover, as opposed to traditional biopsy, liquid biopsy is amenable to real-time analysis with multiple samples over time to monitor tumor progression and response to therapy. Finally, it has the potential to represent the biomarkers of all clones of the primary, metastatic deposits, and subclinical disease.

The current manuscript will review the most commonly used types of liquid biopsy and how these apply to surgical patients in terms of diagnosis, prediction of outcome, and guiding therapy.

Current methods of liquid biopsy for cancer

Liquid biopsy is the procurement of any bodily fluid for analysis of cellular or molecular markers of cancer. Most commonly used fluids to perform liquid biopsy include saliva, urine, blood, plasma, and serum. The common use of these is based on the minimally invasive method and ease of collection of the specimen. However, other tissue fluids such as cerebral spinal fluid, cyst fluid, enteric secretions, bile, pancreatic juice, and nipple secretions, among others, are amenable to liquid biopsy. The most globally important liquid biopsies for cancer patients are derived from blood, plasma, or serum and will be the focus of this review.

The ability to perform liquid biopsies for the management of cancer is based on the principle that either cells or molecular markers unique to the tumor are found in the plasma. These include intact cells, free DNA, RNA, and proteins. Currently, the best studied forms of liquid biopsy are circulating tumor DNA (ctDNA) and circulating tumor cells (CTC). We will focus our attention on these two techniques.

(1) Circulating tumor DNA

Circulating tumor DNA represents short cell-free DNA fragments released into blood by primary tumor or metastatic sites. Both normal and cancer cells release free DNA into circulation by secretion or as a consequence of cell death (necrosis and apoptosis) [4, 5]. This occurrence was first described in 1948 [6], but the ability to identify cancer-specific circulating tumor DNA within a larger population of normal circulating DNA has only recently become feasible through the development of next-generation sequencing.

Circulating tumor DNA can be physically separated from total circulating DNA from normal cells based on size—ctDNA fragments tend to be shorter (~ 166 bp). More commonly, affinity column, magnet, or polymer-based methods are employed [7–9]. Once ctDNA is isolated, whole genome amplification (WGA) methods may be performed to amplify genomic material for further analysis. Genome analysis technologies include untargeted methods for mapping and identification of new aberrations harbored in the genetic material under study as well as targeted technologies to screen for known mutations [10–12]. In addition, the evaluation of DNA fragmentation and methylation patterns was introduced as a relevant method in the detection of cancers [13].

(2) Circulating tumor cells

Cells shed from tumors find their way into circulation and are presumed to be one mechanism of systemic cancer spread [14]. These so-called circulating tumor cells (CTC) are released from primary tumor or metastatic sites. A small subset of CTCs has been shown to have the ability to invade,

intravasate, migrate, and survive in plasma environment. They may be found in circulation as single cells or in clusters [10]. These cells are the probable source of metastatic lesions; hence, they provide the potential for direct assessment of tumor biology [15]. They were first described by Thomas Ashworth in 1869 following identification of cells that resembled those in the primary tumor in post-mortem blood samples [16]. Many current research efforts are focused on optimizing identification and processing of these cells in bodily fluid samples.

CTCs can be isolated through a variety and combination of methods. Enrichment and selection can be broadly categorized as antibody-dependent and non-antibody-dependent-based methods. In the non-antibody-based techniques, CTCs are separated taking advantage of their physical properties.

The use of antibody-based methods for the isolation of CTCs is based on the use of cancer-specific antibodies or antibodies directed at cell-surface antigens of non-hematological cells. Epithelial markers are usually employed for lung and gastrointestinal cancers. Common antigens include epithelial cell adhesion molecule (EpCAM, membrane protein) and cytokeratins (cytoskeletal proteins). Mesenchymal markers such as N-cadherin (membrane protein) and vimentin (cytoskeletal protein) have been introduced as target markers as well in an effort to identify cells that have undergone epithelial to mesenchymal transformation (EMT)—now known to be a critical step for dissemination. Those cells would otherwise risk being missed leading to false negative results [17–20]. Following this first step for isolation, negative enrichment is used to deplete the sample from normal leukocytes by identifying other markers such as antibodies against CD45 [17].

Great efforts have been made to develop platforms that will optimize this process, making it more widely available and cost-effective. One of them, CellSearch, has gained FDA approval in 2004 for breast, prostate, and colorectal cancer. It uses EpCAM antibody-coated ferromagnetic beads to enrich CTCs followed by confirmation through CK, CD45 and DAPI staining with subsequent removal of leukocytes [21, 22]. Other promising platforms include AdnaGEN, RosetteSep, IsoFlux, and HB_Chip [15]. Another technology worth mentioning is GILUPI cell detector, which was developed to enable enrichment directly from arm vein, providing the opportunity to process larger blood volumes with the isolation of more CTCs as a result [23].

Physical properties utilized for enrichment include cell size, density, deformability, and electric charges. These are usually more inexpensive and faster [24]. They also have the ability to identify cells that underwent EMT in addition to those which continue to express epithelial markers. Thus, false negative rates are lower at the expense of higher false positive results. Available technologies such as Dean Flow Fractionation are based on cell size [25], while others like ApoCell/ApoStram or CellCare take advantage of CTCs electrical properties and compressibility, respectively [26, 27].

Once a pool of CTCs has been isolated, not only the cell itself, but its DNA, RNA, and proteins become available for analysis of relevant mutations and molecules that might become target of therapeutic agents. Genome analysis follows the same principles as with ctDNA with the added need for extraction of the genetic material. Regarding RNA and proteins, they allow for a more functional profiling of tumor cells. A single cell provides multiple copies of mRNA for analysis through single-cell RNA sequencing technologies: Smart-Seq2, FISSEQ, Cyto-Seq [28–30]. A relevant advantage of protein analysis relies on the fact that it allows for direct evaluation of potential therapeutic targets. EPISPOT (epithelial immune SPOT) assay is a test designed to identify proteins secreted by viable tumor cells with the aid of immunofluorescence microscopy. Finally, CTCs offer the possibility to conduct functional analysis through CTC culture and xenografting; these techniques are still under development [10].

Both ctDNA and CTCs offer advantages and pose unique challenges in the characterization of patients' tumors. Despite recent developments, most challenges are due to the limited sensitivity and specificity in current isolation and sequencing methods. Hence, its limitations may become less significant as these technologies continue to evolve.

CTC vs. ctDNA

Circulating tumor DNA represents an average of all clones of the primary tumor and metastatic sites, providing a more complete understanding of the tumor genome status than CTC or traditional biopsy [31, 32]. The large volume of circulating genetic material makes this a more sensitive marker than CTCs: ctDNA is found in more patients and in higher concentration than CTCs, with a proportion estimated at 50 to 1 [8]. Nonetheless, ctDNA represents only a small percentage of free plasma DNA, which is mainly derived from normal hematopoietic cells and subject to fluctuation with physical activity, pregnancy, infection [33]. For this reason, isolation of sufficient ctDNA still requires analysis of large blood volumes. In addition, ctDNA allows for the identification of driver gene mutations, but it is not tissue-specific, making it a less than ideal screening tool. Moreover, the half-life of ctDNA is about 16 min, but can be affected by renal function and lead to false positive results if used for screening in patients with renal dysfunction [34]. Finally, it mostly represents the genome of dying cells, not those that are currently dividing and potentially homing new mutations that could anticipate future resistance patterns or therapeutic targets.

On the other hand, CTCs not only provide DNA for analysis, but also RNA and proteins. This is why the isolation of CTCs provides the opportunity to identify more markers and potential therapeutic targets. They are also a direct

manifestation of "relapse", permitting direct assessment of treatment failure. Despite these advantages, CTC analysis faces multiple challenges. Compared to ctDNA, CTCs are less prevalent in plasma, making it less sensitive both for screening purposes and tracking of the evolution of disease. This is particularly relevant in localized disease for which intervention is the most effective and, in some cases, the only curative chance. In addition, each CTC has the potential to represent a unique clone present in the tumor, and as such, is not an "average" of all mutations as is the case with ctDNA. As single clones, CTCs do not reflect tumor heterogeneity as accurately as ctDNA and the limited amount of DNA isolated from them makes amplification methods necessary, introducing the risk of amplification bias [35]. Last, the recognition of the role of epithelial to mesenchymal transition in tumor cell migration and survival in circulation, makes most currently available methods for isolation, based on epithelial markers, insufficient [17].

For the reasons discussed above, there is not currently one method that is superior to the other. The information provided by ctDNA and CTCs is complementary and the isolation and analysis of both provides the opportunity for a more accurate and extensive understanding of tumor biology in general and potential therapeutic options and prediction of outcome in an individual patient.

Liquid biopsy for use in diagnosis and screening

For many cancers, the five-year cancer survival has increased significantly in the past three decades. These advancements can be explained by novel therapies and earlier detection. However, despite the enormous progress made, cancer remains the second leading cause of death in the USA and still represents an area with large potential for new developments [36].

It is well established that early detection of cancer is key for better outcomes, with higher cure rates and longer survival. Proof of this is the fact that despite great improvements in outcomes for most cancers, advances have been slow for lung and pancreatic tumors, mostly diagnosed at advanced stages. Screening is an effective practice for diagnosis of cancer in early stages. After the implementation of screening, lung cancer mortality among current and former smokers with a smoking history of 30 or more pack-years has experienced a 20% reduction [36]. Currently available screening methods include colonoscopy for colon and rectal cancers, mammogram for breast cancer, pap smear for cervical cancer, and PSA for prostate cancer. Ideal screening options should display high sensitivity and specificity, be safe, available, convenient, and inexpensive. Even today, there are no screening tests for many relevant malignancies. Attempts have been made to introduce tumor markers such as CEA, CA 19-9, CA 125 as less invasive alternatives for screening that would increase patient compliance and decrease costs, but they have shown poor performance [37, 38].

A blood test based on the detection of CTCs or mutant DNA would be a unique adjunct to current screening methods, allowing for evaluation of many different cancers at the same time. It could also revolutionize the diagnosis and treatment of many tumor types where no screening exists, such as ovarian and pancreatic cancers. Studies have shown that migration of CTCs into the blood stream is an early event in the course of cancer, even years prior to radiological evidence of disseminated disease [39, 40]. This prompted recent efforts to develop liquid biopsy based screening tests with the goal of finding more cancers in localized stages. In this regard, Ilie et al. found that CTCs could be isolated in the blood of patients with COPD 1 to 4 years prior to CT could detect lung nodules. This led to detection of early-stage lung cancer with prompt resection in these patients [41].

Liquid biopsy-based screening methods would allow for frequent repeated testing in at-risk patients in a minimally invasive way that would likely increase compliance and detection of tumors in resectable stages. It could also guide the need for more expensive or invasive methods, such as additional imaging, hence decreasing exposure to radiation in the majority of patients.

The application of liquid biopsies in screening has limitations based on the current sensitivity and specificity. This remains the Achilles heel of the use of liquid biopsy as a screening method. Both CTCs and ctDNA are present in low concentration within circulation. This is especially true in early stage cancers, making it difficult to detect these lesions and raising concern for a high rate of false negative results. Similarly, low specificity, with high false positive results and inability to determine the tissue of origin of detected mutations would expose patients to unnecessary anxiety, possibly radiation, and even invasive interventions, leading to harm, increasing costs, and introducing inefficiencies to the healthcare system [39]. An example of this phenomenon is the spike in the incidence of asymptomatic prostate cancer in the late 1980s as a result of widespread prostate-specific antigen (PSA) testing. This initial rise was followed by a significant drop that can be explained by the US Preventive Services Task Force recommendations against routine use of the test to screen for prostate cancer due to concerns about overdiagnosis and overtreatment [36].

A new blood test, CancerSEEK, was developed to address these limitations with a cost-effective technology. This is a PCR and ELISA-based assay designed to assess multiple regions of driver genes that are commonly mutated in select cancer types: ovary, liver, stomach, pancreas, esophagus, colorectum, lung and breast. It localizes ctDNA through a panel gene biomarkers (61 amplicon panel) and preselected proteins (CA 125, CEA, CA 19-9, PRL, HGF, OPN, MPO, TIMP-1) with the objective of pointing towards the tissue of

origin. Cohen et al. report a predicted detection capability of 60% of liver cancers to 100% of ovarian cancers, with a sensitivity of 98% for ovarian cancer to 33% in breast cancer and specificity over 99%. Sensitivity of the test was 55% among all eight cancers. Not unexpectedly, it improved as stage increased, from 43% for stage I up to 78% for stage III [39]. Even if imperfect, this test shows that there is great potential for the diagnosis of early cancer and improvement in survival through liquid biopsy.

Liquid biopsy for predicting outcome

Tumor staging is a critical step after the diagnosis of cancer, allowing for classification of the status of disease. It helps clinicians determine treatment options and provide patients and families a reasonable estimate of predicted disease course and outcome. Currently, most tumors are staged based on imaging and surgical pathology. However, this provides limited information on the behavior of a tumor on an individual level. The ongoing discovery of molecular markers of tumor biology makes a more sophisticated staging possible in the near future. Many of these markers could be assessed through a liquid biopsy.

There is substantial published evidence that demonstrates a correlation between ctDNA or CTC levels and tumor burden and cancer progression. Consequently, serial measurements of levels of these markers have been widely investigated as a proxy for disease status and response to treatment [12, 37, 42, 43].

Madhavan et al. studied circulating DNA integrity and concentration in plasma of 383 individuals, 82 with primary breast cancer, 201 with metastatic breast cancer, and 100 healthy controls. A hierarchical decrease in DNA integrity and increase in cfDNA concentration from healthy controls to primary breast cancer and further onto metastatic breast cancer patients was observed. This has turned circulating tumor DNA integrity into an attractive candidate for blood-based multi-marker assays and a prognostic marker for metastatic disease [44].

Gemenetzis et al. recently presented a prospective longitudinal study in which 136 patients with pancreatic cancer were followed with liquid biopsies. Measurement of CTC concentration in peripheral blood was performed at fixed intervals, starting prior to surgical resection, at 4 and 6 postoperative days and every 2 to 3 months thereafter. CTCs were isolated based on size (> 8 μm) and then stratified into epithelial if only expressing cytokeratin or mixed epithelial/mesenchymal if also expressing vimentin. Tumor cells were identified in blood of 131 (96%) patients. Chemotherapy-naïve patients at the time of surgery (58%) had significantly higher CTC numbers before resection when compared to patients post neoadjuvant therapy (42%). Both groups had a significant decrease in the number of CTCs after surgery. However, patients that developed early disease recurrence within 1 year from surgery had significantly higher pre and postoperative CTC counts with a higher proportion mixed epithelial/mesenchymal phenotype CTCs, indicating more aggressive biology. In line with these findings, patients who underwent exploration with aborted resection due to occult abdominal metastatic disease had significantly higher number of CTCs than patients in whom resection was completed [40].

Similar work has been conducted on multiple other cancers. These studies are summarized on Table 1.

Liquid biopsy for guiding therapy

A potential role in guiding therapy is one of the most exciting aspects of liquid biopsies. The isolation of CTCs and ctDNA provides DNA, RNA, and proteins for analysis that may provide valuable information regarding tumor behavior and therapeutic targets at the time of diagnosis and throughout the course of the disease. For example, Maheswaran et al. report that EFGR mutations in CTCs may explain differences in response to treatment with tyrosine kinase inhibitors in non-small cell lung cancer [53]. Similarly, Jiang et al. describe androgen receptor mutations on CTCs obtained from patients with castration-resistant prostate cancer [54]. In the case of melanoma, proto-oncogene BRAF mutations were detected in CTCs and ctDNA, which may guide BRAF-directed therapies in the future [55, 56].

The nature of cancer determines that for any given treatment, there is high likelihood that a small population of cells within the tumor will be resistant to the effects of a drug. When this drug is instituted as first line treatment, those cells will survive and continue to multiply unopposed, generating new resistant clones. This will ultimately lead to treatment failure. For this reason, current regimens consist of a combination of two or more drugs to offer a higher chance of cure [57]. Regardless of the treatment regimen of choice, assessment of disease status and progression during and after treatment is of vital importance to define next steps in the course of this dynamic disease, but remains challenging. In this regard, studies on breast cancer have shown that HER 2 expression can change during the course of the disease [58]. Likewise, tracking KRAS mutations in ctDNA of colorectal cancer patient can predict both treatment response and acquired resistance to epidermal growth factor receptor (EGFR) blockade [59].

Liquid biopsy allows the treating team to assess real-time response to treatment through quantitative and qualitative evaluation tumor cells and genes at set intervals. It provides the opportunity to determine therapy response or failure and adjust strategies prior to it reflecting in changes in currently used markers. Unfortunately, image evidence of treatment failure usually happens with delay and most biomarkers (CA19-9, CEA, chromogranin) are weak predictors of

Table 1 Research efforts aimed at determining the role of liquid biopsy on cancer prognosis and choice of optimal treatment across multiple organ systemsand tumor types

Type of cancer	Marker	Technology	Author	Results
Breast				
	CTC	CellSearch	Cristofanilli [45]	Number of CTCs before treatment is an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer.
	CTC	CellSearch	Rack et al. [46]	Presence of CTCs associated with poor disease-free survival, breast cancer-specific survival and overall survival.
	CTC and ctDNA		Madic et al. [47]	ctDNA levels had no prognostic impact on time to progression or overall survival, whereas CTC numbers were correlated with overall survival and marginally with time to progression.
	CTC		Zhang et al. [48]	CTCs may contain "brain metastases selective markers" (HER2+ / EGFR+ / HPSE+ / Notch1+) suggestive of CTC metastatic competency to the brain.
Lung				
	ctDNA	CAPP-Seq	Newman et al. [12]	Levels of ctDNA were highly correlated with tumor volume for NSCLC
Esophagus				
	CTC		Vashist et al. [49]	DTC in bone marrow is a strong and independent prognostic factor in patients with resectable EC.
Liver				
	CTC	CellSearch	Schulze et al. [50]	Presence of EpCAM-positive CTC in patients with intermediate or advanced HCC and its prognostic value for OS
Pancreas				
	CTC	Size, cytokeratin antibodies, vimentin, and CD45.	Poruk et al. [51]	Cytokeratin-positive CTCs are a significant independent predictor of survival. CTCs expressing both vimentin and cytokeratin was predictive of recurrence.
Colorectal				
	ctDNA	BEAMing	Schmidt and Diehl [33]	Mutant DNA from colorectal tumors can be found in the bloodstream and quantity increases with tumor-stage. Extremely low quantities of mutant DNA can be detected in more than 60% of patients with early, curable CRC.
Bladders				
	CTC	CellSearch	Gazzaniga et al. [52]	CTC presence was the strongest independent predictor of disease progression to muscle invasive disease

progression and outcome [40]. Not unexpectedly, mutations or loss of surface proteins used to detect CTCs could lead to failure to identify the emergence of resistant clones. The use of surface-enhanced Raman spectroscopy (SERS) to monitor the expression levels of multiple surface markers simultaneously has been described in an effort to increase sensitivity in the detection of CTCs and track changes in cell populations in response to molecular targeted therapy with early recognition of resistant ones [60].

Earlier detection of failure in first line treatment creates the opportunity for the introduction of early changes in the choice chemotherapy or immunotherapy agents. Following this premise, several studies have assessed the effectiveness of early adjustments in therapeutic regimens in breast cancer patient. SWOG S0500 trial looked at CTC levels in patients with metastatic breast cancer and whether changing to an alternative chemotherapeutic regimen might improve outcomes for patients whose CTCs were not reduced after one cycle of first-line chemotherapy. CTC level at baseline and after introduction of therapy was found to be an accurate prognostic factor, but unfortunately, early changes in therapy did not improve either overall survival or progression-free survival [61]. These results are evidence of the variability in tumor behavior and highlight the need for novel targeted therapeutic agents and regimens for the treatment of this complex disease. Other ongoing trials, DETECT III and Treat CTC, seek to determine the benefits of HER2-targeted treatment in patients with HER2-negative primary tumors and HER2-positive CTCs [62, 63].

Summary and concluding remarks

In the light of new technologies, liquid biopsy arises as an opportunity to revolutionize cancer care with a highly tailored, minimally-invasive and cost-effective method to screen and monitor response to treatment. However, many challenges still need to be overcome before liquid biopsy becomes a reliable and widely available option. Sensitivity and specificity need to be refined and procedures standardized before clinicians

can commit to make decisions that will impact patient care based on its results.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Literature review article—not applicable.

Informed consent Literature review article—not applicable.

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