Editorial

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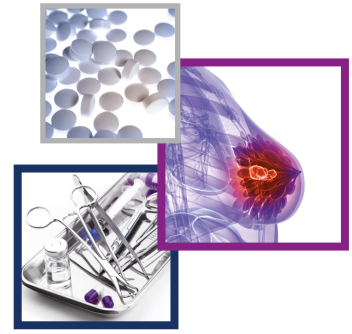
Breast Cancer Management



The role of circulating tumor DNA testing in breast cancer liquid biopsies: getting ready for prime time

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"ctDNA technologies are emerging to enable us to measure molecular aberrations in body fluids with high degrees of detection sensitivity, specificity and reproducibility."

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Invasive tissue biopsies of tumors are associated with several limitations, such as patient surgical risk, inaccurate biopsy sampling of nontumor regions, sample preparation for downstream analysis and procedural costs. Furthermore, a significant limitation of tissue biopsies is the failure to capture intra/intertumoral heterogeneity, thus impacting test accuracy. To overcome the many limitations related to traditional tumor biopsies, molecular circulating tumor DNA (ctDNA) testing may serve as noninvasive liquid biopsies for cancer patients to reflect the same mutations and genetic aberrations as those of primary tumors. As a form of liquid biopsy, ctDNA refers to the tiny fraction (0.01–10%) of short cell-free DNA (cfDNA) fragments that originate from tumors and are detectable in almost all body fluids such as blood, urine or saliva [1–3].

The feasibility of routine ctDNA testing has been assessed in recent programs for various aspects of breast cancer (BrCa) management, including early diagnosis, longitudinal screening of disease progression and treatment response [4]. In the setting of early-stage BrCa, the clinical validity of using ctDNA for molecular relapse detection in a main cohort of 101 patients has been assessed in a prospective multicenter study [5]. This study found that ctDNA detection during follow-up (every 3 months for first year and every 6 months subsequently) is associated with a high risk of future relapse in all BrCa subtypes. The evaluation of ctDNA detection for BrCa relapse further opens up possibilities of profiling ctDNA mutations to guide targeted therapies in advanced BrCa.

For instance, prospective plasma ctDNA detection of somatic mutations in *PIK3CA*, *ESR1*, *ERBB2* and *AKT1* from 234 metastatic BrCa patients has been reported at the recent ESMO 2019 Congress (Barcelona, Spain; 27 September to 1 October 2019) [6]. In this study, actionable mutations (classified using the OncikB database) were identified in 63 patients (39.6%). A notable case within this study was the identification of HER2 amplification in a patient initially diagnosed with ER+/HER2− BrCa through ctDNA analysis, leading to targeted HER2 treatment and a near complete metabolic treatment response.

Using serial ctDNA testing to study CDK4/6 inhibitor resistance in clinical samples, the PALOMA-3 Phase III clinical trial performed retrospective ctDNA analysis in 195 randomized patients with ER+/HER2− metastatic BrCa [7]. The study identified three driver mutations (*RB1*, *PIK3CA*, *ESR1*) of resistance to fulvestrant and palbociclib and presented a proof-of-potential for ctDNA analysis to monitor targeted drug resistance in the clinic [8]. In a bid to expand upon this study, the clinical utility of detecting rising *ESR1* mutation levels in ctDNA of ER+/HER2− metastatic BrCA patients to inform switching to palbocibib–fulversant combination therapy is currently being investigated in a large randomized multicentric Phase III trial (PADA-1) [9].

As discussed in the above selected studies, the potential of ctDNA testing in cancer is increasingly being demonstrated. Yet, while a certain degree of clinical validation (correlation of score/classifier with clinical state/outcome) has been shown, there is still limited concrete evidence of clinical utility (actionable and affects cancer treatment

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positively) that ctDNA analysis is of benefit to cancer patients to support widespread clinical use [10]. Therefore, there are several important factors to be considered to provide clear clinical utility for ctDNA analysis in the clinic to positively enhance BrCa management in patients. These include: clonal hematopoiesis, preanalytical workflow and ctDNA technologies.

Clonal hematopoiesis

The process of clonal hematopoiesis of indeterminate potential (CHIP) is a common aging-related phenomenon by which hematopoietic stem cells acquire mutations, leading to the formation of a genetically distinct subpopulation of blood cells that share these mutations [11,12]. As normal blood cells also release cfDNA into circulation, there is a need to differentiate between CHIP-related mutations and tumor-associated mutations during ctDNA data analysis. In a recent study based on cfDNA mutation sequencing of 124 people with metastatic breast, lung or prostate cancer on MSK-IMPACT (MSK's sequencing platform), it was found that more than half of the identified mutations originated from CHIP in white blood cells, not from cancerous tumors [13]. To ensure the accuracy of ctDNA testing is not being confounded by nontumor biological signals, it is encouraged that matched cfDNA white blood cell and tumor tissue biopsy sequencing be included during ctDNA analysis.

Preanalytical workflow

There is an increasing realization that we need to improve the preanalytical workflow to maximize recovery of cfDNA for analysis. The key preanalytical parameters that can affect ctDNA analysis outcomes include sample collection and processing, transport, as well as processing and storage [14,15]. Currently, there is still a lack of standardization of such preanalytical parameters without clear guidelines and standard operating procedures in a clinical setting. For example, there is still no agreement in the field on the best way to process blood samples prior to any ctDNA analysis. As such technical parameters can completely change the interpretation of ctDNA results, preanalytical parameters should be evaluated in depth and optimized for standardization to aid the clinical utility of ctDNA testing in clinical oncology [16,17].

ctDNA technologies

There currently exists a myriad of technologies for ctDNA testing applications, each with its own unique working mechanism, benefits and shortfalls [18–20]. In terms of comprehensively screening unknown mutations, deep next-generation sequencing technologies such as Ion AmpliSeq, TAm-Seq™ or CAPP-Seq can provide outstanding detection limits (for mutant allele fractions down to ~0.01%) at the expense of longer turnaround time. In contrast, quicker PCR-based technologies such as qPCR, ddPCR or BEAMING are more amenable for predetermined specific mutation analysis which may not require extreme detection sensitivity and information. Furthermore, ctDNA technologies are in a state of constant advancements with regards to detection sensitivity, speed and miniaturization [21–24]. When evaluating ctDNA technologies for feasible implementation in clinical practice, the most likely features to be taken into account are turnaround time, detection limit and cost–effectiveness. Thus, it is imperative that an established ctDNA technology be optimally selected for the intended application to reduce assay time and cost for patients. For the latest ctDNA technologies, the paradigm of laboratory validity, clinical validity and clinical utility must be robustly demonstrated in the process of clinical translation [25].

In summary, the use of ctDNA analysis as a form of liquid biopsy testing in BrCa management is at present highly promising with exciting positive data from current clinical trials. ctDNA technologies are emerging to enable us to measure molecular aberrations in body fluids with high degrees of detection sensitivity, specificity and reproducibility. On a final note, to correctly show the clinical utility of ctDNA analysis, it is of paramount importance to: discriminate nontumor mutations from inherent biological CHIP mechanism; standardize preanalytical workflows to reduce bias; ensure ctDNA technologies are accurate, easy to use and feasible to deliver for affordability to all patients.

Financial & competing interests disclosure

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