Curr. Treat. Options in Oncol. (2019) 20: 68
DOI 10.1007/s11864-019-0667-9

Breast Cancer (EA Comen, Section Editor)

Emerging Role of Genomics and Cell-Free DNA in Breast Cancer

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Published online: 29 June 2019

This article is part of the Topical Collection on *Breast Cancer*

Keywords Circulating tumor DNA · Biomarkers · Translational medicine · Precision medicine · Breast cancer

Opinion statement

Precision Medicine is gaining momentum as the future gold standard healthcare strategy as it enables treatment optimization and consequently a potential improvement for quality of life and survival. This paradigm shift was possible thanks to new high-throughput genomics technologies, which provide prognostic and predictive information on tumor biology and potential treatment options, as standard pathological procedures are unable to capture both spatial and temporal tumor heterogeneity. As a result of decreasing costs, both solid and liquid-based genomics have an increasingly important role in clinical trials' screening procedures and are gradually being incorporated into clinical practice. Notwithstanding the great potential, its clinical utility is still a matter of debate and clinicians need to be aware of caveats in interpreting resulting data.

Introduction

The clinical potential for NGS has expanded, as the cost of genomic sequencing has decreased. The first human genome sequencing cost was approximately $2.7 billion dollars [1]. Soon after completion of the Human Genome Project, the cost to sequence the human genome decreased to less than $100 million dollars [2]. With considerable technological advances, sequencing costs have dropped dramatically with commercial tissue and blood-based NGS comprehensive-genomic profiling less than $6000 in the USA and actual costs of less than $1000. Costs of these tests are approaching the costs of imaging for diagnosis and monitoring treatment response. In 2012, tissue sequencing of primary breast tumors DNA, exome, RNA, and protein-enabled researchers to define the four distinct breast cancer subtypes through a collaboration with the Cancer Genome Atlas Network, further emphasizing the complex heterogeneity of these tumors [3].

Because of the growing interest in understanding the clinical applications of these technologies and their fast-paced innovation, having a clear overview of the state of the art is crucial. The aim of this review is to summarize the main applications of genomics in the management of breast cancer, with a special focus on their clinical application.

Mutational landscape of breast cancer

Data regarding the genomic landscape in metastatic breast cancer (MBC) has rapidly expanded, providing a more comprehensive view of genomic aberrations in breast cancer. This can inform our understanding of breast cancer biology and shape strategies for tailored treatments in the context of clinical trials or standard targeted therapies in MBC.

Estimated frequencies of genomic aberrations vary by dataset based on the type of sample (primary vs metastatic), tumor subtype (luminal A/B vs HER2-enriched vs basal), depth and extent of sequencing (targeted vs whole genome vs whole exome), type of genomic information evaluated (DNA vs RNA vs protein expression), and type of aberration (single nucleotide variants, indels, copy number variants or amplifications, translocation).

Primary breast cancers

There is an urgent need to identify new targetable mutations and to characterize how the well-characterized driver mutations impact response to therapy. To this end, The Cancer Genome Atlas (TCGA) reflects the collaborative efforts of the National Cancer Institute and the National Human Genome Research Institute to utilize NGS to profile the genomics, epigenomics, and proteomics of over 11,000 patients with 33 different types of cancers, including 825 patients with breast cancer [4, 5]. The initial analysis of this data in primary breast cancers confirmed known driver mutations such as *TP53*, *AKT1*, PIK3*CA*, and *GATA3*, and uncovered novel mutated genes including *RUNX1*, *CBFB*, and *PIK3R1* [4].

It furthermore demonstrated that missense mutations were the most common somatic changes in hormone-receptor-positive (ER-positive) breast cancers, while triple negative breast cancers (TNBCs) were enriched for nonsense, frameshift, and complex mutations. With regard to changes in the *TP53* pathway, *TP53* mutation and gain of *MDM2* were seen in 12% and 14% of luminal A tumors, 32% and 31% of luminal B tumors, 84% and 14% of basal-like

tumors, and 75% and 30% of HER2 enriched tumors, respectively [5]. PI3K pathway aberrations were frequently seen, with *PIK3CA* mutation, *PTEN* mutation/loss, and *INPPB4* loss seen in 49%, 13%, and 10% of luminal A tumors; 32%, 24%, and 16% of luminal B tumors; 7%, 35%, and 30% of basal-type tumors; and 42%, 19%, and 30% of HER2-enriched tumors, respectively. *RB1* pathway changes were seen more frequently in luminal B tumors with 58% having cyclin D1 amplification compared with 29% in luminal A and 38% in HER2-enriched tumors. Basal-like tumors were more likely to have *RB1* mutation or loss (20%) and low *RB1* expression. Defects in DNA repair mechanisms were more frequent in basal-like tumors with germline or somatic *BRCA1* or 2 variants that noted 20% of these tumors. *GATA3* and *MAPK* mutations were also frequently seen in luminal breast cancers [5].

Among luminal tumors, *ESR1* was highly expressed but infrequently mutated, *PIK3CA* mutations often did not result in increased markers of pathway activation by RPPA. Among HER2-positive tumors, those that were HER2 enriched by mRNA subtype had higher expression of receptor tyrosine kinases including FGFR4, HER1/EGFR, and HER2, whereas those HER2 positive with luminal mRNA subtype had higher expression of luminal genes including *GATA3*, BCL2, and *ESR1*. Basal-like tumors showed frequent amplification often without mutation in PI3K and RAS-RAF-MEK pathway components (*PIK3CA* (49%), *KRAS* (32%), *BRAF* (30%), and *HER1*/*EGFR* (23%)). Amplifications were also seen in *FGFR1*/*2*, *IFGR1*, c-Kit, met, and *PDGFRA*. Similar data regarding trends in genomic abnormalities based on tumor subtype in primary breast cancers have been shown in sequencing of hundreds of primary breast cancers [6–8].

Multiple other groups have also applied high-throughput techniques to further elucidate the molecular landscape of breast cancer [9–11].

Metastatic breast cancer

More recently presented data have highlighted the differences in mutational landscapes between primary tumor and metastatic recurrent sites. Comparison of frequency of gene abnormalities between datasets of primary versus metastatic tumor demonstrated some aberrant genes including *TP53*, *PIK3CA*, *ERBB2*, *GATA3*, *PTEN*, and others are seen at similar frequencies in primary and metastatic tumors, while *ESR1*, *NF1*, *FOXA1*, *CDKN1B*, and *AKT1* occur more frequently in metastatic tumors [12]. Several mutations have been shown to arise after treatment exposures, including *ESR1* mutations after endocrine therapy, *PIK3CA* after HER2 therapy, and *RB1* mutations with CDK4/6 [13].

Estimates of *ESR1* mutations prevalence in metastatic disease vary significantly based on type of sequencing, prior therapies, and sample size. Larger studies evaluating *ESR1* mutations in cfDNA in patients who progressed on prior endocrine therapy showed that 25–40% harbor these mutations [14••, 15–17].

Other mutations occur infrequently but when observed have been considered a therapeutic target for approved drugs or further study in clinical trials. Germline *BRCA1* and *2* mutations occur in about 5% of unselected breast cancer cases and are enriched in some subgroups depending on ancestry, breast cancer subtype, and age. When detected, these mutations suggest sensitivity to PARP inhibition [18–20]. The frequency of somatic *BRCA1*/*2* mutations in

MBC is not well known; however, small cohorts suggest around 3% with additional cases having defects in DNA damage response genes [21]. *HER2* mutations were observed in 2.4% of 5605 cases of metastatic breast cancer that were sequenced and seem to occur more frequently in lobular carcinomas [22]. *HER2* mutations in the absence of *HER2* amplification detected by traditional IHC and FISH criteria have been associated with response to anti-HER2 therapy, and several clinical trials are ongoing to evaluate the best therapies in this subgroup [23]. *NTRK* fusions have been of interest recently since the approval of a targeted tyrosine kinase inhibitor, larotrectinib, across multiple tumor types. However, screening large cohorts of advanced breast cancers showed that only 0.13% of tumors harbored the target fusion protein [24]. The prevalence of microsatellite instability-high breast tumors is estimated about 1.7%, and when found signifies potential benefit from immune checkpoint inhibition.

Higher tumor mutational burden (TMB) has been associated with improved response to immune checkpoint blockade, and breast cancer ranks near the middle of cancers with regard to TMB [25, 26]. However, this varies by subtype. In the TCGA analysis of primary tumor tissue, the average rate of mutations per megabase in sequenced tumors was 0.84 in luminal A, 1.38 in luminal B, 2.05 in HER2-enriched, and 1.68 in basal-like [5]. Another larger cohort of primary breast cancer found TMB to be 1 [8]. This compares to a recent study of over 300 metastatic tumors, and the median TMB was 2.9, with 11% of cases having a high TMB (TMB > 10) [12].

Although the genomic landscape of metastatic breast cancer is complex and diverse, detecting specific mutations or signatures may provide insight into tumor biology or therapeutic targets (Fig. 1).

Tissue biopsy genomics

Next-generation sequencing (NGS) refers to a number of innovative technologies that allow massively parallel interrogation of nucleic acid sequences. While traditional Sanger sequencing, also called "first-generation" sequencing, has been instrumental in numerous achievements in genomics, including the Human Genome Project, its major disadvantage is its limited throughput, which results in a labor-intensive operation [27, 28]. NGS adapts existing sequencing by synthesis techniques to be simultaneously performed on millions of DNA fragments, typically immobilized over a solid substrate [29, 30]. These high-throughput sequencing methods aim to address the increasing demands in the scientific and medical community for a faster and more economical means of detecting pathogenic variants in the human genome, a critical component in the development of precision medicine.

Next-generation sequencing methodologies are diverse but share similarities in their key steps: sample collection, library preparation, cluster generation, sequencing, and data analysis. In solid tumors, the biopsy samples originate from either the primary tumor or a metastatic focus, which are then formalin-fixed and paraffin-embedded [31]. A minimum tumor nuclei percentage of 20% is typically required for adequate processing [32]. The testing company completes the specimen retrieval process for the intended nucleic acid sample (e.g., genomic DNA or RNA), which is synthesized into double-stranded DNA, the starting point for most NGS reactions [29, 33]. The DNA sample

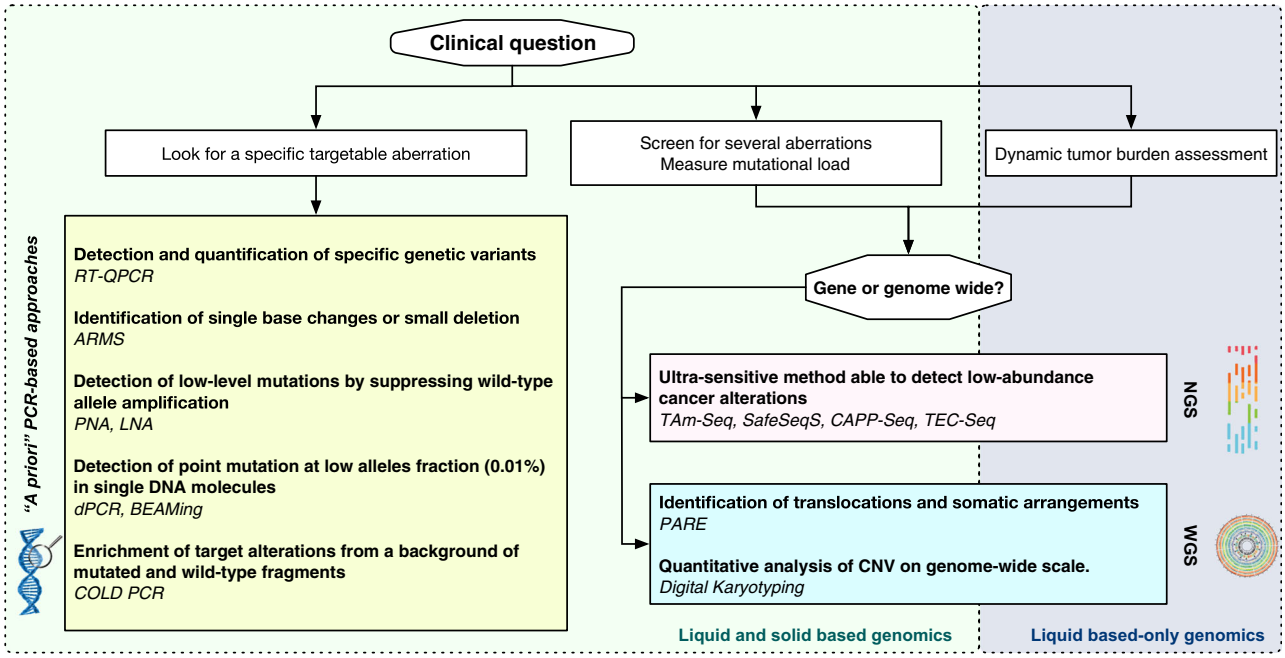

Fig. 1. How cancer-applied genomics can help the clinical workflow. Schematics of the main clinical needs in the treatment of breast cancer and how each genomics assay can be integrated in the clinical workflow

subsequently undergoes fragmentation, size selection, and adapter ligation to form the library of DNA fragments [34]. In the cluster generation step, surface-bound oligonucleotides anneal to the adapter ends of the DNA fragments, allowing for spatially separated amplification of DNA templates. During the sequencing phase, the specific sequencing reactions and detection methods vary depending on the platform, though most NGS techniques use variations of the sequencing by synthesis method [35]. Some newer platforms feature single molecule sequencing techniques that obviate the need for the amplification step [36, 37]. Finally, various bioinformatics tools and reference genomes are employed to construct the genomic map and identify genetic variants, such as single nucleotide polymorphisms (SNPs), small insertions and deletions (indels), and copy number variants (CNVs).

Next-generation sequencing technology can be utilized to interrogate all 3.3 billion bases of the human genome in whole genome sequencing (WGS) or to analyze just the 22,000 protein-encoding regions in whole exome sequencing (WES). The advantage of WGS lies in its comprehensive scope, which can detect alterations in both coding and non-coding regions such as regulatory domains. However, it has a higher cost and a relative deficiency in its depth of coverage (~ 30×), which is sufficient to identify most SNPs and indels but may miss rarer variants, thus limiting its clinical utility [38, 39]. In contrast, WES offers a depth of coverage averaging around 100×—120× and, when combined with transcriptome sequencing, is considered the "gold standard" in evaluating the cancer genome [40, 41]. Interestingly, as WGS technology advances to become more cost-efficient, there is growing evidence that WGS set to a comparable coverage may even surpass WES in its ability to detect exon alterations [42, 43].

There are also targeted gene panels available that focus only on genes with known functional relevance in the tumor of interest. These panels are rapidly interpretable, cost-efficient, and offer the highest depth of coverage, which permits evaluation of low-frequency mutations and even intratumoral heterogeneity, albeit at the expense of much lower sensitivities for CNVs and genomic rearrangements [44]. Therefore, targeted gene panels offer one possibility for a rapid (but restricted) genomic evaluation in the clinical setting. Unfortunately, their inability to uncover new therapeutic targets severely limits their applicability in research. Finally, NGS methods can be applied to non-DNA-based genetic assessments as well, including the evaluation of transcriptomes through RNA sequencing and epigenomes through methylation and ChIP sequencing, thus providing further insight into the complex pathogenesis of breast cancer [45, 46].

The advent of NGS technology with its ability to overcome the throughput limitations of Sanger sequencing is an important step in establishing the foundation for precision medicine. Nevertheless, there are limitations to these techniques. Most clinically relevant are a turn-around time of a few weeks which can delay decision making for treatment and clinical trials, and an inability to capture disease heterogeneity and evolution overtime. Additionally, sample acquisition is a barrier, and patients must undergo biopsies with their associated risks. Furthermore, acquiring an adequate sample that is not compromised in processing is more challenging in bony metastases, which can often be the only site of metastases in luminal breast cancer. Ultimately, the goal is to customize treatments based upon the presence of actionable mutational targets identified through rapid and cost-efficient genomic screening.

Liquid biopsy

Genome-driven oncology is gaining momentum as a crucial aspect of precision medicine in cancer treatment, as the feasibility and cost-effectiveness of genome-sequencing is increasing. As clonal selection occurs as a consequence of treatment resistance, the tumor's genetic and epigenetic landscape changes dynamically. Therefore, *real-time* capable assays aimed at supporting clinical decision-making and tailoring treatment are needed [47]. Although tissue-based analyses are the state-of-the-art source of prognostic and predictive factors, tissue NGS can only provide a spatially and temporally limited overview of tumor heterogeneity.

Liquid biopsy is mainly based on the enrichment of circulating tumor cells (CTCs), cell-free DNA (cfDNA), and circulating tumor DNA (ctDNA), and it is gradually being incorporated in clinical practice and clinical trials for minimal residual disease detection, disease monitoring, and resistance assessment. When compared to tissue-biopsy, liquid biopsy seems to overcome feasibility issues, including inaccessible primary or metastatic tumors, patients' acceptance to invasive procedures, and inadequate material for molecular analysis [48]. Moreover, blood samples can be easily obtained at baseline and at any subsequent time-point, not only providing a tool for tumor characterization, but also for longitudinal monitoring of the tumor. These key strengths make liquid biopsy suitable for a dynamic evaluation of MBC's evolving biology.

Genetic alterations arise stochastically under selection pressure and promote cell proliferation and survival. These mutations include, but are not limited to, point mutations, translocations and rearrangements, loss of heterozygosity, microsatellite instability, methylation, copy number variations, and single nucleotide polymorphisms (SNPs) and can be used as biomarkers to guide targeted therapies [49, 50]. Notably, the use of NGS permits the detection of multiple genetic alterations at the same time, in a faster and more cost-effective way with respect to traditional sequencing. This technique can be applied to tumor tissue analysis, as well as CTCs and ctDNA.

CfDNA is released by primary and metastatic tumor cells. Notably, cfDNA is also derived from normal cells, such as hematopoietic and stromal cells that have undergone necrosis or apoptosis, requiring a careful clinical evaluation especially at low allelic frequencies. The relative amount of cfDNA released by tumor cells varies widely, and the detection of tumor-specific genomic abnormalities on liquid biopsy-based analyses allows the discrimination of ctDNA, which consists of a very small proportion of overall cfDNA (< 5%). One of the most promising application of ctDNA characterization is to identify actionable mutations which can guide treatment strategies [51].

In blood, about 30% of BC patients show detectable *ESR1* mutations after exposure to endocrine therapies (ET), resulting in treatment resistance and aggressive clinical behavior. Aromatase inhibitor (AI) administration beyond progression (HR 3.7; $p = 0.008$) or as maintenance therapy after chemotherapy (HR 3.1; $p = 0.0041$) has been associated with worse progression-free survival (PFS) in the presence of $ESR1^{D538G/Y537S}$ [52]. Chu et al. validated the use of ctDNA-based analyses to predict resistance to therapies; however, some analytical issues seem to have determined different sensitivity in the detection of *ESR1* mutations across different studies [52, 53].

Notably, a prospective-retrospective analysis of the SoFEA trial confirmed the negative impact of *ESR1* mutations on PFS in patients receiving exemestane versus fulvestrant (HR 0.52; $p = 0.02$), while a retrospective analysis of the BOLERO 2 highlighted an unfavorable impact of $ESR1^{D538G/Y537S}$ on overall survival (OS) (*ESR1*wt 32.1 vs $ESR1^{D538G}$ 25.99 vs $ESR1^{Y537S}$ 19.98 vs $ESR1^{D538G/Y537S}$ 15.15 months) [54, 55]. However, a similar benefit in terms of PFS, compared with that of *ESR1* wild type BC, was observed when $ESR1^{D538G}$-mutated tumors were treated with everolimus in combination with exemestane [55]. Conversely, *ESR1* methylation and gene silencing were associated with a lack of response to the combination everolimus/exemestane ($p = 0.023$, Fisher exact test) [56].

Along with *ESR1*, the potential predictive role of ctDNA detected *PIK3CA* mutations has been investigated [57••, 58]. *PIK3CA* mutations occur in about 40% of ER-positive MBC, determining an abnormal activation of the hormone-independent PI3K pathway. Its dynamics on ctDNA analysis reflects response to treatments. Longitudinal evaluation of *PIK3CA* ctDNA levels was observed to strongly predict PFS (HR 3.94; log-rank $p = 0.0013$) after 15 days of treatment with fulvestrant (FASLODEX®) and the CDK 4/6 inhibitor palbociclib (IBRANCE®). Conversely, *ESR1* mutations dynamics did not show an impact on PFS, mostly reflecting clonal selection induced by prior treatments [59]. A retrospective analysis of the FERGI study showed no statistically significant difference in terms of PFS in patients receiving fulvestrant or fulvestrant plus a pan-PI3K inhibitor [60].

The onset of other genetic alteration is currently under the spotlight, including the emergence of *ESR1* fusion proteins, somatic *RB1* mutations and *FGFR1* amplifications in patients progressing on CDK 4/6 inhibitors, or *ERBB2* mutations in HER2-negative patients, offering intriguing future clinical perspectives [61, 62, 63•].

Potential clinical applications

Recently, the Foundation CDx platform was approved by the Food and Drug Administration (FDA) on November 30, 2017, and by the Center for Medicare & Medicaid Services (CMS) for patients with advanced solid tumors [64]. In addition, in Europe, several countries including France and England have established centralized sequencing efforts over the next decade [65, 66]. These efforts would complement existing publicly available genomic databases with examples including TCGA, TARGET, and ENCODE to promote data sharing and collaboration. Clearly, greater availability of these technologies warrants thoughtful consideration of optimal utilization (Table 1).

In breast cancer, the most common mutations observed in tissue and blood NGS include *TP53*, *PIK3CA*, and *GATA3*, among others. To date, high level of evidence exists for *PIK3CA*, *ERBB2* amplification, and germ-line *BRCA1* and *BRCA2* mutations both on liquid and solid-based assays [64]. Although rare, *NTRK* fusions (present in less than 1% of metastatic patients and even rarer in breast cancer patients) and *MSI*-high tumors have targeted drugs leading to high objective response rates, larotrectinib, and pembrolizumab, respectively, with tissue agnostic approvals [67]. These genomic alterations all have clear targeted therapies as previously discussed. Additional targeted therapies are in development with promising results in clinical trials, including AKT1 inhibitors in TNBC and PI3-kinase inhibitors in hormone-receptor-positive breast cancer [57••, 68].

Beyond initial treatment, the emergence of resistance to targeted therapies is a near certainty in metastatic disease. Identification of amplifications and mutations in *ERBB2* and emergence of *ESR1* mutations have been reported as mechanisms of endocrine resistance. In fact, surface markers and genomic alterations for these two genes have been reported on CTCs and ctDNA [69, 70]. Clinical approaches currently being evaluated include HER2-directed therapy and avoidance of AIs in combination with CDK4/6 inhibitors in advanced HR+, HER2– disease. In fact, in a series of 5605 cases of metastatic breast cancer, 12.5% had *ERBB2* alterations, which included both *ERBB2* amplifications and mutations [71]. Although detected in a small percentage of patients, these mutations could not be detected using traditional IHC or FISH methods.

For tissue NGS, initial upfront sequencing in the metastatic setting could be useful to capture the landscape of alterations, prior to genomic evolution with treatment. This approach is typically feasible for advanced breast cancer patients, although it may have some limitations in bone-only metastatic disease. The advantage of an initial tissue biopsy is that the technique allows for diagnosis, immunohistochemical staining, and determining the DNA alterations and potentially RNA sequencing data, as well. However, there are limitations with respect to serial tissue biopsies and the ability to capture tumor spatial heterogeneity in response to treatment.

Table 1. Tissue versus cell-free DNA sequencing in breast cancer. Comparison of the main current and future applications and the current available techniques with clinical practice implications

Characteristic	Tissue sequencing	cfDNA sequencing
Biology	Site specific	•Capable of capturing tumor heterogeneity •Requires tumor shedding
Invasive	Invasive	Minimally invasive
Sampling	•More time intensive to obtain •Complications can occur (e.g., bleeding, infection, pneumothorax) •Requires adequate tissue •Turnaround time ~ 1 month	•Easy to obtain •Timing of sample collection with respect to treatment matters •Turnaround time typically < 2 weeks
Techniques	•Fine needle aspiration •Core needle biopsy •Excisional biopsy •Sequencing of hotpot mutations •Next-generating sequencing typically of formalin-fixed paraffin-embedded (FFPE) tissue	•Peripheral blood draw •Candidate gene "a priori" PCR-based and BEAMing methods •Next-generation sequencing -SCODA -TAm-Seq -SafeSeqS -CAPP-Seq -TEC-Seq
Sequencing length	•Targeted panels •Comprehensive-genomic profiling (> 300 genes, typically > 1 Mbp) •Whole-exome •Whole-genome	•Targeted panels •Comprehensive panels (up to 73 genes, up to ~ 200 kbp) •Longer panels (at least 500 genes) in commercial development
Germline testing	Sometimes inferred based on allele frequency, but not explicitly tested	Can be performed if patient provides additional consent
Current applications	•Histology and tissue architecture •Staging •Genomic alterations •Tumor resistance •Microsatellite instability •Tumor mutational burden	•Prognosis •Minimal residual disease •Genomic alterations •Tumor burden •Response to therapy •Tumor resistance
Challenges	•Accurately representing spatial tumor heterogeneity •Accessing small tumors or sites that are difficult to biopsy •Feasibility of serial biopsies	•Cost and insurance coverage associated with serial biopsies •Determining the optimal platform and sequencing depth for the particular clinical situation •Blood processing and quality control
Future applications	Quality improvement to minimize complications	•Early cancer detection •Monitoring response to treatment •Tumor mutational burden

In terms of resistance, the emergence of *ESR1* mutations indicates resistance to endocrine therapy either when detected in tissue or blood. Given its potential, liquid biopsy has begun to rise interest as a highly promising tool. Ongoing clinical studies have been designed to explore the clinical utility of ctDNA

analysis, such as the Aiming to Understand the Molecular Aberrations in Metastatic Breast Cancer "AURORA" trial (NCT02102165), the UK Plasma Based Molecular Profiling of Advanced Breast Cancer to Inform Therapeutic CHoices "plasmaMATCH" trial (NCT03182634), and a Phase II Trial Testing Oral Administration of Lucitanib in Patients with Fibroblast Growth Factor-Receptor (FGFR)1-amplified or Non-amplified Estrogen Receptor Positive Metastatic BC "FINESSE" (NCT02053636). Interestingly, the ongoing Palbociclib and Circulating Tumor DNA for *ESR1* Mutation Detection "PADA-1" trial (NCT03079011) has been designed to investigate a potential clinical benefit of maintaining palbociclib in combination with fulvestrant after prior therapy with palbociclib and an AI and after the detection of *ESR1* mutations in ctDNA-based analyses.

Despite some limitations that have conditioned its application, several key strengths can be identified in favor of the use of liquid biopsy as a tissue surrogate in clinical practice [72].

Among them, this minimally invasive procedure is a reproducible source of reliable tumor-derived materials that are not contaminated by preservatives, especially in poorly accessible tumors and at any tumor stages. In early stage BC, efforts are being made to use liquid biopsy for the detection of the tumor before it becomes clinically evident, both with respect to early disease detection and minimal residual disease. However, several caveats still exist due to the high sensitivity that is needed and the uncertain predictive value [73]. In the metastatic setting, ctDNA might overcome the difficulties in detecting the heterogeneity between primary tumor and metastases [74].

In addition to CTCs and ctDNA, which can provide complementary information, other components released by tumor cells have gained recent attention as a supplement for liquid biopsy diagnostics. Micro-RNAs (miRNAs) have demonstrated higher stability with respect to mRNA, and their alterations might reflect cancer progression. Furthermore, the analyses of exosomal DNA might improve information on tumor heterogeneity and treatment efficacy. Although promising, these techniques still need to be improved [75–77].

Due to its lower morbidity with respect to conventional biopsy, liquid biopsy enables the longitudinal assessment of tumor characteristics which could be dynamically influenced by treatments such as mutational burden and resistance. Notwithstanding the potential game-changing role, ctDNA analyses still need a better understanding of the full potential and limitations of this technologies before being fully introduced into clinical practice. One of the main shortcomings is the variability of ctDNA levels. In patients with high disease burden, ctDNA fraction is considerable. Nevertheless, even among patients with the same disease stage, ctDNA levels are affected by multiple variables including not only tumor microenvironment and the access to blood vessels but also inflammatory status and other benign conditions that might reduce the accuracy and reliability of the results [78]. A standardization of ctDNA analyses in terms of sample collection, processing and molecular evaluation seems critical to reduce pre-analytical and analytical issues and to improve the accuracy and sensitivity, and an optimization of the costs is required. Another key point is to validate specific time-points for liquid biopsy to be performed, aiming towards an anticipation of disease relapse and progression before it is clinically evident. Eventually, it is crucial to provide clinicians evidence

to support treatment strategies when genomic aberrations of unknown significance are detected through ctDNA evaluation.

Despite these limitations, liquid biopsy is a highly promising tool. Well designed, prospective studies are ongoing to further validate these methods in larger cohorts of patients, providing sufficient statistical power to obtain results regarding the possible implementation of liquid biopsy in routine clinical diagnostics.

To date, use of routine tissue and blood NGS in advanced breast cancer management remains provider-dependent outside of clinical trials and national consortiums. There are many ongoing clinical trials that enable matching tissue NGS genomic alterations with targeted therapies to assess potential clinical benefit of this precision medicine approach. Examples of representative trials basket and umbrella trials with breast cancer patients include NCI-MATCH, TAPUR, MyPathway, and other novel designs including approaches to use RNA sequencing as a functional assay to assess gene expression.

Although different benchmarks currently demonstrate analytical validity of both tissue and blood-based genomics, their clinical utility will ultimately be enhanced as the repertoire of targeted drugs and the evidence on resistance mechanisms and how to overcome them with combination therapy expand.

Compliance with Ethical Standards

Conflict of Interest

Lorenzo Gerratana has received investigator-initiated study support from Eisai, has received compensation from Eli Lilly & Co. for participation on an advisory board, and has received reimbursement for travel expenses from Menarini Silicon Biosystems.
Andrew A. Davis has received reimbursement for travel expenses from Menarini Silicon Biosystems.
Ami N. Shah declares that she has no conflict of interest.
Chenyu Lin declares that he has no conflict of interest.
Carla Corvaja declares that she has no conflict of interest.
Massimo Cristofanilli declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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Applicability of liquid biopsy for disease monitoring and early resistance detection.

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