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Routine molecular applications and recent advances in breast cancer diagnostics

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ABSTRACT

Cancer stands as one of the most common and lethal diseases, imposing a substantial burden on global mortality rates. Breast cancer is distinct from other forms of cancer in which it is the primary cause of death for women. Early detection of breast cancer can significantly lower the risk of mortality, improving the prognosis for those who are affected. The death rate of breast cancer has been steadily rising, according to epidemiological data, especially since the COVID-19 pandemic. This emphasizes the necessity of sensitive and precise technologies that can be utilized in early breast cancer diagnosis. In this process, biomarkers play a pivotal role by facilitating the early detection and diagnosis of breast cancer. Currently, a wide variety of cancer biomarkers have been identified, improving the accuracy of cancer diagnosis. These biomarkers can be applied in liquid biopsies as well as on solid tissues. In the context of breast cancer, biomarkers are particularly valuable for determining who is predisposed to the disease, predicting prognosis at the time of diagnosis, and selecting the best course of therapy. This review comprehensively explores the recently developed gene-based biomarkers from biofluids that are used in the context of breast cancer, as well as the conventional and cutting-edge techniques that have been employed for breast cancer diagnosis.

1. Introduction

Breast cancer (BC) is the most prevalent malignant tumor worldwide; 2.3 million new BC cases were estimated in 2020, according to the World Health Organization's (WHO) statistics (Sung et al., 2021). Typically, a core needle biopsy or tissue biopsy is performed to validate pathological characteristics in order to identify BC (Zhang et al., 2022). However, up to 30% of women diagnosed with cancer at an early stage might develop metastases or exhibit resistance to chemotherapy (Bonotto et al., 2014). For locally advanced BC, the conventional treatment approaches include surgery or mastectomy, with optional radiation therapy or neoadjuvant chemotherapy. It is necessary to classify the pathological subtypes in order to treat BC patients appropriately. Several studies have underscored the importance of delineating the molecular subtypes of BC, considering its heterogeneity in molecular characteristics and cellular composition (Salemme et al., 2023). These subtypes are essential for predicting the prognosis and therapeutic responses as well as treating the disease (Fragomeni et al., 2018). The expression of hormone receptors, including human epidermal receptor 2 (HER2), progesterone receptors (PR), and estrogen receptors (ER), determines the molecular subtypes of BC in contemporary pathological diagnoses (Foulkes et al., 2010; Fragomeni et al., 2018).

The discovery of actionable biomarkers and the improvement of diagnostic tools have led to a constant evolution in the available alternatives for BC diagnosis. Traditionally, biomarker assessment relies on analyses which are conducted on tissue samples obtained with invasive surgical procedures or biopsies (Tomar et al., 2023). However, these analyses face challenges related to tumor heterogeneity and sampling constraints. The revolutionary idea of liquid biopsy has provided an innovative approach to overcome the limitations of conventional tumor specimens, particularly in the context of detecting circulating tumor DNA (ctDNA) which are DNA fragments derived from tumor cells (Santini et al., 2023). Liquid biopsy is a rapidly developing procedure for patients with BC, which encompasses the analysis of various

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components in biofluids, such as DNA, RNA, circulating tumor cells, and extracellular vesicles (Armakolas et al., 2023). In particular, ctDNA analysis provides valuable insights into genetic alterations and tumor dynamics, which is beneficial for managing various diseases. ctDNA testing demonstrates its usefulness in various aspects, including early detection of disease progression, monitoring treatment response, biomarker testing, and identification of resistance mechanisms (Keller et al., 2021). Besides ctDNA, a number of recent studies have highlighted the great potential that microRNA (miRNA) classification from blood sera holds for the diagnosis of BC (Borsos et al., 2022; Davey et al., 2021). Several miRNAs have been hitherto reported with altered expression in human BC. In general, they play a crucial role in controlling gene expression by influencing protein synthesis at the post-transcriptional levels. Numerous miRNAs with abnormal sera levels have been recently identified (Borsos et al., 2022). With some miRNAs being upregulated, indicating a role as putative oncogenes, while others being downregulated, suggesting potential tumor suppressor activity, this discovery hinted at their prospects in BC management (Iorio et al., 2005). These studies emphasize both the diagnostic and therapeutic potential of miRNAs in BC patients. Furthermore, correlated miRNA expression profiles across different malignancies were demonstrated to be associated with long-term survival outcomes (Bouz Mkabaah et al., 2023; Davey et al., 2022).

2. Histopathological classification of breast cancer

Precise pathological reports are essential for patient management in all malignant tumors, including appropriate oncological treatment and decisions about surgical interventions. In the conventional assessment of BC, prognostic and predictive parameters that can define harsh molecular subtypes are included in addition to the precise and thorough inspection of the specimen. The WHO still relies on precise morphological criteria despite an inclination to appropriate molecular subtyping based on routine histopathological examination (Allison et al., 2019).

According to the WHO, histological classification of breast invasive carcinomas can be divided into two main categories: no special and special types. In order to more precisely characterize the biological behavior, additional histological features are described even though the subgroup has a high prognostic value. These features include the associated in situ component, the Nottingham Prognostic Index (NPI), and the presence or absence of either peritumoral lymphovascular or vascular invasion. In addition to histological grade, NPI also includes certain clinical data, such as tumor size and stage (Allison et al., 2019).

2.1. Histological subtypes of breast cancer

2.1.1. Invasive breast carcinoma of no special type

Among BC patients, invasive breast carcinoma of no special type represents a large heterogeneous group that cannot be categorized into any other group. Even within the same case, special morphological characteristics and various histological patterns can be observed that are not considered clinically distinct types. These patients had a slightly worse prognosis and a 10-year survival rate than those with BC overall (65–78% vs. 80%) despite the fact that some special morphological patterns may indicate distinct biological behaviors or affected age groups. The management of these patients relies on subgroups defined by ER and/or HER2 status using biomarkers (Fig. 1A and B) (Allison et al., 2019).

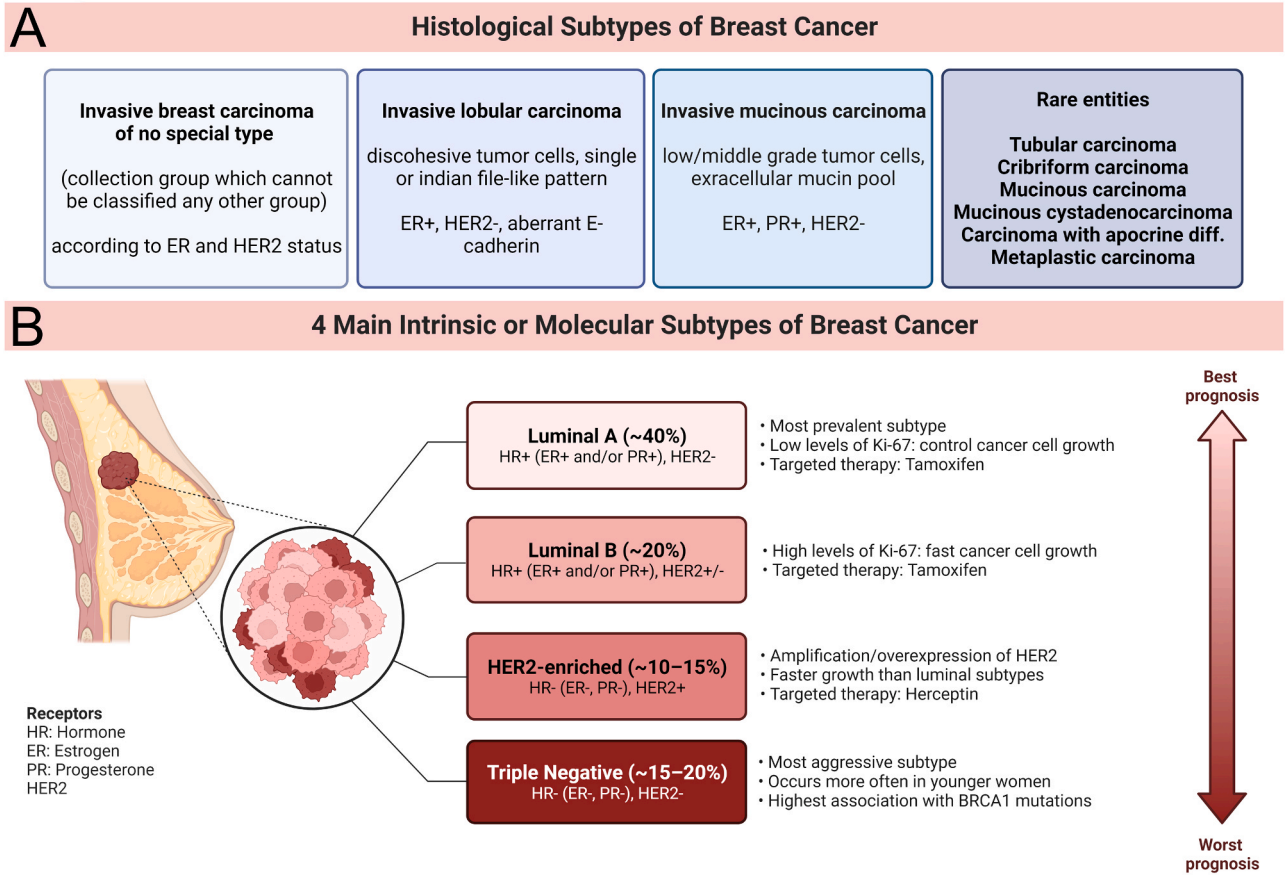

Fig. 1. Breast cancer (BC) subgroups are categorized based on molecular and histological characteristics, with specific histological markers used to identify each subgroup.

2.1.2. Invasive lobular carcinoma

Lobular breast carcinoma, accounting for 5–15% of cases, is the second most common subtype of invasive BC. This special type is characterized by discohesive tumor cells that are typically distributed across desmoplastic stroma or grouped in a single or Indian-file pattern. With a mean age of 60, this type is prevalent in elderly. Invasive lobular carcinoma frequently exhibits ER positivity with HER2 negativity, as well as aberrant expression of E-cadherin (cytoplasmic or lack of expression) which is indicative of a *CDH1* mutation (Fig. 1A and B) (Allison et al., 2019).

2.1.3. Invasive mucinous carcinoma

The third most common type of BC is the mucinous type which accounts for 2% of instances of invasive BC. The morphological characteristics of this type include clusters of low- to middle-grade tumor cells suspended in an extracellular mucin pool. The majority of mucinous carcinomas are positive for ER and PR, while negative for HER2, although in certain cases, the proliferation of micropapillary epithelial cells is associated with HER2 positivity.

The other special types, such as tubular, cribriform, apocrine, and metaplastic breast carcinoma, are uncommon tumors that are represented in 1% or less of BC cases (Fig. 1A) (Allison et al., 2019).

2.2. Molecular subtypes of breast cancer

According to the standard predictive and prognostic biomarkers, including ER, PR, HER2, and Ki-67 proliferation fraction, as well as biological behavior based on survival statistics, BC can be classified into four molecular subtypes: luminal A, luminal B, HER2 positive, and basal-like tumors (Fig. 1B).

ER-positive tumors are defined as those exhibiting expression profiles comparable to those of normal luminal epithelial cells with an active ER pathway (Weigelt et al., 2010). It has been demonstrated that luminal A tumors exhibit poor proliferation and low grade while expressing high levels of ER-activated genes. Although the majority of these tumors have favorable outcomes, luminal B tumors have considerably worse outcomes because they are linked to higher proliferation fractions and grades as well as potential HER2 expression. Albeit the two types of luminal tumors can be separated into subgroups A and B, these cases actually form a spectrum rather than two clearly distinct types (Fig. 1B).

ER-negative tumors are subdivided into HER2-amplified and basal-like groups. Both HER2-amplified and basal-like malignancies are high-grade tumors with a high proliferation rate, high grade, and worse prognosis. While ER and PR negativity and strong HER2 positivity are characteristics of HER2-amplified invasive carcinomas, triple negative (also known as basal-like) tumors represent the worst possible outcome (Fig. 1B) (Allison et al., 2019).

3. Molecular diagnostics of breast cancer tissue

The development of molecular techniques in the past decades has made a significant impact on the detection and management of BC. Translational studies applying advanced molecular techniques have revealed multiple tumor biomarkers that are useful for diagnosis, prognosis, therapeutic response, and monitoring minimally residual diseases. Some of these biomarkers have quickly become integrated into clinical practice. The prognosis and course of treatment of BC are now determined by their molecular classification, which takes into account the gene expression profile of the tumor tissue (Perou et al., 2000). Nonetheless, immunohistochemistry is employed in clinical practice as a cost-effective alternative for gene expression analyses (Cuzick et al., 2011; Ordog et al., 2022; Turkevi-Nagy et al., 2021) (Table1). Classical molecular markers that primarily define the first-line therapy plan include Ki-67, ER, PR, HER2, and p53. These markers are routinely examined by immunohistochemistry (Cuzick et al., 2011). Additionally, testing for specific gene alterations also has predictive and prognostic potential and can be performed using Sanger sequencing or next-generation sequencing (NGS). NGS has the advantage of analyzing multiple genes simultaneously and can identify not only alterations in nucleotide sequences (e.g., point mutations, deletions, and insertions) but also other mutations and gene rearrangements such as translocations, fusions, and amplifications. Our understanding of the genes (and their mutations) linked to BC has expanded thanks to NGS of numerous genes, but the importance of most of them has yet to be clarified. In addition to the well-characterized oncogene mutations, there may be several additional variants of uncertain significance (also known as VUS) due to tumor heterogeneity in the tumor tissue. Since we are currently unable to associate these variants with any clinical data, their existence can be confounding.

Although NGS assays are more sensitive (and expensive) than Sanger sequencing, they are also more susceptible to preanalytical parameters such as native tissue handling, preparation conditions of formalin-fixed paraffin-embedded samples, and DNA isolation methods. Technical parameters, including sequencing depth, fraction of on-target reads, read quality, error rates, the quality of DNA samples, and the precise measurement of tumor cell content, are also crucial factors that affect the results. For multigene sequencing, there are currently only recommendations and guidelines; standardized validation techniques are still lacking (Mosele et al., 2020a). This method will become more frequently incorporated into daily clinical practice if NGS sequencing execution, data analysis, and interpretation are adequately regulated and standardized.

3.1. ESMO guidelines

Currently, the European Society for Medical Oncology (ESMO) guidelines for early BC recommend employing immunohistochemistry to assess classical molecular markers such as ER, PR, HER2, and Ki-67 (both classical and other molecular markers discussed below) (Fig. 1B) (Cardoso et al., 2019; Gennari et al., 2021). Testing for the presence of germline *Breast Cancer gene 1* (*BRCA1)* and *Breast Cancer gene 2* (*BRCA2)* mutations (referred to as gBRCAm) is limited to patients with a family history, a personal history of ovarian cancer, BC that develops before the age of 50, triple-negative breast cancer (TNBC) that develops before the age of 60, or male patients (Gennari et al., 2021). Regarding traditional biomarkers, the guidelines for metastatic breast cancer (MBC) are unchanged. Patients with TNBC, or ER-positive and HER2-negative BC can undergo gBRCAm assessment; however, *Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase Catalytic Subunit Alpha* (*PIK3CA)* mutation testing is optional in either case. Assessments of *ESR1* mutations (ER) and somatic BRCA mutations are also optional, but only if the findings have the potential to influence the treatment choice. Genomic profiling in further tumor tissues or ctDNA diagnostic tests is only suggested if the results allow for a modification of the treatment plan or if patients are eligible to be enrolled in appropriate clinical trials (Table 1) (Gennari et al., 2021).

The following genes must have their mutation status examined for diagnostic purposes. Higher levels of the nuclear and nucleolar protein Ki-67, whose expression is related to cell proliferation, indicate the aggressiveness of the tumor in patients with BC. In clinical practice, immunohistochemistry is used to determine protein expression (Dowsett et al., 2011). The nuclear ER is a ligand-activated transcription factor (Hilton et al., 2018). ERα isoform regulates the expression of genes associated with cell survival and proliferation in BC. One of the primary biomarkers in the recently adopted molecular classification of BC is ER, a well-established prognostic marker employed in the disease, which is determined using immunohistochemistry. A positive ER status is associated with a favorable outcome and indicates the use of endocrine therapy (ER antagonists). However, mutations in *Estrogen receptor 1 (ESR1)* gene can cause tumor cells to acquire resistance during endocrine therapy (Fribbens et al., 2016). Similar to ER, PR is a nuclear

Table 1
Diagnostics methods have been employed for both hereditary and somatic BC, highlighting their advantages.

Nucleic acid-based molecular diagnostics of the breast cancer			
	Hereditary cancer-linked gene mutations	Non-hereditary tumor genomic mutations	Tumor gene-expression profile
Method	NGS sequencing	NGS sequencing	RNA expression assay
Applicable samples	Tissue (isolated DNA) Blood (isolated DNA from leukocytes)	Tumor tissue (isolated DNA) Blood (isolated circulating tumor or cell-free DNA)	Tumor tissue (isolated RNA)
Genes or number of genes tested	*BRCA1*, *BRCA2*, *P53*, *PTEN*, *CDH1*, *PALB2*, and other genes	From 2 to over 400	Depends on assay 7–80
Gained biological information	Germline DNA mutations, deletions, amplifications, and fusions	Mutations, deletions, amplifications, and fusions in tumor DNA	Alterations of gene expression in tumor tissue
Clinical relevance	Identification of patients for targeted therapy	Prognostic information, possible gene targets for targeted therapy and information about recurrence or resistance to treatment	Prognostic information and prediction of benefit from chemotherapy

ligand-activated transcription factor that controls the expression of genes associated with cell differentiation and proliferation (Nicolini et al., 2018). In clinical practice, immunohistochemistry is used to measure PR simultaneously with ER. Better response to endocrine therapy is predicted if the patient also has PR positivity. *Receptor tyrosine kinase 2 (ERBB2)*, encoding HER2 protein, amplification, and the subsequent HER2 overexpression occur in 13–15% of the cases and are indicative of a worse prognosis and high risk of metastasis (Martinez-Saez and Prat, 2021). Immunohistochemistry and/or in situ hybridization (ISH) are typically used for detection. HER2 positivity drives proliferation, promotes adhesion, ensures cell survival, and facilitates metastasis. Targeted therapies against HER2 primarily involve the use of anti-HER2 antibodies such as trastuzumab and pertuzumab, as well as tyrosine kinase inhibitors such as lapatinib, tucatinib, and neratinib. The p53 tumour suppressor protein is encoded by the gene TP53. It has versatile role, through binding to specific DNA elements p53 is capable to induce the expression of different genes which regulates cellular processes such as cell cycle arrest, differentiation, apoptosis, senescence, DNA repair, cell growth and metabolism. Its mutation or dysfunction is observable in many if not all cancer type, in BC pathogenic p53 function is present in 30–35% of all cases (Duffy et al., 2018). p53 mutation status is assessed by immunohistochemistry in daily practice, and although there is no approved drug at the moment for TP53 mutated BC, but there are ongoing clinical trials with compounds which restore the activity of the wild type p53 (Nishikawa and Iwakuma, 2023).

3.2. Nucleic acid-based molecular diagnostics

Furthermore, it is vital to take into account the number of important hereditary and non-hereditary cancer-linked genes whose mutations promote tumor progression in BC. These genes can be categorized based on the roles they perform in the cell cycle, proliferation, cell growth, and apoptosis. They also participate in maintaining genome integrity and contribute to metastasis development. Via the homologous recombination DNA repair pathway, *BRCA1* and *BRCA2* play a fundamental role in double-strand DNA break repair. Because of the loss of genome integrity and elevated mutation rates that result in tumor growth, germline mutations in the *BRCA1* and *BRCA2* genes are associated with an increased risk of developing breast and ovarian cancer (Ben Ayed-Guerfali et al., 2021). Tumors carrying *BRCA* mutations are more sensitive to DNA-damaging agents such as platinum, alkylating agents, topoisomerase II inhibitors, or PARP inhibitors (e.g., olaparib or talazoparib). *PTEN* (phosphatase and tensin homolog) gene is frequently mutated in human cancer and plays a role in cell cycle progression, cell growth, and survival (Carbognin et al., 2019; De Talhouet et al., 2020). The predictive and prognostic significance of *PTEN* for clinical outcomes and response to various treatments in BC is still unclear. The serine/threonine kinase CHK2 (Checkpoint Kinase 2), encoded by *CHEK2* gene, is involved in DNA damage repair and functions as a tumor suppressor, playing a role in apoptosis, cell cycle regulation, and prevention of cell proliferation. Germline mutations in this gene enhance the probability of BC. *ATM* (Ataxia-telangiectasia mutated) gene is involved in double-strand DNA repair and cell cycle regulation (Kleiblova et al., 2019). Mutations in *ATM* increase the relative risk of BC development by 2–5 times (Moslemi et al., 2021). Patients with *ATM* mutations tend to have high-grade disease, more aggressive tumors, and a worse prognosis. PALB2 (Partner and Localizer of BRCA2 protein) acts as a tumor suppressor and also aids in preserving genome integrity. Patients carrying germline mutations in this gene are substantially more likely to develop BC and have a worse prognosis overall (Nepomuceno et al., 2017). Finally, by interacting with damaged DNA and BRCA1 protein, BRIP1 (Breast cancer 1 interacting helicase 1), plays a crucial role in the process of DNA repair. It is a potential candidate gene for hereditary BC in conjunction with *BRCA1–2* (Ouhtit et al., 2016). CDH1 (Cadherin 1 or E-cadherin) protein, encoded by the *CDH1* gene, is vital for epithelial adhesion. The diminished function or abnormal expression of *CDH1* caused by mutations increases the metastatic potential of BC protein functions as a partner for BRCA1 in DNA damage repair (Corso et al., 2020). In vitro and in vivo studies suggest that patients carrying germline mutations in this gene may benefit from therapy with PARP inhibitors. PI3K (Phosphatidylinositol 3-kinase), encoded by *PIK3CA* (the Catalytic Subunit alpha of Phosphatidylinositol 3-kinase) gene, is a family of lipid kinases involved in various cellular processes, including cell growth, proliferation, and differentiation (Reinhardt et al., 2022; Thorpe et al., 2015). PI3K mutations are observed in several types of cancer, including BC, and are associated with chemoresistance and poor prognosis (Mosele et al., 2020b; Sobhani et al., 2018). Using the PI3K inhibitor alpelisib in combination with fulvestrant has demonstrated significant efficacy in patients with *PIK3CA* mutation(s), ER/PR-positive and HER2-negative BC (Table 1).

In addition to determining gene expressions (by immunohistochemistry in clinical practice), BC tumor samples can be classified using multigene expression tests. These tests are used to predict not only the prognosis in BC but also the benefit of chemotherapy (Duffy et al., 2017). The currently available primary gene expression assays include MammaPrint, Oncotype DX, Prosigna, Breast Cancer Index, and EndoPredict. Depending on the particular assay that is employed, the expression of 7–80 genes can be examined. Beyond molecular classification, these assays provide additional information about the patient's prognosis; these include the likelihood of a distant recurrence (at 5 and 10 years), the benefit of chemotherapy, long-term prognosis, and response to systemic therapy.

4. Serum diagnostics opportunities in breast cancer

4.1. Circulating tumor DNA

Tumors can release various DNA fragments present in different bodily fluids. These fragments originated from processes such as apoptosis, necrosis, or active excretion of tumor cells, are often enclosed within extracellular vesicles, or can be freely found in bodily fluids. The amount of ctDNAs in bodily fluids depends on the size and development

rate of the tumor. ctDNAs have a half-life of approximately 2 hours; therefore, they are rapidly eliminated from the bloodstream (Kim and Park, 2023). They are present in blood plasma and are useful clinical indicators since they can reveal subclonal alterations and clonal diversity in real time. Droplet digital PCR (ddPCR) and NGS provide precise molecular detection and a comprehensive picture of genetic modifications and alterations. While ctDNA assays are useful for selecting targeted treatments, they lack simultaneous information on protein expression and single-cell resolution for characterizing genomic diversity. It is well known that BC progression correlates with higher levels of ctDNA (Rohanizadegan, 2018). Consequently, the analysis of circulating tumor-derived material has emerged as an innovative approach for patient management regardless of BC stage. Additionally, it has been reported that the level of ctDNA contains both qualitative and quantitative information since elevated ctDNA levels suggest that the tumor is growing aggressively or that a recurrence is possible (Bittla et al., 2023). It has also been demonstrated that ctDNA levels, even in early-stage BC, can be used as a marker for early detection of the disease and also provide valuable information on treatment adaptation (Davidson et al., 2021).

Numerous methods have been recently employed to identify and quantify ctDNA in BC patients. Plasma samples from patients obtained both before and after surgery can be used for ddPCR to identify *PIK3CA* mutations. ddPCR is a useful technique for assessing tumor mass and monitoring the development of resistant clones via the identification of the pathogenic mutation in blood (Gezer et al., 2022). Additionally, a correlation was also found between ctDNA and response to anti-HER2 treatment; HER2-positive tumors without baseline ctDNA exhibited higher rates of pathological complete response (pCR), suggesting that ctDNA might be a useful biomarker for evaluating the response to neoadjuvant chemotherapy in HER2-positive BC (Ciriaco et al., 2022). Moreover, a large incidence of lymph node metastases was also associated with the elevated ctDNA concentrations after surgery, suggesting the possibility of recurrence and distant metastasis, although with limited sensitivity (Cailleux et al., 2022). Accurate diagnostic assays are crucial in BC management. However, because of their low sensitivity as compared to conventional diagnostic techniques, they require a therapeutic response biomarker that can discriminate between the tumorous and healthy tissues, ensuring that patients receive the best possible care. These findings underscore the potential of serial ctDNA measurements in characterizing patients who are at risk of recurrence and providing valuable insights for personalized treatment plans (Fig. 2).

Advanced sequencing techniques have been employed as a backup method to detect ctDNA in bodily fluids. These techniques have greatly improved the analytical tools available for analyzing ctDNA in patients with BC. Among them, Tagged-Amplicon Deep Sequencing, exhibits impressive sensitivity and specificity of approximately 100% and a detection rate of over 2%, allowing for detailed analysis of the transcripts and genomes of various species (Cailleux et al., 2022). Another innovative approach, Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq), utilizes NGS technology to analyze ctDNA. CAPP-Seq is capable of detecting low amounts of DNA in a cost-effective manner. Tumor heterogeneity can be evaluated by using this method to identify various mutations in patients with the same cancer type. CAPP-Seq has proven to be able to identify tumor burdens prior to medical imaging. This method can detect a variety of mutations, including single nucleotide polymorphisms (also known as SNPs), and significant rearrangements involving insertions, deletions, and copy number variations (CNVs) (Newman et al., 2014, 2016).

Besides the targeted panel sequencing, Whole Genome Bisulfite Sequencing (WGBS-Seq) is the gold-standard technique for obtaining a comprehensive base-pair resolution and quantitative information about methylated cytosines in the genome. By identifying individually methylated cytosines throughout the genome, this method provides unbiased genome-wide DNA methylation profiling. Because it includes exonic alterations, WGBS-Seq may be less sensitive than some other methods, but it is still an affordable and high-yield approach for a thorough analysis of DNA methylation patterns (Olova et al., 2018). Additionally, Whole Genome Sequencing and Whole Exome Sequencing enable the detection of genome-wide rearrangements, somatic chromosomal aberrations, and CNVs (Imperial et al., 2019).

Several reports have demonstrated that this method plays a pivotal role in various aspects of BC management using NGS-based ctDNA detection. It has the potential to identify tumor heterogeneity, multiple druggable mutations in a tumor, to monitor response to a treatment, but it also has a limited screening capacity for detecting early-stage tumors (Sun et al., 2021). For instance, Priskin and colleagues demonstrated that therapeutic resistance can be identified months before image-based diagnostics reveal a certain metastasis by monitoring ctDNA (Priskin et al., 2021). Despite the absence of a standard baseline level of ctDNA for BC diagnosis, fluctuations in ctDNA levels over time are valuable indicators. These variations not only reflect the burden of the disease but

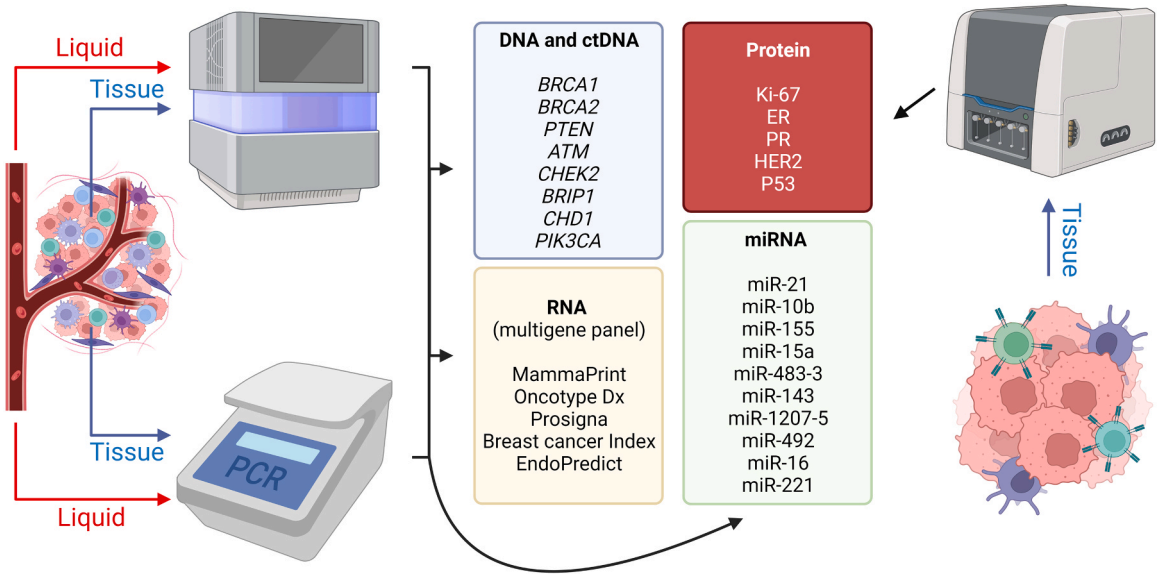

Fig. 2. Biomarkers have been utilized in the routine diagnostics of BC, including future predictive biomarkers. The diagnostic platforms and types of biomarkers are indicated.

also contribute to determining the prognosis of tumorous patients and predicting their response to therapeutic interventions (Fig. 2).

In a prospective study by Garcia-Murillas and colleagues, sequencing of 14 driver gene mutations in primary tumor biopsies revealed that 45 out of 55 patients had at least one of these mutations which can be detected in ctDNA (Garcia-Murillas et al., 2019). Similarly, Olsson and co-researchers conducted a retrospective study involving 20 patients, 14 of whom experienced relapse, and Coombes' team performed a prospective study with 49 patients, 18 of whom relapsed (Coombes et al., 2019; Olsson et al., 2015). Serial monitoring of ctDNA in these studies enabled the detection of metastatic progression on average 11 months earlier (range from 0.5 to 37 months) compared to detection using clinical signs, imaging, CA 15–3, or liver function tests (Olsson et al., 2015). These ctDNA-based methods have a sensitivity that varies from 86% to 93% and a specificity of 100%. These findings highlight the potential of ctDNA analysis as a powerful tool with improved sensitivity and specificity than traditional techniques for detecting early relapse.

Analyzing the proportion of ctDNA is indispensable for understanding tumor dynamics, gauging treatment response, and assessing the risk of relapse. This metric is of particular importance in triple-negative BC patients, where the ctDNA percentage shows a correlation with progression-free survival (Stecklein et al., 2023). Moreover, it provides information on particular gene mutations crucial for metastatic BC management. Among the genes listed in Table 1, several genes, including *TP53, PIK3CA, ESR1, GATA3, ARID1A*, and *PTEN*, are frequently altered in metastatic BC (Kingston et al., 2021). These mutations can be categorized as either truncal which are present in all cancer cells of the patient, or subclonal which are randomly distributed throughout the genome. Notably, the ctDNA dynamics of subclonal mutations have limited potential in predicting clinical outcomes. Understanding the interplay between these genetic alterations and ctDNA levels provides essential information on tailoring treatment strategies and predicting disease progression in BC patients.

4.2. RNA

Although mRNA-based diagnostics provide a more comprehensive snapshot, other types of markers may also be taken into consideration. In BC research, miRNAs have emerged as powerful tools for predicting various aspects of the disease. Initially, a set of particular miRNAs associated with hormone receptor status was discovered, which has a potential in molecular subtyping of BC (Arun et al., 2022; Lowery et al., 2009). In this study, several miRNAs were found to predict ER, PR, and HER2 status (Lowery et al., 2009). Moreover, miRNAs are promising biomarkers, which is supported by a number of studies, demonstrating that patients with BC had higher levels of miRNA-21, miRNA-10b, and miRNA-155 than healthy controls. Interestingly, these miRNAs exhibited significant declines after surgery, radiotherapy, and chemotherapy, indicating their role as response biomarkers (Borsos et al., 2022; Khalighfard et al., 2018). Moreover, miRNAs have proven to be valuable in predicting responses to neoadjuvant chemotherapy (Zhang et al., 2021). Studies revealed specific miRNA expression profiles associated with pCR in HER2-positive patients who received neoadjuvant chemotherapy and adjuvant trastuzumab (Fig. 2) (Xing et al., 2021).

miRNAs, a class of small RNA molecules present in bodily fluids, have emerged as promising tools for disease diagnosis and surveillance due to their stability and broad distribution. They have the power to regulate approximately one-third of all protein-coding genes, influencing vital processes such as cell proliferation and apoptosis. BC is characterized by abnormal cell growth in breast ducts and glands; it can be classified into different subtypes, such as luminal A, luminal B, HER2, and basal-like. Several studies have revealed that patients with BC have distinct patterns of miRNA expression when compared to healthy individuals, with significant alterations observed in particular miRNAs. Certain miRNAs have been identified as key players in BC development, either promoting or hindering cell proliferation due to their interactions with genes involved in cell cycle regulation (Yu et al., 2010). Notably, miRNA-10b was discovered to be upregulated in metastatic breast tumors, indicating their prognostic potential (Khalighfard et al., 2018). miRNAs act at the post-transcriptional level by regulating gene expression. In BC development, miRNAs can function as either oncogenes or tumor suppressors, influencing vital pathways such as angiogenesis, proliferation, metastasis, and evading cell death. Due to their extreme stability and presence in bodily fluids, these small RNA molecules are promising tools for disease diagnosis and progression. Roughly one-third of all protein-coding genes are believed to be under the control of miRNAs, which play a significant role in processes including cell proliferation and apoptosis.

Numerous miRNAs have been demonstrated to affect BC in several studies. miRNA-15a is downregulated in BC, indicating that it functions as a tumor suppressor by regulating Cyclin E1 expression and hampering cell migration (Luo et al., 2013). Another miRNA that has been linked to the growth of BC cells is miRNA-483–3p. When upregulated, miRNA-483–3p prevents cancer cells from entering the S phase of the cell cycle by targeting Cyclin E1. Additionally, miRNA-143 exhibits tumour-suppressive properties in BC because its overexpression reduces cell viability by targeting Extracellular signal regulated kinase 5 (ERK5) and Mitogen-activated protein kinase kinase kinase 7 (MAP3K7) (Huang and Lyu, 2018). Conversely, certain miRNAs demonstrate oncogenic characteristics in BC. For instance, miRNA-1207–5p promotes the growth of BC cells by downregulating cell cycle suppressors Cyclin Dependent Kinase Inhibitor 1A (CDKN1A) and Cyclin Dependent Kinase Inhibitor 1B (CDKN1B) via the *Signal transducer and activator of transcription 6 (STAT6)* gene (Yan et al., 2017). Similarly, miRNA-492 exhibits oncogenic behavior, and its overexpression leads to decreased levels of SRY-box transcription factor 7 (SOX7), a key component of the Wnt/B-catenin pathway (Shen et al., 2015). These findings underscore the complex regulatory roles of miRNAs in BC, with both suppressive and oncogenic effects on the onset and course of the disease. Indeed, miRNAs hold significant promise as biomarkers in BC diagnosis. In a study conducted by Borsos and colleagues, the researchers monitored the expressional changes of 15 pre-selected miRNAs. Their findings revealed a potential advancement in diagnostics by focusing on the expression levels of miR-15a, miR-16, and miR-221 in BC management. The miR-15a, miR-16, and miR-221 combination emerged as the most promising set of multiple miRNAs in their research, suggesting its potential utility for advancing diagnostic applications in breast cancer. Based on their findings, the clinical integration of miR-15a, miR-16, and miR-221 holds significant importance for improving the management and reducing the recurrence of breast cancer (Borsos et al., 2022). This research suggests that analyzing these specific miRNAs might enhance the accuracy and efficiency of BC diagnosis, marking a significant step forward in the field of cancer management (Fig. 2).

Circular RNAs (circRNAs) have emerged as crucial regulators in BC, aiding in subtype classification and therapeutic decisions (Loganathan and Doss, 2023). They play a pivotal role in distinguishing between different BC subtypes, providing rapid insights into suitable therapy protocols, and contributing to improved patient outcomes. CircRNAs with prognostic significance hold therapeutic promise; silencing their expression or utilizing them as therapeutic targets could potentially enhance tumor prognosis (Dawoud et al., 2023). CircRNAs exert their influence through variable splicing which involves the preferential selection of alternative splice sites and the utilization of various splicing mechanisms and mRNA isomers, thereby directly impacting the transcription of linear isoforms. Additionally, circRNAs influence transcription by inducing DNA hypomethylation in the promoter region of the parental gene or regulating intronic enhancers. For instance, circTADA2A-E6 has been identified as a tumor suppressor, with Suppressor of cytokine signaling 3 (SOCS3) as its downstream target gene, indicating its potential therapeutic relevance. Another circRNA, circTADA-E5/E6, exhibited downregulation in BC (Xu and Zhang, 2021). Circ_000911 also acted as a tumour suppressor in BC, as reported

in a study (Wang et al., 2018). CircEPSTI1, which is upregulated in BC, serves as a prognostic marker and mediator in triple-negative BC (TNBC) (Chen et al., 2018). CircANKS1B, which is upregulated in TNBC with lymph node metastases and progressive clinical stages, was found to inhibit cancer metastasis, highlighting its potential role in mitigating disease progression (Zeng et al., 2018). Our knowledge of circRNAs's participation in the pathophysiology of BC has greatly increased as a result of their various functions in drug resistance as well as promoting, regulating, and inhibiting BC (He et al., 2021). These findings underscore the complexity of circRNA-mediated regulatory networks and highlight their potential as therapeutic targets in BC treatment strategies.

5. Conclusions

Recent advancements in molecular research have greatly expanded our understanding of tumor characteristics, leading to a broader knowledge of BC biomarkers and improved personalized patient therapies. This review provides a comprehensive overview of traditional, novel, and potential biomarkers, encompassing gene expression profiling of solid tumors as well as emerging diagnostic opportunities that use serum samples for ctDNA and miRNA profiling. The next generation of liquid biopsy research will be pivotal in determining the clinical feasibility of blood-based genomic profiling, even if this field offers a plethora of opportunities. However, further research is essential before tracking ctDNA mutations, and miRNA profiling can become a standard procedure for patients with early-stage BC. It is indispensable to address challenges such as standardizing the blood collection procedure to enhance sample stability, defining novel quantification methods, standardizing isolation techniques, and improving detection sensitivity.

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Pankotai-Bodó Gabriella: Conceptualization, Writing – original draft, Writing – review & editing. **Pankotai Tibor:** Conceptualization, Writing – original draft, Writing – review & editing. **Sükösd Farkas:** Conceptualization, Writing – original draft, Writing – review & editing. **Oláh-Németh Orsolya:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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References

Allison, K.H., Brogi, E., Ellis, I.O., Fox, S.B., Morris, E.A., Sahin, A., Salgado, R., Sapino, A., Sasano, H., Schnitt, S., Sotiriou, C., van Diest, P.J., members, S., Carneiro, F., Chan, J.K.C., Cheung, A.N.-Y., Cree, I.A.E.B.C., Fitzgibbons, P.L., Gill, A.J., Goldblum, J.R., Lakhani, S.R., Lax, S.F., Lazar, A.J., Moch, H., Ochiai, A., Oliva, E., Rous, B., Singh, R., Soares, F.A., Srigley, J.R., Tan, P.H., Thompson, L.D.R., Tsao, M. S., Tsuzuki, T., Washington, M.K., (2019) WHO Classification of Tumours Editorial Board. Breast tumours [Internet]. International Agency for Research on Cancer WHO classification of tumours series, 5th ed.; vol. 2.

Armakolas, A., Kotsari, M., Koskinas, J., 2023. Liquid Biopsies, Novel Approaches and Future Directions. Cancers (Basel) 15.

Arun, R.P., Cahill, H.F., Marcato, P., 2022. Breast Cancer Subtype-Specific miRNAs: Networks, Impacts, and the Potential for Intervention. Biomedicines 10.

Ben Ayed-Guerfali, D., Ben Kridis-Rejab, W., Ammous-Boukhris, N., Ayadi, W., Charfi, S., Khanfir, A., Sellami-Boudawara, T., Frikha, M., Daoud, J., Mokdad-Gargouri, R., 2021. Novel and recurrent BRCA1/BRCA2 germline mutations in patients with breast/ovarian cancer: a series from the south of Tunisia. J. Transl. Med 19, 108.

Bittla, P., Kaur, S., Sojitra, V., Zahra, A., Hutchinson, J., Folawemi, O., Khan, S., 2023. Exploring Circulating Tumor DNA (CtDNA) and Its Role in Early Detection of Cancer: A Systematic Review. Cureus 15, e45784.

Bonotto, M., Gerratana, L., Poletto, E., Driol, P., Giangreco, M., Russo, S., Minisini, A.M., Andreetta, C., Mansutti, M., Pisa, F.E., Fasola, G., Puglisi, F., 2014. Measures of outcome in metastatic breast cancer: insights from a real-world scenario. Oncologist 19, 608–615.

Borsos, B.N., Pahi, Z.G., Ujfaludi, Z., Sukosd, F., Nikolenyi, A., Banko, S., Pankotai-Bodo, G., Olah-Nemeth, O., Pankotai, T., 2022. BC-miR: Monitoring Breast Cancer-Related miRNA Profile in Blood Sera-A Prosperous Approach for Tumor Detection. Cells 11.

Bouz Mkabaah, L., Davey, M.G., Lennon, J.C., Bouz, G., Miller, N., Kerin, M.J., 2023. Assessing the Role of MicroRNAs in Predicting Breast Cancer Recurrence-A Systematic Review. Int J. Mol. Sci. 24.

Cailleux, F., Agostinetto, E., Lambertini, M., Rothe, F., Wu, H.T., Balcioglu, M., Kalashnikova, E., Vincent, D., Viglietti, G., Gombos, A., Papagiannis, A., Veys, I., Awada, A., Sethi, H., Aleshin, A., Larsimont, D., Sotiriou, C., Venet, D., Ignatiadis, M., 2022. Circulating Tumor DNA After Neoadjuvant Chemotherapy in Breast Cancer Is Associated With Disease Relapse. JCO Precis Oncol. 6, e2200148.

Carbognin, L., Miglietta, F., Paris, I., Dieci, M.V., 2019. Prognostic and Predictive Implications of PTEN in Breast Cancer: Unfulfilled Promises but Intriguing Perspectives. Cancers (Basel) 11.

Cardoso, F., Kyriakides, S., Ohno, S., Penault-Llorca, F., Poortmans, P., Rubio, I.T., Zackrisson, S., Senkus, E., clinicalguidelines@esmo.org, E.G.C.E.a., 2019. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. Ann. Oncol. 30, 1194–1220.

Chen, B., Wei, W., Huang, X., Xie, X., Kong, Y., Dai, D., Yang, L., Wang, J., Tang, H., Xie, X., 2018. circEPSTI1 as a Prognostic Marker and Mediator of Triple-Negative Breast Cancer Progression. Theranostics 8, 4003–4015.

Ciriaco, N., Zamora, E., Escriva-de-Romani, S., Miranda Gomez, I., Jimenez Flores, J., Saura, C., Sloane, H., Starus, A., Fredebohm, J., Georgieva, L., Speight, G., Jones, F., Ramon, Y.C.S., Espinosa-Bravo, M., Peg, V., 2022. Clearance of ctDNA in triple-negative and HER2-positive breast cancer patients during neoadjuvant treatment is correlated with pathologic complete response. Ther. Adv. Med Oncol. 14, 17588359221139601.

Coombes, R.C., Page, K., Salari, R., Hastings, R.K., Armstrong, A., Ahmed, S., Ali, S., Cleator, S., Kenny, L., Stebbing, J., Rutherford, M., Sethi, H., Boydell, A., Swenerton, R., Fernandez-Garcia, D., Gleason, K.L.T., Goddard, K., Guttery, D.S., Assaf, Z.J., Wu, H.T., Natarajan, P., Moore, D.A., Primrose, L., Dashner, S., Tin, A.S., Balcioglu, M., Srinivasan, R., Shchegrova, S.V., Olson, A., Hafez, D., Billings, P., Aleshin, A., Rehman, F., Toghill, B.J., Hills, A., Louie, M.C., Lin, C.J., Zimmermann, B.G., Shaw, J.A., 2019. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin. Cancer Res 25, 4255–4263.

Corso, G., Figueiredo, J., De Angelis, S.P., Corso, F., Girardi, A., Pereira, J., Seruca, R., Bonanni, B., Carneiro, P., Pravettoni, G., Guerini Rocco, E., Veronesi, P., Montagna, G., Sacchini, V., Gandini, S., 2020. E-cadherin deregulation in breast cancer. J. Cell Mol. Med 24, 5930–5936.

Cuzick, J., Dowsett, M., Pineda, S., Wale, C., Salter, J., Quinn, E., Zabaglo, L., Mallon, E., Green, A.R., Ellis, I.O., Howell, A., Buzdar, A.U., Forbes, J.F., 2011. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J. Clin. Oncol. 29, 4273–4278.

Davey, M.G., Davies, M., Lowery, A.J., Miller, N., Kerin, M.J., 2021. The Role of MicroRNA as Clinical Biomarkers for Breast Cancer Surgery and Treatment. Int J. Mol. Sci. 22.
Davey, M.G., Feeney, G., Annuk, H., Paganga, M., Holian, E., Lowery, A.J., Kerin, M.J., Miller, N., 2022. MicroRNA Expression Profiling Predicts Nodal Status and Disease Recurrence in Patients Treated with Curative Intent for Colorectal Cancer. Cancers (Basel) 14.
Davidson, B.A., Croessmann, S., Park, B.H., 2021. The breast is yet to come: current and future utility of circulating tumour DNA in breast cancer. Br. J. Cancer 125, 780–788.
Dawoud, A., Ihab Zakaria, Z., Hisham Rashwan, H., Braoudaki, M., Youness, R.A., 2023. Circular RNAs: New layer of complexity evading breast cancer heterogeneity. Noncoding RNA Res 8, 60–74.
De Talhouet, S., Peron, J., Vuilleumier, A., Friedlaender, A., Viassolo, V., Ayme, A., Bodmer, A., Treilleux, I., Lang, N., Tille, J.C., Chappuis, P.O., Buisson, A., Giraud, S., Lasset, C., Bonadona, V., Tredan, O., Labidi-Galy, S.I., 2020. Clinical outcome of breast cancer in carriers of BRCA1 and BRCA2 mutations according to molecular subtypes. Sci. Rep. 10, 7073.
Dowsett, M., Nielsen, T.O., A'Hern, R., Bartlett, J., Coombes, R.C., Cuzick, J., Ellis, M., Henry, N.L., Hugh, J.C., Lively, T., McShane, L., Paik, S., Penault-Llorca, F., Prudkin, L., Regan, M., Salter, J., Sotiriou, C., Smith, I.E., Viale, G., Zujewski, J.A., Hayes, D.F., International Ki-67 in Breast Cancer Working, G., 2011. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J. Natl. Cancer Inst. 103, 1656–1664.
Duffy, M.J., Harbeck, N., Nap, M., Molina, R., Nicolini, A., Senkus, E., Cardoso, F., 2017. Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). Eur. J. Cancer 75, 284–298.
Duffy, M.J., Synnott, N.C., Crown, J., 2018. Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. Breast Cancer Res Treat. 170, 213–219.
Foulkes, W.D., Smith, I.E., Reis-Filho, J.S., 2010. Triple-negative breast cancer. N. Engl. J. Med 363, 1938–1948.
Fragomeni, S.M., Sciallis, A., Jeruss, J.S., 2018. Molecular Subtypes and Local-Regional Control of Breast Cancer. Surg. Oncol. Clin. N. Am. 27, 95–120.
Fribbens, C., O'Leary, B., Kilburn, L., Hrebien, S., Garcia-Murillas, I., Beaney, M., Cristofanilli, M., Andre, F., Loi, S., Loibl, S., Jiang, J., Bartlett, C.H., Koehler, M., Dowsett, M., Bliss, J.M., Johnston, S.R., Turner, N.C., 2016. Plasma ESR1 Mutations and the Treatment of Estrogen Receptor-Positive Advanced Breast Cancer. J. Clin. Oncol. 34, 2961–2968.
Garcia-Murillas, I., Chopra, N., Comino-Mendez, I., Beaney, M., Tovey, H., Cutts, R.J., Swift, C., Kriplani, D., Afentakis, M., Hrebien, S., Walsh-Crestani, G., Barry, P., Johnston, S.R.D., Ring, A., Bliss, J., Russell, S., Evans, A., Skene, A., Wheatley, D., Dowsett, M., Smith, I.E., Turner, N.C., 2019. Assessment of Molecular Relapse Detection in Early-Stage Breast Cancer. JAMA Oncol. 5, 1473–1478.
Gennari, A., Andre, F., Barrios, C.H., Cortes, J., de Azambuja, E., DeMichele, A., Dent, R., Fenlon, D., Gligorov, J., Hurvitz, S.A., Im, S.A., Krug, D., Kunz, W.G., Loi, S., Penault-Llorca, F., Ricke, J., Robson, M., Rugo, H.S., Saura, C., Schmid, P., Singer, C.F., Spanic, T., Tolaney, S.M., Turner, N.C., Curigliano, G., Loibl, S., Paluch-Shimon, S., Harbeck, N., clinicalguidelines@esmo.org, E.G.C.E.a., 2021. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann. Oncol. 32, 1475–1495.
Gezer, U., Bronkhorst, A.J., Holdenrieder, S., 2022. The Clinical Utility of Droplet Digital PCR for Profiling Circulating Tumor DNA in Breast Cancer Patients. Diagn. (Basel) 12.
He, A.T., Liu, J., Li, F., Yang, B.B., 2021. Targeting circular RNAs as a therapeutic approach: current strategies and challenges. Signal Transduct. Target Ther. 6, 185.
Hilton, H.N., Clarke, C.L., Graham, J.D., 2018. Estrogen and progesterone signalling in the normal breast and its implications for cancer development. Mol. Cell Endocrinol. 466, 2–14.
Huang, X., Lyu, J., 2018. Tumor suppressor function of miR-483-3p on breast cancer via targeting of the cyclin E1 gene. Exp. Ther. Med 16, 2615–2620.
Imperial, R., Nazer, M., Ahmed, Z., Kam, A.E., Pluard, T.J., Bahaj, W., Levy, M., Kuzel, T. M., Hayden, D.M., Pappas, S.G., Subramanian, J., Masood, A., 2019. Matched Whole-Genome Sequencing (WGS) and Whole-Exome Sequencing (WES) of Tumor Tissue with Circulating Tumor DNA (ctDNA) Analysis: Complementary Modalities in Clinical Practice. Cancers (Basel) 11.
Iorio, M.V., Ferracin, M., Liu, C.G., Veronese, A., Spizzo, R., Sabbioni, S., Magri, E., Pedriali, M., Fabbri, M., Campiglio, M., Menard, S., Palazzo, J.P., Rosenberg, A., Musiani, P., Volinia, S., Nenci, I., Calin, G.A., Querzoli, P., Negrini, M., Croce, C.M., 2005. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 65, 7065–7070.
Keller, L., Belloum, Y., Wikman, H., Pantel, K., 2021. Clinical relevance of blood-based ctDNA analysis: mutation detection and beyond. Br. J. Cancer 124, 345–358.
Khalighfard, S., Alizadeh, A.M., Irani, S., Omranipour, R., 2018. Plasma miR-21, miR-155, miR-10b, and Let-7a as the potential biomarkers for the monitoring of breast cancer patients. Sci. Rep. 8, 17981.
Kim, H., Park, K.U., 2023. Clinical Circulating Tumor DNA Testing for Precision Oncology. Cancer Res Treat. 55, 351–366.
Kingston, B., Cutts, R.J., Bye, H., Beaney, M., Walsh-Crestani, G., Hrebien, S., Swift, C., Kilburn, L.S., Kernaghan, S., Moretti, L., Wilkinson, K., Wardley, A.M., Macpherson, I.R., Baird, R.D., Roylance, R., Reis-Filho, J.S., Hubank, M., Faull, I., Banks, K.C., Lanman, R.B., Garcia-Murillas, I., Bliss, J.M., Ring, A., Turner, N.C., 2021. Genomic profile of advanced breast cancer in circulating tumour DNA. Nat. Commun. 12, 2423.
Kleiblova, P., Stolarova, L., Krizova, K., Lhota, F., Hojny, J., Zemankova, P., Havranek, O., Vocka, M., Cerna, M., Lhotova, K., Borecka, M., Janatova, M., Soukupova, J., Sevcik, J., Zimovjanova, M., Kotlas, J., Panczak, A., Vesela, K., Cervenkova, J., Schneiderova, M., Burocziova, M., Burdova, K., Stranecky, V., Foretova, L., Machackova, E., Tavandzis, S., Kmoch, S., Macurek, L., Kleibl, Z., 2019. Identification of deleterious germline CHEK2 mutations and their association with breast and ovarian cancer. Int J. Cancer 145, 1782–1797.
Loganathan, T., Doss, C.G., 2023. Non-coding RNAs in human health and disease: potential function as biomarkers and therapeutic targets. Funct. Integr. Genom. 23, 33.
Lowery, A.J., Miller, N., Devaney, A., McNeill, R.E., Davoren, P.A., Lemetre, C., Benes, V., Schmidt, S., Blake, J., Ball, G., Kerin, M.J., 2009. MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. Breast Cancer Res 11, R27.
Luo, Q., Li, X., Li, J., Kong, X., Zhang, J., Chen, L., Huang, Y., Fang, L., 2013. MiR-15a is underexpressed and inhibits the cell cycle by targeting CCNE1 in breast cancer. Int J. Oncol. 43, 1212–1218.
Martinez-Saez, O., Prat, A., 2021. Current and Future Management of HER2-Positive Metastatic Breast Cancer. JCO Oncol. Pr. 17, 594–604.
Mosele, F., Remon, J., Mateo, J., Westphalen, C.B., Barlesi, F., Lolkema, M.P., Normanno, N., Scarpa, A., Robson, M., Meric-Bernstam, F., Wagle, N., Stenzinger, A., Bonastre, J., Bayle, A., Michiels, S., Bieche, I., Rouleau, E., Jezdic, S., Douillard, J.Y., Reis-Filho, J.S., Dienstmann, R., Andre, F., 2020a. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Ann. Oncol. 31, 1491–1505.
Mosele, F., Stefanovska, B., Lusque, A., Tran Dien, A., Garberis, I., Droin, N., Le Tourneau, C., Sablin, M.P., Lacroix, L., Enrico, D., Miran, I., Jovelet, C., Bieche, I., Soria, J.C., Bertucci, F., Bonnefoi, H., Campone, M., Dalenc, F., Bachelot, T., Jacquet, A., Jimenez, M., Andre, F., 2020b. Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. Ann. Oncol. 31, 377–386.
Moslemi, M., Moradi, Y., Dehghanbanadaki, H., Afkhami, H., Khaledi, M., Sedighimehr, N., Fathi, J., Sohrabi, E., 2021. The association between ATM variants and risk of breast cancer: a systematic review and meta-analysis. BMC Cancer 21, 27.
Nepomuceno, T.C., De Gregoriis, G., de Oliveira, F.M.B., Suarez-Kurtz, G., Monteiro, A. N., Carvalho, M.A., 2017. The Role of PALB2 in the DNA Damage Response and Cancer Predisposition. Int J. Mol. Sci. 18.
Newman, A.M., Bratman, S.V., To, J., Wynne, J.F., Eclov, N.C., Modlin, L.A., Liu, C.L., Neal, J.W., Wakelee, H.A., Merritt, R.E., Shrager, J.B., Loo Jr., B.W., Alizadeh, A.A., Diehn, M., 2014. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat. Med 20, 548–554.
Newman, A.M., Lovejoy, A.F., Klass, D.M., Kurtz, D.M., Chabon, J.J., Scherer, F., Stehr, H., Liu, C.L., Bratman, S.V., Say, C., Zhou, L., Carter, J.N., West, R.B., Sledge, G.W., Shrager, J.B., Loo Jr., B.W., Neal, J.W., Wakelee, H.A., Diehn, M., Alizadeh, A.A., 2016. Integrated digital error suppression for improved detection of circulating tumor DNA. Nat. Biotechnol. 34, 547–555.
Nicolini, A., Ferrari, P., Duffy, M.J., 2018. Prognostic and predictive biomarkers in breast cancer: Past, present and future. Semin Cancer Biol. 52, 56–73.
Nishikawa, S., Iwakuma, T., 2023. Drugs Targeting p53 Mutations with FDA Approval and in Clinical Trials. Cancers (Basel) 15.
Olova, N., Krueger, F., Andrews, S., Oxley, D., Berrens, R.V., Branco, M.R., Reik, W., 2018. Comparison of whole-genome bisulfite sequencing library preparation strategies identifies sources of biases affecting DNA methylation data. Genome Biol. 19, 33.
Olsson, E., Winter, C., George, A., Chen, Y., Howlin, J., Tang, M.H., Dahlgren, M., Schulz, R., Grabau, D., van Westen, D., Ferno, M., Ingvar, C., Rose, C., Bendahl, P.O., Ryden, L., Borg, A., Gruvberger-Saal, S.K., Jernstrom, H., Saal, L.H., 2015. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol. Med 7, 1034–1047.
Ordog, N., Borsos, B.N., Majoros, H., Ujfaludi, Z., Pankotai-Bodo, G., Banko, S., Sukosd, F., Kuthi, L., Pankotai, T., 2022. The clinical significance of epigenetic and RNAPII variabilities occurring in clear cell renal cell carcinoma as a potential prognostic marker. Transl. Oncol. 20, 101420.
Ouhtit, A., Gupta, I., Shaikh, Z., 2016. BRIP1, a potential candidate gene in development of non-BRCA1/2 breast cancer. Front Biosci. (Elite Ed.) 8, 289–298.
Perou, C.M., Sorlie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Rees, C.A., Pollack, J.R., Ross, D.T., Johnsen, H., Akslen, L.A., Fluge, O., Pergamenschikov, A., Williams, C., Zhu, S.X., Lonning, P.E., Borresen-Dale, A.L., Brown, P.O., Botstein, D., 2000. Molecular portraits of human breast tumours. Nature 406, 747–752.
Priskin, K., Polya, S., Pinter, L., Jaksa, G., Csanyi, B., Enyedi, M.Z., Sagi-Zsigmond, E., Sukosd, F., Olah-Nemeth, O., Kelemen, G., Nikolenyi, A., Uhercsak, G., Santha, D., Dobi, A., Szilagyi, E., Valicsek, E., Tordai, L., Toth, R., Kahan, Z., Haracska, L., 2021. BC-Monitor: Towards a Routinely Accessible Circulating Tumor DNA-Based Tool for Real-Time Monitoring Breast Cancer Progression and Treatment Effectiveness. Cancers (Basel) 13.
Reinhardt, K., Stuckrath, K., Hartung, C., Kaufhold, S., Uleer, C., Hanf, V., Lantzsch, T., Peschel, S., John, J., Pohler, M., Bauer, M., Burrig, F.K., Weigert, E., Buchmann, J., Kantelhardt, E.J., Thomssen, C., Vetter, M., 2022. PIK3CA-mutations in breast cancer. Breast Cancer Res Treat. 196, 483–493.
Rohanizadegan, M., 2018. Analysis of circulating tumor DNA in breast cancer as a diagnostic and prognostic biomarker. Cancer Genet 228-229, 159–168.
Salemme, V., Centonze, G., Avalle, L., Natalini, D., Piccolantonio, A., Arina, P., Morellato, A., Ala, U., Taverna, D., Turco, E., Defilippi, P., 2023. The role of tumor microenvironment in drug resistance: emerging technologies to unravel breast cancer heterogeneity. Front Oncol. 13, 1170264.
Santini, D., Botticelli, A., Galvano, A., Iuliani, M., Incorvaia, L., Gristina, V., Taffon, C., Foderaro, S., Paccagnella, E., Simonetti, S., Fazio, F., Scagnoli, S., Pomati, G., Pantano, F., Perrone, G., De Falco, E., Russo, A., Spinelli, G.P., 2023. Network approach in liquidomics landscape. J. Exp. Clin. Cancer Res 42, 193.

Shen, F., Cai, W.S., Feng, Z., Li, J.L., Chen, J.W., Cao, J., Xu, B., 2015. MiR-492 contributes to cell proliferation and cell cycle of human breast cancer cells by suppressing SOX7 expression. Tumour Biol. 36, 1913–1921.
Sobhani, N., Roviello, G., Corona, S.P., Scaltriti, M., Ianza, A., Bortul, M., Zanconati, F., Generali, D., 2018. The prognostic value of PI3K mutational status in breast cancer: A meta-analysis. J. Cell Biochem 119, 4287–4292.
Stecklein, S.R., Kimler, B.F., Yoder, R., Schwensen, K., Staley, J.M., Khan, Q.J., O'Dea, A. P., Nye, L.E., Elia, M., Heldstab, J., Home, T., Hyter, S., Isakova, K., Pathak, H.B., Godwin, A.K., Sharma, P., 2023. ctDNA and residual cancer burden are prognostic in triple-negative breast cancer patients with residual disease. NPJ Breast Cancer 9, 10.
Sun, Y., Liu, F., Fan, C., Wang, Y., Song, L., Fang, Z., Han, R., Wang, Z., Wang, X., Yang, Z., Xu, Z., Peng, J., Shi, C., Zhang, H., Dong, W., Huang, H., Li, Y., Le, Y., Sun, J., Peng, Z., 2021. Characterizing sensitivity and coverage of clinical WGS as a diagnostic test for genetic disorders. BMC Med Genom. 14, 102.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 71, 209–249.
Thorpe, L.M., Yuzugullu, H., Zhao, J.J., 2015. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat. Rev. Cancer 15, 7–24.
Tomar, U., Grover, N., Tomar, S., Bhalla, K., Singh, S., 2023. Liquid biopsy and its significance in tumour - Detection in the field of pathology. J. Oral. Maxillofac. Pathol. 27, 195–200.
Turkevi-Nagy, S., Bathori, A., Bocz, J., Krenacs, L., Cserni, G., Kovari, B., 2021. Syntaxin-1 and Insulinoma-Associated Protein 1 Expression in Breast Neoplasms with Neuroendocrine Features. Pathol. Oncol. Res 27, 1610039.
Wang, H., Xiao, Y., Wu, L., Ma, D., 2018. Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-000911/miR-449a pathway in breast carcinogenesis. Int J. Oncol. 52, 743–754.
Weigelt, B., Geyer, F.C., Reis-Filho, J.S., 2010. Histological types of breast cancer: how special are they? Mol. Oncol. 4, 192–208.
Xing, A.Y., Wang, B., Li, Y.H., Chen, X., Wang, Y.W., Liu, H.T., Gao, P., 2021. Identification of miRNA Signature in Breast Cancer to Predict Neoadjuvant Chemotherapy Response. Pathol. Oncol. Res. 27, 1609753.
Xu, C., Zhang, J., 2021. Mammalian circular RNAs result largely from splicing errors. Cell Rep. 36, 109439.
Yan, C., Chen, Y., Kong, W., Fu, L., Liu, Y., Yao, Q., Yuan, Y., 2017. PVT1-derived miR-1207-5p promotes breast cancer cell growth by targeting STAT6. Cancer Sci. 108, 868–876.
Yu, Z., Baserga, R., Chen, L., Wang, C., Lisanti, M.P., Pestell, R.G., 2010. microRNA, cell cycle, and human breast cancer. Am. J. Pathol. 176, 1058–1064.
Zeng, K., He, B., Yang, B.B., Xu, T., Chen, X., Xu, M., Liu, X., Sun, H., Pan, Y., Wang, S., 2018. The pro-metastasis effect of circANKS1B in breast cancer. Mol. Cancer 17, 160.
Zhang, Y., Li, G., Bian, W., Bai, Y., He, S., Liu, Y., Liu, H., Liu, J., 2022. Value of genomics- and radiomics-based machine learning models in the identification of breast cancer molecular subtypes: a systematic review and meta-analysis. Ann. Transl. Med 10, 1394.
Zhang, Z., Zhang, H., Li, C., Xiang, Q., Xu, L., Liu, Q., Pang, X., Zhang, W., Zhang, H., Zhang, S., Duan, X., Liu, Y., Cui, Y., 2021. Circulating microRNAs as indicators in the prediction of neoadjuvant chemotherapy response in luminal B breast cancer. Thorac. Cancer 12, 3396–3406.