short review

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# Biomarkers and translational research approaches in breast cancer—an update

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**Summary** Diagnosis and decision-making in the treatment of breast cancer patients is vastly dependent on the exploration of biomarkers. Estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are long-standing biomarkers, which determine the breast cancer subtype. In current practice, gene expression analyses further define the molecular breast cancer subtype and give additional information on disease characteristics. Prognostic biomarkers provide information regarding recurrence risk and survival. Predictive biomarkers, such as programmed cell death ligand 1 expression, are tools for identifying patients who can benefit from specific therapy regimens in order to choose the best treatment option for the patient. While some biomarkers are affordable and readily available, others remain technically complex to access. Translational research builds the bridge from discovering novel biomarkers in preclinical studies to testing their application utility in the clinical setting. Integrating translational studies into clinical trials is therefore essential to find novel and reliable biomarkers for an optimal personalized treatment approach for patients with breast cancer.

**Keywords** Breast neoplasms · Biomarker · Translational research · Prognostic markers · Predictive markers

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## Introduction

Biomarkers, essential tools in the treatment of cancer patients, are used to make precise diagnoses and determine the optimal treatment approach for each patient. Prognostic biomarkers give patients and caregivers an estimated survival prognosis. Predictive biomarkers are used to identify patients who can benefit from specific therapies in order to spare patients from non-effective and potentially toxic treatments. Demands on feasible biomarkers are high specificity, technical validity, wide and timely availability across cancer centers, and a cost-effective and material-sparing analysis.

More and more biomarkers are discovered by genome sequencing techniques. The European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) criteria was implemented to rank biomarkers according to their clinical relevance [1]. In breast cancer (BC), ER, PR, HER2, PD-L1, *PIK3CA*, and *gBRCA* have the highest ESCAT score tier I-A [2].

In BC patients, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are long-standing biomarkers determining the BC subtype, prognosis, and therapy options. Nowadays multiple additional biomarkers, summarized in Table 1, exist to guide the optimal treatment of BC patients and are discussed in this short review.

## Hormone receptors

The hormone receptors (HR) ER and PR were the first relevant biomarkers discovered in BC. Approximately 70–80% of BC patients express HR, defining the most frequent BC subtype [3]. HR are prognostic and predictive for endocrine therapy (ESCAT tier I-A) [4]. On

**Table 1** Biomarkers in breast cancer

| Name | Status | Assessment | ESCAT tier | Function | Relevance for BC subtype | Reference |
|---|---|---|---|---|---|---|
| *Estrogen receptor (ER)* | Expression, mutation | IHC, sequencing | I-A | Prognostic and predictive, classification of BC | HR+ BC (Luminal A/B) | [5] |
| *Progesterone receptor (PR)* | Expression, mutation | IHC, sequencing | I-A | Prognostic and predictive, classification of BC | HR+ BC (Luminal A/B) | [4] |
| *Human epidermal growth factor receptor 2 (HER2)* | Overexpression, gene amplification, mutation | IHC, in situ hybridization, sequencing | I-A, (HER2 low: II-B) | Prognostic and predictive, classification of BC | HER2+ BC (Luminal B) | [9, 11] |
| *Programmed cell death ligand 1 (PD-L1)* | Expression (IC, CPS) | IHC, sequencing | I-A | Prognostic and predictive | TNBC | [13] |
| *Tumor-infiltrating lymphocytes (TIL)* | Expression | IHC | NA | Prognostic and predictive | All BC subtypes | [17] |
| *Molecular Intrinsic subtype* | Multi gene expression (recurrence risk, chemotherapy benefit (assay dependent)) | Gene expression profiling | NA | Prognostic and predictive | All BC subtypes | [18] |
| *Germline BRCA1/2 (somatic BRCA1/2)* | Mutation | Sequencing | I-A (II-A) | Prognostic and predictive | HR+ BC, TNBC | [20, 21] |
| *PALB2* | Mutation | Sequencing | II-A | Prognostic and predictive | HR+ BC, TNBC | [21] |
| *Phosphatidylinositol 3-kinase (PI3K) catalytic subunit (PIK3CA)* | Mutation | Sequencing | I-A | Prognostic and predictive | HR+/HER2– BC | [24] |
| *Microsatellite instability (MSI)* | MSI-high/low | Sequencing | I-C | Predictive | HR+, HER2+, TNBC | [25] |
| *Tumor mutational burden (TMB)* | TMB-high/low | Sequencing | I-C | Predictive | HR+, HER2+, TNBC | [25] |
| *Neurotrophic tyrosine receptor kinase (NTRK)* | Fusion | IHC, in situ hybridization, sequencing | I-C | Predictive | HR+, HER2+, TNBC | [27] |
| *Circulating tumor DNA (ctDNA)* | Expression | Isolation from liquid biopsies | NA | Predictive and prognostic | All BC subtypes | [28] |
| *Homologous recombination deficiency (HRD)* | HRD-high/low | Sequencing | NA | Predictive and prognostic | All BC subtypes | [22] |

*BC* breast cancer, *HR+* hormone receptor positive, *HER2+* human epidermal growth factor receptor 2 positive, *TNBC* triple negative breast cancer, *IHC* immunohistochemistry, *NA* not applicable

the molecular level, estrogen receptor 1 (ESR1) mutations occur in around 40% of BC patients. ESR1 mutations were shown to be predictive for therapy resistance to aromatase inhibitors (ESCAT tier II-A) and prognostic for a worse progression-free survival (PFS) [5].

Furthermore, the expression of androgen receptor is investigated which occurs in 50–90% of BC patients, predominantly in the HR+/HER2– subtype [6]. Androgen receptor expression (ESCAT tier II-B) was associated with lower pathologic complete response rates after neoadjuvant chemotherapy in HR+/HER2– BC patients but conversely with a better overall survival (OS) [7]. First reports of phase II trials investigating antiandrogen receptor-targeting therapies with enzalutamide in BC patients suggested limited clinical activity in a population selected by androgen receptor status [8].

## Human epidermal growth factor receptor 2

HER2 overexpression or amplification is used to determine the HER2+ BC subtype (luminal B in case of co-expression of HR) and is prevalent in approximately 20% of BC patients. HER2 is prognostic and predictive for HER2-targeting therapies (ESCAT tier I-A). Furthermore, a translational study investigating HER2 hotspot mutations (tier II-B) postulated that it could be beneficial in identifying patients who are resistant to HER2-targeting agents [9]. HER2 hotspot mutation was a negative prognostic factor for PFS [9].

Tumors with HER2 low expression (1+ or 2+ on IHC staining without amplification in in situ hybridization; ESCAT II-B) were previously classified as HER2 negative. While not a separate subtype defined by a distinct biological behavior, HER2 low status has recently gained clinical importance as the novel HER2-directed antibody drug conjugate trastuzumab deruxtecan has shown clinical activity with prolonged survival in pretreated HER2 low BC patients in a phase III study [10, 11]. Trastuzumab deruxtecan is therefore proposed as a new therapy approach in this patient population.

Notably, ongoing investigations are exploring targeting HER3 in BC given HER3 expression has been described in all BC subtypes and could potentially bear a new treatment strategy particularly for patients

who have thus far had limited therapeutic options such as TNBC (triple-negative breast cancer) [12].

## Immune cells and immune checkpoint molecules

Immune checkpoint inhibitors (ICI) have shown clinical activity in TNBC patients and are now used in the early setting in addition to the metastatic setting [13, 14]. Programmed cell death ligand 1 (PD-L1) expression on tumor and on immune cells such as tumor-infiltrating lymphocytes (TIL) and macrophages serves as a predictive biomarker (ESCAT tier I-A) which guides the application of PD-L1-targeting therapies in TNBC patients. PD-L1 expression (prevalence 20–40%) is a prognostic and predictive biomarker in metastatic TNBC but has no predictive role in early TNBC in which pembrolizumab yielded survival benefits regardless of PD-L1 status [14]. There are some limitations regarding PD-L1 as biomarker, for example in ICI pivotal studies, different applied staining antibodies resulted in the implementation of different scores (for atezolizumab: antibody SP142, immune cell score [IC]; for pembrolizumab: antibody 22C3, combined positive score [CPS]). Furthermore, divergences in PD-L1 positivity rates according to the applied antibody were described [15]. Further, PD-L1 positivity rates vary according to the organ site with lower PD-L1 positivity rates in metastatic lesions (42.2%), such as in liver (17.4%), skin (23.8%), and bone (16.7%) metastases, compared to primary tumor sites (63.7%) which should be taken into account when planning a biopsy [16].

TILs in the inflammatory tumor microenvironment have been shown to be a strong prognostic biomarker in HER2+ and TNBC patients, with higher TIL counts being associated with better prognosis [17]. Furthermore, TILs were shown to serve as predictive biomarkers for response to chemotherapy regimens as well as PD-1/PD-L1-directed therapies [17].

## Genomic signatures in breast cancer

### *Molecular intrinsic subtype*

Multigene sequencing assays (i.e., Mamma Print [Agendia, Agendia NV, Amsterdam, The Netherlands], Oncotype DX [Exact Sciences Corporation, Madison, WI, USA], Prosigna [Vercyte, San Francisco, CA, USA], Endopredict [MYRIAD SERVICE GmbH, Munich, Germany]) are developing and identifying molecular intrinsic BC subtypes as prognostic and predictive biomarkers in BC patients [18]. Molecular intrinsic subtypes are currently being clinically applied in early stage diseases to refine the prognosis and to help identify high-risk patients that should receive (neo)adjuvant chemotherapy.

### *BRCA mutation status and homologous recombination deficiency*

BRCA1 and BRCA2 are involved in maintaining genomic stability by repairing DNA double-strand breaks and are the most frequently affected mutated genes causing the development of BC. Germline BRCA1/2m occur in around 5% of BC patients with the highest incidence in TNBC, followed by HR+/HER2– BC patients [19]. Patients with gBRCA1/2m (tier I-A) are eligible for poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitor therapy [20]. A phase II study looked at homologous recombination-related genes aside from gBRCA1/2m and showed clinical activity of PARP inhibitors in partner and localizer of BRCA2 mutation (PALB2m) carriers (ESCAT tier II-A) and in patients harboring somatic BRCA1/2 mutations (ESCAT tier II-A) [21].

The homologous recombination deficiency (HRD) score emerged as a novel biomarker for genomic instability and BC was classified as HRD-high among multiple tumor types [22]. HRD score can be determined by multigene sequencing techniques and is primarily determined by BRCA1/2 mutation and loss of heterogeneity. The HRD-high genotype showed an immune-sensitive tumor microenvironment with increased TIL count, higher tumor mutational burden, and higher neoantigen load compared to the HRD-low genotype. Furthermore, HRD-high genotype was associated with ICI therapy response [22].

### *Phosphatidylinositol 3-kinase catalytic subunit mutation status*

Around 40% of HR+/HER2– BC patients harbor an activating phosphatidylinositol 3-kinase catalytic subunit (PIK3CA) hotspot mutation [23]. Alpelisib, a PI3K inhibitor, is a therapeutic option in combination with endocrine therapy as second-line treatment in HR+/HER2– BC patients with PIK3CA (ESCAT I-A) mutated tumors in exons 7, 9 and 20 [24].

Another targetable gene alteration of the PI3K/AKT/mTOR signaling pathway is AKT serine/threonine kinase 1 (AKT1) mutation (ESCAT tier II-B), and phase III trials are currently ongoing (i.e., NCT03337724, NCT04305496).

### *Microsatellite instability, tumor mutational burden, and NTRAK fusion*

Further molecular biomarkers investigated with regard to ICI therapy response are microsatellite instability (MSI; ESCAT tier I-C) and tumor mutational burden (TMB; ESCAT tier I-C). MSI-high and TMB-high tumors (cut off of 10 mut/Mb) were associated with better response to ICI therapy but their prevalence in BC is low with 1–2% for MSI-high and 5% for TMB-high [25].

Neurotrophic tyrosine receptor kinase (NTRK) fusions can be detected across a wide range of cancers but are very rare (<0.1%); however, NTRK fusions were found to be oncogenic drivers in secretory breast carcinoma with a prevalence of up to 92% [26]. With an ESCAT tier I-C, NTRK fusions can serve as predictive biomarkers for TRK inhibitors [27]. MSI-high and NTRK fusions are approved as agnostic biomarkers for targeted therapies.

### *Circulating tumor DNA*

Another biomarker currently being widely explored in cancer types is circulating tumor DNA (ctDNA). It negates the need for invasive procedures as it is procured from liquid biopsies [28]. Hence, multiple samplings of ctDNA are feasible for patients and ctDNA can potentially be utilized for longitudinal disease monitoring. Measuring ctDNA was shown to serve as predictive biomarker and its measurement is part of many translational exploratory analyses of clinical trials.

### *Tumor DNA methylation*

Tumor DNA methylation as an epigenetic phenomenon is investigated as a predictive and prognostic biomarker in multiple tumor types, including BC [29]. Tumor DNA methylation profiling was associated with response to PD-1/PD-L1-targeting therapies in translational studies of sarcomas, head and neck, and lung cancers and is also being investigated in TNBC [30].

## Conclusion

Translational research builds the bridge from discovering novel biomarkers in preclinical studies to testing their utility in the clinical setting that directly affects patient care. Biomarker research is a rapidly emerging field with novel approaches such as liquid biomarkers and omics methods. Integrating translational studies in clinical trials is essential to identify novel, clinically relevant biomarkers with the aim to work towards a personalized treatment strategy in BC patients.

## Take home message

Biomarkers are important for diagnosis, treatment, and prognosis. Translational research is needed to identify biomarkers for optimal personalized therapy strategies in breast cancer patients.

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