Breast Cancer: Targets and Therapy

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REVIEW

"Diagnostic and Prognostic Biomarkers of Luminal Breast Cancer: Where are We Now?"

Anna Höller[1,*], Bich Doan Nguyen-Sträuli[1,2,*], Heike Frauchiger-Heuer[1,*], Alexander Ring[1–3,*]

[1]Department of Gynecology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; [2]Department of Biology, Institute of Molecular Health Sciences, Swiss Federal Institute of Technology (ETH) Zurich, Zurich, Switzerland; [3]Department of Medical Oncology and Hematology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

*These authors contributed equally to this work

Correspondence: Heike Frauchiger-Heuer, Tel +41 44 255 42 37, Email Heike.Frauchiger-Heuer@usz.ch; Alexander Ring, Tel +41 76 327 8287, Email Alexander.Ring@usz.ch

Abstract: Luminal breast cancers are hormone receptor (estrogen and/or progesterone) positive that are further divided into HER2-negative luminal A and HER2-positive luminal B subtypes. According to currently accepted convention, they represent the most common subtypes of breast cancer, accounting for approximately 70% of cases. Biomarkers play a critical role in the functional characterization, prognostication, and therapeutic prediction, rendering them indispensable for the clinical management of invasive breast cancer. Traditional biomarkers include clinicopathological parameters, which are increasingly extended by genetic and other molecular markers, enabling the comprehensive characterization of patients with luminal breast cancer. Liquid biopsies capturing and analyzing circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) are emerging technologies that envision personalized management through precision oncology. This article reviews key biomarkers in luminal breast cancer and ongoing developments.

Keywords: breast cancer, luminal A and B, biomarker, circulating tumor cells, circulating tumor DNA

Introduction

Breast cancer is the most common non-cutaneous cancer among women, with an estimated 2.1 million new cases and 627,000 deaths globally in 2020.[1] Luminal breast cancer is the most common subtype of breast cancer, accounting for approximately 70% of cases,[2] and is considered less aggressive compared to other subtypes (ie, human epidermal growth factor receptor 2 (HER2) positive and triple negative). Luminal A breast cancers are characterized by the expression of estrogen receptor (ER) and progesterone receptors (PR), low proliferation and a better prognosis, while luminal B breast cancers also express HER2 and are characterized by high proliferation and a poorer prognosis.[3]

Early diagnosis and appropriate treatment are key factors in improving patient outcomes and survival.[4,5] Several biomarkers are used to guide treatment decisions in luminal breast cancer. Biomarkers are molecular, cellular, or functional characteristics that can be used to identify the presence or progression of a disease or predict response to therapy, and have played a crucial role in the clinical management of breast cancer.[6,7]

In this review, we present a comprehensive overview of the current state of knowledge on diagnostic, prognostic and predictive biomarkers in luminal breast cancer, highlighting key biomarkers and their performance characteristics and limitations. We also evaluate the potential clinical utility of emerging biomarkers in the management of luminal breast cancer, current gaps or hurdles for implementation and suggest potential directions for further research.

Current Landscape of Established Biomarkers – a Chronological Perspective

In luminal breast cancer, biomarkers are indispensable for diagnosis, prognosis, and treatment selection, and have led to significant improvements in patient outcomes. Broadly speaking, biomarker can be divided into diagnostic biomarkers that

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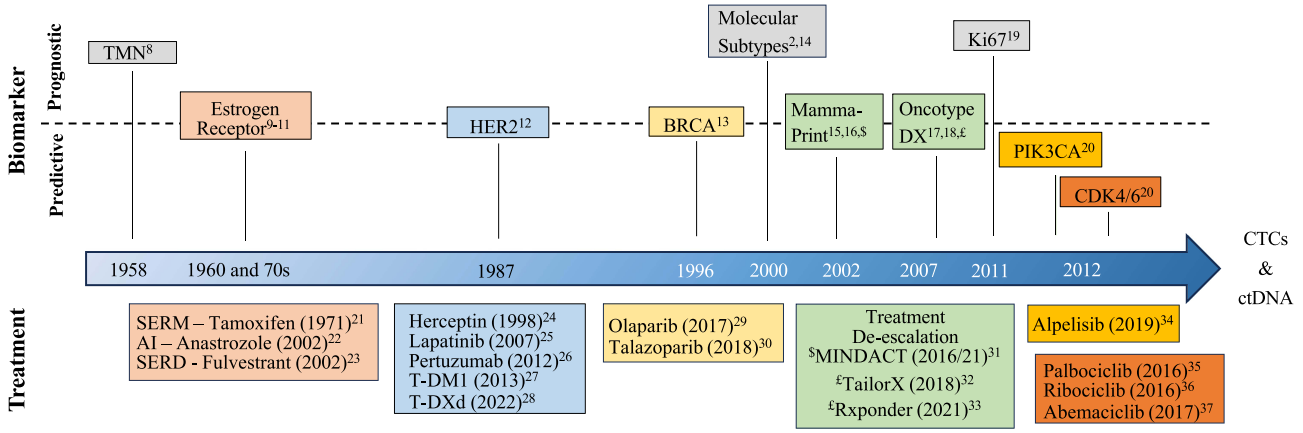

Figure 1 Timeline of major prognostic and predictive biomarker development in luminal breast cancers. Biomarker and associated treatment are shaded in the same color.
Abbreviations: TMN, Tumor, Nodes, Metastases classification; SERM, selective estrogen receptor modulator; AI, aromatase inhibitor; SERD, selective estrogen receptor degrader; HER; HER2, human epidermal growth factor receptor 2, T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; BRCA, breast cancer 1 or 2, early onset; Ki67, MKI-67, marker of proliferation Ki-67; CDK4/6, cyclin-dependent kinase 4/6; PIK3CA –phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

help to establish the presence of cancer, and to determine subtype or stage, prognostic biomarkers that are used to estimate patient outcomes, and predictive biomarkers which are used as a measure of expected response to guide treatment decisions.[6,7] While valid as a general systematic classification, the lines between prognostic and predictive are often blurred. Several types of clinical, histological and molecular biomarkers have been developed and are currently used for the diagnosis, classification and treatment decision in breast cancer (Figure 1, Table 1).

Table 1 Selected Biomarkers in Breast Cancer

Biomarker	Patients	Prognostic/ Predictive	Level of Evidence (LOE)[38]	Strength of Recommendation (SORT)[39,40]
ER	All (obligatory)	Both	IA	A
PR	All (obligatory)	Both	IB	A/B
HER2	All (obligatory)	Both	IA	A
Ki67	All (recommended)	Prognostic (predictive for NACT)	IB	A/B
Oncotype DX	ER-positive, HER2-negative and lymph node–negative or positive (1–3 lymph nodes)	Pro/Pre	IB	A
MammaPrint		Pro/Pre	IA	A
Prosigna		Pro/Pre	IB	A
EndoPredict		Pro/Pre	IB	A
(BCI)		Pro/Pre	IB	A
(uPA/PAI-1)	ER-positive, HER2-negative and lymph node–negative	Pro/Pre	IA	A

Notes: Adapted from SORT: A – based on consistent and good-quality patient-oriented evidence, B – based on inconsistent and limited-quality patient-oriented evidence. Adapted from American Society of Clinical Oncology LOE scale: 1 - Evidence from a single, high-powered, prospective, controlled study that is specifically designed to test marker or evidence from strong meta-analysis; A – prospective, B – prospective using archived samples. In parenthesis (not routinely used in the clinic).
Abbreviations: NACT, neoadjuvant chemotherapy; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; BCI, breast cancer index.

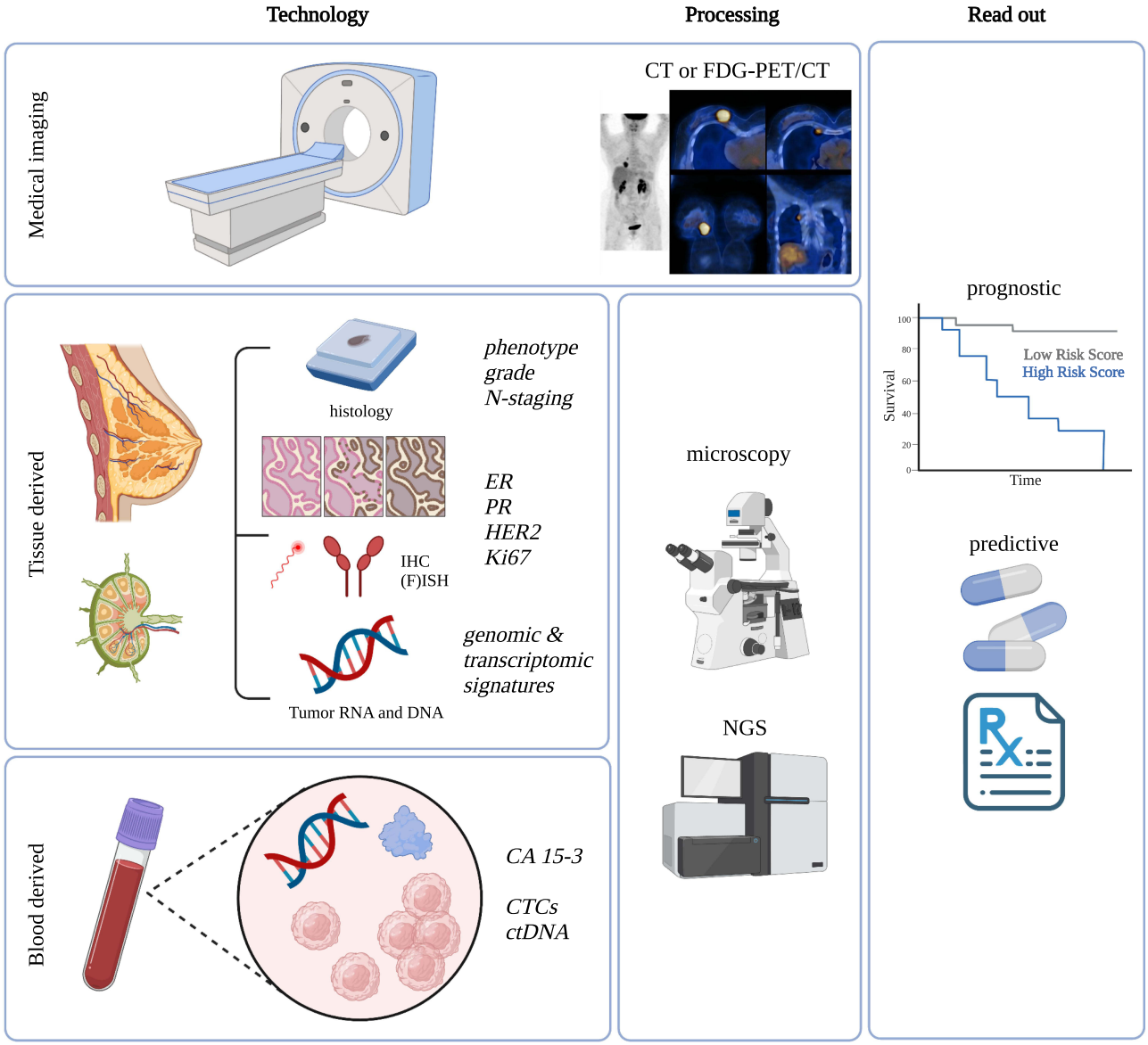

Figure 2 Overview of established and evolving biomarkers and their assessment as well as clinical read out in luminal breast cancer.
Abbreviations: CT, computed tomography; FDG-PET/CT, fluorodeoxyglucose F 18-positron emission tomography/CT; N-staging, nodal or lymph node staging; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry, (F)ISH, (fluorescence) in-situ hybridization; CA 15–3, cancer antigen 15–3; Ki67, MKI-67, marker of proliferation Ki-67; CTCs, circulating tumor cells, ctDNA, circulating tumor (derived) deoxyribonucleic acid, NGS, next-generation sequencing.

The assessment of a minimal subset of biomarkers at first diagnosis is indispensable for patient management in luminal breast cancer (Figure 2). Whenever feasible, measurements should be repeated on recurrent lesions. All laboratories should use validated assays and established criteria for soundness of results (such as the REMARK criteria),[41] and should perform regular quality audits for accreditation by external organizations.

The TNM Classification and Grading

The TNM classification and grading system for breast cancer is used to determine the disease stage based on tumor spread, lymph node involvement, and distant metastasis.[42] Both imaging and biopsies are used for staging (Figures 1 and 2). *Tumor size (T)* is important for prognosis and treatment options, where larger tumors have a worse prognosis and usually require more aggressive treatment.[42,43] *Nodal status* (N) indicates the extent of breast cancer spreading to lymph nodes.[42,44] Positive lymph nodes indicate a more aggressive biology, and diagnosis of lymph node involvement is often achieved on

surgical specimen. The extent of lymph node involvement determines the surgical dissection, ranging from sentinel lymph node (SN) to axillary lymph node dissection (ALND).[45] Concepts for de-escalation are being studied[46,47] (NCT03513614). *Distant metastasis (M)* refers to the presence or absence of hematogenous distant metastases. It has strong implications for prognosis and treatment decisions. Distant or organ metastasis renders most patients incurable, with reduced 5-year survival probability.[48] Palliative treatment options are available for metastatic disease. Staging with CT or PET is reserved for patients with high-risk early-stage disease or clinical suspicion[49–51] (Figure 2). Tumor grade is a prognostic biomarker that defines cancer cell differentiation[52,53] (Figure 2). Grade represents a semi-quantitative evaluation of morphological characteristics, including tubule or gland formation, nuclear pleomorphism, and mitotic count. The Nottingham Grading System (NGS) is widely used, assigning a grade from 1 to,[54,55] and has been validated as an independent prognostic factor,[22,26,27] regardless of stage, tumor size, and lymph node involvement.[58]

Hormone Receptors

Measuring the expression of the steroid hormone receptors (HR) for estrogen and progesterone is recommended by all breast cancer treatment guideline and constitutes routine clinical practice (Figure 2 and Table 1).[48,59–61]

The *estrogen receptor* counts among the most important biomarkers in breast cancer. It was identified in the late 1960s and has been used in clinical practice since the mid 1970s[9–11] (Figure 1). ER is expressed in approximately 80% of breast cancers and is considered as a strong prognostic factor and primary indicator of response to endocrine therapy (ET) (ie, selective estrogen receptor modulators – SERMs (tamoxifen), third-generation aromatase inhibitors (anastrozole, letrozole, exemestane), LH-RH agonists (leuprolide, goserelin), pure estrogen receptor down regulators – SERDs (fulvestrant))[62–65] (Figure 1). A meta-analysis of individual data from 20 randomized clinical trials including 21,457 patients found that in early-stage disease, adjuvant tamoxifen treatment reduced the 15-year recurrence rate by 39% and the mortality rate by 30%[62] in HR positive breast cancer. On the molecular level, a distinction is made between ERα and ERβ receptors (encoded by the ESR1 and ESR2 genes, respectively),[66] however only ERα evaluation is currently routinely clinically assessed.[63]

Progesterone receptor (PR) exists in two isoforms (alpha and beta), is a transcriptional target of ER and therefore strongly estrogen dependent, but also modulates ERα action in breast cancer.[67,68] The proportion of PR-positive breast cancers is lower than ER-positive breast cancers, representing about 20% of cases.[69] While both ER and PR are prognostic, the predictive value PR is controversial.[62,70,71] ER- and PR-positive breast cancers tend to be less aggressive and are associated with a better prognosis compared to HR-negative breast cancers,[63,72] though the impact of endocrine therapy on prognostic is difficult to exclude.

HR expression correlates with tumor grade in luminal BC, with low-grade tumors exhibiting higher ER and PR expression, whereas intermediate- and high-grade tumors may have lower levels of ER and may lack PR expression.[73] The presence of both receptors can be detected using immunohistochemistry (IHC) techniques and is mandatory to diagnose and classify breast cancer subtypes (Table 1), including luminal A and luminal B (Figure 2). While traditionally ≥1% positive nuclei was used as a cut off for positivity for ER or PR, recent evidence suggests that tumors with low ER positivity (1–9%) should be considered separately as these tumors seem biologically and prognostically closer to ER-negative or basal-like cancers.[74] The following subdivision is recommended: ER-/PR-positive: >10% positive tumor cells, ER-/PR-low positive: 1–9% positive tumor cells, ER-/PR-negative: <1% positive tumor cells.[48,59–61,75] Further research is needed to translate these findings into robust clinical application. To avoid ambiguity and better distinguish borderline cases, additional scores have been developed such as the Allred score,[76] which considers staining intensity in addition to the proportion of positive cells, or the immunoreactive score (IRS) according to Remmele and Stenger.[77]

Molecular investigations demonstrated brisk crosstalk of both hormone receptors with other receptor pathways. PR interaction with growth receptors leads to co-activation and co-regulation of common transcriptional targets.[78] The cyclin D1/CDK4/6/RB/E2F1 and ER/PR pathways are tightly linked in luminal breast cancer and together with other findings, such as loss of ER expression, mutations in the ligand binding domain, overexpression of ER co-activators or down-regulation of co-repressors, the interaction of ER with growth factor receptors, and/or the regulation of ER by miRNAs offer explanations for therapy resistance as well as opportunities for targeting.[79,80] The immune system also effects ER

activity, for example activation of breast cancer NFκB/STAT3 via tumor-associated macrophages can drive ER ligand-independent phosphorylation.[81]

Molecular markers, including gene signatures such as Prosigna/PAM50, Endopredict (described in more detail below), breast cancer index (BCI), HOXB13/IL17BR[82] or the immunohistochemical 4 (IHC4) score[83] enable a differentiation of patients based on relapse risk after endocrine therapy and are discussed further below.

Ki-67

Ki-67 was first discovered in 1983 as a nuclear protein expressed in proliferating cells and is widely used to predict patient prognosis and guide treatment decisions (Figures 1 and 2, Table 1).[84–87] Ki-67 is expressed as a percentage or proportion score of tumor cells showing positive nuclear staining, independent of the intensity of coloration.[87] A high Ki-67 index as determined by immunohistochemistry (IHC) is used to distinguish luminal B from A breast cancer.[19,88,89] Results from various trials support the prognostic role of Ki67, with higher expression indicating a more aggressive cancer and a worse prognosis.[38,90] Regarding predictive power, available data suggest that high Ki67 expression can provide important information in the neoadjuvant setting concerning pathological complete response.[91] On the contrary, a predictive role in the adjuvant setting could not be established.[91–94]

Reproducibility issues are an important caveat and were addressed by the International Ki67 in Breast Cancer working group or the Swiss Working Group of Breast- and Gynecopathologists.[19,95,96] This also partially explains why Ki67 is not yet standard in all hospitals and medical centers.

Human Epidermal Growth Factor Receptor HER2

The expression of HER2 promotes the growth and spread of breast cancer cells and was first described in 1987 by Slamon et al[12,97] (Figures 1 and 2, Table 1). Determining HER2 status is crucial due to its impact on prognosis and treatment. Traditionally, HER2-positive breast cancer was defined overexpression according to ASCO/CAP guidelines, with an IHC score of 3+ or HER2 gene amplification.[98] Approximately 15–20% of breast cancers show HER2 overexpression, predicting aggressive biology and response to HER2-targeted therapies (eg, trastuzumab, pertuzumab, lapatinib, trastuzumab-emtansine/T-DM1).[25–28,97,99,100] In 2022, the DESTINY-Breast04 trial demonstrated benefit with trastuzumab deruxtecan/T-DXd in traditionally HER2-positive patients and those with IHC scores of 1+ or 2+[28] T-DXd targets HER2 as an epitope to deliver high concentrations of chemotherapy causing cell death (bystander effect).[101] Including HER2-low cases thereby expands the potential population for HER2-targeted therapies to around 75% of all breast cancer cases.[28,102] Novel drugs leveraging the bystander effect are under investigation.[103] For completion, we mention here circular HER2 RNA as a novel concept for predictive biomarkers.[104]

Importantly, co-expression of HER2 and HR has important implications for prognosis and response to receptor-targeted therapies. HER2/HR-positive tumors have a lower likelihood of response when treated with either ET or HER2-targeted therapy alone.[105] Several studies demonstrated crosstalk between HR and HER2 modulating both anti-HER2–directed and endocrine therapy via compensatory escape pathways due to the bi-directional signaling.[105]

Further Molecular Predictive Biomarker

ET represents the corner stone for systemic treatment in luminal breast cancers. However, resistance to these agents poses a major obstacle, in particular in the metastatic setting.[106] Acquired activating mutations in *ESR1* under the selective pressure of ET lead to constitutive activation and diminished efficacy of AI treatment.[107] Epigenetic changes and upregulation of alternative pathways further predicate resistance to ET in luminal breast cancer.[106] The presence of *FOXA1* is crucial for ER-chromatin interactions and gene expression alterations, while also impacting chromatin accessibility throughout the genome.[108] Aberrant *c-Myc* expression contributes to ET resistance via decrease of the cell cycle regulator p21.[109] *Cyclin D1* activates ER-mediated transcription even in the absence of estrogen.[110]

Phosphatidylinositol 3-kinase (PI3K) is involved in AKT–mammalian target of rapamycin (mTOR) signaling, which is related to HR signaling in breast cancer.[20,111] The aberrant activation of *PI3K/AKT/mTOR* signaling, driven by *PI3K* mutations, can lead to increased ER transcriptional activity and enhanced cell survival, thereby reducing the effectiveness of endocrine therapies.[112] Mutations occur in up to 40% of breast cancers and predict response to *PI3K* inhibitors

(ie, alpelisib).[34,113] Related to this pathway, the inhibition of mTOR (everolimus) improves PFS (as demonstrated in the BOLERO-2 trial),[114] and mTOR alterations can be assessed by next-generation sequencing such as FoundationOne CDx.

Cyclin-dependent kinases 4 and 6 (CDK4/6) are important regulators of cell-cycle progression and have emerged as valuable predictive biomarkers in the metastatic setting.[115] Several pivotal randomized prospective trials (PALOMA-3, MONALEESA-7 and MONARCH-2) demonstrated both PFS and OS benefit among both premenopausal and postmenopausal women and established CDK inhibitors (ie, palbociclib, ribociclib, or abemaciclib) in combination with ET (AI or SERD) as the gold standard in metastatic hormone receptor-positive tumors.[35–37,115–118] Later this approach was also proven effective in the (neo-) adjuvant setting.[119,120]

Breast cancer type 1 susceptibility protein 1 (BRCA1), BRCA2 and *partner and localizer of BRCA2 (PALB2)* are involved in the repair of double strand breaks in DNA.[121] Germline mutations in BRCA1/2 occur in 5% of unselected patients and are culprits in the hereditary breast-ovarian cancer syndrome.[122,123] Therapeutically, these alterations predict a higher likelihood of response to platinum-based chemotherapy and are used in the adjuvant setting to predict response to poly (adenosine diphosphate–ribose) polymerase (PARP) inhibitors such as olaparib and talazoparib in germline mutation carriers.[29,30,113]

The biomarkers urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) have demonstrated promising prognostic and predictive validity for adjuvant chemotherapy in lymph node-negative HR-positive early breast cancer[124,125] (Table 1).

Among serum biomarker the cancer antigen CA 15–3 is the most widely used serum biomarker in breast cancer but is only established to monitor disease course and treatment efficacy in advanced stages and not for initial diagnosis or early detection due to low sensitivity[126,127] (Figure 2).

Gene Expression Signatures

Gene expression profiling was the key to refine breast cancer into molecular subtypes, including luminal A and B, HER2-enriched, basal, and normal like, which have different clinical characteristics and treatment responses.[2,14] Further development and validation of multiparametric gene expression profiles (MammaPrint, Oncotype DX, Endopredict, and Prosigna/PAM50) established their role as predictive tools able to stratify early-stage HR-positive patients according to relapse risk and benefit of additional (chemo)therapy (Figures 1 and 2, Table 1).[15,31,33,128–130] The signatures can help to identify patients with ER-positive, HER2-negative and lymph node–negative or positive (1–3 lymph nodes) breast cancer that may benefit from or could avoid (neo-)adjuvant chemotherapy.[16,59] The most widely used signatures, Oncotype DX and MammaPrint, were initially validated retrospectively including in the NSABP B14/B20,[131,132] SWOG-8814,[133] TransATAC[134] or RASTER[135] trials. Several landmark prospective trials subsequently confirmed their prognostic and predictive power. Oncotype DX uses 21 genes and is the only NCCN accredited multigene test.[17,32] A score of 26 or higher correlates with higher risk of distant recurrence in both pre- and post-menopausal women with N0 or N1 lymph node status, and addition of chemotherapy to endocrine therapy is recommended.[18,32,33,129] The MammaPrint 70-gene signature was first developed and validated as a prognostic tool.[31,136,137] The MINDACT trial subsequently showed that almost half of all women (46%) at high clinical high risk but low genomic risk for relapse may forgo adjuvant chemotherapy.[138] The GEICAM 9906, ABCSG-6 and −8 trials validated EndoPredict as an independent prognostic parameter in node-positive, luminal BC patients treated with adjuvant chemotherapy followed by hormone therapy.[139,140] The Prosigna assay was validated to predict potential benefit from extended endocrine therapy or the addition of chemotherapy.[140,141]

A couple of aspects are noteworthy regarding gene expression signatures: 1) all use different technologies and show little overlap in regard of genes tested and, more importantly, a considerable level of discordance within the same patient.[142] Biologically and technically, it is not surprising that tests measuring fundamentally different genes with different technologies give dissimilar results. Clinically, the discordance might be explained by the absence of any clinical or molecular agreement as to the true boundary between a luminal A and luminal B cancer. 2) Oncotype DX, Prosigna, EndoPredict and BCI do not correlate with tumor size or nodal status, but with tumor grade, perhaps reflecting the proliferative status of tumors as the major criterion for response to chemotherapy.[143]

Emerging Biomarkers and Personalized Oncology

In recent years, there have been significant advances in the discovery and validation of new biomarkers in breast cancer. One prominent example is liquid biopsies, which involve the analysis of blood and other body fluids to identify cellular or molecular changes associated with breast cancer.[144,145] Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) represent the most advanced among these emerging biomarkers, holding significant potential as non-invasive diagnostic tools for breast cancer and will be reviewed in more detail below.[75,144] Other circulating biomarkers include microRNAs, non-coding RNA molecules or exosomes, which have been comprehensively reviewed elsewhere.[146–148]

CTCs

Pathological analysis of tissue biopsies is routinely utilized to predict therapy response and guide further drug selection[48] (Figure 2). However, inter- and intra-tumor heterogeneity, poor accessibility of metastatic lesions, and challenges in repeatedly asking a patient to undergo invasive or even surgical procedures during disease progression can hamper precise therapy decisions. In this context, CTCs have emerged as promising liquid analytes (Figure 3). They are living

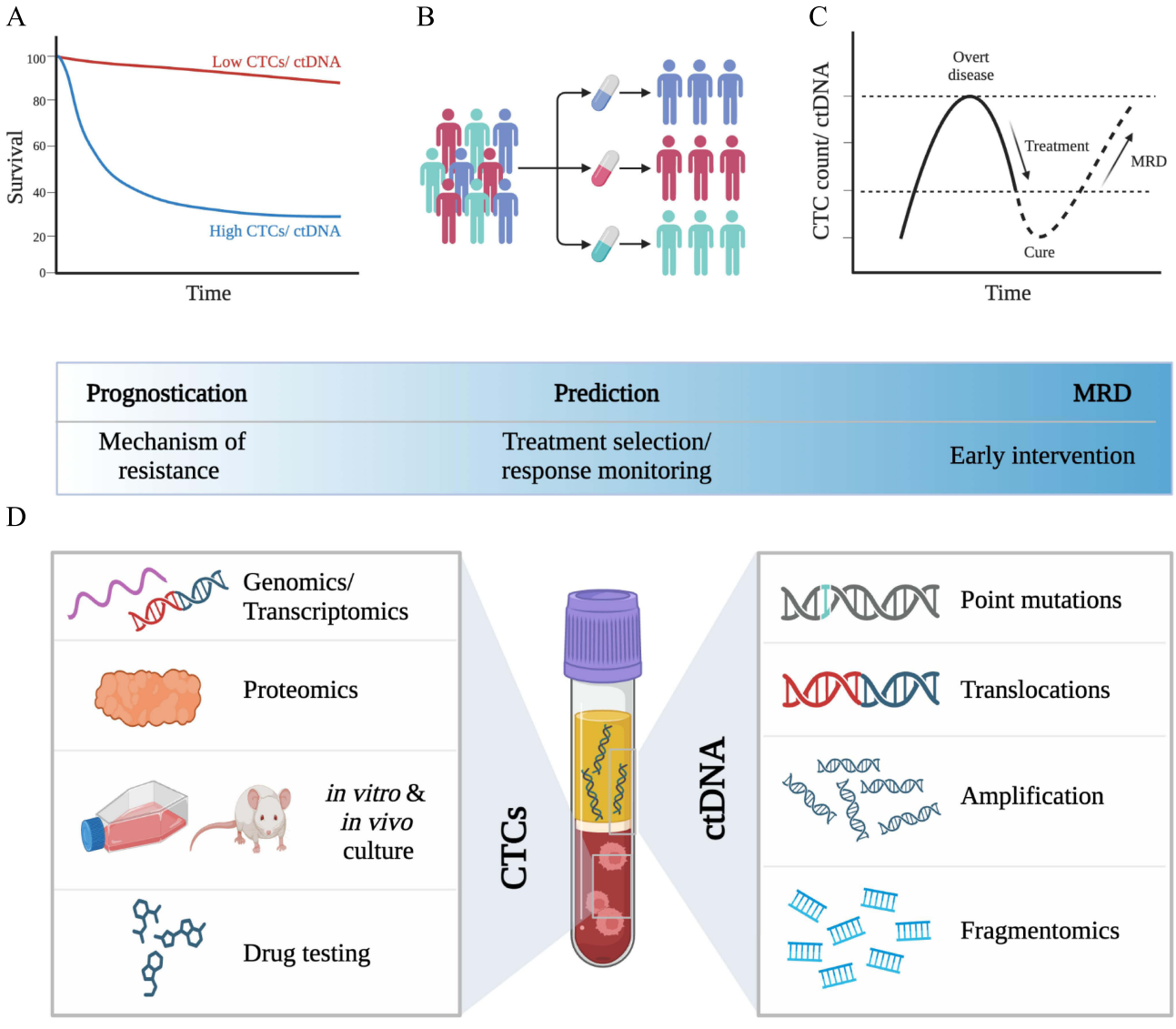

Figure 3 CTCs and ctDNA as liquid biopsies in breast cancer. CTCs and ctDNA as independent (**A**) prognostic (PFS, OS) and (**B**) predictive biomarkers. (**C**) CTCs and ctDNA as sensitive and minimally invasive biomarkers for minimal residual disease (MRD). (**D**) Potential application of molecular and multi-omics analysis, disease modeling (avatar models) and drug testing of CTCs, and extensive genomics profiling of ctDNA.

Abbreviations: CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; PFS, progression-free survival; OS, overall survival.

cells that have broken off from a primary tumor or from a metastatic lesion and are shed into the bloodstream to seed distant metastasis.[149] One of the main advantages is their minimally invasive detection in peripheral blood which allows serial blood sampling and longitudinal monitoring during tumor progression. Further, CTCs as metastatic precursors might hold the potential to capture all biologically and therapeutically relevant aspects of a cancer, which might not be fully reflected by tissue biopsies that only provide a snapshot of mutations present at a given time and location. Further, tissue biopsies might not address evolutionary changes within the tumor and its metastases which can alter the genetic landscape and its responsiveness to therapies during cancer progression.[150] As of May 1st, 2023 there are 215 trials on the ClinicalTrials.gov database related to CTCs in breast cancer, indicating the immense interest in developing CTCs into both prognostic and predictive biomarkers.

Numerous studies have already confirmed the clinical prognostic validity of CTCs, foremost in metastatic breast cancer,[151–155] but also in early-stage disease.[156–158] A large meta-analysis of N = 2436 patients with breast cancer by Cristofanilli et al suggests a classification of metastatic disease into aggressive ≥5 CTCs vs indolent (<5 CTCs), which is supported by robust OS data.[159] While CTCs are not yet routinely used in the clinical management of breast cancer, they have been incorporated into the latest version of the WHO Classification of Tumors: Breast Tumors and AJCC Cancer Staging Manual.[42]

The predictive value of CTCs, including potential de-escalation of systemic treatment, has been investigated, with mixed results. In the early-stage setting, the GeparQuattro and REMAGUS 02 trials showed no association between pathological response of the primary tumor and changes in CTC numbers before and after neoadjuvant chemotherapy.[160,161] Nonetheless, the presence of CTCs post therapy was an independent prognostic factor for early relapse,[151,161] suggesting that the detection of persistent CTCs after completion of treatment might provide superior information on therapy response and risk of relapse than the observed chemosensitivity of the primary tumor. In one Phase II-study, trastuzumab decreased the incidence of clinical relapses in patients with early breast cancer presenting chemotherapy-resistant CK-19mRNA-positive circulating tumor cells.[162]

In the metastatic setting, early switching to an alternate treatment regime in breast cancer patients where a positive CTC count was recorded after one cycle of first-line chemotherapy showed no impact on overall survival.[163] Two proof-of-concept studies investigated HER2-targeted therapies (T-DM1 and lapatinib) in metastatic breast cancer patients with HER2- negative primary tumors and HER-2 positive CTCs. They revealed only marginal benefit in a subset of patients with a HER2-negative primary tumor presents with HER2-positive CTCs during disease progression.[164,165] On the contrary, the STIC CTC trial demonstrated that CTCs were helpful to distinguish a subset of HR-positive, HER2-negative patients who benefit from chemotherapy rather than endocrine therapy.[166] The ongoing DETECT III study (NCT01619111) compares standard therapy ± lapatinib in HER2-negative metastatic breast cancer and HER2-positive CTCs.[167] A recent meta-analysis of N = 1944 breast cancer patients showed the CTC counts before and after treatment have a strong and independent predictive value for survival outcome.[151]

Several challenges and opportunities remain. Currently, only two FDA-approved devices (CELLSEARCH® and Parsortix®) can be used to monitor the presence and number of CTCs in a person's blood.[168,169] CELLSEARCH® uses antigen-dependent immunomagnetic positive selection to capture CTCs based on EpCAM expression, while Parsortix® uses antigen-independent, size-based microfluidics technology. The main shortcoming of these technologies is limited detection due to the rare nature of CTCs in peripheral blood (roughly one CTC in one billion blood cells) and their short circulation time (10–30 minutes).[149] Efficient, robust and reliable CTC enrichment is critical for reproducible down-stream analysis and clinical applications.[149,170] Hence, novel approaches to overcome that limitation have been developed including implantable devices such as direct intravascular coated guidewires,[171] cytapheresis[172] which allows cell fraction enrichment from large blood volumes, or the concept of gaining access to tumor draining vessels to enhance CTC enrichment.[173,174] Although important, these technologies are not yet routine, and the latter scenario will not be suitable for patients with advanced-stage disease who do not qualify for surgery. Recently, a study showed that temporal dynamics of CTC intravasation vary dramatically based on circadian rhythm, both in mouse models and in patients with breast cancer, emphasizing the time-critical aspect of biomarker assessment.[175]

Several high-profile studies have elucidated molecular aspects of CTCs and the role of CTC clusters for metastasis, including the importance of hypoxia, cell–cell junctions, and heterotypic clustering with other cell types in circulation

(such as neutrophils),[176–179] or demonstrated the feasibility of drug testing on CTCs.[180] These findings have not yet found their way into clinical application but hold significant potential as therapeutic targets (Figure 3D).

ctDNA

All cells in the human body release DNA into the bloodstream as circulating cell free DNA (cfDNA). The fraction that is released by cancer cells is called ctDNA and represents a sensitive method to detect and monitor tumor-specific aberration via minimally invasive blood draws as liquid biopsies (Figures 2 and 3).[144] Several studies demonstrated the potential role of ctDNA as a clinically relevant tool for early detection, diagnosis, prediction of pathologic complete response (pCR), monitoring of minimal residual disease or relapse and to guide targeted therapies or detect resistance (eg, ESR1 mutations, PI3K mutations).[181–187] In early-stage breast cancer, sub analysis of the NeoALTTO trial demonstrated that ctDNA detection can stratify patients with HER-amplified tumors that are at risk for failure to achieve pCR after NACT.[183] The neoadjuvant I-SPY 2 trial demonstrated that persistent ctDNA was a predictor of poor response and metastatic recurrence, while clearance of ctDNA after NACT predicted improved survival independent of pCR.[188] The CHiRP trial demonstrated the ability of ctDNA to detect MRD in patients with late adjuvant HR+ breast cancer with a median lead time of 12.4 months before overt disease recurrence.[189] Ongoing trials are intended to further validate the role of ctDNA as a prognostic and predictive tool in breast cancer. The TRAK-ER trial investigates whether therapy escalation in ER+ patients with ctDNA-based molecular relapse can prevent clinical relapse (NCT04985266). The STRIVE trial is testing whether ctDNA be used for early detection of breast cancer (and other solid tumors) that will occur within one year (NCT03085888). In the advanced setting, both the PlasmaMATCH and SOLAR-1 trial demonstrated the accuracy of ctDNA-based predictive biomarker assessment for mutation-directed therapy.[34,184] SOLAR-1 showed that PIK3CA-targeted therapy prolonged survival in patients with HR-positive, HER2- advanced breast cancer.[34] Targeted assays such as FoundationOne Liquid CDx or the Guardant360 have been prospectively validated and received FDA approval as companion diagnostics.[34,184] Other approaches such as fragmentomics combined with mutation-based analysis hold significant potential to improve diagnosis and management of breast cancer[190] (Figure 3D).

Limitations of Current Biomarkers and Future Developments

Survival rates of early-stage breast cancer are excellent, reaching 90% within 5 years.[191] Yet roughly one-third of patients experience distant recurrence up to 32 years after initial diagnosis and standard of care treatments.[192,193] The prognosis of metastatic disease is drastically reduced to 30% 5-year relative survival.[191] We suggest two major shortcomings of current biomarkers and treatments that represent urgent clinical needs to further improve outcomes.

Firstly, the inability of current biomarkers and treatment strategies to efficiently detect and eradicate micro-metastatic disease. Estimates suggest that up to 75% of BC patients harbor micro-metastases or disseminated tumor cells (DTCs) at the time of diagnosis.[194,195] DTCs may enter a (initially indolent) state of dormancy that protects them from detection and eradication with current clinical standard of care strategies.[196,197] Novel strategies for detection (including liquid biopsies) and targeting of dormant disease (eg, enforcing a dormant state, awakening or targeted eradication of dormant tumor cells) are subject to intensive research efforts.[197,198]

Secondly, the dearth of therapies that specifically target the metastatic processes such as intra- and extravasation, circulating tumor cell dissemination, homing of tumor cells at distant sites.[94] To address this shortcoming, CTCs as the progeny of metastatic tumors could serve as ideal prognostic and predictive biomarkers and targeting of CTCs could directly interrupt the metastatic progression.[149] The finding that dissociation of CTC clusters via Na+/K+-ATPase inhibitors (eg, digoxin) dramatically reduces distant metastasis in pre-clinical models underlines this notion and is currently being tested in a phase I/II clinical trial (NCT03928210).

Conclusion

The increasing combination of traditional histopathological and molecular biomarkers, such as genomic alterations and transcriptional signatures, continues to improve clinical management and outcomes in patients with luminal breast cancers. However, there are still limitations to the clinical utility of current biomarkers regarding early detection and follow-up with minimal harm to patients. Emerging technologies such as CTC and ctDNA analysis as liquid biopsies have the potential to improve upon these shortcomings and foster the implementation of precision oncology in the

management of luminal breast cancer. The discovery and validation of new biomarkers is an active area of research that will improve our understanding of breast cancer and guide highly personalized treatment approaches in the future.

Disclosure

Anna Höller and Bich Doan Nguyen-Sträuli are co-first authors for this study. Heike Frauchiger-Heuer and Alexander Ring are co-last authors for this study. The authors report no conflicts of interest in this work.

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