Review Article

Advances in molecular medicine for breast cancer practice: a narrative review

Ravi Velaga, Masakazu Toi

Department of Breast Surgery, Kyoto University Hospital, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan

Contributions: (I) Conception and design: Both authors; (II) Administrative support: Both authors; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

Correspondence to: Masakazu Toi. Department of Breast Surgery, Graduate School of Medicine, Kyoto University Hospital, Kyoto University, 54 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan. Email: toi@kuhp.kyoto-u.ac.jp.

Background and Objective: Breast cancer subtype identification using immunohistochemistry is used in biological profiling of primary breast cancer and as predictive markers for adjuvant or neoadjuvant therapies such as cytotoxic multi-drug chemotherapy, endocrine therapy and anti-human epidermal growth factor receptor 2 (HER2) therapy. Multiple genomic signatures predict prognosis and therapeutic impact, especially in luminal diseases. Prospective studies clarified their use. More profound characterisation and comparison of these genomic signatures have also been carried out in the past decade. Immunotherapy in combination with chemotherapy is now considered not only for metastatic diseases but also for treating primary breast cancers. PD-L1 status alone is currently assessed to indicate the immunotherapy benefit in current practice. Thus, additional immune signatures could help predict the therapeutic efficacy. We also touched upon liquid biopsy and hereditary breast cancer diagnosis as molecular medicine. These assays are now expanding from laboratory use to clinical use quite rapidly. This review presents advances in breast cancer intrinsic subtyping, gene expression profiling as a prognostic, predictive and stratification tool, immune-based cell signatures, genetic medicine, and liquid biopsy.

Methods: Key studies that contributed to international guidelines are reviewed and presented in this narrative review. We focused on how molecular medicine has improved and incorporated into clinical practice in recent days as precision medicine in primary breast cancer treatment. In March 2021, we concluded the literature presented in this review. Keywords such as breast cancer intrinsic subtypes, gene expression signatures such as Oncotype DX, MammaPrint, PAM50, Breast Cancer Index (BCI), EndoPredict and breast cancer immune subtype signatures are used to search the literature in PubMed between 2008 to 2021. Articles published only in the English language were included.

Key Content and Findings: Genomic signatures like Oncotype DX with strong evidence and other (MammaPrint, BCI) with medium evidence are used to guide adjuvant endocrine and chemotherapy in women with more than 50 years and/or postmenopausal with hormone receptor positive and HER2 negative tumours.

Conclusions: Advances in molecular medicine promise to improve accurate prognostication, therapeutic outcomes, optimal escalation and de-escalation of treatment, and offer comprehensive breast cancer precision medicine.

Keywords: Breast cancer intrinsic subtypes; multigene expression assays; predisposition genes; pathogenic germline variants (PGVs); liquid biopsy

Received: 12 April 2021; Accepted: 05 August 2022; Published online: 21 August 2022.
doi: 10.21037/abs-21-64
View this article at: https://dx.doi.org/10.21037/abs-21-64

Introduction

In 2015, Cristian Tomasetti and Bert Vogelstein reported a paper on stem cell divisions and the resulting majority of cancer is more because of "bad luck (1)". Despite remarkable advances in molecular and sequencing technologies, the focus remains on understanding how cancer is caused and how it can be detected early for better prevention and treatment. An increase in the survival of breast cancer patients has been attributed to an increase in awareness, screening modalities (imaging and potential use of liquid biopsy), and the development of genomic profiling assays. Conventionally, clinicopathological characteristics such as tumour size, nodal status, and metastasis are associated with prognosis. Histological tumour grade and breast cancer hormone receptors; oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status, and proliferation rate are associated with both prognosis and sensitivity to treatment modalities. Reproducibility of these markers after immunohistochemistry (IHC) is still a major issue in daily practice among different laboratories and clinical investigations. Even with multi-gene signatures like Oncotype DX, MammaPrint, PAM50, EndoPredict (EP) and Breast cancer index (BCI) that are proven to successfully identify and/or stratify breast cancer patients for improved treatment modalities, both inter and intra tumour heterogeneity, accuracy and sensitivity play a significant role in further improving the efficacy of gene signatures. Along with the above, immune cell signatures could also contribute to identifying and/or stratifying breast cancer patients for escalation and de-escalation strategies. This review presents advanced breast cancer studies that reported use of gene signatures based on next generation sequencing technologies, microarray and machine learning. This review also presents advanced clinical trials that have been reported to be beneficial using known breast cancer gene signatures like Oncotype DX, MammaPrint, PAM50, EP, BCI and also an advanced multivariate algorithm that incorporated machine learning along with the genomic and clinicopathological features in identifying patients who may not need further treatment. We present the following article in accordance with the Narrative Review reporting checklist (available at https://abs.amegroups.com/article/view/10.21037/abs-21-64/rc).

This review aims to provide significant recent outcomes on the breast cancer clinical trials and research studies, which reported improved identification and stratification of breast cancer intrinsic subtypes, predisposition genes and the potential of liquid biopsy in cancer detection.

Methods

As to the methodology, breast cancer clinical trials and research studies were selected based on the positive outcomes and beyond the already known prognostic and predictive signatures to stratify breast cancer intrinsic subtypes and cancer detection. Trial reports and research study publications are mainly from the past ten years. Breast cancer intrinsic subtypes, gene expression signatures, Oncotype DX, MammaPrint, PAM50, EP, BCI, and breast cancer immune subtype signatures are used as keywords to search for the literature from PubMed. Studies covering the aforementioned search terms and that we thought have made impactful finding until March 2021 were only used to present in this narrative review (*Table 1*). Key historical studies were also cited in the main text. We may have missed some very important studies to include in this review. Only publications in the English language were selected. Where ever necessary, reports from earlier years were cited.

Precision medicine to assess breast cancer intrinsic subtypes

With tumour evolution and heterogeneity revolutionising precision oncology, it becomes increasingly significant for the clinicians and patients to become aware of ever-changing tumour markers. A classic example of this is HER2-positive breast cancer, which evolves during the cancer progression with a chance to detect different subtypes of breast cancer, including the basal like or luminal A (Lum A) subtypes. Though, breast tumour classification has become more robust from the pioneering work by Perou *et al.* by monitoring relevant signalling pathway activities via gene expression profiling (2), due to selective clonality within developing neoplasms and distinct clones representing the intratumour heterogeneity might still complicate the subtype classification. Using cDNA microarrays of gene expression patterns, the ER^+/luminal-like subtype was further categorised into two distinctive Lum A and luminal B (Lum B) subtypes (3). Practical and clinical implications of gene expression based intrinsic molecular subtyping and the potential for comprehensive tumour analysis beyond IHC markers to translate into clinical practice were very well covered by Prat *et al.* (4,5). The efficacy of multimodality therapies depends on the precise estimation of the level of

Table 1 The literature search strategy summary

Items	Specification
Date of search	February–April 2021
Databases and other sources searched	PubMed
Search terms used	Breast cancer intrinsic subtypes, gene expression signatures, Oncotype DX, MammaPrint, PAM50, EndoPredict, Breast Cancer Index, breast cancer immune subtype signatures
Timeframe	2008–2021, where necessary, earlier impact studies were included
Inclusion and exclusion criteria	Studies/reports only in English language were included, clinical trials and clinical research studies with an impact were only included
Selection process	The authors equally contributed in selecting the literature. Clinical and molecular research studies that contributed to advancing the understanding, use and significance of breast cancer signatures were included

risk and response of an individual patient and subtype (6,7). PAMELA trial showed that approximately 20–60% of HER2-enriched (HER2-E) within HER2 positive (HER2+) breast cancers did not achieve a complete response following anti-HER2 therapies. The study identified biological changes to be more evident in hormone receptor positive (HR+) disease. *In vitro* breast cancer cell line analysis in the same study reported that discontinuation of HER2-targeted therapy *in vitro*, or acquired resistance to anti-HER2 therapy, leads to restoration of the original HER2-E phenotype (8). Thus, supporting the use and maintenance of anti-HER2 treatment in HER2+ breast cancer sensitive patients and the need for further research into identifying underlying genetic and molecular subtypes changes to improve patient outcomes. The use of microarray made it possible to find gene expression signatures by which it can be established whether a group of genes (multigene assays) correlate with clinical variables like diagnosis or prognosis (9).

Multigene genomic or panel assays are now recommended by professional organizations like the American Society of Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN) guidelines and St. Gallen consensus conference as information that could help patients and physicians to make appropriate therapeutic decisions and to identify high risk individuals for breast cancer in oncology and genetic clinic.

Multigene expression assays

Multigene expression assays are developed by taking robustness, clinical validation, clinical utility and economic value into consideration and with an aim to have no or little inter-sample and inter-test variation to classify prognosis and chemotherapy indication. van't Veer *et al.* and van de Vijver *et al.* first validated a 70-gene signature in breast cancer patients while classifying them into good or poor prognosis groups (10,11). Among the multi-gene panels that were (*Table 2*) developed over the last two decades to predict the risk of distant recurrence and response to adjuvant therapy in early breast cancer, Oncotype DX and MammaPrint have been shown to benefit early-stage oestrogen-receptor positive and HER2-negative breast cancer patients in prospectively designed randomised studies (12). Gene expression-based assays such as Oncotype DX (13-15) identify low- and high-risk patients who do or do not need adjuvant or neoadjuvant chemotherapy. Oncotype DX provides a "Recurrence Score (RS)" based on a 21-gene signature that stratifies patients into low/intermediate/high-risk groups. The benefit of adjuvant chemotherapy using RS was demonstrated in retrospective (16) and prospective (17) trials. Like TAILORx, a more recent RxPONDER trial (18) made it clear that adjuvant chemotherapy can be avoided in post-menopausal patients with 1–3 lymph nodes. The PAM50/Prosigna (19) identifies patients who may not need or achieve any improved response benefits due to chemotherapy from those who are likely to benefit with a few years of follow-up. Five intrinsic molecular subtypes (IMS) defined by PAM50 include Lum A, Lum B, HER2-E, basal-like (Basal), and normal-like (Normal). TransATAC study shows that PAM50 is driven more by the ER status, which often may not be available in genome-wide studies. Also, as highlighted by Parker *et al.* (19) and Sørlie *et al.* (20) gene centring could be another challenge with PAM50. A recent review by Qian *et al.* (12) covered the latest studies, current status, present and future challenges of all the five

Table 2 Commercially available gene expression assays used in risk assessment and treatment benefits

Gene signature	No. of genes	Tissue	Analysis	Approach	Tumour type	Prognostic or Predictive	Company	Ref.
MammaPrint	70	FFPE	mRNA	Microarray	ER positive and negative	Prognostic and predictive	Agendia	(10,11)
Oncotype DX	21	FFPE	mRNA	qRT-PCR	ER positive	Prognostic and predictive	Genomic health	(12-18)
Prosigna/PAM50	50	FFPE	mRNA	NanoString nCounter	Distinguishes between luminal A, luminal B, HER2-enriched, normal-like and basal-like breast cancer subtypes	Prognostic	Nanostring	(19)

FFPE, formalin-fixed, paraffin-embedded; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

breast cancer prognostic gene signatures.

In addition to the recent studies covered by Qian *et al.*, here we summarised the outcomes of a prospective, randomised phase 3 MINDACT trial with an exploratory analysis by age that tested the genomic risk (using the MammaPrint 70-gene signature) and clinical risk among 6,693 patients to determine a breast cancer patient's need for chemotherapy. The study was reported as a positive de-escalation study. For women over 50, no difference was found in distant metastasis free survival (DMFS) between women who received adjuvant chemotherapy and those who did not, indicating that they could avoid chemotherapy and still achieve similar results. DMFS at 5 years in women with breast cancer who were clinically-high risk but with low genomic risk and were not treated with chemotherapy was 95.1%, which was above the predefined non-inferiority boundary of 92%. Thus, demonstrating that MammaPrint® low-risk patients have excellent outcomes without adjuvant chemotherapy (21).

Limitations of multi-gene panel tests & advances with HER2DX prognostic score and others

When multiple tests are available, it is inevitable to raise questions of accuracy and use of one over the other while deciding the risk, treatment and cost burden. Even the uncertainty involving the cost-effectiveness of the gene expression assays has been reported (22). While one study (23) reported a decrease in cost burden using MammaPrint, two other studies (24,25) found an increase in direct cost burden compared with St Gallen guidelines. From the pharmacoeconomic viewpoint the National Institute for Health and Care Excellence (NICE), UK, recommends considering Oncotype DX. The current multigene assays are limited to early recurrence scoring but are suboptimal for predicting late recurrent in HR+/HER2– early breast cancers. Though PAM50 and BCI (26) scores help precise prediction for the late recurrence (27), it is still not the consensus. However, further studies to establish a consensus on this could help. Also, the prognostic or predictive values of these assays are limited to HR+/HER2– breast cancer but no other breast cancer subtypes (28). Comparative studies indicate that risk prediction frequently differs when different prognostic assays are tested in the same case (28-31). Therefore, an opportunity to develop more accurate prognostic and predictive breast cancer biomarkers as predictors for late recurrence and chemotherapy benefit in early breast cancer patients could help provide precision treatments and improved outcomes.

To overcome the limitations of these gene expression signatures for clinical utility, HER2DX, a combined novel prognostic score based on 17 clinicopathological and genomic variables in early-stage HER2+ breast cancer, was reported (32). A combination of tumour size, nodal status, number of tumour infiltrating lymphocytes, PAM50 subtypes, and expression of 13 genes obtained from patients, tumour samples, showed a significant association of DMFS and identified patients with early-stage, HER2+ breast cancer. HER2DX prognostic score successfully identified a significant proportion of patients who might not need additional therapies and could be candidates for escalated or de-escalated systemic treatment. Further validation of the potential of HER2DX could be evaluated in different breast cancer subtypes.

Another study aimed to evaluate similarity among the four tests and characterise the molecular features that drive these tests, leading to inter-test differences. The same batch of RNA extracts from the samples from tamoxifen or anastrozole arms of the ATAC trial (33) were used. TransATAC study (34) used only molecular information like

Table 3 Stromal and immune-related gene prognostic and predictive signatures

Gene signature	Predictive or prognostic	Number of genes	Clinical outcomes	Ref.
SDPP	Prognostic	26	Differential immune responses Angiogenic & hypoxic responses	(36)
Stromal 50-gene signature	Predictive	50	Response to anthracycline-based neoadjuvant chemotherapy	(37)
12-immune-gene signature	Prognostic	12	Survival risk & possibility of immunotherapy incorporation	(39)
10-immune-gene & 7-transcription-factor signature	Prognostic	10 and 7 transcription factors	Degree of immune infiltration & the expression of immune checkpoint genes	(40)
17-immune-gene signature	Prognostic & predictive	17	Enriched immune microenvironment-related pathways	(41)

SDPP, stroma-derived prognostic predictor.

oestrogen, proliferation, invasion and HER2 scores from Oncotype DX RS but no clinicopathological features were included. Interestingly the study reported that Oncotype DX RS is mainly driven by the oestrogen module in the majority of the TransATAC cohort, while proliferative features determined the risk of recurrence (ROR), EP and BCI.

Raj Kumar *et al*. (35) reported a principle component analysis based on PAM50 subtyping when using in-house, The Cancer Genome Atlas (TCGA) breast cancer and METABRIC cohorts to overcome ER status in unbalanced cohorts. By doing so, the authors reported that introducing protein expression-based ER status can be avoided mixing into gene expression based subtyping methods. By applying PCA-PAM50, a more aggressive subset of Lum A tumours were reclassified as Lum B, increasing the Lum B subtype consistency with IHC by 25–49%.

Advances in breast cancer immune biomarkers

Owing to the prognostic ability of both tumour and stromal cells, Finak *et al*. in 2008 reported a 26-gene stroma-derived prognostic predictor in breast cancer patients (36). Following that a 50-gene stromal signature was reported to predict poor responses to anthracycline-based neoadjuvant chemotherapy (37). Kwon summarised different immune-related prognostic or predictive breast cancer gene signatures reported and suggested how incorporating immune gene signatures could help improve the prognostic or predictive ability of multigene assays and response to breast cancer therapies (38). The heterogeneity of the tissue poses one other challenge for accurate prognostic estimation. Recently, a 12-gene signature was reported using LASSO Cox regression analysis in a combination of gene expression profiles and clinical data of breast cancer patients collected from TCGA and Gene Expression Omnibus (GEO). The 12-gene signature significantly stratified patients into high and low immune risk groups associated with overall survival and assessed the possibility of immunotherapy incorporation in personalized breast cancer management (39). Another recent study built a prognostic index based on the TCGA dataset, transcriptional factor regulatory network and gene set enrichment pathway differences between high- and low-risk groups. The study reported 10 prognosis related immune genes and 7 prognostic transcription factors as having a stronger predictive ability than the tumour pathological stage while reflecting on the immune infiltration of breast cancer patients (40). The study also found that the expression levels of LAG-4, TIM-3, and PD-L1 were higher in the low-risk group and showed a significant negative correlation with risk score, thus highlighting their role in tumour immunotherapy. Interestingly another study using the TCGA and ImmPort dataset found 17 most immune-related representative genes were selected to establish a breast cancer risk score based on a prognostic prediction model. The study found that the 17 genes were enriched in numerous breast cancer and immune microenvironment related pathways demonstrated high predictive accuracy (41). Other than *CCR7*, *HSPA2* & *SEMA3B* genes are common in two of the three studies (*Table 3*) that used the TCGA dataset to identify a more accurate prognostic and predictive immune-related breast cancer gene signature showed unique list of genes. It is interesting how the analysis model, datasets and inclusion of different data points could result in different

gene signatures. However, using big datasets and varying statistical models reveals opportunities to propose and test different signatures and build on the current models in predicting survival and treatment efficacy in all subtypes of breast cancer.

Significance of breast cancer risk genes

The earliest report of breast cancer genetic predisposition can be traced back to 1866 by Paul Broca in 15 members of his wife's family (42). To cancer patients, the discovery of two significant breast cancer susceptibility genes, *BRCA1* and *BRCA2* in 1994 and 1995 brought the promise of genetic testing (43,44). Immediately after the discovery of *BRCA1/2*, a report in Philadelphia Inquirer (45) debated the benefits of gene testing for cancer risk in women. While the debate was ongoing, a year later in 1996, reports of the *BRCA1/2* commercial genetic tests for clinical use in detecting predisposition to breast and ovarian cancer were reported (46,47). On the one hand, multi-gene panel tests are advancing; hereditary breast cancer genesis has also been extensively analysed for improved screening and risk identification. In parallel, coupled with progress in bioinformatics, the use of multi-gene panels for detecting pathogenic germline variants (PGVs), such as *BRCA1/2* and other genes, for breast cancer has expanded in the clinical setting. These genetic assays assist in identifying high-risk individuals for developing breast cancer and other cancers like ovarian cancer, pancreas cancer and prostate cancer.

Recently two large studies involving a panel of 34 and 28 susceptible genes performed sequencing of 60,466 women with breast cancer and 53,461 controls. These studies aimed to estimate the overall risks of breast cancer and tumour subtypes associated with germline protein-truncating, rare missense and PGVs. In both studies, the variations in *BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *RAD51C*, *RAD51D*, *ATM*, and *CHEK2* had a significant association with breast cancer risk. While one study involving 113,000 cohort defined the genes that are most clinically useful for breast cancer risk prediction with estimated risk, the other US only cohort provided estimates of the prevalence and risk of breast cancer associated with pathogenic variants in known breast cancer-predisposition genes (48,49). A few months prior to these studies, a study in 1995, Japanese breast cancer patients analysed germline variants in 11 breast cancer susceptibility genes. The median age of 53 years was reported at the time of diagnosis of patients with PGVs, while 60 years in patients with no pathogenic variants (60 years). This study interestingly reported that *BRCA1/2* tumours without biallelic inactivation were indistinguishable from those without germline variants (50). With a combination of larger cohorts and improved sensitivity in identifying pathogenic mutations among high, moderate and low-risk breast cancer genes, NCCN guidelines and criteria are also evolving (NCCN, ver.1, 2020) to stratify and identify patients who could be at higher risk (51). Identification of genes with enrichment of rare but significant germline truncating mutations, tumour specific loss of heterozygosity, and homology-directed repair variants in *BRCA1/2* genes could help establish the role of PGVs affecting an individual's risk for tumour progression. From a cancer patient's perspective, understanding what caused their condition could help relieve their stress and offer relief. Knowledge of cancer predisposition genes could also help in improved diagnosis and clinical management while deciding on treatment options.

Liquid biopsy

Over the past few decades, cancer has been characterised as a systemic disease with clonal evolution, tumour heterogeneity and environment playing their role. In reality, elucidating one layer of complexity could only underscore other complex features that account for cancer progression. More comprehensive information could be obtained with longitudinal analysis from both tissue and liquid biopsies. From a biomarker assay point of view, it was in 1869 that Thomas Ashworth first observed the presence of circulating tumour cells and suggested that the circulating tumor cells (CTCs) released into the bloodstream lead to metastatic cancer. Fast forward to 1994, scientists, for the first time, detected specific mutations using cell-free DNA (cfDNA) found in the blood. After 3 years, in 1997, Dennis Lo successfully detected fetal cfDNA in the blood. Almost a decade and a half later, in 2013, Dawson *et al.*'s proof-of-concept analysis showed circulating tumour DNA (ctDNA) as an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer with changing tumour burden (52). Three years earlier, Klaus Pantel and Catherine Alix-Panabieres introduced the concept of a "liquid biopsy (53)". It was in June 2016 that the Food and Drug Administration (FDA) approved the first liquid biopsy test, the cobas® *EGFR* mutation test, as a cfDNA test. With *EGFR* exon 19 deletions or L858R mutations in metastatic non-small cell lung cancer (NSCLC) patients, plasma samples are the candidates for treatment with

Tarceva (erlotinib). Over the past decade, multiple reviews covered the advantages, challenges and future applications of liquid biopsies in early breast cancer detection and relapse. With the rapid development of technologies, advances in methods to accurately measure the amounts of ctDNA and analysis of low-abundance ctDNA and cfDNA; characterisation and understanding of the complex cancer phenotypes (e.g., mutational burden, clonal expansion), evaluation of early treatment response, relapse and discovery of acquired resistance have also improved. Multiple reviews have addressed the benefits and challenges of liquid biopsy in omics driven early and late-stage breast cancers (54-58).

Potential use of ctDNA in early and advanced trials

To take advantage of the potential of ctDNA in cancer diagnostics, ultrasensitive technologies are developed to detect low (<0.1%) mutant allele frequencies. With specificity >99%, CancerSEEK looked for 16 genes that are highly mutated in cancer and 11 protein markers that are often released into the blood in patients with non-metastatic, clinically detected cancers of the ovary, liver, stomach, pancreas, oesophagus, colorectum, lung, or breast patient. The median sensitivity reported for stage II tumours was 73% and 43% for stage I cancers (59). CancerSEEK also pinpoints cancer's tissue of origin and differs by cancer type, with a median of 83% among all the study participants. Recently, the TARGET (part A) study, a molecular profiling program with the primary aim to match patients with advanced cancers to early phase clinical trials, was reported. Somatic mutations and copy number alterations (CNA) across a 641 cancer-associated-gene panel in a single ctDNA assay (60) were used to identify the patients suited to the respective clinical trial group. Having demonstrated the robust workflow in supporting clinical decision-making and compatible data turnaround time in accordance with clinical practice, the TARGET part B study was initiated in 2017. In part B, the primary aim was to improve the matching of patients to clinical trials according to the molecular profile of their cancer while shortening the data turnaround time to 15–20 days. Findings reported by the TARGET study encourage routine implementation of ctDNA testing as supplementary to tumour testing. Another study, plasmaMATCH, analysed the potential use of ctDNA genomic profiling to direct therapy in an advanced breast cancer trial without needing repeated tumour biopsy. The study demonstrated an excellent 96–99% accordance between ctDNA digital PCR and targeted sequencing. plasmaMATCH also demonstrated that ctDNA testing offers accurate, rapid genotyping that enables the selection of mutation-directed therapies suitable for licensed targeted therapies, such as *PIK3CA*-mutant breast cancer, with the transformative potential of efficient and rapid screening for clinical trials (61).

ctDNA and longitudinal monitoring

ctDNA analysis also enables longitudinal, dynamic assessment of tumour evolution throughout the clinical course, thus serving as a non-invasive, real-time molecular tool to monitor treatment response and clonal evolution and guide for subsequent precision therapies. PALOMA-3 trial was performed using ctDNA exome sequencing to investigate the mechanisms leading to resistance to the CDK4/CDK6 inhibitor palbociclib plus fulvestrant versus fulvestrant. Contrary to earlier studies, the PALOMA-3 trial identified that *RB1* mutations are rare (4.7%) and often subclonal (mutations that are present in a fraction of cells but contribute to heterogeneity and sometimes lead to resistance), suggesting the potential activity of subsequent endocrine-based therapy after progression on the combination (62). BEECH trial also demonstrated the use of assessment of the dynamic changes of ctDNA levels in predicting progression-free survival and drug efficacy. A study like PALOMA-3 demonstrated the potential of early ctDNA dynamics in early drug development, evaluated drug efficacy early, and assessed whether a biomarker (*PIK3CA* mutation in BEECH trial) predicts targeted therapy efficacy (63). With an aim to identify whether ctDNA will serve as a biomarker in predicting treatment response to anti-HER2-targeted therapy, the NeoALTTO trial analysed *PIK3CA* and *TP53* mutations during digital PCR (64). Interestingly, the trial concluded that undetectable ctDNA at baseline had the highest pathological complete response (pCR) rates, suggesting the best candidates for treatment de-escalation strategies. With many studies demonstrating the translational potential of cfDNA and ctDNA for improved cancer management, another study developed a tailor made targeted cfDNA sequencing approach for breast cancer. Using unique molecular identifiers (UMIs) for error correction the study aimed to identify low-frequency variants and reliable identification of copy number variations (CNVs) from plasma DNA (65).

Immunotherapy, breast cancer and liquid biopsies

Impassion130 trial reported a 40% reduced risk of disease progression or death in patients receiving atezolizumab plus nab-paclitaxel or placebo (66). Following this report, the FDA approved the first checkpoint inhibitor immunotherapy drug [anti-PD-L1 antibody atezolizumab (Tecentriq®), in combination with chemotherapy (Abraxane®)] for the treatment of metastatic triple negative breast cancer (TNBC) patients who had positive PD-L1 protein expression. A pre-specified second interim overall survival analysis of phase 3 IMpassion130 study did not report a significant overall survival benefit. Efficacy and safety of atezolizumab plus nab-paclitaxel in patients reported a clinically meaningful overall survival benefit which was improved in patients with PD-L1 immune-cell positive metastatic TNBC who received nab-paclitaxel plus atezolizumab when compared with the control group (67). However, with no significant difference in overall survival between the treatment groups, also treatment related deaths in two (<1%) patients and other adverse events such as neutropenia, endocrinopathies, and others remain challenges for the success of combinational immunotherapy treatment. This justifies further exploration and understanding of the need for new combination therapeutic strategies. In the context of immunotherapy, serial ctDNA testing may perform as a predictive biomarker in patients with advanced solid tumours treated with pembrolizumab (68). Sixteen clonal somatic mutations were selected for personalized ctDNA assay, and baseline ctDNA ($ctDNA_B$) was detected in 92 of 94 (98%) samples. The study suggested selecting a bespoke ctDNA assay as a strength, which allowed to apply the test to all patients whose whole exome sequencing data was available. $ctDNA_B$ concentration correlates with progression-free survival, overall survival, clinical response and clinical benefit. This shows the benefits of ctDNA-based surveillance among patients treated with immune checkpoint blockade in a clinical setting.

Although HER2DX served as a reliable prognostic score that identified patients who may not need additional treatment, its broader applicability in different ethnic groups still needs to be addressed. Going forward, multi-ethnic cross-cultural inclusive breast cancer clinical and translational research studies could address such limitations. Along with it, consortium studies like the TCGA, International Cancer Genome Consortium (ICGC) and others could help maximise the use of big data and machine learning algorithms resulting in a more accurate and reliable breast cancer prognostic and/or predictive gene signatures.

Conclusions

The combined use of anatomical and biomarker staging, deep learning algorithms, and tumour genomic assays could guide us to precisely predict prognostic outcomes and consider optimal therapeutic plans for each patient. High-throughput technologies like microarray and next-generation sequencing, along with machine learning approaches, could close the gap between clinical and research intrinsic subtyping, thus helping advance breast cancer management.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Annals of Breast Surgery* for the series "Cuting-edge Surgical Research". The article has undergone external peer review.

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://abs.amegroups.com/article/view/10.21037/abs-21-64/rc

Peer Review File: Available at https://abs.amegroups.com/article/view/10.21037/abs-21-64/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://abs.amegroups.com/article/view/10.21037/abs-21-64/coif). The series "Cuting-edge Surgical Research" was commissioned by the editorial office without any funding or sponsorship. MT served as the unpaid Guest Editor of the series and serves as an unpaid editorial board member of *Annals of Breast Surgery* from July 2020 to June 2024. MT is supported by the abovementioned grants from Chugai, Takeda, Pfizer, Kyowa-Kirin, Taiho, JBCRG association, Eisai, Daiichi-Sankyo, AstraZeneca, Astellas, Shimadzu, Yakult, Luxonus, AFI technologies, Nippon-Kayaku, Shionogi, and GL Science; receives consulting fees from Kyowa-Kirin, Daiichi-Sankyo, AstraZeneca, BMS, Eli Lilly, Athenex

Oncology, Bertis, Terumo, Luxonus, and Kansai Medical Net, lecture fee from Takeda, Chugai, Pfizer, Kyowa-Kirin, Taiho, Eisai, Daiichi-Sankyo, Eli Lilly, AstraZeneca, Exact Science, Novartis, Konica Minolta, Shimadzu, Yakult, and Nippon Kayaku; and holds leadership roles at JBCRG, JBCRN & OOTR. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 2015;347:78-81.
2. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
3. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.
4. Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. Nat Rev Clin Oncol 2011;9:48-57.
5. Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 2015;24 Suppl 2:S26-35.
6. Osborne CK, Yochmowitz MG, Knight WA 3rd, et al. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980;46:2884-8.
7. Rouzier R, Perou CM, Symmans WF, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005;11:5678-85.
8. Brasó-Maristany F, Griguolo G, Pascual T, et al. Phenotypic changes of HER2-positive breast cancer during and after dual HER2 blockade. Nat Commun 2020;11:385.
9. Chibon F. Cancer gene expression signatures - the rise and fall? Eur J Cancer 2013;49:2000-9.
10. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-6.
11. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999-2009.
12. Qian Y, Daza J, Itzel T, et al. Prognostic Cancer Gene Expression Signatures: Current Status and Challenges. Cells 2021;10:648.
13. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817-26.
14. Toi M, Iwata H, Yamanaka T, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. Cancer 2010;116:3112-8.
15. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2018;379:111-21.
16. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010;11:55-65.
17. Sparano JA, Gray RJ, Makower DF, et al. Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2015;373:2005-14.
18. Kalinsky K, Barlow WE, Meric-Bernstam F, et al. First results from a phase III randomized clinical trial of standard adjuvant endocrine therapy (ET) +/- chemotherapy (CT) in patients (pts) with 1-3 positive nodes, hormone receptor-positive (HR+) and HER2-negative (HER2-) breast cancer (BC) with recurrence score (RS) <25: SWOG S1007 (RxPonder). Cancer Res 2021;81:abstr GS3-00.
19. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160-7.
20. Sørlie T, Borgan E, Myhre S, et al. The importance of gene-centring microarray data. Lancet Oncol 2010;11:719-20; author reply 720-1.
21. Piccart M, van 't Veer LJ, Poncet C, et al. 70-gene signature as an aid for treatment decisions in early

breast cancer: updated results of the phase 3 randomised MINDACT trial with an exploratory analysis by age. Lancet Oncol 2021;22:476-88.
22. Kelly CM, Bernard PS, Krishnamurthy S, et al. Agreement in risk prediction between the 21-gene recurrence score assay (Oncotype DX®) and the PAM50 breast cancer intrinsic Classifier™ in early-stage estrogen receptor-positive breast cancer. Oncologist 2012;17:492-8.
23. Retèl VP, Joore MA, Knauer M, et al. Cost-effectiveness of the 70-gene signature versus St. Gallen guidelines and Adjuvant Online for early breast cancer. Eur J Cancer 2010;46:1382-91.
24. Chen E, Tong KB, Malin JL. Cost-effectiveness of 70-gene MammaPrint signature in node-negative breast cancer. Am J Manag Care 2010;16:e333-42.
25. Kondo M, Hoshi SL, Ishiguro H, et al. Economic evaluation of the 70-gene prognosis-signature (MammaPrint®) in hormone receptor-positive, lymph node-negative, human epidermal growth factor receptor type 2-negative early stage breast cancer in Japan. Breast Cancer Res Treat 2012;133:759-68.
26. Sgroi DC, Chapman JA, Badovinac-Crnjevic T, et al. Assessment of the prognostic and predictive utility of the Breast Cancer Index (BCI): an NCIC CTG MA.14 study. Breast Cancer Res 2016;18:1.
27. Sgroi DC, Carney E, Zarrella E, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst 2013;105:1036-42.
28. Prat A, Parker JS, Fan C, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. Ann Oncol 2012;23:2866-73.
29. Varga Z, Sinn P, Seidman AD. Summary of head-to-head comparisons of patient risk classifications by the 21-gene Recurrence Score® (RS) assay and other genomic assays for early breast cancer. Int J Cancer 2019;145:882-93.
30. Iwamoto T, Lee JS, Bianchini G, et al. First generation prognostic gene signatures for breast cancer predict both survival and chemotherapy sensitivity and identify overlapping patient populations. Breast Cancer Res Treat 2011;130:155-64.
31. Sestak I, Buus R, Cuzick J, et al. Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol 2018;4:545-53.
32. Prat A, Guarneri V, Paré L, et al. A multivariable prognostic score to guide systemic therapy in early-stage HER2-positive breast cancer: a retrospective study with an external evaluation. Lancet Oncol 2020;21:1455-64.
33. Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 2010;11:1135-41.
34. Buus R, Sestak I, Kronenwett R, et al. Molecular Drivers of Oncotype DX, Prosigna, EndoPredict, and the Breast Cancer Index: A TransATAC Study. J Clin Oncol 2021;39:126-35.
35. Raj-Kumar PK, Liu J, Hooke JA, et al. PCA-PAM50 improves consistency between breast cancer intrinsic and clinical subtyping reclassifying a subset of luminal A tumors as luminal B. Sci Rep 2019;9:7956.
36. Finak G, Bertos N, Pepin F, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 2008;14:518-27.
37. Farmer P, Bonnefoi H, Anderle P, et al. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med 2009;15:68-74.
38. Kwon MJ. Emerging immune gene signatures as prognostic or predictive biomarkers in breast cancer. Arch Pharm Res 2019;42:947-61.
39. Xie P, Ma Y, Yu S, et al. Development of an Immune-Related Prognostic Signature in Breast Cancer. Front Genet 2020;10:1390.
40. Yu X, Guo J, Zhou Q, et al. A novel immune-related prognostic index for predicting breast cancer overall survival. Breast Cancer 2021;28:434-47.
41. Mu G, Ji H, He H, et al. Immune-related gene data-based molecular subtyping related to the prognosis of breast cancer patients. Breast Cancer 2021;28:513-26.
42. Broca PP. Traite des tumeurs. Paris: P. Asselin, 1866.
43. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66-71.
44. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789-92.
45. McCullough M. Debating the benefits of genetic testing for cancer risk in women. Philadelphia Inquirer 1995:D1-2. December 11, 1995.
46. Zoler ML. BRCA1 gene test goes commercial. Internal Medicine News 1995:1-2.
47. Kolata G. Breaking ranks, lab offers test to assess risk of breast cancer. N Y Times Web 1996;A1-A15.
48. Breast Cancer Association Consortium; Dorling L, Carvalho S, et al. Breast Cancer Risk Genes - Association

Analysis in More than 113,000 Women. N Engl J Med 2021;384:428-39.
49. Hu C, Hart SN, Gnanaolivu R, et al. A Population-Based Study of Genes Previously Implicated in Breast Cancer. N Engl J Med 2021;384:440-51.
50. Inagaki-Kawata Y, Yoshida K, Kawaguchi-Sakita N, et al. Genetic and clinical landscape of breast cancers with germline BRCA1/2 variants. Commun Biol 2020;3:578.
51. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology—genetic/familial high-risk assessment: breast, ovarian, and pancreatic. Version 1. 2020.
52. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.
53. Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med 2010;16:398-406.
54. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223-38.
55. Heitzer E, Haque IS, Roberts CES, et al. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20:71-88.
56. Alimirzaie S, Bagherzadeh M, Akbari MR. Liquid biopsy in breast cancer: A comprehensive review. Clin Genet 2019;95:643-60.
57. Alba-Bernal A, Lavado-Valenzuela R, Domínguez-Recio ME, et al. Challenges and achievements of liquid biopsy technologies employed in early breast cancer. EBioMedicine 2020;62:103100.
58. Cheng ML, Pectasides E, Hanna GJ, et al. Circulating tumor DNA in advanced solid tumors: Clinical relevance and future directions. CA Cancer J Clin 2021;71:176-90.
59. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359:926-30.
60. Rothwell DG, Ayub M, Cook N, et al. Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study. Nat Med 2019;25:738-43.
61. Turner NC, Kingston B, Kilburn LS, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. Lancet Oncol 2020;21:1296-308.
62. O'Leary B, Cutts RJ, Liu Y, et al. The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial. Cancer Discov 2018;8:1390-403.
63. Hrebien S, Citi V, Garcia-Murillas I, et al. Early ctDNA dynamics as a surrogate for progression-free survival in advanced breast cancer in the BEECH trial. Ann Oncol 2019;30:945-52.
64. Rothé F, Silva MJ, Venet D, et al. Circulating Tumor DNA in HER2-Amplified Breast Cancer: A Translational Research Substudy of the NeoALTTO Phase III Trial. Clin Cancer Res 2019;25:3581-8.
65. Kleftogiannis D, Ho D, Liew JX, et al. Detection of genomic alterations in breast cancer with circulating tumour DNA sequencing. Sci Rep 2020;10:16774.
66. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med 2018;379:2108-21.
67. Schmid P, Rugo HS, Adams S, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2020;21:44-59.
68. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 2020;1:873-81.

doi: 10.21037/abs-21-64
Cite this article as: Velaga R, Toi M. Advances in molecular medicine for breast cancer practice: a narrative review. Ann Breast Surg 2023;7:38.

