



# The evolution and advances of biomarker use in clinical trials for breast cancer treatment—a narrative review

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**Abstract:** Cancer is characterized with enormous heterogeneity, including intertumoral heterogeneity and intratumor heterogeneity, which represents the main hurdle and challenges for therapy. The great success in the precision medicine and personalized treatment among all cancer types has been noted in breast cancer. Treatment for breast cancer has evolved in the past decades starting from CMF regimen (cyclophosphamide, methotrexate and fluorouracil) chemotherapy as the standard of care regardless of histological type in 1970s to the current stage of molecular subtype-based therapy which can also be known as biomarker-driven therapy. In response to this great shift of paradigm, clinical trials in breast cancer have evolved during the past several decades, while biomarkers are becoming prominently valuable in driving breast cancer research and drug development. By enabling early detection, identifying treatment responders and monitoring treatment response and therapeutic effects, biomarker-based clinical trial design will speed the development process and allow evaluation of multiple patient groups while biomarker-driven personalized treatment approaches will lead to better patient outcomes. This review provides an overview of the biomarker landscape in different molecular subtypes of breast cancer and the use of prognostic biomarkers in assisting in predicting therapeutic outcomes and monitoring recurrence in patients with breast cancer.

**Keywords:** Biomarker; clinical trials; breast cancer; molecular subtypes; disease recurrence; therapeutic outcomes

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## Introduction

With the approval of newer drugs based on efficacy in patients stratified by the presence or absence of molecular biomarkers, we are in an era of precision medicine and biomarker guided therapy (1). Oncology, especially solid tumors, have been considered as a potential focus area for the development of biomarkers (2). This is substantiated by the increase in the number of Food and Drug Administration (FDA) approved drugs for oncologic indications that could be used for biomarker guided therapy. From 2006 to 2018, a total of 31 drugs have been approved for 38 oncologic indications by the FDA based on efficacy in patients with the presence or absence of a

particular biomarker. Among them, 5 drugs, trastuzumab, lapatinib, pertuzumab, ado-trastuzumab emtansine and olaparib were approved for treating patients with breast cancer (3). This is further substantiated by the growing list of companion diagnostics approved by the FDA in which a total of 17 companion diagnostic assays have been approved for use in patients with breast cancer (4). This suggests that, among different types of cancers, breast cancer might represent the most robust type for the development of biomarker guided therapeutics and biomarker-based clinical trials.

The categorization of breast cancer into distinct histological and molecular subtypes had undergone paradigm shift in the last few decades. In 2003, the World

Health Organization (WHO) categorized breast cancer into 17 histological types (5,6). Since the histological categorization did not have any therapeutic implications, it was not subsequently used in clinical practice (7). Classification of breast cancer based on predictive biomarkers like expression of oestrogen receptor (ER), progesterone receptors (PR) and assessment of human epidermal growth factor receptor 2 (HER2) are clinically relevant for therapeutic decision making (8-10). Although breast cancers are frequently classified based on the hormone receptor (HR) (ER+ and/or PR+) and HER2 status, they are molecularly and histologically heterogeneous (7). Microarray based gene expression studies have expanded the scope of conventional molecular classification and had also established the correlation between molecular and histological types (11). Despite the availability of microarray based gene panels, immunohistochemistry (IHC) based identification of molecular subtypes (HR+ and/or HER2+) is considered as gold standard (12). Cellular proliferation markers like Ki-67 which has been reported to be of prognostic value can also be considered as surrogate markers in clinical trials (13).

The previous approach to treatment of breast cancer is an “one size fits all” modality had undergone, and a paradigm shift towards a more personalized treatment based on genetic predisposing factors (14). Despite the identification of established biomarkers, the search for additional biomarkers for prediction of prognosis in breast cancer has not ceased. This is in part due to the newer targeted therapeutics, which by definition, performs better in distinct patient subgroups. The availability of biomarkers with good prognostic ability will help in selecting patient subgroups who could potentially benefit from a particular treatment regimen and shortening the drug development process. This also minimizes over-treatment, reduces the economic burden of unnecessary therapeutic regimens and improves quality of life in patients with breast cancer (15,16). The purpose of this review is to provide an overview of the biomarker landscape in different molecular subtypes of breast cancer and the use of prognostic biomarkers updated information on the current status of prognostic biomarkers and their efficacy in assisting in predicting therapeutic outcomes and monitoring recurrence in patients with breast cancer. We present the following article in accordance with the Narrative reporting checklist (available at <http://dx.doi.org/10.21037/tbcr-20-66>).

## Biomarker landscape in different molecular subtypes of breast cancer

### *HER2 positive breast cancer*

Among patients with breast cancers, 15–30% of patients experience HER2 positive breast cancer which is known for its poor prognosis (17,18). Trastuzumab is a landmark drug for the anti-HER2 treatment. Prior to the approval of trastuzumab, HER2 positive breast cancer was associated with high recurrence rates and poor survival outcomes (19-21). Trastuzumab was approved by the FDA in 1998 for the treatment of HER2 overexpressing, node positive or node negative breast cancer in adjuvant setting and for the systemic treatment of HER2 overexpressing metastatic breast cancer. It is recommended to be used either as a monotherapy or in combination with chemotherapy (22). Subsequent to the approval of trastuzumab, 7 other drugs targeting HER2 positive breast cancer have been approved by the FDA. Pertuzumab (HER2 targeted monoclonal antibody) (23), lapatinib (HER1 and HER2 tyrosine kinase inhibitor) (24), neratinib (HER tyrosine kinase inhibitor) (25), tucatinib (HER2 tyrosine kinase inhibitor) (26), trastuzumab emtansine (27) and trastuzumab deruxtecan (28) (antibody drug conjugate) were the currently available drugs targeting HER2 positive breast cancer. They change the treatment pattern and prognosis in HER2-positive breast cancer patients.

Until now, HER2 is still the only validated biomarker for the treatment with these targeted therapies. Trastuzumab has been evaluated both as monotherapy and in combination with chemotherapy in early and metastatic breast cancers. Owing to the mechanism of action of trastuzumab, it is effective only in patients with HER2 positive breast cancer. In early clinical trials with trastuzumab monotherapy, an overall response rate of 26% was observed which increased to 35% and 41% for patients with IHC defined HER2 levels of 3+ and gene amplification by fluorescent in situ hybridization (FISH) confirmed HER 2 positive patients, respectively (29). Similarly, in patients with metastatic breast cancer, overall response rates of 26% was observed with significantly better response rate in patients with IHC3+ HER2 levels. Clinical benefit rates were also better for patients with higher HER2 expression levels (IHC3+ vs. IHC2+: 48% vs. 7%). HER2 gene amplification by FISH also revealed similar improvement in efficacy for HER2 positive patients (29). These results suggest that, irrespective of HER2 detection method (FISH vs. IHC),

patients with good HER2 levels most likely experiences a favorable therapeutic response to trastuzumab. Similar results were also observed with trastuzumab in combination with chemotherapy (30). Although the survival outcomes were significantly better in trastuzumab containing arm, a sub-population of patients seems to have no clinical benefit despite the presence of HER2 overexpression. A probable reason for the observed discrepancy with respect to the poor survival outcomes in a few patients could be the development of resistance to HER2 directed therapies (31).

Other molecular markers related to the HER2 pathway have also been evaluated for their role in predicting therapeutic response. The landmark Clinical Evaluation of Pertuzumab and Trastuzumab (CLEOPATRA) trial evaluated the efficacy of dual HER2 targeted therapy in combination with chemotherapy in HER2 positive, metastatic breast cancer patients (32,33). In an exploratory analysis from the CLEOPATRA trial, serum markers, ligands and receptors and intracellular pathway markers were assessed using appropriate assays to investigate their predictive therapeutic effects and prognostic effects independent of treatment arm. The biomarkers included amphiregulin, betacellulin, epidermal growth factor (EGF), transforming growth factor alpha, EGF receptor, HER2, HER3, insulin-like growth factor 1 receptor, PTEN, phosphorylated AKT, PIK3CA, CMYC, serum HER2 extracellular domain (sHER2), and FCR. These markers were estimated by IHC, FISH and reverse transcription polymerase chain reaction. Among the markers analyzed, high HER2 mRNA and HER3 mRNA, low sHER2 were significantly associated with a prolonged PFS. The analysis of prognostic effect of the biomarkers in the patients pooled from both the arms revealed PIK3CA mutation to be the most significant prognostic factor in patients with HER2 positive, metastatic breast cancer treated with either drug regimens. Patients with mutated PIK3CA had a worse PFS (8.6 months for mutated *vs.* 13.8 months for wild type in control group; 12.5 months for mutated *vs.* 21.8 months for wild type in pertuzumab group) (34). The identical results obtained in both the treatment groups in the CLEOPATRA trial suggested that mutation in PIK3CA could be a marker for HER2 resistance.

The efficacy of dual HER2 targeted therapy has also been evaluated in the neoadjuvant settings in the Chemotherapy, Herceptin and Lapatinib in Operable Breast Cancer (CHER-LOB) Trial. The trial evaluated the efficacy of chemotherapy in combination with trastuzumab or lapatinib and chemotherapy in combination with

trastuzumab plus lapatinib in patients with HER2 positive, stage II to IIIA operable breast cancer. The addition of dual HER2 targeted drugs improved pCR rates In comparison to either drug alone (25% *vs.* 26.3% *vs.* 46.7%, P=0.019) (35). In the subsequent predictive biomarker study, patients with wild type and mutated PIK3CA had similar pCR rates (33.7% *vs.* 22.7%, P=0.323) in the pooled cohort while PIK3CA mutated patients had a better pCR rate in patients treated with dual HER2 targeted therapy (48.4% *vs.* 12.5%, P=0.06) (36). The similar rates of pCR among patients with PIK3CA wild type and mutated patients in this study could be due to the low number of PIK3CA mutated patients in the study (n=5). However, the role of PIK3CA mutation in predicting resistance to HER2 directed therapies have been confirmed in multiple trials with single or dual HER2 directed therapies in neoadjuvant settings (37,38). In a pooled stratified analysis of multiple trials with dual HER2 targeted therapy, patients with PIK3CA wild type have better pCR (45.5% *vs.* 21.4%) in comparison to patients with mutated PIK3CA (39).

The results of the above studies suggested a worse prognosis for patients with PIK3CA mutated, HER2 positive breast cancer in neoadjuvant, adjuvant and advanced metastatic settings. Hence inhibition of PI3K pathway might enhance clinical benefit in patients with HER2 positive breast cancer. The phase IB, PIKHER2 study (NCT01589861) evaluated the efficacy of a pan-isoform, class I, PI3K inhibitor, buparlisib, in combination with lapatinib in patients with HER2 positive, trastuzumab resistant, advanced breast cancer. The study reported a disease control rate of 79% (95% CI, 57% to 92%) and clinical benefit rate of 29% (95% CI, 12% to 51%) with one patient reporting complete response (40). In another similar phase II study (NCT01132664), buparlisib was evaluated in combination with trastuzumab in patients with trastuzumab resistant, HER2 positive, advanced breast cancer. The study reported an objective response rate (ORR) of 10% which was much lower than the predefined, primary endpoint (ORR ≥25%). Among the 4 patients with partial response, 2 patients had PI3K pathway activated tumors (41). Nevertheless, both the studies did not select for the patients with PIK3CA mutation. Neoadjuvant PI3K inhibition in HER2 Over Expressing Breast canCer (NeoPHOEBC) trial (NCT01816594), evaluated the efficacy of buparlisib or placebo in combination with trastuzumab and paclitaxel in patients with HER2 positive breast cancer in neoadjuvant setting. The study was terminated due to liver toxicity. But the premature results suggested a lack of enhanced efficacy

with the addition of buparlisib with respect to the pCR rates (32% vs. 40%, P=0.811). However, in a small sub-group of ER+ patients, addition of buparlisib led to significant improvement in ORRs (68.8% vs. 33.3%, P=0.03) (42).

The effectiveness of PI3K inhibition in combination with HER2 targeted therapy was evaluated in a phase I study (NCT02038010) with the  $\alpha$ -specific PI3K inhibitor, alpelisib. Alpelisib in combination with trastuzumab emtansine in trastuzumab resistant patients revealed an ORR of 43% which increased to 53% in patients with anomalies in the PI3K pathway. The clinical benefit rate was 56% in patients with mutation in any of the genes involved in the PI3K pathway including 3 patients who progressed on previous trastuzumab emtansine treatment (43). All the completed studies evaluating PI3K inhibition in combination with HER2 targeted drugs have included patients not selected for mutation in PI3K pathway which makes it difficult to interpret the precise role of PI3K mutation as a biomarker in selecting patients eligible for combination therapy with PI3K inhibitors and HER2 targeted drugs. An ongoing phase III study (NCT04208178) is currently evaluating the efficacy of alpelisib in combination with dual HER2 targeted therapy (trastuzumab and pertuzumab) in patients with HER2 positive, PIK3CA mutated, advanced breast cancer who have been previously treated with trastuzumab and pertuzumab. The results of this study will provide further insights on the prognostic value of PI3KCA mutation in predicting response to HER2 targeted drugs and for selecting patients for PI3K inhibitor treatment in the advanced breast cancer setting.

Biomarkers of immunity may also have potential prognosis significance in HER2-positive advanced breast cancer. A retrospective analysis of the CLEOPATRA study assessed the prognostic ability of tumor infiltrating lymphocytes (TILs) in predicting therapeutic outcomes. TILs from the available tumor samples were assessed by histopathology and a cut-off of 20% stromal TILs were used for stratified regression analysis. The median PFS values were similar in patients with TILs  $\leq$ 20% and  $>$ 20% (HR: 0.85; 95% CI, 0.70 to 1.03) whereas median OS was significantly shorter in patients with TIL  $\leq$ 20% (HR 0.76; 95% CI, 0.60 to 0.96). However, there was significant interaction between stromal TIL values and drug treatment with respect to both PFS and OS (44). This suggests that stromal TIL might be potential prognostic marker for predicting OS in patients with HER2 positive, metastatic breast cancer irrespective of the treatment regimen. This finding has also been substantiated in a recent meta-analysis of 5 randomized controlled trials involving patients with

HER2 positive, non-metastatic breast cancer. The pooled odds of pathologic complete remission (pCR) in high TIL expressing patients from 4 studies involving trastuzumab as the only HER2 targeted agent, in combination with chemotherapy, was found to be 1.90 (95% CI, 1.21 to 3.00). Similar results were also obtained from studies with lapatinib as the sole HER2 targeted agent [odds ratio (OR): 1.79, 95% CI, 0.99 to 3.23]. Surprisingly, despite the lack of interaction between treatment arms and TIL expression in predicting pCR, studies with dual HER2 targeted therapies revealed a higher odds of pCR in high TIL expressing patients (OR: 3.04; 95% CI, 0.84 to 11.20), although there was no statistical significance (P=0.147) (45).

#### ***HR positive (HR+) breast cancer***

Hormone receptors including ER and PR are expressed in approximately 80% of breast cancer (46). Owing to the expression of HRs, endocrine therapy has been the mainstay of treatment in patients with HR positive breast cancer in neoadjuvant, adjuvant and advanced treatment settings (29). Determination of HR status is mandatory for selecting patients who are likely to respond to endocrine therapies like the selective ER modulators (tamoxifen), aromatase inhibitors (anastrozole, letrozole and exemestane), luteinising-hormone releasing hormone agonists (goserelin), selective ER degrader (fulvestrant) and oophorectomy, across different treatment settings (47-49). The prognostic ability of HR in predicting response to endocrine therapies have been reported in multiple studies. In a patient level meta-analysis on the efficacy of adjuvant tamoxifen, the relative risk (RR) for disease recurrence was significantly low for up to 10 years in patients with ER positive breast cancer (RR: 0.62, P<0.00001). In patients with ER positive breast cancer, PR status was not predictive factor for disease recurrence (10). This suggests that there could be more heterogeneity with respect to predictive biomarkers with other genetic factors also influencing the disease free survival in patients with HR positive disease (50-52). Multiple retrospective studies have reported superior outcome in ER or PR positive tumors. But the observed favourable outcome seems to be negated after 5-10 years of adjuvant treatment (51,52). This suggests that HR receptor status alone cannot predict therapeutic outcome in patients with HR positive, early breast cancer, treated with endocrine therapies. In a previous retrospective study, patients with PIK3CA mutation had a better PFS

when treated with everolimus (53). To address the issue, the updated guidelines from the European group on tumor markers recommended future research focusing on the development of biomarkers with increased positive predictive value and those that could assist in selection of patients who could potentially benefit from specific endocrine therapy (54).

Patients with HR positive breast cancer respond differentially with temporal variation to endocrine therapy which suggest role of other molecular pathways especially the ones involved in cell cycle. Cyclin-dependent kinases (CDK) 4 and 6 promote cell cycle entry and *in vitro* studies confirm their role in breast cancer proliferation (55,56). Further *in vitro* studies reported that breast cancer resistant to endocrine therapy might still be dependent on CDK4/6 (57,58). Based on these observations, 3 CDK4/6 inhibitors, palbociclib, ribociclib and abemaciclib have been approved by the FDA for the treatment of patients with HR positive, HER2 negative, early and metastatic breast cancer (59-61). The approval of CDK4/6 inhibitors has changed the treatment landscape of patients with HR positive breast cancer. CDK4/6 inhibitors have been used in combination with different endocrine therapy regimens like aromatase inhibitors, fulvestrant and tamoxifen and the probable biomarkers predicting therapeutic outcome have been evaluated in the landmark trials.

In the PALOMA-3 trial (NCT01942135), fulvestrant in combination with palbociclib was found to be effective in prolonging PFS in the overall patients and also in stratified subgroups in the advanced treatment settings. The combination was found to be equally effective in both ER+/PR- and ER+/PR+ subgroups (HR: 0.46 for both the subgroups) (46). Further, the study also reported that a subset of patients treated with fulvestrant alone elucidated a prolonged progression free survival (62). Subsequently, an exploratory biomarker analysis was performed. The predictive ability of mutations in PI3KCA and the gene encoding the main endoplasmic reticulum isoform (ESR1) was assessed in long-term ( $\geq 18$  months) and short-term responders ( $< 18$  months). The incidence of ESR1 and PI3KCA mutation at baseline was lower in long-term responders in both the treatment arms. However, a more pronounced difference in mutation rates were observed in the fulvestrant plus placebo arm. The incidence of ESR1 mutation was 6% and 33% in long-term and short-term responders while the corresponding incidence of PI3KCA mutation was 6% and 39% in long- and short-term responders, respectively (63). This suggested that

the baseline mutation in these genes may attenuate the resistance to endocrine therapy and by extension, the survival outcomes in patients with HR+, HER2- locally advanced/metastatic breast cancer. In a pooled analysis of two different trials, patients with ESR1 mutation and ESR1 wild type in the PALOMA-3 trial reported better PFS when treated with palbociclib in combination with fulvestrant than fulvestrant alone (HR: 0.43 for ESR1 mutated and 0.49 for wild type,  $P < 0.05$  for both). Stratified analysis from the SoFEA study evaluating fulvestrant and exemestane revealed that patients with ESR1 mutation had a significantly better PFS (HR: 0.52,  $P = 0.02$ ) when treated with fulvestrant which corroborated with the exploratory analysis of the PALOMA-3 study (64-66). This suggested that ESR1 mutation could be used to select patients for treatment with fulvestrant over aromatase inhibitors. An analysis stratified by molecular subtype of breast cancer (luminal A vs. luminal B) in patients treated in the PALOMA-3 trial revealed similar effect estimates for treatment with fulvestrant plus palbociclib (HR for PFS for luminal A and B: 0.23 and 0.26,  $P < 0.0001$  for both). Baseline mRNA levels of CDK6 and cyclin E1 (CCNE1) were significantly associated ( $P < 0.05$  for both) with treatment outcome in patients with bone only and visceral only metastases, respectively. CCNE1 was also associated significantly with therapeutic outcomes in patients with non-visceral (excluding bone) metastasis in patients analysed in the PALOMA-3 trial (29). In another study employing paired baseline and end of study sequencing of ctDNA, from the PALOMA-3 study, newer driver mutations in the PIK3CA and ESR1 genes emerged during treatment in both the arms (67). This suggested that mutations in these 2 loci might have therapeutic implications for treatment with other endocrine drugs like aromatase inhibitors. The clinical significance of these mutations with respect to stage of disease and line of treatment was assessed by a study by Takeshita *et al.* They analysed the ctDNA of early and metastatic BC patients and identified that the prevalence of mutations in PIK3CA and ESR1 genes were high in late treatment settings. This suggested the role of endocrine therapy in driving the mutations leading to resistance to endocrine therapy. The same study also revealed shorter time to treatment failure in PIK3CA mutated patients treated at early lines of treatment while there was no statistically significant difference in patients with ESR1 mutations (68). Hence the current evidence suggests a role for mutations in PIK3CA and ESR1 genes to impact treatment outcomes with endocrine therapy. This should be considered while designing future trials to

evaluate the efficacy of drugs with similar molecular targets.

The PALOMA-2 trial (NCT01740427) evaluated the efficacy of the aromatase inhibitor, letrozole in combination with palbociclib in comparison to monotherapy with letrozole. The study revealed superior efficacy of letrozole in combination with palbociclib (HR for PFS: 0.58,  $P<0.001$ ) as against therapy with letrozole alone (69). In an extensive biomarker analysis of the PALOMA-2 study, subgroups stratified by above and below median expression of multiple genes at baseline was performed. The results revealed significant improvement in PFS in patients treated with letrozole and palbociclib irrespective of the basal mRNA levels of all the analysed genes. Whereas, changes in expression of CDK4 significantly altered the PFS in patients treated with letrozole alone. This substantiated the role of CDK4 expression as a prognostic marker for resistance to endocrine therapy. Similarly high ESR1 expression was associated with better PFS irrespective of treatment groups. But the addition of palbociclib seems to bring benefits to patients with both low and high ESR1 expression. Molecular subtype of breast cancer (luminal A vs. B) was also not associated with differential treatment effect. Extensive analysis with large gene panels revealed only increased expression of fibroblast growth factor receptor 2 (FGFR2) ( $P=0.05$ ), Erb-B2 receptor tyrosine kinase 3 (ERBB3) ( $P=0.07$ ) and programmed cell death protein 1 (PD-1) ( $P=0.024$ ) to be associated with PFS in patients treated with palbociclib and letrozole (70). In a statistically adjusted analysis of the PALOMA-2 trial, the expression of CDK4 was associated with treatment outcome only in patients with visceral metastases ( $P=0.010$ ) (71).

The results of the biomarker analysis from PALOMA-2 and PALOMA-3 studies did not reveal conclusive findings with respect to patients who may derive more benefits from the addition of CDK4/6 inhibitors to endocrine therapy in HR+, HER2- advanced breast cancer. Further exploration of biomarkers which can predict therapeutic effects and with prognostic value in patients treated with endocrine therapy is warranted. Similar to HER2 positive breast cancer, triple therapy with PI3K inhibitors like alpelisib, endocrine therapy and CDK4/6 inhibitors is also being explored in patients with HR +, HER2-, PI3K mutations (72,73).

### **Triple negative breast cancer (TNBC)**

Among the molecular types of breast cancer, TNBC constitutes the most heterogeneous tumor type which is

difficult to be treated because of the difficulties in finding the targeted therapies with current available therapeutic options (74). Unlike patients with HR positive and HER2 positive breast cancer, the molecular mechanism that drives the development of cancerous cell mass has not been identified in TNBC (75,76). Based on genomic expression profiles, TNBC is classified into 4 broad types, namely, basal-like subtype 1, basal-like subtype 2, mesenchymal (M) and luminal androgen receptor (LAR), which also has therapeutic implications (77). Based on gene expression profiling, Lehmann *et al.* reported that 41% of patients with the BL1 subtype experienced pCR while 18% and 29% of patients with BL2 and LAR subtype experienced pCR after treatment with neoadjuvant chemotherapy (77).

Among the different cellular proteins, the tumor protein gene 53 (*TP53*) has been assessed for its prognostic ability in previous studies. In a study by Kim *et al.*, TNBC patients with missense mutation in the *TP53* gene had poor 5-year distant recurrence free survival. In another retrospective study, TNBC patients with detectable TP53 by IHC had higher OS than patients without TP53 expression (78,79). The observational nature of these studies may have introduced confounding biases which needs to be addressed in prospective studies. Although previously considered as a difficult therapeutic target, TP53 has been explored as a drug target in invitro studies with TP53 mutated cell lines which were significantly more sensitive to the investigative drug, PRIMA-1 and PRIMA-1MET (80).

Although numerous other biomarkers have been found to be differentially expressed in patients with TNBC, none of the biomarkers have been established to be associated with therapeutic outcomes. Even cell proliferation related prognostic markers like Ki-67 have been reported to be of limited value in patients with TNBC (74,81). A main reason for the lack of prognostic biomarkers is the absence of well-defined targeted therapies. Among the available therapeutic options, apart from chemotherapy, poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) are a promising option for therapy for patients with TNBC (82). Germline mutation in *BRCA1* gene has been reported to be a predisposing factor for TNBC and olaparib, a PARPi, has been reported to be effective in *BRCA1/2* mutated TNBC patients (83). In the pivotal phase 3 trial (NCT02000622) that evaluated the efficacy and safety of olaparib in comparison to standard of care treatment in patients with germline mutation in *BRCA1/2*, olaparib exhibited superior efficacy. The study was performed in metastatic setting. The overall HR for PFS was 0.58 (95% CI, 0.43 to 0.80) which

was improved to 0.43 (95% CI, 0.29 to 0.63) in TNBC patients as against 0.82 (95% CI, 0.55 to 1.26) in HR positive patients. Similarly, patients with BRCA1 mutation had a significantly better HR when treated with olaparib in comparison to patients BRCA2 mutated patients (HR of 0.54, P<0.05 vs. HR of 0.68, P>0.05) (84). A subsequent sub-group analysis of patients with TNBC and BRCA1 mutation has not been published yet. But the observed improvement in HR in TNBC and BRCA1 mutated patients suggests that BRCA1 mutation could be a potential prognostic biomarker in patients with TNBC treated with PARPi.

A previous registry-based real-world study (PREGNANT registry) from Germany in HER2 negative patients in metastatic setting reported a lack of significant association between BRCA status and prognosis after treatment with chemotherapy in patients with TNBC (85). This suggests that BRCA mutation may not be significant independent prognostic factor for patients with TNBC, but could be used to select for patients who could derive potential benefit from PARPi treatment.

In a recent abstract presented at the annual meeting for European Society for Medical Oncology, the effectiveness of olaparib monotherapy in neoadjuvant treatment of patients with TNBC was reported. As per the study, the ORR in overall patients was 64% (5 complete response and 15 partial response), while in patients with somatic or germline BRCA1/2 mutations, the ORR was 100% (3/5 complete response and 2/5 partial response). All the non-responders were negative for all known mutations in the DNA damage repair genes (*ATRX*, *BRCA1/2*, *EMSY*, *MSH6*, *PARP10*, *PPM1D*) and mutation in TP53 was not associated with response to olaparib (86).

Among other potential targeted therapy regimens in TNBC, the use of immune checkpoint inhibitors, especially programmed cell death 1 (PD-1) and programmed cell death-ligand 1 (PD-L1) seems to be promising. The IMpassion130 phase 3 clinical trial (NCT02425891) evaluated the efficacy of atezolizumab, a PD-L1 inhibitor, with nab-paclitaxel in TNBC patients with metastatic disease. The study reported a statistically non-significant OS advantage for atezolizumab in combination with nab-paclitaxel (HR: 0.86, 95% CI: 0.73 to 1.02). But in the PD-L1 expression positive patients, the OS significantly favoured atezolizumab (HR: 0.71, 95% CI, 0.54 to 0.94) (87). PD-L1 expression which is considered as a surrogate marker for the prediction of PD-1/PD-L1 inhibitor efficacy in other cancer types, could

also be considered in TNBC. The issues pertaining to PD-L1 expression testing like testing platform, desired target cell population (immune cells vs. tumor cells), threshold and antibody clones needs to be resolved for clinical decision making. In the biomarker exploratory analysis of the IMpassion study, PD-L1 expression in the tumor cells and the immune cells were separately evaluated. Patients with PD-L1 positive tumor cells had a relatively better PFS (HR of 0.62 in immune cells vs. 0.51 in tumor cells) and similar OS (HR of 0.62 in immune cells vs. 0.63 in tumor cells) in comparison to immune cell defined PD-L1 positivity. CD8 ≥0.5 (HR for PFS: 0.74, HR for OS: 0.66, P<0.05 for both) and TILs ≥10% (HR for PFS: 0.66, P<0.05; HR for OS: 0.75, P>0.05) were also associated with improvement in PFS and OS (88). But the clinical utility of TILs and CD8 levels require further studies for confirmation.

Atezolizumab has also been evaluated as monotherapy in patients with metastatic TNBC. In a early phase clinical study, treatment with atezolizumab led to stable clinical response and patients with PD-L1 positive expression had higher ORR (12%) and OS (10.1 months). High levels of tumor infiltrating immune cells (>10%) was an independent factor predicting objective response and longer OS (89). The phase II KEYNOTE-086 study evaluated the effectiveness of pembrolizumab in PD-L1 positive patients with metastatic TNBC. The study reported a moderate ORR (21.4%), PFS (2.1 months) and OS (18 months) (90). Despite selecting patients for PD-L1 expression, the lower response rates and OS in metastatic setting suggest a limited role for PD-L1 in predicting therapeutic outcome with PD-1/PD-L1 drugs in metastatic TNBC.

Apart from the above studies, capivasertib, a kinase inhibitor with activity against PI3K/AKT activated tumors was tested in metastatic TNBC patients. There was no statistically significant improvement in PFS and OS in the overall patients. But in the sub-group of patients with PIK3CA/AKT1/PTEN-altered tumors, PFS was significantly better for capivasertib (HR, 0.30; 95% CI, 0.11 to 0.79) (91). Similarly, ipatasertib, an AKT inhibitor, in combination with paclitaxel, also improved PFS in comparison to paclitaxel alone (HR: 0.60, 95% CI, 0.37 to 0.98) (92). Similarly, MK-2206, which is an allosteric AKT inhibitor, has been evaluated in a recent phase-2 study. The study revealed limited clinical activity for MK-2206 in patients with PIK3CA/AKT1 mutation (ORR: 5.6%; 1/18 partial response) and PTEN loss/mutation (ORR: 0%). At present, three different clinical studies are in progress, and it is expected that satisfactory curative effect may be

obtained in biomarker-defined subgroups.

### Biomarker landscape in early detection of recurrence

Early detection of disease relapse before overt recurrence assist in prompt change in therapeutic intervention to extend the disease free survival. It could also improve the ability of biomarkers to predict outcome with subsequent therapeutic regimens. Among the available biomarkers for predicting recurrence, circulating tumor DNA (ctDNA) had been reported to predict recurrence providing sufficient lead time for changing therapeutic regimen. A multicenter prospective study, published in *Clinical Cancer Research* in 2019, monitored the disease recurrence of patients by analysing ctDNA. The study included 49 breast cancer patients who completed surgery and adjuvant therapy, including 34 patients with HR+/HER2-, 8 patients with HR+/HER2+, and 7 patients with TNBC. During the whole study period, 18 patients relapsed and 31 patients remained disease free. The plasma samples were estimated for the presence of ctDNA every 6 months by ultra deep sequencing. A total of 208 plasma samples were obtained, and the results showed that of the 18 patients with recurrence, 16 had positive ctDNA before clinical or imaging diagnosis of recurrence. In HR+/HER2- breast cancer patients, the lead time to report recurrence was 301 days before clinical diagnosis of recurrence. However, HR+/HER2+ breast cancer patients reported that the lead time to detect early recurrence was 164 days and that of TNBC was 258 days (93). The insights provided by this study suggest that ctDNA detection could be incorporated into routine clinical practice for delaying progression in early breast cancer patients. But the practical challenges might include determining the optimum frequency, timing, threshold of ctDNA and sensitivity of the testing platform, for clinical practice. Nevertheless, future studies could provide further insights on the utility of determining ctDNA levels.

Apart from detecting early recurrence, ctDNA could also monitor drug efficacy in real-time. The PALOMA 3 trial that evaluated the efficacy of palbociclib in combination with fulvestrant also reported the probable genetic markers for predicting early recurrence. The study reported higher baseline tumor purity (HR: 1.20), baseline TP53 (HR: 1.84), baseline FGFR1 amplification (HR: 2.91) to be associated with worse PFS (67). In another exploratory study from the PALOMA 3 trial, ctDNA analysis was performed during the

treatment process from day 1 to day 15. The results showed that the median PFS was only 4.1 months in patients with less decline in PIK3CA ctDNA within 15 days, while the median PFS of patients with more change in PIK3CA ctDNA was 11.2 months. One group of patients with low circulating DNA ratio (CDR) (the ratio of ctDNA on the 15<sup>th</sup> day to the 1<sup>st</sup> day) can continue to receive palbociclib treatment, while the other group of patients with higher CDR could be treated with a different therapeutic regimen (94). This study suggests that changes in ctDNA on day 15 can help to identify those patients who may potentially benefit from palbociclib early and change treatment options for those who may not experience favourable treatment outcome after treatment with palbociclib and fulvestrant. Based on the results of this study, a possible future clinical study design can be made to stratify patients by ctDNA level on the 15<sup>th</sup> day.

The difference in genetic mutation landscape within sub-clonal cell populations needs further studies for providing conclusive results. In RCTs, mutation landscape is either assessed in stored tumor samples or in ctDNA both of which may not provide the complete mutation landscape. This could be the reason for outliers in exploratory studies and in observational studies evaluating the role of mutations on the therapeutic outcomes. In future, patients may have to be assessed for mutation periodically from ctDNA for improving the clinical care. But assessment of mutation landscape in metastatic sites and continuous monitoring of tumor samples may not be practically possible.

### Biomarkers landscape in therapeutic outcome prediction

Long-term exposure to therapeutic doses of chemotherapeutic drugs may lead to severe adverse events. With the usage of combination chemotherapy and targeted therapy, de-escalation of chemotherapy could be achieved without reducing therapeutic outcome. Biomarkers could also assist in selecting patients for appropriate de-escalation therapy. In case of HER2 positive breast cancer, dual HER2 targeted therapy in combination with chemotherapy is the standard of care in metastatic breast cancer. The PerELISA study evaluated Ki-67 guided usage of dual HER2 targeted therapy (trastuzumab and pertuzumab) in combination with letrozole and chemotherapy (paclitaxel). The patients (HR positive, HER2 positive) were tested after 2 weeks of letrozole treatment. Patients whose Ki-67 level decreased by more than 20% relative to baseline (molecular remission) continued treatment with trastuzumab

+ pertuzumab + letrozole, while patients without molecular response were treated with paclitaxel (13 weeks) + trastuzumab + pertuzumab for one week. The results showed that 20.5% of patients with molecular remission achieved pCR, while 81.3% of patients with molecular non-response achieved pCR with additional chemotherapy. In addition, PAM50 subtypes were significantly associated with Ki-67 levels and pCR. In patients with molecular remission, the pCR of HER2-enriched breast cancer was significantly higher than that of non-HER2 enriched patients (45.5% vs. 13.8%,  $P=0.042$ ) (95). This study suggests that Ki-67 levels could assist in selecting patients for de-escalation of chemotherapy in patients with HR+, HER2+ breast cancer. However, the study recruited a relatively small number of patients and did not have any comparator. Hence the precise role of the additional chemotherapy regimen is still uncertain.

## Conclusions

In oncology specifically breast cancer, the posture toward biomarker development for innovative therapies is becoming the norm versus exception which will be adopted to this evolving paradigm of precision medicine and personalized therapy. A shift has been brought to the design and conduct of clinical trials in breast cancer by the current era of targeted therapy. Understanding the biomarker landscape, including the clinical research activity, is critical in drug development. This new knowledge will help us classify patients into even smaller subgroups, customize treatment, monitor efficacy, predict therapeutic outcome based on their specific biomarkers, leading to a brand new drug development paradigm.

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