Molecular Profiling of Breast Cancer in Clinical Trials: A Perspective

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12.1 Introduction

Breast cancer is one of the most common malignancies and accounts for more than 30% of cancer diagnosis among women throughout the world [1]. Increased breast cancer incidence rate can be evidenced from the findings that every eighth women in the United States is at risk of developing this brutal disease. Women not only from underdeveloped or developing countries become victim of this disease and struggle for survival, but also women from developed countries are also facing the same issue [2–5]. Breast cancer has heterogeneous nature in histological, pathological, and clinical investigations, and it is always a challenge for surgeons/oncologists to identify suitable treatment for every patient [6, 7]. Conventionally, breast tumors were categorized by using slide-based techniques and histopathological attributes responsible for diagnosing ductal or lobular breast carcinoma and characterizing

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tumor size, grade, and involvement of lymph nodes [8, 9]. With the advancement in molecular biology-related knowledge, different breast cancer molecular subtypes have been recognized based on the status of HR and HER2, which differ in chemotherapeutic responsiveness and disease prognosis [10]. Epithelial carcinoma is the most commonly diagnosed breast cancer type and, therefore, gathers greater attention in this chapter. Basic intrinsic epithelial breast carcinoma types are "luminal A, luminal B, $HER2^{+}$, and basal-like cancer" [11, 12]. Androgen receptor-based epithelial breast carcinoma types have also been reported [13].

Identifying precise molecular breast carcinoma subtypes could lead to more personalized method for breast cancer treatment via targeted therapies [14]. Furthermore, the clinical advantage experienced with agents targeting HER2/hormone signaling has opened new ways to identify and test more molecular targets [15]. Advancement in the molecular profiling-related knowledge has revealed many novel genetic and epigenetic alterations/modifications as possible drivers of breast carcinoma biology [16]. Some of these genetic alterations that can help in characterizing currently available breast cancer molecular subtypes are shown in Fig. 12.1.

After BRCA1/BRCA2, many other genetically targeted agents were explored in breast cancer and now in progress to become clinically important markers. Most important factor in recognizing some molecular marker is its role in treatment and patient's overall survival. To address the potential of various biomarkers, response to treatment was evaluated with the help of clinical trials as a best source of confirmation and many are still in progress. The current chapter will highlight recent advancements in the molecular profiling of breast cancer leading to better disease diagnosis and treatment.

12.2 Molecular Profiling in Breast Cancer

Breast cancer molecular profiling is capable of monitoring and predicting treatment response in different ways [23] and can be determined with different techniques including RT-PCR [24, 25], immunohistochemistry [26, 27], fluorescence in situ hybridization (FISH) [28], DNA hybridization-based analysis [29], and *next-generation sequencing* (*NGS*) [30].

12.2.1 Genomic Tools for Detection of Breast Cancer

Genomics refers to the analysis of sequence and structural variations in DNA. It also involves investigation of gene expression and functional element annotation at a genomic scale. Genomic tools are used to detect indels, single nucleotide polymorphisms, and epigenetic modifications [31]. Genomic analyses lead to the development of diagnostic tests which provided patients personalized diagnostic information [32]. It also helped for the development of personalized treatment plans, consequently preventing resistance, toxicity, and nonresponsiveness. Due to lack of knowledge involved in carcinogenesis, we are still targeting one drug, one gene, and one organ site model [33].

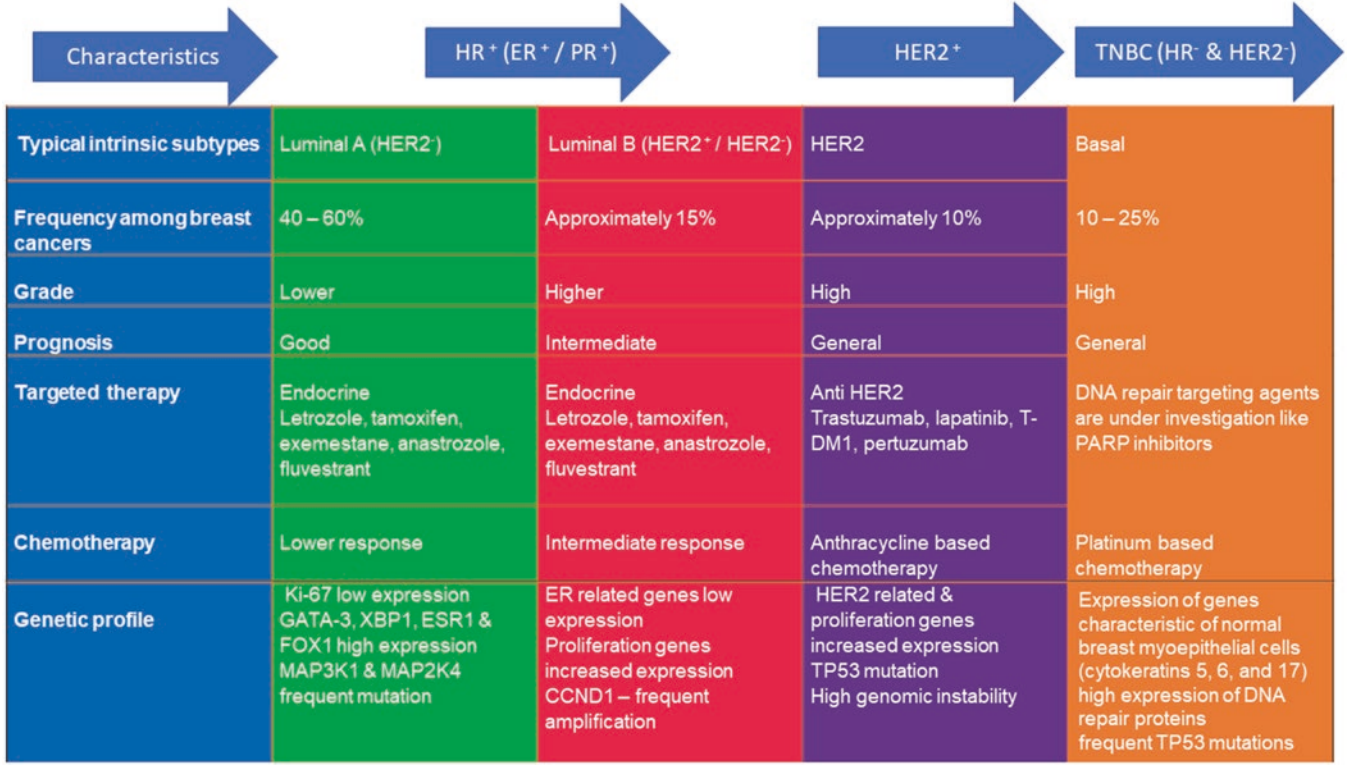

Fig. 12.1 Breast cancer tumor's molecular subtypes [17–22]

12.2.1.1 Oncotype DX

Oncotype DX is RT-PCR-based genomic assay, optimized for FFPE biopsy specimens [34]. The assay was established to predict recurrence score in breast cancer patients of stage I and II, lymph node-negative, hormone receptor-positive, and metastatic cancer, treated with tamoxifen [35]. It utilizes set of important genes customized after data evaluation form 447 patients. During the project, 250 genes were studied, and panel of 21 genes was derived for HR+ breast cancer patients, likely considered to be the prognostic for breast cancer. In this panel, 16 genes are related to cancer and 5 are reference genes as internal control [36]. The cancer-related gene panel is associated with the genes of known functions involved in basic tumorigenesis pathways, i.e., cell proliferation, invasion, hormone response, and other oncogenes. Genes specifically related to breast cancer, incorporated on Oncotype DX, are shown in Fig. 12.2. It stratifies recurrence score between 0 and 100 [37]. Score correlates to disease recurrence possibility among patients successfully treated with chemotherapy within 10 years of diagnosis. The significance of this assay was evaluated and validated by using cohort study from the National Surgical Adjuvant Breast and Bowel Project (NSABP) and trials B-14 and B-20 [38]. Oncotype Dx predicts potential benefit from adjuvant chemotherapy. To date, Oncotype DX is the only multigene assay for breast cancer and incorporated in the guidelines of National Comprehensive Cancer Network (NCCN), highlighting its use and ability to predict the risk of recurrence and benefits from adjuvant chemotherapy [39–41]. According to guidelines, once patients treated with tamoxifen have been classified to lower risk of recurrence by the Oncotype DX assay, they can be spared from adjuvant chemotherapy [42].

Oncotype DX has become the most commonly used clinical assay, but few studies showed that immunohistochemistry (IHC) score provides similar prognostic information which is a less expensive and simpler alternative [36]. Other reports

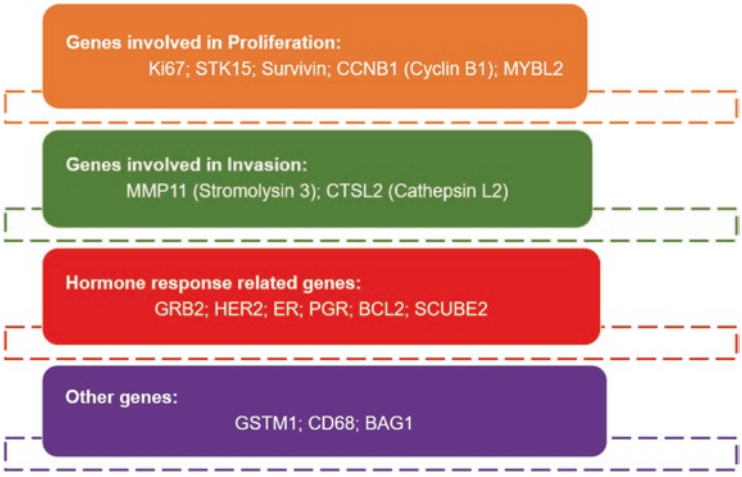

Fig. 12.2 Gene profile of Oncotype DX assay [44–46]

show that Oncotype DX also provides information which can predict benefits from adjuvant chemotherapy [43].

12.2.1.2 MammaPrint

MammaPrint is a molecular diagnostic assay which involves microarray-based approach to predict tumor recurrence in breast cancer patients [44]. It consists of a customized panel of 70 genes that has been displayed sovereign prognostic value for lymph node-negative breast cancer patients and is associated with tumor development and metastasis [47, 48]. These genes are the hallmarks of cancer and play roles in regulation of cell cycle, metastasis, invasion, proliferation, extravasation, adaptation to microenvironment, survival in circulation, and angiogenesis [36]. MammaPrint was initially established from expression arrays of whole genome using a cohort of breast cancer patients who had gone through definitive surgery only, with known clinical outcomes and with no systemic therapy [49].

In MammaPrint gene expression levels are determined by the probe-specific hybridization of complementary DNA [50]. In 2007, US FDA approved MammaPrint for freshly frozen tissue samples. During the process, RNA after extraction from tissues is amplified, co-hybridized is carried out using a standard reference, and 70-gene expression profile is obtained [51]. MammaPrint has been shown as prognostic indicator, independent of clinicopathologic features such as size of tumor, HER2 status, and hormone receptor status [52, 53]. This method has been reported to have significantly higher correlation of prognostic prediction to tumor recurrence [42]. In MammaPrint, patients are classified into low-risk and high-risk groups corresponding to a 10-year distant metastasis-free survival rate.

MammaPrint is a useful diagnostic tool, but there are many limitations that must be considered. The patient recommended for MammaPrint screenings should be of stage I or II lymph node-negative invasive breast cancer with tumor size less than 5 mm^3 [50, 54]. Further, MammaPrint is restricted to patients with less than 65 years of age, and it also needs large amount of specimen. Collection of tissue samples and handling make this assay hard for use in normal clinical practice [55]. Collection is very critical for optimum results and requires regions clear of both stromal and necrotic tissue with at least 30% of malignant cells, which may be impossible to obtain from a biopsy [56]. For these limitations, ASCO required further data and recommendations for usage of MammaPrint in clinical settings. To date, only Agendia laboratory (Amsterdam) performs this assay [57, 58].

12.2.1.3 PAM50 (Prosigna)

PAM50 is also a molecular test for tumor profiling which helps to evaluate chemotherapy benefits in addition to hormone therapy for ER-positive and HER2-negative breast cancers. It investigates the activity of 50 genes to predict the risk of distant recurrence from 5 to 10 years. It is based on qRT-PCR that has been recommended for FFPE tissue specimens of ER-positive, HER2-negative, basal, luminal A- and luminal B-like breast cancers [36]. It estimates the chances of metastasis for postmenopausal women with stage I and II lymph node-negative breast cancers. However, multivariate analyses have revealed this assay also provides information

that is independent of clinicopathologic variables [59]. PAM50 provides the detailed quantitative information about luminal gene expression, proliferation, PGR, ESR1, and ERBB2 and, consequently, can be used for opting proper treatment decisions [36]. Different work is in progress to assess the efficacy of this test and has been reported to be superior to IHC and Oncotype DX for predicting the emergence of late relapses following adjuvant endocrine therapy. Prosigna is manufactured by NanoString Technologies, distributed to different pathology labs and is approved for use in the European Union [60].

12.2.1.4 Genomic Grade Index (GGI) (Ipsogen)

The GGI is a microarray-based test which includes 97 genes, created by Sotiriou et al., with the intention of making tumor grading system more precise. It was developed from the data of 189 breast cancer cohort and validated in different subtypes of 597 tumors [61]. GGI grades tumor into high risk and low risk instead of 1, 2, and 3 grades of histopathology. GGI provides valuable information for estimation of breast cancer prognosis in ER-positive breast cancers and is also shown to help in prediction of relapse in endocrine-treated cancers [62] and prognosis in the patients with neoadjuvant therapy. The FDA has authorized the marketing of GGI to ipsogen JAK2 RGQ PCR Kit, manufactured by QIAGEN GmbH [63].

12.2.1.5 Breast Cancer Index (BCI)

Biotheranostics' Breast Cancer Index (BCI) is a quantitative RT-PCR-based prognostic test. For BCI formalin-fixed and FFPE tissue blocks are used. There are two outputs of this assay, based on unique gene signatures, which include BCI predictive and BCI prognostic. BCI prognostic helps for assessment of patient's individualized risk for distant recurrence, while BCI predictive provides possibility of benefit from extended endocrine therapy, possibly more than 5 years. BCI includes two independent biomarkers, IL17BR:HOXB13 and five cell cycle-associated gene index, which helps to assess tumor grade. The test is limited to patients with ER^+ and lymph node-negative cancer. So far, BCI has not added value information to other available prognostic tests limiting its clinical utility [64].

12.2.1.6 Theros H/I^{SM} and MGI^{SM}

These are transcriptomic-based biomarkers. In Theros H/I^{SM} clinical output of breast cancer individuals is determined who were treated with tamoxifen by evaluating the expression of two genes HOXB13 and IL17BR. If the expression ratio of these mentioned genes is high, then it represents no response to tamoxifen and tumor aggressiveness [65]. MGISM is also a molecular diagnostic test. This test is carried out to check the recurrence risk by using five-gene expression index for ER-positive breast cancer individuals [66]. Thus, more data is required for superiority of Theros H/I^{SM} and MGI^{SM} compared with other conventional methods.

To date, many genomic tests have been developed to improve the diagnosis and therapy of breast cancer. The IMPAKT 2012 group assessed the effectiveness of different available tests, i.e., MammaPrint, Oncotype DX, Genomic Grade Index, PAM50, and EndoPredict. They reported that MammaPrint and Oncotype DX have

considerable validity and significance for both analytical and clinical aspects, in ER^+ breast cancer patients. Unfortunately, no significant association of other tests with prognosis was observed, and further studies are required for their convincing clinical validity [64].

12.3 Immunohistochemistry in Molecular Profiling of Breast Cancer

12.3.1 Significance of Immunohistochemistry as a Diagnostic Tool

Personalized cancer therapy demands use of several biomarkers during histopathological diagnosis [67]. Surgical pathology heavily relies on immunohistochemistry for diagnosing various malignancies. Protein localization and tumor classification can be done by IHC [68], although molecular profiling assists immunohistochemistry (IHC) which is currently performed with the conventional markers for breast cancer prognosis. However, only ER-positive cancer patients get benefit from this information [69]. Immunohistochemistry is used to measure the expression level of predictive markers including estrogen receptor/progesterone receptor (ER/PR) and human epidermal growth factor receptor 2 (HER2) during clinical assessment of tumors [70]. Treatment approaches with antiestrogen or anti-HER2-based therapies are followed for subgroups of patients selected based on these predictive markers. In addition, this approach also aids in analyzing the recurring risk of cancer in such patients [71].

12.3.2 Advancements and Limitations of IHC Techniques

There are several limiting factors due to which conventional methods of IHC are not well acknowledged recently. These include extra labor, time expenditure, expenses, and the large amount of sample tissue required for the procedure. This can be explained by example of Oncotype Dx test used for identification and prognosis of breast cancer. It demands much time and labor as more than 20 genes need to be examined for their role in breast cancer [72, 73]. Although these issues are assumed to be resolved by using an automated IHC machine, expenditures of both money and time still remain major issues while dealing with a large number of biomarkers and tissue sample, respectively. Additionally, limitations like variations in results, qualitative evaluation, and subjective decision make this technique a less reputable proteomic tool [72].

12.3.2.1 Multicolored-Based Immunohistochemistry

In recent investigations, multiplexing method with molecular dyes and quantum dots (QDs) is used for multicolored-based IHC assays [74, 75]. Multicolor IHC has advantage that it facilitates co-expression of several biomarkers with both direct and

indirect sequential staining. However, several drawbacks are associated with multicolor staining [76]. These include increased labor and time expenditure, higher reagent costs, and sensitive procedure of probe conjugation using less stable primary antibodies and non-specific binding of secondary probes. These undesired factors lessen the effectiveness of multicolored immunohistochemistry [77].

12.3.2.2 Microfluidic-Based Multiplexed Immunohistochemistry (MMIHC)

Integration of IHC-based assays with an appropriate multiplexing method can prove an efficient diagnostic method for cancer patients [78]. Immunohistochemistry has been further modified with microfluidic parallel multiplexed design for diagnosing breast cancer quantitatively. This methodology provides an enclosed microenvironment in which fluids can be easily and timely manipulated [79]. Development of MMIHC platform demonstrates the enhanced IHC performance with accurate diagnosis, time, and cost-effectiveness as compared to previous methods which employ analysis of whole sections of breast cancer tissues [80]. Usually microfluidic devices are designed in such a way that glass slide and microchannel are permanently bonded together, and introduction of an interface between a microfluidic device and tissue slide has not been commonly reported by previous studies. Thus, it can be assumed that use of microfluidic design is not frequently practiced in studies with human clinical specimens [80–82].

Structural Design of Microfluidic Devices

Kim et al. had designed a microfluidic device by taking into consideration of solution number, biomarker count, and adequate reaction channel dimensions. Four biomarkers were used including estrogen (ER), Ki-67, progesterone (PR), and human epidermal growth factor 2 (HER2) receptors. The device contained six and four reservoirs for reagents and biomarkers, respectively. In addition, microvalves for both reagent and biomarker reservoirs, four reaction channels, and one outlet were included in the design. Lastly, to maintain constant pressure and creating a temporary seal, a weight was put on the top of the device [80, 83].

Preparation and Assembly of MMIHC Assay

The procedure employed for the preparation of MMIHC device involved two-step soft lithography, poly(dimethylsiloxane) (PDMS; Sylgard 184; Dow Corning, MA) replica molding and aligning processes. To minimize tissue damage, an appropriate interface between MMIHC device and tissue slide was prepared. To assemble, bottom plate of device was loaded with tissue slide. Afterward, tissue was treated with washing buffer, and four reaction channels containing MMIHC device were placed on it. Buffer was filled in microchannels carefully to avoid creation of micro-bubbles. Lastly, upper plate of the device was loaded with a weight so any leakage could be avoided, and tissues would be pressed with walls of microchannels [84, 85].

12.3.2.3 Analysis of Human Breast Cancer Tissue with MMIHC

After initial testing and trials of MMIHC device, Kim et al. used this platform for examination of tumor tissues of patients. This modified technique minimizes need of additional externally connected equipment. A major advantage of using MMIHC platform is that probability of assay failure is reduced under 1%, which is frequently observed in case of clinically rare samples. Immunohistochemical staining can be easily repeated in this setup due to an enclosed microenvironment and semi-automation of the staining process, and antibody consumption is reduced up to 200-fold along with speedy immunological reaction. Additionally, comparison of MMIHC results with those of western blotting revealed that this technique can give better results for semiquantitative analysis of cell blocks. Its effectiveness is exhibited by the fact that more accurate results are obtained during relative quantification due to single site biomarker staining which enables direct comparison and eliminates undesired variation as observed in multistep conventional IHC [80].

Quantification with image analyses needs further advancements and improvements in algorithms for clear scoring. Although MMIHC was considered more advantageous than earlier techniques, reliability of its results was doubted when compared to conventional whole tissue analysis [85]. These concerns are primarily based on scoring discrepancy probably caused by inborn errors of IHC due to variation in laboratory conditions or observer's skills. Other reasons include selection of specimens, processing errors, representation methods of MMIHC results, etc. In conclusion, after required modifications, a more applicable, fast, and easy to quantify MMIHC platform can improve the patient care conditions by facilitating clinical diagnosis of breast cancer [85, 86].

12.4 High-Throughput Sequencing (NGS) Technologies

Human genome consists around 3 billion nucleotides and 22,000 genes comprising on 23 chromosomes. Conventional methods took 10–12 weeks for genetic testing of known genes involved in breast cancer. This turnaround time, along with cost and area of genome studied, improved with the advent of new technologies, i.e., next-generation sequencing [87]. It has also helped to achieve new treatment avenues and make patient's lives better. Next-generation sequencing (NGS) has played very important role for investigations in such a heterogeneous and complex disease like breast cancer [88]. Firstly, it helped to characterize genome and exome of cancer patients. Along with unraveling the mutational processes, large-scale studies have discovered new genes associated with the disease. Advanced tools allow deep investigations of whole genome data and its correlation with disease stage, prognosis, and treatment options [89, 90].

12.4.1 Identification of New Genes

NGS has led to the discovery of new "driver" and "passenger" mutations. It all particularly was at the highest peak in 2012, with exceptional unraveling of mutational landscape. Some of the mutations newly identified in 2012 are shown in Table 12.1.

12.4.2 Delineating the Mutational Steps in Cancer

Many studies illustrated the mutational process underlying the cause and propagation of breast cancer, out of which the study published by Nik-Zainal [95] was the most appealing one of that time. According to this most important driver mutation in case of breast cancer patients occur in genes like TP53, *GATA3*, *PIK3CA*, *MAP2K4*, *SMAD4*, *MLL2*, *MLL3*, etc., duration and strength of each mutation determine the mutational process or pathway to the disease.

12.4.3 Detecting Minimal Residual Disease (MRD)

Generally, circulating tumor cell in blood and bone marrow has impact in development of breast cancer [96]. Nested real-time PCR has been used to detect tumor DNA in serum of relapsed breast cancer patients and to detect MRD. Early diagnosis can also be made by detecting serum DNA using NGS [97].

12.4.4 Drug Response Prediction

Various prognostic markers have been recognized which can not only identify patients with better or worse outcome of disease but can also predict response of patients to a certain treatment. It can not only reduce cost but save time as well. The most important markers studied till today in case of breast cancer are ER and HER2, having both prognostic and predictive roles. Oncotype Dx or recurrence score is used to estimate the expression level of 21 genes for stratifying ER breast cancer

Table 12.1 Mutated genes identified through next-generation sequencing (NGS)

S. No	Study	Mutated genes
1	Stephens et al. [91]	AKT2, TBX3, ARID1B, CDKN1B, NCOR1, MAP3K1, MAP3K13, SMARCD1, CASP8
2	Banerji et al. [92]	RUNX1, CBFB
3	Shah et al. [93]	USH2A, COL6A3, MYO3A, NRC31, PRKCE, PRKCQ, PRKG1, PRPS2, PRKCZ
4	Cancer Genome Atlas [94]	AFF2, OR6A2, PIK3R1, PTPRD, NF1, RPGR, SF3B1, CCND3, CTCF, TBL1XR1, NCOR1, ZFP36L1, GPS2, CLEC19A, RYR2, HIST1H2BC, GPR32, SEPT13, PTPN22, DCAF4L2, OR6A2

into high- and low-risk groups, using microarray analysis [98]. The use of whole genome sequencing techniques has given the insight into intra-tumor heterogeneity. Firstly, it showed the different subtypes of tumor with changed rearrangement patterns and mutations; secondly, metastasis is altered in case of primary tumors. Thirdly, it has been proven that tumor can progress using distinct pathways.

12.5 Biomarkers in Randomized Clinical Trials (RCT)

Biomarkers are naturally occurring molecules, characteristics, or genes used to perform a clinical assessment (prediction, identification, and monitoring the health states of individuals) and planning new therapeutics. In clinical trials of different tumor types, the relationship between drug response to presence, absence, or any kind of change in biomarker was tested. This consists of proof-of-concept trials, which include integral and integrated biomarkers. In integral biomarkers trials, patients with presence or absence of specific biomarkers were included only, while in integrated biomarkers trials, biomarkers effect mainly on drug response was tested [99]. Main goal of biomarkers incorporation into clinical trials was specific selection of patients who were expected to be benefitted from some specific therapies and to give more inclusive sight of how novel therapies function. But, incorporation of biomarkers into clinical trials is still challenging, because there is a need for considering some assays which can act as standards in different countries and clinical practices. A study of phase Ib/randomized phase II trial (double-blind clinical trial of tamoxifen plus taselisib or placebo) for HR+ metastatic breast cancer patients found that clinical outcomes can be improved by combining PI3K-AKT-mTOR pathway inhibitors with prior endocrine therapy. Taselisib is PI3K inhibitor having higher selectivity for mutant (MUT) PI3Kα isoforms than wild type. POSEIDON phase Ib data with tamoxifen (TAM) plus taselisib revealed greater performance in metastatic Ca breast individuals with an acceptable toxicity profile. Patients were grouped based on histology, menopausal status, no prior chemotherapy history, and treatment centers [100]. First randomized double-blind controlled clinical trial MANTICORE (Multidisciplinary Approach to Novel Therapies in Cardiology Oncology Research) was carried out on 100 early breast cancer patients at 2 centers. It was carried out in HER2+ early breast cancer (EBC) patients for evaluation of heart failure pharmacotherapy in the prevention of adjuvant trastuzumab-mediated left ventricular (LV) dysfunction. Adjuvant trastuzumab (TRZ) is mostly done for HER2+ overexpressing EBC patients with survival rates of 5 years. However, it has fivefold increased clinical heart failure rate. For prevention of such negative sequelae, LV remodeling is recognized as an early indicator of heart diseases. One of the methods used for quantifying LV remodeling and function is cardiac magnetic resonance imaging (CMR). So, MANTICORE trial was designed for evaluation of heart failure pharmacotherapy in the prevention of adjuvant trastuzumab-mediated left ventricular (LV) dysfunction. Patients were randomized to receive perindopril, bisoprolol, or placebo prior to initiating TRZ. So,

this study has the potential to implement change in clinical practices with TRZ-based adjuvant therapy [101].

Programmed cell death-1 receptor and its ligands (PD-L1) are considered as therapeutic targets in reactivation of immune responses against cancer. Avelumab, an anti-PD-L1 antibody in clinical trials of metastatic breast cancer or locally advanced cancer, is being investigated (a phase Ib JAVELIN solid tumor trial). Immunohistochemistry was used to assess tumor PD-L1 with various cutoff criteria. Total 168 metastatic patients with HER2+, HER2−/ER+ or PR+, triple negative (TNBC = HER2−/ER−/PR−), or unknown biomarker were treated with avelumab. It showed a significant safety profile and had clinical activity in a subgroup of metastatic breast cancer patients. In patients with triple negative breast cancer, clinical response to avelumab is associated with the presence of PD-L1-expressing immune cells within tumor cells [102].

A single-arm clinical trial (phase II) with only one agent platinum was conducted on TNB patients along with correlated biomarkers. In case of metastatic TNBC, with germline BRCA1/BRCA2 mutations, platinum is used as active chemotherapeutic agent. Patients can be identified who could benefit from platinum therapy based on measurement of tumor DNA repair functions. Well-designed potential controlled trials that use diagnostically certified assays and predefined criteria are warranted to assess the clinical utility of DNA repair measurement for analyzing responsiveness to DNA-damaging agents and platinum [103]. These enrichment biomarkers, presently in clinical trials, may become predictive biomarker in the future after being clinically proven. Some examples are *RAS* mutations for both MAPK and PI3K pathway inhibitors, IGF mutations with IGF-1R antibodies and PTEN loss, and *PIK3CA* mutations for PI3K-Akt-mTOR pathway inhibitors. Various biomarker panels have been developed, like TruSeq Amplicon—Cancer Panel (TSACP) to assist identification of significant breast cancer-associated biomarkers for research and for clinical practices [104] (Table 12.2).

12.6 Conclusion

Advancement in molecular profiling of breast tumor types has showed differential molecular features that affect responsiveness, prognosis, and resistance to therapy. In this new era, importance of molecular profiling for breast cancer diagnosis and treatment can be evidenced with the emergence of vast variety of techniques and assays in clinical practice. These technologies have proven to solve various diagnostic issues, increased the information available from clinical trials, and paved toward personalized medicine overcoming the challenges of traditional techniques. Research is still in progress via clinical trials incorporating biomarkers to secure maximum benefits for breast cancer patients.

Table 12.2 Completed and ongoing biomarker-driven clinical trials of breast cancer (mentioned in text)

Trial name/ID	Agents	Phase	Patient population	Status
NCT02530424	Palbociclib, fulvestrant, trastuzumab, and pertuzumab expression of Ki67	Phase II	Triple targeting of ER, HER2, and RB1 in HER2- and ER-positive Ca breast, *n* = 36 patients	Ongoing
NCT02032277	Veliparib plus carboplatin or carboplatin PARP inhibitor, neoadjuvant chemotherapy	Phase III	*n* = 634 patients triple-negative breast cancer, clinical stage II–III	Ongoing
NCT02162719 LOTUS	Ipatasertib plus paclitaxel versus placebo plus paclitaxel, PI3K/AKT pathway inhibitor	Phase II	Metastatic triple-negative breast cancer, *n* = 166 patients	Ongoing
NSABP B-42 Double-blinded, randomized trial	Placebo-controlled trial of extended adjuvant endocrine therapy (tx) with letrozole (*L*) (aromatase inhibitor (AI))	Phase II	Stage I–III, postmenopausal, and hormone receptor (+) Ca breast, *n* = 3966	Completed
Nanoparticle albumin-bound (nab) paclitaxel	ab-paclitaxel followed by FEC (5-FU [fluorouracil], epirubicin, and cyclophosphamide)	Phase II	HER2-negative breast cancer *n* = 25 with no previous chemotherapy	Completed
NCT01889238 MDV3100 open label trial	Enzalutamide	Phase II	Androgen receptor-positive TNBC	Not recruiting anymore
NCT01990209	Orteronel	Phase II	Androgen receptor positive with metastatic breast cancer; *n* = 86	Ongoing
NCT01528345	Dovitinib, dovitinib placebo and fulvestrant	Phase II	Her− and HR+ metastatic postmenopausal individuals having progression after endocrine therapy, *n* = 97	Completed
NCT01791985	AZD4547 activity with either anastrozole or letrozole or both	Phase I/II	ER+ breast cancer patients with disease progression by letrozole and anastrozole, *n* = 56	Ongoing
NCT02437318 Double-blind randomized trial	Placebo controlled study of faslodex and alpelisib in combination	Phase III	HER2−, hormone receptor+, postmenopausal females and men with disease progression after aromatase inhibitor therapy, *n* = 572	Ongoing

(continued)

Table 12.2 (continued)

Trial name/ID	Agents	Phase	Patient population	Status
NCT00773695 Multicenter randomized study	Avastin activity was evaluated in combination with neoadj therapy. (antiangiogenic therapy done in this trial)	Phase II	Effect of this treatment was evaluated in primary tumors of HER2− Ca breast patients, $n = 150$	Completed
NCT01965522	Anti-proliferative effects of vitamin D3 (2000 IU daily) or placebo, and to melatonin (20 mg/day) or placebo in breast cancer (MELO-D) as measured by Ki67	Phase II	144 women with histologically confirmed invasive breast cancer (ductal, lobular, or mixed) Change in microRNA blood serum was studied	Completed
NCT01612871	Anastrozole, tamoxifen, exemestane and letrozole (hormonal therapy)	Phase IV	Metastatic Ca breast $n = 39$, specific circulating microRNAs were detected before and after given treatment	Completed
NCT02656589	Capecitabine, trastuzumab drugs given to patients, microRNAs expression was analyzed		300 participants, microRNA of HER2+ individuals treated with Herceptin having stage IV	Ongoing
NCT01907529	Neoadjuvant docetaxel, epirubicin, cyclophosphamide, and human recombinant endostatin (endostar)	Phase II/III	300 participants with histologically confirmed invasive breast cancer having stage III breast cancer and belonging to age group 18–70. This trial is designed on hypothesis that active angiogenesis agent combined to chemotherapy could enhance the pathological response rate	Completed

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