

REVIEW

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Emerging therapies for breast cancer

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Abstract

HER2 and CDK4/6 are undoubted two most important biological targets for breast cancer. Anti-HER2 treatments enhance objective response and progression-free survival/disease-free survival as well as overall survival. Three CDK4/6 inhibitors consistently improve objective response and progression-free survival; however, overall survival data are waited. Optimization of chemotherapy and endocrine strategies remains an unmet need. Check point inhibitor-based immunotherapy combined with chemotherapy is a promising field, especially for triple-negative breast cancer.

Keywords: Breast cancer, CDK4/6 inhibitors, SERD, PD1 and PD-L1 antibodies, PARP inhibitors, Fulvestrant, ADC, Nab-paclitaxel

Background

The median overall survival of metastatic breast cancer (MBC) is about 2 to 3 years. Although it is still an incurable disease for more than 90% of MBC patients, much progress has been made in the past decade. The longest reported median overall survival was 56.5 months for HER2-positive MBC patients with first-line treatment of docetaxel, trastuzumab, and pertuzumab, while it was 54.1 months for luminal subtype MBC patients with first-line fulvestrant treatment. This review will focus on drug developments in metastatic setting.

CDK4/6 inhibitors

Estrogens [1] and antiestrogens [2] act on sensitive populations of cells in early to mid-G1 phase of ER-positive breast cancer cell lines. CDK4 and CDK6 are activated by binding to D-type cyclins and act early in G1 phase [3–6]. CDK in G1 phase mainly targets the retinoblastoma susceptibility gene product (pRb), which mediates G1 arrest through sequestration of transcriptional factors of the E2F-DP family and then transcription of requisite genes for S-phase entry [6, 7].

Inhibitors of CDK4 and CDK6 entering clinical trials include palbociclib, ribociclib, and abemaciclib [8]. Palbociclib blocks ATP binding to the CDK4/6 enzymes with half-maximal inhibitory concentration (IC50)

0.01 $\mu\text{mol/L}$ for CDK4/cyclin D1, 0.009 $\mu\text{mol/L}$ for CDK4/cyclin D3, and 0.015 $\mu\text{mol/L}$ for CDK6/cyclin D2 complexes [9]. The combination of palbociclib with endocrine therapy has synergistic effects in ER+ human breast cancer cell lines [10] as well as xenograft models, and in tamoxifen-resistant breast tumor, the synergy is also present between palbociclib and selective estrogen receptor downregulator [11]. Therefore, endocrine therapies and inhibitors targeting CDK4/6 activity are the core treatment modality in patients with HR+ advanced breast cancer [12].

Besides their antiproliferative activity, palbociclib has shown strong antimetastatic activity in a dose-dependent manner through reducing cyclooxygenase-II expression in MDA-MB-231 (ER α -) and T47D (ER α +) breast cancer cells [13]. Cyclooxygenase-II gene is associated with the activation of epithelial-to-mesenchymal transition process, which helps the epithelial cells to lose their epithelial characteristics and gains mesenchymal characteristics, therefore increasing their invasive and metastatic potentials [14]. Additionally, palbociclib-induced CDK4/6 inhibition can lead to senescence of melanoma cell lines via promoting Forkhead Box M1 degradation [15]. Moreover, cyclin D1 is one of the ER transcriptional targets, thus rationalizing the use of CDK4/6 inhibitors in ER α + breast cancer [16].

Predictive factors for CDK4/6 inhibitors may include cyclin D1 and phosphorylated Rb for sensitivity [8, 17] and p16 for resistance. The cyclin D-CDK4/6-INK4-Rb pathway is frequently deregulated in breast cancer via *CCND1* (cyclin D1) amplification (29–58%), *CDK4* (14–25%) and

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CDK6 amplification [18], p16 loss (49%) [19], and *TP53* inactivation (12–84%) [18]. Cyclin D1 overexpression and Rb phosphorylation in ER α + cancers contribute to the drug resistance to the hormonal therapy [20–24]. Abemaciclib effectively induces the G1 cell cycle arrest, which is dependent upon the presence of Rb. p16 overexpression in Rb-deficient breast cancer cells might account for the resistance to palbociclib, as CDK4/6 enzymes might be already inhibited by the overexpressed p16 [25]. Moreover, ER α + subtype shows the highest sensitivity to CDK inhibitors, possibly due to the hyperactivation of CDK4/6, while palbociclib showed no antiproliferative effect in Rb-deficient MDA-MB-468 (ER α -) human breast cancer cell lines [8, 26–28]. However, the value of any of these biomarkers was not confirmed in translational studies of clinical trials.

Palbociclib

Phase I studies using single-agent palbociclib 2/1 (2-week on and 1-week off) schedule [29] and 3/1 (3-week on and 1-week off) schedule [30] were done to identify the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the first-in-class, oral CDK4/6 inhibitor in Caucasian and Japanese patients [31]. The MTD of 3/1 schedule was 125 mg once daily and recommended for further development. Palbociclib was well tolerated, and neutropenia was the only significant DLT.

Phase II study of palbociclib used a single agent in advanced breast cancer [32]. Eligible patients had Rb-positive MBC. Of the 37 enrolled patients, 33 patients were HR+ (7% ER α +, 4% PR+, and 22% ER α +/PR+). Clinical benefit rate was 21% for patients with HR+ and 29% for patients with HR+/HER2- who were exposed to at least two prior lines of hormonal therapy. Progression-free survival (PFS) was significantly longer for patients with HR+ rather than HR- ($p = 0.03$). Most adverse events were myelosuppression. Neutropenia (grade 3/4) was common with 46% requiring dose modifications.

The first combination trial is a randomized, multicenter active-controlled phase I/II study (PALOMA-1) designed to assess the efficacy, safety, and pharmacokinetics of letrozole 2.5 mg QD (continuously) in combination with palbociclib 125 mg QD (schedule 3/1) vs single-agent letrozole 2.5 mg QD (continuously) for the first-line treatment of ER(+) and HER2 (-) ABC in postmenopausal women. Phase I results showed no drug–drug interactions between letrozole and palbociclib. The recommended phase II dose is at 125 mg once daily on 3/1 schedule [33]. The phase II portion consisted of two cohorts. In cohort 1, patient selection was based only on ER/HER2 status. In cohort 2, tumor CCND1 amplification and/or p16 loss were eligibility criteria. The final analysis reported results from both cohorts combined (cohort 1, $n = 66$; cohort 2,

$n = 99$). At data cutoff for final analysis (November 29, 2013), PFS was significantly improved with palbociclib (20.2 vs 10.2 months), leading to its conditional FDA approval in February 2015 [34]. PALOMA-2 is a confirmative phase III trial. The study met its primary endpoint [35].

In the second- or later-line setting, the value of palbociclib was assessed in the prospective, multicenter, double-blind phase III PALOMA-3 study in pre/perimenopausal women with hormone receptor-positive/HER2-negative MBC after progression on endocrine therapy. Patients were randomized to receive fulvestrant with either palbociclib or placebo. Palbociclib was associated with longer progression-free survival (PFS; 11.2 vs 4.6 months, HR = 0.497, $p < 0.0001$) [36]. Its efficacy is independent on PIK3CA mutation status. On February 19, 2016, the US Food and Drug Administration (FDA) approved a new indication for palbociclib based on this result [37]. Overall, findings across the studies suggest palbociclib has synergy with endocrine therapy in both endocrine-naive and endocrine-resistant settings.

Ongoing clinical trials include the HR+/HER2+ study (PATINA) exploring in the HER2+ first-line MBC setting (NCT02947685), adjuvant study PALLAS and PENELOPE-B (NCT01864746, NCT02513394), and PALOMA-4, PEARL, PARSIFAL and NCIC MA-38 (PALESTRA) study (NCT02630693, NCT02491983, NCT02028507, and NCT02297438, respectively) in the MBC setting. Future research directions include deep diving of translational markers, mechanisms, and treatment strategies after endocrine or CDK4/6 inhibitor resistance, Asia population.

Ribociclib

Ribociclib (LEE011) is an orally bioavailable, highly selective CDK4/6 inhibitor which is also in later stages of clinical development. In preclinical studies, ribociclib caused inhibition of tumor growth and cell cycle arrest in several Rb-proficient cell lines and in a dose-dependent manner [38]. The antitumor activity is confirmed in a variety of xenograft tumor models, including *PIK3CA*-mutant breast cancer, *NRAS*- and *BRAF*-mutant melanoma, and neuroblastoma [39, 40].

MONALEESA-1 (NCT01919229) [41] is a phase II study to assess the biological activity of 14 days of neoadjuvant treatment with ribociclib (400 or 600 mg, daily) plus letrozole (2.5 mg, daily), compared with single-agent letrozole (2.5 mg, daily) in postmenopausal patients with newly diagnosed, resectable, HR+, HER2-early breast cancer. The results suggested absence of a drug–drug interaction between ribociclib and letrozole and showed that ribociclib plus letrozole significantly reduced Ki-67 expression in HR+, HER2- breast cancer. MONALEESA-2 (NCT01958021) [42] is a phase III study

to evaluate ribociclib as first-line therapy for HR+/HER2- advanced breast cancer. The study group is ribociclib (600 mg/day, 3-week on/1-week off) plus letrozole, compared to single-agent letrozole. Primary endpoint was investigator-assessed progression-free survival. The duration of progression-free survival was significantly longer in the ribociclib group than in the placebo group (hazard ratio, 0.56; 95% CI, 0.43 to 0.72; $p = 3.29 \times 10^{-6}$ for superiority). Ribociclib received FDA breakthrough therapy designation in combination with letrozole on August 3, 2016, and finally approved on March 13, 2017.

In addition, there are two ongoing phase III trials: MONALEESA-3 (NCT02422615) and MONALEESA-7 (NCT02278120).

MONALEESA-3 is a randomized double-blind, placebo-controlled study of ribociclib in combination with fulvestrant for the treatment of postmenopausal women with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer who have received no or only one line of prior endocrine treatment. The phase III MONALEESA-7 study is investigating the combination of ribociclib with goserelin and tamoxifen or nonsteroidal aromatase inhibitor (NSAI) in premenopausal women with HR+/HER2- advanced breast cancer. Patients will be randomly assigned in a 1:1 ratio to two treatment arms: ribociclib or placebo plus goserelin and tamoxifen/NSAI. Furthermore, clinical trials of ribociclib in combination with targeted therapies and/or hormonal therapies are ongoing as well. A phase II trial was conducted based on the preliminary results from a phase I study of ribociclib combined with exemestane and everolimus showing improved efficacy and manageable toxicity [43].

Abemaciclib

Findings from the phase I study I3Y-MC-JPBA (JPBA) indicate that the abemaciclib single-agent MTD of 200 mg administered orally every 12 h (Q12H) demonstrates an acceptable safety profile. Abemaciclib has demonstrated evidence of clinical activity in women with MBC at doses of both single-agent 150 and 200 mg Q12H, and the range of steady-state exposures is comparable for the two doses. In this study, among 36 patients with HR+ MBC receiving abemaciclib, the median PFS was 8.8 months and there were 12 confirmed partial responses (PRs), for an objective response rate of 33.3%. In the same study, the combination of abemaciclib plus fulvestrant was also evaluated and demonstrated an acceptable safety profile in 19 women with four confirmed PRs observed [44]. Safety and tolerability of abemaciclib in combination with endocrine therapies (including anastrozole and letrozole) are being further evaluated in patients with HR+, human epidermal growth factor

receptor 2-negative (HER2-) MBC, in the ongoing phase Ib study I3Y-MC-JPBH (JPBH) [45].

Phase II study (MONARCH-1) has investigated in 132 HR+ metastatic breast cancer patients, further confirming that single-agent abemaciclib at a dose level of 200 mg Q12H is clinically active. In MONARCH-1 study, 19.7% of the overall population has achieved an overall response rate (ORR). The median PFS was 6 months, and the median overall survival (OS) was 17.7 months. 90.2% of the patients had grade 1–3 diarrhea with 70.5% in grade 1 or 2. The decrease in white blood cell had been observed in 90.8% patients, 63.1% of which in grade 1 or 2 [46].

Phase II neoadjuvant study (neoMONARCH) comparing the biological effects of abemaciclib plus anastrozole vs abemaciclib monotherapy vs anastrozole monotherapy in women with early-stage HR+, HER2- BC. Patients (pts) were stratified by progesterone receptor status and tumor size and randomized 1:1:1. The results indicated that abemaciclib, along or in combination with anastrozole, significantly reduced Ki-67 expression compared to anastrozole along after 2 weeks of treatment based on geometric mean change (63.2 vs 92.6 vs 90.6%) and complete cell cycle arrest (14.8 vs 66.1 vs 58.8%). The majority of patients experienced an objective response [47].

Ongoing clinical trials include two randomized, double-blind, placebo-controlled, phase III studies, I3Y-MC-JPBM (MONARCH-3) and I3Y-MC-JPBL (MONARCH-2), to further confirm the safety and efficacy of abemaciclib in combination with current standard endocrine therapies (either NSAIs or fulvestrant) in HR+, HER2- breast cancer. Future directions also include using the CDK4 and CDK6 inhibitors in the adjuvant and neoadjuvant therapy settings. A neoadjuvant trial investigating the combination of abemaciclib and aromatase inhibitor in locally advanced ER-positive, HER2-negative breast cancer (neoMONARCH, NCT02441946) is ongoing [48]. More exciting, abemaciclib distributes efficiently to the brain in nonclinical species, potentially providing a unique opportunity to treat primary brain tumors as well as cancers that have metastasized to the brain (Lilly internal data).

PD1 and PD-L1 antibodies

In the past several years, immunotherapy has been established as a new standard of care, with remarkable activity and curative potential in patients with a broad range of tumor types. Antibodies to cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death-1 (PD-1), and programmed death-ligand 1 (PD-L1), all of which increase the immune response against the tumor by blocking immune-regulating proteins that downregulate the immune system, have increased response rates and OS in

melanoma, non-small cell lung cancer, renal cell carcinoma, Hodgkin lymphoma, urothelial carcinoma, and squamous cell carcinoma of the head and neck [49–55].

The role of immunotherapy in breast cancer has yet to be defined, but increasing evidence points to triple-negative breast cancer (TNBC) as possibly having unique characteristics that may make them more responsive to checkpoint inhibition. The higher genomic instability and mutational burden of TNBC result in a higher propensity to generate neoantigens, which can be recognized as “nonself” by the adaptive immune system [56].

TNBC have a higher amount of tumor-infiltrating lymphocytes (TILs) [57] and higher PD-L1 protein [58, 59] or messenger RNA (mRNA) [60, 61] expression compared with other breast cancer subtypes. Higher levels of TILs generally are associated with poor-prognostic clinicopathologic features, including ER negativity, higher grade, higher proliferative rate, and lymph node positivity [57, 62–65]. However, despite worse clinical features, higher levels of TILs are associated with improved DFS and OS, independent of systemic therapy [65–67]. An association between greater tumor infiltrative lymphocytes (TILs) and better prognosis in breast cancer has been recognized for some time; newer studies have shown the specific relevance in TNBC, which has been shown to have substantial infiltration with TILs [57, 68–70]. This apparent paradox highlights the role that the immune system may play in a subset of TNBC and suggests that TILs may be a surrogate for an adaptive immune response in these cancers.

PD-L1 expression is significantly associated with the presence of TILs [58–60], correlates with higher histologic grade, greater tumor size, and higher expression of the proliferation marker Ki-67 [71]. Data from The Cancer Genome Atlas (TCGA) have confirmed higher PD-L1 mRNA expression in TNBC vs non-TNBC samples [60]. This and other studies have shown that PD-L1 is not detected in normal breast tissue but is expressed in about half of all breast cancers, including approximately 20 to 30% of TNBCs [72, 73], which suggests that the most common mechanism of regulation of PD-L1 expression in TNBC is regulatory feedback (acquired resistance) to immune engagement. In addition, the loss of PTEN expression in TNBCs is associated with PD-L1 overexpression [60], confirming an association between increased PI3K signaling and the presence of PD-L1 [74]. These findings suggest that, in addition to acquired resistance mechanisms, PD-L1 expression can also be regulated by molecular alterations and oncogenic pathways (intrinsic resistance), linking molecular and immune heterogeneity.

More recently, analysis of gene expression profiles of 587 TNBC samples identified six distinct subtypes, including an immunomodulatory (IM) subtype characterized by

high expression of immune-related genes. This subtype is rich of immune-activated and associated signaling components contributed from both the tumor and the infiltrating lymphocytes, and it has been associated with improved relapse-free survival compared with other subtypes [75]. RNA sequencing also showed this subtype to have substantially higher expression of PD-L1, PD-1, and CTLA-4. These and other data provide evidence that IM subset is mostly likely to benefit from checkpoint inhibition.

Pembrolizumab

Pembrolizumab is a high-affinity, highly selective, humanized monoclonal IgG4 antibody against PD-1 that prevents PD-1 from binding to its ligands, PD-L1 and PD-L2. Pembrolizumab is approved in several countries for the treatment of advanced melanoma [76], non-small cell lung cancer in certain situations [77], and as a second-line treatment for head and neck squamous cell carcinoma [78]. Additionally, clinical studies with pembrolizumab have demonstrated promising efficacy with durable responses and a manageable safety profile in many advanced malignancies, including gastric cancer [79] and urothelial cancer [80].

KEYNOTE-012 (NCT01848834) was a multicenter, non-randomized phase Ib trial of single-agent pembrolizumab given at 10 mg/kg every 2 weeks to patients with advanced PD-L1-positive (expression in stroma or $\geq 1\%$ of tumor cells by immunohistochemistry) malignancies. Among 111 patients with TNBC whose tumor samples were screened for PD-L1 expression, 58.6% had PD-L1-positive tumors; 32 women were enrolled and assessed for safety and antitumor activity. All patients had metastatic TNBC at study entry, and most were heavily pre-treated, having received therapy in both the early and advanced disease settings. Among the 27 patients who were evaluable for antitumor activity, the ORR was 18.5% (1 CR and 4 PR). The median time to response was 17.9 weeks (range, 7.3 to 32.4 weeks). The most common treatment-related AEs of any grade included arthralgia (18.8%), fatigue (18.8%), myalgia (18.8%), and nausea (15.6%), including 5 (15.6%) patients with grade ≥ 3 toxicity and 1 treatment-related death [81].

KEYNOTE-028 (NCT02054806) is an ongoing multicohort, open-label phase Ib study evaluating the safety and efficacy of pembrolizumab in patients with PD-L1-positive advanced solid tumors. Of the 248 patients with ER+/HER2-negative breast cancer who had evaluable tumor samples screened for PD-L1 expression, 48 (19%) had PD-L1-positive tumors. Of these, 25 patients were enrolled. Patients were heavily pretreated, with 76% having received ≥ 3 prior lines of therapy for advanced disease, including 48.0% who received ≥ 5 prior lines. In the 22 patients with at least one scan after baseline, ORR was 14% and CBR was 23%. The safety profile was

similar to KEYNOTE-012 with 16.0% grade 3–4 AEs including grade 3 autoimmune hepatitis (4%) [82].

A randomized, phase III study of single-agent pembrolizumab vs single-agent chemotherapy per physician's choice for metastatic TNBC (KEYNOTE-119/NCT02555657) is ongoing. Estimated 600 metastatic triple-negative breast cancer (mTNBC) patients with central determination of PD-L1 tumor status who has previously received either one or two prior systemic treatments for metastatic setting will receive pembrolizumab 200 mg intravenously every 3 weeks or receive capecitabine, eribulin, gemcitabine, and vinorelbine per physician's choice. The primary endpoint is PFS and OS. The primary completion date will be this June. Future directions also include using pembrolizumab in the neoadjuvant therapy settings. A neoadjuvant trial investigating pembrolizumab in combination with chemotherapy as neoadjuvant treatment for participants with TNBC is ongoing (KEYNOTE-173/NCT02622074). Estimated 100 previously untreated, locally advanced TNBC will be allocated to receive pembrolizumab combined or sequentially with paclitaxel/nab-paclitaxel/doxorubicin/cyclophosphamide/carboplatin. The primary endpoint is DLTs with secondary endpoints being pathologic complete response (pCR) and ORR.

Atezolizumab

Atezolizumab is an engineered, humanized IgG1 monoclonal antibody that targets PD-L1 and inhibits the interaction between PD-L1 and these two receptors, PD-1 and B7-1. It has been approved in the USA for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic non-small cell lung cancer. Atezolizumab has shown activity in TNBC both as single-agent and combination treatment with chemotherapy in early phase trials and is now being tested in phase III setting.

In a phase I study (NCT01375842, PCD4989g), clinical activity analyses have been performed in 21 patients with PD-L1-selected (IC2/3) TNBC who received atezolizumab treatment at 1200 mg Q3W [73]. Unconfirmed responses were recorded for 5 patients, of whom two experienced a complete response and 3 patients experienced a partial response. As of 2nd September 2014, 4 of these 5 patients were still responding and 1 patient experienced disease progression. The Kaplan–Meier estimated overall 24-week PFS rate was 33% (95% CI 12 to 53%).

Another phase Ib multi-arm study (NCT01633970, GP28328) evaluates the safety and preliminary efficacy of a number of combinations of atezolizumab in patients with locally advanced or metastatic solid tumors. Arm F of the study is testing the combination of atezolizumab and nab-paclitaxel in female patients with metastatic TNBC. Patients received 800 mg of atezolizumab on

days 1 and 15 of every 28-day cycle plus nab-paclitaxel (125 mg/m²) on days 1, 8, and 15 of every 28-day cycle. Up to two prior cytotoxic regimens for metastatic disease were allowed. By 14 January 2016, safety and preliminary efficacy data were available for 32 patients [83]. Of the efficacy-evaluable patients, 13 received the treatment combination as first-line therapy and 19 had received ≥ 1 prior cytotoxic regimens for metastatic disease; 88% had previously received taxanes. In the overall efficacy-evaluable population, 12 patients (38%) achieved objective responses. Clinical responses were observed in patients with PD-L1 IC1/2/3 expression tumors as well as in those with PD-L1 IC0 expression. Six of 13 patients (46%) who received atezolizumab plus nab-paclitaxel as first-line therapy achieved responses, comprising one complete response and five partial responses. The combination was well tolerated and consistent with the known risks of nab-paclitaxel and atezolizumab [83]. The most frequent AEs attributed to atezolizumab ($\geq 10\%$) included fatigue, pyrexia, diarrhea, nausea, alopecia, pruritis, headache, peripheral neuropathy and peripheral sensory neuropathy, and decreased neutrophil count. Based on these results, the combination of atezolizumab and nab-paclitaxel is being evaluated in a phase III study (NCT02425891) of patients with previously untreated mTNBC.

Nivolumab

As the first PD-1 blocking antibody approved for clinical practice in worldwide, nivolumab has got its indications in unresectable or metastatic melanoma, metastatic non-small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, and recurrent/metastatic squamous cell carcinoma of the head and neck [49, 52–54, 84, 85]. But the clinical data in breast cancer is rare reported. Up to date, no clinical result of significance published on nivolumab-treated breast cancer, but indeed, there are many ongoing trials to assess the safety and efficacy of nivolumab as monotherapy or combined therapy in this disease.

A phase I/II, open-label study of nivolumab as monotherapy or combined with ipilimumab in advanced or metastatic solid tumors with a cohort of TNBC is currently recruiting [86]. The primary purpose is to analyze the safety and efficacy (ORR, PFS). Additionally, evaluation of putative biomarkers such as PD-1 and PD-L1 expression will be performed. The study uses a modified Simon 2-stage design. In stage 1, 36 patients for each tumor type will be assigned 1:1 to treatment with either nivolumab (*N*) or nivolumab + ipilimumab (*N + I*) for 4 doses and then nivolumab maintenance until progression or toxicity. Treatment arms will proceed independently into stage 2 if ≥ 2 patients in a given arm for each tumor type have an OR. In stage 2, an additional

22 patients per tumor type will be assigned to each arm (N or $N + I$) and receive the stage 1 dosing regimen. The estimated completion date will be December 2018.

In another phase I trial, nivolumab (nivo) is tested combined with nab-paclitaxel (nab-P) in HER2-negative MBC [87]. Patients with MBC will be treated in 2 arms: nab-P 100 mg/m² on days 1, 8, and 15 of each 28-day cycle plus nivo 3 mg/kg on days 1 and 15 starting at cycle 3 or nab-P 260 mg/m² on day 1 of each 21-day cycle plus nivo 5 mg/kg on day 15 starting at cycle 3. The primary endpoint is the DLTs. Secondary study endpoints include treatment-emergent adverse events (TEAEs), PFS, OS, disease control rate, ORR, and duration of response. Exploratory endpoints include tumor-associated PD-L1 expression, modulation of immune activation in the tumor and peripheral blood in response to nivo treatment, serum nivo levels, and development of antiglobulin antibodies. The estimated completion date will be October 2017.

Other ongoing clinical trials besides those mentioned above are listed in Table 1.

Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 IgG1 antibody recently approved by FDA for the treatment of metastatic Merkel cell carcinoma [88]. In a cohort of phase Ib JAVELIN study (NCT01772004), 168 patients with locally advanced or metastatic breast cancer refractory to or progressing after standard-of-care therapy received avelumab at 10 mg/kg Q2W [89]. The ORR in the entire cohort was 5.4%, including 5 PRs in TNBC ($n = 57$). Among all patients with PD-L1-expressing immune cells within the tumor, 33.3% (4 of 12) had PRs. In patients with TNBC who had PD-L1+ immune cells within the tumor, 44.4% (4 of 9) had PRs, compared with 2.6% (1 of 39) for TNBC and PD-L1-immune cells. Contrastively, out of the 72 patients with HR+ disease, an objective response was seen in only 2.8%, but 54% were found to have PD-L1 expression.

This brings into question the antibodies used for PD-L1 testing as well as potential differences in efficacy between different subsets of breast cancer.

A phase III randomized trial is ongoing to test adjuvant treatment for high-risk TNBC patients with avelumab (A-Brave, NCT02926196). Patients with high-risk primary TNBC (all comers, PD-L1-positive or unselected for PD-L1 status) who have completed treatment with curative intent including surgery of the primary tumor, neo- or adjuvant chemotherapy, and radiotherapy (if indicated) are recruited and randomized to experimental arm (avelumab for 1 year) or no further intervention arm. The primary endpoint is DFS. The primary completion date will be June 2021.

PARP inhibitors

Healthy cells defend themselves against the deleterious effects of DNA damage through an interrelated series of molecular pathways, the DNA damage response (DDR), that recognize DNA damage, stall the cell cycle, and mediate DNA repair. Poly(ADP-ribose) polymerase (PARP) are nuclear enzymes that catalyze the transfer of ADP ribose from NAD⁺ to target proteins and facilitate DNA repair [90]. At sites of DNA damage, PARP1 binds damaged DNA at single-strand DNA breaks (SSBs) and other DNA lesions, an event that causes a series of allosteric changes in the structure of PARP1 that activate its catalytic function PARP and activates intracellular signaling pathways that modulate DNA repair and cell survival through poly(ADP)-ribosylation of several nuclear proteins involved in chromatin architecture and DNA metabolism [91–93]. PARP inhibition results in double-strand breaks in replicating cells [94]. In cells with wild-type BRCA1/2, double-strand breaks are repaired via homologous recombination, but in BRCA1/2-deficient cells with homologous recombination deficiency (HRD), DNA strand breaks rely on PARP1 functionality for repair [94, 95]. Therefore, inhibition of PARP1 by RNA interference or with chemical inhibitors

Table 1 Clinical trials testing nivolumab in patients with breast cancer

Disease setting	Phase	Clinical trial reference number	Cancer type	Estimated Enrollment	Primary Endpoint	Regimens	Control arm
Metastatic	II	NCT02892734	HER2-	29	PFS	Nivolumab + ipilimumab	None
Metastatic	I	NCT02309177	HER2-	20	MTD	Nivolumab + nab-paclitaxel	None
Metastatic	II	NCT02499367	TNBC	84	PFS	Nivolumab + doxorubicin/ cyclophosphamide/cisplatin/radiation	Active Comparator
Metastatic	I	NCT02453620	HER2-	45	Safety	Nivolumab + entinostat + ipilimumab	None
Metastatic	I/II	NCT01928394	TNBC	58	ORR	Nivolumab ± ipilimumab	None
Metastatic	I	NCT02834247	TNBC	36	MTD/ORR	Nivolumab ± TAK659	None

TNBC, triple negative breast cancer; HER2, human epidermal growth factor receptor-2; PFS, progress free survival; MTD, maximum tolerance dose; ORR, objective response rate

leads to severe, highly selective toxicity in BRCA1/2-deficient cells [96], the so-called synthetic lethality [97]. In breast cancer, the presence of germline mutations in BRCA1/2 is characterized by features of basal-like sporadic breast tumors, including a greater likelihood of being high-grade, ER/PgR-negative, HER2-negative, and a high frequency of TP53 mutations [98]. The presence of germline mutations in BRCA1/2 increases the lifetime risk of breast cancer to 60–70% [99] and occurs in about 10% of patients with TNBC [18, 100, 101].

Up to date, three typical PARP inhibitors—olaparib [102, 103], rucaparib [104, 105], and niraparib [106], have all received their FDA approval for advanced ovarian cancer and/or primary peritoneal cancer with or without germline and/or somatic mutations in BRCA1/2. In the setting of breast cancer, a proof of concept study was conducted to assess the efficacy, safety, and tolerability of olaparib alone in women with BRCA1 or BRCA2 mutation advanced breast cancer. Patients had been given a median of three previous chemotherapy regimens. Overall responses ranged from 22% (6 out of 27, 100 mg twice per day) to 41% (11 out of 27, 400 mg twice per day) with tolerable toxicity [107]. However, a phase II study evaluating olaparib 400 mg twice a day as a single agent for patients with advanced breast cancer ($n = 26$, 81% TNBC) did not report any confirmed responses in BRCA1/2 mutation neither positive ($n = 10$) nor negative ($n = 16$) subjects, even though the target lesions were reduced in size by >30% in 5 out of 10 (50%) patients with BRCA1 or BRCA2 mutations, but were not confirmed objective responders because of absence of confirmation at the next visit (three patients) or progression of nontarget or new lesions at the same visit (two patients) [108]. A phase I/Ib study tested the effects, safety, and activity of the combination of olaparib and carboplatin. Olaparib tablets were introduced in a 3 + 3 dose escalation with carboplatin q21 days, up to 8 cycles, followed by olaparib 300 mg bid maintenance. Fourteen patients with breast cancer (11 TNBC, 7 germline BRCA mutation carriers) were enrolled. One patient with BRCA1 mutation TNBC achieved CR and another 6 achieved PR [109].

Several randomized phase III trials investigating the use of olaparib in the metastatic (NCT02000622) and neoadjuvant (NCT02032823) setting are ongoing. Germline mutation in BRCA1 or BRCA2 is essential as an inclusion criterion in these trials. The primary endpoints are PFS and invasive disease-free survival (IDFS), respectively. Another PARP inhibitor, talazoparib, is studied in neoadjuvant setting. Thirteen early-stage breast cancer patients with germline mutations in either BRCA1 or BRCA2 were treated for 2 months with talazoparib. All patients displayed a reduction in tumor volume from 30 to 98% after 2 months [110]. This study is now being expanded to

assess the effects of 4 to 6 months of neoadjuvant talazoparib therapy. Similar neoadjuvant studies assessing rucaparib in breast cancer are also under way.

Fulvestrant

Fulvestrant is a new type of endocrine treatment—an ER antagonist with a novel mode of action. Fulvestrant is a 7a-alkylsulphonyl analog of 17b-oestradiol, which is distinctly different in chemical structure from the non-steroidal structures of tamoxifen, raloxifene, and other SERMs [111].

Fulvestrant competitively inhibits binding of estradiol to the ER, with a binding affinity of 89% of estradiol, while tamoxifen affinity is only 2.5% of that of estradiol [112, 113]. Fulvestrant-ER binding impairs receptor dimerization, and energy-dependent nucleo-cytoplasmic shuttling, thereby blocking nuclear localization of the receptor [114, 115]. Additionally, any fulvestrant-ER complex that enters the nucleus is transcriptionally inactive and has no demonstrable agonist activity because both AF1 and AF2 are disabled. Finally, the fulvestrant-ER complex is unstable, resulting in accelerated degradation of the ER protein, compared with estradiol- or tamoxifen-bound ER [116], leading to complete inhibition of estrogen signaling [117–119]. Therefore, it is also called as selective estrogen receptor downregulator.

The phase I clinical trials in postmenopausal women with primary breast cancer have shown that fulvestrant significantly downregulates ER expression in ER-positive tumors in a dose-dependent manner. There was also a significant decrease in progesterone receptor (PR) expression (a marker of estrogen action) consistent with the preclinical data demonstrating that fulvestrant lacks intrinsic estrogen agonist activity. These changes in ER and PR expression were accompanied by reductions in expression of Ki-67, a marker of tumor cell proliferation [120]. Neoadjuvant NEWEST trial showed that in 211 early breast cancer patients, the 500 mg regimen of fulvestrant resulted in a significantly ($p < 0.0003$) greater reduction in ER expression compared with the 250-mg dose at week 4 (22 vs 15%) [121]. At week 16, ER expression was reduced by 34 and 25%, respectively [121].

Four phase III clinical trials (studies 9238IL/0020 and 9238IL/0021) [122, 123], EFECT [124] and SoFEA [125] showed that fulvestrant 250 mg is as effective as the conventionally used drugs. Fulvestrant 250 mg combined with AI gave contradictory results compared with AI only in two phase III trials [126, 127].

Increase of dose of fulvestrant improves efficacy. CONFIRM trial [128] was completed in 736 postmenopausal women with advanced breast cancer who had disease recurrence on or after adjuvant endocrine therapy or progression following endocrine therapy for advanced disease. Fulvestrant 500 mg significantly

improves median PFS, which translating into a 4.1-month increase in median OS and a 19% reduction in the risk of death. First-line randomized phase II trial in 205 postmenopausal women with fulvestrant 500 mg treatment had a median PFS and OS of 23.4 and 54.1 m, respectively [129]. The confirmative phase III FALCON (NCT01602380) trial showed that patients treated with fulvestrant had a statistically significant 21% improvement in progression-free survival compared to those treated with anastrozole (16.6 vs 13.8 months, $p = 0.048$) [130]. Fulvestrant ongoing clinical studies are listed in

Table 2. Other SERDs, such as AZD9291, AZD9496, and GDC810 are under clinical investigations.

T-DM1 and other ADCs

Approximately 18–20% of invasive breast cancers are HER2-positive subtype with poor prognosis in the absence of anti-HER2 treatment. Trastuzumab emtansine (T-DM1) is a complex compound produced by the conjugation of trastuzumab, a stable thioether linker, and the potent cytotoxic drug maytansine derivate (DM1). It is the first antibody-drug conjugate (ADC)

Table 2 Fulvestrant ongoing clinical studies

CT.gov No.	Name	Investigational Agent	Trial
NCT02646735	FRIEND	Fulvestrant Exemestane	A Randomized, Open label, Parallel-group, Multi-Centre, Pilot study to compare the Efficacy and Tolerability of Fulvestrant 500mg with Exemestane as First line endocrine therapy for Postmenopausal Hormone Receptor Positive HER2 negative Advanced Breast Cancer patients relapse after adjuvant Non-steroidal Aromatase Inhibitors
NCT02072512	PROOF	Goserelin Fulvestrant Anastrozole	A Phase III, Randomized, Open label, Parallel-group, Multi-Centre study to compare the Efficacy of Goserelin combined with Fulvestrant 500 mg and Anastrozole 1mg as First line endocrine therapy for Pre- or Peri-menopausal patients having Hormone Receptor Positive Advanced Breast Cancer After or During Adjuvant Endocrine therapy
NCT02107703	MONARCH 2	Fulvestrant Abemaciclib	A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study of Fulvestrant With or Without Abemaciclib, a CDK4/6 Inhibitor, for Women With Hormone Receptor Positive, HER2 Negative Locally Advanced or Metastatic Breast Cancer
NCT02422615	MONALEESA 3	Ribociclib Fulvestrant	A Randomized Double-blind, Placebo-controlled Study of Ribociclib in Combination With Fulvestrant for the Treatment of Men and Postmenopausal Women With Hormone Receptor Positive, HER2-negative, Advanced Breast Cancer Who Have Received no or Only One Line of Prior Endocrine Treatment
NCT02690480	FLIPPER	Fulvestrant Palbociclib	A Randomized, Double-blind, Parallel-group, Multicentre, Phase II Study to Compare the Efficacy and Tolerability of Fulvestrant 500mg With Placebo and Fulvestrant 500mg in Combination With Palbociclib as First Line Treatment for Postmenopausal Women With Hormone Receptor-positive Metastatic Breast Cancer, Who Have Completed at Least 5 Years of Adjuvant Endocrine Therapy and Remained Disease Free for More Than 12 Months Following Its Completion or Have "de Novo" Metastatic Disease
NCT02028507	PEARL	Palbociclib Exemestane Fulvestrant Capecitabine	Phase III Study of Palbociclib in Combination With Endocrine Therapy (Exemestane or Fulvestrant) Versus Chemotherapy (Capecitabine) in Hormonal Receptor (HR) Positive/HER2 Negative Metastatic Breast Cancer (MBC) Patients With Resistance to Non-steroidal Aromatase Inhibitors
NCT02491983	PARSIFAL	Palbociclib Fulvestrant Letrozole	A Randomized, Multicenter, Open-label, Phase II Trial to Evaluate the Efficacy and Safety of Palbociclib in Combination With Fulvestrant or Letrozole in Patients With HER2 Negative, ER+ Metastatic Breast Cancer
NCT02536742	PYTHIA	Palbociclib Fulvestrant	A Phase II Study of Palbociclib Plus Fulvestrant Versus Placebo Plus Fulvestrant for Pretreated Patients With ER+/HER2- Metastatic Breast Cancer
NCT01633060	BELLE 3	BKM120 Fulvestrant	A Phase III Randomized, Double Blind Placebo Controlled Study of BKM120 With Fulvestrant, in Postmenopausal Women With Hormone Receptor-positive HER2-negative Locally Advanced or Metastatic Breast Cancer Which Progressed on or After Aromatase Inhibitor Treatment
NCT02340221	SANDPIPER	Taselisib Fulvestrant	A Phase III, Double-Blind, Placebo Controlled, Randomized Study of Taselisib plus Fulvestrant vs Placebo plus Fulvestrant in Postmenopausal women with Estrogen Receptor-Positive and HER2-Negative Locally Advanced or Metastatic Breast Cancer who have Disease Recurrence or Progression during or after Aromatase Inhibitor Therapy
NCT02216786	MANTA	Fulvestrant AZD2014 Everolimus	A Randomized Phase II Study of Fulvestrant in Combination With the Dual mTOR Inhibitor AZD2014 or Everolimus or Fulvestrant Alone in Estrogen Receptor-positive Advanced or Metastatic Breast Cancer ()

developed specifically for the treatment of HER2-positive breast cancer [131, 132]. The binding of T-DM1 to HER2-positive cells allows internalization of this complex by endocytosis, subsequent intralysosomal proteolytic degradation, and then release of potent DM1, a derivative of the antimitotic drug maytansine [133, 134].

The maximum tolerated dose (MTD) determined by the phase I and II clinical trials is 3.6 mg/kg every 3 weeks with bone marrow suppression and liver toxicity being dose-limiting toxicity, the clearance of the drug is 12.9 ml/day/kg (± 3.4 ml/day/kg) and its half-life is 3.5 days [135, 136].

The first-line MARIANNE trial (NCT01120184) is a large three-arm phase III study which randomized patients with previously untreated HER2-positive MBC to receive T-DM1 plus pertuzumab, T-DM1 plus placebo, and combination of trastuzumab plus a taxane (paclitaxel or docetaxel). T-DM1 is concluded to be non-inferior to trastuzumab + taxanes but with a better toxicity profile [137].

The two pivotal trials, EMILIA and TH3RESA trials conducted in second line and third- and later lines, respectively, demonstrated that T-DM1 is better than lapatinib/capecitabine and treatment of physician's choice (TPC), respectively, in terms of ORR and PFS as well as OS. These two phase III trials suggest that T-DM1 is standard of choice in second- and later-line management of HER2-positive MBC.

The ongoing studies, including phase Ib and II studies, STELA, BP22572, TDM4529g/BO25430, and TDM4874g/BO22857 in adjuvant/neoadjuvant setting and TEAL in neoadjuvant setting, and phase III studies, MO28231 in metastasis setting, KAITLIN (BO28407) and KATHERINE (BO27938) in adjuvant setting, and KRISTINE (BO28408/TRIO021) in neoadjuvant setting, will help to elucidate if T-DM1 could have a role in first-line treatment of metastatic breast cancer as well as in the adjuvant and neoadjuvant setting.

ADCs are biological drugs containing a monoclonal antibody linked by a covalent bond to a cytotoxic drug via a synthetic coupler. The ADC is designed such that when it reaches the target cell, it releases the cytotoxic agent inside them, thus sparing non-tumor cells from damage. In preclinical experiment, the new triple conjugate, T-DM1 with another antibody, such as pertuzumab or atezolizumab, was successful and works in cell lines as well as animal models.

Innovative chemotherapies

Nab-paclitaxel

Taxanes are widely used as antitumor agents. Albumin-bound paclitaxel (nab-paclitaxel; Abraxane) is a second generation of taxanes, that has been developed to

improve the therapeutic index of paclitaxel, also reducing the toxicities associated with Taxol and the CrEL and ethanol vehicle. Nab-paclitaxel is a good candidate since it can be given without steroid or antihistamine premedication. Due to its safety, nab-paclitaxel can be delivered at higher doses, in a shorter infusion time, thus enabling a higher drug C_{max} and plasma area under the curve (AUC). Upon intravenous infusion, nab-paclitaxel dissociates into its albumin and paclitaxel on small particles of 8–30 nm and then distributes rapidly to extravascular compartment and selectively delivers larger amounts of nab-paclitaxel to tumors by exploiting endogenous albumin transport pathways [138, 139].

Nab-paclitaxel was approved for metastatic breast cancer by FDA in 2005. Since then, it has been studied in a variety of breast cancer patient populations and with different doses and schedules. The GeparSepto (GBG 69) trial assessed weekly nab-paclitaxel on improving pathological complete response rate compared with weekly solvent-based paclitaxel, both followed by epirubicin plus cyclophosphamide as neoadjuvant treatment. Results showed that 12 continuous weekly doses of nab-paclitaxel 125 mg/m² for neoadjuvant therapy is both well tolerated and associated with significant superior pCR rates (38%) vs weekly paclitaxel 80 mg/m² (29%) [140]. This result is consistent with that of another phase III ETNA study [141].

In metastatic setting, the phase II tnAcity study results were presented in 2016 SABCS meeting. One hundred ninety-one women with mTNBC were randomized to receive one of three weekly regimens: nab-paclitaxel + carboplatin (nab-P/C), nab-paclitaxel + gemcitabine (nab-P/G), or gemcitabine + carboplatin (G/C) as first-line treatment. The trial found that an investigational weekly combination regimen of nab-P/C had significantly longer PFS (7.4 months) compared to weekly regimens of either nab-P/G (5.4 months; $p = 0.02$) or G/C (6.0 months; $p = 0.03$) [142]. The approval in MBC was based on a randomized phase III trial of nab-paclitaxel 260 mg/m² vs paclitaxel 175 mg/m² every 3 weeks. Nab-paclitaxel demonstrated a significantly higher overall response rate (ORR 33 vs 19%; $p = 0.001$) and longer time to tumor progression (23 vs 17 weeks; HR 0.75; $p = 0.006$) vs paclitaxel in the intention-to-treat (ITT) population [143].

A systematic review discussed recent studies and ongoing trials of nab-paclitaxel in breast cancer and provides perspectives on the future role of nab-paclitaxel in breast cancer. Sixty-three studies of nab-paclitaxel in breast cancer published between 2013 and 2015 were analyzed, including 23 in early stage and 30 in metastatic setting. Among phase II and III studies of neoadjuvant nab-paclitaxel (majority administered weekly) that did not select for specific disease subtype, the pCR rate ranged

from 22 to 40%. And for HER2-negative breast cancer or TNBC, the overall pCR rate ranged from 5.7 to 53% with the highest pCR rate achieved in TNBC treated by nab-paclitaxel + carboplatin. Four studies of nab-paclitaxel in MBC of unselected subtype reported median OS ranging from 10.8 months with nab-paclitaxel 260 mg/m² q3w to 26.9 months with nab-paclitaxel 125 mg/m² qw 3/4 combined with cisplatin. Response rate by subgroup demonstrated a higher response in TNBC [144].

Nab-paclitaxel is continuously being investigated in different stages and settings of aggressive breast cancer listed in Table 3. Immune checkpoint inhibitors and their optimal combination partners are hot topics [145].

Eribulin

Eribulin mesylate (E7389) is a structurally simplified synthetic analog of halichondrin B, which was first isolated more than 20 years ago from two unrelated species of sponge, *Halichondria okadai* Kadota, and *Aninella* sp. [146, 147]. It is a nontaxane inhibitor of microtubule dynamics and the only cytotoxic agent in the last decade to improve overall survival in heavily pretreated patients with MBC. Eribulin inhibits microtubule polymerization

(or growth), through an eribulin-specific binding site on β -tubulin, without any effect on microtubule depolymerization (or shortening) unlike conventional anti-tubulin agents, like taxanes, epothilones, and vinca alkaloids [148]. It may have additional antitumor mechanism through effects on epithelial-to-mesenchymal transition [149] and tumor vasculature remodeling [150, 151].

The first reported phase III study was the EMBRACE (the Eisai Metastatic Breast Cancer Study Assessing Physician's Choice Versus E7389) [152], the pivotal phase III trial that led to the regulatory approval of eribulin for the treatment of MBC. In this study, 762 women were randomly assigned (2:1) to either eribulin ($n = 508$) or treatment of physician's choice (TPC; $n = 254$). OS and PFS were the co-primary endpoints. Median overall survival was significantly improved in women assigned to eribulin compared with TPC (13.1 vs 10.6 months, $p = 0.041$). In the early-line MBC setting, eribulin did not improve PFS or OS than capecitabine. Subgroup analysis of the two trials showed that TNBC patients might benefit more from it [153, 154]. A recent trial comparing eribulin head to head with vinorelbine conducted in Chinese population showed that it improved progression-free survival.

Table 3 Nab-paclitaxel's ongoing trials phase III and important phase II trials as listed below

CT.gov No.	Phase	Investigational Agent	Trial	Setting	n	Completion Date
NCT02620280	III	Atezolizumab	Neo-Adjuvant Study With the PDL1-directed Antibody in Triple Negative Locally Advanced Breast Cancer Undergoing Treatment With Nab-paclitaxel and Carboplatin (NeoTRIPaPDL1)	Neoadjuvant TNBC	272	2022
NCT02425891	III	Atezolizumab	A study of atezolizumab in combination with nab-paclitaxel compared with placebo with nab-paclitaxel for participants with previously untreated metastatic triple negative breast cancer (IMpassion130)	mTNBC	900	2020
NCT02819518	III	Pembrolizumab	A Randomized, Double-Blind, Phase III Study of Pembrolizumab (MK-3475) Plus Chemotherapy vs Placebo Plus Chemotherapy for Previously Untreated Locally Recurrent Inoperable or Metastatic Triple Negative Breast Cancer - (KEYNOTE-355)	Locally recurrent or metastatic BC	858	2019
NCT01690702	III	Epirubicin, Cyclophosphamide Docetaxel	Adjuvant Phase III Trial to Compare Intense Dose-dense Adjuvant Treatment With EnPC to Dose Dense, Tailored Therapy With dtEC-dtD for Patients With High-risk Early Breast Cancer (GAIN-2)	Adjuvant high risk BC	2886	2020
CBCSG018	II	Gemcitabine Cisplatin	A randomized phase 2 trial of weekly nab-paclitaxel plus cisplatin versus gemcitabine plus cisplatin as first-line treatment for patients with metastatic triple-negative breast cancer	mTNBC	254	2018
NCT02685059	II	Durvalumab	A Randomized Phase II Study to Investigate the Addition of PD-L1 Antibody MEDI4736 to a Taxane-anthracycline Containing Chemotherapy in Triple Negative Breast Cancer (GeparNuevo)	Neoadjuvant TNBC	174	2018
NCT02783222	II	nab-paclitaxel	A Randomized Phase II Study to Evaluate the Efficacy and Impact on Function of Two Different Doses of Nab-paclitaxel in Elderly Patients With advanced breast Cancer (EFFECT)	$\geq 65y$ Locally recurrent or metastatic BC	156	2017

Eribulin is currently being studied in several clinical trials. A phase III study comparing eribulin with paclitaxel in the first-line and second-line treatment of HER2-negative MBC is currently recruiting patients in the USA. A phase II study of eribulin in combination with trastuzumab and pertuzumab is currently recruiting (NCT01912963). PD-L1 is expressed in approximately 60% of TNBC tumors, suggesting that PD-L1 may be a therapeutic target for this disease [81]. The combination of pembrolizumab and eribulin demonstrated a 33.3% ORR for patients with metastatic triple-negative breast cancer (TNBC) who received 0 to 2 prior lines of therapy [155]; a further confirmative phase III trial is warranted.

Future research is needed to optimize the role of eribulin in the treatment of MBC, in terms of both patient selection and its position in the therapeutic sequence. Eribulin should also be further tested as first-line treatment in advanced breast cancer, in the adjuvant and neoadjuvant setting alone and in combination with a variety of agents, particularly biologics.

Utidelone

Refractory to anthracycline and taxane remains a main cause of disease progression for metastatic breast cancer. Epothilones are a class of naturally existing microtubule inhibitors produced by the myxobacterium *Sorangium cellulosum*. The molecular structure and mechanism of action of epothilones differ from those of taxanes. Thus, patients with tumors resistant to taxanes remain sensitive to epothilones [156]. Utidelone is a genetically engineered epothilone analog which attempts to achieve better efficacy, more favorable safety profile, and lower cost than ixabepilone, a semisynthetic epothilone analog which is the only drug in this class that has been approved by the US FDA.

A series of trials have shown promise efficacy for utidelone as a potential treatment for heavily pretreated drug-resistant, advanced breast cancer.

The pivot study is a phase III open-label, superiority, randomized study to enroll patients with metastatic breast cancer refractory to anthracycline and taxane chemotherapy regimens. Four hundred five patients were randomized by 2:1 to treatment with utidelone (30 mg/m² once per day on days 1–5) plus capecitabine (1000 mg/m² twice per day on days 1–14) or capecitabine alone (1250 mg/m² twice per day on days 1–14). The primary endpoints centrally assessed by a masked independent radiology review committee showed improved ORR in the utidelone plus capecitabine group than in the capecitabine alone group (40.4 vs 21.5%; $p = 0.0002$). Median PFS was 8.44 months compared with 4.27 months, respectively (HR 0.46; $p < 0.0001$). The

analysis of OS is immature, and analysis with available data by the cutoff date showed longer OS in the utidelone plus capecitabine group compared with the capecitabine alone group (16.13 vs 12.78 months; HR 0.63 $p = 0.0059$). No significant between-group differences were noted for safety outcomes, except for peripheral neuropathy which was significantly higher with utidelone plus capecitabine compared with capecitabine alone (grade 3: 22 vs <1%). Notably, utidelone caused only very mild myelosuppression (leucopenia 48 vs 47% in all grade) and no liver toxicities [157]. Further research is needed to optimize the formulation of utidelone for more convenient administration and to reduce the incidence of peripheral neuropathy. Future development for the role of utidelone in earlier settings of breast cancer, combination studies with other biological immunotherapies, and targeted agents are warranted.

Other potential agents/therapies

Chimeric antigen receptor (CAR) is a modular fusion protein comprising extracellular target-binding domain usually derived from the single-chain variable fragment (scFv) of antibody, spacer domain, transmembrane domain, and intracellular signaling domain [158]. CAR-engineered T cells (CAR-T cells) have yielded unprecedented efficacy in B cell malignancies, most remarkably in anti-CD19 CAR-T cells for B cell acute lymphoblastic leukemia (B-ALL) with up to a 90% complete remission rate [159, 160]. However, this success has encountered significant hurdles in translation to solid tumors.

Folate receptor-alpha (FR α) is a glycosyl-phosphatidyl inositol (GPI)-anchored protein that is overexpressed at both the protein and mRNA levels in TNBC [161], where it serves a biological role in TNBC cell growth and folate uptake. Strong FR α immunohistochemical staining is highly associated with poor outcome in breast cancer patients [162]. FR α also expressed at low levels on the apical surface of a subset of polarized epithelial cells including the parotid, kidney, lung, thyroid, and breast. Specific overexpression of FR α in certain malignancies, including TNBC, with low coordinate expression in normal tissue, makes FR α an attractive target. The transfer of T cells genetically redirected with a CAR specific for FR α is an attractive technology that is actively being investigated. The CAR approach combines the antigen specificity of an antibody with the ability of T cells to mediate the killing of tumor cells in a single fusion molecule. CAR-modified T cells actively and specifically target their specified antigen and have the capacity to persist as memory cells in vivo [163, 164]. Song et al. demonstrated that FR α -specific CAR-T cells have the capacity to inhibit human TNBC growth in vivo: infused FR α -specific CAR-T cells mediated significant,

albeit modest, reduction in tumor progression compared to the control mice treated with untransduced T cells ($p = 0.01$) or with anti-CD19 CAR-T cells ($p = 0.035$), as measured by caliper-based tumor size. The same dose of FR α CAR-T cells mediated more effective tumor regression in mice with MDA-231. FR α tumors, despite larger initial tumor burden, suggest that the regression of TNBC mediated by CAR-T cells is dependent on a sufficient level of surface tumor antigen expression [165]. Future studies will be required to determine the minimal and maximal threshold of FR α expression for activation and effective lysis by FR α CAR-T cells upon stimulation with the TNBC cell lines or autologous tumor cells. Such results might aid in determining which patients may best benefit from FR α CAR-T cell therapy.

MicroRNAs (miRNAs) are small non-coding RNAs and negatively regulate protein-coding gene expressions by promotion of mRNA degradation or inhibition of translation. Overexpressions of oncogenic miRNAs that inhibit tumor suppressor genes are associated with cancer development. On the other hand, reduction or loss of expression of tumor-suppressive miRNAs induce upregulated expression of their target oncogenes [166]. In breast cancer, some miRNAs have been shown to upregulate the functions of oncogenes while others stimulate tumor suppressors. And the various breast cancer subtypes exhibit different molecular miRNA signatures. For instance, miR-342 was expressed most strongly in the ER-positive/HER2-positive tumors [167]. miR-342 influences the ER expression level and the response to tamoxifen [168, 169]. MiR-10b, miR-26a, and miR-153 have been suggested to be potential biomarkers of TNBC [170]. Lehmann et al. revealed that TNBC can be classified into at least six distinct molecular subtypes with differing biological characteristics based on mRNA profiling, including two basal-like types (BL1 and BL2), an immunomodulatory type (IM), a mesenchymal type (M), a mesenchymal stem-like type (MSL), and a luminal androgen receptor type (LAR) [75]. miRNAs also have important roles in endocrine resistance, and some studies have attempted to identify miRNAs that contribute to the clinical benefits of hormonal therapies. The miR-221/222 cluster is associated with tamoxifen resistance in breast cancer cells [171, 172]. Masri et al. suggested that miR-128a modulates the transforming growth factor- β signaling and survival of letrozole-resistant cell lines [173]. Jung et al. suggested that the plasma miR-210 level is useful for predicting and/or monitoring the therapeutic response to treatments involving trastuzumab, and the upregulation of miR-21 expression has been reported to be associated with trastuzumab resistance in HER2-positive breast cancer [174]. Furthermore, Moskwa et al. suggested that miR-182 downregulates BRCA1 expression

and found that the manipulation of miR-182 expression in breast cell lines affected their sensitivity to PARP1 inhibitors [175]. miRNA might also contribute to the immune system in breast cancer. Iliopoulos et al. demonstrated that miR-21 expression was upregulated by ovalbumin stimulation in T cells and also that the inhibition of PD-1 increased miR-21 expression [176]. Modulating miRNA expression appears to be a promising strategy for cancer therapy. Specific knockdown of miR-20b in a breast cancer nude mice model has shown to suppress tumor growth in vivo. Systemic delivery of poly-lactico-glycolic acid-based miR-21 and miR-10b antagonists in a breast cancer model caused dramatic effects on tumor regression [177]. Further biological research into the ability of novel agents to regulate miRNA expression is warranted, and miRNA is expected to become a therapeutic target of treatments for breast cancer.

Conclusions

Undoubtedly, HER2 and CDK4/6 are the two most important targets for breast cancer; biologicals targeted against the two targets not only increase objective response rates but also prolong PFS. Overall survival improvement is documented for anti-HER2 treatments and has not been determined with CDK4/6 inhibitors. Chemotherapy and endocrine therapy are still the basic treatments, although optimization of dosage remains an unmet need. For triple-negative breast cancer where anti-HER2 and endocrine treatment fail, immunotherapy based on check point inhibitors is promising, especially when combined with chemotherapy.

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