Drugs (2017) 77:1313–1336
DOI 10.1007/s40265-017-0774-5

REVIEW ARTICLE

# Neoadjuvant Therapy for Breast Cancer: Established Concepts and Emerging Strategies

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Published online: 14 June 2017


**Abstract** In the last decade, the systemic treatment approach for patients with early breast cancer has partly shifted from adjuvant treatment to neoadjuvant treatment. Systemic treatment administration started as a 'one size fits all' approach but is currently customized according to each breast cancer subtype. Systemic treatment in a neoadjuvant setting is at least as effective as in an adjuvant setting and has several additional advantages. First, it enables response monitoring and provides prognostic information; second, it downstages the tumor, allowing for less extensive surgery, improved cosmetic outcomes, and reduced postoperative complications such as lymphedema; and third, it enables early development of new treatment strategies by using pathological complete remission as a surrogate outcome of event-free and overall survival. In this review we give an overview of the current standard of neoadjuvant systemic treatment strategies for the three main subtypes of breast cancer: hormone receptor-positive, triple-negative, and human epidermal growth factor receptor 2-positive. Additionally, we summarize drugs that are under investigation for use in the neoadjuvant setting.

**Key Points**

Neoadjuvant treatment is increasingly preferred over adjuvant treatment in patients with early breast cancer.

The best neoadjuvant regimens differ between breast cancer subtypes.

Drugs under investigation in the neoadjuvant setting include cyclin D-cyclin-dependent kinase (CDK) 4/6 inhibitors, mammalian target of rapamycin (mTOR) inhibitors, phosphoinositide 3-kinase (PI3K) inhibitors, poly(ADP-ribose) polymerase (PARP) inhibitors, immune checkpoint inhibitors, vascular endothelial growth factor receptor (VEGF) inhibitors, antibody drug conjugates, and various new combined treatment approaches.

## 1 Introduction

Breast cancer is the most common cancer among women worldwide. An estimated 2.4 million women were diagnosed with breast cancer in 2015 and 523,000 patients died of the disease [1]. Breast cancer can be divided into three main subtypes based on expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), with differences in prognosis, treatment options, and responses [2]. The hormone receptor-positive subtype expresses ER, PR, or both receptors, and has no HER2 overexpression or amplification (i.e. HER2-negative). The HER2-positive subtype shows overexpression or amplification of HER2 with or

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without co-expression of ER and/or PR. Triple-negative breast cancer (TNBC) refers to the absence of ER and PR expression, and HER2 overexpression (or HER2 amplification) in tumor cells.

The variety in these phenotypic subtypes is a reflection of variation in gene expression. Perou and colleagues defined at least five different molecular subtypes that are clinically relevant: luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like [3–5]. For the purpose of this review, we focus on the three major histological subtypes mentioned earlier.

The main aim of systemic treatment in addition to local treatment is to eradicate micrometastases in order to maximize the chance of cure. Systemic treatment may include chemotherapy, endocrine therapy, and targeted therapy, and is either administered before surgery (neoadjuvant) or after surgery (adjuvant). Neoadjuvant chemotherapy is at least as effective as adjuvant chemotherapy, but the neoadjuvant approach has several additional advantages [6, 7]. First, it enables response monitoring with the opportunity to stop ineffective treatment and switch to a non-cross-resistant regimen [8–13]. Second, it enables downstaging of the tumor and involved lymph nodes and allows more conservative surgery of the breast and axilla [14, 15]. Additionally, it creates time to await results of genetic tests and decide on type of surgery, including preventive and reconstruction surgery. Third, it facilitates research in identifying radiological, histological, and molecular predictors for response [16, 17]. In addition, the neoadjuvant approach expedites the evaluation of new treatment strategies by using early surrogate endpoints. Pathological complete response (pCR) is most widely used as a surrogate endpoint and correlates with recurrence-free survival (RFS) and overall survival (OS) [12, 18–21]. The preferred and most commonly used definition of pCR is absence of residual invasive tumor cells in the breast and lymph nodes; we refer to this definition in this review unless stated otherwise. The association between pCR and long-term outcome varies across subtypes and remains subject to some debate [19–21]. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recognize pCR as a valid endpoint of neoadjuvant trials and as the basis for accelerated drug approval, although full approval still requires a demonstrated benefit in long-term outcome [18, 22]. Other surrogate endpoints such as the residual cancer burden (RCB) score need further evaluation and validation per subtype [23].

The recommendation for systemic treatment is based on tumor characteristics, extent of breast cancer, and patient characteristics. The current European Society for Medical Oncology (ESMO) [24], National Comprehensive Cancer Network (NCCN) [25], and St. Gallen [26] guidelines advise endocrine therapy for all patients with hormone receptor-positive tumors. In addition, chemotherapy is recommended for hormone receptor-positive, HER2-negative tumors larger than 5 cm, or when more than three lymph nodes are involved. For patients with hormone receptor-positive breast cancer, chemotherapy may be withheld based on a low clinical risk or low genomic risk profile [24–28]. Nearly all patients with TNBC should be treated with chemotherapy. In addition, for almost all HER2-positive breast cancers, chemotherapy in combination with HER2-directed treatment is recommended [24–26, 29].

If systemic treatment is recommended, this can be administered either in the adjuvant or neoadjuvant setting. Neoadjuvant treatment is preferred over adjuvant therapy in cases of locally advanced, inoperable breast cancer, or if breast-conserving surgery (BCS) is desired but is not yet possible. Primary surgery is advised if uncertainty exists about the extent of the breast cancer, which potentially has implications for the systemic treatment [16, 25].

In this review, we discuss established neoadjuvant strategies in hormone receptor-positive, triple-negative, and HER2-positive breast cancer. Additionally, we provide an overview of recently approved and investigational drugs for breast cancer treatment and discuss whether these strategies are likely to have a future place in the neoadjuvant management of breast cancer.

## 2 Hormone Receptor-Positive Breast Cancer

Approximately 70% of breast cancers are hormone receptor-positive [30, 31]. Most tumors with >50% ER expression and a low proliferation index respond well to endocrine therapy [32–35]. We describe currently applied neoadjuvant treatment regimens (summarized in Table 1) and promising strategies with new drugs.

### 2.1 Chemotherapy

The chemotherapy regimens to be used in the neoadjuvant setting are the same as those used in the adjuvant setting [24, 25]. A chemotherapy regimen containing an anthracycline, cyclophosphamide, and a taxane is mostly recommended in the neoadjuvant setting. A meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) in 44,000 patients showed that the addition of a taxane to a fixed anthracycline-based regimen improves breast cancer-specific survival (BCSS), with a hazard ratio (HR) of 0.86 [standard error (SE) 0.04, $p = 0.0005$]. However, when anthracycline dose in the non-taxane control arm was also increased, no added effect of taxanes was seen (HR 0.94, SE 0.06, $p = 0.1$) [36]. Taxanes are

**Table 1** Established neoadjuvant treatment regimens per subtype

| | | |
|---|---|---|
| ER+/PR+ low risk[a] | Endocrine therapy | Premenopausal: tamoxifen or aromatase inhibitor + LHRH agonist |
| | | Postmenopausal: aromatase inhibitor |
| ER+/PR+ high risk[b] | Chemotherapy | 4× ddAC → 12× P weekly, or 4× T 3-weekly |
| | | 6× TAC 3-weekly |
| | | 6× FEC → 4× T 3-weekly |
| | | Anthracycline free: 4× TC |
| HER2+ | Chemotherapy + anti-HER2[c] | Anthracycline free: 6–9× taxane[d] + Cb + Tzt + Ptz |
| | | 6× T (3-weekly) + Cb + Tzt + Ptz |
| | | Anthracycline containing: (F)EC + Tzt + Ptz → taxane[d] + Tzt + Ptz |
| Triple-negative | Chemotherapy | 4× ddAC → 12× P weekly (+4× Cb) |
| | | 6× TAC |

*BCSS* breast cancer-specific survival, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *LHRH* luteinizing hormone-releasing hormone, *dd* dose-dense (2-weekly), *A* adriamycin, *C* cyclophosphamide, *P* paclitaxel, *T* docetaxel, *Cb* carboplatin, *Tzt* trastuzumab, *Ptz* pertuzumab, *F* 5-Fluorouracil, *E* epirubicin

[a] Low risk is considered a predicted 10-year BCSS without systemic treatment >88% for hormone receptor-positive breast cancer

[b] High risk is considered a predicted 10-year BCSS without systemic treatment ≤88% for hormone receptor-positive breast cancer

[c] In cases of T1 and N0, paclitaxel plus trastuzumab can be considered

[d] Either weekly paclitaxel or 3-weekly docetaxel

equally effective if administered concurrently or sequentially with anthracyclines, although concurrent regimens such as docetaxel/doxorubicin/cyclophosphamide (TAC) show increased toxicity and require prophylactic administration of granulocyte colony-stimulating factor [37–39]. The choice between various anthracycline/taxane regimens is therefore mainly a matter of toxicity and duration (see Table 1). Within the sequential regimens, weekly paclitaxel improves disease-free survival (DFS) and OS compared with 3-weekly paclitaxel. Three-weekly docetaxel also improves DFS compared with 3-weekly paclitaxel, but not OS [38, 40].

More frequent administration of cytotoxic therapy (dose-dense) is a more effective way of minimizing residual tumor burden than dose-escalation [41]. In a meta-analysis of ten randomized controlled trials (RCTs), dose-dense-administered chemotherapy improved OS by 16% [HR 0.84, 95% confidence interval (CI) 0.72–0.98, $p = 0.03$] and DFS by 17% (HR 0.83, 95% CI 0.73–0.94, $p = 0.005$) [42]. In the few trials that were designed to analyze the pure effect of dose-dense compared with standard-dose chemotherapy, the benefit on both OS and DFS was largest for hormone receptor-negative tumors [39, 42–45]. A recent pooled analysis of two Italian trials showed a larger benefit of the dose-dense regimen for premenopausal women [46].

Patients unfit for anthracyclines (e.g. due to cardiac symptoms) may benefit from four cycles of docetaxel/cyclophosphamide (TC) every 3 weeks. This regimen improved OS compared with four cycles of adriamycin/cyclophosphamide (AC) after a median follow-up of 7 years (HR 0.69, 95% CI 0.50–0.97, $p = 0.032$) [47]. A joint analysis of the three ABC trials comparing six cycles of TC with six cycles of TAC in HER2-negative breast cancer patients after a median follow-up time of 3.3 years was unable to demonstrate non-inferiority for the non-anthracycline regimen (HR 1.23, 95% CI 1.01–1.50, $p = 0.04$). To determine the effect on survival, longer follow-up is needed [48].

## 2.2 Endocrine Therapy

### *2.2.1 Premenopausal Patients*

Neoadjuvant endocrine therapy for premenopausal women is largely unstudied [34]. In the only neoadjuvant study performed, more patients had a clinical response with anastrozole plus ovarian function suppression (OFS) than in the tamoxifen group (70 vs. 51%, respectively; estimated difference between groups 20%, 95% CI 7–33%, $p = 0.004$) [49]. Whether this is partly due to the longer time required to achieve steady-state drug concentrations for tamoxifen (±2 months) than for an aromatase inhibitor [(AI); ±2 weeks] is unknown. Longer duration of treatment (24 weeks) showed a higher response rate than shorter duration (16 weeks) in both groups, but the optimal duration of endocrine therapy has yet to be determined [49].

OFS is required for premenopausal patients treated with an AI as an AI does not suppress gonadal estrogen production. OFS added to adjuvant tamoxifen in premenopausal women showed non-significant improvement in both DFS and OS in a combined analysis of two

randomized trials (SOFT [50] and ECOG-3193 [51]) [52]. In a combined analysis of the adjuvant SOFT [50], TEXT [53], and ABCSG-12 [54] trials, numerically better DFS was seen in the AI plus OFS group compared with the tamoxifen plus OFS group, but was not statistically significant (HR 0.89, 95% CI 0.57–1.39). OS was numerically, but not statistically significant, worse in the AI plus OFS group (HR 1.31, 95% CI 0.93–1.84) [52]. In general, the addition of OFS results in more pronounced endocrine side effects (such as hot flushes and sweats), which are more frequently reported in patients using tamoxifen plus OFS compared with those using an AI plus OFS. In both groups, the side effects improved over time but did not reach baseline. Persistent vaginal dryness, sexual issues, and short-term bone or joint pain are more often reported for an AI plus OFS. No difference in quality of life was reported between the two groups [55].

Based on higher response rates with the addition of OFS to endocrine therapy in the metastatic setting, OFS may improve pCR rates when added to neoadjuvant endocrine therapy. In concordance with the updated American Society of Clinical Oncology (ASCO) guideline, the choice for an AI versus tamoxifen in both the adjuvant and neoadjuvant setting should be based on adverse events (AEs) [56].

#### 2.2.2 *Postmenopausal Patients*

For postmenopausal women, an AI is preferred over tamoxifen. A subset-analysis within a larger meta-analysis comprising seven prospective RCTs and approximately 1400 patients demonstrated a highly statistically significant benefit favoring an AI over tamoxifen for the clinical response rate [CRR; odds ratio (OR) 1.69, 95% CI 1.36–2.10, $p < 0.001$], radiological response rate (OR 1.49, 95% CI 1.18–1.89, $p < 0.001$), and BCS rate (OR 1.62, 95% CI 1.24–2.12, $p < 0.001$) [34]. Several trials compared 3–4 months of treatment with an AI with treatment duration up to 8–12 months. In these trials, longer treatment increased pCR and BCS rates [57–60]. One study reported 7.5 months of treatment with an AI as optimal duration to achieve maximum tumor reduction sufficient for BCS [61]. Taken together, for postmenopausal women who will be treated with neoadjuvant endocrine therapy, an AI is recommended and longer treatment duration showed higher pCR and BCS rates.

### 2.3 Overcoming Endocrine Resistance

De novo resistance to endocrine therapy occurs in a small group of patients with hormone receptor-positive early breast cancer (EBC). Additionally, approximately one-third of patients eventually relapse and are considered to be resistant to endocrine therapy [62–66]. For these patients, alternative endocrine strategies are crucial. Several mechanisms are described to play a role in endocrine resistance, including dysregulation of the cyclin D-cyclin-dependent kinase (CDK) 4/6-INK4-retinoblastoma (Rb) pathway and activation of the mammalian target of rapamycin/protein kinase B/phosphoinositide 3-kinase (mTOR/Akt/PI3K) pathway (Fig. 1) [66–70]. Below we describe promising strategies targeting these pathways and their potential role in the neoadjuvant setting.

#### 2.3.1 *Cyclin D-Cyclin-Dependent Kinase (CDK) 4/6 Inhibitors*

When intact, CDK4/6 functions as an important switch in the progression from G1 to S-phase via the cyclin-D-CDK4/6-INK-Rb-pathway (Fig. 1) [71]. However, in many cancers this pathway is disrupted through mutations or amplifications of CDK4/6, which in turn promotes cell proliferation and resistance to endocrine therapy [34, 72]. Targeting CDK4/6 with selective inhibitors (palbociclib or ribociclib) combined with endocrine treatment in first- and second-line treatment improves progression-free survival (PFS) in patients with hormone receptor-positive metastatic breast cancer (MBC); median PFS for ribociclib and letrozole was not reached (95% CI 19.3–not reached) in the Monaleesa-2 trial and was 24.8 months (95% CI 22.1–not reached) in the Paloma-2 trial [73–75]. Subgroup analyses of the Paloma-2 and Monaleesa-2 trials showed that the benefit of CDK4/6 inhibition (with palbociclib and ribociclib, respectively) was also seen in patients with de novo MBC [73]. The objective response rate (ORR; complete response + partial response) for ribociclib and letrozole was 47% (38–57%), compared with 34% (25–42%) without ribociclib [76].

Most common AEs for palbociclib and ribociclib include neutropenia, which resolves more rapidly than neutropenia caused by cytological agents, and results less often in febrile neutropenia and non-hematological AEs such as nausea, fatigue, diarrhea, and asthenia [77, 78]. The third developed CDK inhibitor, abemaciclib, is more selective in targeting CDK6 and has a slightly distinct toxicity profile than the other CDK4/6 inhibitors. It is less myelotoxic but induces more gastrointestinal-related AEs. Results from the combination of abemaciclib and letrozole or anastrozole for locally recurrent breast cancer or MBC in the phase III Monarch-3 (NCT02246621) study are still awaited. At this moment, palbociclib in combination with letrozole or fulvestrant [77] is approved by the FDA and EMA for treatment of women with hormone receptor-positive/HER2-negative MBC. Ribociclib in combination with an AI is also approved by the FDA and is currently under review by the EMA [74].

Given the benefit of CDK4/6 inhibition in patients with MBC, implementation in the neoadjuvant setting seems a

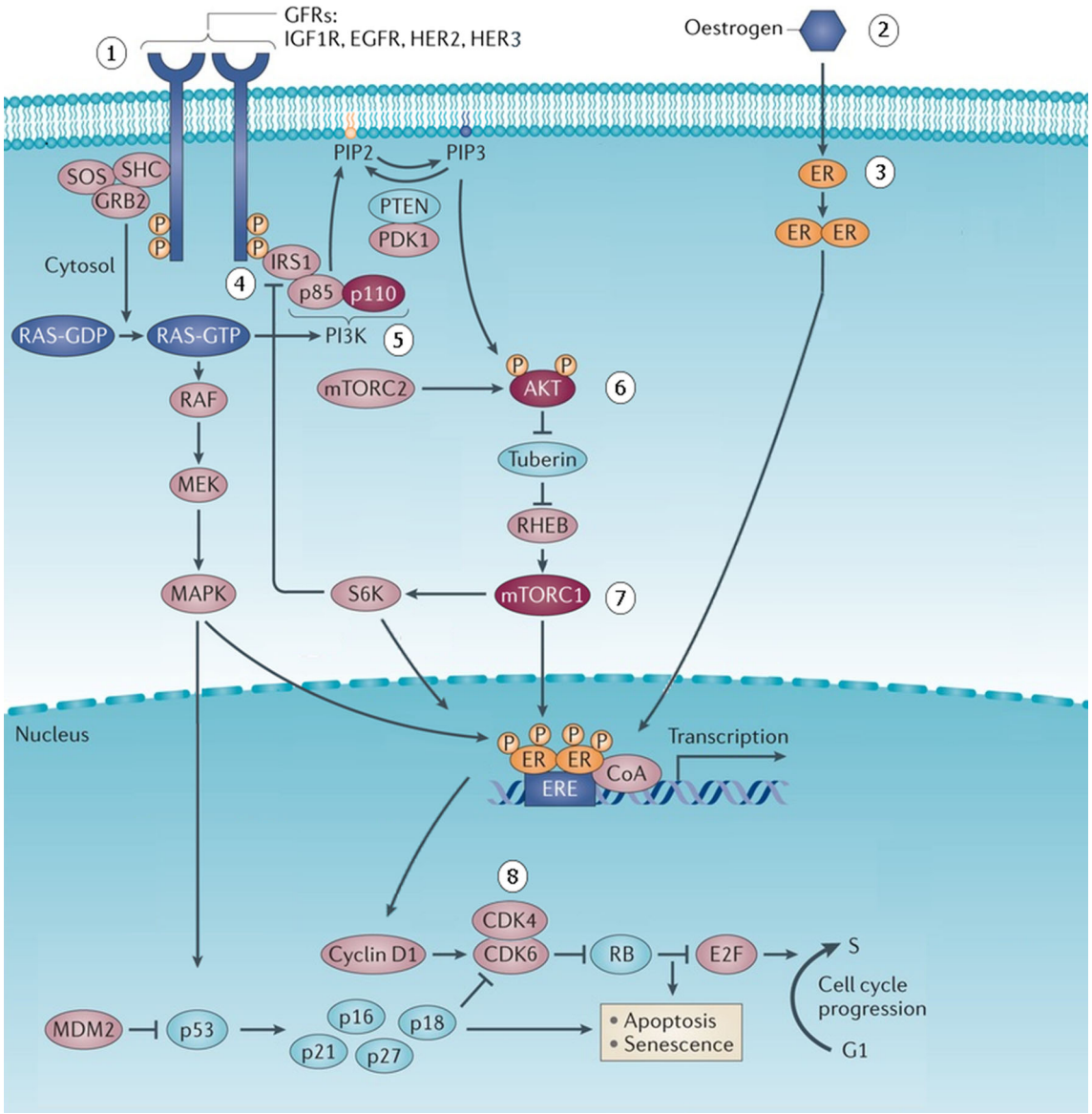


**Fig. 1** Schematic overview of drug class targets in breast cancer. *GFRs* growth factor receptors, *IGF1R* insulin-like growth factor 1 receptor, *EGFR* epidermal growth factor receptor, *HER2* human epidermal growth factor receptor 2, *HER3* human epidermal growth factor receptor 3, *SOS* Son of Sevenless, *SHC* SHC adaptor protein, *GRB2* growth factor receptor-bound protein 2, *P* phosphorylation, *ER* estrogen receptor, *IRS1* insulin receptor substrate 1, *PI3K* phosphoinositide 3-kinase, *p85* regulatory subunit of PI3K, *p110* catalytic subunit of PI3K, *RAS-GDP* rat sarcoma guanosine diphosphate, *RAS-GTP* rat sarcoma guanosine triphosphate, *RAF* rapidly accelerated fibrosarcoma, *MEK* MAPK/ERK kinase, *MAPK* mitogen-activated protein kinase, *mTORC2* mechanistic target of rapamycin complex 2, *AKT* protein kinase B, *RHEB* RAS homolog enriched in brain, *mTORC1* mechanistic target of rapamycin complex 1, *S6K* ribosomal S6 kinase, *ERE* estrogen receptor response element, *CoA* coactivators, *CDK4* cyclin-dependent kinase-4, *RB* retinoblastoma protein, *E2F* E2 factor family of transcription factors, *MDM2* mouse double minute 2 homolog, *LHRH* luteinizing hormone-releasing hormone. *1* Antibodies against HER2 (trastuzumab, pertuzumab), against HER3 (e.g. patritumab, seribantumab), and antibody-drug conjugates (e.g. T-DM1, SYD985). *2* Therapy that reduces systemic estrogen levels, including aromatase inhibitors (e.g. letrozole, anastrozole, exemestane) and ovarian function suppression (e.g. LHRH agonists, oophorectomy). *3* Drugs targeting the estrogen receptor (e.g. tamoxifen, fulvestrant). *4* Tyrosine kinase inhibitors (e.g. lapatinib, neratinib, afatinib). *5* PI3K inhibitors (e.g. buparlisib, pictilisib, alpelisib, taselisib). *6* Akt inhibitors (e.g. MK-2206). *7* mTOR inhibitors (e.g. everolimus, temsirolimus). *8* CDK4/6 inhibitors (e.g. palbociclib, ribociclib, abemaciclib). Adapted with permission from Macmillan Publishers Ltd. [273]. Copyright (2015)

promising approach. All three CDK4/6 inhibitors are currently evaluated in the neoadjuvant setting. In the NeoMonarch trial (NCT02441946), postmenopausal patients with hormone receptor-positive/HER2-negative breast cancer were randomized between anastrozole, abemaciclib, or the combination for 2 weeks, followed by 14 weeks combination treatment in all patients. Abemaciclib alone and in combination with anastrozole significantly

decreased Ki-67 levels after 2 weeks of treatment compared with anastrozole alone. Therewith, the study met its primary endpoint [79]. Results from a neoadjuvant single-arm, phase II study ($n = 50$) showed that palbociclib added to anastrozole completely arrested cell cycle in 87% of the patients with stage II–III breast cancer after 15 days of treatment compared with 26% of patients in the first cycle with anastrozole alone, regardless of *PIK3CA, PTEN* or *TP53* mutation status. However, Ki67 levels at surgery after a median washout period of 29 days were lower than after 15 days of treatment within the same patients. This was not observed for patients ($n = 8$) who continued with palbociclib until surgery. None of the patients achieved pCR [80]. Results from other trials evaluating the efficacy, optimal duration of treatment, and predictive biomarkers for the combination of a CDK4/6 inhibitor and endocrine treatment in the neoadjuvant setting are eagerly anticipated [examples include NCT01723774, NCT02296801 (Pallet), NCT02712723 (Feline), and NCT02520063].

#### 2.3.2 *Phosphoinositide 3-Kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/Akt/mTOR) Inhibitors*

The most intensively studied inhibitors of the PI3K/Akt/mTOR pathway are inhibitors of the mTOR. They inhibit tumor growth and restore sensitivity to endocrine treatment in tumors with upregulated Akt signaling [81, 82]. Everolimus, the first approved mTOR inhibitor, in combination with exemestane, substantially improved DFS (HR 0.36, 95% CI 0.27–0.47, $p < 0.001$) in postmenopausal patients with hormone receptor-positive/HER2-negative MBC who progressed on treatment with an AI [82]. However, the improvement in DFS with everolimus plus exemestane in a similar group of patients in the BOLERO-2 trial did not translate into a significantly improved OS (HR 0.89, 95% CI 0.73–1.10, $p = 0.14$) [83, 84]. Everolimus combined with tamoxifen resulted in a similar improvement in DFS in AI-resistant MBC patients (HR 0.54, 95% CI 0.36–0.81, $p = 0.0021$) and improvement in OS after a median follow-up of 24 months (HR 0.45, 95% CI 0.24–0.81, $p = 0.007$). Exploratory subgroup analysis showed a larger benefit in patients with acquired endocrine resistance compared with primary resistance [85]. Side effects of mTOR inhibitors include stomatitis, rash, hyperglycemia, diarrhea, nausea, and anorexia, and are usually mild to moderate, but can be life-threatening in cases of non-infectious pneumonitis [82, 84, 86–89].

A trial with temsirolimus combined with letrozole in patients with AI-naive MBC was prematurely stopped as no improvement in DFS was seen at the second interim analysis [89]. Several reasons may explain the disappointing results, including the high number of HER2-positive tumors (23% and an additional 36% of tumors with unknown HER2 status in the temsirolimus arm), selection of AI-naive patients, and the intermittent schedule of temsirolimus. Perhaps dual mTOR1/mTOR2 inhibitors can overcome incomplete inhibition seen with mTOR inhibitors. Phase I/II trials with dual mTOR1/mTOR2 inhibitors [Sapanisertib (Tak228): NCT02619669, NCT02988986; and AZD2014: NCT01597388, NCT02216786] are still ongoing [90].

Everolimus combined with letrozole as neoadjuvant treatment for postmenopausal women improved the response rate measured by ultrasound (58 vs. 47%, $p = 0.035$) compared with letrozole plus placebo. However, only two patients had a pCR compared with one in the placebo group [86]. Long-term efficacy results are still awaited. The small benefit with the addition of everolimus came along with more grade 3–4 AEs (23 vs. 4%), and subsequent dose reductions were necessary in 53% of patients treated with the combination compared with 8% in the placebo group.

The PI3K/Akt/mTOR pathway may also be targeted more upstream. A diverse set of PI3K inhibitors is explored in early-phase clinical trials, including pan-class I PI3K inhibitors (buparlisib, pictilisib) and selective PI3Kα inhibitors (alpelisib, taselisib). In the two BELLE trials, buparlisib in combination with fulvestrant improved PFS modestly in postmenopausal women with MBC who progressed on endocrine treatment [91, 92]. The small benefit with PI3K inhibitors observed in the above-mentioned trials suggests a biological response in a subset of patients. In both trials, patients with a *PIK3CA*-mutation derived the most benefit [92]. Nevertheless, a validated biomarker to select patients for these inhibitors is still lacking [67, 93]. Noteworthy, treatment with buparlisib induced serious AEs, including transaminitis, hyperglycemia, rash, mood disorders, and suicidal attempts [86, 91, 92]. Toxicity profiles of the PI3Kα inhibitors seem to be much more favorable [94, 95]. First results of studies that directly compare pan-PI3K inhibitors with PI3Kα inhibitors (both in combination with letrozole) (NCT01923168) and large phase III studies with a PI3Kα inhibitor [e.g. NCT02340221 (Sandpiper)] are eagerly awaited.

Lastly, the mTOR/PI3K/Akt pathway may be targeted via Akt inhibitors. Akt kinase activity is increased in up to 55% of breast cancers [96] and is associated with worse outcome in ER-positive breast cancer [97]. One potent Akt inhibitor is MK-2206, which, combined with anastrozole or fulvestrant, resulted in a clinical benefit rate (CBR) of 37% (including two patients with partial response and nine with stable disease for >6 months) in a phase I study for patients with ER-positive MBC [93]. A neoadjuvant trial with the same Akt inhibitor is now ongoing (NCT01776008).

Taken together, the modest benefit seen in endocrine treatment-naive patients, lack of data on survival, serious

AEs, and lack of good biomarkers currently limit the role of mTOR/PI3K/Akt pathway inhibitors in the neoadjuvant setting. Studies to further explore the mechanism of action and biomarkers for mTOR/PI3K/Akt pathway inhibitors and the most optimal combinational approach, including triple combinations with endocrine therapy (tamoxifen or an AI), an mTOR/PI3K/Akt pathway inhibitor, and a CDK4/6 inhibitor in the neoadjuvant setting, are ongoing (e.g. NCT02520063).

### 2.4 Bisphosphonates

Bisphosphonates are established drugs for the prevention of skeletal-related events in MBC. In the last decade, interest has grown in their antitumor effects. In a meta-analysis of 26 trials, including the AZURE trial [98, 99], zoledronic acid added to standard adjuvant treatment improved the risk of distant recurrence, bone recurrence, and breast cancer mortality in postmenopausal women [100]. As bisphosphonates have no effect on pCR rates [101–104], their role as adjunct to neoadjuvant treatment seems limited.

## 3 Triple-Negative Breast Cancer

The triple-negative subtype accounts for 15% of breast cancers [105, 106]. This subtype is associated with a higher risk of recurrence and breast-cancer-related death than other subtypes in the first years after diagnosis. After 5–7 years, very few recurrences are seen [107–110]. Patients who develop TNBC at a young age (<50 years) or who have a family history of breast and/or ovarian cancer have a higher risk of harboring deleterious *BRCA1* or *BRCA2* germline mutations, with incidences ranging from 12 to 29% for *BRCA1* and 9 to 17% for *BRCA2* [111–113]. In TNBC patients unselected for *BRCA1/2* mutation risk, the prevalence is 11–16% for *BRCA1* mutations and 4% for *BRCA2* mutations [114, 115]. Besides a mutation in the *BRCA1/BRCA2* gene, hypermethylation of the *BRCA1* promotor, or hypermethylation of the Fanconi anemia gene *FANCF*, results in a BRCA-like phenotype [116, 117]. Approximately 50% of the triple-negative tumors in young women are BRCA-like [118, 119], and incidence declines with age [118]. BRCA-mutated and BRCA-like tumors share homologous recombination deficiency (HRD), which makes them more sensitive to DNA double-strand break (DSB)-inducing agents, such as anthracyclines, cyclophosphamide, and platinum salts. However, tumors can adapt during treatment and regain their ability to repair DNA DSBs. HRD can be determined with various genomic tests, which partially, but not completely, overlap [118, 120–125].

TNBC has a significantly higher percentage of tumor-infiltrating lymphocytes (TILs), higher expression of programmed death-ligand 1 (PD-L1) [126–131], and higher mutational load compared with other breast cancer subtypes [69, 132]. These findings provide a basis for studying immunotherapy approaches in TNBC. Nevertheless, the mainstay treatment for TNBC is chemotherapy, while many efforts are made to optimize chemotherapeutic regimens, targeted strategies, and immune checkpoint blockade for this aggressive breast cancer subtype. Current standard treatment and promising new drugs are discussed below.

### 3.1 Chemotherapy

Similar to what we described under the chemotherapy section for hormone receptor-positive breast cancer, the preferred chemotherapy regimen in TNBC is dose-dense anthracyclines plus cyclophosphamide followed by a taxane (Table 1) [42–44]. In TNBC, the addition of carboplatin to 12-times-weekly paclitaxel increased pCR from 41 to 54% (OR 1.71, one-sided $p = 0.003$). This beneficial effect translated into improved BCS rates [133]. Although underpowered, the addition of carboplatin did not result in a survival benefit after a median follow-up of 39 months. A significantly improved 3-year event-free survival [(EFS); HR 0.30, 95% CI 0.19–0.45] and 3-year OS (HR 0.20, 95% CI 0.11–0.36) was observed for patients who achieved pCR compared with patients who did not [134]. In the GeparSixto trial, the addition of carboplatin to doxorubicin, paclitaxel and bevacizumab in patients with stage II–III breast cancer increased the pCR rate to 53%, compared to 37% without carboplatin (OR 1.94, 95% CI 1.24–3.04, $p = 0.005$) [135]. The GeparSixto trial has not yet published OS data on the effect of carboplatin; however, pCR is strongly associated with OS in TNBC and this argument is often used to add carboplatin to neoadjuvant treatment in TNBC [21]. The addition of carboplatin comes at a cost of increased grade 3–4 neutropenia, thrombocytopenia, anemia, and diarrhea [133, 135]. Several studies evaluating the addition of carboplatin to neoadjuvant chemotherapy in TNBC are ongoing, including one specifically for tumors harboring HRD (NCT01042379).

Patients with a tumor harboring HRD may benefit more from intensified alkylating chemotherapy supported by autologous peripheral stem cell transplantation (PSCT) than from standard chemotherapy. A meta-analysis including 15 RCTs and 6211 breast cancer patients (including 379 TNBC patients) showed the greatest reduction in risk of death (33%) compared with other subtypes [136]; however, the included trials have limitations. Among the most important, patients in the control arms of 5 of the 15 trials received a higher cumulative chemotherapy dose than patients in the 'intensified' arms. In a retrospective analysis of one of the studies, a significant benefit for intensified

chemotherapy was seen among patients with HRD (adjusted HR 0.19, 95% CI 0.08–0.48, $p = 0.001$) [120]. Other studies confirmed this striking observation [137, 138]. The predictive value of HRD for intensified chemotherapy benefit is now being prospectively tested in two RCTs (NCT01057069 and NCT02810743) [139]. While awaiting the results of these trials, intensified chemotherapy should not be used outside the context of a clinical trial.

Based on the beneficial effect and acceptable safety profile of capecitabine in MBC, interest has grown in using capecitabine in both the neoadjuvant and adjuvant setting [140]. In a recent meta-analysis of seven trials in the adjuvant and neoadjuvant setting, the addition of capecitabine to a anthracycline/taxane chemotherapy regimen improved DFS in patients with TNBC (HR 0.73, 95% CI 0.59–0.91, $p = 0.005$) and in patients with three or more positive axillary lymph nodes regardless of subtype (HR 0.74, 95% CI 0.59–0.94, $p = 0.012$) [141]. However, an OS benefit for TNBC with neoadjuvant or adjuvant capecitabine has only been reported in two trials—the FinXX and Create-X trials [142–144]. Preliminary results of the FinXX trial showed significantly improved OS in 202 patients with TNBC after a median follow-up of 10 years (HR 0.55, 95% CI 0.31–0.96, $p = 0.037$) with the addition of capecitabine to an anthracycline/taxane-based regimen (T-CEF) [144]. The Create-X trial addresses the issue of residual disease, which is one of the strengths of a neoadjuvant treatment approach. In that trial, Asian patients with residual disease after neoadjuvant chemotherapy were randomized to receive adjuvant capecitabine for six to eight 14-day treatment cycles (with a 7-day break) or no chemotherapy. Patients with hormone receptor-positive disease received adjuvant endocrine therapy. Adjuvant capecitabine improved 5-year DFS (HR 0.70, 95% CI 0.53–0.93, $p < 0.005$) and OS (HR 0.60, 95% CI 0.40–0.92, $p < 0.01$). Patients with TNBC benefitted most from adjuvant capecitabine (HR 0.58, 95% CI 0.39–0.87). More cases of neutropenia, hand-foot syndrome, and gastrointestinal-related AEs were observed with capecitabine [142]. The incorporation of capecitabine in neoadjuvant or adjuvant strategies seems beneficial for at least the subset of TNBC patients with residual disease after standard systemic therapy.

### 3.2 Poly(ADP-Ribose) Polymerase (PARP) Inhibitors

Poly(ADP-Ribose) polymerase (PARP) inhibitors disturb the repair of single-strand DNA breaks (SSB), mainly via two mechanisms. The first is inhibition of the PARP-1 enzyme, preventing binding to SSBs and accumulation of repair enzymes by PARP-1. Second, PARP inhibitors trap PARP-1 enzymes onto the DNA, which obstructs the replication fork necessary for DNA repair [145]. The subsequent persisting SSBs are converted to DSBs during DNA replication. If repair via homologous recombination is not possible, error-prone repair mechanisms take over (e.g. non-homologous end-joining), leading to accumulation of DSBs, which ultimately become lethal to the cell. *BRCA1*- and *BRCA2*-mutated tumors are specifically sensitive to PARP inhibitors; this concept is known as synthetic lethality (Fig. 2) [146]. The PARP inhibitor olaparib is approved for the treatment of relapsed BRCA-mutated ovarian cancer, and in BRCA-mutated MBC showed an ORR of 41% and a median PFS of 5.7 months [147]. In the neoadjuvant setting, the addition of both carboplatin and veliparib to a neoadjuvant anthracycline/taxane-containing regimen was evaluated in the I-Spy-2 trial [148]. The addition led to a doubling of the pCR rate in patients with TNBC, from 26 to 52%; however, more patients in the veliparib group had a *BRCA1/2* mutation (17 vs. 7%) [148] and further research is necessary to evaluate whether this gain in pCR rate could also be reached with the addition of carboplatin alone. Exploratory analysis showed that HRD [149] and a PARP1–7 signature [124] could predict sensitivity to veliparib [139]. Whether these results can be translated to all patients with an HRD breast tumor (including hormone receptor-positive tumors), and which test can best identify these patients, remains to be seen. In vivo experiments with HRD tumors showed conflicting results, indicating there might be a gradient in HRD influencing the response to PARP inhibitors [150, 151]. HRD as a predictive biomarker for the more potent PARP inhibitor niraparib will be evaluated in the ABC study (NCT02826512). Talazoparib is currently being evaluated in the neoadjuvant setting, followed by standard (neo)adjuvant treatment (NCT02282345). Preliminary results show a mean decrease in tumor volume of 78% (range 30–98%), assessed by ultrasound in all 13 patients, and no grade 4 toxicities [152]. Olaparib added to standard neoadjuvant chemotherapy and carboplatin will be evaluated in three trials—NCT02561832, NCT02789332 (GeparOla), and NCT02624973. The combination rucaparib and cisplatin will be evaluated in patients with residual disease after standard neoadjuvant chemotherapy, in a phase II trial [NCT01074970 (BRE09-146)]. Lastly, olaparib will be evaluated as adjuvant treatment up to 12 months after completion of (neo)adjuvant treatment in the Olympia trial (NCT02032823).

The main toxicities with olaparib and veliparib are mild (grade 1–2) hematological toxicities, nausea and vomiting, diarrhea, dyspepsia, fatigue, and dizziness. Grade 3–4 toxicities occurred in 18–25% of patients, mainly consisting of nausea, vomiting, and anemia [147, 153, 154]. A similar rate of grade 3–4 toxicities were reported in patients using olaparib maintenance therapy for more than 2 years

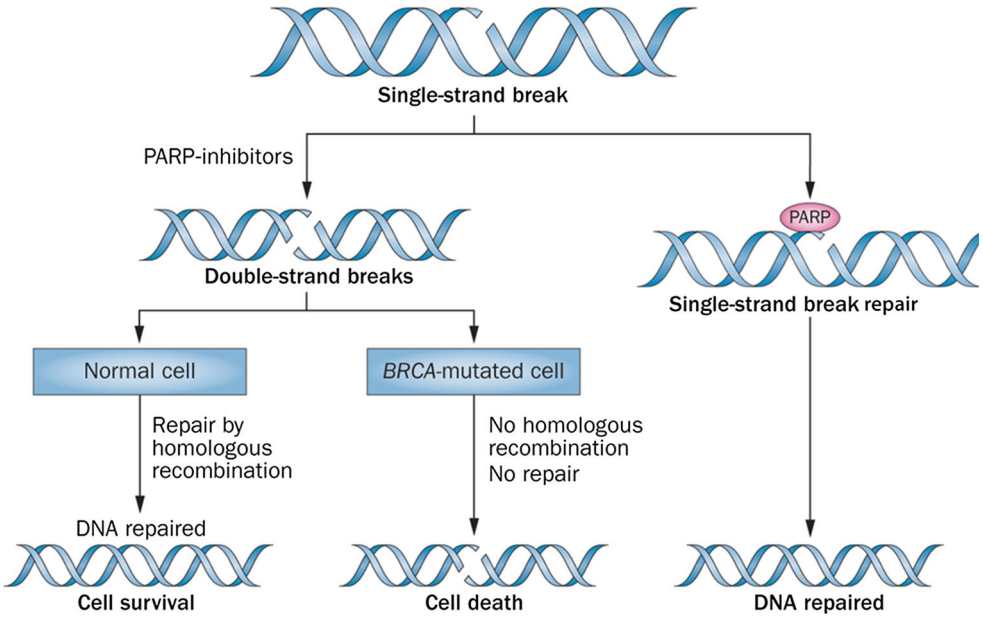


**Fig. 2** Simplified demonstration of synthetic lethality by PARP inhibitors in BRCA-mutated tumors. *PARP* Poly (ADP-ribose) polymerase. Adapted with permission from Macmillan Publishers Ltd. [274]. Copyright (2014)

[155, 156]. Concerns were raised on the development of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) during maintenance treatment. Until now, the effect of prior chemotherapy on the development of MDS/AML cannot be distinguished from a possible effect of PARP inhibition. Patients receiving maintenance therapy need to be carefully monitored. Given the favorable toxicity–benefit ratio in BRCA-mutated breast cancers, PARP inhibitors offer a promising additive to neoadjuvant treatment for this subgroup.

### 3.3 Immune Checkpoint Inhibitors

Immunotherapeutic interventions that reactivate the endogenous T-cell compartment represent the most significant development in oncology in the past decade. There are several 'immune checkpoints' that regulate the immune system. Programmed death-1 (PD-1) is an immune checkpoint that is found on the surface of T cells. When PD-1 is bound by its ligand PD-L1, the function of T cells is inhibited. Cancer cells express PD-L1 and can thereby efficiently suppress T-cell activity. Antibodies against the checkpoint molecules cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1 have now been approved for melanoma, lung cancer, and renal cell cancer.

Early trials with anti-PD-(L)1 show durable responses in approximately 3–19% of MBC patients [157–160]. With response rates around 9–19%, patients with TNBC seem to benefit relatively more often from anti-PD-(L) compared with patients with other breast cancer subtypes [157–159]. In Keynote-012, a phase Ib study with pembrolizumab (anti-PD1) in patients with advanced PD-L1-positive TNBC, the ORR was 18.5% (5 of 27 evaluable subjects), with one complete response and four partial responses [159]. Limited preliminary data from phase I/II trials suggest that response rates are higher in metastatic TNBC patients receiving chemotherapy plus PD-1 blockade [161, 162]. Numerous clinical trials with (combination)immunotherapy are ongoing in metastatic TNBC (e.g. NCT02499367 and NCT02425891), and we expect that immune checkpoint blockade will become part of the standard treatment for a subset of patients with metastatic TNBC.

No data on the efficacy of neoadjuvant immune checkpoint blockade in TNBC are available yet, but many neoadjuvant trials are ongoing. Most trials evaluate anti-PD-(L)1 together with standard chemotherapy (e.g. NCT02489448 and NCT02622074) or in patients with residual disease after neoadjuvant chemotherapy (NCT02530489 and NCT02954874). Given the power of immunotherapy to induce durable responses, it is possible that in a small subgroup of high-risk primary TNBC patients, anti-PD-(L)1 becomes standard of care. However, the central research goal for the coming years will be to determine which high-risk TNBCs will benefit from immunotherapy.

### 3.4 Angiogenesis Inhibitor

Bevacizumab is a monoclonal antibody against the vascular endothelial growth factor (VEGF), especially isoform VEGF-A, which is a ligand for the VEGF receptor and which promotes angiogenesis [163–165]. The addition of bevacizumab to an anthracycline/taxane chemotherapy regimen increased pCR rates in some studies [166, 167] but not in all [133, 168]. Moreover, the increase in pCR has,

until now, not translated into improved RFS or OS [134]. In addition, bevacizumab, when added to adjuvant chemotherapy (anthracyclines with or without taxanes), did not result in long-term clinical benefit in terms of either invasive DFS or OS (HR 0.87, 95% CI 0.72–1.07 for invasive DFS, and HR 0.84, 95% CI 0.64–1.12 for OS) [169]. Lastly, bevacizumab added to chemotherapy causes serious toxicities, including febrile neutropenia, hypertension, and mucositis [133, 167, 170, 171]. Taken together, these data leave little evidence to incorporate bevacizumab or other VEGF inhibitors in neoadjuvant treatment regimens.

### 3.5 Anti-Androgens

The androgen receptor (AR) is expressed in approximately 70–80% of all breast cancers, including one-third of TNBCs [172–174]. The anti-androgens bicalutamide and enzalutamide induced a CBR of 19 and 38%, respectively (including two complete responses and five partial responses in the latter), in a small group of women with AR-positive/triple-negative, pretreated MBC [175, 176]. Although the response rate was relatively low, the efficacy of anti-androgen therapy is noteworthy in this subtype for which chemotherapy is still the mainstay of treatment and chemotherapy resistance is a major problem. Moreover, anti-androgen treatment has mild AEs, with the only grade 3 event being fatigue.

Enzalutamide is the first anti-androgen to be evaluated in the neoadjuvant setting in combination with paclitaxel in patients with TNBC (NCT02689427). Interestingly, combinations of bicalutamide and palbociclib (NCT02605486) and enzalutamide and taselisib (NCT02457910) are now under investigation in patients with AR-positive/triple-negative MBC [177]. Optimization of such combinations might be an interesting approach for a subset of patients with TNBC.

## 4 Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Breast Cancer

Approximately 15–20% of breast cancers are HER2-positive [178–181]. Without HER2-directed treatment, HER2-positive breast cancer is characterized by an aggressive course of disease and a poor prognosis [182, 183].

### 4.1 Current Standard Neoadjuvant Therapy

#### *4.1.1 HER2 Antibodies*

Trastuzumab, the first approved HER2-targeted agent, is a monoclonal antibody directed towards the extracellular domain (subdomain IV) of HER2. Trastuzumab blocks HER2 activation, inhibits intracellular signaling pathways, initiates antibody-dependent cellular cytotoxicity (ADCC), and prevents the formation of the constitutive active p95HER2 fragment (Fig. 2) [184–188]. The introduction of trastuzumab has substantially improved the outcome of patients with HER2-positive breast cancer [189–193]. In a meta-analysis including eight randomized trials in EBC, trastuzumab reduced the risk of recurrence or death by 40% at an overall median follow-up of 2.4 years [194]. A sustained benefit was seen at 10 years, with DFS rates of 69–74% with trastuzumab compared with 62–67% without [195–197]. In two trials, neoadjuvant-administered trastuzumab at least doubled the pCR rate compared with chemotherapy alone, and improved EFS [198–200]. Therefore, trastuzumab-based therapy is the standard of care in HER2-positive breast cancer. In EBC, trastuzumab is administered for 1 year as a longer duration has not shown to be superior and the non-inferiority of a shorter duration could not be demonstrated [201–203]. Results of other trials examining shorter treatment duration are still awaited [NCT00712140 (Persephone), NCT00629278 (Short-HER), and NCT00593697 (SOLD)]. Furthermore, trastuzumab concurrent with, at least the taxane part of, chemotherapy is preferred over sequential use [204].

Pertuzumab is a monoclonal antibody directed towards the extracellular dimerization domain (subdomain II) of HER2. Binding prevents heterodimerization with other HER receptors, of which HER2/HER3 blockade seems clinically the most relevant; ADCC might also play a role [184]. In the Neosphere trial, docetaxel plus dual HER2 blockade with trastuzumab and pertuzumab was superior to trastuzumab or pertuzumab alone, with pCR rates of 46% compared with 29 and 24%, respectively [205]. More recently, the survival results also revealed a numerically higher efficacy of dual blockade, with a 5-year PFS (definition equivalent to EFS) of 86% compared with 81% with trastuzumab alone (HR 0.69, 95% CI 0.34–1.40), although lacking statistical significance [206]. The trastuzumab/pertuzumab combination with different chemotherapy backbones resulted in pCR rates of 55–64% in the Tryphaena trial [207] and 63–69% in the GeparSepto trial [208]. A pCR rate of 91% has been reported with trastuzumab/pertuzumab plus paclitaxel in 42 HER2-positive/hormone receptor-negative tumors [209]. Pertuzumab received accelerated approval for neoadjuvant use in combination with trastuzumab-based chemotherapy [25]; however, definitive approval in EBC awaits survival results of the large ($n$ = 4806) randomized adjuvant Aphinity trial (NCT01358877). Neoadjuvant pertuzumab can be considered for patients with a high risk of recurrence, which the NCCN guideline defines as primary tumors measuring more than 2 cm, and/or node-positive disease [25].

Of major public interest are trastuzumab biosimilars. Although they do not convey new drug targets, they will impact price development and are expected to reduce the costs of healthcare. As such, they are an important development for the treatment of patients. A high degree of similarity with regard to efficacy and safety has been observed between biosimilars and original trastuzumab [210–212]. The first biosimilars are under review at regulatory health agencies.

#### 4.1.2 *Chemotherapy Backbone for HER2-Targeted Therapy*

Although trastuzumab is generally well-tolerated, its use is associated with an increased risk of cardiotoxicity, especially when combined with anthracyclines [189, 213–215]; therefore, anthracycline-free regimens were investigated. In the adjuvant BCIRG-006 trial, 10-year DFS and OS were similar with a carboplatin/taxane/trastuzumab regimen compared with a sequential anthracycline, taxane and trastuzumab regimen (DFS 73 vs. 75%, and OS 83 vs. 86%). However, the study was not powered to test a statistical difference between the two trastuzumab-containing arms. Cardiotoxicity and secondary hematological malignancies were less frequent with the anthracycline-free regimen [190, 195]. The neoadjuvant Tryphaena trial compared the cardiac tolerability of carboplatin/docetaxel with two sequential epirubicin-taxane arms in the presence of trastuzumab and pertuzumab. Significant decline in left ventricular ejection fraction ($\geq$10% points from baseline to <50%) during treatment was seen in 4–6% of patients [207].

In a lower-risk population, with the majority having node-negative disease, adjuvant TC plus trastuzumab resulted in 2-year DFS and OS estimates of 98 and 99%, respectively [216]. Similarly, a 3-year DFS of 99% was seen with adjuvant trastuzumab plus paclitaxel in patients with predominantly stage I HER2-positive disease [217]. In an attempt to further reduce treatment-related toxicity, this paclitaxel-trastuzumab regimen is being compared with adjuvant trastuzumab-emtansine (T-DM1) in the ongoing Atempt trial, which has recently completed enrollment (NCT01853748). Patients with stage I disease may receive their systemic treatment as neoadjuvant therapy, just as patients with stage II–III disease do, although the need for downstaging is less obvious; however, these patients may still benefit from other advantages of neoadjuvant therapy, including monitoring of the response. Different chemotherapy backbones with dual HER2 blockade are currently being evaluated in ongoing neoadjuvant studies, including weekly paclitaxel/carboplatin regimens [e.g. NCT01996267 (Train-2), NCT02789657 (BrUOG308), and NCT02436993] and T-DM1 [e.g. NCT02073487 (Teal) and NCT02326974].

In conclusion, in stage I HER2-positive EBC, anthracyclines can be safely withheld in the presence of trastuzumab, and the addition of pertuzumab is not likely to improve outcome further. While neoadjuvant systemic treatment is a reasonable option in stage I disease, most of these patients will receive their systemic treatment in an adjuvant setting. Currently available data suggest that carboplatin/taxane regimens are good alternatives for anthracycline-containing regimens in combination with trastuzumab plus/minus pertuzumab for stage II–III disease. Results of trials directly comparing anthracycline-containing and anthracycline-free regimens are soon expected [e.g. NCT01996267 (Train-2), NCT02510781, NCT02041338].

#### 4.1.3 *Anti-HER2 Therapy in Hormone Receptor-Positive/HER2-Positive Breast Cancer*

Significantly lower pCR rates in HER2-positive breast cancer are observed in hormone receptor-positive tumors compared with hormone receptor-negative tumors. A possible explanation is that these tumors use the ER pathway as an escape mechanism when HER2 is blocked (Fig. 1). In the randomized NSABP-B52 trial, the addition of concurrent endocrine therapy to trastuzumab-based chemotherapy plus pertuzumab increased the pCR rate from 41 to 46%, which was non-significant as the study was powered to detect an absolute increase of 15% [218]. Of note, the addition of endocrine therapy to chemotherapy was also not antagonistic, as had been observed in preclinical studies [219, 220].

#### 4.1.4 *De-Escalation*

Currently available treatment regimens with dual HER2 blockade are highly effective and pCR without the use of chemotherapy has been observed. In the Neosphere study, 17% of patients achieved a pCR after 12 weeks of trastuzumab plus pertuzumab without chemotherapy [205]. In hormone receptor-negative tumors, the same treatment resulted in a pCR rate of 34% [209], and pCR breast rates of 18–43% were seen with 12–24 weeks of trastuzumab plus lapatinib [221–223]. Strikingly, a pCR breast rate of 11% has been observed after only 10–12 days of trastuzumab plus lapatinib [224]. These results raise the question whether we can de-escalate treatment and omit or reduce chemotherapy. Research focusing on reducing treatment instead of adding additional compounds and prolonging treatment duration is desired.

In hormone receptor-positive tumors, 12 weeks of trastuzumab plus endocrine therapy resulted in a pCR rate of 15% [225], and 12–24 weeks of trastuzumab and endocrine therapy plus lapatinib resulted in pCR breast rates of

9–18% [221–223]. A pCR rate of 27% was observed with dual HER2 blockade with pertuzumab plus endocrine therapy and the CDK4/6 inhibitor palbociclib [226]. Several ongoing studies are evaluating different chemotherapy-free regimens [e.g. NCT02689921 (NeoAdapt) and NCT02907918 (Paltan)]. Withholding chemotherapy while maintaining efficacy is highly desirable, although it has yet to be discovered which patients and tumors qualify for this approach. Image-guided treatment adaptation may be a strategy to selectively reduce treatment. Intensifying or switching to an alternative regimen based on interim-imaging has received most attention in studies to date [10, 11, 13], but it is worth evaluating whether chemotherapy can be stopped prematurely in case of an early complete response on imaging. Starting off with biologicals alone and adding chemotherapy only in patients with insufficient response is another reasonable approach that deserves further exploration [221].

## 4.2 Antibody Drug Conjugates (ADCs)

Antibody drug conjugates are drugs composed of a cytotoxic agent linked to an antibody that targets antigens that are specific to or overexpressed in tumor cells. Additionally, the cytotoxic agent should be inactive when bound. After tumor binding, the complex is internalized and the cytotoxic agent is released intracellularly. This so-called 'targeted chemotherapy' improves selective drug delivery while minimizing systemic exposure to the cytotoxic agent [227].

### *4.2.1 Trastuzumab-Emtansine (T-DM1)*

T-DM1 is an ADC composed of trastuzumab and the microtubule inhibitor emtansine, a derivate of maytansine. In this composite form, trastuzumab retains its mechanisms of action [227, 228]. Due to its activity and apparent favorable toxicity profile in MBC [229–231], T-DM1 has also been evaluated in EBC. Twelve weeks of neoadjuvant T-DM1 after three to four cycles of AC induced a pCR rate of 56%, with good cardiac tolerability [232].

In the neoadjuvant I-Spy-2 trial, 83 patients received either T-DM1 plus pertuzumab, or paclitaxel plus trastuzumab, both followed by AC. The pCR rate was higher in the T-DM1-pertuzumab arm (52%) than in the paclitaxel-trastuzumab arm (22%) [233]. However, in the Kristine trial, pCR rates were significantly lower with T-DM1 plus pertuzumab compared with docetaxel/carboplatin plus trastuzumab and pertuzumab (44 vs. 56%, $p = 0.015$), although the safety profile was better with T-DM1 (grade 3–4 AEs in 13% of patients vs. 64%) [234]. In HER2-positive/hormone receptor-positive tumors, pCR rates were 42% with T-DM1 plus endocrine therapy, 41% with T-DM1 alone, and 15% with trastuzumab plus endocrine therapy [225]. The randomized Katherine trial ($n = 1487$) will provide more insight into the relative efficacy of T-DM1 versus trastuzumab as adjuvant therapy in 'no pCR' cases after completion of neoadjuvant therapy (NCT01772472).

In conclusion, neoadjuvant T-DM1 is not superior to standard polychemotherapy with trastuzumab and pertuzumab, but harbors a favorable toxicity profile when compared with trastuzumab–docetaxel combinations. T-DM1 currently has no role in the neoadjuvant or adjuvant setting, but this may change if non-inferiority with significantly less toxicity can be demonstrated compared with the taxane plus trastuzumab part of therapy in the presence of pertuzumab in both arms [e.g. NCT01966471 (Kaitlin)].

### *4.2.2 ADCs Under Investigation*

SYD985, composed of trastuzumab plus the DNA-alkylating agent duocarmycin (seco-DUBA), has shown promising preclinical activity and is currently being tested in metastatic cancers (NCT02277717) [235]. More ADCs are under development or are currently in clinical trials for advanced disease, including XMT-1522, which includes a HER2 antibody plus the antimitotic agent auristatin (NCT02952729), and U3-1402, consisting of a HER3-antibody and a topoisomerase I inhibitor (NCT02980341).

## 4.3 Other Antibodies

### *4.3.1 Anti-Vascular Endothelial Growth Factor*

In the randomized adjuvant Beth trial (>3500 patients), the addition of bevacizumab to trastuzumab-based chemotherapy did not improve DFS (92% in both arms, stratified HR 1.00, 95% CI 0.79–1.26) or OS (97 vs. 96%, stratified HR 0.87, 95% CI 0.60–1.25) at a median follow-up of 38 months. Grade 3–4 AEs were significantly more common with bevacizumab, including hypertension, bleeding, proteinuria, gastrointestinal perforations, and chronic heart failure [236]. In another trial, patients with insufficient response at first positron emission tomography evaluation randomized to bevacizumab added to neoadjuvant docetaxel plus trastuzumab, had a pCR rate of 44% compared with 24% without bevacizumab [11]. In a third trial, the pCR rate was similar with and without bevacizumab combined with a trastuzumab-based anthracycline-taxane regimen (57 vs. 58%), but higher if combined with a taxane-only regimen and trastuzumab (41 vs. 36%) [237].

Although bevacizumab has shown to increase the pCR rate in some subgroups in the neoadjuvant setting, it lacks survival benefit in the adjuvant setting and is associated

with potentially severe side effects. Therefore, bevacizumab should not be incorporated in current neoadjuvant regimens for HER2-positive EBC.

#### *4.3.2 Anti-HER3*

Antibodies against HER3 have been developed in view of the importance of HER3 in oncogenic signaling and its potential role in trastuzumab resistance. These antibodies capture HER3 in the inactive conformation by preventing ligand-binding (AMG888/patritumab, MM-121/seribantumab, AV-203) or by other means (LJM716) [238]. Of these, only MM-121 is currently evaluated in the neoadjuvant setting, although in HER2-negative EBC (NCT01421472).

#### *4.3.3 Bispecific Antibodies*

Bispecific antibodies have two different antigen-binding sites. MM-111 binds HER2 and HER3 with formation of a trimeric complex [238]. In a phase I study, patients with different HER2-positive tumors received MM-111 combined with other HER2-directed agents and/or chemotherapy. Depending on the treatment arm 17-44% had complete or partial response [239]. Ertumaxomab is a trifunctional, bispecific antibody targeting HER2 on tumor cells and CD3 on T cells, and recruits FcY receptor-positive cells, with subsequent activation of these immune cells [240]. Although it has shown activity in a phase I trial [241] and is theoretically promising, no ongoing study currently evaluates ertumaxomab in breast cancer. ZW25 is a bispecific antibody targeting two different epitopes of the HER2 receptor, with promising preclinical tumor activity. It is currently under investigation in a phase I trial (NCT02892123) [242].

### 4.4 Tyrosine Kinase Inhibitors

Lapatinib is an orally available, reversible inhibitor of the tyrosine kinase activity of HER1 and HER2. It has been evaluated extensively in the metastatic, adjuvant, and neoadjuvant settings. A meta-analysis of six neoadjuvant trials showed a significant absolute 13% (95% CI 8–19%) increase in pCR rate (either pCR breast or pCR breast and axilla) with trastuzumab plus lapatinib compared with trastuzumab alone [243]. Lapatinib results in lower pCR rates than trastuzumab [244–250]. Neoadjuvant lapatinib plus trastuzumab improved 3-year EFS non-significantly compared with trastuzumab alone (84 vs. 76%, HR 0.78, 95% CI 0.47–1.28) [251]. In addition, no significant DFS benefit was seen after a median follow-up of 4.5 years with adjuvant lapatinib plus trastuzumab over trastuzumab alone (HR 0.84, 95% CI 0.70–1.02) [252]. In the Teach trial, 1-year lapatinib after completion of chemotherapy without trastuzumab did not significantly improve DFS or OS, although a significant DFS benefit was seen in the hormone receptor-negative subgroup [253].

The TKI afatinib irreversibly targets HER1, HER2, and HER4. In the neoadjuvant setting, 6 weeks of afatinib monotherapy resulted in a partial response in 70% of patients (7/10) versus 75% (6/8) with lapatinib and 36% (4/11) with trastuzumab; a complete response was not seen [254]. In the neoadjuvant Dafne trial, 65 patients received 12 weeks of afatinib with concurrent paclitaxel and trastuzumab from week 6 followed by epirubicin/cyclophosphamide and trastuzumab. With 71% hormone receptor-positive tumors, the pCR rate was 49%, which was lower than the predefined lower boundary of 55%. Additionally, eight patients developed clinical progression during afatinib monotherapy and treatment was discontinued in 28% of patients [255].

Neratinib is another irreversible TKI that binds to the ATP site of the tyrosine kinase domain of HER1, HER2, and HER4. The neoadjuvant I-Spy-2 trial compared neratinib and trastuzumab with a sequential taxane-anthracycline regimen; pCR rates were higher with neratinib (39 vs. 23%) [256]. In another neoadjuvant trial, neratinib did not increase the pCR rate compared with trastuzumab (33 vs. 38%), while the combination of trastuzumab and neratinib had the highest pCR rate (50%) [257]. The randomized phase III I-Spy-3 trial will evaluate the addition of neratinib to trastuzumab and pertuzumab [256]. Neratinib after completion of chemotherapy and trastuzumab in high-risk EBC patients ($n$ = 2840) resulted in a marginal increase in 2-year DFS of 94% with neratinib versus 92% with placebo (HR 0.68, 95% CI 0.50–0.91, $p$ = 0.010). In the 721 neoadjuvantly treated patients with residual disease, DFS was not significantly different between the treatment arms (HR 0.78, 95% CI 0.50–1.21) [258]. The most common grade 3–4 toxicity of neratinib is diarrhea, occurring in 31–40% of patients [256–258].

Tucatinib (ONT-380) is a TKI with high selectively for HER2, which could potentially reduce the side effects associated with HER1 inhibition. Tucatinib plus T-DM1 has an ORR of 41% (all partial responses) in previously treated MBC patients, of whom 60% had CNS metastases [259]. Another study also demonstrated activity for CNS metastases [260]. Less than 5% experienced grade 3–4 diarrhea [259].

Varlitinib (ASLAN001/ARRAY-334543) is a reversible TKI against HER1, HER2, and HER4. It is currently investigated in combination with capecitabine in HER2-positive MBC in a single arm [261] and a randomized phase II trial (NCT02338245), and also in combination with weekly paclitaxel/carboplatin as neoadjuvant therapy in EBC (NCT02396108).

Overall, the available data do not justify the routine use of any of the above-described TKIs in the neoadjuvant setting, neither as a single blockade nor as dual blockade with trastuzumab. The selective use of a TKI (or other agent) in patients with residual disease after completion of neoadjuvant treatment is an interesting and clinically relevant new research field. Neratinib has been examined in this setting but failed to show a convincing benefit in light of its associated toxicity. However, further research may identify a subgroup with substantial benefit of this approach with either neratinib or another drug.

### 4.5 PI3K/mTOR Inhibitors

Everolimus has been examined thoroughly in HER2-positive breast cancer as a constitutively active PI3K/mTOR pathway and has been described to be involved in trastuzumab resistance [262, 263]. Everolimus added to trastuzumab-based chemotherapy as second-line treatment or higher modestly increased PFS in women with MBC (median PFS 7.0 vs. 5.8 months; HR 0.78, 95% CI 0.65–0.95, $p = 0.007$) [262]. However, everolimus added to first-line trastuzumab-based chemotherapy did not improve DFS (median PFS 15.0 vs. 14.5 months; HR 0.89, 95% CI 0.73–1.08, $p = 0.12$) [263]. In both studies, the effect of everolimus was more pronounced in hormone receptor-negative tumors [262, 263], and more on-treatment and AE-related deaths occurred with everolimus [263]. Trials of everolimus in HER2-positive EBC are scarce. One randomized trial reported lower pCR breast rates after 6 weeks of neoadjuvant everolimus plus trastuzumab compared with trastuzumab monotherapy (8 vs. 15%) [264].

Buparlisib, a pan-PI3K inhibitor, added to trastuzumab and paclitaxel did not increase the pCR breast rate (32 vs. 40%), but significantly increased grade 3–4 rash and hepatotoxicity [265]. There are currently no ongoing neoadjuvant studies with buparlisib, or any other PI3K inhibitor, in HER2-positive EBC.

Due to the absence of a clear treatment benefit and potentially severe toxicity, everolimus and buparlisib have no role in the neoadjuvant treatment of HER2-positive EBC.

### 4.6 Immune Checkpoint Inhibitors

Together with the TNBC subtype, HER2-positive breast cancers have more TILs [128] and a relatively high mutational load [69]. Based on the presence of these putative predictive markers and recent preclinical work [266–268] showing that anti-HER2 treatment in combination with checkpoint blockade results in significant tumor control, it could well be that HER2-positive breast cancer has a special benefit when treated with checkpoint blockade in combination with anti-HER2 treatment. In the Javelin phase I trial with avelumab (anti-PD-L1), 26 patients with HER2-positive MBC were included and only one objective response was observed, however this was in the absence of HER2-directed treatment [157]. Many clinical trials evaluating the efficacy of anti-PD-(L)1 together with HER2-blockade in the metastatic disease setting are ongoing. For example, in the Panacea phase Ib/II trial (NCT02129556), pembrolizumab (anti-PD1) combined with trastuzumab will be administered to trastuzumab-resistant, HER2-positive MBC patients. Awaiting the efficacy data in the metastatic disease setting, some immunotherapy trials are already ongoing in the neoadjuvant setting. For example, in a phase Ib trial with three neoadjuvant cohorts, atezolizumab (anti-PD-L1) is combined with T-DM1 or trastuzumab and pertuzumab (NCT02605915). In cohort 2A, EBC patients with a tumor greater than 2 cm will receive atezolizumab in combination with trastuzumab/pertuzumab, followed by docetaxel/carboplatin/trastuzumab/pertuzumab. Cohort 2B researches atezolizumab plus T-DM1 followed by standard treatment, and cohort 2C investigates the effect of neoadjuvant atezolizumab plus T-DM1. Since no efficacy data on anti-PD-(L)1 plus HER2-directed therapy are available yet, and given the already favorable outcome of most primary HER2-positive breast cancers, it is too early to speculate on the future role of immune checkpoint inhibition in HER2-positive EBC.

### 4.7 Heat Shock Protein 90 Inhibitors

Heat shock protein 90 (HSP90) is a regulatory protein involved in the maturation and stabilization of several proteins, including HER2. By inhibiting HPS90, HER2 becomes unstable and undergoes degradation [269–271]. Several HSP90 inhibitors, including ganetespib, tanespimycin, and AUY22, have been evaluated in phase I/II trials with ORRs (complete response + partial response) when applied as monotherapy in 15% of patients [270], and when combined with trastuzumab in 22% of patients [269, 272] with previously treated HER2-positive MBC. Despite these results, the field of HSP-90 inhibitors has become quiet and a role in the neoadjuvant setting seems unlikely.

## 5 Discussion and Conclusions

Adjuvant systemic treatment has greatly improved survival for EBC patients. Shifting systemic treatment from the adjuvant to the neoadjuvant setting has several additional benefits, including an accelerated evaluation of new drugs. This strategy has opened a window of opportunity to

facilitate patient access to promising new drug combinations; however, new studies that incorporate sufficiently powered long-term outcome measures are still needed for definitive approval [19].

With the wide range of available drugs, combinational strategies, and better predictive biomarkers, we are increasingly able to tailor treatment according to breast cancer subtypes. This approach can be further optimized by identification of specific molecular drug targets and predictive markers. Predictive markers will allow better patient selection to increase the benefit while safely withholding toxic drugs for others. The future place of the described new and investigational therapies in the neoadjuvant setting awaits results from neoadjuvant trials. The introduction of CDK4/6 inhibitors as an addition to standard treatment for patients with high-risk, ER-positive tumors seems appealing. In addition, for patients with TNBC the treatment options are gradually expanding to less toxic-targeted therapies, including PARP inhibitors for patients with a *BRCA* mutation and anti-PD-(L)1.

Whether residual tumor after neoadjuvant treatment can reliably identify patients that may benefit from further adjuvant treatment with known or new drugs must be further evaluated. In contrast, for some subtypes, excellent outcomes have redirected focus to de-escalation strategies. In particular, the high pCR rates in HER2-positive breast cancer have led to several trials being conducted to explore strategies to de-escalate chemotherapy.

**Compliance with Ethical Standards**

**Funding** The authors thank the Dutch Cancer Society (KWF) for the fellowship grant to Marleen Kok.

**Conflict of interest** Gabe S. Sonke received institutional research support funding from Roche, AstraZeneca, Merck and Novartis. Sabine C. Linn reports grants and non-financial support from AstraZeneca and Roche, grants from Genentech, advisory support (paid to the institution) from Novartis, Philips Health BV and IBM, and unpaid advisory support from Cergentis outside the submitted work. In addition, Dr. Linn has a patent pending for the BRCA-like signature (WO/2015/080585 and PCT/NL2014/050813). Marleen Kok receives an unrestricted research grant from Bristol-Myers Squibb. Tessa G. Steenbruggen, Mette S. van Ramshorst and Carolien H. Smorenburg declare that they have no competing interests.

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