HER2-positive breast cancer: new therapeutic frontiers and overcoming resistance

Sonia Pernas and Sara M. Tolaney

Abstract: The introduction of anti-HER2 therapies to the treatment of patients with HER2-positive breast cancer has led to dramatic improvements in survival in both early and advanced settings. Despite this breakthrough, nearly all patients with metastatic HER2-positive breast cancer eventually progress on anti-HER2 therapy due to de novo or acquired resistance. A better understanding not only of the underlying mechanisms of HER2 therapy resistance but of tumor heterogeneity as well as the host and tumor microenvironment is essential for the development of new strategies to further improve patient outcomes. One strategy has focused on inhibiting the HER2 signaling pathway more effectively with dual-blockade approaches and developing improved anti-HER2 therapies like antibody–drug conjugates, new anti-HER2 antibodies, bispecific antibodies, or novel tyrosine kinase inhibitors that might replace or be used in addition to some of the current anti-HER2 treatments. Combinations of anti-HER2 therapy with other agents like immune checkpoint inhibitors, CDK4/6 inhibitors, and PI3K/AKT/mTOR inhibitors are also being extensively evaluated in clinical trials. These add-on strategies of combining optimized targeted therapies could potentially improve outcomes for patients with HER2-positive breast cancer but may also allow de-escalation of treatment in some patients, potentially sparing some from unnecessary treatments, and their related toxicities and costs.

Keywords: breast cancer, drug–antibody conjugates, HER2-positive, new anti-HER2 therapies, novel combinations, resistance, tyrosine kinase inhibitors

Received: 25 October 2018; revised manuscript accepted: 23 January 2019.

Introduction

A better understanding of tumor biology and HER2 signaling has led to the development and approval of new HER2-targeted agents that, together with the use of continued anti-HER2 therapy beyond progression, have resulted in unprecedented survival outcomes in patients with advanced HER2-positive breast cancer. The addition of trastuzumab to standard therapy dramatically improved prognosis for patients with HER2-positive breast cancer, and became a landmark in the treatment of these patients. The second anti-HER2 agent that was incorporated into routine practice of advanced HER2-positive disease was lapatinib, an oral tyrosine kinase inhibitor (TKI) that reversibly inhibits HER1 or epidermal growth factor receptor (EGFR) and HER2 kinases. The approval of lapatinib was based on the improvement in progression-free survival (PFS) found in a phase III trial when combined with capecitabine versus capecitabine alone though no improvement in overall survival (OS) was observed. Pertuzumab is a humanized monoclonal antibody that binds to HER2 on extracellular domain II, a different domain than trastuzumab, preventing homo- and heterodimer formations and blocking one of the most powerful heterodimers, HER2/HER3, that activates several intracellular signaling cascades including cell proliferation and survival. The addition of pertuzumab to a taxane and trastuzumab combination
compared with taxane and trastuzumab therapy alone as a first-line treatment in advanced HER2-positive breast cancer resulted in an improvement not only in PFS but also in OS by almost 16 months, reaching a median survival of nearly 5 years and establishing this regimen as the preferred regimen in the first-line setting. Finally, trastuzumab emtansine (T-DM1) is an antibody–drug conjugate (ADC) comprised of trastuzumab covalently linked to a maytansine derivate (DM1), a potent antimitotic agent that binds microtubules. After selectively binding to HER2, the conjugate is internalized within endocytic vesicles and degraded in the lysosomes, releasing the active payload within the cell. This results in cell death by mitotic catastrophe. T-DM1 significantly improved both PFS and OS compared with lapatinib plus capecitabine as a second-line treatment and as a later line in patients with advanced HER2-positive breast cancer previously treated with trastuzumab. Based on those results, T-DM1 is currently the only ADC approved to treat breast cancer and the standard second-line therapy for advanced HER2-positive disease. To date, there is no standard of care treatment for patients with advanced HER2-positive tumors following treatment with trastuzumab, pertuzumab and T-DM1. Treatment options at this point include lapatinib plus capecitabine, combinations of trastuzumab with other chemotherapies (such as vinorelbine or gemcitabine), or dual-blockade combinations without chemotherapy, such as trastuzumab with lapatinib or endocrine therapy with either trastuzumab or lapatinib in patients with hormone receptor (HR)-positive disease.

Despite the outstanding improvement in survival with the introduction of anti-HER2 therapies alone or as dual HER2-blockade in the standard treatment of advanced disease, most patients ultimately develop progressive disease and die. Furthermore, up to 40–50% of patients with advanced HER2-positive breast cancer will develop brain metastases during their disease course. Better options for the prevention and treatment of brain metastases are clearly needed. A growing understanding of the underlying mechanisms of primary and acquired resistance to anti-HER2 therapies and compensatory pathways as well as tumor heterogeneity and the tumor microenvironment is essential for the development of novel therapeutic strategies. A substantial number of novel anti-HER2 treatments are being investigated extensively in the preclinical and clinical settings to further improve patient outcomes. Here, we review the rationale and latest evidence of those novel treatments and approaches to overcome resistance in advanced HER2-positive breast cancer.

Mechanisms of resistance and response heterogeneity to anti-HER2 therapy

Many potential resistance mechanisms to anti-HER2 therapy have been described that ultimately lead to reactivation of the HER2 pathway or its downstream signaling, through pathway redundancy or stimulation of alternative survival pathways. Some of these mechanisms include incomplete blockade of the HER2 receptor that activates compensatory mechanisms within the HER family (such as HER3), activation of alternative receptor tyrosine kinases (RTKs) or other membrane receptors outside of the HER family (such as insulin-like growth factor 1 receptor [IGF-1R]) and MET, and alterations in downstream signaling pathways, such as hyperactivation of the PI3K/AKT/mTOR pathway by reduced levels of tumor suppressor genes (like PTEN and INPP4-B), or by activating mutations in PIK3CA (phosphatidylinositol-4,5’-bisphosphate 3-kinase catalytic subunit). Several other biologic features have been associated with response heterogeneity to anti-HER2 therapy, including HER2 mRNA or protein levels, tumor intrinsic subtype, alterations in the HER2-receptor (such as p95HER2), and host and tumor microenvironment components, such as tumor infiltrating lymphocytes (TILs) and FCγR polymorphisms. In the CLEOPATRA trial for instance, high HER2 protein and high HER2 and HER3 mRNA levels were associated with a significantly better outcome (p < 0.05). In contrast, PIK3CA mutation was identified as a strong negative prognostic biomarker, despite deriving benefit from pertuzumab and trastuzumab treatment. In the EMILIA trial, a greater benefit in OS was also observed in patients treated with T-DM1 and high HER2 mRNA expression. Notably, PIK3CA mutations were associated with significantly shorter PFS and OS in patients treated with capecitabine plus lapatinib, but not in T-DM1 treated patients (median PFS 10.9 vs. 9.8 months; OS, not reached in mutant or wild type). Regarding TILs, an increased quantity of stromal TILs was significantly associated with improved OS in patients with advanced HER2-positive breast cancer treated with docetaxel, trastuzumab, and pertuzumab or placebo in the CLEOPATRA trial.
It has also been demonstrated that the cyclin D1-CDK4 pathway can mediate resistance to HER2-targeting therapies \textit{in vitro} and \textit{in vivo} and that targeting resistant tumor cells with CDK 4/6 inhibitors re-sensitizes them to anti-HER2 therapy and delays tumor recurrence in HER2-driven breast cancers \textit{in vivo} in patient-derived xenograft tumors.\textsuperscript{23} As discussed below, trials are currently underway to evaluate the efficacy of combined HER2 and CDK4/6 inhibition in HER2-positive breast cancer.

Substantial preclinical and clinical studies support the bidirectional cross-talk between HER2 and estrogen receptor (ER) signaling when both receptors are expressed in breast cancer cells.\textsuperscript{24} Tumors that express both ER and HER2 are less sensitive to endocrine therapy than ER-positive and HER2-negative tumors, and ER can act as an escape pathway to HER2 inhibition.\textsuperscript{25,26} Concurrent inhibition of ER together with dual anti-HER2 therapy can improve outcomes, as demonstrated in several trials in early and advanced HER2-positive breast cancer.\textsuperscript{27–29}

The HER2\textsubscript{Δ16} splice variant is a major oncogenic driver that promotes trastuzumab resistance. Preclinical data suggest trastuzumab-resistant HER2\textsubscript{Δ16} cells are sensitive to the SRC kinase inhibitor dasatinib and data from a phase I/II (GEICAM/2010-04) study suggest there may be a signal for activity when dasatinib is combined with trastuzumab and paclitaxel in the first-line treatment for patients with advanced HER2-positive breast cancer.\textsuperscript{30,31} In addition, SRC activation by itself has been associated with trastuzumab resistance.\textsuperscript{32}

The mechanisms that contribute to T-DM1 resistance are not fully understood. There are multiple components to consider when identifying mechanisms of resistance for ADCs, such as the ones related to the antibody, the linker or the payload. Preclinical studies have shown that CDK1/cyclin B1 activity is needed for T-DM1 action. Silencing cyclin B1 induces resistance to T-DM1 while increasing the levels of cyclin B1 in resistant cells partially restores sensitivity.\textsuperscript{33}

Other potential mechanisms of T-DM1 resistance have been proposed including the reduction of the intracellular DM1 payload due to upregulation of multidrug resistance proteins (e.g. MDR1),\textsuperscript{34} impaired lysosomal proteolytic activity\textsuperscript{35} or lysosomal transporter loss (e.g. SLC46A3).\textsuperscript{34} Interestingly, molecular imaging seems promising not only to further our understanding of tumor heterogeneity in advanced HER2-positive breast cancer but also to identify patients who will unlikely benefit from T-DM1.\textsuperscript{36} In the prospective ZEPHIR trial, striking levels of inter- and intrapatient heterogeneity in HER2 expression were observed, with one-third of patients having little or no trastuzumab-zirconium uptake (HER2-Positron emission tomography (PET)/computed tomography (CT) scan [PET/CT scan]) across their metastatic sites. Moreover, the combined use of HER2-PET/CT scan and early fluorodeoxyglucose-PET/CT scan discriminated patients treated with T-DM1 with a median time to treatment failure (TTF) of 2.8 months from those with 15 months of TTF.\textsuperscript{36} Despite the extensive translational research being conducted, most of the mechanisms of HER2 resistance and potential biomarkers of response or resistance either have not been clinically validated, or the results are contradictory.\textsuperscript{37} To date, no biomarker beyond HER2 exists for patient selection for anti-HER2 therapy in HER2-positive breast cancer. Of note, the interpretation of mechanisms of resistance based solely in preclinical models can be challenging due to tumor heterogeneity, the complex nature of drug resistance and compensatory pathways, and the use of different tumor cell lines. Moreover, multiple mechanisms of resistance may coexist in the same cell.

**Novel strategies to overcome resistance to HER2-targeted therapy**

**Replacement of current anti-HER2 therapies for improved anti-HER2 drugs ADCs.** ADCs are a therapeutic class that provide wider therapeutic window by more efficient and specific drug delivery. ADCs exploit target selectivity of monoclonal antibodies (MAbs) to deliver cytotoxic drugs to antigen-expressing cells to improve tumor selectivity and reduce damage to normal cells.\textsuperscript{38} The success observed with the first-in-class T-DM1 has led to a rapid and extensive development of new ADCs. Table 1 lists several anti-HER2 ADCs in clinical development.\textsuperscript{7,39–41}

**Trastuzumab deruxtecan** (DS-8201, Daiichi Sankyo, Inc.) is an ADC comprising trastuzumab, a cleavable drug linker, and a topoisomerase I payload that has a high drug to antibody ratio (7–8). In preclinical studies, DS-8201a showed a broader anti-tumor activity than T-DM1, including...
Therapeutic Advances in Medical Oncology 11

efficacy against low HER2-expressing tumors. In an updated subgroup analysis from a phase I study with multiple expansion cohorts, DS-8201a demonstrated an overall response rate (ORR) of 54.5% (54/99 patients) in patients with HER2-positive metastatic breast cancer pretreated with T-DM1 (as well as trastuzumab and pertuzumab in the majority of cases). Median duration of response and median PFS had not yet been reached.39 Interestingly, in patients with HER2 low-expressing metastatic breast cancer [defined as immunohistochemistry (IHC) 1+ or 2+ and in situ hybridization (ISH)-negative], the ORR was 50% (17/34 patients). Trastuzumab deruxtecan was relatively well tolerated; common adverse events (AEs) included nausea (73.5%; 3.5% grade ≥3), decreased appetite (59.5%; 4.5% grade ≥3) and vomiting (39.5%; 1.5% grade ≥3). In August 2017, trastuzumab deruxtecan received a United States Food and Drug Administration (US FDA) Breakthrough Therapy designation for the treatment of patients with HER2-positive, locally advanced, or metastatic breast cancer who have been treated with trastuzumab and pertuzumab and, have progressed to T-DM1. DS-8201a is being evaluated in numerous trials, including two phase III studies: the DESTINY-Breast02 (ClinicalTrials.gov identifier: NCT03523585) is evaluating DS-8201a versus investigator’s choice (capecitabine with trastuzumab or lapatinib) for patients with HER2-positive, unresectable or metastatic breast cancer pretreated with prior T-DM1, and the DESTINY-Breast03 (ClinicalTrials.gov identifier: NCT03529110) is a randomized, open-label study of DS-8201a versus T-DM1 for patients with HER2-positive, metastatic breast cancer previously treated with trastuzumab and taxane.

SYD985 (Synthon Biopharmaceuticals BV) is a third generation ADC based on trastuzumab and a ‘cleavable’ linker – duocarmycin payload, which is present as an inactive prodrug (valine-citrulline-seco – DUocarmycin – hydroxyBenzamide – Azaindole –vc-seco-DUBA). Proteases present in endosomes result in the linker cleavage in SYD985 and the release of the membrane-permeable active toxin. It then binds to the minor groove of DNA, causing irreversible DNA alkylation. This results in cell death in both dividing and nondividing cells in the tumor microenvironment but also in neighboring tumor cells due to the bystander effect. SYD985 has shown impressive preclinical results in breast cancer (even more potent than T-DM1) and encouraging clinical activity. Results from a dose-escalation phase I trial showed encouraging activity in heavily pretreated patients43,44 and led the US

| Table 1. HER2-directed ADCs in clinical development. |
|--------------------------------------------------|
| **Agent** | **Anti-HER2 MAb/payload (target)** | **Drug to antibody ratio** | **Linker drug** | **Phase of development** | **ORR in HER2-positive** | **ORR in HER2 low (IHC1+ /2+/ISH-)** |
|-----------|-----------------------------------|---------------------------|-----------------|-------------------------|--------------------------|----------------------------------|
| T-DM1 (Trastuzumab-DM1) | Trastuzumab/DM1 (anti-tubulin) | 3.5 | Noncleavable | US FDA Approved | 43.6% | — — — |
| DS-8201a (Trastuzumab duruxtecan) | Trastuzumab/ exatecan derivative [topoisomerase I inhibitor] | 8 | Cleavable | II/III NCT03248492 NCT03529110 NCT03523585 | 54.5% | 50% |
| SYD985 | Duocarmycin derivative [alkylating agent] | 2.8 | Cleavable | III NCT03262935 | 33% | HR + 27% | HR − 40% |
| XMT-1522 | XMT-1519/ monomethyl auristatin (anti-tubulin) | 12 | Cleavable | I NCT02952729 | unknown | unknown |
| ARX788 | Anti-HER2 MAb/ auristatin analog 269 [AS269] (anti-tubulin) | 1.9 | Non-cleavable | I NCT03255070 | unknown | unknown |
| DHES0815A | Trastuzumab/ alkylator 2 | Cleavable | I NCT03451162 | unknown | unknown |

ADC, antibody–drug conjugate; HR+, hormone receptor positive; HR−, hormone receptor negative; IHC, immunohistochemistry; ISH, In Situ Hybridization; MAb, monoclonal antibody; NCT, ClinicalTrials.gov identifier; ORR, overall response rate; US FDA, United States Food and Drug Administration.
FDA to grant the agent Fast Track designation in January 2018. Results from the expansion cohorts recently reported an ORR of 33% and a median PFS of 9.4 months\(^4\) in a cohort of patients with advanced HER2-positive disease, previously treated with a median of six lines of therapy for metastatic disease (\(n=50\)). Interestingly, SYD985 was also effective in patients with HER2-low metastatic breast cancer, with an ORR of 27% and 40% in the HR-positive, HER2-low, and in the HR-negative, HER2-low cohorts, respectively. Enrollment was based on central HER2 analysis by IHC and ISH and HER2 low was defined as IHC 1\(^+\)/2\(^+\) and fluorescence in situ hybridization (FISH) negative. Most of the adverse drug reactions were mild or moderate, with ocular toxicity and fatigue being most frequently reported. A phase III pivotal study (TULIP) is ongoing (ClinicalTrials.gov identifier: NCT03262935) which compares SYD985 with the treatment of the physician’s choice in patients with HER2-positive metastatic breast cancer in the third line and beyond.

**MEDI4276** (Medimmune) is a bispecific ADC that targets two different domains of the HER2 receptor, resulting in crosslinking followed by internalization of the complex, cleavage of the linker, and release of the payload. MEDI-4276 comprises the single-chain variable fragment (scFv) of trastuzumab, which binds to domain IV of HER2, and the anti-HER2 Mab 39S, which binds to domain II of HER2. The bispecific antibody is then conjugated, via a cleavable linker, to the cytotoxic anti-microtubule agent tubulysin. Results from the phase I study (ClinicalTrials.gov identifier: NCT02576548) in patients with advanced HER2-positive breast or gastric cancer\(^4\) showed clinical activity, but also considerable toxicity with 28% of patients (12/43) having grade 3–4 AEs; most common were grade 3 elevated aspartate transaminase (AST; 19%) and grade 3 elevated alanine transaminase (ALT; 12%). Given the challenges with toxicity, clinical testing with this agent has been discontinued for breast and gastric cancers.

**ADCT-502** (ADC Therapeutics) is also a novel pyrrolobenzodiazepine (PBD)-based ADC that targets HER2-expressing solid tumors, including breast cancers.\(^4\) However, based on data from the phase I study (ClinicalTrials.gov identifier: NCT03125200) that showed that ADCT-502 did not meet the necessary efficacy and safety profile required for patient benefit, clinical testing of this drug was recently halted.

In preclinical models, another bivalent biparatopic HER2-targeting ADC that targets two non-overlapping epitopes on HER2 and is conjugated with microtubule inhibitor demonstrated superior activity than T-DM1 in breast cancer models and was able to overcome T-DM1 resistance. This biparatopic ADC also demonstrated bystander killing activity.\(^4\)

In contrast with T-DM1, most of these new ADCs have a cleavable drug linker (see Table 1) that mediates the bystander killing effect. This is the passive diffusion of the free cytotoxin from target-positive cancer cells into the tumor microenvironment, killing neighboring cancer cells that are insensitive to the ADC because of the lack or limited target expression. This desired feature of those novel HER2-targeting ADCs, given that heterogeneity is frequent in HER2-positive breast cancer, may be however, a double-edged sword with an increased toxicity.

**Novel anti-HER2 antibodies.** Margetuximab (MGAH22, MacroGenics) is an Fc-optimized chimeric monoclonal antibody that binds to the same epitope as trastuzumab. Margetuximab has enhanced Fc\(\gamma\) receptor-binding properties with an increased affinity for CD16A polymorphisms and a decreased affinity for Fc\(\gamma\)RIIB (CD16B), an inhibitory receptor, which allows it to bind more tightly to effector cells and increase antibody-dependent cell-mediated cytotoxicity (ADCC); it also preserves the antiproliferative properties of trastuzumab.\(^4\) A first-in-human phase I study demonstrated promising single-agent activity of margetuximab in heavily pretreated patients with HER2-positive solid tumors. Among 24 patients with metastatic breast cancer, the ORR was 17% and 3 out of the 4 responders remained on treatment for 39–54 months.\(^4\) The most common AEs were grade 1–2 constitutional symptoms and no cardiotoxicity was observed. Margetuximab is currently being evaluated in the randomized phase III SOPHIA trial (ClinicalTrials.gov identifier: NCT02492711) that compares margetuximab plus chemotherapy with trastuzumab plus chemotherapy as a third-line therapy in patients with HER2-positive breast cancer after prior treatment with trastuzumab, pertuzumab, and T-DM1. The US FDA has granted Fast Track designation for the investigation of margetuximab for the treatment of patients with metastatic or...
locally advanced HER-positive breast cancer previously treated with anti-HER2-targeted therapy.

**Bispecific antibodies.** Bispecific antibodies (BsAbs) combine the functionality of two MAbs that target two different targets or epitopes, either in the same or in different receptors. BsAbs can interfere with two or more RTK signaling pathways, by inactivating either the RTKs or their ligand. Several are currently being studied in patients with advanced HER2-positive disease.

**MCLA-128** (Merus) is a full-length immunoglobulin (Ig)G1 BsAb with enhanced ADCC activity targeting both HER2 and HER3. Preliminary results from a phase I/II study in solid tumors (ClinicalTrials.gov identifier: NCT02912949) that include eight patients with heavily pretreated (median of five prior lines in the metastatic setting) HER2-positive metastatic breast cancer showed an encouraging clinical benefit rate of 70%.50 A phase II study (ClinicalTrials.gov identifier: NCT03321981) is ongoing to evaluate the activity of MCLA-128 in two metastatic breast cancer populations: in HER2-positive/amplified patients (cohort 1) in combination with trastuzumab ± chemotherapy, and in ER-positive/low-HER2 expression (cohort 2) in which MCLA-128 is administered in combination with endocrine therapy.

**ZW25** (Zymeworks Inc.) is a novel Azymetric bispecific antibody biparatopic that binds to two different epitopes on the extracellular domain of HER2 ECD2 and ECD4. This results in increased tumor cell binding, blockade of ligand-dependent and independent growth, and improved receptor internalization and downregulation relative to trastuzumab. Also, in vivo studies demonstrate anti-tumor activity in HER2-low to high expressing models. Results from the phase I study (ClinicalTrials.gov identifier: NCT02892123) evaluating the safety and efficacy of single-agent ZW25 in separate expansion cohorts, including HER2-high (IHC 3+ or 2+/FISH+) breast, gastric/esophageal, and other cancers, demonstrated a promising anti-tumor activity and no dose-limiting toxicities were observed. In patients with heavily pretreated HER2-expressing breast cancers that had progressed to a median of five HER2-targeted regimens for metastatic disease, a partial response rate of 33% (6/18 patients) was observed with a disease control rate of 50%. The most common AEs were diarrhea and infusion reaction, all grade 1 or 2, with no treatment-related discontinuations.51

**GBR1302** (Glenmark Pharmaceuticals) is a HER2xCD3 bsAb developed to direct T-cells to HER2-expressing tumor cells. Preclinically, GBR1302 has demonstrated potent killing of HER2-positive human cancer cells, as well as growth suppression of the trastuzumab-resistant cell line JIMT-1. In contrast, the GBR1302 concentration required to kill primary cardiomyocytes with normal HER2 levels was up to 1000 times greater than the concentration needed to kill HER2 3+ tumor cell lines. A first-in-human phase I study of single-agent GBR1302 in progressive HER2-positive solid tumors is ongoing (ClinicalTrials.gov identifier: NCT02829372). Preliminary biomarker and pharmacodynamic data from this study were recently presented, demonstrating modulation of peripheral T-cell populations and cytokines.52

**PRS-343** (Pieris Pharmaceuticals, Inc.) is a monoclonal antibody-bispecific protein targeting HER2 and the immune receptor CD137. CD137 is a key costimulatory immunoreceptor and a member of the tumor necrosis factor receptor superfamily. PRS-343 is designed to promote CD137 clustering by bridging CD137-positive T-cells with HER2-positive tumor cells, thereby providing a potent costimulatory signal to tumor antigen-specific T-cells that has demonstrated tumor inhibition and TIL expansion in a humanized mouse model.53 The two clinical studies evaluating PRS-343 in HER2-positive solid tumors are ongoing, either as a single-drug agent (ClinicalTrials.gov identifier: NCT03330561) or in combination with atezolizumab (ClinicalTrials.gov identifier: NCT03650348).

Expression of the tumor-specific antigen p95HER2, a truncated form of HER2, occurs in about 40% of HER2-positive tumors. Rius Ruiz and colleagues have developed a TCB against p95HER2 (p95HER2-TCB) that has a potent anti-tumor effect on breast tumors expressing...
p95HER2, both in vitro and in vivo.\textsuperscript{54} In contrast with HER2, p95HER2 is not expressed in normal tissues, therefore, it has no effect on nontumor cells that do not overexpress HER2. Those findings support further investigation with this compound.

**Novel TKIs.** TKIs are orally bioavailable small molecules developed to further block the HER receptor family, acting on the intracellular domain. Those HER-directed TKIs have a lower molecular weight compared with MAbs, allowing them a more efficacious penetration through the blood–brain barrier and therefore, theoretically may be more effective for the treatment of HER2 brain metastases. Lapatinib was the first TKI approved in HER2-positive advanced breast cancer (and to date, remains the only one) based on the results described above. Dual blockade with lapatinib plus trastuzumab without chemotherapy also demonstrated benefit in OS in heavily pretreated patients with advanced disease, when compared with lapatinib alone.\textsuperscript{55} Initial results with these molecules, however, have not been the ones initially expected, even in the treatment of brain metastases.\textsuperscript{56,57} As a first-line therapy, lapatinib was found to be inferior to trastuzumab when combined with paclitaxel.\textsuperscript{58} In the same way, afatinib was found to be not as effective as trastuzumab and less tolerated when each was combined with vinorelbine in a phase III trial (LUX-Breast1 study).\textsuperscript{59} No difference was detected between lapatinib-capecitabine and trastuzumab-capecitabine for the incidence of brain metastases in the phase III CEREBEL (EGFR111438) study.\textsuperscript{56} In the phase II LUX-Breast3 trial, patients with HER2-positive breast cancer and progressive brain metastasis previously treatment with trastuzumab, lapatinib or both, were randomized to afatinib alone, afatinib plus vinorelbine or the investigator’s choice of treatment. Similarly, afatinib-containing regimens not only did not show better activity than investigator-selected treatments but also seemed to be less tolerated. No further development of afatinib for HER2-positive breast cancer is currently planned. There are, however, several novel TKIs in clinical development (Table 2).\textsuperscript{60–66}

**Neratinib** is an irreversible pan-HER TKI that has been recently approved as an extended adjuvant therapy in early HER2-positive breast cancer based on the results of the phase III ExteNET study.\textsuperscript{67} In previously untreated patients with HER2-positive advanced breast cancer, neratinib-paclitaxel was not superior to trastuzumab-paclitaxel in terms of PFS in the randomized phase II NEfERT-T trial that included 479 women.\textsuperscript{68} Median PFS was 12.9 months in both arms [hazard ratio, 1.02; 95% confidence interval (CI), 0.81–1.27; \( p = 0.89 \)]. However, the incidence of central nervous system (CNS) recurrences was significantly lower (relative risk, 0.48; 95% CI, 0.29–0.79; \( p = 0.002 \)) and time to CNS metastases significantly delayed with neratinib-paclitaxel (hazard ratio, 0.45; 95% CI, 0.26–0.78; \( p = 0.004 \)). Diarrhea and gastrointestinal toxicity (i.e. nausea, vomiting) were more common with neratinib-paclitaxel (30.4% versus 3.8% of diarrhea grade 3); however, primary prophylaxis for diarrhea was not mandatory in this trial. In patients with HER2-positive breast cancer brain metastases, the combination of neratinib and capecitabine demonstrated a reduction of CNS lesions in 49% of patients in the phase II trial TBCRC 022.\textsuperscript{60} This regimen has now been endorsed by the National Comprehensive Cancer Network for the treatment of HER2-positive brain metastases.\textsuperscript{69} The phase III NALA study (Clinical Trials.gov identifier: NCT01808573) is directly comparing the combination of neratinib plus capecitabine to lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer who have received at least two prior HER2-directed regimens in the metastatic setting. This study has already completed recruitment and it will provide valuable data on the real efficacy of lapatinib and neratinib after the current standard treatments of pertuzumab and T-DM1.

**Tucatinib (ONT-380)** is an oral HER2-selective small molecule TKI with nanomolar potency and is approximately1000-fold more potent for HER2 than EGFR. Because of its selectivity for HER2, there are fewer EGFR-related toxicities, such as rash and diarrhea, which are common with many of the other anti-HER TKIs. In a phase Ib study, in which tucatinib was combined with capecitabine or trastuzumab in heavily pretreated patients with HER2-positive metastatic breast cancer (including patients with brain metastases), the triple combination demonstrated an ORR of 61% (in 14 patients out of 23 with measurable disease) and a median duration of response of 10 months (95% CI: 2.8–19.3). In patients with measurable brain metastases at baseline, an encouraging ORR of 42% (5/12) in the brain was observed.\textsuperscript{62} Median PFS and median duration of response were 7.8 months, and 10 months, respectively. In June 2017, tucatinib was granted US FDA
Orphan Drug status for HER2-positive patients with brain metastases. An ongoing phase II trial, HER2CLIMB (ClinicalTrials.gov identifier: NCT02614794), is randomizing patients with HER2-positive breast cancer with or without brain metastases to capecitabine and trastuzumab with or without tucatinib. The combination of tucatinib and T-DM1 was evaluated in another phase Ib study (ClinicalTrials.gov identifier: NCT01983501), showing promising activity in patients with HER2-positive metastatic breast cancer who had undergone a median of two prior HER2 therapies. ORR was 48% and the median PFS of 8.2 months (95% CI, 4.8–10.3 months). Among the 30 patients with brain metastases included in this study, the median PFS was 6.7 months (95% CI, 4.1–10.2 months) and the brain-specific ORR among patients with measurable disease was 36% (including 2 patients with complete response). Importantly, the combination of tucatinib with T-DM1 appeared to have an acceptable toxicity, with most AEs attributable to T-DM1 and consistent with those observed in other studies that used T-DM1 as a single agent.

**Pyrotinib** is a novel irreversible pan-HER TKI that has demonstrated promising activity and acceptable tolerability in a phase II trial in patients with advanced HER2-positive breast cancer treated with at least two previous lines of therapy. Patients were randomized to receive pyrotinib plus capecitabine or lapatinib plus capecitabine. ORR was 78.5% in the pyrotinib/capecitabine arm and 57.1% in the capcitabine/lapatinib arm, with a median PFS of 18 months and 7 months, respectively (hazard ratio \(=0.36; p<0.001\)). In contrast, pyrotinib was associated with higher rates of AEs, which included hand-foot syndrome (25 versus 21%), diarrhea (15 versus 5%) and neutropenia (9 versus 3%). Biomarker analyses suggest that PIK3CA and TP53 mutations in

| Agent | Target | Reported results of efficacy in HER2-positive advanced disease | CNS ORR (monotherapy) | CNS ORR in combination with capecitabine | Phase of development |
|-------|--------|---------------------------------------------------------------|-----------------------|------------------------------------------|---------------------|
| Neratinib<sup>60,61</sup> | Irreversible pan-HER | Single-agent ORR 56% (phase II) | 8% | 49% (phase II) | US FDA approved only in the adjuvant setting III (metastatic) NALA-NCT01808573 |
| Tucatinib<sup>62–64</sup> | Selectively inhibits HER2 relative to EGFR | In combination with capecitabine and trastuzumab: ORR 61% PFS of 7.8m In combination with T-DM1: ORR 48% PFS 8.2 m (phase Ib) | 5–9% (+trastuzumab) | 42% (+trastuzumab) | II HER2CLIMB-NCT02614794 |
| Pyrotinib<sup>65</sup> | Irreversible pan-HER | Single-agent ORR 50%, CBR 61%, PFS 35.4 w (phase I) In combination with capecitabine ORR 78.5% PFS 18 m (phase II) | NA | NA | III NCT003080805 |
| Poziotinib<sup>66</sup> | Irreversible pan-HER | Single-agent DCR 75% PFS 4 m (phase II) | NA | NA | II |

CBR, clinical benefit rate; CNS, central nervous system; DCR, disease control rate; EGFR, epidermal growth factor receptor; m, months; NA, not applicable; NCT, ClinicalTrials.gov identifier; ORR, overall response rate; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; US FDA, United States Food and Drug Administration; w, weeks.
circulating tumor DNA rather than in archival tumor samples may predict response to pyrotinib.\textsuperscript{65,70} An ongoing phase III trial (ClinicalTrials.gov identifier: NCT03080805) is comparing pyrotinib plus capecitabine \textit{versus} lapatinib plus capecitabine in patients with HER2-positive breast cancer previously treated with trastuzumab and taxane.

\textbf{Poziotinib} is also an oral pan-HER kinase showing potent activity through irreversible inhibition of these kinases. Results from a single-arm, phase II trial (ClinicalTrials.gov identifier: NCT02418689) evaluating the efficacy and safety of poziotinib as monotherapy in heavily pretreated patients with HER2-positive metastatic breast cancer showed a disease control rate of 75.5\% (77/102), including 20 patients with confirmed partial response and a median PFS of 4 months (95\% CI, 2.9–4.4 months). The most common AEs were diarrhea and stomatitis (being grade $\geq 3$ 15\% and 26\%, respectively).\textsuperscript{66}

\textit{Combinations of anti-HER2 agents with other drugs}

\textbf{Immunotherapy.} Preclinical and clinical data suggest that HER2-positive breast cancer is immunogenic.\textsuperscript{71} In contrast with luminal tumors, HER2-positive tumors have a higher mutational burden, and harbor higher numbers of TILs and programmed cell death protein 1 ligand (PD-L1) positivity.\textsuperscript{72} In addition, mechanisms of action of anti-HER2 MAbs include not only ADCC but also the generation of adaptive immunity.\textsuperscript{73} Together, these data support the rationale of combining anti-HER2 therapies with immune checkpoint blockade (anti-PD-1 or anti-PD-L1 agents). Results from the JAVELIN phase I study,\textsuperscript{74} however, were disappointing and no responses were seen with single-agent avelumab in the subgroup of patients with advanced pretreated HER2-positive breast cancer. The first study to evaluate the addition of pembrolizumab to trastuzumab in patients with trastuzumab-resistant HER2-positive breast cancer was the PANACEA (IBCSG 45-13/BIG 4-13/KEY-NOTE-014) study. This phase Ib/II study demonstrated that the combination was associated with an ORR of 15.2\% and a median of PFS and OS of 2.7 months and 16 months, respectively, in PD-L1 positive patients.\textsuperscript{75} However, these data also highlight the limitations of this combination, as no responses were seen in the PD-L1 negative cohort and most of the PD-L1 positive patients who initially responded eventually developed resistant disease. Several randomized studies are ongoing to further evaluate the role of immune checkpoints inhibitors in HER2-positive metastatic breast cancer. Results from the phase II KATE2 trial (ClinicalTrials.gov identifier: NCT02924883) assessing the efficacy and safety of T-DM1 in combination with atezolizumab or placebo in pretreated patients with HER2-positive advanced breast cancer were recently presented.\textsuperscript{76} In this trial, the addition of atezolizumab to T-DM1 did not demonstrate a significant PFS benefit in the ITT population (8.2 \textit{versus} 6.8 months; hazard ratio 0.82; 95\% CI 0.55–1.23). However, an exploratory endpoint demonstrated promising PFS in the PD-L1 positive (PD-L1 IHC expression >1\%) and stromal TIL subgroups.\textsuperscript{76} The phase III NRG-BR004 trial (ClinicalTrials.gov identifier: NCT03199885) is investigating the combination of paclitaxel, trastuzumab, pertuzumab with or without atezolizumab as a first-line treatment. In addition, trastuzumab deruxtecan (DS-8201a) is also being evaluated in combination with nivolumab in a phase Ib study (ClinicalTrials.gov identifier: NCT03523572) in patients with advanced breast (with high and low HER2 expression) and urothelial cancers.

Immunotherapy with HER2-targeting vaccines are also being currently investigated in clinical trials. The HER2 vaccine NeuVax\textsuperscript{TM} (Nelipepimut-S or E75 peptide combined with granulocyte macrophage-colony stimulating factor)\textsuperscript{77} is being evaluated in two phase II clinical trials in combination with trastuzumab in breast cancer patients with HER2–3+ (ClinicalTrials.gov identifier: NCT02297698) and in HER2–1+/2+ (ClinicalTrials.gov identifier: NCT02976989), respectively. ETBX-021 is another HER2-targeting vaccine comprising an Ad5 vector and a modified HER2 gene insert that is being evaluated in a phase I clinical trial with locally advanced or metastatic HER2-low-expressing (IHC 1+/2+) breast cancer.

\textbf{CDK4/6 inhibitors}

There is a strong rationale to evaluate CDK4/6 inhibitors in HER2-positive breast cancer. Activity of CDK4/6 is regulated by several mechanisms that include mitogenic signaling pathways (such as HER2) by increasing CCND1 expression or increasing cyclin D1 protein stability.\textsuperscript{23,78} In addition, mouse models have shown that cyclin D1/CDK4 plays an important role in the
formation and growth of breast tumors driven by ERBB2. Preclinical studies demonstrate a clear synergy between anti-HER2 therapy and CDK4/6 inhibitors, and that CDK4/6 inhibition can specifically overcome acquired resistance to anti-HER2 therapy. Moreover, CDK4/6 inhibition delayed recurrence of HER2-driven breast cancers in mouse models. Early clinical data also support the use of CDK4/6 inhibitors in HER2-driven breast cancers, especially in the subset of patients with ER-positive, HER2-positive disease. Currently, there are many clinical trials evaluating the role of CDK4/6 inhibitors in advanced HER2-positive breast cancer, including two global, randomized trials: the MonarcHER study (ClinicalTrials.gov identifier: NCT02675231), which evaluates the role of abemaciclib with trastuzumab in pretreated metastatic disease, and the PATINA study (ClinicalTrials.gov identifier: NCT02947685), which explores the benefits of adding palbociclib to trastuzumab, pertuzumab and an aromatase inhibitor after an induction of standard first-line therapy. Initial reports from the phase II SOLTI-1303 PATRICIA trial (ClinicalTrials.gov identifier: NCT02448420), which evaluates the combination of palbociclib, trastuzumab ± letrozole in heavily pretreated (up to 2–4 prior lines in the metastatic setting) patients with HER2-positive breast cancer, suggest that the combination is active particularly in the luminal subtype by PAM50, with a better PFS compared with nonluminal (12.4 versus 4.1 months, hazard ratio 0.30, 95% CI 0.11–0.86 p = 0.025). Thus, identification of the nonluminal subtypes by PAM50 might help to identify those patients who might not derive a great benefit from this treatment strategy, regardless of HR status. Other nonrandomized phase Ib/II studies in advanced HER2-positive breast cancer are ongoing, including those combining palbociclib and T-DM1 (ClinicalTrials.gov identifier: NCT01976169); palbociclib, trastuzumab, pertuzumab and anastrozole (ClinicalTrials.gov identifier: NCT03304080); ribociclib with trastuzumab or T-DM1 (ClinicalTrials.gov identifier: NCT02657343); and palbociclib with tucatinib and letrozole (ClinicalTrials.gov identifier: NCT03054363). The JPBO trial (ClinicalTrials.gov identifier: NCT02308020) is testing abemaciclib as a single agent in patients with brain metastasis secondary to HR-positive breast cancer, non-small cell lung cancer, or melanoma, including a cohort of patients with HR+, HER2-positive breast cancer. However, within the cohort of HR-positive, HER2-positive patients, there were no objective responses seen at the time of the interim analysis, and the cohort was not able to move to the second stage.

PI3K/Akt/mTOR inhibitors

As mentioned previously, dysregulations in the PI3K/AKT/mTOR pathway seem to play an important role in trastuzumab resistance. PI3K inhibition results in an enhanced HER2 signaling in HER2-overexpressing breast cancer, especially in an increased expression of HER2 and HER3. Targeting both pathways could prevent the development of resistance. However, results of two phase III trials evaluating the role of everolimus, an mTOR inhibitor, in combination with either trastuzumab plus paclitaxel as first-line treatment (BOLERO-1) or in combination with trastuzumab plus vinorelbine in trastuzumab-resistant (BOLERO-3) advanced HER2-positive breast cancer were quite disappointing with a significant increase in toxicity. Although in the PFS subgroup analysis of both studies, the benefit of adding everolimus to the standard therapy seemed greater in patients who had HR-negative disease. Moreover, the combined biomarker analyses of the BOLERO-1 and BOLERO-3 trials demonstrate an improved PFS in patients harboring PIK3CA mutations or PTEN loss when treated with everolimus. Current efforts have focused on evaluating α-specific PI3K inhibitors, the isoform encoded by the PIK3CA gene, such as alpelisib (BYL719) in combination with anti-HER2 therapies. Alpelisib was combined with LJM716 (a HER3 inhibitor) and trastuzumab in patients with HER2-positive advanced breast cancer with a PIK3CA mutation and prior pertuzumab and T-DM1 (ClinicalTrials.gov identifier: NCT02167854). Preliminary results of this combination showed a high toxicity profile including diarrhea, hyperglycemia, hypokalemia, mucositis and transaminitis, and limited activity (best response was stable disease (SD) in five of six evaluable patients). A phase I study of alpelisib and T-DM1 in heavily pretreated HER2-positive patients showed significant activity, with an ORR of 43% and median PFS of 8.1 months (95% CI 3.9–10.8). Furthermore, activity was observed in T-DM1-resistant patients with an ORR and clinical benefit rate of 30% and 60%, respectively, and median PFS of 6.3 months (95% CI 1.6–10.5). The dose-limiting toxicity (DLT) was a maculopapular rash. Most frequently reported toxicities included fatigue, rash, gastrointestinal
side effects, thrombocytopenia, anemia, elevated liver enzymes, and hyperglycemia.\textsuperscript{93} Taselisib (GDC-0032), a β-sparing PI3K inhibitor, is being evaluated in an ongoing phase Ib dose-escalation trial in combination with different anti-HER2 therapies in patients with advanced HER2-positive breast cancer (ClinicalTrials.gov identifier: NCT02390427). Copanlisib is a pan-class I PI3K inhibitor with particular activity against PI3Kα that is being evaluated in combination with trastuzumab. Results from a phase Ib (ClinicalTrials.gov identifier: NCT02705859; PantHER trial) in pretreated metastatic HER2-positive breast cancer (with a median of four prior lines) showed no DLTs but grade 3 hypertension was reported in 33% (n = 4) of patients. The best response was stable disease in 9/12 patients and 6 patients continued treatment ≥16 weeks. The PIK3CA mutation was present in 6/12 (50%) of tumors.\textsuperscript{94} MEN1611 is a potent, selective, orally available class I PI3k inhibitor showing high activity against p110α mutant isoforms, and minimal inhibition of the δ isoform that is going to be evaluated in a phase Ib study in combination with trastuzumab with or without fulvestrant (B-PRECISE-01 study). The study will enroll patients with PIK3CA-mutated, HER2-positive advanced breast cancer pretreated with anti-HER2 based therapy.

### Conclusion

Although the use of anti-HER2-targeted therapy has dramatically changed the outlook for patients with advanced HER2-positive breast cancer, almost all patients ultimately experience disease progression. Most of them advance to the point where no approved HER2-targeting treatment controls their disease. This might change in the near future as many promising anti-HER2 therapies are being developed in this setting. The newer HER2 ADCs such as DS-8201a and SYD985 may replace T-DM1 in the second-line treatment space or may be utilized in the third-line and beyond setting after progression on T-DM1. Moreover, these newer ADCs, as opposed to T-DM1, are active not only in patients with HER2-positive breast cancer but also in patients with HER2 low-expressing tumors (IHC1+ or 2+/FISH) in whom to date, there are no current anti-HER2 therapies specifically indicated. This desired feature of its bystander effect may be particularly useful in heterogeneous cancer cell populations among HER2-positive disease. On the other hand, the new TKIs, such as neratinib or tucatinib, are being explored with capecitabine and may demonstrate activity in the third-line setting, though may be associated with increased toxicity relative to trastuzumab. And finally, marglituximab plus chemotherapy is being compared with trastuzumab plus chemotherapy also in the third-line setting in a registrational study.

These strategies of combining optimized HER2-targeted therapies could potentially improve outcomes for HER2-positive breast cancer patients but may also allow de-escalation of treatment in selected patients, potentially sparing some from unnecessary treatments and their related toxicities. Hence, potential biomarkers of response or resistance such as intrinsic subtypes, might be helpful to better select patients for these strategies. Moreover, specific strategies for HR-positive, HER2-positive breast cancer are needed, such as the current studies exploring the use of CDK 4/6 inhibitors in the first and later line setting with anti-HER2 therapy and specific trials allowing patients with progressive brain metastases (who are generally excluded from clinical trials) should be enhanced. The underlying mechanisms of resistance to anti-HER2 therapies and compensatory pathways are indeed complex and a wide range of mechanisms of resistance may coexist in the same cell. Therefore, combining clinical strategies and strengthening international collaborations in the translational setting might be needed to validate predictive biomarkers beyond HER2, which will help us to better select patients and improve their outcomes.

### Acknowledgements

We thank Kaitlyn T. Bifolck, BA, for her editorial support. Fundación AECC (Asociación Española Contra el Cáncer) and the Spanish Society of Medical Oncology (SEOM) grants (to S. Pernas) for their support.

### Funding

This manuscript did not receive specific funding.

### Conflict of interest statement

S. Pernas has received honoraria for talks and travel grants from Roche, outside of the submitted work and has served on advisory boards for Polyphor. S. Tolaney receives institutional research funding from Eli Lilly, Pfizer, Novartis, Exelixis, Eisai, Merck, Bristol Meyers Squibb, AstraZeneca, Nektar, Nanostring, Cyclacel, and Immunomedics. S. Tolaney has served on advisory boards or as a consultant for Eli Lilly, Pfizer, Novartis, Eisai, Merck, AstraZeneca, Nektar, Nanostring, Immunomedics, and Puma.
ORCID iD
Sonia Pernas https://orcid.org/0000-0002-1485-5080

References
1. Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015; 372: 724–734.
2. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177–182.
3. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783–792.
4. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355: 2733–2743.
5. Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 2008; 68: 9280–9290.
6. Barok M, Tanner M, Koninki K, et al. Trastuzumab-DM1 causes tumour growth inhibition by mitotic catastrophe in trastuzumab-resistant breast cancer cells in vivo. Breast Cancer Res 2011; 13: R46.
7. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012; 367: 1783–1791.
8. Krop IE, Kim SB, Martin AG, et al. Trastuzumab emtansine versus treatment of physician’s choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol 2017; 18: 743–754.
9. Lin NU. Better treatments needed for breast cancer brain metastases. Lancet Oncol 2015; 16: 1583–1584.
10. Rimawi MF, Schiff R and Osborne CK. Targeting HER2 for the treatment of breast cancer. Annu Rev Med 2015; 66: 111–128.
11. Nahta R, Yuan LX, Zhang B, et al. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer Res 2005; 65: 11118–11128.
12. Minuti G, Cappuzzo F, Duchnowska R, et al. Increased MET and HGF gene copy numbers are associated with trastuzumab failure in HER2-positive metastatic breast cancer. Br J Cancer 2012; 107: 793–799.
13. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004; 6: 117–127.
14. Berms K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007; 12: 395–402.
15. Chandarlapaty S, Sakr RA, Giri D, et al. Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. Clin Cancer Res 2012; 18: 6784–6791.
16. Nuciforo P, Thyparambil S, Aura C, et al. High HER2 protein levels correlate with increased survival in breast cancer patients treated with anti-HER2 therapy. Med Oncol 2016; 10: 138–147.
17. Carey LA, Berry DA, Cirrincione CT, et al. Molecular heterogeneity and response to neoadjuvant human epidermal growth factor receptor 2 targeting in CALGB 40601, a randomized phase III trial of paclitaxel plus trastuzumab with or without lapatinib. J Clin Oncol 2016; 34: 542–549.
18. Scaltriti M, Rojo F, Ocana A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007; 99: 628–638.
19. Luen SJ, Salgado R, Fox S, et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol 2017; 18: 52–62.
20. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 2008; 26: 1789–1796.
21. Baselga J, Cortes J, Im SA, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 2014; 32: 3753–3761.
22. Baselga J, Lewis Phillips GD, Verma S, et al. Relationship between tumor biomarkers
and efficacy in EMILIA, a phase III study of trastuzumab emtansine in HER2-positive metastatic breast cancer. *Clin Cancer Res* 2016; 22: 3755–3763.

23. Goel S, Wang Q, Watt AC, *et al.* Overcoming therapeutic resistance in HER2-positive breast cancers with CDK4/6 inhibitors. *Cancer Cell* 2016; 29: 255–269.

24. Giuliano M, Hu H, Wang YC, *et al.* A phase III, randomized, open-label phase II trial of trastuzumab and lapatinib with pertuzumab in women with HER2-enriched subtype as a predictor of pathological complete response following trastuzumab emtansine (T-DM1): the ZEPHIR trial. *Ann Oncol* 2016; 27: 619–624.

25. Gebhart G, Lamberts LE, Wimana Z, *et al.* Molecular imaging as a tool to investigate heterogeneity of advanced HER2-positive breast cancer and to predict patient outcome under trastuzumab emtansine (T-DM1): the ZEPHIR trial. *Ann Oncol* 2016; 27: 619–624.

26. Rimawi M, Ferrero JM, de la Haba-Rodriguez J, *et al.* First-line trastuzumab plus an aromatase inhibitor, with or without pertuzumab, in human epidermal growth factor receptor 2-positive and hormone receptor-positive metastatic breast cancer (PERTAIN): a randomized, open-label phase II trial. *J Clin Oncol* 2018; 36(Suppl.): abstract 1014.

27. Liu P, Huang WC, Li P, *et al.* Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med* 2011; 17: 461–469.

28. Sabbagh M, Gil-Gomez G, Guardia C, *et al.* Defective cyclin B1 induction in trastuzumab-eminansine (T-DM1) acquired resistance in HER2-positive breast cancer. *Clin Cancer Res* 2017; 23: 7006–7019.

29. Trail PA, Dubowchik GM and Lowinger TB. Antibody drug conjugates for treatment of breast cancer: Novel targets and diverse approaches in ADC design. *Pharmacol Ther* 2017; 17: 126–142.

30. Ocana Fernandez A, Ruiz Borrego M, Gil Martin M, *et al.* A phase II trial of dasatinib (D) in combination with trastuzumab (T) and paclitaxel (P) in the first line treatment of HER2 positive metastatic breast cancer (MBC) patients (pts): GEICAM/2010-04 [abstract]. *Ann Oncol* 2017; 28(Suppl. 5): abstract 239PD.

31. Ocana Fernandez A, Ruiz Borrego M, Gil Martin M, *et al.* A phase II trial of dasatinib (D) in combination with trastuzumab (T) and paclitaxel (P) in the first line treatment of HER2 positive metastatic breast cancer (MBC) patients (pts): GEICAM/2010-04 [abstract]. *Ann Oncol* 2017; 28(Suppl. 5): abstract 239PD.

32. Zhang S, Huang WC, Li P, *et al.* Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med* 2011; 17: 461–469.

33. Sabbagh M, Gil-Gomez G, Guardia C, *et al.* Defective cyclin B1 induction in trastuzumab-eminansine (T-DM1) acquired resistance in HER2-positive breast cancer. *Clin Cancer Res* 2017; 23: 7006–7019.

34. Li G, Guo J, Shen BQ, *et al.* Mechanisms of acquired resistance to trastuzumab emtansine in breast cancer cells. *Mol Cancer Ther* 2018; 17: 1441–1453.

35. Liu P, Huang WC, Li P, *et al.* Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med* 2011; 17: 461–469.

36. Gebhart G, Lamberts LE, Wimana Z, *et al.* HER2-positive breast cancer is lost in translation: time for patient-centered research. *Nat Rev Clin Oncol* 2017; 14: 669–681.

37. Trail PA, Dubowchik GM and Lowinger TB. Antibody drug conjugates for treatment of breast cancer: Novel targets and diverse approaches in ADC design. *Pharmacol Ther* 2018; 181: 126–142.

38. Gebhart G, Lamberts LE, Wimana Z, *et al.* HER2-positive breast cancer is lost in translation: time for patient-centered research. *Nat Rev Clin Oncol* 2017; 14: 669–681.

39. Trail PA, Dubowchik GM and Lowinger TB. Antibody drug conjugates for treatment of breast cancer: Novel targets and diverse approaches in ADC design. *Pharmacol Ther* 2018; 181: 126–142.

40. Saura C, Thistlethwaite F, Banerji U, *et al.* A phase I expansion cohorts study of SYD985 in heavily pretreated patients with HER2-positive or HER2-low metastatic breast cancer [abstract]. *J Clin Oncol* 2018; 36(Suppl.): abstract 1014.

41. Yurkovetskiy A, Gumerov D, Ter-Ovanesyan E, *et al.* Non-clinical pharmacokinetics of XMT-1522, a HER2 targeting auristatin-based antibody
drug conjugate [abstract]. Proc Am Assoc Cancer Res 2017; 58: abstract 48.

42. Doi T, Shitara K, Naito Y, et al. Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastroesophageal tumours: a phase 1 dose-escalation study. Lancet Oncol 2017; 18: 1512–1522.

43. van Herpen CM, Banerji U, Mommers EC, et al. First-in-human phase 1 study of SYD985, a novel anti-HER2 antibody-drug conjugate SYD985 [abstract]. Eur J Cancer 2015; 51(Suppl. 3): S65.

44. Aftimos PG, van Herpen CM, Mommers EC, et al. SYD985, a novel anti-HER2 ADC, shows promising activity in patients with HER2-positive and HER2-negative metastatic breast cancer [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2016.

45. Pegram M, Hamilton E, Tan AR, et al. Phase 1 study of bispecific HER2 antibody-drug conjugate MEDI4276 in patients with advanced HER2-positive breast or gastric cancer [abstract]. Ann Oncol 2018; 29: mdy048.05.

46. Zammarchi F, Chivers S, Williams DG, et al. ADCT-502, a novel pyrrolobenzodiazepine (PBD)-based antibody–drug conjugate (ADC) targeting low HER2-expressing solid cancers [abstract]. Eur J Cancer 2016; 69: S28.

47. Li JY, Perry SR, Muniz-Medina V, et al. A biparatopic HER2-targeting antibody-drug conjugate induces tumor regression in primary models refractory to or ineligible for HER2-targeted therapy. Cancer Cell 2016; 29: 117–129.

48. Nordstrom JL, Gorlatov S, Zhang W, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcγ receptor binding properties. Breast Cancer Res 2011; 13: R123.

49. Bang YJ, Giaccione G, Im SA, et al. First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. Ann Oncol 2017; 28: 855–861.

50. Alsina M, Boni V, Schellens JH, et al. First-in-human phase 1/2 study of MCLA-128, a full length IgG1 bispecific antibody targeting HER2 and HER3: final phase 1 data and preliminary activity in HER2+ metastatic breast cancer (MBC) [abstract]. J Clin Oncol 2017; 35 (Suppl.): abstract 2522.

51. Meric-Bernstam F, Beeram M, Ignacio Mayordomo J, et al. Single agent activity of ZW25, a HER2-targeted bispecific antibody, in heavily pretreated HER2-expressing cancers [abstract]. J Clin Oncol 2018; 36 (Suppl.): abstract 2500.

52. Wermke M, Alt J, Kauh JS, et al. Preliminary biomarker and pharmacodynamic data from a phase I study of single-agent bispecific antibody T-cell engager GBR 1302 in subjects with HER2-positive cancers [abstract]. J Clin Oncol 2018; 36: 69.

53. Hinner MJ, Bel Aiba R, Wiedenmann A, et al. Costimulatory T cell engagement via a novel bispecific anti-CD137/anti-HER2 protein [abstract]. J Immunother Cancer 2015; 3: P187.

54. Rius Ruiz I, Vicario R, Moranco B, et al. p95HER2-T cell bispecific antibody for breast cancer treatment. Sci Transl Med 2018; 10.

55. Blackwell KL, Burstein HJ, Storniolo AM, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. J Clin Oncol 2012; 30: 2583–2592.

56. Pivot X, Manikhas A, Zurawski B, et al. CEREBEL (EGF111438): a phase III, randomized, open-label study of lapatinib plus capecitabine versus trastuzumab plus capecitabine in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol 2015; 33: 1564–1573.

57. Cortes J, Dieras V, Ro J, et al. Afatinib alone or afatinib plus vinorelbine versus investigator’s choice of treatment for HER2-positive breast cancer with progressive brain metastases after trastuzumab, lapatinib, or both (LUX-Breast 3): a randomised, open-label trial. Lancet Oncol 2015; 16: 1700–1710.

58. Gelmon KA, Boyle FM, Kaufman B, et al. Lapatinib or trastuzumab plus taxane therapy for human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol 2015; 33: 1574–1583.

59. Harbeck N, Huang CS, Hurvitz S, et al. Afatinib versus vinorelbine versus trastuzumab plus vinorelbine in patients with HER2-overexpressing metastatic breast cancer who had progressed on one previous trastuzumab treatment (LUX-Breast 1): an open-label, randomised, phase 3 trial. Lancet Oncol 2016; 17: 357–366.

60. Freedman RA, Gelman RS, Melisko M, et al. TBCRC 022: phase II trial of neratinib +
capecitabine for patients (Pts) with human epidermal growth factor receptor 2 (HER2+) breast cancer brain metastases (BCBM) [abstract]. J Clin Oncol 2017; 35: 1005.

61. Freedman RA, Gelman RS, Wefel JS, et al. Translational breast cancer research consortium (TBCRC) 022: a phase II trial of neratinib for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases. J Clin Oncol 2016; 34: 945–952.

62. Hamilton E, Borges V, Conlin A, et al. Efficacy results of a phase 1b study of ONT-380, an oral HER2-specific inhibitor, in combination with capecitabine (C) and trastuzumab (T) in HER2+ metastatic breast cancer (MBC), including patients (pts) with brain metastases (mets) [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2016.

63. Borges VF, Ferrario C, Aucoin N, et al. Tucatinib combined with ado-trastuzumab emtansine in advanced ERBB2/HER2-positive metastatic breast cancer: a phase 1b clinical trial. JAMA Oncol 2018; 4: 1214–1220.

64. Metzger O, Barry W, Krop I, et al. Phase I dose-escalation trial of ONT-380 in combination with trastuzumab in patients (pts) with HER2+ breast cancer brain metastases [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2016.

65. Ma F, Li Q, Chen S, et al. Phase I study and biomarker analysis of pyrotinib, a novel irreversible pan-ErbB receptor tyrosine kinase inhibitor, in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol 2017; 35: 3105–3112.

66. Park YH, Lee KH, Sohn JH, et al. A phase II trial of the pan-HER inhibitor poziotinib, in patients with HER2-positive metastatic breast cancer who had received at least two prior HER2-directed regimens: results of the NOV120101-203 trial. Int J Cancer 2018; 143: 3240–3247.

67. Martin M, Holmes FA, Ejlertsen B, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017; 18: 1688–1700.

68. Awada A, Colomer R, Inoue K, et al. Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEIERT-T randomized clinical trial. JAMA Oncol 2016; 2: 1557–1564.

69. National Comprehensive Cancer Network. NCCN Guidelines for Patients: Breast Cancer Metastatic. Plymouth Meeting, PA: National Comprehensive Cancer Network, 2018.

70. Xu B, Ma F, Ouyang Q, et al. Abstract PD3-08: A randomized phase II trial of pyrotinib plus capecitabine versus lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer previously treated with taxanes, anthracyclines and/or trastuzumab. Cancer Res 2018; 78: Abstract nr PD3-08.

71. Bianchini G and Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. Lancet Oncol 2014; 15: e58–e68.

72. Kim A, Lee SJ, Kim YK, et al. Programmed death-ligand 1 (PD-L1) expression in tumour cell and tumour infiltrating lymphocytes of HER2-positive breast cancer and its prognostic value. Sci Rep 2017; 7: 11671.

73. Stagg J, Loi S, Divisekera U, et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci U S A 2011; 108: 7142–7147.

74. Dirix LY, Takacs I, Jerusalem G, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat 2018; 167: 671–686.

75. Loi S, Giobbie-Hurda A, Gombos A, et al. Phase Ib/II study evaluating safety and efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant HER2-positive metastatic breast cancer: results from the PANACEA (BCSG 45-13/BIG 4-13/KEYNOTE-014) study [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2017.

76. Emens LA, Esteva F, Beresford M, et al. Results from KATE2, a randomized phase 2 study of atezolizumab (atezo) + trastuzumab emtansine (T-DM1) vs placebo (pbo) + T-DM1 in previously treated HER2+ advanced breast cancer (BC) [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2018.

77. Mittendorf EA, Clifton GT, Holmes JP, et al. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. Ann Oncol 2014; 25: 1735–1742.

78. Diehl JA, Cheng M, Roussel MF, et al. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 1998; 12: 3499–3511.
79. Yu Q, Geng Y and Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. Nature 2001; 411: 1017–1021.

80. Choi YJ, Li X, Hydbring P, et al. The requirement for cyclin D function in tumor maintenance. Cancer Cell 2012; 22: 438–451.

81. Finn RS, Dering J, Conklin D, et al. PD0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009; 11: R77.

82. Witkiewicz AK, Cox D and Knudsen ES. CDK4/6 inhibition provides a potent adjunct to Her2-targeted therapies in preclinical breast cancer models. Genes Cancer 2014; 5: 261-72.

83. DeMichele A, Clark AS, Tan KS, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015; 21: 995–1001.

84. Patnaik A, Rosen LS, Tolaney SM, et al. Efficacy and safety of abemaciclib, an inhibitor of CDK4 and CDK6, for patients with breast cancer, non-small cell lung cancer, and other solid tumors. Cancer Discov 2016; 6: 740–753.

85. Ciruelos E, Villagrasa P, Pare L, et al. PAM50 intrinsic subtype predicts survival outcome in HER2-positive/hormone receptor-positive metastatic breast cancer treated with palbociclib and trastuzumab: a correlative analysis of the PATRICIA (SOLTI 13-03) trial [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2017.

86. Ciruelos E, Villagrasa P, Paré L, et al. SOLTI-1303 PATRICIA phase II trial (STAGE 1) — Palbociclib and trastuzumab in postmenopausal patients with HER2-positive metastatic breast cancer [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2018.

87. Bachelot T, Kabos P, Yardley D, et al. Abemaciclib for the treatment of brain metastases secondary to hormone receptor positive breast cancer [abstract]. San Antonio, TX: San Antonio Breast Cancer Symposium, 2017.

88. Serra V, Scaltriti M, Prudkin L, et al. PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 2011; 30: 2547–2557.

89. Hurvitz SA, Andre F, Jiang Z, et al. Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1): a phase 3, randomised, double-blind, multicentre trial. Lancet Oncol 2015; 16: 816–829.

90. Andre F, O'Regan R, Ozguroglu M, et al. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 2014; 15: 580–591.

91. Andre F, Hurvitz S, Fasolo A, et al. Molecular alterations and everolimus efficacy in human epidermal growth factor receptor 2-overexpressing metastatic breast cancers: combined exploratory biomarker analysis from BOLERO-1 and BOLERO-3. j Clin Oncol 2016; 34: 2115–2124.

92. Shah PD, Chandrarlapaty S, Dickler MN, et al. Phase I study of LJM716, BYL719, and trastuzumab in patients (pts) with HER2-amplified (HER2+) metastatic breast cancer (MBC) [abstract]. j Clin Oncol 2015; 33: 590.

93. Jain S, Shah AN, Santa-Maria CA, et al. Phase I study of alpelisib (BYL-719) and trastuzumab emtansine (T-DM1) in HER2-positive metastatic breast cancer (MBC) after trastuzumab and taxane therapy. Breast Cancer Res Treat 2018; 171: 371–381.

94. Keegan NM, Walsh JM, Toomey S, et al. A phase Ib trial of copanlisib and trastuzumab in pretreated recurrent or metastatic HER2-positive breast cancer “PantHER” [abstract]. j Clin Oncol 2018; 36: 1036.