

The changing treatment of metastatic her2-positive breast cancer (Review)

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Abstract. Human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer has been historically associated with an aggressive disease course with common distant metastasis and poor prognosis. HER2-targeting therapies have significantly changed treatment and drastically improved outcomes for this group of patients. However, primary or acquired resistance to anti-HER2 regimens leads almost universally to disease progression, often with difficult to treat central nervous system (CNS) metastases. The current review summarized the existing therapeutic options for HER2-positive metastatic disease in the first, second and further line setting. Furthermore, novel agents currently under development were presented, which have demonstrated encouraging results in heavily pretreated patients or specific subgroups, such as HR-positive/HER2-positive tumors and CNS disease.

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1. Introduction

Breast cancer is the most common malignancy and first cause of cancer-related death among women worldwide (1). In the last years it has become clear that breast cancer is a heterogeneous disease with distinct molecular characteristics and clinical behavior. Human epidermal growth factor receptor 2 (HER2) gene amplification resulting in overexpression of the HER2 protein can be detected in approximately 15-20% of invasive breast tumors and is, historically, associated with an aggressive disease course with common distant metastases and poor prognosis (2-4).

HER2, a member of the epidermal growth factor family, is a transmembrane protein consisting of an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain with tyrosine kinase activity. Homo- and heterodimerization of the HER family proteins [epidermal growth factor receptor (EGFR), HER2, HER3, HER4], mostly involving HER2 as the preferred dimerization partner, leads to activation of various downstream signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) that promote cell proliferation, migration, survival and invasion (5,6).

The introduction of therapies inhibiting the HER2 signaling has drastically altered the landscape of treatment with significantly improved outcomes in this group of patients (7). However, primary or acquired resistance to anti-HER2 regimens leads almost universally to disease progression, often with development of difficult to treat central nervous system (CNS) metastases. In this review, we will summarize the existing therapeutic options for HER2-positive metastatic disease as well as novel agents currently under development that are likely to further improve prognosis.

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CDK, cyclin dependent kinase; CNS, central nervous system; CTLA4, cytotoxic T-lymphocyte antigen 4; DCR, disease control rate; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FcR, fc-receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; NK, natural killer cells; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression free survival; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; T-DM1, ado Trastuzumab emtasine; TILs, tumor-infiltrating lymphocytes; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden; TTP, time to progression

Key words: metastatic breast cancer, HER2-positive, HER2-targeting agents, triple-positive breast cancer, immunotherapy

2. Established HER2-targeting agents

Trastuzumab. Trastuzumab is a recombinant humanized IgG monoclonal antibody directed against the extracellular domain of HER2 with various molecular mechanisms (8). Upon binding to its receptor, trastuzumab induces internalization and degradation of HER2, inhibits the HER2-dependent downstream proliferation signaling and activates natural killer (NK) cells in the tumor microenvironment through antibody-dependent cellular cytotoxicity (ADCC). Trastuzumab was the first targeted agent to demonstrate impressive efficacy as monotherapy as well as in combination with chemotherapy in the first-line setting and the first monoclonal antibody to be approved for the treatment of metastatic HER2-positive breast cancer in 1998 (9,10).

Trastuzumab and chemotherapy in the first line setting. According to the landmark study of Slamon *et al* (10), the addition of trastuzumab to taxane or anthracycline/cyclophosphamide resulted in higher objective response rate (ORR) (50 vs. 32%, $P<0.001$), longer progression free survival (PFS) (7.4 vs. 4.6 months, HR=0.5, 95% CI: 0.41-0.63, $P<0.001$) and overall survival (OS) (25.1 vs. 20.3 months, HR=0.8, 95% CI: 0.64-1.00, $P=0.046$) compared to chemotherapy alone in patients with HER2-positive metastatic breast cancer (10). Because of the cardiotoxicity attributed to the combination of trastuzumab with anthracycline (cardiac dysfunction in 27 vs. 13% of the patients), trastuzumab and taxane was since adopted as the preferred treatment.

It has been suggested that vinorelbine could be an alternative option for taxanes in this setting, demonstrating similar PFS (12.4 vs. 15.3 months HR=0.94, 95% CI: 0.71-1.25; $P=0.67$) and OS rates (35.7 vs. 38.8 months, HR=1.01, 95% CI: 0.71-1.42; $P=0.98$) in a phase III trial (11). On the other hand, triple therapy with the addition of carboplatin (12,13) or non-pegylated liposomal doxorubicin (14) to trastuzumab and taxane showed no OS benefit and was associated with excessive toxicity.

Sequential trastuzumab and chemotherapy. Sequential treatment with initial single-agent trastuzumab followed by the addition of chemotherapy upon progression has always been considered an appealing strategy, given that it delays the initiation of chemotherapy. It has been evaluated in three randomized trials which, however, failed to demonstrate non-inferiority.

In the phase II HERTAX trial (15), patients received either trastuzumab monotherapy and switched to docetaxel at progression or upfront trastuzumab and docetaxel combination. ORR was better in the combination group (79 vs. 53%, $P=0.016$) and, despite similar PFS in both groups (9.4 vs. 9.9 months, HR=1.33, 95% CI: 0.86-2.06, $P=0.2$), OS was longer in the combination arm, with a trend towards statistical significance (30.5 vs. 19.7 months, HR=1.49, 95% CI: 0.91-2.47, $P=0.12$).

The phase III JO17360 trial (16) evaluated single-agent trastuzumab followed by the addition of docetaxel after progression vs. upfront docetaxel and trastuzumab combination. PFS after both trastuzumab and docetaxel was comparable (12.4 vs. 14.6 months, HR=1.35, 95% CI: 0.79-2.30, $p=0.27$). Nevertheless, patient accrual was terminated prematurely due to better PFS in the combination arm compared to trastuzumab monotherapy (14.6 vs. 3.7 months, HR=4.24, 95% CI:

2.48-7.24, $P<0.01$), which was the primary endpoint of the study. OS was also significantly better in the upfront combination arm (median OS not available due to small number of deaths, HR=2.72, 95% CI: 1.03-7.18, $P=0.04$).

SAKK 22/99 (17), a phase III study with similar design to JO17360, randomized patients to receive either trastuzumab followed by trastuzumab plus chemotherapy or upfront trastuzumab plus chemotherapy. Time to progression (TTP) and OS did not differ significantly between the two groups (12.2 vs. 10.3 months, HR=0.7, 95% CI: 0.5-1.1, $P=0.10$ and 35.6 vs. 36.3 months, HR=0.9, $P=0.55$, respectively). In subgroup analysis of the trastuzumab alone arm, patients without visceral disease had a significantly longer TTP compared to the ones with visceral metastases (21.8 vs. 10.1 months, $P=0.03$). Based on these findings, the authors suggest that single-agent trastuzumab could be a sufficient treatment option for individual patients without visceral involvement.

Pertuzumab. Like trastuzumab, pertuzumab is a recombinant monoclonal antibody against HER2. It binds to a different extracellular dimerization domain than trastuzumab and prevents HER2/HER3 heterodimerization, thereby acting complementarily to trastuzumab in inhibiting the downstream proliferation pathways (6).

Pertuzumab, trastuzumab and docetaxel in the first-line setting. Dual HER2 inhibition with trastuzumab and pertuzumab in combination with chemotherapy has been introduced as the current standard-of-care in first-line treatment based on the results of the phase III CLEOPATRA study (18,19). Previously untreated patients were randomized to receive trastuzumab and docetaxel with or without pertuzumab. The addition of pertuzumab led to significant improvement of ORR (80 vs. 69%, $P=0.001$), PFS (18.5 vs. 12.4 months, HR=0.62, 95% CI: 0.51-0.75, $P<0.001$) and OS (56.5 vs. 40.8 months, HR=0.68; 95% CI: 0.56-0.84, $P<0.001$). Recently, the end-of-study analysis further confirmed the durable superiority of the dual blockade with 8-year OS rates of 37 and 23%, respectively (20). According to a post-hoc analysis, continuing the administration of docetaxel after 6 cycles did not add clinical benefit in terms of PFS or OS (21). Patients who had previously received trastuzumab in the neoadjuvant or adjuvant setting were also found to experience similar benefit from the three-agent combination.

Pertuzumab, trastuzumab and other chemotherapy-regimens in the first-line setting. Chemotherapy regimens other than docetaxel have been evaluated in combination with trastuzumab and pertuzumab. As shown in the preliminary results of the PERUSE trial (22), paclitaxel-containing regimens are valid alternative options to docetaxel. ORR was 79% with docetaxel, 83% with paclitaxel and 77% with nab-paclitaxel, while PFS was 19.6, 23.0 and 18.1 months, respectively. Toxicity was in accordance with known safety profiles, with docetaxel being associated more frequently with febrile neutropenia (11 vs. 1%) and mucositis (25 vs. 14%) and less frequently with peripheral neuropathy (16 vs. 31%). Smaller single-arm studies have demonstrated efficacy for vinorelbine (ORR 74.2%, PFS 14.3 months) (23) and eribulin (ORR 80%) combined with dual HER2 blockade (24).

Pertuzumab, trastuzumab and chemotherapy in the second-line setting. Efficacy of dual HER2-blockade with trastuzumab and pertuzumab in patients previously treated with trastuzumab for advanced disease was investigated in the phase III PHEREXA trial (25). Patients who had progressed on trastuzumab and taxane in the first-line setting were randomized to receive trastuzumab and capecitabine with or without the addition of pertuzumab. The primary endpoint of PFS (11.1 vs. 9 months, HR=0.82, 95% CI: 0.65-1.02, P=0.07) was not reached, although there was a non-significant improvement in the pertuzumab arm. Similarly, there was a numerical survival benefit in the pertuzumab group (OS 36.1 vs. 28.1 months, HR=0.76; 95% CI: 0.60-0.98), but statistical significance was not claimed as the trial was underpowered for OS analysis (26). Although the combination of trastuzumab and pertuzumab does seem to offer clinical benefit beyond the first-line setting and the reported median PFS is comparable to the one demonstrated with ado trastuzumab emtasine (T-DM1), PHEREXA is a negative trial and T-DM1 remains the current standard-of-care in the second-line setting.

Ado Trastuzumab emtasine (T-DM1). T-DM1 is an antibody-drug conjugate composed of trastuzumab and the potent microtubule-inhibitory agent DM1. In binding HER2, the cytotoxic component is selectively delivered to HER2⁺ cells and leads to apoptosis upon intracellular release (27).

T-DM1 in the second- and third-line setting. Efficacy of T-DM1 as third- and second-line treatment was demonstrated in TH3RESA (28,29) and EMILIA (30,31) trials, respectively. TH3RESA compared T-DM1 to treatment of physician's choice after previous exposure to trastuzumab, lapatinib and taxane and demonstrated a significantly longer PFS (6.2 vs. 3.3 months, HR=0.53, 95% CI: 0.42-0.66, P<0.0001) and OS (22.7 vs. 15.8 months, HR=0.68, 95% CI: 0.54-0.85, P=0.0007) in the T-DM1 group, thereby establishing T-DM1 as preferred therapy in the third line. T-DM1 was also associated with a lower incidence of grade 3 or worse adverse events (32 vs. 43%).

In EMILIA, patients previously treated with trastuzumab and a taxane were randomized to receive T-DM1 or lapatinib plus capecitabine-the standard-of-care at the time. T-DM1 was associated with significantly improved PFS (9.6 vs. 6.4 months, HR=0.65, 95% CI: 0.55-0.77, P<0.001) and OS (30.9 vs. 25.1 months, HR=0.68, 95% CI: 0.55-0.85, P<0.001) and lower toxicity (grade 3/4 adverse events in 41 vs. 57% of the patients). Based on these results, T-DM1 was rapidly adopted as the preferred regimen in the second line.

T-DM1 in the first-line setting. T-DM1 in the first-line was evaluated in the MARIANNE trial (32,33). In this randomized phase III study, previously untreated patients were divided in three treatment arms: T-DM1, T-DM1 plus pertuzumab and trastuzumab plus taxane. The study demonstrated non-inferiority-but also no superiority - concerning the primary endpoint of PFS for both the T-DM1 and T-DM1 plus pertuzumab arms (14.1 and 15.2 months, respectively vs. 13.7 months in the trastuzumab plus taxane group, P=0.31 and 0.14). OS exceeded 50 months and was also similar across three treatment arms. T-DM1-containing arms were associ-

ated with lower treatment discontinuation rates due to adverse events and better quality of life. However, since comparison was not made to a group receiving dual HER2 blockade, which is the current standard-of-care, T-DM1 remains an alternative first-line option mainly for patients unsuitable to receive the preferred regimen.

Lapatinib. Lapatinib is a small-molecule dual tyrosine kinase inhibitor (TKI) reversibly targeting the intracellular domain of both EGFR and HER2 and blocking activation of downstream proliferation pathways and was, until recently, the only TKI approved in the treatment of HER2⁺ breast cancer (34).

Historically, lapatinib in combination with capecitabine was a commonly used treatment upon progression on trastuzumab plus chemotherapy (35). However, since superiority of T-DM1 and, more recently, neratinib was demonstrated, this regimen is reserved for later lines of therapy.

Lapatinib in the first-line setting. Lapatinib is inferior to trastuzumab in the first-line setting, as shown in the MA.31 trial (36), in which previously untreated patients with metastatic disease received taxane and HER2 blockade with either trastuzumab or lapatinib. Lapatinib was associated with shorter intention-to-treat PFS (9.0 vs. 11.3 months, HR=1.37, 95% CI: 1.13-1.65, P=0.001), which was the primary endpoint of the study. Toxicity was also higher in the lapatinib arm, with more incidents of grade 3/4 diarrhea and rash (P<0.001).

Lapatinib +/- trastuzumab in pretreated patients. According to the findings of the EFG104900 trial (37), combination of lapatinib and trastuzumab in trastuzumab-exposed patients is superior to lapatinib alone in terms of PFS (11.1 vs. 8.1 weeks, HR=0.74, 95% CI: 0.58-0.94, P=0.011) and OS (14 vs. 9.5 months, HR=0.74, 95% CI: 0.57-0.97, P=0.026), with the exception of hormone receptor (HR)-positive tumors, which are known to demonstrate higher levels of HER2 resistance (38). Therefore, combination of lapatinib plus trastuzumab can be considered a chemotherapy-free alternative for heavily pretreated patients.

3. Triple positive breast cancer

Approximately 50% of HER2-positive breast tumors also express HR (38). There is a known bidirectional crosstalk between the estrogen receptor (ER) and HER receptor families, resulting in overexpression of ER in the presence of acquired HER2 resistance and resistance to hormonal treatment through HER2 overexpression (39). The obvious therapeutic strategy for this group of patients includes simultaneous blocking of both signaling pathways with a combination of HER2-targeted agents and endocrine therapy. It has been previously shown that addition of trastuzumab or lapatinib to an aromatase inhibitor prolongs PFS, although not OS, compared to endocrine therapy alone in the metastatic setting (40-42). Addition of endocrine therapy to HER2-targeted treatment is much less studied although widely used - there is to date no randomized trial.

Historically, clinical trials did not distinguish the HR status in HER2⁺ disease. Dual HER blockade plus chemotherapy has been the standard of care for these patients, although the idea

of chemotherapy-free regimens has always been appealing. Efficacy of endocrine treatment combined with single-agent or dual HER2 blockade in the absence of chemotherapy has been recently investigated in the PERTAIN and ALTERNATIVE trials (43,44).

In the phase II PERTAIN trial, treatment-naïve patients (with exception of endocrine therapy) were randomized to receive trastuzumab and an aromatase inhibitor with or without Pertuzumab (43). The primary endpoint of the study was PFS, which was shown to be significantly better with the dual blockade (18.9 vs. 15.8 months, HR=0.65, 95% CI: 0.48-0.89, P=0.007). ORR was similar between the two arms (63.3 vs. 55.7%, P=0.254), nevertheless, duration of response was significantly longer in the pertuzumab group (27.1 vs. 15.1 months, HR=0.57, 95% CI: 0.36-0.91, P=0.018).

ALTERNATIVE (44), a randomized, phase III trial, evaluated first-line treatment with an aromatase inhibitor combined with trastuzumab or lapatinib or both in postmenopausal women. Patients in the dual blockade arm had a significantly longer PFS compared to trastuzumab (11.0 vs. 5.7 months, HR=0.62, 95% CI: 0.45-0.88, P=0.0064), which was the primary endpoint of the study. Median PFS with lapatinib was 8.3 months (HR vs. trastuzumab 0.71, 95% CI: 0.51-0.98, P=0.036). Adverse events like diarrhea, rash and paronychia were more common in the lapatinib containing regimens, mostly grade 1 and 2.

Both trials suggest encouraging efficacy of dual HER2 blockade and endocrine therapy in previously untreated HER2-positive, HR-positive disease, thereby offering an alternative chemotherapy-free option for selected patients unfit to receive chemotherapy.

4. Novel agents

New generation TKIs. Novel TKIs currently adopted in clinical practice include neratinib and pyrotinib, two irreversible pan-HER TKIs targeting EGFR, HER2 and HER4, as well as tucatinib, a highly selective HER2 inhibitor with minimal inhibition of EGFR, therefore with decreased potential for EGFR-related toxicities.

Neratinib in later-line setting. The phase III NALA trial (45) compared neratinib plus capecitabine to lapatinib plus capecitabine after two or more anti-HER2 containing treatment lines. Treatment with neratinib resulted in significantly longer PFS (8.8 vs. 6.6 months, P=0.0003) and a trend towards improved OS (24.0 vs. 22.2 months, HR=0.88, 95% CI: 0.72-1.07, P=0.2086). Grade 3 diarrhea was more common in the neratinib arm (24 vs. 13%), although discontinuation of treatment due to adverse events was lower with neratinib (10.9 vs. 14.5%). Based on these findings, the combination of neratinib and capecitabine has been approved from the Food and Drug Administration (FDA) in April 2020 as third-line treatment in metastatic HER2-positive breast cancer.

Neratinib in the first-line setting. In the phase II NefERT-T trial (46), neratinib failed to demonstrate superiority against trastuzumab as first-line therapy. Patients with previously untreated advanced disease were randomized to receive paclitaxel with either trastuzumab or neratinib. There was no

difference in the median PFS (12.9 months in both arms), while neratinib was associated with higher incidence of grade 3 diarrhea (30.4 vs. 3.8%). However, a sub-analysis of NefERT-T showed neratinib to be more effective than trastuzumab in preventing CNS disease (relative risk of brain metastases 0.48, P=0.002).

Neratinib in CNS disease. CNS activity of neratinib has also been tested outside the NefERT-T trial. In one cohort of the single-arm multi-cohort TBCRC 022 trial (47), forty patients with progressive CNS disease after at least one line of CNS-directed treatment were treated with neratinib monotherapy. ORR was only 8%, resulting in a median PFS of 1.9 months. However, combination of neratinib and capecitabine in another cohort of the same trial (48) resulted in more promising outcomes. Patients with or without prior exposure to lapatinib were treated with neratinib and capecitabine, with an ORR of 33 and 49%, PFS of 3.1 and 5.5 months and OS of 15.1 and 13.3 months, respectively. As noted in the study, these findings support a synergy between HER2-targeted agents and chemotherapy.

Pyrotinib in second-line setting. Efficacy of pyrotinib has been recently evaluated in a Chinese open-label, randomized phase II study (49). Patients enrolled in the study had been previously treated with chemotherapy and trastuzumab and were randomized to receive either pyrotinib or lapatinib - both drugs in combination with capecitabine. ORR was the primary endpoint of the study and was significantly better in the pyrotinib group (78.5 vs. 57.1%, treatment difference 21.3%, 95% CI: 4.0-38.7, P=0.01). Pyrotinib demonstrated significant superiority in terms of PFS as well (18.1 vs. 7.0 months, HR=0.36, 95% CI: 0.23-0.58, P<0.001). As far as toxicity is concerned, grade 3/4 hand-foot syndrome and diarrhea were more common in the pyrotinib arm (24.6 vs. 20.6% and 15.4 vs. 4.8%, respectively). These findings were confirmed in the phase III PHOEBE trial (50) comparing pyrotinib plus capecitabine to lapatinib plus capecitabine after exposure to trastuzumab and taxane. PFS was significantly longer with pyrotinib (12.5 vs. 6.8 months, HR=0.39, 95% CI: 0.27-0.56, P<0.0001), while the toxicity profile was in accordance with the one of the phase II trial.

Another randomized phase III trial (51) evaluated capecitabine with pyrotinib or placebo in Chinese patients after failure of trastuzumab and taxane. PFS was significantly longer in the pyrotinib arm (11.1 vs. 4.1 months). According to the study design, patients who progressed on placebo plus capecitabine received pyrotinib monotherapy. In this subgroup of patients, ORR was 38% and PFS 5.5 months. The most common adverse event in the pyrotinib arm was grade 3 diarrhea, which was observed in 30.8% of patients.

The biggest limitation of these trials is that, among the study population, there was no prior exposure to current standards of care in earlier lines such as pertuzumab and T-DM1. However, pyrotinib in combination with capecitabine has already received approval in China for HER2⁺ breast cancer after anthracycline or taxane chemotherapy (52).

Tucatinib in later-line setting. Tucatinib was recently granted Breakthrough Therapy designation by the FDA in combina-

tion with trastuzumab and capecitabine as second or later line treatment, based on the positive results of the HER2CLIMB trial (53). In this randomized trial, patients pretreated with trastuzumab, pertuzumab and T-DM1 received a combination of trastuzumab and capecitabine with or without tucatinib. The addition of tucatinib resulted in improved PFS at 1 year (33.1 vs. 12.3%, HR for progression or death 0.54, 95% CI: 0.42-0.71, $P<0.001$). ORR (40.6 vs. 22.8%) and OS at 2 years (44.9 vs. 26.6%, HR for death 0.66, 95% CI: 0.50-0.88, $P=0.005$) were significantly better in the tucatinib group as well.

Tucatinib in CNS disease. Interestingly, patients with brain metastases were included in HER2CLIMB, composing almost half of the study population (47.5%), and seemed to benefit particularly from the triplet therapy (PFS at 1 year 24.9 vs. 0%, HR=0.48, 95% CI: 0.34-0.69, $P<0.001$, median CNS-PFS 9.9 vs. 4.2 months, median OS 18.1 vs. 12.0 months, HR=0.58, 95% CI: 0.40-0.85, $P=0.005$) (54). Since brain metastases appear often in the course of the disease, these findings make tucatinib an extremely promising later-line option.

Antibody-drug conjugate Trastuzumab deruxtecan (DS-8201). Trastuzumab deruxtecan is an antibody-drug conjugate comprised of an anti-HER2 antibody bound to a cytotoxic topoisomerase I inhibitor (a derivative of irinotecan) (55). It has been recently granted accelerated approval by the FDA for patients who have received at least two anti-HER2-based treatment lines. Efficacy was evaluated in the phase II single-arm DESTINY-Breast01 trial (55) enrolling patients pretreated with trastuzumab and T-DM1. Patients included in the study had received a median of six previous treatments, however achieved an impressive ORR of 60.9% (95% CI: 53.4-68.0), with a median duration of response of 14.8 months (95% CI: 13.8-16.9). As far as tolerability is concerned, among myelosuppression and nausea, interstitial lung disease occurred in 13.6% of the patients, including one death. Thus, careful monitoring for pulmonary symptoms is considered mandatory during treatment.

Interestingly, DESTINY-Breast01 included 24 patients with pretreated asymptomatic brain metastases. ORR was similar to the whole patient population (58.3%, 95% CI 36.3-77.9), with a median duration of response of 16.9 months (95% CI: 5.7-16.9) (56). In fact, one patient experienced a >50% regression of in-brain disease.

Antibody-drug conjugate trastuzumab duocarmazine (SYD985). Trastuzumab duocarmazine is composed of trastuzumab linked, via a cleavable linker, to a prodrug of the alkylating agent duocarmycin. Evaluation of the drug in a phase I dose-escalation and dose-expansion study (57) including patients with different solid tumors showed an ORR of 33% (16 of 48 patients) in HER2-overexpressing breast cancer and 29% (9 of 32 patients) in hormone-receptor positive, HER2-low breast cancer. A phase III trial comparing trastuzumab duocarmazine to physician's choice treatment in patients who have progressed after two anti-HER2 therapies is currently recruiting (NCT03262935).

Several other antibody-drug conjugates in earlier stages of development are being investigated in preclinical and clinical trials (A166, ALT-P7, ARX788, DHES0815A, MEDI4276, RC48, XMT-1522) (58).

HER2-targeting monoclonal antibody Margetuximab (MGAH22). Fc-receptor (FcR) polymorphism is considered one of the mechanisms of resistance to trastuzumab by reducing the trastuzumab-mediated cellular cytotoxicity. Margetuximab is a chimeric IgG monoclonal antibody with similar antiproliferative effects as trastuzumab, but greater affinity to variants of the CD16A receptor (59).

Margetuximab in later-line setting. Based on the rationale that increased affinity can enhance antibody-mediated immunity, margetuximab versus trastuzumab in heavily pretreated patients has been investigated in the phase III SOPHIA trial (60). Patients exposed to two or more HER2-targeting regimens were randomized to margetuximab plus chemotherapy or trastuzumab plus chemotherapy. PFS was significantly longer in the margetuximab arm in the ITT population (5.8 vs. 4.9 months, HR=0.76, $P=0.033$) and, interestingly, in patients harboring the CD16A-158 F allele (6.9 vs. 5.1 months, HR=0.68, $P=0.005$), which is associated with poor clinical response to trastuzumab. Furthermore, OS in the second interim analysis of patients carrying the CD16A-158F allele favored margetuximab, although not significantly (23.7 vs. 19.4 months, $P=0.087$). Trastuzumab performed better in the homozygous patients, although clinical characteristics of the population were not balanced, with more patients with visceral and brain metastasis and older age being assigned to margetuximab. Final OS analysis is awaited in order to better define the role of margetuximab in this setting.

Bispecific antibody ZW25. ZW25 is a bispecific HER2-targeting antibody that simultaneously binds two non-overlapping HER2 epitopes, resulting in dual HER2 blockade with increased tumor cell binding and internalization (61). Effectiveness and safety of ZW25 in HER2-expressing tumors are being currently investigated in a 3-part study (NCT02892123). Part 1 has recently demonstrated clinical benefit in heavily pretreated patients with a variety of solid tumors, with a median PFS of 5.2 months and ORR >30% (62), while no dose-limiting adverse events occurred. Parts 2 and 3 of the study evaluating ZW25 as monotherapy and combined with chemotherapy in specific tumor types are still ongoing. Furthermore, a currently recruiting phase II trial evaluates ZW25 in combination with palbociclib and fulvestrant in HR⁺/HER2⁺ breast cancer (NCT04224272).

Bispecific antibody MCLA-128. MCLA-128 is a bispecific antibody with high antibody-dependent cellular cytotoxicity binding HER2 and HER3. Preliminary clinical activity in solid tumors has been confirmed in a phase I trial (62), where, among 10 patients with heavily pretreated metastatic breast cancer, clinical benefit rate was 70% and the drug showed a favorable toxicity profile (only one infusion related reaction grade 4 in a total of 15 patients). An ongoing phase II trial investigates the addition of MCLA-128 to trastuzumab and vinorelbine in HER2⁺ tumors, as well as to endocrine therapy in HR⁺/HER2 low tumors (NCT03321981).

Cyclin dependent kinases (CDK)4/6 inhibitors. CDK4/6 inhibitors target the cell proliferative activity of CDK4/6 in the estrogen signaling pathway and are currently, in combi-

nation with endocrine therapy, the standard-of-care in the treatment of hormone receptor positive/HER2 negative breast cancer (63). Potential for integration of CDK4/6 inhibitors in HER2-positive disease was first confirmed in the phase II monarchHER trial (64), in which patients were randomized to receive either abemaciclib, fulvestrant and trastuzumab (arm A), or abemaciclib and trastuzumab (arm B) or trastuzumab plus chemotherapy (arm C). The triple combination significantly improved PFS (8.3 vs. 5.5 months, HR=0.67, P=0.051) compared to trastuzumab plus chemotherapy. On the other hand, no benefit was demonstrated for the abemaciclib/trastuzumab arm. According to an exploratory analysis, median overall survival is to date not significantly different between arms A and C (24 vs. 21 months).

CDK4/6 inhibition in ER⁺/HER2⁺ disease is being further studied in ongoing clinical trials. The phase II PATRICIA trial (65) evaluates the combination of palbociclib and trastuzumab in postmenopausal patients after 2-4 lines of HER2-directed treatments. During phase I of the trial, 60 patients were divided in 3 cohorts: ER-negative (cohort A), ER-positive (cohort B1) and ER-positive receiving letrozole (cohort B2). Phase I confirmed the efficacy of the regimen for both the luminal A and luminal B subtypes (median PFS 12.4 vs. 4.1 months in cohort A, p=0.025). Recruitment in phase II is ongoing with a target of a total of 232 patients (NCT02448420). In accordance with the known safety profile of CDK4/6 inhibitors, high grade neutropenia was observed in at least 80% of the participants. In the phase III PATINA trial (66), patients receive 6-8 cycles of chemotherapy with taxane or vinorelbine plus dual HER2-directed treatment with trastuzumab and pertuzumab and are then randomized to trastuzumab/pertuzumab and endocrine treatment with or without palbociclib. Primary endpoint of the study is PFS (NCT02947685).

PI3K/AKT/mTOR inhibition. A potential mechanism of resistance to HER2-targeted therapy is the permanent upregulation of the PI3K/AKT/mTOR pathway, often due to mutations of the PIK3CA gene or loss of function of the PTEN tumor suppressing phosphatase (67). Various studies have tested the hypothesis that combined targeting of PIK/AKT/mTOR and HER2 could overcome HER2 resistance.

Everolimus. Everolimus is an mTOR inhibitor that showed promising results in combination with trastuzumab and chemotherapy in early studies (68), nonetheless failed to demonstrate clinically significant benefit in the phase III BOLERO-3 (69) and BOLERO-1 (70) trials.

BOLERO-3 assessed the addition of everolimus to trastuzumab and chemotherapy in patients with disease progression under HER2-inhibition. Everolimus led to a median PFS of 7.0 vs. 5.8 months (HR=0.78, 95% CI: 0.65-0.95, P=0.0067), a statistically significant but moderate benefit. In BOLERO-1, a study including patients in the first-line setting, hazard of progression was similar in both groups (15.0 vs. 14.5 months, HR=0.89, 95% CI: 0.73-1.08, P=0.1166), while everolimus was associated with increased toxicity. However, according to a combined exploratory analysis of both studies, the subpopulation of patients with hyperactivation of the PI3K pathway seemed to profit from everolimus in terms of PFS (71).

1st line setting

Trastuzumab + Pertuzumab ± Taxane (mainstay)

Contraindication to chemotherapy:
Trastuzumab + Pertuzumab

Selected cases* of HR-positive disease:
AI ± Trastuzumab or Lapatinib

2nd line setting

T-DM1

Further line setting

If not previously received: T-DM1

If not previously received: Pertuzumab in
combination with Trastuzumab

If T-DM1 / Pertuzumab previously
received multiple options available
(insufficient evidence to recommend one
regimen over another)

Trastuzumab + Chemotherapy

Lapatinib + Trastuzumab

Lapatinib + Capecitabine

Lapatinib + AI (HR-positive disease)

Preferred in CNS disease

Neratinib + Capecitabine

Tucatinib + Trastuzumab + Capecitabine

Pyrotinib + Capecitabine

Trastuzumab deruxtecan

Figure 1. Proposed treatment algorithm for patients with metastatic HER2-positive breast cancer. *Low burden disease, long disease-free interval and contraindication for HER2-targeted treatment. HR, hormone receptor; AI, aromatase inhibitor; CNS, central nervous system.

PI3K inhibitors. Efficacy of PI3K blockage was initially tested using non-specific anti-PI3K drugs (68). A phase Ib trial evaluated treatment with the pan-PI3K inhibitor buparlisib and lapatinib in trastuzumab-pretreated patients (72). The combination led to a disease control rate of 79% (95% CI: 57-92) and clinical benefit rate of 29% (95% CI: 12-51) in a total of 24 patients, although high toxicity rates were found to be an important limiting factor.

More recently, newer agents managed to demonstrate clinical activity in small studies, while offering a better tolerated toxicity profile. For instance, the combination of alpelisib, a PI3K α isoform-specific inhibitor, and T-DM1 in trastuzumab-refractory disease resulted in an ORR of 43% in the total population (n=17) and 30% in T-DM1-resistant patients (n=10) (73). Alpelisib combined with dual HER2-blockade is currently under evaluation as maintenance treatment in patients harboring a PIK3CA mutation in a phase III trial (NCT04208178). Other selective PI3K inhibitors such as taselisib and MEN1611 are also being tested in combination with anti-HER2 treatment in early-phase trials (NCT02390427, NCT03767335) (68).

Immunotherapy. Although breast cancer is historically considered poorly immunogenic, HER2⁺ tumors have more

Table I. Molecular targets of the various therapeutic agents in metastatic HER2-positive breast cancer

Drug Name(s)	Target
Anti-HER2 agents	
Trastuzumab, pertuzumab ^a	HER2
Margentuximab ^a	HER2 (greater affinity to variants of the CD16A receptor)
ZW25 ^b	Two non-overlapping HER2 epitopes
MCLA-12 ^b	HER2, HER3
Lapatinib ^c	EGFR, HER2
Neratinib, pyrotinib ^c	EGFR, HER2, HER3, HER4
Tucatinib ^c	HER2
Antibody-drug conjugates (trastuzumab + cytotoxic agent)	
T-DM1	HER2 + microtubules
Trastuzumab deruxtecan	HER2 + topoisomerase 1
Trastuzumab duocarmazine	HER2 + DNA (alkyl group binding)
CDK4/6 inhibitors (estrogen signaling pathway)	
Abemaciclib, palbociclib	CDK 4/6
PI3K/AKT/mTOR inhibitors	
Everolimus	mTOR
Buparlisib, alpelisib	PI3K
Immunotherapeutic agents	
Atezolizumab	PD-L1
Pembrolizumab	PD-1

^aMonoclonal antibodies. ^bBispecific antibodies. ^cTyrosine kinase inhibitors. EGFR, epidermal growth factor receptor; CDK, cyclin dependent kinase; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositol-4,5-biphosphate 3-kinase; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1.

often higher expression of possible predictive biomarkers such as programmed death-ligand 1 (PD-L1), tumor-infiltrating lymphocytes (TILs) and tumor mutational burden (TMB) compared to luminal subtypes (74). Trastuzumab is also believed to enhance immunogenicity due to ADCC (59). Based on this rationale, immunotherapy with immune checkpoint-inhibitors has been evaluated in HER2⁺ breast cancer with considerable results in certain subgroups of patients.

Atezolizumab immunotherapy. Atezolizumab is a monoclonal IgG1 antibody directed against PD-L1. Efficacy in metastatic HER2⁺ breast cancer has been tested in the phase II KATE2 trial (75,76), where patients with progressive disease after trastuzumab and taxane were randomized to receive T-DM1 with or without atezolizumab and were stratified in two cohorts based on PD-L1 expression. There was no statistical significance in PFS in the intention-to-treat population (HR=0.82, P=0.3332), but an exploratory analysis of the PD-L1-positive subgroup (PD-L1 $\geq 1\%$) revealed a numerical PFS benefit in the atezolizumab arm (HR=0.60, 95% CI: 0.32-1.11). 1-year OS also favored not significantly the atezolizumab group (HR=0.55, 95% CI: 0.22-1.38). However, generalizability of the results remains uncertain due to the limited number of patients (n=19).

Pembrolizumab. Combination of pembrolizumab, a programmed cell death protein-1 (PD-1) inhibitor, with trastuzumab in patients pretreated with trastuzumab was evaluated in the phase Ib-II PANACEA trial (77). In PD-L1-positive tumors, ORR was 15% (6 of 40 patients) and disease control rate (DCR) 25%, while there were no objective responders

among PD-L1-negative patients. ORR reached 39% and DCR 47% in a subgroup of PD-L1-positive patients with TILs $>5\%$ in the metastatic lesion, further underlying the need for reliable biomarkers allowing efficient patient selection.

Anyhow, more data are necessary to define the role of immune checkpoint inhibitors in HER2 positive metastatic breast cancer. Several phase I and II trials evaluating immunotherapy with PD-1/PD-L1 and CTLA4-blockade in HER2 positive breast cancer are currently ongoing (78).

5. Proposed treatment algorithm

Based on the data presented above, a proposed treatment algorithm for patients with metastatic HER2-positive breast cancer is shown in (Fig. 1). Distinct molecular targets of the different drugs assessed in the treatment of these patients are presented in (Table I).

6. Conclusion

Prognosis of HER2-positive metastatic breast cancer has improved drastically since the introduction of HER2-targeted therapies. However, acquired resistance to treatment is common in the course of the disease, with the development of brain metastases remaining a difficult problem in clinical practice. Various novel agents are currently under investigation either as monotherapy or in combination with existing regimens with encouraging results in pretreated patients. Integration of new treatments for specific subgroups such as patients with HR-positive/HER2-positive tumors or CNS disease could

further improve prognosis. Most importantly, identification of predictive factors is crucial to better determine the most appropriate therapeutic approach for different patients.

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MM and IPT wrote the manuscript, GG, SI and NKS critically revised the manuscript and EAK revised the manuscript and supervised the work. All authors read and approved the final manuscript. MM and EAK confirm the authenticity of all the data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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