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Management of advanced-stage HER2+ breast cancer: current evidence and future perspectives

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Abstract

Human Epidermal Growth Factor Receptor 2 (*HER2*) overexpression or amplification is found in 15–20% of invasive breast cancers. Relentless research efforts in molecular biology and drug development have led to the implementation of several HER2-targeting agents, including monoclonal antibodies, tyrosine kinase inhibitors and antibody-drug conjugates, representing one of the best examples of bench-to-bedside translation in oncology. While the individual classes of drugs have each brought improvements, the combinatorial and sequential use of different anti-HER2 therapies have increased cure rates in the early stage setting and substantially prolonged survival for patients with metastatic disease. In this review, we will describe key steps and pivotal studies in the development of the modern paradigm for the treatment of HER2+ advanced-stage breast cancer, including selection and sequencing of new generation HER2-targeted therapies. Lastly, we will outline the factors that are known today to be related to resistance to anti-HER2 therapies including intratumoral heterogeneity, activation of alternative signaling pathways, and immune escape mechanisms, and the potential strategies that might be used in the future to overcome these mechanisms and further improve patient outcomes.

INTRODUCTION

The discovery of the *Human Epidermal Growth Factor Receptor 2* (*HER2*, also known as *ERBB2*) gene has been one of the major breakthroughs in cancer biology over the last

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50 years[1,2]. In patients with breast cancer (BC), *HER2* amplification or overexpression is detected in approximately 15–20 % of cases and has been historically associated with aggressive disease behavior, poor survival, and decreased response to standard treatments such as chemotherapy and endocrine therapy (ET)[3].

The discovery and clinical characterization of HER2 as a biomarker in BC[4–11] spurred the development of monoclonal antibodies (mAbs) that showed promising anti-tumor activity in preclinical models[12–14]. One murine mAb directed against HER2^{p185} demonstrated remarkable preclinical activity and was subsequently humanized into humAb4D5–8, which was later named trastuzumab[15,16]. Trastuzumab has multiple postulated mechanisms of action, foremost of these is binding to the extracellular domain (ECD) IV of the HER2 receptor and thus preventing its cleavage and inhibiting HER2-mediated intracellular signaling cascades[17]. Specifically, inhibition of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling pathways leads to cell cycle arrest and suppression of cell growth and proliferation[18]. Trastuzumab also mediates the activation of antibody-dependent cell-mediated cytotoxicity (ADCC) by attracting immune cells within the tumor microenvironment[19–23]. The anti-tumor effects of trastuzumab were confirmed in Phase I and II clinical studies[24–27], which were followed by definitive Phase III trials that led to its regulatory approval for the treatment of BCs driven by the *HER2* oncogene, which we call HER2-positive (HER2+) BC today[28,29]. Since then, the survival of patients with this BC subtype has substantially changed and represents one of the most striking examples of biomarker-driven drug development in oncology. The success of trastuzumab also catalyzed the development of additional novel HER2-targeting therapies, including mAbs, tyrosine kinase inhibitors (TKIs) and antibody-drug conjugates (ADCs), and today we have eight Food and Drug Administration (FDA)-approved HER2-directed agents (Fig. 1). As a result, overall survival (OS) of patients with HER2+ metastatic BC (MBC) continues to improve over time[30–32].

Given the landscape of therapies and recent introduction of new options, herein, we will discuss the current evidence that supports the selection and sequencing of HER2 targeted therapies by line of treatment for patients with HER2+ MBC. The sensitivity of HER2 as a therapeutic target in BC suggests that ongoing research aimed at enhancing and optimizing neutralization of this protein target has potential to offer further improvement in clinical outcomes. As such, in addition to the advancements in drug discovery, we will also highlight the factors that have been associated with development of resistance to anti-HER2 therapies, including intratumoral heterogeneity, (re)-activation of alternative signaling pathways, and immune escape mechanisms, along with potential novel strategies that might be used to overcome these and improve patient outcomes for the future.

SEQUENCING OF HER2-TARGETED AGENTS FOR THE TREATMENT OF ADVANCED BREAST CANCER

First Line Treatment

In 2001, a pivotal Phase III trial demonstrated that the addition of trastuzumab to first-line chemotherapy (either anthracycline-cyclophosphamide or paclitaxel) resulted in a

significantly improved time to disease progression (7.4 vs 4.6 months, P<0.001) and OS (25.1 vs 20.3 months; P=0.046) in patients with HER2+ MBC compared with standard chemotherapy alone[28]. These results not only had a dramatic impact on the lives of patients with this subtype of BC, but also revolutionized the field and established the paradigm we still follow today, that targeting HER2 is a critical component in the treatment of HER2+ BC. The cross over design of this study (crossover rate around 66%) further demonstrated that patient survival was improved with the up-front or early use of trastuzumab, and given its more favorable cardiac safety (13% and 27% of cardiac disfunction in the paclitaxel-trastuzumab and the anthracycline-trastuzumab groups, respectively), the combination of paclitaxel with trastuzumab was ultimately selected and established as the preferred first-line treatment for HER2+ MBC[28]. Almost a decade later, the Phase III CLEOPATRA trial set a new standard of care, demonstrating substantial clinical benefit with the use of dual HER2 blockade, adding pertuzumab to trastuzumab and docetaxel (THP) as new first-line therapy[33] (Fig. 2). Unlike trastuzumab which binds to the ECD IV of HER2, pertuzumab binds to ECD II and suppresses HER2 heterodimerization with HER1, HER3 and HER4, thereby more effectively blocking downstream signaling[34,35]. The addition of pertuzumab resulted in a significantly improved median progression-free survival (PFS) from 12.4 months to 18.5 months[33] (hazard ratio [HR] 0.69, 95% confidence interval [CI] 0.58–0.81, P<0.001) and improvement in median OS from 40 months to 56 months[36] (HR 0.69, 95% CI 0.58–0.82; Table 1). With regards to toxicity, treatment-related adverse events, including febrile neutropenia, neutropenia, diarrhea, pneumonia and cellulitis, were similar between the treatment arms and were largely detected during concurrent use with chemotherapy[33,36]. The rate of left ventricular dysfunction was somewhat lower in the pertuzumab group than in the control group (6.6% vs 8.6%)[36] (Fig. 3). An updated and final analysis of the CLEOPATRA study revealed an 8-year landmark OS rate of 37% for those on the THP arm, and most remarkably 16% of these patients were alive without disease progression[37]. This large population of exceptional responders has raised exciting questions about the possibility of curing some patients with HER2+ MBC (Box 1).

In clinical practice today, the current recommendation is to co-administer docetaxel for six-eight cycles, if tolerated, with trastuzumab and pertuzumab (HP), then followed by maintenance HP alone until disease progression. The use of an alternative taxane to docetaxel, either paclitaxel or nab-paclitaxel, is associated with safety and efficacy comparable to that seen in the CLEOPATRA trial and these combinations have also been widely adopted into routine care[38,39]. In the subgroup of patients with hormone receptor (HR)+, HER2+ disease, the addition of ET to trastuzumab and pertuzumab maintenance is supported by several trials[40–43] (**see triple-positive BC section**; Table 1). It is important to underline, however, that none of the trials has directly evaluated the benefit of adding ET to maintenance HP versus no ET in this setting, even though this approach is in common practice today.

In patients where the administration of taxanes may not be feasible, other chemotherapy options can be considered. The Phase II open-label VELVET trial investigated the combination of intravenous vinorelbine plus pertuzumab and trastuzumab in patients with previously untreated locally advanced or MBC, reporting a median PFS of 14.3

(95% CI 11.2–17.5) and 11.5 (95% CI 10.3–15.8) months in Cohort 1 (pertuzumab and trastuzumab administered sequentially) and Cohort 2 (pertuzumab and trastuzumab co-infused), respectively[44,45]. Similarly, in the Phase II open-label EORTC 75111–10114 trial, which enrolled patients who were aged either ≥70 years or ≥60 years with confirmed functional restrictions, the addition of metronomic oral cyclophosphamide (50 mg per day) to trastuzumab plus pertuzumab was reported to numerically but not statistically increase median PFS compared to dual HER2 blockade alone (6-month PFS 73.4% vs 46.2%, P=0.12), with an acceptable safety profile[46].

In clinical practice, around 30% of patients with MBC experience oligoprogressive disease[47], defined as documented progression in up to 5 individual metastatic lesions[48]. In this specific clinical scenario, no consensus on the best strategy to apply (local ablative therapy plus continuation of systemic treatment vs change of the systemic therapy) exists[49]. In HER2+ MBC, several patients nowadays are generally managed with local treatment(s) and maintenance of the same anti-HER2 therapy, scant data from prospective trials support this attitude. Preliminary analysis of a Phase II clinical trial (CURB), which randomized patients with BC or lung cancer with up to 5 extracranial sites to the standard of care systemic therapy and stereotactic body radiation therapy (SBRT) up-front or delayed at further progression, reported PFS improvement for SBRT to oligoprogressive lesions in the lung cancer cohort, but not in patients with BC[50]. Although these data suggest that SBRT may not be associated with major changes in the natural history of MBC, it is worth noting that very few patients with HER2+ disease were enrolled in the trial. For this reason, the option to continue systemic therapy and add local ablative therapy to oligoprogressive lesions is often considered on an individualized basis and deserves further definitive prospective evaluation.

Other anti-HER2 agents and regimens have been tested in the first-line setting, but none to date has been successful in surpassing the efficacy of the THP regimen. Notably, T-DM1 (ado-trastuzumab emtansine), a HER2 targeting ADC, was tested against standard of care trastuzumab plus taxane in the randomized Phase III MARIANNE trial and failed to show superior PFS or OS in the first line patient population (Table 1)[51]. Novel generation HER2 ADCs, such as trastuzumab deruxtecan (T-DXd; discussed below), are now being tested in this setting. The Phase III DESTINY-Breast09 (NCT04784715) is assessing the efficacy and safety of T-DXd with or without pertuzumab compared to THP as first-line treatment for patients with HER2+ MBC. Similarly, there are trials currently exploring the potential of adding novel targeted agents, such as the anti-HER2 TKI tucatinib (HER2CLIMB-05, NCT05132582) or the cyclin-dependent kinase 4 and 6 inhibitor (CDK4/6i) palbociclib (PATINA, NCT02947685), to maintenance HP with the aim of further prolonging the efficacy of first-line treatment.

Second Line Treatment

The notion that anti-HER2 therapy could still work after tumor progression on anti-HER2 treatment was demonstrated in a series of clinical trials. In a Phase III study of patients with HER2+ MBC and disease progression on prior taxane and trastuzumab, the combination of lapatinib plus capecitabine produced superior PFS (8.4 vs 4.4 months, HR 0.49, 95% CI

0.34–0.71, P<0.001) compared to capecitabine alone[52]. In a second study, the combination of lapatinib and trastuzumab showed an increased PFS compared to trastuzumab alone in a Phase III trial of patients with HER2+, trastuzumab-refractory MBC (12 vs 8 weeks, HR 0.73, 95% CI 0.57–0.93, P=0.008), with a trend toward an improved OS[53]. As a result, lapatinib was the second HER2 targeted therapy and first HER2 TKI approved for HER2+ MBC. Similarly, the randomized Phase III PRECIOUS trial evaluated the re-administration of pertuzumab plus trastuzumab and physician's choice chemotherapy in patients with HER2+ MBC who had disease progression following pertuzumab-containing therapy, and reported an improved PFS for the rechallenge over trastuzumab and chemotherapy alone (median PFS 5.3 vs 4.2 months, HR 0.76 one-side 95% CI 0.97, P=0.022)[54]. Taken together, these trials helped to establish a role for continued targeting of HER2 beyond progression on trastuzumab. Limited evidence, however, is available regarding the optimal number of lines of HER2 blockade beyond progression to initial trastuzumab. For instance, the Phase III GBG 26/BIG 3–05 trial, which randomized patients with HER2+ MBC with disease progression on trastuzumab to either capecitabine with or without trastuzumab, did not demonstrate a significant OS benefit (24.9 vs 20.6 months, HR 0.94 95% CI 0.65–1.35, P=0.73) for treatment on the trastuzumab-containing arm[55], although a post-hoc analysis revealed that patients receiving anti-HER2 treatment after second disease progression had a better post-progression survival[55]. Hence the concept of continued sequential blockade of HER2 has been widely adopted into clinical practice.

T-DM1, the prototype anti-HER2 ADC, composed of trastuzumab connected via non-cleavable linkers to the tubulin-targeting payload DM1,[56] was a novel and groundbreaking HER2-targeting therapy when first introduced into the clinic. ADCs offered the unique potential to combine potent cytotoxicity with targeted delivery in one treatment, and clinically T-DM1 fulfilled the promise of the ADC paradigm. The pivotal study that led to the approval of T-DM1 for HER2+ MBC was the EMILIA trial. Patients who were previously treated with trastuzumab and taxane for HER2+ MBC, were randomly assigned to either standard of care second line lapatinib plus capecitabine or T-DM1. Not only was T-DM1 associated with a significantly prolonged 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) (Table 1), but it was also associated with a more favorable overall safety profile with less high-grade toxicities[57]. The most common grade 3/4 adverse events with T-DM1 were thrombocytopenia (12.9%) and elevated serum concentration of transaminases (2.9–4.3%), while grade 3/4 diarrhea (20.7%) and palmar-plantar erythrodysesthesia (16.4%) were most commonly observed in the lapatinib-capecitabine group[57] (Fig. 3). An updated analysis of EMILIA, which included patients who crossed over from the control arm to T-DM1, confirmed the OS benefit (29.9 vs 25.9 months, HR 0.75, 95% CI 0.64–0.88)[58]. Although, as previously noted above, T-DM1 could not supersede first line therapy in the MARIANNE trial[51] and was never specifically tested in a post-pertuzumab population, it was ultimately established as the preferred second-line treatment for HER2+ MBC.

More recently, introduction of the new generation HER2 ADC T-DXd, has once again transformed the treatment landscape of HER2+ BC. T-DXd is differentiated from T-DM1 most significantly by its novel cleavable linker and potent topoisomerase I inhibitor payload, with a higher drug-to-antibody ratio (DAR) at 8 which is twice that of T-DM1. In particular,

when the linker is cleaved, it allows the payload to retain membrane permeability and diffuse from cells that express the target antigen to neighboring cells where it can exert its cytotoxic effects irrespective of target antigen expression. This 'bystander effect' is considered a unique and crucial aspect of T-DXd efficacy against tumors with heterogeneity of HER2 surface expression and differentiates T-DXd from all other HER2 targeted therapies available today[59].

In the first dedicated study of T-DXd for patients with HER2+ MBC, DESTINY-Breast01, T-DXd showed a striking response rate (60.9%, 95% CI 53.4–68.0), PFS (16.4 months, 95% CI 12.7-not reached) and OS (28.4 months, 95% CI 24.6–37.2) in this heavily pretreated cohort of patients with a median of 6 lines of prior therapy, including prior T-DM1[60,61]. The meaningful activity observed in Phase I/II trials[60–63], ultimately led to the DESTINY-Breast03 trial, a randomized Phase III study designed to compare T-DXd with T-DM1 in patients with HER2+ BC who had been previously treated with trastuzumab and taxane in the advanced or metastatic setting[64]. Approximately 60% of patients had also received prior pertuzumab and half the patients had only one prior line of therapy in the metastatic setting, hence the trial included a large second line population. Importantly, this was the first clinical trial to directly compare two ADCs targeting the same antigen, against each other. Not only did the study meet its primary PFS endpoint with an HR of 0.33 and an improvement in median PFS from 7 months with T-DM1 to 29 months with T-DXd, there was also a significant OS advantage with a HR of 0.64, convincingly establishing T-DXd as the more efficacious HER2 ADC[64] (Table 1). Based on the DESTINY-Breast03 results, T-DXd is now endorsed by various international guidelines as the preferred second line option for the treatment of HER2+ MBC today (Fig. 2). Moreover, with a complete response rate of 21% and therefore a potential for durable remissions, there is significant interest in the long-term survival outcomes from this trial as it matures[65]. It is important to note that a limited number of patients in the control arm (17%) crossed over to T-DXd, which still leaves open the question of optimal treatment sequencing and whether a similar OS may be achieved by delaying T-DXd to further lines, where it also was associated with an OS advantage in the DESTINY-Breast02 trial (discussed afterwards). Longer follow-up of these pivotal studies and additional information on other potential advantages with earlier use of T-DXd (e.g., long term remission rates and prevention of CNS disease) may ultimately help resolve this question. Also relevant to this issue is the ongoing DESTINY-Breast09 trial (NCT04784715) which is comparing T-DXd against the CLEOPATRA regimen in the first-line setting and has the potential to be another practice-changing study for the field.

An important toxicity of T-DXd that is essential to highlight is the risk for interstitial lung disease (ILD). In BC trials the incidence of T-Dxd-related ILD has ranged from 10 to 15% with most cases being low grade in severity and reversible[66] (Fig. 3). However, there have been cases of fatal ILD, as high as 2.7% as was observed in the DESTINY-Breast01 trial[60]. Despite limited understanding of the pathophysiology of this toxicity, a heightened awareness and the widespread adoption of monitoring and management guidelines has resulted in a decrease in the incidence of these high-grade events in recent trials[67]. In an update from DESTINY-Breast03, the overall incidence of adjudicated ILD events was 15%, however, there were no Grade 4 events and no deaths attributable to ILD in this trial[65]. As such, vigilance with prompt and adequate multidisciplinary management of lung toxicity

during T-DXd treatment is essential in clinical practice to deliver this therapy safely[67–69]. The development of asymptomatic ILD (radiological findings only; grade 1) requires T-DXd interruption until fully resolved[67]. T-DXd can be resumed at the same dose if ILD resolves ≤28 days from date of onset. If it resolved in >28 days from date of onset, the dose should be reduced by one dose level. Conversely, in patients who develop ILD of grade 2 or higher T-DXd should be permanently discontinued[67]. No definitive recommendation currently exists on the administration of other anti-HER2 agents in patients who discontinue T-DXd due to ILD and do not have concurrent disease progression. In the DESTINY trials, these patients were required to receive clinical-radiological follow-up without starting a new therapy before the evidence of disease progression. In clinical practice, one could consider a similar approach, however it is common practice to re-introduce HER2 blockade with or without other low toxicity therapies to maintain remission, even in absence of data from prospective trials. Considering there is up to a 15% of patients who experience ILD on T-DXd, this is an area where further research could identify an optimal algorithm. In addition to ILD, other common treatment-related adverse events included nausea (72.8%), fatigue (44.7%), vomiting (44.0%), and neutropenia (42.8%)[64], deserving appropriate prophylaxis and proper management when T-DXd is administered in clinical practice[70] (Fig. 3).

Third Line and Beyond

Several trials have investigated different and novel anti-HER2 agents in pre-treated populations with HER2+ MBC. Among these new therapies, TKIs targeting the intracellular catalytic kinase domain of HER2 have been extensively investigated[71]. By competing with adenosine triphosphate, TKIs block kinase phosphorylation and downstream signaling activation[71]. As discussed above, lapatinib was the first HER2-targeting TKI to be tested and approved for HER2+ MBC after disease progression on trastuzumab-based therapy[52,53]. However, given its modest overall efficacy it had a limited clinical role, and ultimately lapatinib was replaced by more potent new generation TKIs.

Tucatinib, an orally bioavailable small molecule TKI that is highly selective for HER2, has been particularly notable for its safety profile and efficacy in the treatment of brain metastases[72,73]. The HER2CLIMB trial compared the activity of trastuzumab and capecitabine with either tucatinib or placebo as treatment for patients with HER2+ MBC, including patients with brain metastases, previously receiving trastuzumab, pertuzumab and T-DM1[74]. This trial met its primary and key secondary endpoints as median PFS (7.8 vs 5.6 months, HR 0.54, 95% CI 0.42–0.71) and OS (21.9 vs 17.4 months, HR 0.73, 95% CI 0.59–0.90) were significantly longer in the tucatinib-treated group and compared to placebo (Table 1). Diarrhea was the most common adverse event in the tucatinib-combination arm (80.9% overall), although largely grades 1 and 2 and manageable (68%). Grade 3/4 reversible liver enzyme elevations were also observed in ~5% of patients necessitating monitoring for this potential side effect (Fig. 3). Overall, only 5.9% of patients discontinued tucatinib for toxicity[74] and hence it is generally considered to be a relatively well tolerated regimen. Importantly, about half (47%) of the patients enrolled to HER2CLIMB had central nervous system (CNS) metastases at baseline, including treated and stable (n=117, 40.2%) as well as progressing or active brain metastases (n=174, 59.8%) (Box 2). Overall, the PFS and OS benefit of tucatinib was maintained in these subgroups[74–77]. In patients with

measurable, active brain metastases at baseline, the intracranial objective response rate was higher for tucatinib than control (47% vs 20%)[76,77]. In addition, the study demonstrated strong activity for tucatinib over placebo to reduce the risk of CNS disease progression for both stable (2-year CNS PFS 24% vs 0%) and active brain metastases groups (2-year CNS PFS 12% vs 0%)[76,77]. In summary based on HER2CLIMB, the regimen of tucatinib plus capecitabine and trastuzumab received approval after one line of therapy for HER2+ MBC, and is widely accepted as a preferred treatment post T-DXd with an endorsement for use in an earlier line for those patients with active brain metastases[78] (Fig. 2). It is important to highlight, however, that little is known on the efficacy of tucatinib combination after T-DXd exposure. A recent real-world experience has reported that survival outcomes in this setting mirror those described in the HER2CLIMB trial[79], while additional studies on larger cohorts of patients treated with tucatinib after T-DXd exposure are warranted.

Neratinib is an irreversible pan-HER (HER1, HER2, and HER4) TKI which has also demonstrated promising efficacy in combination with capecitabine in metastatic HER2+ BC[80–82]. On this basis, the combination of neratinib and capecitabine was compared to lapatinib and capecitabine in the Phase III NALA trial in patients with metastatic HER2+ BC who received two or more prior anti-HER2-based regimens[83]. The study demonstrated an improvement in mean PFS for the neratinib arm (8.8 vs 6.6 months, HR 0.76, 95% CI 0.63–0.93), however this was in the absence of an OS benefit (24 vs 22.2 months, HR 0.88, 95% CI 0.72–1.07)[83] (Table 1). Uniquely the NALA trial allowed a direct comparison of two HER2 targeting TKIs, and additionally revealed pronounced differences in their efficacy against brain metastases. Approximately 16% of patients had asymptomatic or stable brain metastases (treated or untreated) at baseline. In this subgroup, similar median PFS (7.8 vs 5.5 months) and OS (16.4 vs 15.4 months) were observed between the two treatment arms[84]. In contrast however, the cumulative incidence of interventions for CNS disease (25.5% vs 36.0%), the development of progressive CNS disease (26.2% vs 41.6%), and the intracranial objective response rates (26.3% vs 15.4%) favored neratinib over lapatinib[84]. Unfortunately, diarrhea has been a prohibitive toxicity of neratinib and was the most common adverse event in the neratinib-capecitabine arm (83.2% overall), with 24% of patients experiencing grade 3 or 4 events despite prophylaxis[83](Fig. 3). It is noteworthy that the management of neratinib-induced diarrhea can substantially be improved with a gradual dose-escalation approach and prophylactic antidiarrheal use as established in the CONTROL trial[85]. Nevertheless, the better safety profile of tucatinib along with a statistically significant OS advantage has positioned tucatinib as the preferred anti-HER2 TKI for the treatment of HER2+ MBC [78]. Pyrotinib is another irreversible TKI that targets EGFR, HER2, and HER4. In the Phase III PHOEBE trial, pyrotinib combined with capecitabine led to improved PFS (12.5 vs 6.8 months, HR 0.39, 95% CI 0.27–0.56)[86] and OS (not-reached vs 26.9 months, HR 0.69, 95% CI 0.48–0.98)[87] when compared to lapatinib plus capecitabine in patients with HER2+ MBC who have previously received trastuzumab and taxane therapy (Table 1). Given that the trial was conducted solely in Chinese patients, pyrotinib is currently an approved option in China but is not yet approved elsewhere.

In addition to anti-HER2 TKIs, ADC therapies have also been tested in more heavily pre-treated patients with disease progression after two or more lines of therapy for HER2+ MBC. The Phase III DESTINY-Breast02 trial compared T-DXd to the treatment

of physician's choice (capecitabine plus trastuzumab or lapatinib) in patients with HER2+ MBC previously treated with T-DM1 therapy, demonstrating a clear PFS and OS benefit for the ADC (median PFS 17.8 vs 6.9 months, HR 0.36, 95%CI 0.28–0.45, P<0.0001) (Table 1)[88]. Given that DESTINY-Breast03 results have already positioned T-DXd as preferred second line therapy, the DESTINY-Breast02 results while unlikely to change clinical practice, do confirm the activity of T-DXd as one of the most promising new therapies for HER2+ MBC today and establishes it as a proven option post T-DM1 for those unable to receive it in an earlier setting. It is also important to highlight that DESTINY-Breast02's results clearly support the notion that a HER2-targeting ADC can overcome resistance to a previous one, fostering further clinical development of HER2 ADCs with novel payloads. In this regard, trastuzumab duocarmazine (SYD985) is a new generation HER2-targeting ADC comprised of trastuzumab bound to a DNA alkylator payload, duocarmycin, via a cleavable linker[89]. In the phase III TULIP trial, trastuzumab duocarmazine was compared to standard chemotherapy plus trastuzumab in patients with HER2+ MBC who had received either T-DM1 or at least 2 prior therapies for metastatic disease. The trial demonstrated an improvement in PFS for patients receiving trastuzumab duocarmazine (7 vs 4.9 months, HR 0.64, 95% CI 0.49–0.84, P=0.002), however this was in the absence of a survival benefit and at the cost of ocular and lung toxicity that led to drug discontinuation in 20.8% and 6.3% of patients, respectively[90]. Currently this ADC is under review by the regulatory agencies and has the potential to be the next approved HER2 ADC for the advanced setting.

Finally, margetuximab is a new mAb with similar HER2 targeting properties of trastuzumab, however in contrast, it has a modified Fc Domain which is engineered to have greater binding affinity to activating CD16A receptors and reduced affinity for inhibitory CD32B receptors on immune Natural Killer (NK) cells [91]. Based on promising activity seen in early-phase clinical trials[92], the Phase III SOPHIA study was designed and conducted to compare margetuximab in combination with chemotherapy versus trastuzumab plus chemotherapy in patients with HER2+ MBC who had received two or more prior anti-HER2 regimens[93]. While the study showed a marginal benefit in terms of PFS gain for the margetuximab arm (median PFS 5.8 vs 4.9 months, HR 0.76, 95% CI 0.59–0.98, P=0.03)[93], this was in absence of a statistically significant OS benefit[94] (Table 1). Moreover, an exploratory analysis of treatment response in relation to CD16A receptor genotypes, revealed differential benefits for margetuximab based on possession of an F allele, hence necessitating the requirement for a genetic biomarker to optimally select patients for this novel therapy. Overall, given its modest efficacy and the concurrent development of other potent anti-HER2 agents, margetuximab has been relegated to the later line setting for HER2+ MBC. Despite this, its excellent safety profile and potential for immune anticancer effects (which are often more pronounced in less pre-treated populations), margetuximab is undergoing further exploration in the neoadjuvant setting for HER2+ BC (MARGOT trial, NCT04425018).

Given the success with continuous targeting of the HER2 receptor, there exists a robust pipeline of novel HER2- targeting agents in clinical development, some of which are already in late phases of testing (Tables 2 and 3). As the field continues to move forward, more than ever before, there is a critical need to understand mechanisms of resistance. For example, with T-DXd as the preferred second line therapy today, T-DM1 has now defaulted to a later line option but its precise efficacy in this setting is unknown. Similarly, it is not clear how

efficacious other late line therapy options will be in this post-TDXd setting. Without data to guide a rational treatment selection, we are left today to empirically sequence treatments based on availability rather than knowledge of disease biology. This is an area where research efforts must be prioritized.

It is also important to underline that the advancements in the armamentarium of anti-HER2 drugs has also profoundly impacted the landscape of patients with early stage HER2+ BC. Approximately 90% of the patients with HER2+ early BC are cured by systemic therapy and multimodality treatments, leading to a progressive overall decrease in the prevalence of patients with MBC, particularly in countries where the latest therapies are readily available[30–32]. Accordingly, the characteristics of the MBC patient population today is also changing, with fewer patients having metastatic recurrence from prior early stage HER2+ BC, and the proportion of de novo stage IV cancers is increasing[95,96]. Complicating things further, many of the promising therapies developed for late stage HER2+ BC are eventually tested and often become incorporated into early-stage curative regimens and hence this has implications for their potential efficacy when re-considered for use in the metastatic setting. Due to these evolving trends, most of the patients currently diagnosed with HER2+ MBC in western countries today have either de novo disease or have highly resistant tumors, which have recurred after initial exposure to our most effective HER2-targeting therapies including trastuzumab, pertuzumab, adjuvant T-DM1 and in some cases neratinib. These factors taken together, add complexity and challenge our current approach to therapy based on “line of treatment” which can miss the nuances of the patient population and prior drugs and regimens received. These are highly important considerations and will need to be addressed as we plan future trials for HER2+ breast cancer.

TREATMENT OF TRIPLE-POSITIVE ADVANCED BREAST CANCER

Approximately half of HER2+ BCs will have co-expression of the estrogen receptor (ER) and/or progesterone receptor (HR+/HER2+)[97,98] and are commonly referred to as triple-positive BC. Older trials have explored the efficacy of antiestrogen therapy alone in HR+/HER2+ BC but found it to be ineffective, essentially identifying the first proven mechanism of resistance to hormone therapy[99]. Considering the biologic crosstalk between the HER2 and ER pathways, several trials have tested the addition of ET to HER2 inhibition, especially as maintenance therapy, reporting improved outcomes and providing compelling evidence for dual ER-HER2 targeting in triple-positive BC[40–43,100,101]. In the Phase II PERTAIN trial, patients with HR+/HER2+ MBC were randomized to receive trastuzumab plus aromatase inhibitor with or without pertuzumab as first-line treatment, with a provision to provide taxane-based induction chemotherapy at the investigator’s discretion[40]. The study showed a statistically significant PFS improvement for the addition of pertuzumab (median PFS 20.6 vs 15.8 months, HR, 0.67; 95% CI 0.50–0.89, P=0.006), without an OS benefit (60.2 vs 57.2 months, HR 1.05, 95% CI 0.73–1.52) (Table 1)[42]. Similarly, the Phase III ALTERNATIVE trial tested trastuzumab and aromatase inhibitor therapy +/− lapatinib in patients with HR+/HER2+ MBC who had progressed on at least one previous trastuzumab-containing therapy in the early or metastatic setting[41]. The combination of lapatinib with trastuzumab and aromatase inhibitor proved to be superior to trastuzumab plus aromatase

inhibitor alone in prolonging median PFS (11 vs 5.6 months, HR 0.62, 95% CI 0.45–0.88, P=0.0063), while mature OS data are pending[41].

To date, there is no definitive first line trial of ET with HER2 blockade versus THP for HR+/HER2+ MBC, however an exploratory analysis of the PERTAIN trial of the patients who received ET and HER2 blockade without induction chemotherapy, revealed PFS and OS rates similar to the CLEOPATRA regimen, supporting the notion that some selected patients may benefit from a chemotherapy-free regimen[42]. This approach was also tested in the Phase III SYSUCC-002 trial which investigated whether trastuzumab plus ET was non-inferior to trastuzumab plus chemotherapy[43]. The study met its primary PFS endpoint demonstrating non-inferiority for the endocrine arm, particularly in patients with a disease-free interval longer than 24 months (Table 1), and additionally this combination was associated with a more favorable toxicity profile[43]. Considering however that the control arm of the trial was substandard (without pertuzumab and taxane), one cannot consider this a practice-changing result.

In the past decade, CDK4/6is have revolutionized the treatment paradigm for patients with HR+ BC[102], given that preclinically the cyclin D1-CDK4/6 axis can drive resistance to ET[103]. Similarly, upregulation of the cyclin D1-CDK4/6 axis can contribute to resistance to anti-HER2 therapy and the combination of CDK4/6is with either trastuzumab or lapatinib has been shown to overcome resistance in preclinical models[104–106]. Based on this, several studies are clinically testing the combination of CDK4/6i with HER2 blockade. The phase II single-arm SOLTI-1303 PATRICIA trial enrolled patients with HER2+ MBC who had received 2–4 lines of prior therapy and treated them with the combination of palbociclib and trastuzumab and the same with or without letrozole if ER-positive[107]. PFS at 6 months was 33.3%, 42.8% and 46.4% for ER-negative, ER-positive without letrozole and ER-positive with letrozole, respectively[107]. Biomarker analyses revealed that patients with Luminal A and B tumors had longer PFS, hence the follow-up PATRICIA II trial (NCT02448420) is testing the same combination in patients specifically with Luminal intrinsic subtype HER2+ MBC. Similarly, the Phase II randomized monarcHER trial compared the combination of abemaciclib plus trastuzumab (with or without fulvestrant) versus chemotherapy plus trastuzumab in patients with pre-treated ER+/HER2+ MBC, showing a statistically significant improvement in median PFS for the triple therapy arm[108]. In the first-line setting, the Phase III PATINA trial (NCT02947685) is investigating the addition of palbociclib to trastuzumab, pertuzumab and ET as maintenance therapy for HR+/HER2+ MBCs; and the Phase III DETECT V/CHEVENDO (NCT02344472) is evaluating the combination of ribociclib, ET, trastuzumab and pertuzumab as a chemo-free regimen in patients with ER+ HER2+ MBC.

RESISTANCE TO ANTI-HER2 THERAPIES IN BREAST CANCER AND NOVEL THERAPIES IN DEVELOPMENT

HER2 intratumoral heterogeneity

Various definitions of HER2 heterogeneity have been proposed over time[109–113], however fundamentally, HER2 spatial heterogeneity is characterized by variation in the distribution

and expression of the HER2 protein among subpopulations of cells within a single tumor; similarly, the HER2 gene itself may be variably altered. This type of diversity in HER2 expression may reflect different levels of dependence on HER2 as a driver and differing levels of responsiveness to treatments. The existence of HER2 heterogeneity may also complicate the precise classification of the HER2 status of a tumor and the identification of the optimal treatment strategy[114]. Therefore, understanding spatial HER2 heterogeneity is of critical importance in the clinical management of patients with BC.

In a post-hoc analysis of the MARIANNE trial, which failed to demonstrate the superiority of T-DM1 over taxane plus trastuzumab[51], heterogeneity of HER2 immunohistochemistry (IHC) staining was associated with numerically shorter median PFS compared to tumors exhibiting homogeneous expression[115]. Similar observations have been made in early-stage HER2+ BC where heterogeneity is identified as a biomarker of poor response and outcome to anti-HER2 systemic therapy[116–120]. In a Phase II neoadjuvant trial of T-DM1 plus pertuzumab, HER2 heterogeneity was assessed on pre-treatment biopsies from two locations of each tumor and defined as an area with *ERBB2* amplification in >5% but <50% of tumor cells, or a HER2-negative area by FISH. By this definition heterogeneity was detected in 10% (16/157) of evaluable cases. The pathologic complete response rate was 55% in the non-heterogeneous subgroup and 0% in the heterogeneous group ($P<0.0001$, adjusted for hormone receptor status)[117].

These cases illustrate the limited potential of highly specific HER2-targeted agents such as T-DM1 in cases of tumoral heterogeneity; hence combination therapies incorporating anti-HER2 agents with non-selective cytotoxics remain the preferred first line treatment regimens today. Conversely the new generation of ADCs, typified by T-DXd, with advanced pharmaceutical properties including high DARs and cleavable linkers, exploiting a bystander effect, have been able to overcome the barriers of heterogeneity[121]. In this regard, T-DXd has shown striking efficacy in cases of HER2+ MBC refractory to T-DM1[60,88]. In fact, the possibility to target cancer cells with low levels of HER2 has paved the way for clinical trials of T-DXd in patients with “HER2-low” BCs. The results of the phase III DESTINY-Breast04 trial were ground breaking in showing unprecedented activity and a survival benefit for T-DXd compared to chemotherapy of physician’s choice in patients with pre-treated advanced HER2-low expressing BCs[122]. Recently, the results of the phase II DAISY trial, which enrolled patients with advanced BCs with variable HER2 status, added complexity to this scenario, reporting a response rate to T-DXd of about 30% in the HER2 IHC-0 cohort[123]. As a result, novel and more sensitive methodologies to characterize the minimum threshold of HER2 expression required to activate T-DXd therapy may be warranted.

Overall, based on the transformative results that have been obtained with T-DXd, several other new-generation anti-HER2 ADCs are currently in clinical development for either HER2+ or HER2-low BCs (Table 2).

Dysregulation of intracellular signaling pathways

Activation of signaling pathways that can promote tumor progression upon HER2 inhibition, has also been linked to resistance to anti-HER2 therapies. In most cases, this is related to compensatory reactivation of PI3K/AKT and RAS/MAPK pathways (Fig. 4).

Preclinical studies have established that the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT pathway serves as the dominant signaling cascade downstream of activated HER2, and inhibition of PI3K/AKT signaling can induce major antitumor effects[35,124,125]. Not surprisingly, mutational activation of PI3K/AKT signaling is a postulated mechanism of resistance to some HER2 targeted therapies such as trastuzumab and lapatinib[126–128]. Activating *PIK3CA* mutations are detected in approximately 30% of HER2+ cancers[129,130], and have been associated with poorer clinical outcomes in patients who had been treated with anti-HER2 therapies in both early[131] and metastatic[132,133] settings. In the CLEOPATRA trial, *PIK3CA* mutations were identified in 32% of patients and this was as an independent risk factor of poor prognosis and a shorter median PFS. [132] Similarly, in the EMILA trial, *PIK3CA* mutations were also correlated with shorter median PFS and OS (17.3 vs. 27.8 months) for the patients treated with capecitabine plus lapatinib[133]. Based on this, several trials have investigated the combination of HER2 blockade and PI3K pathway-targeting agents. The BOLERO-3 study evaluated the efficacy of the selective mammalian target of rapamycin (mTOR) inhibitor everolimus in combination with trastuzumab and vinorelbine in patients with HER2+ advanced BC resistant to trastuzumab. Although in the overall analysis there was only a marginal PFS improvement in the everolimus arm[134], tumors with *PIK3CA* mutations, *PTEN* loss or hyperactivation of the PI3K pathway had more pronounced benefits from everolimus treatment[135]. In spite of this, the triplet combination was associated with significant toxicity that dampened its uptake in the clinical setting. The advent of more potent and selective PI3K pathway inhibitors, has opened the door for further investigation of dual HER2- and PI3K pathway-blockade[136]. The PI3Kα-selective inhibitor alpelisib is now under investigation in combination with trastuzumab in pre-treated HER2+ MBCs that harbor *PIK3CA* mutations (ALPHABET, NCT05063786).

While inhibition of MAPK signaling by itself did not prove effective in HER2-driven BC models, activating mutations in the RAS-MAPK pathway such as *NF1* loss were identified in patients who had developed resistance to anti-HER2 therapies[137,138] (Fig. 4). Preclinical work established that such acquired alterations in the MAPK pathway could render tumors insensitive to HER2 inhibitors such as trastuzumab or tucatinib. These studies also suggested that combination blockade of HER2 and the MAPK pathway, or the use of ADC therapies that bypass these alterations could prove efficacious in this context[139]. No active clinical trials, however, are currently testing this hypothesis.

The co-occurrence of activating point mutations in *ERBB2* and *HER2* amplification has also been associated with resistance to both mAbs (trastuzumab/pertuzumab) as well as the first-generation generation TKI lapatinib[140,141]. In fact, recent findings from plasmaMATCH demonstrate that the incidence of *HER2* mutations in HER2+ cancers increases with the number of lines of HER2-directed therapy[142]. Preclinical and clinical research however has revealed that irreversible TKIs such as afatinib and neratinib are active against many of these studied *HER2* point mutants[141,143]. In the SUMMIT basket clinical trial, neratinib was

demonstrated to be effective against *HER2*-mutant/*HER2* non-amplified MBC[143] suggesting this could be a strategy for testing in *HER2* mutated/amplified cancers as well.

Targeting HER2 partners of dimerization

HER3 is the preferred dimerization partner of HER2, promoting potent intracellular pathway activation and cell proliferation[144], hence inhibition of HER3 is an attractive therapeutic strategy for treating HER2+ BCs (Fig. 4). The two main strategies that currently exploit HER3 as a therapeutic target are bispecific antibodies (BsAbs), targeting multiple epitopes of HER2 or HER2 and HER3, and anti-HER3 ADCs. Bispecific antibodies have been conceived with the aim of targeting either two binding sites of two different antigens or two distinct epitopes on the same antigen[145]. Zenocutuzumab (MCLA-128) is an IgG1 BsAb that targets HER2 and HER3, preventing HER2–HER3 dimerization and activation of downstream signalling[146]. In patients with HER2+ MBC who have had progressed on prior anti-HER2 therapy including T-DM1, MCLA-128 in combination with trastuzumab and vinorelbine demonstrated only marginal clinical benefit[147], hence deferring its further development in this setting. On the other hand, BsAbs targeting two different domains of HER2, such as zanidatamab (ZW25) and KN026, are currently being tested in HER2+ BC[148].

Next-generation ADC targeting HER3 are also in clinical development in BC, including HER2+ disease. Patritumab deruxtecan (HER3-DXd) is a novel ADC composed of a human anti-HER3 mAb with the same linker-payload technology as T-DXd as indicated by its name. In a Phase I/II trial of patients with HER3-expressing heavily pretreated MBCs, HER3-DXd reported an ORR of 42.9% in the cohort of HER2+ MBC expressing high levels of HER3[149]. These encouraging results suggest that targeting HER3 with novel ADCs in the context of HER2+ MBC might represent an appealing strategy.

Immune escape mechanisms and immunotherapy

The host immune system response is known to play a relevant role in the pathogenesis and treatment response of HER2+ BCs. High tumor mutational burden and levels of tumor-infiltrating lymphocytes[150] have been observed in HER2+ BC, with the latter conferring an improved prognosis [151,152], potentially regardless of type of therapy[153]. In addition, anti-HER2 mAbs, such as trastuzumab and pertuzumab, exert part of their antitumor effects by activating ADCC[20–23]. It is important to underline that HER2 overexpression can itself induce a host immune response against the tumor. Indeed, when compared to healthy subjects, patients with HER2+ BC have a progressive decrease of anti-HER2 Th1 function, which may represent an immune evasion mechanism in HER2-driven tumors[154]. On this basis, immune checkpoint inhibitors (ICIs) targeting either the programmed cell death protein 1 (PD-1) or its ligand (PD-L1) have been tested in combination with HER2-targeting drugs in patients with pretreated HER2+ MBC (Fig. 4). Disappointing results, however, have been obtained in early-phase clinical trials that have investigated ICIs alone or in combination with trastuzumab in unselected, heavily pretreated MBC, with no observed clinical responses[155–157]. Some degree of activity was registered in the single-arm, Phase II PANACEA trial, where the combination of trastuzumab and the PD-1 inhibitor pembrolizumab resulted in an ORR of 15% in patients with HER2+ MBC carrying a PD-L1

combined positive score ≥1%[157]. Similarly, the randomized Phase II KATE2 trial, which overall did not show any survival improvement when the anti-PD-L1 mAb atezolizumab was added to T-DM1 in patients with previously treated HER2+ MBC, reported a trend toward longer PFS in the PD-L1-positive subpopulation[158]. This signal prompted to the design of the Phase III KATE-3 trial (NCT04740918) that is testing the combination of atezolizumab and T-DM1 in patients with HER2+ and PD-L1-positive MBC. The same combination is also being investigated in the early setting in patients with HER2+ BC and residual disease after neoadjuvant systemic therapy (ASTEFANIA; NCT04873362). It is worth noting that the inconclusive results that have been achieved thus far with immunotherapy in HER2+ MBC may be related to the use of ICIs in a late line setting. Indeed, as was observed in triple-negative BC and other solid tumors, ICIs seem to be more effective in earlier stages of cancer[159,160]. To this end, the Phase III NRG BR004 trial (NCT04538742) was designed to investigate whether the addition of the PD-L1 inhibitor atezolizumab to THP would improve PFS when compared to THP plus placebo in patients with untreated HER2+ MBC. Regrettably, the study was discontinued early due to toxicity concerns[161].

The scant benefit that has been obtained with the use of immunotherapy in HER2+ MBC, mainly based on the inhibition of the PD-1/PD-L1 axis, encouraged the conduction of preclinical and clinical studies aimed at investigating addition mechanisms of immune escape that can be potentially targetable in the HER2+ disease[162]. For instance, the proportion of immunosuppressive myeloid cells tends to increase in invasive tumors compared to pre-invasive lesions, while the fraction of antigen-presenting cells decreases as tumors progress[163]. In HER2+ BC models, tumor-associated macrophages can be recruited by tumor-derived chemokine (C-C motif) ligand (CCL2) and promote tumor progression and dissemination[164]. On this basis, the use of novel ICIs targeting CSF1R and CCR2 is speculated to be a rational approach. In addition, trastuzumab can also engage Fc receptors on macrophages promoting antibody-dependent cellular phagocytosis (ADCP)[165]. As result, targeting macrophage signal regulatory proteins that are overexpressed in HER2+ BC, such as CD47[166], might overcome trastuzumab resistance[167], offering a potential treatment strategy to treat HER2+ BC that is either trastuzumab-sensitive or trastuzumab-resistant.

The modest efficacy of immunotherapy in HER2+ BC might be also explained by the loss of expression of MHC class I molecules on metastatic tumors, causing reduced or delayed recovery of the T cell repertoire clones that target BC-specific antigens[168]. Novel immunotherapeutic strategies have been conceived to overcome these mechanisms. For instance, several BsAbs have been developed to express two antigens that bind immune and cancer cells, respectively, favoring immune recognition and killing[169]. There are several such BsAbs under clinical investigation with HER2 as the tumor-specific target. Bispecific T cell engagers (BiTEs), that are directed against CD3 and HER2, can drive T cells to target HER2-expressing tumor cells. The benefit of the BiTE strategy is that a significant portion of the T cells are activated, and T cell activation is independent of antigen specificity. Similarly, bispecific killer cell engagers (BiKEs) connect to HER2 on tumor cells and CD16 on natural killer (NK)/monocytic cells to destroy cancer cells that express HER2. Different leading strategies have been developed to build new BsAbs. The “knobs-in-hole” technology consists of the replacement of a smaller amino acid with a larger one in the CH3 region of an antibody chain to form a “knobs” structure and at the same time replacing a larger amino

acid in the other chain with a smaller amino acid to form a “hole” structure. Runimotamab (BTRC4017A) and M-802 are examples of BsAbs that use this technology and are currently under investigation in early phase clinical trials for HER2+ tumors. Other technologies are based on the use of antibodies that can target antigens expressed by T cells or NK cells. For instance, monalizumab is a BsAb that prevents the interaction between HLA-E, which is overexpressed in malignant tumors, and NKG2A, an inhibitory checkpoint receptor expressed on NK and CD8+ T cells[170]. Monalizumab can boost the cytotoxic potential of other mAbs, like trastuzumab, and was evaluated in combination with trastuzumab in HER2+ MBC in the Phase II MIMOSA trial[171]. The study, however, showed no objective response in this patient population[171].

Additional novel therapeutic strategies targeting the immune system in HER2+ BC that are currently on preclinical and clinical development include immune-stimulating antibody conjugates, engineered toxin bodies, cell therapies based on chimeric antigen receptor (CAR) technology (i.e., CAR-T cells, CAR-Macrophages, CAR-NK cells), cancer vaccines targeting HER2 (i.e., peptide-based, protein-based, cell-based, dendritic cells-based, recombinant DNA-based and virus-based vaccines)[148]. Table 3 summarizes selected novel therapies, including BsAbs and cancer vaccines, that are currently under development for patients with HER2+ MBC.

Conclusions and future directions

The identification of HER2 as an important oncogene and therapeutic target for aggressive BC has brought about unprecedented improvements in patient survival. The remarkable responsiveness of HER2-driven BCs to HER2-targeted therapies that remain effective even after multiple lines of treatment, has contributed to the success in treating this BC subtype. Interestingly, only marginal results have been obtained with treatments targeted against parallel or collaborative pathways (e.g., PI3K or ER/CDK4/6) versus the more direct approaches to targeting HER2 itself. Next-generation HER2 targeting therapies with the potential to overcome limitations of first-generation drugs have improved patient outcomes and have broaden the applicability and have expanded the patient populations eligible for these targeted agents. New therapies on the horizon hold even greater potential in this regard. The overall success of HER2 directed therapies suggests that this paradigm could be applied to other oncoprotein driven malignancies where multiple forms of targeted therapy may be needed to derive a lasting impact that overcomes resistance. As the field continues to evolve, there are exciting novel avenues of investigation yet to explore. For instance, the application of artificial intelligence (AI)-based algorithms to refine the assessment of HER2 or other biomarkers in clinical samples[172] as well as AI-driven multimodal integration of clinical and genomic data[173] have the promise to improve patient selection and treatment decision-making. Similarly, the use of liquid biopsy assays for minimal residual disease (MRD) monitoring could guide in the future treatment escalation/de-escalation in the context of HER2+ MBC[174]. Lastly, single-cell sequencing for genetic heterogeneity assessment as well the development of novel classes of drugs that safely target HER2+ tumor cells while minimizing side effects represents areas of ongoing research. Continued translational research and collaborations will be essential to build on the progress made in HER2+ MBC treatment and achieve further advances.

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KEY POINTS

1. The discovery and clinical characterization of the Human Epidermal Growth Factor Receptor 2 (HER2, also known as ERBB2) gene as biomarker has represented a milestone for breast cancer treatment.
2. Development of several HER2-targeting agents, including monoclonal antibodies, tyrosine kinase inhibitors and antibody-drug conjugates, has increased cure rates in early stages and substantially improved survival for patients with HER2-positive metastatic breast cancer.
3. Incorporating novel anti-HER2 therapies into treatment algorithms of HER2-positive metastatic breast cancer to optimize selection and sequencing represents a current clinical challenge.
4. Several mechanisms, such as intratumoral heterogeneity, activation of alternative signaling pathways, and immune escape mechanisms, can reduce the efficacy of anti-HER2 drugs.
5. Continued translational research and collaborations will be essential to build on the progress made in the treatment of HER2-positive metastatic breast cancer and achieve further advances.

BOX 1.

EXCEPTIONAL RESPONDERS TO HER2-TARGETING THERAPY. CAN WE CURE METASTATIC HER2+ BC?

Metastatic BC is considered an incurable disease. However, the implementation of novel and effective new generation treatments, particularly for the HER2+ subtype, has challenged this assumption, posing new research and clinical questions. As an example of this, the final survival analysis of the CLEOPATRA trial revealed that 16% of patients who had received the combination of docetaxel, trastuzumab and pertuzumab were alive and without disease progression after 8 years of follow-up[37]. Similar findings have been provided by other prospective and retrospective studies[39,185–188], and based on these results, several important questions have emerged.

Can exceptional responders be identified up-front?

Both clinical and molecular features have been correlated with the efficacy of anti-HER2 therapies. Patients with *de novo* HER2+ MBC seem to have a higher probability of achieving long-lasting responses as compared to patients who have relapsed after initial adjuvant therapy[187–190], possibly implicating tumor heterogeneity and existence of overlapping mechanisms of resistance to previous anti-HER2 drugs. Similarly, tumor burden and extent of disease are also linked to long-term outcomes, with lower burden, oligometastatic, and/or non-visceral sites of metastases being associated with better survival [190–192]. Finally, favorable long-term outcomes have been correlated with the achievement of complete tumor response/regression to anti-HER2 systemic therapy[192–194]. To this end, transcriptomic and genomic markers that infer HER2 pathway addiction could be helpful in identifying exceptional responders. For example, elevated *HER2* mRNA expression has been linked to better long-term prognosis with several anti-HER2 agents[133,195,196]. Conversely, oncogenic alterations that activate the Pi3k/Akt pathway, such as *PIK3CA* hotspot mutations and *PTEN* loss-of-function, can reduce the sensitivity to HER2 blockade[128,133,195,196]. Although no single biomarker, outside of HER2 status, has been consistently and clinically proven to identify patients who will most benefit from anti-HER2 therapy, the multimodal integration of clinical and molecular features might have the power of refining patient selection.

Is treatment escalation/intensification needed in all HER2+ MBC?

The recent addition of new and highly effective anti-HER2 therapeutic agents, has dramatically transformed outcomes for patients with HER2+ MBC today[60,64,74,88]. Indeed, moving these novel effective drugs to the earlier lines of therapy for metastatic disease is showing improvements in disease control and survival outcomes, including complete remissions, unlike we have been able to achieve in the past[64,65]. On this basis, some trials are investigating more intensive frontline treatment strategies for metastatic disease (i.e., escalation therapy with the addition of ADCs or immunotherapy) with the hope of further improving the long-term remission or “cure” rates. On the other hand, unselected treatment escalation can result in a significant risk of overtreatment for some along with an increased risk of treatment-related toxicities. In order to provide more rational selection of therapy, the inclusion of novel clinical and molecularly-integrated

assays[197], or the use of ctDNA dynamics[198], may facilitate treatment personalization. For example, cases where there is failure to clear ctDNA or cases where tumors are identified as highly addicted to HER2 pathway activation, and are being treated with the goal of "cure", may derive the greatest benefit from HER2-targeting treatment intensification, given in the setting of favorable clinical and molecular features. Several collaborative efforts are ongoing to address this specific approach.

Can anti-HER2 therapy be safely stopped in patients with HER2+ MBC who have had exceptional tumor response?

With an increasing number of patients achieving profound and long-lasting complete remissions on anti-HER2 therapy for MBC, the question has been raised as to whether treatment can be safely stopped in some selected patients[199]. To date, scant data are available on the potential applicability of this approach in the clinical setting, outside of anecdotal cases where treatment was stopped for patients experiencing unacceptable drug-related adverse events. At present, most guidelines do not specifically address or make recommendations for anti-HER2 therapy interruption or discontinuation[200]. To address this relevant question, the Translational Breast Cancer Research Consortium (TBCRC) has recently launched a Phase II study aimed at assessing whether anti-HER2 treatment can be safely stopped in patients with HER2+ MBC that have had exceptional response to treatment, as defined as PFS longer than 3 years (STOP-HER2; NCT05721248). The study will include two different cohorts. Patients who do not elect to discontinue anti-HER2 maintenance treatment will be included in a non-randomized, observational cohort, while those willing to discontinue maintenance anti-HER2 treatment will be included in a second cohort. Patients will be followed by standard radiological scans as well as serial monitoring of circulating tumor DNA (ctDNA). While ctDNA results will not be used to make treatment decisions in this study, the results will be collected and analyzed to further learn how minimal residual disease (MRD) dynamics may be used in the future to further refine and personalize treatment decisions[201].

BOX 2.

MANAGEMENT OF PATIENTS WITH HER2+ BC AND BRAIN METASTASES

HER2+ BC is highly organotrophic to the central nervous system (CNS) compared to other BC subtypes[202], with a lifetime risk of developing brain metastases that is up to 50%[203–206]. Unfortunately, most therapies that are currently used in the adjuvant setting of HER2+ BC, including trastuzumab, pertuzumab and T-DM1, have not had an impact in reducing the risk of CNS relapse[207]. Conversely, when used in the metastatic setting in patients with pre-existing CNS involvement, many of the same HER2 targeted therapies have demonstrated efficacy in the treatment of established CNS metastases[208,209]. Most successful, to date, in this regard are the anti-HER2 TKIs, which have more efficient penetration through the blood–brain barrier and have provided compelling clinical results[210,211]. The initial studies investigating lapatinib, either as a monotherapy or in combination with capecitabine, for CNS efficacy reported objective intracranial response rates ranging between 18% to 66%[212,213]. The TBCRC 022 trial subsequently examined neratinib alone or in combination with capecitabine[81,214]. Among patients who had not been previously exposed to lapatinib, objective responses in the central nervous system were observed in 49%; in those who received prior lapatinib, the response rate was 33%[81,214]. In the NALA trial, the cumulative incidence of CNS progression in patients receiving lapatinib plus capecitabine versus neratinib plus capecitabine was 41.6% and 26.2%, respectively[84], supporting greater efficacy for neratinib in the CNS compartment. In another cohort of the TBCRC 022 trial, neratinib was tested in combination with T-DM1, demonstrating meaningful intracranial activity, regardless of progression to prior local CNS-directed therapy and/or T-DM1 exposure[215]. Recently, the HER2CLIMB trial was the first prospective and randomized study to demonstrate clinically meaningful benefits for the HER2 selective TKI, tucatinib, in patients with brain metastases, marking an important milestone in the treatment of HER2+ MBC[74–77]. The study showed statistically significant gains in CNS objective response rate, CNS PFS, overall PFS, and OS with tucatinib treatment[74–77]. In patients with measurable, active CNS metastases at baseline, the CNS objective response rate was 47% in the tucatinib arm compared to 20% in the control arm. The responses were more durable with tucatinib, with a median duration of intracranial response of 8.3 months versus 3.0 months in the control arm. The study also showed that only 3.6% of patients experienced progressive disease as a best response, indirectly indicating the safety of this approach in lieu of local therapy. Most importantly, there were substantial differences in OS between the groups, with a median of 21.6 months versus 12.5 months among all 291 patients with brain metastases at baseline, and a median of 21.4 months versus 11.8 months among 174 patients with active brain metastases at baseline, in favor of the tucatinib arm[74–77]. Based on these results, tucatinib is currently being investigated as maintenance therapy in combination with first line pertuzumab and trastuzumab in HER2+ MBC (HER2CLIMB-05; NCT05132582) and as post-neoadjuvant therapy in combination with T-DM1 for patients with residual disease (CompassHER2 RD; NCT04457596) with the aim of delaying or preventing CNS progression.

Although monoclonal antibodies cannot typically cross an intact blood-brain barrier, the disruption induced by tumor cells can allow large molecules to cross in a heterogeneous manner[216]. Multiple lines of evidence now support that monoclonal antibodies and first-generation ADCs might be effective in patients with brain metastases, although with modest response rates[217]. On the other hand, next-generation ADCs, such as T-DXd, have demonstrated promising intracranial activity in patients with CNS involvement from HER2+ MBC. In the DESTINY-Breast03 trial, the CNS objective response rate was 65%, and 27.8% of patients achieved a CNS complete response with T-DXd[218]. Other studies, such as the TUXEDO and DEBBRAH trials, that enrolled patients with active or progressive brain metastases, the intracranial response rates with T-DXd therapy ranged from 44% to 83%[219–221]. The ongoing DESTINY-Breast12 trial (NCT04739761) is a real-world study testing the use of T-DXd in patients with HER2+ MBC, with half of the patients enrolled having brain metastases at baseline (n=250). This trial will provide the largest experience to date for T-DXd in the treatment of brain metastases.

Despite remarkable advancements in the systemic treatment of HER2+ BC, management of patients with brain metastases remains challenging and requires multidisciplinary expertise. In clinical practice, the addition of local ablative therapies, such as stereotactic radiotherapy or surgical resection, to anti-HER2 systemic therapy is generally recommended in case of limited CNS progression (<5–10 progressive lesions) and in presence ofmdisease control outside the brain[78]. Conversely, in case of concurrent disease progression inside and outside the CNS, switching to another anti-HER2 is endorsed by several guidelines[78]. The pattern of CNS involvement (i.e., number, size, and intracranial location of brain metastases), neurological symptoms and likelihood of response to available therapies, including surgical resection, stereotactic radiotherapy, whole brain radiotherapy and systemic therapy, are all taken into consideration when making treatment decisions for an individual. Given the increasing availability of effective systemic options for CNS disease, future studies are needed to prospectively address the optimal approach and sequence of these therapies for patients with HER2+ BC BM. A relevant unmet need is also related to the early diagnosis and monitoring of BMs. Next-generation imaging along with the use of non-invasive diagnostic tool for early diagnosis have the promise of further improvement the management of patients with HER2+ BC and help to prevent SNC disease.

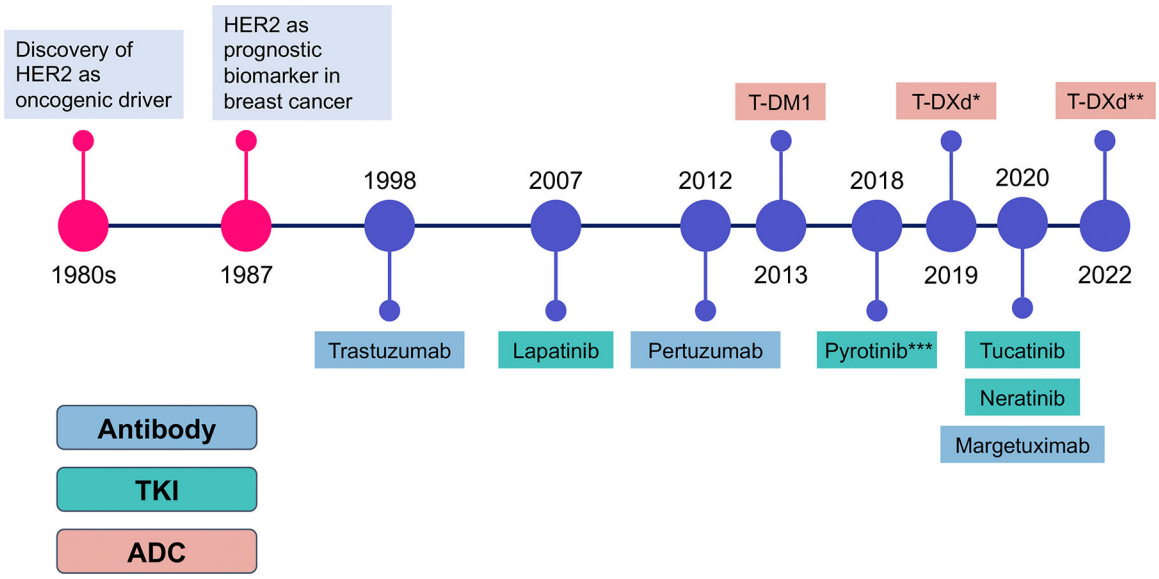

Fig. 1: Timeline of the evolution of HER2 as biomarker in breast cancer and approval of therapeutic agents for HER2+ metastatic breast cancer.

Abbreviations: ADC, antibody-drug conjugate; mAb, monoclonal antibody; TKI, tyrosine kinase inhibitor. * FDA approval for HER2+ advanced breast cancer; ** FDA approval for HER2-low advanced breast cancer; *** pyrotinib is currently approved in China only.

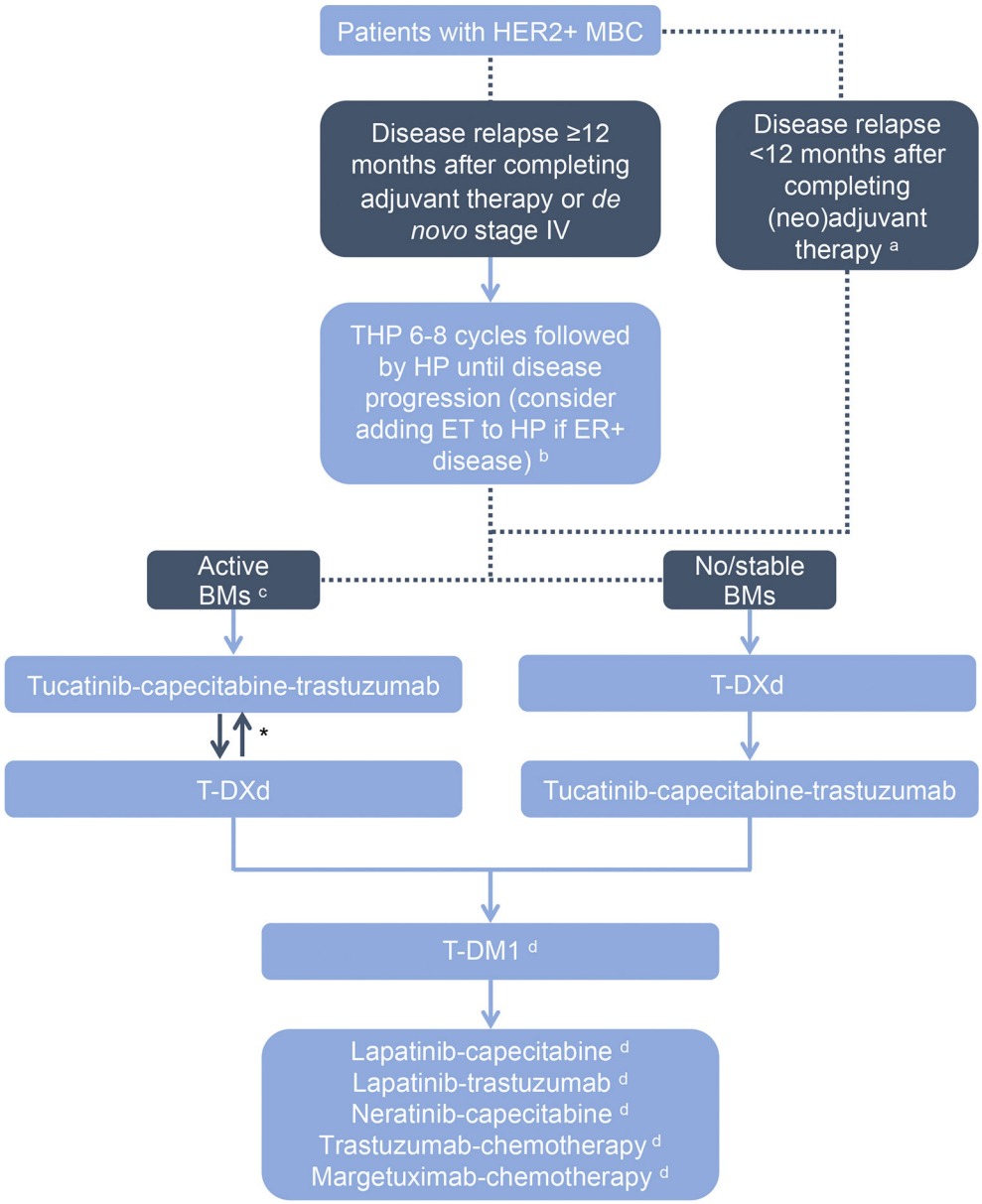

Fig. 2: Treatment algorithm for patients with advanced/metastatic HER2+ breast cancer.

[a] For patients with disease-free interval 6–12 months who did not receive pertuzumab in the early setting, consider THP as first-line regimen. [b] Paclitaxel or nab-paclitaxel can be used to replace docetaxel as chemotherapy backbone. The use of vinorelbine instead of taxanes can be considered in selected cases where the administration of taxanes is deemed to be not safe. [c] Consider adding local intervention (radiotherapy or surgery) depending on brain metastases characteristics. [d] No data after tucatinib- and T-DXd-based regimens. * Bilateral arrows indicate that T-DXd and tucatinib-capecitabine-trastuzumab can be used interchangeably in patients with active brain metastases after progression to first-line therapy. Abbreviations: BM, brain metastases; ER, estrogen receptor; ET, endocrine therapy; MBC, metastatic breast cancer; THP, trastuzumab-pertuzumab-taxane.

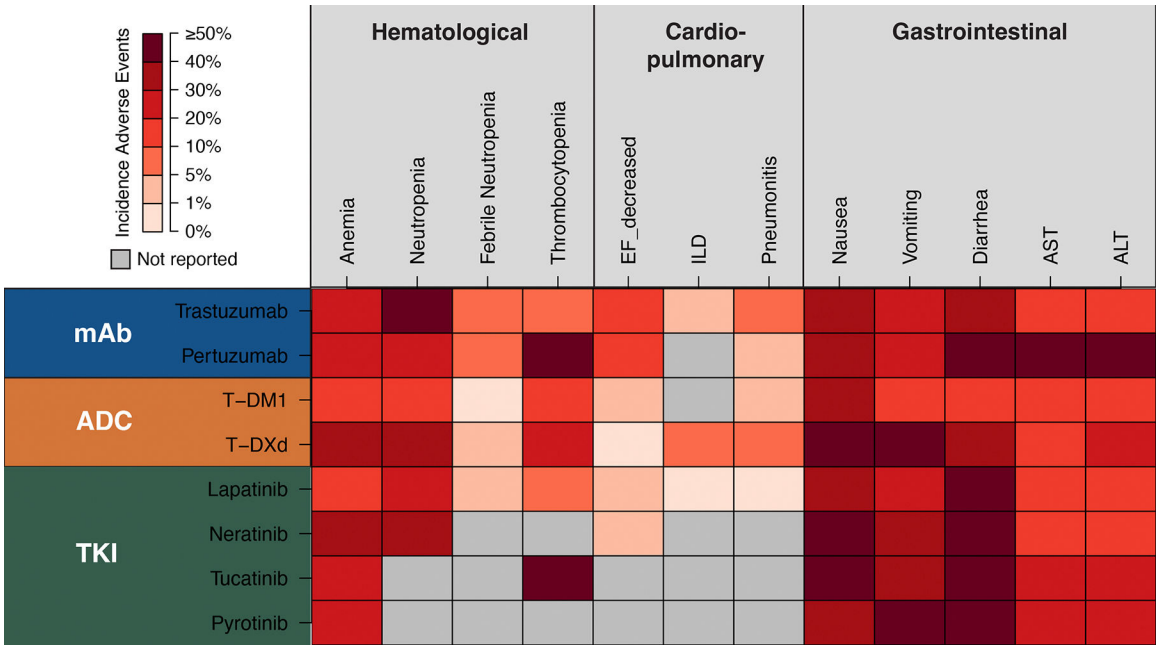

Fig. 3:

Incidence of selected treatment-related adverse events of clinical interest in clinical trials testing anti-HER2 therapies in HER2-positive breast cancer.

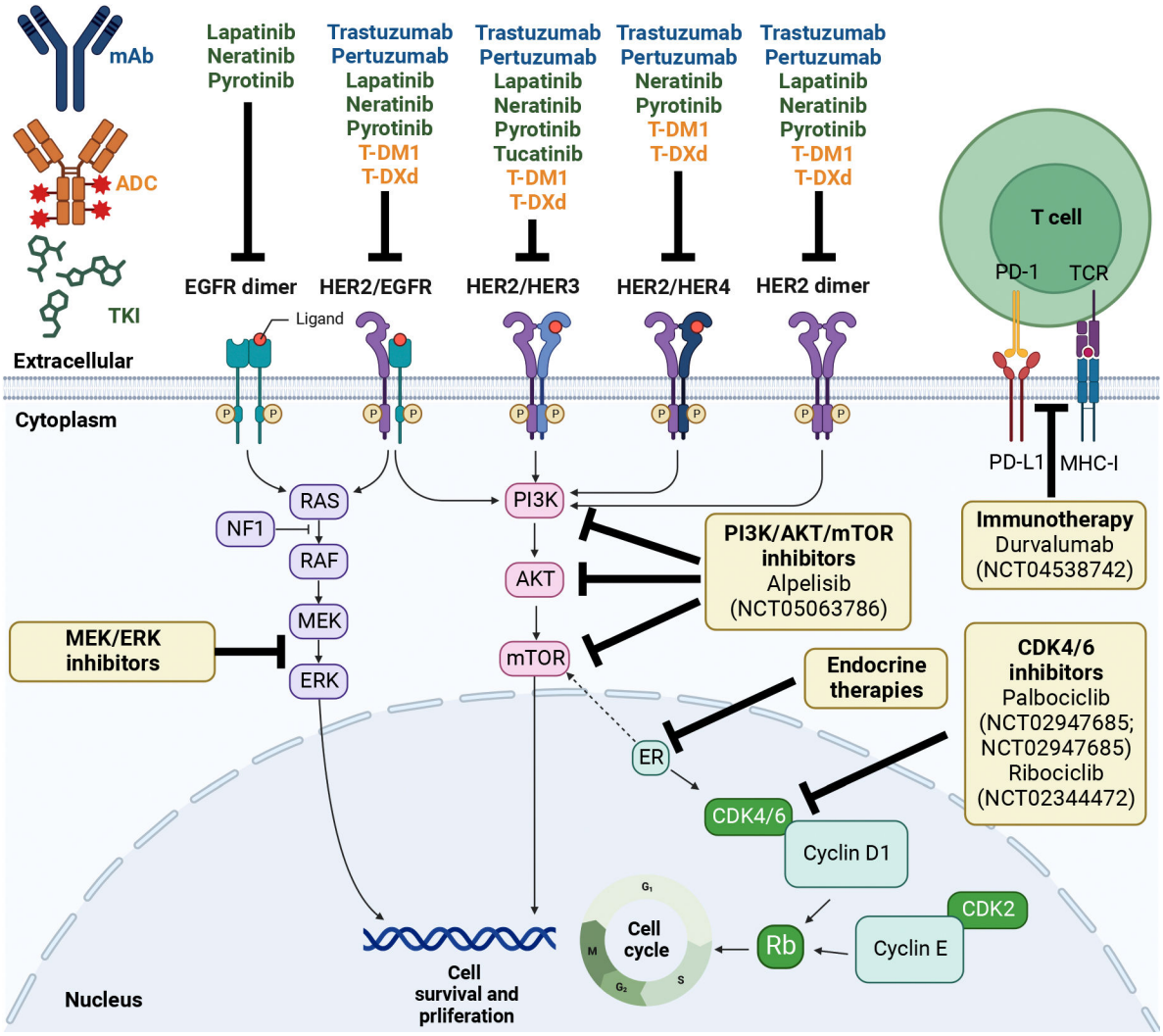

Fig. 4:

Heterodimerization of HER family members and downstream intracellular pathways that can mediate resistance to anti-HER2 therapy.

Table 1.

Pivotal Phase II/III Clinical Trials in Advanced/Metastatic HER2-positive Breast Cancer.

Trial (NCT ID)	Phase	Line	Sample Size	Experimental Arm	Control Arm	mPFS (months)	mOS (months)	Ref
CLEOPATRA (NCT00567190)	III	1st	808	Trastuzumab + docetaxel + pertuzumab	Trastuzumab + docetaxel + PBO	18.7 vs 12.4 (HR 0.69, 95%CI 0.58–0.81)	57.1 vs 40.8 (HR 0.69, 95%CI 0.58–0.82)	33,36,37
PERUSE (NCT01572038)	III	1st	1,436	Trastuzumab + pertuzumab + taxane	N/A	20.7 (95%CI 18.9–23.1)	65.3 (95%CI 60.9–70.9)	38,39
PERTAIN (NCT01491737)	II	1st	258	Trastuzumab + pertuzumab + ET	Trastuzumab + ET	20.6 vs 15.8 (HR 0.67, 95%CI 0.50–0.89)	60.2 vs 57.2 (HR 1.05, 95%CI 0.73–1.52)	40,42
MARIANNE (NCT01120184)	III	1st	1,095	T-DM1 + PBO or pertuzumab	Trastuzumab + taxane	14.1 (T-DM1+PBO) vs 15.2 (T-DM1+P) vs 13.7 (TH) (HR 0.91, 97.5%CI, 0.73–1.13; HR 0.87, 97.5%CI 0.69–1.08)	53.7 (T-DM1+PBO) vs 51.8 (T-DM1+P) vs 50.9 (TH) (HR 0.88, 97.5%CI, 0.67–1.15; HR 0.81, 97.5%CI 0.61–1.08)	51
EMILIA (NCT00829166)	III	2nd	991	T-DM1	Lapatinib + capecitabine	9.6 vs 6.4 (HR 0.65, 95%CI 0.55–0.77)	29.9 vs 25.9 (HR 0.75, 95%CI 0.64–0.88)	57,58
DESTINY-Breast03 (NCT03529110)	III	2nd	524	T-DXd	T-DM1	28.8 vs 6.8 (HR 0.33, 95%CI 0.26–0.43)	NR	64,65
TH3RESA (NCT01419197)	III	>2nd	602	T-DM1	TPC	6.2 vs 3.3 (HR 0.52, 95%CI 0.42–0.66)	22.7 vs 15.8 (HR 0.68, 95%CI 0.54–0.85)	175,176
HER2CLIMB (NCT02614794)	III	>2nd	612 (480 primary endpoint analysis)	Trastuzumab + capecitabine + tucatinib	Trastuzumab + capecitabine + placebo	7.8 vs 5.6 (HR 0.54, 95%CI 0.42–0.71)	24.7 vs 19.2 (HR 0.73, 95%CI 0.59–0.90)	74,75
DESTINY-Breast02 (NCT03523585)	III	>2nd	608	T-DXd	TPC	17.8 vs 6.9 (HR 0.36, 95%CI 0.28–0.45)	NR	88
NALA (NCT01808573)	III	>2nd	621	Neratinib + capecitabine	Lapatinib + capecitabine	8.8 vs 6.6 (HR 0.76, 95%CI 0.63–0.93)	24 vs 22.2 (HR 0.88, 95%CI 0.72–1.07)	83
PHOEBE (NCT03080805)	III	>1st	267	Pyrotinib + capecitabine	Lapatinib + capecitabine	12.5 vs 6.8 (HR 0.39, 95%CI 0.27–0.56)	NR vs 26.9 (HR 0.69, 95%CI 0.48–0.98)	86
SOPHIA (NCT02492711)	III	>2nd	536	Margetuximab + chemotherapy	Trastuzumab + chemotherapy	5.8 vs 4.9 (HR 0.76, 95%CI 0.59–0.98)	21.6 vs 21.9 (HR 0.95, 95%CI 0.77–1.17)	93,94

Abbreviations: CI, confidence interval; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; N/A, not available; NR, not reported; P, pertuzumab; PBO, placebo; TH, trastuzumab-taxane; TPC, treatment of physician choice.

Table 2.

Antibody-drug conjugates in clinical development for HER2-positive breast cancer.

ADC	Antibody	Payload	Payload MoA	Linker	DAR	Preliminary efficacy data	Stage of development	Trial ID
ARX788	Modified trastuzumab	MMAF	Tubulin-targeting agent	Non-cleavable	1.9	ORR 65.5%, DCR 100%, mPFS 17 mo[177]	II	NCT05018676 NCT04829604 NCT05426486 NCT05018702 NCT04983121 NCT01042379
Disitamab vedotin (RC48-ADC)	Hertuzumab	MMAE	Tubulin-targeting agent	Cleavable	4	ORR 15%, DCR 45%[178,179]	II/III	NCT05331326 NCT04400695 NCT05134519 NCT03500380
MRG002	Anti-HER2 IgG	MMAE	Tubulin-targeting agent	Cleavable	3.8	ORR 34.7% DCR 75.5% (HER2-low)[180]	II/III	NCT04924699 NCT04742153 NCT05263869
A166	Trastuzumab variant	MMAF	Tubulin-targeting agent	Cleavable	2.8	ORR 59–71% DCR 85%[200]	II	NCT05346328 NCT03602079
BDC-1001	Trastuzumab biosimilar	TLR 7/8 agonist	TLR7/8 agonist	Non-cleavable	NR	NR	I/II	NCT04278144
SBT6050	Anti-HER2 IgG	Analogue of motolimod	TLR8 agonist	Cleavable	NR	NR	I	NCT04460456
FS-1502	Trastuzumab	MMAF	Tubulin-targeting agent	Beta-glucuronide	2	ORR 52.9% DCR 88.2% mPFS 15.5 mo[181]	III	NCT03944499 NCT05755048
Zanidatamab zovodotin (ZW49)	Biparatopic anti-HER2 IgG (ZW25)	Auristatin	Tubulin-targeting agent	Cleavable	2	ORR 13%, DCR 50%, CBR 25%[202]	II	NCT05035836 NCT01042379
GQ1001	Trastuzumab	DM1	Tubulin-targeting agent	Intelligent ligase dependent conjugation	NR	NR	I/II	NCT04450732 NCT05575804
DP303c	Anti-HER2 IgG	MMAE	Tubulin-targeting agent	Cleavable	2	NR	II/III	NCT05334810 NCT05901935
BB-1701	Anti-HER2 IgG	Eribulin	Microtubule inhibitor	Cleavable	4	DCR 100%[182]	I	NCT04257110
SHR-A1811	Anti-HER2 IgG	SHR9265	Topoisomerase I inhibitor	Cleavable	5.7	ORR 81%[183]	II/III	NCT05845138 NCT05814354 NCT05635487 NCT05353361 NCT05769010 NCT05792410 NCT05424835 NCT05911958 NCT05749588
DB-1303	Anti-HER2 IgG	P1003	Topoisomerase I inhibitor	Cleavable	8	ORR 50% DRC 96%[184]	I/II	NCT05150691

Abbreviations: CBR, clinical benefit rate; DAR, drug-antibody ratio; DCR, disease-control rate; IgG, immunoglobulin G; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; MoA, mechanism of action; NR, not reported; ORR, overall response rate; TLR, toll-like receptor. Disease control rate (DCR) describes the percentage of patients whose experimental treatment has led to a complete response, partial response, or stable disease. Conversely, clinical benefit rate (CBR) is defined as the percentage of patients who achieved complete response, partial response, or at least six months of stable disease because of therapy.

Table 3.

Selected agents in clinical development for HER2-positive metastatic breast cancer.

Compound	Category	Target(s) or MoA	Phase of development	NCT ID
DZD1516	Tyrosine kinase inhibitor	HER2 selective inhibitor	I	NCT04509596
BDTX-189	Tyrosine kinase inhibitor	HER2/EGFR inhibitor	I	NCT04209465
Runimotamab (BTRC4017A)	Bispecific antibody	HER2-CD3	I	NCT03448042
IBI315	Bispecific antibody	HER2-PD-L1	I	NCT04162327
PRS-343	Bispecific antibody	HER2-CD137	I	NCT03650348
SAR-443216	Bispecific antibody	HER2-CD3/CD28	I	NCT05013554
BDC-1001	Bispecific antibody	HER2-TLR7/8	I/II	NCT04278144
NJH395	Bispecific antibody	HER2-TLR7	I	NCT03696771
SBT6050	Bispecific antibody	HER2-TLR8	I/II	NCT04460456 NCT05091528
HER2BATs	Bispecific antibody	HER2− CD3+ activated T cells	I/II	NCT03272334 NCT04158947
TAC01-HER2	Bispecific antibody	HER2− CD3 and CD4 co-receptor domain	I/II	NCT04727151
DF-1001	Bispecific antibody	HER2− NK cells	I/II	NCT04143711 NCT05597839
MVF-HER-2	Peptide/protein-based vaccine	Two chimeric peptides cosynthesized with B cell epitopes derived from HER2 ECDs 2 and 4	I	NCT01376505
ACE1702	Bispecific antibody	HER2− NK cells	I	NCT04319757
GP2	Peptide/protein-based vaccine	HER2-derived HLA-A2- and HLA-A3-restricted epitope	III	NCT05232916
GLSI-100	Peptide/protein-based vaccine	HER2-derived HLA-A2- and HLA-A3-restricted epitope	III	NCT05232916
TPIV100	Peptide/protein-based vaccine	HER2 multiple epitope-based vaccine	II	NCT04197687
pNGVL3-hICD	DNA-based vaccine	HER2 ICD DNA plasmid-based vaccine	I/II	NCT00436254 NCT05163223
WOKVAC	DNA-based vaccine	Plasmid-based DNA with three epitopes: IGFBP2, HER2 and IGF1R	I/II	NCT04329065 NCT03384914 NCT02780401
VRP-HER2	Virus-based vaccine	Alphaviral vector encoding the ECD and transmembrane domains of HER2 (VRP-HER2)	I/II	NCT03632941

Abbreviations: ECD, extracellular domain; MoA, mechanism of action; NK, natural killer.