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REVIEW

Clinical trial data and emerging strategies: HER2-positive breast cancer

Sonia Pernas[1,2] · **Sara M. Tolaney**[3,4]

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Abstract

A deeper insight into tumor biology and HER2 signaling has led to the development of novel anti-HER2 drugs that have significantly improved the prognosis of patients with HER2-positive breast cancer. The breast cancer immune microenvironment has emerged as a potential prognostic factor. Moreover, the host immune system not only seems to play a critical role in the prognosis of HER2-positive breast cancer, but also seems to modulate treatment response to some HER2-targeted agents. Here, we review the latest evidence of the role of immunotherapy in HER2-positive breast cancer and present emerging strategies.

Keywords HER2-positive breast cancer · Immunotherapy · Tumor-infiltrating lymphocytes · Immune checkpoints · Tumor immune microenvironment · Antibody-dependent cellular cytotoxicity

Introduction

The introduction of human epidermal growth factor receptor 2 (HER2)-targeted agents in the treatment of patients with HER2-positive breast cancer has led to significant improvements in survival outcomes in both early and metastatic settings. Treatment of HER2-positive breast cancer has evolved rapidly in recent years. A better understanding of tumor biology and HER2 signaling has been crucial for the development of new strategies to further improve patient outcomes. Current novel HER2-targeted therapies include dual-HER2 inhibition with monoclonal antibodies (mAbs), like trastuzumab plus pertuzumab [1–3]; antibody–drug conjugates (ADCs) such as T-DM1 [4, 5] and trastuzumab-deruxtecan [6]; and tyrosine kinase inhibitors (TKIs) such as tucatinib [7] and neratinib [8, 9].

Nevertheless, HER2-positive breast cancer is a heterogeneous disease, with different treatment sensitivities and survival outcomes [10]. Immune tumor microenvironment (TME) has emerged as a potential prognostic factor but also as a modulator of treatment response in HER2-positive breast cancer. Breast cancer immunogenicity is also heterogeneous, with different rates of immune infiltration depending on tumor subtype [11]. HER2-positive breast cancers are generally considered more immunogenic than hormone receptor (HR)-positive/HER2-negative breast cancers, but less immunogenic than triple-negative breast cancers (TNBC), and differences in immunogenicity exist also among intrinsic molecular subtypes, with HER2-enriched tumors being one of the most immunogenic [12, 13]. Compared to other subtypes, HER2-enriched tumors show the highest levels of tumor-infiltrating lymphocytes (TILs) and they are associated with higher expression of immune activation genes [14]. In addition, some chemotherapies, such as anthracyclines and cyclophosphamide [15], and HER2-targeted treatments can activate the immune system by immunogenic cell death and antibody-dependent cellular cytotoxicity (ADCC), respectively [16]. Moreover, the concurrent administration of taxanes and trastuzumab might increase the immune effect of trastuzumab, by acting on tumor and natural killer (NK) cells [17].

✉ Sara M. Tolaney
Sara_Tolaney@DFCI.HARVARD.EDU

1 Department of Medical Oncology, Catalan Institute of Oncology - ICO, L'Hospitalet de Llobregat, Barcelona, Spain

2 Breast Cancer Group, Institut d'Investigacio Biomedica de Bellvitge - IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain

3 Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA

4 Harvard Medical School, Boston, MA, USA

In this review, we summarize the clinical data on the different approaches exploring the interaction of the immune system and HER2-positive breast cancer, and potential combinations of anti-HER2 agents with immunotherapies to enhance treatment efficacy or overcome HER2 resistance.

Immune system in HER2-positive breast cancer

Immune cells can inhibit tumor growth and progression but can also help to create an immunosuppressive environment in which the tumor can proliferate. $CD8^+$ cytotoxic T-cells, CD4+ T-helper 1 (Th1) cells, and NK cells are usually associated with anti-tumor immune responses, along with M1 tumor-associated macrophages (TAMs) and DC1 dendritic cells. In contrast, CD4+ T-helper 2 (Th2) cells, myeloid derived suppressor cells (MDSC), CD4+ expressing $FOXP3^+$ cells (T-reg cells), M2 macrophages, and DC2 dendritic cells might induce a pro-tumorigenic environment [18]. TILs include different cell types, with T-cells being the most common, but with variable proportions of macrophages, NK cells, dendritic cells, and B-cells. The prevalence of TILs depends not only on tumor subtype, but also on the stage of the disease (with early disease showing the highest rate of TIL infiltration), and on breast cancer metastases sites, with the lung showing the highest rate of TILs, and skin and liver with the lowest [19, 20].

HER2 amplification seems to result in a non-inflamed TME as low infiltration of TILs is observed, compared to TNBC [21]. ERBB family members may play an important role in evading the antitumor immune response by modulating the immunological landscape of the TME. HER2 signaling inhibits Fin responses and downregulates interferon regulatory factors and inflammatory chemokine production via PI3K–AKT pathway, resulting in the reduction of effector CD8+ T-cells and a decrease in the major histocompatibility complex (MHC) class I expression [22]. Moreover, HER2 amplification also causes loss of phosphorylation of TANK-binding kinase 1 (TBK1) and reduces stimulator of interferon genes (STING) signaling, diminishing the interferon and antitumor immune responses [22].

Prognostic and predictive role of tumor-infiltrating lymphocytes (TILs)

Contrary to TNBC, in which a significant and independent association between increased TIL levels and both disease-free survival (DFS) and overall survival (OS) have been reported, results in HER2-positive breast cancer are diverse. As HER2-positive breast cancer is a heterogeneous disease, differences in TIL levels exist among intrinsic molecular intrinsic subtypes with HER2-enriched tumors having the highest levels of immune infiltrates [13, 23].

The prognostic and predictive role of TILs in early-stage HER2-positive breast cancer has been extensively investigated. In the neoadjuvant setting, higher levels of TILs and/or immune-activated RNA signatures have been consistently associated with higher pathologic complete response (pCR) rates as well as with an improved DFS [12, 24–27]. In the adjuvant setting, data from the FinHER trial, evaluating the role of trastuzumab in combination with adjuvant chemotherapy, suggested a positive association between levels of TILs and a more favorable patient outcome [28]. However, results from pivotal trials evaluating the role of adjuvant trastuzumab were diverse. In the NCCTG-N9831 trial, the presence of high TILs was associated with an improvement in DFS only in patients receiving chemotherapy alone, but not among patients treated with trastuzumab [29]. In the NRG/NSABP B-31 trial, increases in stromal TILs (sTILs), as a semicontinuous variable or as lymphocyte-predominant breast cancer with more than 50% s TILs were statistically significantly associated with improved DFS in both arms, the chemotherapy alone arm and in the trastuzumab arm. However, there was no association of sTILs with trastuzumab benefit [30]. The role of TILs has also been studied in the randomized non-inferiority phase III Short-HER trial, evaluating 1 year vs. 9 weeks of adjuvant trastuzumab. In that study, higher levels of TILs were associated with an improved distant-DFS, with a 27% reduction in the risk for each 10% TILs increment. Of note, in patients with low-TILs tumors, a benefit with 1-year trastuzumab over 9 weeks duration was observed [31]. In addition, a combined prognostic score (called HER2DX) based on 17 clinicopathological and genomic variables, which encompasses among others TILs (as a continuous variable), PAM50 intrinsic subtype and 13 genes, was recently developed. HER2DX was significantly associated with distant metastasis-free survival and DFS, identifying potential patients with early-stage HER2-positive breast cancer who might escalate or de-escalate systemic treatment [32].

In the adjuvant phase III APHINITY trial, evaluating the role of the dual blockade with pertuzumab and trastuzumab vs. trastuzumab alone in addition to chemotherapy, both TILs (> 75%) and T-cell-related genes predicted greater benefit in terms of invasive DFS from dual blockade [33]. Importantly, TILs are a dynamic biomarker. In the PAMELA trial, evaluating a chemotherapy-free regimen with the dual-blockade lapatinib-trastuzumab+/− endocrine therapy, TILs during treatment, but not baseline, were associated with pCR [13, 34]. On the other hand, and contrary with what has been observed in TNBC, high TILs in residual disease in HER2-positive breast cancer following neoadjuvant therapy have been associated with worse DFS [35]. This might

be explained by the fact that an increase in FOXP3 levels has been reported in HER2-positive residual disease. An increase in regulatory T-cells in non-pCR samples suggests the development of an immunosuppressive phenotype [36].

In the metastatic setting, the role of TILs has been evaluated in the phase III CLEOPATRA trial, which assessed the addition of pertuzumab to trastuzumab and docetaxel as first-line therapy for HER2-positive disease. In this trial, each 10% increase in TILs was significantly associated with longer OS, but not with a benefit in progression-free survival (PFS) [37]. However, an association in the opposite direction was observed in a retrospective study aiming to characterize the immune infiltrates of breast cancer metastases [20]. Those differences could be explained by the fact that, in the CLEOPATRA trial, most samples analyzed were from primary tumors and the vast majority of patients (89%) had not previously been treated with trastuzumab. Therefore, no definitive conclusions can be drawn in the role of TILs in patients with advanced HER2-positive breast cancer.

Prognostic and predictive role of TIL subsets and other immune cells

High cytotoxic T-cells (CD8+) and a high CD8/FOXP3 ratio have been associated with both an increased pCR rate and a favorable outcome in patients treated with neoadjuvant therapy based on anthracyclines, taxanes, and anti-HER2 drugs [38]. However, other studies have found discrepant results [39]. The predictive value of TILs, TIL subsets, and other immune cells in patients receiving chemotherapy-free lapatinib plus trastuzumab neoadjuvant treatment has been assessed in the TBCRC006 trial. Using multispectral imaging of pre-treatment tumor biopsies by multiplexed immunofluorescence, the authors identified an immune infiltrate profile characterized by high CD4, CD8, and CD20 cells, which was independently associated with pCR [40].

The immune landscape of metastatic breast cancer appears to be unbalanced toward an immunosuppressive phenotype, as compared to early setting, showing not only lower TILs and other immune-activating molecules levels but upregulating immunosuppressive molecules and, therefore, evading the immune surveillance [41]. In the metastatic setting, CD8+, high CD8/FOXP3 ratio, and programmed death-ligand 1 (PD-L1)− group has been associated with a significantly longer PFS and OS than the CD8−, CFRlow, and PD-L1+ group in patients treated with pertuzumab, trastuzumab, and docetaxel [42].

Regarding innate immunity cells, the prognostic and predictive role of TAMs in breast cancer is inconclusive. It seems though that the infiltration of TAMs in the TME has been associated with unfavorable clinicopathologic features in both HR-negative and HER2-positive phenotypes. In a study evaluating the role of immunological markers in patients with advanced HER2-positive breast cancer receiving trastuzumab, high numbers of M1-like TAMs together with high number of CD8+ T-cells were significantly associated with improved survival and long trastuzumab-free periods [43]. On the contrary, high number of M2 TAMs (CD163+) has been correlated with hyaluronan accumulation and a poor prognostic factor [44]. Regarding NK cells, higher levels of NK cells (CD56+) in pre-treatment tumor samples have been associated with higher pCR rates [45]. Moreover, the ADCC-mediated mechanism of action of HER2-targeted monoclonal antibodies is thought to be mainly caused by NK cells.

Clinical relevance of host immunity in small HER2-positive breast cancer

Results from the phase II APT trial, evaluating paclitaxel plus trastuzumab in small, node-negative HER2-positive breast cancers, showed that immune profiles of small HER2-positive breast cancers differ also according to histological grade, HR status, and molecular subtype. High TILs (> 60%) were found more often in high-grade, HR-negative, and in HER2-enriched and basal-like tumors. Conversely, lower TILs levels and lower stromal PD-L1 expression (≤ 1%) were found in low-grade, HR-positive, and in luminal tumors [46]. A significant association between tumor size and an exhausted CD8 T-cell signature was observed, together with a trend of higher TIL and increased sPD-L1 expression in larger tumors, highlighting the hypothesis that adaptive resistance mechanisms play a role in the immunity escape of HER2-positive tumors [46].

Clinical data on enhancing the immune system in HER2-positive breast cancer

The inhibition of HER2 signaling potentially switches non-inflamed profiles of HER2-positive tumors into inflamed ones. Anti-HER2 antibody (mAb) therapy not only interrupts oncogenic signals and induces FcR-mediated cytotoxicity but its therapeutic effect also depends on adaptive immunity [16]. Anti-HER2 mAbs inhibit downstream PI3K-AKT signaling and induce ADCC, leading to upregulation of PD-L1 expression by tumor cells and of PD1 expression by T-cells. ADCs induce immunogenic cell death and MHC class I expression in cancer cells, leading to dendritic cells maturation, differentiation of TAMs into the pro-inflammatory M1 type, and increased T-cell infiltration [22]. In preclinical studies, tucatinib favorably modulated the immune microenvironment and demonstrated enhanced activity in combination with α-PD1 therapy in trastuzumab-resistant HER2-positive murine tumor model. Of note, significantly greater efficacy in tumor growth and survival was observed

with the combination of tucatinib and α-PD1, but not with tucatinib and α-CTLA4 compared to tucatinib alone [47].

Combination of immune checkpoint inhibitors and HER2-targeted agents

The combination of HER2-targeted agents with immune checkpoint inhibitors could increase both the innate and acquired immune activation, and therefore enhance antitumor efficacy. Several trials combining immune checkpoint inhibitors and HER2-targeted therapy are under investigation, both in the advanced (Tables 1, 2) and early settings (Table 3). In the single-arm phase Ib/II PANACEA trial that investigated the addition of pembrolizumab to trastuzumab in trastuzumab-resistant patients with HER2-positive advanced breast cancer, the combination resulted in objective responses in patients with PD-L1-positive, but not in PD-L1-negative, tumors [48]. PD-L1 staining in at least 1% of tumor cells or any staining in stroma were defined as positive. The measure of expression was the combined positive score (CPS), defined as the ratio of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) out of the total number of tumor cells × 100. In another phase Ib trial of patients with heavily pre-treated HER2-positive metastatic breast cancer, no significant clinical activity was observed with the combination of durvalumab with trastuzumab, but all patients in this trial had < 1% PD-L1 expression on their tumors [49].

The KATE2 study was the first randomized phase II trial assessing the efficacy and safety of T-DM1 with or without atezolizumab in patients with HER2-positive advanced breast cancer that had progressed on prior treatment with trastuzumab and a taxane. A meaningful improvement in PFS was not observed in patients who received atezolizumab vs. placebo in the intention-to-treat population, and more adverse events were recorded in the atezolizumab group. However, a prespecified exploratory analysis suggested a benefit with T-DM1 plus atezolizumab in patients with PD-L1-positive tumors (median PFS 8.5 months vs. 4.1 months in the T-DM1 + atezolizumab and T-DM1 plus placebo arms, respectively) [50]. Importantly, other exploratory immune-related biomarker data, including PD-L1 expression, T-effector cell gene signature, CD8 immunohistochemistry expression, and TILs also were associated with PFS benefit [50]. In a post hoc analysis, although the number of events was small, the data suggested an OS benefit with atezolizumab plus T-DM1 in PD-L1-positive patients (1-year survival rate of 94% vs 88%; hazard ratio 0.55 [0.22–1.38]). The randomized double-blind phase III KATE3 study is ongoing (NCT04740918) in order to compare T-DM1 plus atezolizumab versus T-DM1 plus placebo in PD-L1+ patients with advanced HER2-positive breast cancer, and previously treated with trastuzumab, pertuzumab

Table 1 Results from clinical trials assessing the combination of checkpoint inhibitors and HER-targeted treatment in advanced HER2-positive breast cancer

Study	Phase	Setting	*n*	Treatment	Results/Status
PANACEA (NCT02129556)	Phase Ib–II	Advanced	58	Trastuzumab+pembrolizumab	– ORR 6/40 (15%) in PD-L1+ pts – No responses among PD-L1− pts – Median PFS 2.7 m (90%CI 2.6–4.0)
CCTG IND.229 (NCT02649686)	Phase Ib	Advanced	15	Trastuzumab+durvalumab	– No responses by RECIST – 4/14 (29%) SD – All pts < 1%PD-L1 tumor expression
KATE-2 (NCT02924883)	Randomized phase II	Advanced	202	TDM1+atezolizumab/placebo T-DM1+placebo	– Median PFS (range) in the PD-L1+ population (exploratory analyses) – T-DM1+ atezo:8.5 m – T-DM1+ placebo: 4.1 m HR 0.60 (95%CI 0.32–1.11)
NCT03523572 [53]	Phase Ib	Advanced in HER2-positive and HER2-low pts	48	Trastuzumab-deruxtecan+nivolumab	– In the HER2-positive cohort: ORR 59% (19/32) Median PFS 8.6 m – In the HER2-low cohort: ORR 38% (6/16) Median PFS 6.3 m

HR hazard ratio; *m* months; *OR* objective response; *ORR* overall response rate; *PFS* progression-free survival; *pts* patients; *SD* stable disease

Table 2 Ongoing clinical trials assessing the combination of checkpoint inhibitors and HER-targeted treatment in advanced HER2-positive breast cancer

Study	Phase	Setting	*n*	Treatment	Status
NCT03032107	Phase1b	Advanced	27	T-DM1+pembrolizumab	Active, not recruiting
NCT03125928	Single-arm phase II	1st line advanced	50	Paclitaxel+trastuzumab+pertuzumab+atezolizumab	Recruiting
NRG-BR004 (NCT03199885)	Randomized double-blind phase III	1st line advanced	600	Paclitaxel+trastuzumab+pertuzumab with atezolizumab/placebo	Recruiting
KATE3 (NCT04740918)	Randomized double-blind phase III	Advanced in PD-L1+ pts who have received prior trastuzumab+/− pertuzumab, and taxane	320	T-DM1+atezolizumab/placebo	Recruiting
SOLTI-ATREZZO (NCT04759248)	Single-arm phase II	Advanced PAM50 non-luminal/HER2+ breast cancer refractory to trastuzumab/pertuzumab based therapy and T-DM1	110	Atezolizumab+trastuzumab+vinorelbine	Recruiting
AVIATOR (NCT03414558)	Randomized open-label phase II	Advanced HER2+ breast cancer	100	Trastuzumab and vinorelbine or Trastuzumab and vinorelbine with avelumab or Trastuzumab and vinorelbine with avelumab and utomilumab (41BB/CD137agonist)	Suspended
NCT03417544	Single-arm phase II	In brain mets	33	Atezolizumab+pertuzumab+high-dose trastuzumab	Active, not recruiting
TOPAZ (NCT04512261)	Single-arm phase Ib/II	In brain mets	33	Pembrolizumab+tucatinib+trastuzumab	Recruiting

mets metastases; *pts* patients

and a taxane. There are several ongoing phase III studies also investigating the role of atezolizumab in combination with pertuzumab, trastuzumab, and paclitaxel, both in the advanced setting as first-line treatment (NCT03199885), and in the neoadjuvant setting, following dose-dense doxorubicin and cyclophosphamide (AC) in the Impassion050 trial (NCT03726879) or in combination with carboplatin in the APTneo trial (NCT03595592). The primary analysis of the Impassion 050 trial (Table 3) found that adding atezolizumab to neoadjuvant anti HER2 treatment, does not improve pathological complete response. This is the first phase 3 trial to report data comparing a neoadjuvant anti-HER2 based regimen with or without the anti-PD-L1 antibody, in patients with high risk, HER2-positive early breast cancer, receiving dose-dense anthracycline and taxane-based chemotherapy in combination with pertuzumab and trastuzumab [51]. The trial was stopped early when an Independent Data Monitoring Committee considered an unfavorable benefit-risk profile with the intervention. However, longer follow-up is needed to see if there is an impact in EFS and OS, which were secondary endpoints, as pCR may not be the best endpoint for measuring immunotherapy's efficacy.

Recently, the WSG KEYRICHED-1 provided the first results of neoadjuvant chemotherapy-free 12-week anti-HER2 de-escalation therapy with trastuzumab and pertuzumab in combination with the PD-1 inhibitor pembrolizumab in HER2-enriched early breast cancer. This single-arm, phase II trial (NCT03988036) enrolled patients with newly diagnosed HER2 2+ (ISH+) or HER2 3+ breast cancer (stage I–III) and HER2-enriched subtype by PAM50 analysis. In this trial, 65% of patients had tumors ≥ 2 cm and 30% had positive lymph node involvement. Centrally confirmed pCR rate (primary endpoint) in surgical specimens was 46% (95% CI 0.31–0.62) in the 43 patients of the pp population, and 52% (95%CI 0.37–0.67) in all 46 evaluable patients. Negative PR status ($n = 12$) was a significant predictor for pCR ($p = 0.027$). Interestingly, despite HER2-enriched subtype, no pCR was observed in the 4 patients with immunohistochemical (IHC) HER2 2+/ISH+ status in contrast to 20/39 (51%) pCRs in IHC HER2 3+ tumors. No new safety signals were observed [52].

Results from the phase 1b trial evaluating the combination of trastuzumab-deruxtecan with nivolumab in patients with HER2-expressing advanced breast cancer have recently been reported. The combination demonstrated

Table 3 Clinical trials assessing the combination of checkpoint inhibitors and HER-targeted treatment in early-stage HER2-positive breast cancer

Study	Phase	Setting	*n*	Treatment	Primary outcome/status
Impassion050 (NCT03726879)	Randomized double-blind phase III	Neoadjuvant	454	ddAC followed by paclitaxel+trastuzumab+pertuzumab with atezolizumab or placebo	pCR in the PD-L1-positive population and ITT population – In the ITT population, pCR was 62.4% in the atezolizumab arm and 62.7% in the placebo ($p=1.0$) – In the PD-L1 positive population, pCR was achieved by 64.2% of the atezolizumab arm and 72.5% of the placebo arm ($p=0.2$)
KEYRICHED-1 (NCT03988036)	Single-arm, phase II	Neoadjuvant	46	Pembrolizumab plus trastuzumab and pertuzumab × 12 weeks	pCR in patients with PAM 50 HER2-enriched tumors In the per protocol population, pCR was 46% and 52% in all 46 evaluable patients
APTneo (NCT03595592)	Randomized, open-label phase III	Neoadjuvant	650	Pertuzumab and trastuzumab with carboplatin and paclitaxel+/− atezolizumab	EFS Recruiting
NCT03747120	Randomized, open-label phase II	Neoadjuvant	174	Trastuzumab, pertuzumab and weekly paclitaxel or THP+pembrolizumab or trastuzumab+pembro+weekly paclitaxel	pCR Recruiting
Astefania NCT04873362	Randomized, double-blind placebo-controlled phase III	Adjuvant	1700	Atezolizumab or placebo with T-DM1 in patients with residual invasive disease following preoperative therapy	IDFS Recruiting

ddAC dose-dense doxorubicin+cyclophosphamide; *EFS* event-free survival; *ITT* intention-to-treat; *pCR* pathologic complete response; *IDFS* invasive disease-free survival

antitumor activity in both HER2-positive or HER2-low breast cancer patients with an overall response rate of 59.4% and 37.5%, respectively, and a manageable safety profile [53].

Immune-optimized anti-HER2 antibodies (margetuximab)

Another attempt to increase antitumor activity has been made through the design of monoclonal antibodies engineered for increased affinity to some single-nucleotide polymorphisms in Fcγ receptor-binding (FcγR) on effector cells leading to immune cell activation. Margetuximab is an Fc-optimized chimeric monoclonal antibody that binds to the same epitope as trastuzumab but with enhanced FcγR properties: an increased affinity for activating CD16A polymorphisms (FcγRIIIa) and a decreased affinity for inhibitory FcγR CD32B (FcγIIb), resulting in a superior binding to effector cells and increased activation of innate and adaptive anti-HER2 immune responses. It also preserves the antiproliferative properties of trastuzumab. Margetuximab has been evaluated in the SOPHIA phase III randomized trial ($n=536$) that compares margetuximab plus chemotherapy with trastuzumab plus chemotherapy as a third-line therapy in patients with advanced HER2-positive breast cancer previously treated with trastuzumab, pertuzumab, and T-DM1. Margetuximab plus chemotherapy demonstrated a small but statistically significant PFS benefit over trastuzumab plus chemotherapy, meeting the primary endpoint of the study (median PFS of 5.8 months vs 4.9 months; hazard ratio 0.76; 95% confidence interval [CI], 0.59–0.98; $p=0.03$) [54]. The SOPHIA study also tested the hypothesis that altering Fc–FcγR interactions can drive clinical benefit. Exploratory PFS analysis by CD16A genotype suggested that the presence of a CD16A-158F allele may predict margetuximab benefit over trastuzumab (6.9 months versus 5.1 months, respectively; hazard ratio 0.68 95% CI, 0.52–0.90; $p=0.005$). The final OS analysis for the ITT population did not demonstrate a statistically significant advantage for margetuximab plus chemotherapy compared with trastuzumab plus chemotherapy. A prespecified non-α-allocated analysis of CD16A genotyping showed a numerical

OS advantage of 4.4 months in favor of margetuximab in F homozygous patients ($n = 192$, 38%), along with a numerical OS advantage of 9.1 months in favor of trastuzumab in CD16A-158 V homozygotes [55]. The U.S. Food and Drug Administration has approved margetuximab (Margenza) in combination with chemotherapy for the treatment of adult patients with metastatic HER2-positive breast cancer who have previously received 2 or more anti-HER2 regimens, at least one of which for metastatic disease. An ongoing neoadjuvant investigator-sponsored trial is comparing margetuximab vs trastuzumab when given in combination with a taxane and pertuzumab in patients with the low-affinity CD16A genotype (the MARGetuximab Or Trastuzumab trial- MARGOT; NCT04425018).

Bispecific antibodies

Immune activation can be also enhanced using bispecific antibodies (BsAbs) which combine the functionality of two MAbs that target two different targets or epitopes, either in the same or in different receptors. Different BsAbs exist that target both HER2 and T-cells in order to redirect immune effector cell to the tumor. Several BsAbs are currently being studied in patients with advanced HER2-positive disease.

Zanidatamab, also known as ZW25 (Zymeworks Inc.) is a humanized bispecific antibody biparatopic that binds to two different epitopes on the extracellular domain of HER2 ECD2 and ECD4. This results in increased tumor cell binding, blockade of ligand-dependent and independent growth, and improved receptor internalization and downregulation relative to trastuzumab. In vivo studies demonstrate antitumor activity in HER2-low to high expressing models. Results from the phase I/II study (NCT02892123) evaluating the safety and efficacy of single-agent ZW25 in separate expansion cohorts of patients with HER2-positive (IHC 3+ or 2+/FISH+) solid tumors demonstrated a promising anti-tumor activity and no dose-limiting toxicities were observed. In patients with heavily pre-treated HER2-expressing breast cancers who had progressed to a median of five HER2-targeted regimens, an objective response rate of 36.4% and a median PFS of 7.3 months were observed across all treatment regimens (8/22 patients). The most common adverse events were diarrhea and infusion reaction, all grades 1 or 2, with no treatment-related discontinuations [56, 57]. A phase II study of ZW25 in combination with palbociclib and fulvestrant is ongoing in patients with advanced HER2-positive, HR-positive breast cancer (NCT04224272).

GBR1302 (Glenmark Pharmaceuticals) is a HER2xCD3 BsAb developed to direct T-cells to HER2-expressing tumor cells. Preclinically, GBR1302 has demonstrated potent killing of HER2-positive human cancer cells, as well as growth suppression of trastuzumab-resistant cell lines. Preliminary biomarker and pharmacodynamic data from a phase I study of single-agent GBR1302 in progressive HER2-positive solid tumors (NCT02829372) demonstrated modulation of peripheral T-cell populations and cytokines [58]. A phase I/II, open-label, dose escalation study is ongoing in patients with HER2-positive metastatic breast cancer (NCT03983395).

PRS-343 (Pieris Pharmaceuticals, Inc.) is another anti-HER2 BsAbs targeting HER2 and the immune receptor CD137 (4-1BB). CD137 is a key costimulatory immunoreceptor and a member of the tumor necrosis factor receptor superfamily. PRS-343 promotes CD137 clustering by bridging CD137-positive T-cells with HER2-positive tumor cells, leading to a potent costimulatory signal to tumor antigen-specific T-cells [59]. Two clinical studies evaluating PRS-343 in HER2-positive solid tumors are ongoing, either as a single-drug agent (NCT03330561) or in combination with atezolizumab (NCT03650348).

Emerging strategies

Numerous ongoing studies are investigating the combination of immune checkpoint inhibitors with HER2-targeted agents in HER2-positive breast cancer in both the advanced (Table 2) and early settings (Table 3), and results are awaited.

Several clinical trials are also evaluating therapeutic cancer vaccines in HER2-positive breast cancer either alone or in combination with anti-HER2 targeting agents. Different strategies for developing vaccines against breast cancer are being investigated, including the use of specific tumor antigen-derived peptides, proteins, DNA, mRNA, whole tumor cells, and dendritic cells. Peptide-based cancer vaccines are the most studied cancer vaccines, especially E75. E75 is an amino acid-long peptide derived from the HER2 receptor which binds to HLA-A2, thus activating cytotoxic T lymphocytes. The HER2 vaccine NeuVax (nelipepimut-S or E75 peptide combined with granulocyte macrophage-colony stimulating factor) is being investigated in combination with trastuzumab in a phase II clinical trial in the adjuvant setting to prevent recurrences in patients with high-risk HER2-positive breast cancer (NCT02297698) [60]. In a phase 2 clinical trial, patients with residual disease following neoadjuvant chemotherapy are being randomized to the multi-epitope HER2 Vaccine TPIV100 vs. Placebo (NCT04197687). Ongoing clinical trials of therapeutic vaccines are also being developed in HER2-expressing ductal carcinoma in situ (DCIS), for instance with the multi-epitope HER2 Peptide Vaccine H2NVAC before surgery (NCT04144023).

Cellular immunotherapy is also being evaluated in HER2-positive breast cancer. A phase 1/2 study to investigate the safety, tolerability, and clinical activity of HER2-specific

dual-switch CAR-T cells, BPX-603, administered with rimiducid to pre-treated patients with locally advanced or metastatic solid tumors which are HER2 amplified/overexpressed is ongoing (NCT04650451). The combination of trastuzumab plus natural killer immunotherapy in patients with HER2-positive recurrent breast cancer has also been investigated in a phase I/II trial (NCT02843126).

Conclusion

Treatment of HER2-positive breast cancer is evolving rapidly, and the use of anti-HER2-targeted therapy has dramatically changed the outcomes for patients with HER2-positive breast cancer. However, not all patients benefit to the same extent from current anti-HER2 therapies and importantly, HER2-positive breast cancer is a heterogeneous disease. The interaction between breast cancer and the host immune system in HER2-positive disease seems dynamic and complex, playing a role not only in the prognosis, but also in modulating treatment response to some HER2-targeted agents. Results in the advanced setting suggest that the benefit from immune checkpoint inhibitors could be restricted to patients with PD-L1-positive disease. However, research is ongoing to enhance immune activation in HER2-positive breast cancer and better determine which patients can benefit from it.

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Declarations

Conflict of interest SP reports advisor/consultant role for AstraZeneca, Daiichi-Sankyo, Polyphor, Novartis, Eisai, Pierre-Fabre, Seattle-Genetics, and Roche. SMT reports institutional research funding from AstraZeneca, Lilly, Merck, Nektar, Novartis, Pfizer, Genentech/Roche, Immunomedics, Exelixis, Bristol-Myers Squibb, Eisai, Nanostring, Cyclacel, Odonate, Seattle Genetics; and advisor/consultant role for AstraZeneca, Lilly, Merck, Nektar, Novartis, Pfizer, Genentech/Roche, Gilead, Immunomedics, Bristol-Myers Squibb, Eisai, Nanostring, Puma, Sanofi, Celldex, Paxman, Odonate, Seattle Genetics, Silverback Therapeutics, G1 Therapeutics, AbbVie, Anthenex, OncoPep, Outcomes4Me, Kyowa Kirin Pharmaceuticals, Daiichi-Sankyo, Samsung Bioepsis Inc.

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Research involving human and/or animals participants Not applicable.

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