Current Breast Cancer Reports (2019) 11:248–258
https://doi.org/10.1007/s12609-019-00332-4

IMMUNO-ONCOLOGY (S TOLANEY, SECTION EDITOR)

Immunotherapy for HER2-Positive Breast Cancer: Changing the Paradigm

Jesús Soberino[1] · Fabricio Racca[1] · José Pérez-García[1,2] · Luis F. García-Fernández[3] · Javier Cortés[1,4,5]

Published online: 13 November 2019

Abstract

Purpose of Review To summarize and discuss the available evidence and ongoing efforts in order to establish the efficacy and safety of immunotherapeutic approaches in HER2-positive breast cancer.

Recent Findings The introduction into the clinic of anti-HER2–targeted therapies more than 15 years ago resulted in a substantial improvement in the outcome of patients with HER2-overexpressing breast cancer. However, only patients with the highest levels of HER2 expression will potentially benefit from these therapies and, unfortunately, many patients progress or relapse after optimal treatment. As metastatic breast cancer remains an incurable disease, new therapeutic strategies are urgently needed to improve clinical outcomes in these patients. Immunotherapy is emerging as a new treatment modality in breast cancer. Although it has long been regarded as a non-immunogenic disease, new preclinical and clinical studies have emphasized the therapeutic potential of the use of anti-HER2 therapies in combination with immune checkpoint inhibitors in improving outcomes in breast cancer patients.

Summary Emerging results from clinical trials evaluating immunotherapeutic agents, either as monotherapy or in combination with anti-HER2–targeted therapies, are showing promising results in the management of HER2-positive breast cancer.

Keywords Breast cancer · HER2-positive · Tumor-infiltrating lymphocytes · Immunotherapy · Antibody-dependent cellular cytotoxicity

Introduction

The immune system is the hardwired host defense mechanism that acts against pathogens and cancer. Immune cells present in the tumor microenvironment play a critical role in regulating tumor progression and control, and the manipulation of the immune microenvironment has already emerged as a promising tool for novel cancer treatments [1•, 2]. Although the idea of harnessing immune cells to fight cancer is not new, it has only recently gained interest thanks to advances in both basic immunology research [3] and the advent of immuno-oncology [4]. The 2018 Nobel Prize in Physiology or Medicine was awarded to Dr. James P. Allison and Dr. Tasuku Honjo for their respective discoveries of the immune checkpoint proteins cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) [5]. The discovery of immune checkpoints, which regulate immune activation, and their successful inhibition with monoclonal antibodies has led to a surge in new investigational therapies for the treatment of solid tumors and hematological malignancies [6, 7•, 8].

Although breast cancer has not been traditionally considered as an immunogenic malignancy, it is a heterogeneous disease comprising several biologically defined subtypes that are associated with distinct clinical behavior, each requiring specific therapeutic strategies [9–11]. The mutational burden in breast cancer and the presence of tumor-infiltrating lymphocytes (TILs), which have been associated with clinical outcomes, vary by subtype. Both triple-negative breast cancer (TNBC) and HER2-positive (HER2+) breast cancer have

This article is part of the Topical Collection on *Immuno-oncology*

✉ Javier Cortés
jacortes@vhio.net

[1] IOB Institute of Oncology, Quirónsalud Group, Madrid-Barcelona, Spain

[2] Medica Scientia Innovation Research (MedSIR), Barcelona, Spain

[3] Medical Statistics Consulting, Valencia, Spain

[4] Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

[5] IOB Institute of Oncology, Quirónsalud Group Hospital Ruber Internacional, Calle de La Masó 38, 28034 Madrid, Spain

been associated with high rates of cell proliferation and genomic instability as well as significantly higher TIL levels than hormone receptor –positive tumors [9, 12, 13]. This supports the hypothesis that genomic instability can promote antitumor immune responses owing to the availability of a large number of tumor-associated antigens.

Given the superior response to immunotherapy of human malignancies with a high mutational load and immune cell infiltrates, HER2+ breast cancer represents a candidate setting for the exploration of the potential of cancer immunotherapy [14]. Although the development of immunotherapy drugs in breast cancer has been slower than that in other cancers, the number of clinical trials of immunotherapies for breast cancer, either as monotherapy or in combination with other treatments, has grown rapidly in recent years [15•]. Given the findings of the IMpassion130 phase III trial, the U.S. Food and Drug Administration recently granted accelerated approval to atezolizumab (anti-PD-L1 antibody) in combination with chemotherapy for adult patients with unresectable locally advanced or metastatic TNBC PD-L1–positive tumors [16, 17].

Role of TILs as Predictors of Clinical Outcomes in HER2-Positive Breast Cancer

The complex interplay between cancer cells and the tumor microenvironment plays a critical role in tumor growth, pathogenesis, and progression and is continuously evolving across the different stages of disease [18, 19]. The molecular and cellular immune components of the tumor microenvironment influence disease outcome by altering the balance of suppressive versus cytotoxic responses in the surroundings of the tumor [3, 20]. Evidence is accumulating to suggest that the presence of a lymphocytic infiltrate in cancer tissue is associated with favorable long-term prognosis and better response to therapy in various types of cancer [20, 21], including breast cancer, especially more aggressive and proliferative forms, such as TNBC and HER2+ breast cancer [22••].

Overexpression of human epidermal growth factor receptor 2 (ErbB2/HER2) in breast cancer cells, found in approximately 20% of patients, has long been established as a major negative prognostic factor associated with an aggressive form of the disease, reduced responses to traditional therapies, increased risk of disease recurrence, and poor survival [23, 24]. The introduction into the clinic of the anti-HER2–humanized monoclonal antibody (mAb) trastuzumab more than 15 years ago revolutionized the treatment of HER2+ breast cancer, forever changing the clinical landscape of the disease. The combination of anti-HER2 therapies with chemotherapy is the standard treatment for HER2-positive breast cancer in current practice [25, 26].

In a seminal study, conducted nearly 30 years ago, inflammatory cell infiltrates were shown to be related with several clinicopathological features in breast cancer and predicted axillary node metastasis ($p < 0.001$) and were also significant predictors of recurrence-free survival ($p = 0.08$) and breast cancer–related survival ($p = 0.0164$) in rapidly proliferating breast tumors, particularly axillary lymph node–negative tumors. These results clearly demonstrated the presence of efficient immunological antitumor defense mechanisms against breast cancer [27].

TILs in the HER2-Positive Breast Cancer Adjuvant Setting

Several studies have analyzed the prognostic value of TILs in predicting benefit from anti-HER2 therapies in the adjuvant setting. In a prospectively designed retrospective study conducted using samples from the FinHER adjuvant phase III trial, 232 patients with early-stage HER2+ breast cancer were randomized to receive or not receive 9 weeks of trastuzumab in addition to their adjuvant chemotherapy. It was shown that each 10% increment in lymphocytic infiltrate was associated with a significant 18% reduction in the relative risk of distant recurrence in patients who received trastuzumab (HR 0.82; 95% CI 0.58–1.16; $p < 0.025$). This was the first report of an association between higher levels of TILs and increased benefit from the addition of trastuzumab to chemotherapy in HER2+ disease. It was also the first time that an immune biomarker was demonstrated to be predictive of therapeutic benefit [28]. In another study, the NSABP trial B-31, a surrogate gene expression signature of TILs, including 119 genes, was developed and used to evaluate the potential predictive value for the degree of benefit from trastuzumab plus standard adjuvant chemotherapy in early-stage HER2+ breast cancer. It was shown that patients with high TIL-associated gene expression treated with trastuzumab showed a higher benefit in the relapse-free survival, compared to those who received chemotherapy alone (HR 0.06; 95% CI 0.01–0.47; $p = 0.007$). This study confirmed TILs as another complementary predictive marker for trastuzumab benefit in HER2-positive breast cancer [29]. In line with these results, data from a whole transcriptome analysis performed in the North Central Cancer Treatment Group (NCCTG) N9831 adjuvant trastuzumab trial suggested that increased relapse-free survival was linked to a subset of immune function genes. Among patients with immune response–enriched tumors and contrary to findings in patients with non-immune response–enriched tumors, there was a significant difference in relapse-free survival among patients treated with trastuzumab plus chemotherapy compared with those who received chemotherapy alone (HR 0.36; 95% CI 0.23–0.56; $p < 0.001$) [30]. However, an exploratory analysis from a subset of N9831 trial participants with HER2-positive disease found no association between high levels of TILs and trastuzumab therapy benefit. Patients with high levels of lymphocyte infiltration, consisting of at least 60% TILs, did not appear to derive any additional benefit from the

addition of trastuzumab to chemotherapy (HR 2.43; 95% CI 0.58–10.22; $p = 0.22$). These results should be interpreted with caution, since only 94 out of 945 patients (9.9%) were classified as having lymphocyte-predominant breast cancer (LPBC) and only 8 disease recurrence events were registered, probably undermining the power of the study to detect the real treatment effect in this group of patients [31]. According to a recent comprehensive biomarker analysis of data from the randomized phase III APHINITY trial [32], higher levels of TILs are associated with favorable outcomes in patients with HER2+ breast cancer treated with trastuzumab and pertuzumab (HR 0.91; 95% CI 0.86–0.96; $p = 0.001$), with the highest quartiles of TILs predicting the greatest pertuzumab benefit for these patients (HR 0.35; 95% CI 0.19–0.65; $p = 0.003$) [33].

TILs in the HER2-Positive Breast Cancer Neoadjuvant Setting

In the neoadjuvant setting, the prognostic value of TILs for predicting benefit from anti-HER2 therapies was evaluated in different clinical trials, including more than 2000 patients in total. In a prospectively planned secondary analysis of the randomized NeoALTTO phase III trial, the presence of TILs at diagnosis (cutoff value for high TILs was 30%) was seen to be an independent, positive prognostic marker for both pathologic complete response (pCR) and event-free survival (EFS) in early-stage HER2+ breast cancer treated with chemotherapy and neoadjuvant anti-HER2 agents. The relationship between pCR and presence of TILs was nonlinear; i.e., rates of pCR increased sharply when the levels of stromal TILs were greater than 5%, regardless of treatment group (adjusted odds ratio 2.60; 95% CI 1.26–5.39; $p = 0.01$). In contrast, the relationship between the presence of TILs and EFS was found to be linear: the higher the level of TILs in the primary tumor at diagnosis, the better the EFS after treatment with trastuzumab, lapatinib, or the combination of both; each 1% increase in TILs was associated with a 3% decrease in the rate of an event (adjusted HR 0.97; 95% CI 0.95–0.99; $p = 0.002$). This was the first time that high levels of stromal TILs were shown to be associated with improved clinical outcomes, regardless of the anti-HER2 agent administered [34]. Results from the TIL biomarker analysis performed in the randomized CHER-LOB phase II trial showed that TILs predicted the achievement of pCR for early HER2+ breast cancer patients undergoing neoadjuvant chemotherapy plus anti-HER2–targeted therapy. This predictive effect seemed to be limited to hormone receptor–negative patients [35]. In the low-powered survival analysis of this trial, a nonsignificant trend suggested a positive correlation between increased levels of intratumoral TILs and better EFS (5-year EFS rate for patients with intratumoral TILs above vs. patients with intratumoral TILs below the median, 89% vs. 76%, respectively, with borderline significance for the comparisons between the two groups). No differences were found when levels of stromal TILs were compared. Among patients with residual disease, no association was observed between levels of intratumoral TILs and stromal TILs and EFS [36]. The randomized GeparSixto phase II trial prospectively validated TILs as predictive biomarkers of pCR in HER2+ and triple-negative breast carcinomas. Stromal TILs and LPBC phenotype (≥ 60% of either intratumoral or stromal TILs) were seen to be significant predictors of pCR ($p < 0.001$). In patients with HER2+ breast cancer, pCR rates ≥ 75% were observed in those with the LPBC phenotype ($p = 0.006$) [37]. In the randomized NeoSphere phase II trial, an exploratory analysis in patients with operable or locally advanced HER2+ breast cancer who received anti-HER2–targeted therapies (trastuzumab, pertuzumab, or dual blockade) showed that the group with low levels of TILs had the lowest rate of pCR (4.3%), compared to those with intermediate levels (26.9%) and LPBC phenotype (26.7%) ($p = 0.018$ for low-TIL vs. intermediate-TIL/LPBC). These results demonstrated that the immune system modulates response to therapies containing trastuzumab and pertuzumab [38]. The randomized phase II NeoLath study (JBCRG-16) examined the correlation between TILs and treatment outcomes of neoadjuvant dual-HER2 blockade therapy with trastuzumab and lapatinib in HER2+ early breast cancer. Univariate analysis showed that the percentages of TILs and neuropilin 1 (NRP-1)–positive TILs were associated with pCR, and multivariate analysis showed that the percentage of NRP-1–positive TILs was significantly associated with pCR (adjusted OR 1.08; 95% CI 1.04–1.13; $p < 0.0001$) [39].

Data from a meta-analysis of published results from five large clinical trials (a total of 1256 patients), including CHER-LOB, GeparQuattro, GeparQuinto, GeparSixto, and NeoALTTO studies (cutoff value for high TILs was 60% in the first four trials and 30% in NeoALTTO), showed a significant association between pCR rates and high pretreatment levels of TILs (OR 2.46; 95% CI 1.36–4.43; $p = 0.003$). This association was greater in patients from the studies that used a 60% cutoff to define high TILs (OR 2.88; 95% CI 2.03–4.08; $p < 0.001$). On the other hand, no interaction was observed between TIL subgroup (high vs. other) and response to anti-HER2 agents ($p = 0.747$) and chemotherapy ($p = 0.201$). This meta-analysis concluded that, in HER2+ breast cancer, high baseline TILs were associated with an increased probability of pCR, regardless of the anti-HER2 agent(s) and neoadjuvant chemotherapy regimens used. In addition, patients with TIL levels > 60% prior to treatment demonstrated a greater benefit from neoadjuvant chemotherapy combined with anti-HER2 therapy [40].

All these studies showed that the TIL status of primary HER2+ breast tumors might predict pCR to neoadjuvant chemotherapy in combination with trastuzumab and, moreover, that patients with high TIL levels in residual tumors exhibit better prognosis than those with low levels. Therefore,

evaluation of TILs in the residual tumor after neoadjuvant treatment with trastuzumab might be necessary to identify patients with good prognosis among those without pCR.

TILs in Advanced HER2-Positive Breast Cancer

Compared to the previous scenarios, data on the role of TILs in the metastatic setting of HER2+ breast cancer are very limited. The randomized phase III CLEOPATRA study compared the addition of either pertuzumab or placebo to first-line therapy with trastuzumab and docetaxel in patients with locally recurrent, unresectable, or metastatic HER2+ breast cancer. It was found that each 10% increase in stromal TILs was significantly associated with longer overall survival (OS) (adjusted HR 0.89; 95% CI 0.83–0.96; $p = 0.0014$), suggesting that the effect of antitumor immunity extends to the advanced setting. No significant association between TIL values and progression-free survival (PFS) was observed (adjusted HR 0.95; 95% CI 0.90–1.00; $p = 0.063$), and no significant differences were found in the treatment effect of pertuzumab by stromal TIL value for either PFS ($P_{\text{interaction}} = 0.23$) or OS ($P_{\text{interaction}} = 0.21$). Of interest, freshly obtained tumor samples were found to have significantly lower TIL values than archival samples [10.0% (95% CI 5.0–20.0) vs. 15.0% (5.0–35.0); $p = 0.00036$] [41]. Other studies have made contributions to the association between TIL levels and clinical outcomes in the metastatic setting. In a secondary analysis of the randomized Canadian Cancer Trials Group MA.31 phase III trial, in which patients with HER2+ metastatic breast cancer were randomized to receive trastuzumab or lapatinib in combination with a taxane, a low level of preexisting cytotoxic (CD8+) stromal TILs predicted the most benefit from an antibody-based (trastuzumab) versus a small molecular-based (lapatinib) therapy against the same target [42].

In a study performed in the Oulu University Hospital (Finland) between 2009 and 2014, patients who received at least one dose of intravenous trastuzumab for the treatment of metastatic HER2+ breast cancer were retrospectively identified (limited to those who had prechemotherapy tumor samples available, $n = 48$). Using a computer-assisted method to count the immune cells present in tumor samples, the potential prognostic and predictive value of TILs in metastatic HER2+ breast cancer treated with trastuzumab was examined. Interestingly, a positive correlation between time to trastuzumab discontinuation in response and CD8+ T cells was shown ($r_s = 0.541$, $p = 0.046$, for lymphocytes in the invasive margin; $r_s = 0.620$, $p = 0.018$, for lymphocytes in the center of the tumor; and $r_s = 0.623$, $p = 0.017$, for lymphocytes in the whole tumor area). Furthermore, time to trastuzumab discontinuation was found to be associated with high CD8+ T cells ($p = 0.038$, for lymphocytes in the center of the tumor, and $p = 0.007$, for lymphocytes in the whole tumor area). The results suggested a significant role for a high number of lymphocytes in the center of the tumor, especially CD8, in improved survival and benefit from trastuzumab therapy in patients with metastatic HER2+ breast cancer [43].

These data highlight the clinical relevance of antitumor immunity in the efficacy of anti-HER2 therapies, paving the way for the evaluation of novel treatments or combinations that might further improve clinical outcomes in HER2+ breast cancer patients.

Immunotherapy for HER2-Positive Breast Cancer: Recent Advances and Combination Therapeutic Approaches

Role of the Immune Response in HER2-Positive Breast Cancer

Trastuzumab has been thought to act primarily by directly affecting tumor cells, but results from the clinical setting suggest that anti-HER2 therapy may also serve to relieve suppression of antitumor effector immunity. Trastuzumab binding to the extracellular domain of HER2 prevents receptor dimerization, increases receptor internalization and degradation, and inhibits receptor shedding [44]. Collectively, these actions inhibit RAS-MAPK and PI3K-AKT-mTOR signaling pathways, leading to the suppression of cancer cell proliferation and growth [45]. In addition, trastuzumab activity has been shown to be mediated through antibody-dependent cellular cytotoxicity (ADCC) [44–47]. Increased receptor degradation enhances HER2 peptide presentation on major histocompatibility complex (MHC) receptors. Moreover, while the antibody binds to HER2 on the cell surface, the crystalline fragment (Fc) of the immunoglobulin interacts with Fc-gamma receptors (FcγR) on innate immune effector cells, such as natural killer (NK) cells and macrophages, activating ADCC activity [48, 49].

The importance of trastuzumab-mediated ADCC activity was initially shown in preclinical models in which experimental molecules containing only the Fab fraction of trastuzumab did not exert any antitumor effect [50]. In patients, studies in early-stage breast cancer who received single-agent trastuzumab before surgery reported data suggesting a role for ADCC. In particular, patients induced to complete or partial remission by trastuzumab alone were found to have higher ADCC and higher in situ infiltration of cytotoxic lymphocytes, including NK cells [47]. Likewise, even when used in combination with docetaxel, neoadjuvant trastuzumab was associated with significantly increased numbers of tumor-associated NK cells and increased lymphocyte expression of cytotoxic activity markers compared with controls [51••]. In a pilot study in patients with operable HER2+ breast cancer, ADCC activity increased significantly after short-term (4 weeks) preoperative therapy with trastuzumab in 15 of 18 (83%) patients enrolled in

the study and was found to be significantly related with the number of lymphocytes coexpressing CD16 and CD56, but not with trastuzumab serum levels. The association of maximal ADCC activity with pCR and low-to-absent ADCC with a lack of clinical response suggests that high or low ADCC can either increase or limit trastuzumab clinical activity, respectively. Evidence suggests that in some instances, specific FcγR polymorphisms might be associated with higher antibody-binding affinities and improved clinical responses to trastuzumab-based therapy [52]. However, conflicting data have been reported in this regard [53–55]. All these observations suggest that the immunostimulatory effect of monoclonal antibodies may contribute to their superior therapeutic response compared to other treatments.

More important evidence on the fundamental role of ADCC in the mode of action of trastuzumab against HER2+ breast cancer cells came from the randomized phase II EGF104900 trial. In this study, patients with HER2+ metastatic breast cancer (n = 296) whose disease progressed during prior trastuzumab-based therapies were randomly assigned to receive lapatinib monotherapy or lapatinib in combination with trastuzumab. In this study, lapatinib plus trastuzumab showed superiority to lapatinib monotherapy in PFS (HR 0.74; 95% CI 0.58–0.94; p = 0.011) and offered significant OS benefit (HR 0.74; 95% CI 0.57–0.97; p = 0.026) [56]. ADCC could account for the improved clinical activity observed with trastuzumab when administered in combination with lapatinib. By inhibiting phosphorylation of the HER2 tyrosine domain, lapatinib prevents HER2 internalization and degradation, inducing accumulation of HER2 on the cell membrane, thus increasing the number of binding sites for trastuzumab [57] and trastuzumab-dependent ADCC activity [58].

Pertuzumab is another mAb directed against the extracellular dimerization domain of HER2, targeting a different epitope than trastuzumab. Upon binding, pertuzumab inhibits dimerization of HER2 with other receptors of the HER family [59]. Like trastuzumab, pertuzumab can mediate ADCC, and simultaneous binding of both antibodies to the receptor increases the concentration of FcγR binding sites on HER2+ cells, possibly enhancing NK-mediated ADCC responses [60]. Studies in mouse models have shown that adding pertuzumab to trastuzumab increases the total number of tumor-infiltrating NK cells and the percentage that are actively engaged in killing tumor cells, enhancing ADCC activity [61]. In addition, only tumor cells treated with both antibodies are likely to have a sufficient number of cell-bound antibodies to induce efficient C3 opsonization, required to initiate complement-mediated cytotoxicity and macrophage-mediated tumor cell killing [62]. In any case, it is still unclear if the observed synergism and improved clinical efficacy of the pertuzumab plus trastuzumab combination is a consequence of this enhanced immune activity [63].

Immunogenic Cell Death

The new concept of immunogenic cell death (ICD) has emerged as an exciting strategy for the activation of the immune system against cancer [64, 65]. Depending on the triggering stimulus, cancer cell death can be immunogenic or non-immunogenic. ICD involves changes in the composition of the cell surface, as well as the release of damage-associated molecular patterns (DAMPs), such as calreticulin or ATP, among others. These DAMPs operate as natural adjuvants and communicate a state of danger to the organism, although they are unable to initiate an adaptive immune response unless dying cells display antigenic epitopes that have not previously elicited central or peripheral tolerance [66]. Such *neo-epitopes* may be encoded by host genes that mutate in the course of oncogenesis and tumor progression [67]. The interaction of DAMPs with pattern recognition receptors (PRRs) expressed on a variety of cells, such as monocytes, macrophages, dendritic cells (DCs), and other components of the innate immune system, not only establishes a first line of defense (innate response) but also generates the optimal conditions for the initiation of antigen-specific immune responses (adaptive response) [64, 65]. Human malignancies with a high mutational load show a superior response to immunotherapy with checkpoint blockers than tumors with a relatively low number of somatic mutations, and such a response mostly depends on adaptive immunity [14]. Therefore, DAMP signaling constitutes a promising target for the development of therapeutic agents with oncological applications.

In the phase II NeoPHOEBE trial, 50 patients with HER2+ primary breast cancer were randomized to receive neoadjuvant trastuzumab, combined with either the pan-PI3K inhibitor buparlisib or placebo for 6 weeks, followed by the addition of paclitaxel. A preplanned exploratory analysis revealed that tumor samples obtained after just 15 days of therapy showed a higher percentage of stromal TILs and, importantly, the absolute increase from baseline to day 15 in both arms, in the setting of trastuzumab without chemotherapy, was significantly associated with a higher chance of achieving pCR (OR per 10% absolute change 1.94; 95% CI 1.14–3.28; p = 0.014). These results further supported the concept that trastuzumab monotherapy can enhance a functional antitumor immunity, resulting in augmented tumor responses [68, 69].

The recently approved antibody-drug conjugate T-DM1 (trastuzumab emtansine) [70], developed to improve the efficacy of trastuzumab, is composed of trastuzumab linked to the cytotoxic antimicrotubule agent emtansine. Upon binding to HER2, T-DM1 undergoes receptor-mediated internalization and subsequent proteolytic digestion, releasing the cytotoxic catabolite. At the same time, T-DM1 blocks HER2-mediated signal transduction, induces ADCC, and inhibits shedding of the HER2 extracellular domain [71]. It has been reported that ansamitomicin P3, a direct precursor in the synthesis of DM1

which is cytotoxic for tumor cells, induces maturation of DCs and facilitates antigen uptake and migration of tumor-resident DCs to tumor-draining lymph nodes, resulting in a robust activation of antitumor immunity [72, 73].

A study of matched tumor biopsies (pretherapeutic vs. on-treatment) from 28 treatment-naïve patients diagnosed with HER2-positive/estrogen receptor (ER)–positive breast cancer, who underwent a single treatment of T-DM1 monotherapy within a subtrial of the WSG-ADAPT protocol (NCT01745965), showed that stromal TILs increased in response to T-DM1; on average, the percentage of stromal TILs was significantly higher in on-treatment biopsies (pretherapeutic: mean, 13.77% ± 2.7%; on-therapy: mean, 20.84% ± 2.9%). Furthermore, in line with previous observations in human primary breast cancers, the increase in TILs, particularly T cells, with T-DM1 treatment was confirmed in a murine model of human HER2-overexpressing breast cancer. On the other hand, a markedly increased expression of CTLA-4 was observed on both CD4+ and CD8+ T cells upon T-DM1 treatment. Although PD-1 expression was only slightly upregulated, its ligand PD-L1 showed a strong upregulation on tumor-associated macrophages (TAMs). On the basis of the previous findings, the potential synergistic effect of T-DM1 plus blocking antibodies against CTLA-4 and PD-1 has been tested. Although the tumor model presented primary resistance to trastuzumab, the combination of T-DM1 with anti-PD-1/CTLA-4 blocking antibodies resulted in strong in vivo antitumor efficacy, achieving almost 100% complete cure and greatly enhanced T cell responses, including complete tumor rejection and memory formation [74]. Encouraged by these data, the use of checkpoint inhibitors (anti-PD-1 and anti-CTLA-4) to enhance antitumor immunity in HER2+ breast cancer has become an attractive strategy.

Preclinical evidence suggests that immune-mediated resistance to trastuzumab can be overcome by combining it with checkpoint inhibitors, so several trials have been testing the association of checkpoint inhibitors and HER2-targeted treatment. The single-arm phase Ib/II PANACEA trial examined the antitumor activity of pembrolizumab, a PD-1 inhibitor antibody, added to trastuzumab in advanced HER2+ breast cancer patients who progressed while on trastuzumab treatment. Seven patients (15%; 95% CI 7–27) in the PD-L1–positive cohort ($n = 46$) and none in the PD-L1–negative cohort ($n = 12$) achieved objective response with the combined therapy. Likewise, 11 patients (24%; 95% CI 14–36) in the PD-L1–positive cohort and none in the PD-L1–negative cohort achieved disease control. Although the majority of patients had low levels of stromal TILs (median 1.5%, IQR 0–5), significantly higher levels were observed in the PD-L1–positive cohort than in the PD-L1–negative cohort. Furthermore, there was significantly greater lymphocytic infiltration in objective responders and in patients with disease control. This study suggested that a subset of patients whose tumors are PD-L1 positive and have high TILs would be the group most likely to benefit of checkpoint inhibition and trastuzumab or trastuzumab-based therapies [75]. The phase I CCTGIND.229 trial tested the combination of durvalumab, a PD-L1 inhibitor antibody, added to trastuzumab in 15 patients with metastatic HER2+ breast cancer who had received extensive previous HER2-targeted therapies. The best response documented in the trial was stable disease at week 6 of treatment in 4 (29%) of 14 evaluable patients. All patients had PD-L1 expression lower than 1% on archival tissue or prestudy biopsy [76]. The randomized phase II KATE2 trial evaluated the efficacy and safety of atezolizumab (PD-L1 inhibitor antibody) in combination with T-DM1, compared with T-DM1-placebo, in patients with HER2+ locally advanced or metastatic breast cancer previously treated with trastuzumab and taxane-based therapy. Exploratory analyses showed improved time to progression with T-DM1 plus atezolizumab in patients with PD-L1–positive tumors with no improvement seen in patients with PD-L1–negative tumors [77]. A randomized phase III trial, comparing the PFS in patients with HER2+ metastatic breast cancer treated with first-line paclitaxel, trastuzumab, and pertuzumab with or without atezolizumab, is ongoing (NCT03199885). Another trial, the phase III APTneo neoadjuvant study, is comparing the EFS in women with early high-risk and locally advanced HER2+ breast cancer treated with trastuzumab, pertuzumab, and chemotherapy with or without atezolizumab (NCT03595592).

Novel Anti-HER2 Antibodies to Enhance ADCC

New engineered antibodies against HER2 are in development for breast cancer treatment. Margetuximab (formerly MGAH22) is an investigational mAb derived from 4D5, the parent antibody of trastuzumab. It is a chimeric IgG1 anti-HER2 antibody, containing a Fab fragment identical to that of trastuzumab, and an Fc domain engineered to present increased affinity for both high-affinity 158V and low-affinity 158F isoforms of FcγR-IIIA (CD16A), while decreasing affinity for the inhibitory FcγR-IIB (CD16B) in immune cells. It was designed to increase the ability to kill tumor cells through an Fc-dependent mechanism, including ADCC [78]. Margetuximab showed promising activity as a single agent against HER2+ advanced breast cancer in a phase I trial [79] and was evaluated in the randomized phase III SOPHIA trial in heavily pretreated patients (at least two prior lines of anti-HER2 therapies, $n = 536$) with HER2+ metastatic breast cancer. Margetuximab plus chemotherapy led to significant improvements in PFS, response, and clinical benefit compared to trastuzumab plus chemotherapy. The benefits were enhanced in patients with low-affinity CD16A-158F genotypes. The median PFS of patients treated with margetuximab and chemotherapy was 5.8 months versus 4.9 months in patients treated with trastuzumab and chemotherapy (HR 0.76; 95% CI 0.59–0.98; $p = 0.033$). Among the approximately 85% of

patients carrying the CD16A low-affinity 158F allele, a prespecified exploratory subpopulation in the study, PFS was prolonged by 1.8 months in the margetuximab arm compared to the trastuzumab arm (6.9 months vs. 5.1 months; HR 0.68; 95% CI 0.52–0.90; $p = 0.005$). The objective response rate (ORR), a secondary outcome measure in the SOPHIA study, was 22% in the margetuximab arm (95% CI 17.3–27.7%) compared to 16% in the trastuzumab arm (95% CI 11.8–21.0%). At the time of the primary PFS analysis, OS data were immature [80].

T Cell–Bispecific Antibodies

T cell–bispecific antibodies (bsAbs) are engineered molecules that include, within a single entity, binding sites to the invariant CD3 chain of the T cell receptor (TCR) and to tumor-associated or tumor-specific antigens. Binding to the tumor antigen results in crosslinking of the TCR and subsequent lymphocyte activation and tumor cell killing. MCLA-128 is a novel bsAb targeting HER2 and HER3 receptors with enhanced ADCC activity. MCLA-128 has been developed to overcome HER3-mediated resistance to EGFR and HER2-targeted therapies. In vitro preclinical activity of MCLA-128 was shown to be superior to that of anti-HER3 antibodies and trastuzumab, alone or combined with either pertuzumab or anti-HER2 antibodies. In addition, the ADCC activity of MCLA-128 was equivalent to trastuzumab when targeting HER2-overexpressing cell lines, but significantly superior when targeting cells with low expression of HER2 and when low-affinity FcγR-III effector cells were used. In vivo, MCLA-128 reduced tumor burden significantly compared to lapatinib or trastuzumab plus pertuzumab treatment groups. MCLA-128 specifically and potently inhibits ligand-dependent HER2:HER3 signaling resulting in suppression of tumor growth in vitro and in vivo [81]. A first-in-human phase I/II dose-finding study in malignant solid tumors, including relapsed/refractory HER2–amplified breast cancer, is ongoing (NCT02912949). ZW25 is another bsAb that binds to two different epitopes on the extracellular domain of HER2, resulting in increased tumor cell binding, blockade of ligand-dependent and ligand-independent growth, and improved internalization and downregulation relative to trastuzumab. Results from the phase I study demonstrated promising antitumor activity. In patients with heavily pretreated HER2+ breast cancer that had progressed on a median of five anti-HER2 regimens for metastatic disease, a partial response rate of 33% was observed, with a disease control rate of 50% [82]. GBR1302 is a HER2xCD3 bsAb designed to direct T cells to HER2-expressing tumor cells. A first-in-human phase I study of single-agent GRB1302 in progressive HER2+ solid tumors is ongoing (NCT02829372). PRS-343, a bsAb targeting HER2 and the costimulatory immune receptor CD137/4-1BB, is an antibody designed to promote CD137 clustering in HER2+ tumor cells. PRS-343 has demonstrated tumor inhibition and TIL expansion in a humanized murine model. Two clinical studies evaluating PRS-343 in patients with advanced or metastatic HER2+ solid tumors, including breast cancer, are ongoing, either as a single agent (NCT03330561) or in combination with atezolizumab (NCT03650348).

Novel Complex Immunotherapy Combinations

The randomized phase II AVIATOR trial is comparing trastuzumab and vinorelbine in combination with avelumab (PD-L1 inhibitor antibody) or avelumab and utomilumab (CD137/4-1BB agonistic mAb) in patients with advanced HER2+ breast cancer who have progressed on prior trastuzumab and pertuzumab (NCT03414658). The rationale is that CD137 is expressed on activated T cells and NK cells, and trastuzumab upregulates CD137 expression on NK cells. Moreover, CD137 agonists stimulate NK function. The combination of a CD137 agonist with trastuzumab is synergistic in nu/nu HER2+ xenografts models.

Other Immunotherapeutic Approaches for HER2-Positive Breast Cancer Treatments

Promising approaches to harness the immune system against HER2+ breast cancer are in development [46, 83•, 84]. These include (i) interleukin-2 fusion proteins: the anti-erbB2 single-chain (sc) Fv-Fc-IL-2 fusion protein (HFI) is the basis for the development of a novel targeted anticancer agent, in particular for the treatment of HER2+ cancer patients. In vivo, HFI showed significant activity in inhibiting HER2-overexpressing tumor growth and demonstrated potency in initiating a cytotoxic activity on unstimulated peripheral blood mononuclear cells (PBMCs) against human breast and ovarian cancer cells [85]; (ii) vaccination against HER2 peptides to augment antitumor immunity against HER2-overexpressing breast cancers. DCs are considered a master regulator of immune response against various pathogens and are critical for T cell–mediated antitumor immunity. Given their role in mediating antitumor immunity, DCs are being explored for tumor antigen–targeted vaccination in breast cancer. Since complete loss of anti-HER2–specific Th1 immunity in breast cancer patients has been correlated with poor therapy response and diminished disease-free survival rates [86], HER2 is a promising target of interest for the development of DC vaccine immunotherapy in HER2-overexpressing breast cancers [87]; (iii) adoptive transfer of T cells targeting tumors via chimeric antigen receptor T cell (CAR-T) therapy. CAR-T therapy involves the initial removal of the patient's immune cells from the blood via leukapheresis. Antibody-coated beads targeting tumor-associated antigens are added to activate the isolated T lymphocytes which are simultaneously transduced

with a lentiviral vector encoding the CAR into the T cell via reverse transcription. The T cells are then expanded and reinfused into the patient [88].

Conclusions

Despite remarkable progress in understanding the immune landscape of the tumor microenvironment of the different subtypes of breast cancer, immunotherapy has yet to realize its full potential in the management of the disease. Breast cancer has long been regarded as a non-immunogenic disease, but new preclinical and clinical studies have highlighted the potential of immunotherapy in improving outcomes in breast cancer patients.

The immunogenic nature of breast cancer was illustrated by the identification of TILs in breast tumors, which are particularly abundant in HER2+ breast cancer. TILs have been shown to be clinically relevant and are regarded not only as predictive of response but also as significant prognostic markers in HER2+ disease. Blockade of immune checkpoints with monoclonal antibodies has been shown to restore the activity of cell-mediated immunity and promote antitumor response. Preliminary results from early-phase clinical trials have emphasized the therapeutic potential of the use of anti-HER2 therapies in combination with immune checkpoint inhibitors.

However, several aspects must be considered if we are to properly position immunotherapy in HER2+ breast cancer in the near future. First, although the available evidence favors the selection of patients with PD-L1–positive tumors, it is rather early to anticipate a lack of efficacy of these therapies in PD-L1–negative patients; second, our knowledge of the role that chemotherapy might play in this setting is still very scant; third, the effectiveness of immunotherapy in earlier lines of treatment in HER2+ breast cancer is still controversial; fourth, dealing with tumor heterogeneity will probably help us to better understand the exact role of immunotherapy in breast cancer in general, and HER2+ breast cancer in particular; and fifth, the integration of PD-1/PD-L1 expression and TILs in clinical research and the optimal way of measuring them remain to be fully characterized.

While trastuzumab and other anti-HER2 therapies are highly efficacious, only patients with the highest levels of HER2 expression—about 20%—have the potential to respond to these treatments. Furthermore, many patients with HER2-overexpressing tumors progress or relapse even after treatment with optimized HER2-directed therapies. Unfortunately, HER2+ metastatic breast cancer is still an incurable disease and, therefore, new and more effective therapeutic strategies are urgently needed to improve clinical outcomes, particularly for those with advanced disease. Our sincere desire to cure more patients keeps us working to find better treatments to fight this disease, and it will become clear in the very near future whether immunotherapy is a useful approach in achieving this goal.

Compliance with Ethical Standards

Conflict of Interest Jesús Soberino reports personal fees and other from Roche, personal fees from Eisai, and other from Merck Sharp & Dohme (MSD) outside the submitted work. José Pérez-García reports work with Roche and Lilly outside the submitted work. Javier Cortés reports grants, personal fees, and other from Roche; personal fees and other from Celgene; work with Cellestia; grants and other from AstraZeneca; work with Biothera Pharmaceuticals; work with Merus; work with Seattle Genetics; work with Daiichi Sankyo; work with Erytech; work with Athenex; work with Pholypor; work with Lilly; work with Servier; personal fees from Novartis, grants, and personal fees from Eisai; grants and personal fees from Pfizer; personal fees from Samsung; grants from Ariad Pharmaceuticals; grants from Baxalta GmbH/Servier Affaires; grants from Bayer HealthCare; grants from F. Hoffman-La Roche; grants from Guardant Health; grants from Merck Sharp & Dohme (MSD); grants from PIQUR Therapeutics; grants from Puma Biotechnology; grants from Queen Mary University of London; and work with MedSIR outside the submitted work. Fabricio Racca and Luis F. García-Fernández declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• de Visser KE, Eichten A, LM C. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6:24–37 **This review provides an overview on the paradoxical role of adaptive and innate leukocytes as critical regulators of cancer progression and highlights new understanding that has been gained by manipulating immune responses in preclinical models**.
2. Gravitz L. Cancer immunotherapy. Nature. 2013;504(7480):S1. https://doi.org/10.1038/504S1a.
3. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565–70. https://doi.org/10.1126/science.1203486.
4. Sanmamed MF, Chen L. A paradigm shift in cancer immunotherapy: from enhancement to normalization. Cell. 2018;175(2):313–26. https://doi.org/10.1016/j.cell.2018.09.035.
5. The Nobel Prize in Physiology or Medicine 2018 https://www.nobelprize.org/prizes/medicine/2018/summary/. Accessed 8/9/2019.
6. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:3887–95.
7.• Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov. 2018;8:1069–

86 **This article reviews the current state of T cell costimulatory mechanisms and immune checkpoint blockade therapy, mainly of CTLA-4 and PD-1, from a basic biology and immunologic perspective for the cancer research community**.

8. Weintraub K. Drug development: releasing the brakes. Nature. 2013;504(7480):S6–8. https://doi.org/10.1038/504S6a.
9. Luen S, Virassamy B, Savas P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. Breast. 2016;29:241–50. https://doi.org/10.1016/j.breast.2016.07.015.
10. Polyak K. Heterogeneity in breast cancer. J Clin Invest. 2011;121(10):3786–8. https://doi.org/10.1172/jci60534.
11. Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet. 2011;378(9805):1812–23. https://doi.org/10.1016/s0140-6736(11)61539-0.
12. Budczies J, Bockmayr M, Denkert C, Klauschen F, Lennerz JK, Gyorffy B, et al. Classical pathology and mutational load of breast cancer—integration of two worlds. J Pathol Clin Res. 2015;1(4):225–38. https://doi.org/10.1002/cjp2.25.
13. Loi S. Tumor-infiltrating lymphocytes, breast cancer subtypes and therapeutic efficacy. Oncoimmunology. 2013;2(7):e24720. https://doi.org/10.4161/onci.24720.
14. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69–74.
15.• Esteva FJ, Hubbard-Lucey VM, Tang J, Pusztai L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. Lancet Oncol. 2019;20(3):e175–e86. https://doi.org/10.1016/s1470-2045(19)30026-9 **This review provides details of the rationale to evaluate novel combinations based on immunotherapeutic approaches in patients with metastatic breast cancer**.
16. FDA - U.S. Food and Drug Administration. FDA approves atezolizumab for PD-L1 positive unresectable locally advanced or metastatic triple-negative breast cancer 2019 2019. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-atezolizumab-pd-l1-positive-unresectable-locally-advanced-or-metastatic-triple-negative. Accessed 8/30/2019.
17. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018;379(22):2108–21. https://doi.org/10.1056/NEJMoa1809615.
18. Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G. Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol. 2013;23(6 Pt B):522–32. https://doi.org/10.1016/j.semcancer.2013.08.007.
19. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, et al. New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med. 2015;13:45. https://doi.org/10.1186/s12916-015-0278-7.
20. Chew V, Toh HC, Abastado JP. Immune microenvironment in tumor progression: characteristics and challenges for therapy. J Oncol. 2012;2012:608406. https://doi.org/10.1155/2012/608406.
21. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29(8):1093–102. https://doi.org/10.1038/onc.2009.416.
22.•• de Melo Gagliato D, Cortes J, Curigliano G, Loi S, Denkert C, Perez-Garcia J. Tumor-infiltrating lymphocytes in Breast Cancer and implications for clinical practice. Biochim Biophys Acta Rev Cancer. et al., 2017;1868(2):527–37. https://doi.org/10.1016/j.bbcan.2017.10.003 **Several trials have confirmed that tumor-infiltrating lymphocytes in the stroma are associated with favorable long-term outcome and increased chemosensitivity in breast cancer patients. This article summarizes data on the role of lymphocyte infiltration in breast cancer prognosis and response to therapy**.
23. Choritz H, Busche G, Kreipe H. Quality assessment of HER2 testing by monitoring of positivity rates. Virchows Arch. 2011;459(3):283–9. https://doi.org/10.1007/s00428-011-1132-8.
24. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235(4785):177–82.
25. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;244(4905):707–12.
26. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344(11):783–92. https://doi.org/10.1056/nejm200103153441101.
27. Aaltomaa S, Lipponen P, Eskelinen M, Kosma VM, Marin S, Alhava E, et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer. 1992;28a(4-5):859–64. https://doi.org/10.1016/0959-8049(92)90134-n.
28. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50. https://doi.org/10.1093/annonc/mdu112.
29. Kim S-R, Gavin PG, Pogue-Geile KL, Song N, Finnigan M, Bandos H, et al. Abstract 2837: a surrogate gene expression signature of tumor infiltrating lymphocytes (TILs) predicts degree of benefit from trastuzumab added to standard adjuvant chemotherapy in NSABP (NRG) trial B-31 for HER2+ breast cancer. Cancer Res. 2015;75(15 Supplement):2837. https://doi.org/10.1158/1538-7445.am2015-2837.
30. Perez EA, Thompson EA, Ballman KV, Anderson SK, Asmann YW, Kalari KR, et al. Genomic analysis reveals that immune function genes are strongly linked to clinical outcome in the North Central Cancer Treatment Group n9831 adjuvant trastuzumab trial. J Clin Oncol. 2015;33(7):701–8. https://doi.org/10.1200/jco.2014.57.6298.
31. Perez EA, Ballman KV, Tenner KS, Thompson EA, Badve SS, Bailey H, et al. Association of stromal tumor-infiltrating lymphocytes with recurrence-free survival in the N9831 adjuvant trial in patients with early-stage HER2-positive breast cancer. JAMA Oncol. 2016;2(1):56–64. https://doi.org/10.1001/jamaoncol.2015.3239.
32. von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med. 2017;377(2):122–31. https://doi.org/10.1056/NEJMoa1703643.
33. Krop IE, Paulson J, Campbell C, Kiermaier AC, Andre F, Fumagalli D, et al. Genomic correlates of response to adjuvant trastuzumab (H) and pertuzumab (P) in HER2+ breast cancer (BC): biomarker analysis of the APHINITY trial. J Clin Oncol. 2019;37(15_suppl):1012. https://doi.org/10.1200/JCO.2019.37.15_suppl.1012.
34. Salgado R, Denkert C, Campbell C, Savas P, Nuciforo P, Aura C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. JAMA Oncol. 2015;1(4):448–54. https://doi.org/10.1001/jamaoncol.2015.0830.
35. Dieci MV, Bisagni G, Cagossi K, Bottini A, Sarti S, Piacentini F, et al. Abstract PD1-1: tumor infiltrating lymphocytes and correlation with outcome in CHER-LOB study. Cancer Res. 2015;75(9 Supplement):PD1. https://doi.org/10.1158/1538-7445.SABCS14-PD1-1.

36. Dieci MV, Bisagni G, Cagossi K, Generali DG, Sarti S, Piacentini F, et al. Abstract P2-08-03. Survival analysis of the prospective randomized CHER-LOB study: correlation with tumor infiltrating lymphocytes. Cancer Res. 2016;76(4 Supplement):P2-08-3. https://doi.org/10.1158/1538-7445.SABCS15-P2-08-03.
37. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33(9):983–91. https://doi.org/10.1200/jco.2014.58.1967.
38. Bianchini G, Pusztai L, Pienkowski T, Im YH, Bianchi GV, Tseng LM, et al. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol. 2015;26(12):2429–36. https://doi.org/10.1093/annonc/mdv395.
39. Kawaguchi K, Suzuki E, Kataoka TR, Hirata M, Ohno S, Bando H, et al. Analysis of tumor infiltrating lymphocytes in HER2-positive primary breast cancer treated with neoadjuvant lapatinib and trastuzumab: the NeoLath study (JBCRG-16). J Clin Oncol. 2016;34(15 Supplement):599.
40. Colinas C, Ceppi M, Lambertini M, Scartozzi M, Garaud S, Fumagalli D, et al. Tumor infiltrating lymphocytes in HER2-positive breast cancer patients treated with neoadjuvant chemotherapy plus trastuzumab, lapatinib or their combination: a meta-analysis of published randomized clinical trials. Ann Oncol. 2017;28(Suppl_1):27P. https://doi.org/10.1093/an-nonc/mdx138.004.
41. Luen SJ, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol. 2017;18(1):52–62. https://doi.org/10.1016/s1470-2045(16)30631-3.
42. Liu S, Chen B, Burugu S, Leung S, Gao D, Virk S, et al. Role of cytotoxic tumor-infiltrating lymphocytes in predicting outcomes in metastatic HER2-positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol. 2017;3(11):e172085. https://doi.org/10.1001/jamaoncol.2017.2085.
43. Honkanen T, Moilanen T, Karihtala P, Tiainen S, Auvinen P, Vayrynen JP, et al. Prognostic and predictive role of spatially positioned tumor infiltrating lymphocytes in metastatic HER2 positive breast cancer treated with trastuzumab. Sci Rep. 2017;7:18027. https://doi.org/10.1038/s41598-017-18266-1.
44. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1):39–51.
45. Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Ann Oncol. 2007;18(6):977–84.
46. Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: recent advances and combination therapeutic approaches. Breast Cancer (Dove Med Press). 2019;11:53–69. https://doi.org/10.2147/bctt.s175360.
47. Gennari R, Menard S, Fagnoni F, Ponchio L, Scelsi M, Tagliabue E, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res. 2004;10(17):5650–5. https://doi.org/10.1158/1078-0432.ccr-04-0225.
48. Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Trastuzumab JB. (Herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res. 2001;61:4744–9.
49. Baselga J. J. A. Mechanism of action of anti-HER2 monoclonal antibodies. Ann Oncol Off J Eur Soc Med Oncol. 2001;12(Suppl 1):S35–41.
50. Spiridon CI, Guinn S, Vittetta ES. A comparison of the in vitro and in vivo activities of IgG and F(ab′) 2 fragments of a mixture of three monoclonal anti-Her-2 antibodies. Clin Cancer Res. 2004;10:3542–51.
51.•• Arnould L, Gelly M, Penault-Llorca F, Benoit L, Bonnetain F, Migeon C, et al. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer. 2006;94(2):259–67. https://doi.org/10.1038/sj.bjc.6602930 **This study supports an in vivo role for immune responses (antibody-dependent cell-mediated cytotoxicity) as a mechanism of action of trastuzumab**.
52. Varchetta S, Gibelli N, Oliviero B, Nardini E, Gennari R, Gatti G, et al. Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer overexpressing Her2. Cancer Res. 2007;67(24):11991–9. https://doi.org/10.1158/0008-5472.can-07-2068.
53. Gavin PG, Song N, Kim SR, Lipchik C, Johnson NL, Bandos H, et al. Association of polymorphisms in FCGR2A and FCGR3A with degree of trastuzumab benefit in the adjuvant treatment of ERBB2/HER2–positive breast cancer. JAMA Oncol. 2017;3:335.
54. Hurvitz SA, Betting DJ, Stern HM, Quinaux E, Stinson J, Seshagiri S, et al. Analysis of Fc receptor IIIa and IIa polymorphisms: lack of correlation with outcome in trastuzumab-treated breast cancer patients. Clin Cancer Res. 2012;18:3478–86.
55. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu–positive metastatic breast cancer. J Clin Oncol. 2008;26:1789–96.
56. Blackwell KL, Burstein HJ, Storniolo AM, Rugo HS, Sledge G, Aktan G, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. J Clin Oncol. 2012;30(21):2585–92. https://doi.org/10.1200/jco.2011.35.6725.
57. Scaltriti M, Verma C, Guzman M, Jimenez J, Parra JL, Pedersen K, et al. Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. Oncogene. 2009;28:803–14.
58. Maruyama T, Mimura K, Izawa S, Inoue A, Shiba S, Watanabe M, et al. Lapatinib enhances Herceptin-mediated antibody-dependent cellular cytotoxicity by up-regulation of cell surface HER2 expression. Anticancer Res. 2011;4v:2999–3005.
59. Malenfant SJ, Eckmann KR, Barnett CM. Pertuzumab: a new targeted therapy for HER2-positive metastatic breast cancer. Pharmacotherapy. 2014;34(1):60–71. https://doi.org/10.1002/phar.1338.
60. Yamashita-Kashima Y, Iijima S, Yorozu K, Furugaki K, Kurasawa M, Ohta M, et al. Pertuzumab in combination with trastuzumab shows significantly enhanced antitumor activity in her2-positive human gastric cancer xenograft models. Clin Cancer Res. 2011;17:5060–70.
61. Tóth G, Szőőr Á, Simon L, Yarden Y, Szöllősi J, Vereb G. The combination of trastuzumab and pertuzumab administered at approved doses may delay development of trastuzumab resistance by additively enhancing antibody-dependent cell-mediated cytotoxicity. MAbs. 2016;8:1361–70.
62. Mamidi S, Cinci M, Hasmann M, Fehring V, Kirschfink M. Lipoplex mediated silencing of membrane regulators (CD46, CD55 and CD59) enhances complement-dependent anti-tumor activity of trastuzumab and pertuzumab. Mol Oncol. 2013;7:580–94.
63. Scheuer W, Friess T, Burtscher H, Bossenmaier B, Endl J, Hassman M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. Cancer Res. 2009;69:9330–6.

64. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol. 2017;17(2):97–111. https://doi.org/10.1038/nri.2016.107.
65. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51–72. https://doi.org/10.1146/annurev-immunol-032712-100008.
66. Fuchs Y, Steller H. Live to die another way: modes of programmed cell death and the signals emanating from dying cells. Nat Rev Mol Cell Biol. 2015;16:329–44.
67. van Kempen TS, Wenink MH, Leijten EF, Radstake TR, Boes M. Perception of self: distinguishing autoimmunity from autoinflammation. Nat Rev Rheumatol. 2015;11:483–92.
68. Knutson KL, Clynes R, Shreeder B, Yeramian P, Kemp KP, Ballman K, et al. Improved survival of HER2+ breast cancer patients treated with trastuzumab and chemotherapy is associated with host antibody immunity against the HER2 intracellular domain. Cancer Res. 2016;76:3702e10.
69. Loibl S, de la Pena L, Nekljudova V, Zardavas D, Michiels S, Denkert C, et al. Neoadjuvant buparlisib plus trastuzumab and paclitaxel for women with HER2+ primary breast cancer: a randomised, double-blind, placebo-controlled phase II trial (NeoPHOEBE). Eur J Cancer. 2017;85:133–45. https://doi.org/10.1016/j.ejca.2017.08.020.
70. von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N Engl J Med. 2019;380:617–28.
71. Amiri-Kordestani L, Blumenthal GM, Xu QC, Zhang L, Tang SW, Ha L, et al. FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. Clin Cancer Res. 2014;20(17):4436–41.
72. Martin K, Müller P, Schreiner J, Prince SS, Lardinois D, Heinzelmann-Schwarz VA, et al. The microtubule-depolymerizing agent ansamitocin P3 programs dendritic cells toward enhanced anti-tumor immunity. Cancer Immunol Immunother. 2014;63:925–38.
73. Müller P, Martin K, Theurich S, Schreiner J, Savic S, Terszowski G, et al. Microtubule-depolymerizing agents used in antibody-drug conjugates induce antitumor immunity by stimulation of dendritic cells. Cancer Immunol Res. 2014;2:741–55.
74. Muller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, et al. Trastuzumab emtansine (T-DM1) renders HER2+ breast cancer highly susceptible to CTLA-4/PD-1 blockade. Sci Transl Med. 2015;7(315):315ra188. https://doi.org/10.1126/scitranslmed.aac4925.
75. Loi S, Giobbie-Hurder A, Gombos A, Bachelot T, Hui R, Curigliano G, et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b-2 trial. Lancet Oncol. 2019;20(3):371–82. https://doi.org/10.1016/s1470-2045(18)30812-x.
76. Chia SKL, Bedard PL, Hilton J, et al. A phase 1 study of a PD-L1 antibody (durvalumab) in combination with trastuzumab in HER-2 positive metastatic breast cancer (MBC) progressing on prior anti HER-2 therapies (CCTGIND.229/NCT02649686). 1029 (abstr). Proc Am Soc Clin Oncol. 2018;36(suppl):1029.
77. Emens L, Esteva FJ, Beresford MJ, et al., editors. Results from KATE2, a randomized phase 2 study of atezolizumab (atezo)+ trastuzumab emtansine (T-DM1) vs placebo (pbo)+T-DM1 in previously treated HER2+ advanced breast cancer. PD3–01 (abstr). AACR - San Antonio Breast Cancer Symposium; 2018; San Antonio, TX.
78. Nordstrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcgamma receptor binding properties. Breast Cancer Res. 2011;13:R123.
79. Bang YJ, Giaccone G, Im SA, Oh DY, Bauer TM, Nordstrom JL, et al. First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. Ann Oncol. 2017;28(4):855–61. https://doi.org/10.1093/annonc/mdx002.
80. Rugo HS, Im SA, Shaw Wright G, Escriva-de-Romani S, DeLaurentis M, Cortes J, et al., editors. SOPHIA primary analysis: a phase 3 study of margetuximab + chemotherapy versus trastuzumab + chemotherapy in patients with HER2+ metastatic breast cancer after prior anti-HER2 therapies. Abstract 1000. ASCO Annual Meeting; 2019; Chicago, IL.
81. Geuijen C, Rovers E, Nijhuis R, den Blanken-Smit R, Visser T, Bartelink W, et al. Preclinical activity of MCLA-128, and ADCC enhanced bispecific IgG1 antibody targeting the HER2:HER3 heterodimer. J Clin Oncol. 2014;32(15(suppl)):560.
82. Meric-Bernstam F, Beeram M, Mayordomo I, et al. Single agent activity of ZW25, a HER2-targeted bispecific antibody, in heavily pretreated HER2-expressing cancers [abstract]. J Clin Oncol. 2018;36(Suppl):Abstract 2500.
83.• Basu A, Ramamoorthi G, Jia Y, Faughn J, Wiener D, Awshah S, et al. Immunotherapy in breast cancer: current status and future directions. Adv Cancer Res. 2019;143:295–349. https://doi.org/10.1016/bs.acr.2019.03.006 **This article discusses the challenges of breast cancer immunotherapy and future directions for potential ways of improving responses to immunotherapy in breast cancer**.
84. Oiseth S, Aziz M. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat. 2017;3:250–61.
85. Du YJ, Lin ZM, Zhao YH, Feng XP, Wang CQ, Wang G, et al. Stability of the recombinant anti-erbB2 scFv-Fc-interleukin-2 fusion protein and its inhibition of HEr2-overexpressing tumor cells. Int J Oncol. 2013;42(3):507–16. https://doi.org/10.3892/ijo.2012.1747.
86. Nocera NF, Lee MC, De La Cruz LM, Rosemblit C, Czerniecki BJ. Restoring lost anti-HER-2 Th1 immunity in breast cancer: a crucial role for Th1 cytokines in therapy and prevention. Front Pharmacol. 2016;7:356. https://doi.org/10.3389/fphar.2016.00356.
87. Radford KJ, Tullett KM, Lahoud MH. Dendritic cells and cancer immunotherapy. Curr Opin Immunol. 2014;27:26–32. https://doi.org/10.1016/j.coi.2014.01.005.
88. Levine BL, Miskin J, Wonnacott K, Keir C. Global manufacturing of CAR T cell therapy. Mol Ther Methods Clin Dev. 2017;4:92–101. https://doi.org/10.1016/j.omtm.2016.12.006.