Clinical development of immunotherapies for HER2+ breast cancer: a review of HER2-directed monoclonal antibodies and beyond

Ricardo L. B. Costa and Brian J. Czerniecki

INTRODUCTION

The clinical classification of breast cancer is based on the presence of transmembrane receptors, namely estrogen and progesterone, along with the amplification or overexpression of the human epidermal growth factor receptor 2 (HER2) protein/oncogene. HER2 is a tumor-associated antigen (TAA) that is overexpressed or amplified in ~25% of patients with breast cancer and correlates with poor clinical outcomes if not appropriately treated with HER2-targeted therapies.

Passive immunotherapy with HER2-directed monoclonal antibodies (mAbs), such as trastuzumab and pertuzumab, in combination with chemotherapy has led to an improvement in clinical outcomes of patients with HER2-positive (HER2+) metastatic breast cancer (MBC), as these agents have been shown to improve median overall survival (OS) to as much as 57 months. These improvements have been largely credited to the direct targeting of HER2 by mAbs, which leads to the downregulation of oncogenic intracellular pathways being triggered by HER2 activation through homo- and hetero-dimerization in the cancer cell membrane.

Notwithstanding the success of HER2-targeted treatments, the challenge to treat patients with HER2+ breast cancer represents an evolving field that should take into account interactions between different components of the immune system. As HER2 is a TAA, it can be targeted by a wide array of treatment strategies. To be effective, these treatments should ultimately lead to cytotoxicity by stimulating type 1 immunity (Th1). In this context, HER2-adaptive immune response is supported by both CD4+ and CD8+ T cells that secrete Th1 cytokines.

Thus far, early phase clinical trials of new immune agents for the treatment of patients with HER2+ breast cancer have shown modest results. The phase 1b/2 KEYNOTE-014/PANACEA trial assessed the preliminary safety and efficacy of an anti-PD-1 mAb (pembrolizumab) combined with trastuzumab for the treatment of patients with HER2+ progressive MBC. Only 15% of the enrolled patients who showed PD-L1+ tumors (assessed in both cancer and immune cells) achieved a partial response, and there was no evidence of tumor response among patients in the PD-L1− cohort. These results suggest that the development of immunotherapies for HER2+ breast cancer will need to take into account more complex interactions between different components of the immune system and other treatment modalities.

Remarkably, there is mounting preliminary evidence showing that the antitumor activity of HER2-directed mAbs can also be attributed to the broad activation of the immune system, including both its adaptive and innate branches. For instance, FcγR-mediated activation of immune cells other than cytotoxic CD8+ T cells is necessary for antitumor activity. In addition, the activation of CD4+ Th1 response against HER2 by HER2-primed dendritic cell (DC) vaccines has led to breast cancer tumor regression in an early phase clinical trial. Other treatments leading to immune-mediated cytotoxicity via other passive (e.g., adoptive T-cell transfer and cytokine administration) or active (e.g., HER2-directed vaccines) mechanisms are under development. This narrative review aims to summarize the discoveries involving both passive and active immune therapies for the treatment of HER2+ breast cancer and highlight rationales for novel development therapeutic approaches.
HER2-DIRECTED MABS

Both trastuzumab and pertuzumab are HER2-directed mAbs. Trastuzumab is an IgG1 recombinant humanized mAb against the extracellular domain of HER2 (p185) and was developed to abrogate HER2 transmembrane domain activity. Pertuzumab binds to a different epitope of the HER2 dimerization domain than trastuzumab, preventing interactions with other receptors in the HER2 family that lead to cell growth inhibition. The antitumor efficacy of HER2-directed mAbs has been largely attributed to their direct inhibitory action on the extracellular domain of HER2. Relevant interactions between HER2-directed mAbs and the immune system, which lead to antibody-dependent cellular cytotoxicity, have been observed.

Albeit lacking clinical utility in routine clinical practice, the presence of polymorphisms in the immunoglobulin G fragment C receptors (FcγR) present in cytotoxic cells (e.g., natural-killer [NK] lymphocytes, macrophages, and neutrophils) are associated with increased trastuzumab-mediated antitumor activity. FcγRIIlα and FcγRIllα are activating receptors present in these immune cells activated by the Fc portion of trastuzumab. HER2 mice models lacking common γ-chain receptors exhibit significantly less tumor mass reduction when treated with trastuzumab compared to mice models with preserved FcγRs.

In a study including 54 consecutive patients with HER2 MBC who were treated with trastuzumab combined with taxane, it was found that the FcγRIllα-158 V/V genotype (20%) was significantly correlated with an increased objective response rate (ORR) when compared with either 158 V/F (48%) or F/F (31%) genotypes (ORR, 82% vs. 42% vs. 35%, respectively; \(P = 0.03\)). Median progression-free survival (PFS) was not reached among patients with the 158 V/V genotype. Finally, there was a trend toward statistical significance in regards to ORR and PFS for the FcγRIllα-131 H/H genotype.

Significant correlations between improved complete pathological response rates and the FcγRIllα-158 V/V genotype have also been reported among patients receiving neoadjuvant HER2-targeted treatment.

In light of these findings, developmental therapeutics have gained momentum in fostering the clinical development of novel HER2-targeted mAbs. Margetuximab (MGAH22) is a chimeric anti-HER2 mAb with an Fc domain modified for improved binding to FcγRIIa and lower affinity to its inhibitory FcγRIIB counterpart. Antibody-dependent cytotoxicity assays showed enhanced activity of margetuximab against HER2 cancer cells when compared with trastuzumab surrogates in donors with a low-affinity variant of FcγRIIa.

The safety of margetuximab was assessed in a first-in-human phase 1 clinical trial that included 66 patients with metastatic progression of HER2 solid tumors, 27 of whom had MBC. Patients were treated with intravenous infusions of margetuximab at doses of 0.1–6.0 mg/kg for 3 out of every 4 weeks (Regimen A) or once every 3 weeks (Regimen B). All 66 patients were evaluated for safety. Infusion-related reaction and fatigue were the most commonly reported adverse events (AEs), attributed to margetuximab (18% and 14% of patients, respectively). Tumor reduction was observed in 11 out of 28 patients with breast cancer. All study participants had been previously treated with at least one HER2-targeted therapy. Given its favorable toxicity and tolerability profiles, margetuximab is under development in a dose-regimen of 15 mg/kg given every 3 weeks.

The clinical efficacy of margetuximab is being assessed in a phase 3 trial (SOPHIA trial, NCT02492711) comparing margetuximab combined with chemotherapy with trastuzumab plus chemotherapy. This trial includes patients with HER2 MBC who progressed following HER2-targeted therapy. Preliminary results based on 536 patients with HER2 MBC were presented. Patients were randomized 1:1 to chemotherapy (i.e., choice of standard dose of capecitabine, eribulin, gemcitabine, or vinorelbine) with margetuximab or trastuzumab at standard doses. Margetuximab led to only a modest improvement in PFS over trastuzumab (median of 6 months vs 5 months; hazard ratio [HR], 0.76; 95% CI, 0.59–0.98; \(P = 0.033\)). Clinical outcomes were improved in FcγRIllα 158F allele carriers (median PFS, 7 months vs. 5 months; HR, 0.68; 95% CI, 0.52–0.90; \(P = 0.005\)).

Another way to potentially enhance the antitumor efficacy of HER2-targeted mAbs is through the modification of the HER2 single-chain variable fragment (scFv) domain, which binds a target protein other than HER2. Greene et al. reported the results of a breast cancer xenograft model, demonstrating the antitumor efficacy of a HER2 scFv and an IFN-γ engineered protein. This new compound showed increased antitumor activity when
compared to the 4D5 HER2-directed antibody. Clinical development of this agent is awaited.

HER2-DIRECTED ANTIBODY DRUG CONJUGATES (ADCs)

ADCs are being developed under the premise of producing increased cytotoxicity with a concomitant reduction in chemotherapy off-target AEs. ADCs are molecules composed of an antibody linked to a chemotherapy payload. These linkers can be classified into two main groups, cleavable and noncleavable, on the basis of the stability of their bonds with the two molecules. Noncleavable linkers require intracellular degradation before drug release and activity.22,33,34 Aside from the obvious mechanisms of action inherent to the activity of the antibody and chemotherapy choices, ADCs are an attractive treatment modality for HER2+ breast cancer due to possible bystander effects.34 As much as 15% of the HER2+ breast tumors present intratumoral HER2-expression heterogeneity and permeable cytotoxic payloads can exert cytotoxic effects to neighboring cells.35 Nonetheless, the drug-antibody ratio should also be considered when developing ADCs. A higher payload per unit of antibody would lead to increased antitumor efficacy but also to greater toxicity. For example, a recently approved HER2 ADC (DS8201a) showed significant risk of serious AEs and treatment-related deaths in early phase clinical trials.36

T-DM1 is an ADC that is formed by the bonding of trastuzumab to a potent microtubule inhibitor emtansine (DM1)37. T-DM1 is an FDA-approved ADC for the treatment of HER2+ breast cancer. These two molecules are linked by noncleavable linkers (i.e., two disulfides) and have an average drug-antibody ratio of 3.5.31 In a seminal phase 1 trial published in 2010, the safety and tolerability of T-DM1 was firmly established, as patients treated at the maximum tolerated dose (3.6 mg/kg every 3 weeks) level had a very low risk of grade 3/4 adverse events (AEs).38,39 From the study’s primary endpoint showed a clinically significant overall response rate of 60.9%. Notwithstanding the evidence of significant antitumor efficacy, treatment-associated grade 3/4 AEs and two deaths were observed. The two deaths were secondary to ILD. It is also noticeable that a total 25 patients (13.6%) had ILD, with a median time to ILD onset of 193 days (range of 42–535). Furthermore, grade 3 neutropenia was observed in 20.7% of patients, which further suggests that patients should be monitored closely while on treatment. Currently, DS-8201a is being developed in two phase 3 confirmatory clinical trials for the treatment of patients with progressive unresectable or metastatic HER2+ breast cancer (DESTINY-Breast02 [NCT03523585] and DESTINY-Breast03 [NCT03529110], respectively). Furthermore, DS-8201a is under development in the DESTINY-Breast04 (NCT03734029), a phase 3 trial assessing this agent’s efficacy in treating patients with HER2-low breast cancer (i.e., IHC 1+ and IHC 2+/ISH- HER2-expression tumors).

To replicate the successful development of T-DM1 and DS8201a, other ADCs are currently under development for the treatment of HER2+ MBC. SYD985 is a cleavable ADC composed of a duocarmycin, a potent DNA-alkylating agent with two moieties (DNA-alkylating and DNA-binding) that bind into the minor groove of DNA.39 The in vitro antitumor activity of SYD985 and T-DM1 was assessed using HER2-overexpressing cell lines (IHC3+). SK-BR-3 and UACC-893, with each cell line showing similar inhibitory potencies (IC50 6.9 and 15.7 ng/mL in SK-BR-3 and 54.1 and 35.9 ng/mL in UACC-893 for SYD985 and T-DM1, respectively)39. The in vivo activity of SYD985 was tested in cell line-derived xenograft models and in a breast cancer patient-derived xenograft model (PDX) with different HER2-expression statuses. In BT-474 and MAXF162 HER2 IHC3+ models, SYD985 showed significantly more antitumor activity than T-DM1. Remarkably, SYD985 was 3- to 50-fold more cytotoxic than T-DM1 in low HER2-expressing (2þ/1þ) cell lines. An open-label, randomized clinical trial comparing the efficacy of SYD985 with the physician’s treatment of choice for the salvage treatment of patients with HER2+ locally advanced or MBC is ongoing (NCT03362935).

XMT-1522 is another HER2 ADC with nanomolar potency, which is composed of a HER2 antibody (HT-19) and a dolaflexin platform; the latter is able to conjugate auristatin drug payload at a ratio of ~1–13.47 In low HER2 mouse xenograft models, XMT-1522 was found to lead to complete tumor regression, which was not observed with T-DM1 treatment.48 A phase 1 multiple-histology trial of XMT-1522 for the treatment of patients with HER2 IHC3+ progressive MBC is ongoing (NCT02952729). Preliminary showed no dose-limiting toxicities across any of the 6 planned dose levels and no treatment-related serious AEs. A total of 18 patients were evaluable for efficacy. One partial response was observed at the first restaging scans in a patient with HER2+ MBC who was previously treated with T-DM1. Two cases of stable disease were seen in HER2+ BC patients (with a duration of disease stability of 13+ and 12+ weeks)39.

The ADC ARX788 is a dolastatin analog (MMAF) that is coupled via a noncleavable linker to the HER2 IgG, with a mean drug-
antibody ratio of 1.9, and has shown activity against HER2+ ovarian, gastric, and breast cancer cell lines. Furthermore, ARX-788 was shown to induce regression in a trastuzumab-resistant–derived breast xenograft (JIMT-1) model and was found to be significantly more effective than T-DM1 at equivalent doses. ARX788 is in its early stages of development in a multiple-histology phase 1 clinical trial. Only patients with HER2+ MBC are included, and all patients must have received prior treatment with trastuzumab (NCT03255070).

ADCT-502 is another ADC that is directed against human HER2 and is site-specifically conjugated to the highly cytotoxic pyrrolobenzodiazepine-based linker-drug tesirine. The in vivo antitumor activity of ADCT-502 was compared to T-DM1 in both cell line-derived and PDXs. For example, in a HER2+ fluorescent in situ hybridization-negative (FISH-) breast cancer PDX, ADCT-502 had increased dose-dependent antitumor activity when compared to T-DM1. Notwithstanding the favorable preclinical toxicity profile of this novel agent, a phase 1 clinical trial of ADCT-502 in patients with HER2+ expression was terminated due to safety concerns (NCT03125200). The low drug-antibody ratio of 1.7 compared to other ADCs could lead to increased risk of off-target toxicity.

Another treatment strategy being developed is the use of biparatopic antibodies (BpAbs), which have the capacity to bind two different nonoverlapping epitopes on the antigen, leading to increased ADC internalization and cytotoxicity in a broader range of HER2-expressing cancer cells. MEDI4276 is a novel compound composed of variable domain sequences of 39S (IG1 human HER2 mAb) and trastuzumab. MEDI4276 contains four antigen-binding units that target two different HER2 epitopes. This chimeric immunoglobulin is able to block HER2-HER3 interactions, as it blocks both ligand-independent and ligand-dependent HER2 activation. AZ13599185 is a tubulysin variant that inhibits microtubule polymerization during mitosis to induce cell death. MEDI4276 is an ADC produced from the conjugation of this aforementioned novel HER2 antibody with AZ13599185 via a noncleavable linker (i.e., maleimidocaproyl) to the HER2 antibody. Preclinical results are so far promising, with MEDI4276 showing activity in trastuzumab-resistant HER2+ breast cancer cells. The results of a phase 1/2 clinical trial of MEDI4276 for the treatment of patients with progressive HER2+ metastatic solid tumors are forthcoming (NCT02576548).

Moving forward, a possible differential toxicity profile of novel HER2 ADC should also be considered, as the toxicity of novel agents could be mediated by interactions with immune cells through the FcγR. For example, Uppal et al. suggested that T-DM1 inhibits megakaryocyte differentiation through DM1 intracellular accumulation in an FcγRllα-dependent manner. In addition, in an early phase clinical trial, thrombocytopenia was the dose-limiting toxicity of a bispecific mAb targeting HER2 and FcγRIII (2B1).66

Immune Checkpoint Inhibitors

In May 2019, based on results of a phase 3 trial (IMPRESSion 130), the FDA approved the use of atezolizumab in combination with paclitaxel protein-bound for the treatment of patients with PD-L1+ (ie, ≥1% expression by tumor immune cells) triple-negative breast cancer (TNBC). Among patients with PD-L1+ tumors, atezolizumab improved median PFS to 8 months compared with 5 months among patients treated with chemotherapy alone (P = 0.001). In the first interim analysis, the PD-L1+ exploratory analyses also showed an increase in median OS from 15.5 to 25 months, favoring treatment with atezolizumab. Updated OS results have been reported (data cutoff 1 January 2019), showing a median OS of 18 months compared with 25 months, favoring the study arm. Collectively, these results suggest possible late antitumor efficacy. The clinical efficacy of immune checkpoint inhibitors for the treatment of patients with HER2+ breast cancer remains to be determined.

HER2+breast cancer cells use the PD-1/PD-L1 checkpoint axis to evade cytotoxicity by immune cells. Notably, ADCT-502 is another ADC that is directed against human HER2 and is site-specifically conjugated to the highly cytotoxic pyrrolobenzodiazepine-based linker-drug tesirine. The in vivo antitumor activity of ADCT-502 was compared to T-DM1 in both cell line-derived and PDXs. For example, in a HER2+ fluorescent in situ hybridization-negative (FISH-) breast cancer PDX, ADCT-502 had increased dose-dependent antitumor activity when compared to T-DM1. Notwithstanding the favorable preclinical toxicity profile of this novel agent, a phase 1 clinical trial of ADCT-502 in patients with HER2+ expression was terminated due to safety concerns (NCT03125200). The low drug-antibody ratio of 1.7 compared to other ADCs could lead to increased risk of off-target toxicity.

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It is possible that the combination of two checkpoint inhibitors might achieve greater synergistic effect (ie, improved efficacy with no additional toxicity) than either monotherapy. However, the results of an early clinical trial of the combination of ADCT-502 and durvalumab showed no increase in antitumor efficacy as compared to durvalumab alone (NCT03125928, NCT03726879).

Interestingly, Czerniecki et al. showed anti-HER2 CD4+ Th1 immunity to be a relevant component of HER2+ therapy, as loss of CD4+ Th1 responses correlated with poor prognosis and treatment responses. The administration of a class II HER2 peptide-pulsed Type I polarized DC1 vaccine was shown to induce a strong anti-HER2 CD4+ Th1 response, with a pathologic complete response rate (pCR) among HER2+ ductal carcinoma in situ (DCIS) patients. Notably, patients with non-small cell lung cancer with increased circulating dysfunctional CD4+ immunity (i.e., a baseline profile showing a low percentage of CD4+-differentiated T cells) had no objective response to PD-1/PD-L1 blockade therapy in an observational study. Receiver operating curve analyses showed a cut-off value of >40% to identify objective responders with 100% specificity (P < 0.0003). Supporting this notion, preclinical data showed that the activation of anti-HER2 CD4+ Th1 immunity
in TUBO mice models prior to immune checkpoint blockade leads to improved outcomes73.

**CYTOKINE-ACTIVATED MEDIATION**

Passive activation of the cytotoxic branch of the immune system can be accomplished through the administration of Th1 cytokines. IFN-γ is known to stimulate both CD8+ and CD4+ Th1 response31,74. The combination of IFN-γ and an anti-HER2 antibody synergistically reduces tumor growth in HER2-expressing tumors 75. Preliminary results of a phase 1 dose-escalation clinical trial showed that IFN-γ was tolerable for the treatment of patients with HER2+ MBC when combined with paclitaxel administered weekly and HER2-targeted agents, such as pertuzumab and trastuzumab76. Efficacy results from the dose-expansion cohort are forthcoming.

IL-12 is also known to increase IFN-γ levels in mice harboring HER2+ breast cancer cells77. IL-12 in combination with a HER2-mAb (4D5) showed antitumor activity through the activation of NK cells78. In a phase 1 trial, IL-12 was administered twice a week in combination with weekly intravenous infusions of trastuzumab to 15 patients with trastuzumab-naive MBC who either had IHC 2+ or 3+ HER279. Fatigue and nausea were the most common all-grade AEs. One patient achieved a complete clinical response. The favorable toxicity profile of IL-12 when administered in combination with trastuzumab and paclitaxel was also observed in a small multiple-histology phase 1 trial80. These trials not only showed the favorable safety profile of IL-12 treatment combinations; they also showed that IL-12 can lead to increased IFN-γ production.

IL-2 has also been shown to increase IFN-γ production through the activation of NK cells in vivo81. As a proof of concept, in a phase 1 trial including 10 patients with HER2-expressing MBC (IHC 2+ and 3+), IL-2 showed an expansion of NK cells when combined

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**Table 1. HER2-directed immunotherapy trials under development.**

| Compound                  | Classification | Study phase | Patient population | HER2 1-2+, non-amplified allowed, y/n | Lines of therapy, no. | Study population (n), no. | NCT ID       |
|---------------------------|----------------|-------------|--------------------|--------------------------------------|------------------------|---------------------------|--------------|
| HER2-directed mab         |                |             |                    |                                      |                        |                           |              |
| MCLA-128                  | HER2/HER2 mAb  | 2           | HER2+ LAD or MBC   | No                                   | >1                     | 120                       | 03321981     |
| GBR 1302                  | CD3 bispecific mAb | 1/2        | HER2+ LAD or MBC   | No                                   | >1                     | 158                       | 03983395     |
| HER2-directed ADC         |                |             |                    |                                      |                        |                           |              |
| RC48                      | HER2 ADC       | 1b/2        | HER2+ LAD or MBC   | No                                   | >1                     | 165                       | 03052634     |
| DS-8201a                  | HER2 ADC       | 3           | HER2+ LAD or MBC   | No                                   | >1                     | 600                       | 03523585     |
| DS-8201a                  | HER2 ADC       | 3           | HER2+ LAD or MBC   | No                                   | >1                     | 500                       | 03529110     |
| RC48                      | HER2 ADC       | 1b/2        | HER2+ LAD or MBC   | No                                   | >1                     | 165                       | 03052634     |
| FS-1502                   | HER2 ADC       | 1           | HER2+ LAD or MBC   | Yes                                  | >1                     | 92                        | 03944499     |
| SYD985                    | HER2 ADC       | 2           | HER2+ LAD or MBC   | No                                   | >2                     | 345                       | 03262935     |
| ARX788                    | HER2 ADC       | 1           | HER2+ LAD or MBC   | Yes                                  | >1                     | 60                        | 03255070     |
| PD-1/PO-D1 immune checkpoint inhibitors |            |             |                    |                                      |                        |                           |              |
| Pembrolizumab             | PD-1 mAb       | 1b          | HER2+ LAD or MBC   | No                                   | >1                     | 27                        | 03032107     |
| Pembrolizumab             | PD-1 mAb       | 2           | HER2+ stage I-III  | No                                   | 0                      | 174                       | 03747120     |
| Atezolizumab              | PD-L1 mAb      | 1b          | HER2+ LAD or MBC   | Yes                                  | >1                     | 98                        | 02605915     |
| Atezolizumab              | PD-L1 mAb      | 3           | HER2+ LAD or MBC   | No                                   | >1                     | 6000                      | 03199885     |
| Atezolizumab              | PD-L1 mAb      | 2a          | HER2+ LAD or MBC   | No                                   | 0                      | 50                        | 03125928     |
| Atezolizumab              | PD-L1 mAb      | 3           | HER2+ stage I-III  | No                                   | 0                      | 453                       | 03726879     |
| Atezolizumab              | PD-L1 mAb      | 2           | HER2+ MBC with CNS involvement | No | - | 33 | 03417544 |
| KN035                     | PD-L1 mAb      | 2           | HER2+ LAD or MBC   | NR                                   | >1                     | 59                        | 04034823     |
| Cytokine directed therapies |                |             |                    |                                      |                        |                           |              |
| IFN-γ                     | Th1 cytokine   | 2           | HER2+ stage I-III  | No                                   | N/A                    | 43                        | 03112590     |
| Tocilizumab               | IL-6 receptor inhibitor mAb | 1           | HER2+ LAD or MBC   | No                                   | >1                     | 20                        | 03135171     |
| Utomilumab                | Receptor co-stimulatory of TNF mAb | 1b       | HER2+ LAD or MBC   | No                                   | >1                     | 79                        | 03364348     |
| Utomilumab                | Receptor co-stimulatory of TNF mAb | 2           | HER2+ LAD or MBC   | No                                   | >1                     | 100                       | 03414658     |
| CAR-T-cell                | Anti-HER2 CAR T-cell NOS | 1/2        | HER2+ LAD or MBC   | NR                                   | >1                     | 60                        | 02713984     |

Trials listed at www.clinicaltrials.gov as of 22 August 2019.

ADC antibody-drug conjugate, CAR-T Chimeric antigen receptor T-cell, CNS central nervous system, LAD locally advanced disease, mAb monoclonal antibody, MBC metastatic breast cancer, NCT National Clinical Trial, NOS not otherwise specified, NR no response, TNF tissue necrosis factor.
with trastuzumab and was well tolerated; these results support further development of this treatment combination\(^6\). IL-2 in combination with trastuzumab was tested in a Simon 2-stage clinical trial for the treatment of patients with pretreated HER2\(^+\) MBC\(^8\). A ≥2-fold increase in the peripheral level of IFN-\(\gamma\) was detected in 8 out of the 13 patients treated, but no NK cell expansion was observed. No responses were observed, and 12 patients had disease progression with a median time to progression of 51 days (range, 29–326 days).

HER2-DIRECTED VACCINES

Active immunotherapy with HER2-directed vaccines is compelling, warranting further development for several reasons. Among these reasons is wider stimulation of the immune system, including its innate branch. Another reason is the potential for epitope spreading with the activation of immune response against other antigens and HER2 epitopes.

HER2 vaccination is currently under development using a number of different strategies, such as peptide-, protein-, DNA-, and whole cell- or cell lysate-based vaccines. Thus far, HER2 peptide (i.e., E75) and antigen-presenting cell vaccines (i.e., HER2-dendritic cells) are in more advanced stages of clinical development. A full review of the clinical and preclinical data supporting the development of the wide array of HER2 vaccine strategies is outside the scope of this review and has been reviewed elsewhere\(^6\).

E75 VACCINE

The E75 vaccine (i.e., E75 peptide [HER2 369-377]) is a 9-amino acid human leukocyte antigen- (HLA-) restricted peptide located in the HER2 extracellular domain. The E75 vaccine boosts immune response by activating CD8\(^+\) and CD4\(^+\) Th1 responses\(^84-88\). This vaccine has the limitation of HLA class I restriction, which decreases its ability to more broadly activate the immune system and limits the patient population in which it can be used.

Clinical studies on the safety and efficacy of E75 vaccines have been rationally developed in the adjuvant setting, which is a less immune-tolerate environment than the metastatic setting.

In these trials\(^89-91\), 108 women with high-risk HER2-expressing breast cancer were treated with E75\(^92\). After a prespecified follow-up time of 60 months, the disease-free survival rate for vaccinated women was 89.7% compared to 80.2% in the control group (\(P = 0.08\)). More recently, when compared with placebo, E75 vaccination failed to improve the disease-free survival of patients with HER2-expressing, high-risk breast cancer in a randomized phase 3 trial, leading to the conclusion that synergistic combinations may be needed\(^93\).

HER2-dendritic cell vaccines

DCs are part of the antigen-presenting machinery, which costimulates both CD8\(^+\) and CD4\(^+\) T cells into Th1 responses against HER2\(^94\). In vivo models showed that HER2-pulsed DC vaccination boosts anti-HER2 Th1 immunity\(^95\). Autologous DC vaccines pulsed with both class I and II HER2 peptides (Fig. 2) have been more extensively studied in patients with HER2\(^+\) DCIS of the breast\(^96,97\). In an early phase clinical trial, a total of 54 patients with either HER2\(^+\) DCIS or invasive breast cancer received 6 weekly intratumoral and/or intranodal injections of DC1 vaccines pulsed ex vivo with 6 distinct MHC class II HER2\(^+\) peptides. Treatment was well tolerated and all patients completed the planned treatment. It is remarkable that as much as 81% of the patients showed peripheral blood activation of CD4\(^+\) and CD8\(^+\) Th1 response. Furthermore, pCRs were observed (DCIS, 28.6%; invasive breast cancer, 8.3%). This proof-of-concept trial showed that active stimulation of the adaptive immune system can lead to antitumor activity in HER2\(^+\) breast cancer. The efficacy of HER2-pulsed DC vaccines is being compared with WOKVAC vaccines (DNA Plasmid, which encodes for epitopes for HER2, IGFBP2, and IGF-1R) in a randomized phase 2 trial of patients with stages I-III HER2\(^+\) breast cancer (NCT03384914).

ADOPTIVE T-CELL THERAPIES

Several immunotherapy strategies are classified as being adoptive T-cell therapies. These strategies include the genetic modification of T-cell receptors via gene transfer technology that are able to recognize MHC I antigens with high affinity; the creation of chimeric antigen receptor T cells (CARs) by fusing a costimulatory specific antibody protein to the endogenous T-cell receptor; the infusion of ex vivo expanded tumor-infiltrating lymphocytes; and the infusion of peripheral ex vivo tumor antigen-primed and tumor antigen-expanded T cells. These modalities have been more extensively studied in patients with hematologic malignancies.
and these studies have shown encouraging results for the treatment of subsets of patients with aggressive malignancies, such as acute lymphoblastic leukemia98,99. Currently, there are two FDA-approved CD19-directed CAR T-cell therapies: tisagenlecleucel and axicabtagene ciloleucel which are indicated for the treatment of (i) acute lymphoblastic leukemia and diffuse large B-cell lymphoma and (ii) relapsed or refractory B-cell non-Hodgkin lymphoma, respectively.

T cells against HER2 can be successfully expanded ex vivo in mice models and have shown evidence of antitumor activity100,101. One patient with HER2+ (Dako 3+) MBC was treated with autologous HER2+99,377 T cells that were cocultured with HLA2-peptide-loaded DCs in a pilot trial102. There was evidence of tumor cell disappearance in the bone marrow but no penetration of T cells into the tumor.

HER2 CAR T cells containing CD28 costimulatory domain were administered to the central nervous system (CNS) of mice showing regression of HER2+ MBC in the CNS102. Clinical data of adoptive T-cell strategies are lacking for patients with HER2+ MBC, likely as a function of increased cost, needs for specialized centers for treatment development, and concerns related to off-target AEs secondary to the broad stimulation of the immune system against non-tumor specific antigens. Indeed, serious AEs have been reported with regard to HER2-directed CAR T-cell treatment as a function of acute cytokine release103,104.

**IMMUNOTHERAPY-BASED COMBINATION STRATEGIES**

As the complexities of interactions between different components of the immune system become known, other targets have started to emerge. For example, 4-1BB is a costimulatory receptor tissue necrosis factor (TNF) observed on CD8+ CD4+ and NK cells, which, when activated, leads to immune cell proliferation104,105. Utomilumab, a 4-1BB receptor IgG2 mAb agonist, is currently being developed in combination with trastuzumab or T-DM1 for the treatment of patients with advanced HER2+ breast cancer in a phase 1 dose-escalation trial (NCT03364348) and in combination with avelumab in a phase 2 trial (AVIATOR, NCT03414658) (Table 1). Preclinical data show that the combination of utomilumab and an mAb targeting the PD-1/PD-L1 axis leads to increased immune response106.

Modulation of the tumor microenvironment, including the tumor microvasculature, represents a further area of growing interest. It is currently not understood how tumor blood vessels can be altered to optimize drug delivery and facilitate immune cytotoxicity. Until now, antiangiogenic therapy has failed to show clinically significant improvements for the treatment of patients with HER2+ breast cancer using standard therapies107. Proangiogenic stimuli, such as increased vascular endothelial growth factor production, lead to immune evasion through a number of mechanisms, including the suppression of CD8+ T cells and DCs and the induction of PD-L1 expression by immune cells108. The latter mechanism supports the development of antiangiogenic agents in conjunction with immune checkpoint inhibitors.

Indoleamine 2, 3-dioxygenase 1 (IDO1) is an enzyme that catalyzes the metabolism of tryptophan in the tumor microenvironment, high levels of which mediate the inhibition of cytotoxic T cells via macrophages, DCs, and tumor cells109,110. In a TNBC model, IDO inhibitor D-1-methyl-tryptophan was shown to have in vivo antitumor activity. IDO overexpression was observed in a subset of HER2+ breast tumors (40%), which could be used to develop a synergistic treatment strategy, as observed in TNBC preclinical models111,112. Toll-like receptors have also been shown to be associated with an adaptive immune response. Activation of Toll-like receptor 4, which is expressed by DCs, has been shown to increase antigen processing and cross-presentation in vivo113. Oligodeoxynucleotides containing CpG motifs activate Toll-like receptor 9, which has shown to activate immune cytotoxicity in pre-clinical model114. In HER2+ breast cancer preclinical models, activation of Toll-like receptor 2 has been shown to augment trastuzumab-mediated cytotoxicity against HER2+ breast cancer cells115. Toll-like receptor agonists are being developed in combination with HER2-directed vaccines (NCT02276300).

**IMMUNOTHERAPY FOR CNS DISEASE**

Through the course of their disease, patients with HER2+ MBC face a high risk of CNS involvement with an absolute risk (AR) as high as 40%116-118. Patients are usually treated with CNS-directed therapies, including surgery and or radiation therapy, and systemic therapies are offered upon CNS disease progression. Lapatinib (an oral tyrosine kinase inhibitor) in combination with capecitabine is commonly used for the treatment of patients with HER2+ CNS involvement, and phase 2 trial data support an objective CNS response of 66%119,120. Interestingly, there are emerging data showing that the antitumor efficacy of oral tyrosine kinase inhibitors may be secondary to not only direct abrogation of HER2 signaling but also to activation cytotoxicity Th1 immune response, as suggested by results observed in mice models121.

As oral HER2-targeted agents continue to show signs of clinical efficacy for the treatment of CNS disease, interactions between these agents and the immune system should be considered in the development of future treatments122,123. Nonetheless, immunotherapies present a potential solution, as treatment with checkpoint inhibitors has shown CNS antitumor efficacy in patients with solid tumors124. Indeed, CNS breast tumor tissue analysis showed expression of PD-L1 in 53% of the cases (n = 84)125. A clinical trial assessing the antitumor efficacy of atezolizumab in combination with trastuzumab and pertuzumab for the treatment of patients with HER2+ breast cancer with progressive brain metastases is forthcoming (NCT03417544). The safety and preliminary efficacy of tremelimumab, a mAb against CTLA4, was assessed in combination with radiation therapy and trastuzumab in six women with HER2+ breast cancer with brain metastases126. Two women had non-CNS disease control lasting over 12 weeks, and treatment was well tolerated. Further studies are needed to assess for a possible abscopal effect, which may be the cause of the observed improved outcomes.

**DISCUSSION**

HER2-directed mAbs have significantly improved the outcomes of patients with localized or metastatic HER2+ breast cancer. The clinical efficacy of second generation of treatments, including the ADC T-DM1, further supports HER2 as being a robust target for future treatment development. Immunotherapy has gained momentum for the treatment of a wide array of solid tumors using mAbs that target the PD-1/PD-L1 axis, immune checkpoint inhibitors. However, these agents have not shown clinically relevant results in unsellected patients with HER2+ breast cancer. These findings, along with emerging clinical and preclinical data, suggest that more complex interactions between different components of the immune system, such as innate and adaptive branches, will have to be considered to develop treatments with clinically relevant efficacy.

From the preclinical standpoint, for optimal testing of new strategies, models will have to reconcile the ability to assess the preliminary efficacy of developing therapies with dealing with an immune-tolerant environment necessary for tumor growth. Nonetheless, the standardization of measures of immune activation and biomarkers predictive of benefit from therapy are lacking for both preclinical and clinical use. In addition, developing immunotherapies will need to account for synergistic interactions with chemotherapies. For a large subset of patients with HER2+ breast
cancer, these interactions are an important component of treatment, as there are modest response rates associated with trastuzumab as monotherapy compared with combination treatments with chemotherapy (11% vs. 60%)\textsuperscript{128,129}.

The clinical development of HER2 immunotherapies will have to surpass the challenge of the favorable efficacy to toxicity ratio of currently approved HER2-targeted agents used to treat MBC in the first-, second-, and third-line settings (overall response rates, 43–80%) and localized disease\textsuperscript{130,131}. In the latter scenarios, another caveat is the possible additional or differential toxicity profile of new treatments aiming to broaden the activation of the immune system, such as the development of mAbs\textsuperscript{132}. It should be noted that HER2-directed vaccines have shown favorable toxicity profiles, with negligible AR for grade 3/4 treatment-related AEs, but adoptive T cell–based therapies likely present with greater AR for AEs. This finding will need to be considered, along with increased patient treatment burden, for the development of future treatments\textsuperscript{133}.

Notwithstanding these hurdles, activation of the immune system has the potential for self-sustained and prolonged antitumor activity. In exploratory analyses, it is remarkable that atezolizumab in combination with abraxane improved the survival rates of patients with PD-L1\textsuperscript{+} metastatic TNBC in the first-line setting when compared to chemotherapy alone (25 months vs 15 months; HR, 0.62)\textsuperscript{134}.

It should be considered that HER2 immunotherapies could potentially be developed in a broader patient population, such as patients with an intermediate HER2-expression level who are not candidates for treatment with approved HER2 mAbs (e.g., HER2 2\textsuperscript{+}, FISH/dual in situ hybridization-negative). It is also more likely that HER2 immunotherapies are successful for the treatment of patients with low tumor burden as a function of having lower immune tolerance than patients with high tumor burden undergoing later lines of systemic therapies. There are numerous scenarios in which immunotherapies could be developed in the future with the goal of improving outcomes while deescalating chemotherapy to avoid significant AEs and improve clinical outcomes (Fig. 3). For instance, data suggest that CD8\textsuperscript{+} T–cell–mediated immunity can take place against tumors located in the CNS\textsuperscript{135}, as evidenced in other solid tumors with brain metastases. The clinical relevance of developing strategies to prevent and treat HER2\textsuperscript{+} metastatic disease to the brain cannot be overstated, as these patients have a high risk of brain involvement (~40%)\textsuperscript{136–139}.

CONCLUSIONS

HER2 overexpression/amplification is a biomarker predictive of strong correlations between improved clinical outcomes and treatments with HER2-targeted agents. Recent discoveries support the development of immunotherapies (both passive and active) aimed to activate different components of the immune system. In addition, passive therapies with novel mAbs and ADCs are in the advanced stages of development, as phase 3 clinical trial are ongoing.

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REFERENCES

1. Hammond, M. E. et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J. Clin. Oncol. 28, 2784–2795 (2010).
2. Wolff, A. C. et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. 31, 3997–4013 (2013).
3. Slamon, D. J. et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235, 177–182 (1987).
4. Slamon, D. J. et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med. 344, 783–792 (2001).
5. Swain, S. M. et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N. Engl. J. Med. 372, 724–734 (2015).
6. Costa, R. L. B., Soliman, H. & Czerniecki, B. J. The clinical development of vaccines for HER2\textsuperscript{+} breast cancer: current landscape and future perspectives. Cancer Treat. Rev. 61, 107–115 (2017).
7. de Melo Gagliato, D., Jardim, D. L., Marchesi, M. S. & Hortobagyi, G. N. Mechanisms of resistance and sensitivity to anti-HER2 therapies in HER2\textsuperscript{+} breast cancer. Oncotarget 7, 64431–64446 (2016).
8. Diers, V. et al. Trastuzumab emtansine versus capcitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer (EMILIA): a descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. Lancet Oncol. 18, 732–742 (2017).
9. Kropp, I. E. et al. Trastuzumab emtansine versus treatment of physician’s choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol. 18, 743–754 (2017).
10. Borchiae, H. et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N. Engl. J. Med. 373, 1627–1639 (2015).

Fig. 3 Possible areas of HER2 immunotherapy clinical development. Th1 response against HER2 breast cancer cells supported by CD8\textsuperscript{+}, CD4\textsuperscript{+}, NK lymphocytes, and DC.
75. Nagai, Y. et al. Disabling of the erbB pathway followed by IFN-gamma modulation of T cells in breast cancer patients. Cancer Immunol. Immunother. 67, 1842–1852 (2007).

76. Fukosy, G. K. et al. A novel dendritic cell-based immunization approach for the induction of durable Th1-polarized anti-HER2/neu responses in women with early breast cancer. J. Immunother. 35, 54–62 (2012).

77. Harada, S. et al. The significance of HER-2/neu receptor positivity and immunophenotype in ductal carcinoma in situ with early invasive disease. J. Surg. Oncol. 104, 458–465 (2011).

78. Roses, R. E. et al. HER-2/neu overexpression as a predictor for the transition from in situ to invasive breast cancer. Cancer Epidemiol. Biomark. Prev. 18, 1336–1339 (2009).

79. Mau, M. V. & June, C. H. Making better chimeric antigen receptors for adoptive T-cell therapy. Clin. Cancer Res. 22, 1875–1884 (2016).

80. Ott, P. A., Dotti, G., Yee, C. & Goff, S. L. An update on adoptive T-cell therapy and chimeric antigen receptors. Mol. Cancer Ther. 15, 325–343 (2016).

81. Knudson, K. L. & Disis, M. L. Expansion of HER2/neu-specific T cells ex vivo following immunization with a HER2/neu peptide-based vaccine. Clin. Breast Cancer 2, 73–79 (2001).

82. Bernhard, H. et al. Adoptive transfer of autologous, HER-2-specific, cytototoxic T lymphocytes for the treatment of HER2-overexpressing breast cancer. Cancer Immunol. Immunother. 61, 2171–2173 (2012).

83. Westwood, J. A. et al. Combinatorial PD-1 blockade and CD137 activation has therapeutic efficacy in murine cancer models and synergizes with cisplatin. PLoS ONE 8, e84927 (2013).

84. Gianni, L. et al. AVEREL: a randomized phase III trial evaluating bevacizumab in combination with docetaxel and trastuzumab as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer. J. Clin. Oncol. 31, 1719–1725 (2013).

85. Fukumura, D., Kloepper, J., Amoozgar, Z., Duda, D. G. & Jain, R. K. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat. Rev. Clin. Oncol. 15, 325–340 (2018).

86. Wei, H. et al. Combinatorial PD-1 blockade and CD137 activation has therapeutic efficacy in murine cancer models and synergizes with cisplatin. PLoS ONE 8, e84927 (2013).

87. Zhai, L. et al. Molecular pathways: targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy. Clin. Cancer Res. 21, 5427–5433 (2015).

88. Munn, D. H. et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297, 1687–1690 (2002).

89. Hwu, P. H. et al. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. J. Immunol. 164, 3596–3599 (2000).

90. Theate, I. et al. Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. Cancer Immunol. Res. 3, 161–172 (2015).
113. Hou, D. Y. et al. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antigen responses. Cancer Res. 67, 792–801 (2007).

114. Soliman, H. et al. Analysis of indoleamine 2-3 dioxygenase (IDO1) expression in breast cancer tissue by immunohistochemistry. Cancer Immunol. Immunother. 62, 829–837 (2013).

115. Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat. Med. 13, 1050–1059 (2007).

116. Krieg, A. M. Development of TLR9 agonists for cancer therapy. J. Clin. Invest. 117, 1184–1194 (2007).

117. Lu, H. et al. TLR2 agonist PSK activates human NK cells and enhances the antitumor effect of HER2-targeted monoclonal antibody therapy. Clin. Cancer Res. 17, 6742–6753 (2011).

118. Olson, E. M. et al. Clinical outcomes and treatment practice patterns of patients with HER2-positive metastatic breast cancer in the post-trastuzumab era. Breast 22, 525–531 (2013).

119. Brufsky, A. M. et al. Central nervous system metastases in patients with HER2-positive metastatic breast cancer: incidence, treatment, and survival in patients from registHER. Clin. Cancer Res. 17, 4834–4843 (2011).

120. Gori, S. et al. Central nervous system metastases in HER-2 positive metastatic breast cancer patients treated with trastuzumab: incidence, survival, and risk factors. Oncologist 12, 766–773 (2007).

121. Olson, E. M. et al. Incidence and risk of central nervous system metastases as site of first recurrence in patients with HER2-positive breast cancer treated with adjuvant trastuzumab. Ann. Oncol. 24, 1526–1533 (2013).

122. Bachelot, T. et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. Lancet Oncol. 14, 64–71 (2013).

123. Hannesdottir, L. et al. Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response. Eur. J. Immunol. 43, 2718–2729 (2013).

124. Murthy, R. K. et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N. Engl. J. Med. https://doi.org/10.1056/NEJMoa1914609 (2019).

125. Margolin, K. et al. Iplimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. Lancet Oncol. 13, 459–465 (2012).

126. Duchnowska, R. et al. Immune response in breast cancer brain metastases and their microenvironment: the role of the PD-1/PD-L axis. Breast Cancer Res. 18, 43 (2016).

127. McArthur, H. et al. Abstract 4705: CTLA4 blockade with HER2-directed therapy (H) yields clinical benefit in women undergoing radiation therapy (RT) for HER2-positive (HER2+) breast cancer brain metastases (BCBM). Cancer Res. 77, 4705–4707 (2017).

128. Baselga, J. et al. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J. Clin. Oncol. 14, 737–744 (1996).

129. Esteva, F. J. et al. Phase II study of weekly dacetaxel and trastuzumab for patients with HER2-2 overexpressing metastatic breast cancer. J. Clin. Oncol. 20, 1800–1808 (2002).

130. Baselga, J. et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N. Engl. J. Med. 366, 109–119 (2012).

131. Verma, S. et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N. Engl. J. Med. 367, 1783–1791 (2012).

132. Oberg, H. H. et al. Tribody (HER2xCD16) is more effective than Trastuzumab in enhancing gammadelta T cell and natural killer cell cytotoxicity against HER2-expressing cancer cells. Front. Immunol. 9, 814 (2018).

133. Costa, R. et al. A brief report of toxicity end points of HER2 vaccines for the treatment of patients with HER2(+) breast cancer. Drug Des. Devel. Ther. 13, 309–316 (2019).

134. Sampson, J. H. et al. Subcutaneous vaccination with irradiated, cytokine-producing tumor cells stimulates CD8+ cell-mediated immunity against tumors located in the "immunologically privileged" central nervous system. Proc. Natl Acad. Sci. USA 93, 10399–10404 (1996).

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R.L.B.C. researched data for the article, provided substantial contribution to the discussion of content, wrote the manuscript, and reviewed/edited the manuscript before submission. B.J.C. provided substantial contribution to the discussion of content, wrote the manuscript, and reviewed/edited the manuscript before submission.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to R.L.B.C.

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