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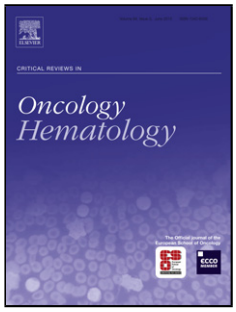

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PII: S1040-8428(20)30065-2

DOI: https://doi.org/10.1016/j.critrevonc.2020.102927

Reference: ONCH 102927

To appear in: *Critical Reviews in Oncology / Hematology*

Received Date: 24 May 2019

Revised Date: 6 February 2020

Accepted Date: 2 March 2020

Please cite this article as: Musolino A, Boggiani D, Pellegrino B, Zanoni D, Sikokis A, Missale G, Silini EM, Maglietta G, Frassoldati A, Michiara M, Role of Innate and Adaptive Immunity in the Efficacy of anti-HER2 Monoclonal Antibodies for HER2-positive Breast Cancer, *Critical Reviews in Oncology / Hematology* (2020), doi: https://doi.org/10.1016/j.critrevonc.2020.102927

Role of Innate and Adaptive Immunity in the Efficacy of anti-HER2 Monoclonal Antibodies for HER2-positive Breast Cancer

Running Title: Immune response to anti-HER2 monoclonal antibodies

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Highlights

- Anti-HER2 monoclonal antibodies (mAbs) are effective for all stages of HER2-positive breast cancer.
- However, intrinsic or acquired resistance to these drugs may occur.
- Innate and adaptive immunity play a key role in the efficacy of anti-HER2 mAbs.

- We report known and novel strategies for optimizing anti-HER2 therapies.

Abstract

Anti-HER2 monoclonal antibodies (mAbs) such as trastuzumab are effective for all stages of HER2-positive breast cancer (BC). However, intrinsic or acquired resistance to these drugs may occur in a significant number of patients (pts) and, except for HER2 status, no validated predictive factors of response/resistance have been identified to date. This lack is in part due to the not yet fully elucidated mechanism of action of mAbs *in vivo*. Increasing evidence suggests a significant contribution of both innate and adaptive immunity to the antitumor effects of mAbs. The aim of this review was to describe the role of innate and adaptive immunity in the efficacy of anti-HER2 mAbs and to report known and novel strategies to be used for optimizing immune effects of anti-HER2 therapies for HER2-positive BC.

Keywords: Innate immunity; adaptive immunity; breast cancer; HER2; monoclonal antibodies.

1. Introduction

HER2 (Her-2/neu, c-erbB-2) is a 185-kDa transmembrane tyrosine kinase protein giving higher aggressiveness in breast cancers (BCs). In humans, HER2 overexpression occurs in 15–20% of primary breast tumors, and is associated with diminished disease-free (DFS) and overall survival (OS) [1]. The humanized immunoglobulin G1 (IgG1) anti-HER2 monoclonal antibody (mAb) trastuzumab in combination with chemotherapy is an effective treatment for all stages of HER2-positive BC [2]. Other anti-HER2 mAbs have demonstrated efficacy in patients (pts) with HER2-positive tumors, either in combination with trastuzumab [3,4], or after trastuzumab progression [5].

After treatment with adjuvant trastuzumab, relapse can occur in up to 25.4% of the pts at 10 years of follow-up [6]. Moreover, only 25–30% of HER2-positive metastatic BC (MBC) pts will respond to single-agent anti-HER2 mAbs, and most of them will experience disease progression during the first year of treatment [5,7]. These findings are consistent with the occurrence of intrinsic or acquired resistance to HER2-targeting antibodies in a significant number of pts [5-8].

Preclinical studies have suggested that anti-HER2 mAbs work at different levels by blocking the dimerization of HER2 by inhibiting intracellular signaling pathways, inducing apoptosis, or activating host immune response [8-11]. With the exception of HER2 status, no validated predictive factors of either response or resistance to anti-HER2 mAbs have been identified to date [9-13]. This is partly due to the not yet fully elucidated mechanism of action of mAbs *in vivo* [14].

Innate and adaptive immune responses are components of an integrated system of antitumor host defense in which numerous cells and molecules function cooperatively [14-16]. Natural killer (NK) cells, monocytes and neutrophils recognize and kill tumor cells in an antigen-independent manner (innate immunity) [15]. Breast cancer antigens including HER2 have been identified, and the T and B lymphocytes specific for these antigens may recognize and destroy tumor cells (adaptive immunity) [15]. Evasion of innate and adaptive immunity is thought to be critical for breast cancer growth and progression [14]. Increasing evidence also suggests a significant contribution of both innate and adaptive immunity to the antitumor effects of mAbs [14-17].

The aim of this review was to describe the role of innate and adaptive immunity in the efficacy of anti-HER2 mAbs and to report known and novel strategies to be used for optimizing immune effects of anti-HER2 therapies for HER2-positive BC.

2. Innate immune response to anti-HER2 mAbs

2.1. *Antibody-dependent cell-mediated cytotoxicity*

To date, all of the currently approved mAbs are of the IgG isotype [15]. The structure of an IgG antibody comprises two antigen-binding fragments (Fabs) linked to a single crystalline fragment (Fc) domain via the hinge region. This structural arrangement allows antibodies to link a bound antigen with the humoral and cellular components of the immune system [17]. Fc gamma receptors (FcγRs) are expressed on a number of cells in the immune system including phagocytes such as macrophages and monocytes, granulocytes such as neutrophils and eosinophils, and lymphocytes of the innate immune system (NK cells) or adaptive immune system (e.g., B cells) [11,15,17]. FcγRIIa and FcγRIIIa are activating FcγRs that are expressed on monocytes/macrophages and on both monocytes/macrophages and NK cells, respectively. Intracellular signaling through the activating FcγRs leads to immune effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) [11,14-17].

The role of ADCC in mediating response to trastuzumab has been supported by several preclinical observations: in animal models, growth of HER2-positive cells was blocked by trastuzumab but not by trastuzumab-F(ab')2 fragments [18]; trastuzumab had a significantly reduced antitumor effect in FcγR-deficient mice [18,19]; *in vivo* activity of trastuzumab correlated with significantly increased numbers of peritumoral lympho-monocytes [19,20].

2.2. *Fc gamma receptor polymorphisms*

Single-nucleotide polymorphisms (SNPs) valine (V) 158 phenylalanine (F) and histidine (H) 131 arginine (R), located, respectively, in the extracellular domains of the FcγRIIIa and FcγRIIa, have been associated with differential antibody-binding affinities and ADCC [11,20]. In vitro studies of these polymorphisms demonstrated that peripheral blood mononuclear cells (PBMCs) homozygous for the high-affinity *FcγRIIIa*-V158 or *FcγRIIa*-H131 alleles induced significantly higher trastuzumab-mediated ADCC than PBMCs with other genotypes [11,21,22]. The largest retrospective analysis to date evaluating FcγR polymorphisms in the adjuvant setting of HER2-

positive BC was performed in a subset of 1286 pts enrolled in the randomized phase III Breast Cancer International Research Group (BCIRG)-006 trial [23]. In that study, no correlation was observed between *FcγRIIIa*-V158F and *FcγRIIa*-H131R SNPs and DFS in pts treated with trastuzumab [23]. These results differed from those of a previous pilot study showing an association between *FcγRIIIa*-158 V/V and/or *FcγRIIa*-131 H/H genotypes and trastuzumab efficacy in the metastatic setting [11]. More recently, two studies tested for the association of FcγR polymorphisms with DFS in the N9831 and NSABP B-31 clinical trials of pts with early-stage HER2-positive BC treated with chemotherapy alone or chemotherapy plus trastuzumab [24,25]. The *FcγRIIb*-I232T polymorphic variant, which is associated with loss-of-function of the inhibitory FcγRIIb [26], was predictive of adjuvant trastuzumab benefit in the N9831 study [24]. Furthermore, analysis of *FcγRIIIa*-V158F SNP in the NSABP B-31 trial indicated that pts with the low-affinity 158 F/F genotype received less benefit from the addition of trastuzumab in comparison with pts with 158 F/V or V/V genotypes [25].

3. Strategies to enhance innate immune response to anti-HER2 mAbs

3.1. *Dual anti-HER2 therapy*

Pertuzumab is a humanized IgG1 mAb that binds an epitope on HER2 that is distinct from that of trastuzumab. It targets the extracellular sub-domain 2 of HER2, which is a region necessary for dimerization with other HER family receptors [13]. Dual anti-HER2 therapy with pertuzumab and trastuzumab in combination with chemotherapy has been tested in HER2-positive BC providing clinical benefit in metastatic, neoadjuvant and adjuvant setting [3,4,13]. Increased ADCC may account for the synergistic effects of pertuzumab and trastuzumab *in vivo*. As pertuzumab and trastuzumab are not competing for the same binding epitope on HER2, their combination may lead to higher antibody load on tumor cells resulting in increased ADCC [26]. Compared to monotherapy, combination of the two mAbs enhanced the recruitment of NK cells responsible for ADCC, and significantly delayed the outgrowth of xenografts from intrinsically trastuzumab-

resistant cells [13,17]. Several antineoplastic drugs currently combined or sequentially administered with anti-HER2 mAbs (i.e., anthracyclines, cyclophosphamide, taxanes) induce the release of tissue damage-associated molecular pattern molecules (DAMPs), which have been shown to activate the immune system [15]. In mouse xenograft models, the triple-drug combination of pertuzumab plus trastuzumab plus docetaxel increased NK cell activation and recruitment to the tumor, thus suggesting that this combination strategy cooperatively enhances ADCC activity and contributes to tumor shrinkage [26].

Preclinical data suggest that lapatinib, a small molecule inhibitor of both epidermal growth factor receptor (EGFR) and HER2 tyrosine kinases, is able to prevent the internalization of HER2 protein in HER2-overexpressing BC cells, thus increasing the number and the intensity of the exposition of this protein to the ADCC activity of trastuzumab [27]. The same observation was made in HER2-positive gastric cancer cell lines and esophageal squamous-cell carcinomas [28]. A recent study reported an association between *FcγRIIIa* polymorphism and pathologic complete response (pCR) to the combination of chemotherapy plus trastuzumab and lapatinib in pts with operable HER2-positive BC: pts with favorable *FcγRIIIa* genotypes (V carriers) derived the most benefit from the combination of trastuzumab and lapatinib through enhancement of trastuzumab-mediated ADCC [12].

Tucatinib is an oral tyrosine kinase inhibitor (TKI) that is highly selective for the kinase domain of HER2 with minimal inhibition of EGFR. In the phase II randomized HER2CLIMB trial, 480 heavily pretreated pts with HER2-positive MBC were randomly assigned to receive either tucatinib or placebo, in combination with trastuzumab and capecitabine. The primary endpoint of progression-free survival (PFS) at 1 year was 33.1% in the tucatinib-combination group and 12.3% in the placebo-combination group (hazard ratio [HR], 0.54; 95% confidence interval [CI], 0.42 to 0.71; $P<0.001$). OS at 2 years was 44.9% in the tucatinib-combination group and 26.6% in the placebo-combination group (HR, 0.66; 95% CI, 0.50 to 0.88; $P=0.005$). Furthermore, among pts with previously untreated, treated and stable, or treated and progressing brain metastases, PFS at 1

year was 24.9% in the tucatinib-combination group and 0% in the placebo-combination group (HR, 0.48; 95% CI, 0.34 to 0.69; *P*<0.001) [29].

3.2. *Anti-HER2 mAbs combined with cytokines*

Several preclinical studies have demonstrated that interleukin (IL)-2, augments NK cell-mediated ADCC against breast cancer cells coated with trastuzumab [15,30,31]. However, in a phase II trial of trastuzumab in combination with low-dose IL-2 in pts with HER2-positive MBC who had previously failed trastuzumab, there were neither objective anti-tumor responses nor evidence of NK cell expansion or increase in ADCC [32]. Other trials with trastuzumab and low dose IL-2 reported conflicting results [15,32,33]. IL-12, as well as IL-15 and IL-21, have also been observed to enhance the immune-mediated effects of trastuzumab in preclinical models [15,34]. Nonetheless, clinical trials of trastuzumab plus those cytokines in BC, as well as of other mAbs in other tumor types, failed to support a role for recombinant ILs in the therapeutic effects of mAbs [15,35,36].

3.3. *mAb Fc engineering*

Due to the potential benefits of augmenting binding to FcγRs with resultant enhanced innate immune cell function, several approaches have been utilized to engineer the Fc region of mAbs [37]. Margetuximab is an Fc-optimized anti-HER2 mAb with mutations of 5 amino acid residues resulting in increased binding to FcγRIIIa and enhanced ADCC activity [38]. In particular, binding to the low-affinity allelic variant of FcγRIIIa (*FcγRIIIa*-158F) is increased in a proportionally greater fashion than binding to the high-affinity allele (*FcγRIIIa*-158V) [37,38]. Moreover, the optimized Fc domain of margetuximab reduces the binding to the inhibitory receptor FcγRIIb, leading to activation of monocytes/macrophages and consequent induction of HER2 antigen presentation [39,40]. In a pivotal phase III clinical trial of margetuximab plus chemotherapy vs. trastuzumab plus chemotherapy for previously treated HER2-positive MBC (SOPHIA,

NCT02492711), margetuximab demonstrated superior PFS over trastuzumab (HR, 0.76, *P*=0.033), particularly in carriers of the *FcγRIIIa*-158F allele (HR, 0.68, *P*=0.005) [41].

4. Adaptive immune response to anti-HER2 mAbs

4.1. *T cell-mediated immunity*

Antigen-presenting cells (APCs) are groups of cells that are widely distributed in tissues and include B cells, macrophages, and dendritic cells (DCs). DCs are the most efficient APCs, and appear to be crucial for induction of tumor-specific, T-cell-mediated, adaptive immune response [14,15]. Cancer cells express tumor antigens but are poor for antigen presentation and for providing costimulatory signals for T-cell activation following primary antigen recognition (T-cell priming) [14,15,42]. Antigen transfer to DCs and their surrogate presentation on major histocompatibility complex (MHC) class I and class II molecules is paramount to stimulate CD8+ cytotoxic T cells as well as CD4+ helper T cells [42,43].

Several evidences support the importance of antitumor T cell immunity for the clinical benefit of anti-HER2 mAbs [15,17,42]. MAbs can facilitate the uptake of tumor antigens by DCs. NK cell tumor cytolytic activity induced by, e.g., trastuzumab increases the availability of antigen–antibody immune complexes, which are internalized by DCs through FcγRs expressed on their surface membrane [17,42-45]. In preclinical models, trastuzumab-dependent NK cell activation results in the production of IFNγ and chemokines (e.g., IL-8), which have been shown to prime DC polarization for IL-12 production [42,44]. IL-12 secretion by DCs enhances the cross-presentation of tumor antigens to cytotoxic CD8+ T cells, and the differentiation of naive CD4+ T cells into tumor-specific CD4+ T helper cell type 1 (Th1) subsets, which also reinforce cytotoxic immune response [45]. In some tumor vaccination clinical trials, pts receiving the combination of vaccine and trastuzumab had better HER2-specific CD8+ T-cell response compared with that of vaccine alone [42].

Some studies have also provided evidence for the induction of a humoral anti-HER2 immune response during treatment with trastuzumab [46-48]. Pts with HER2-positive MBC showed elevated levels of anti-HER2 antibodies prior to initiation of treatment with trastuzumab and chemotherapy [46,47]. Interestingly, anti-HER2 humoral response was increased after treatment initiation and correlated with better PFS and OS [46]. Similarly, in the adjuvant setting, the generation of anti-HER2 antibodies was observed in pts treated with chemotherapy and trastuzumab and was significantly associated with improved DFS [47]. Trastuzumab administration has also been shown to induce the occurrence of anti-trastuzumab anti-idiotype (anti-ID) antibodies that mimic HER2, and may elicit anti-HER2 antibody response [48]. Such an idiotype-specific immunity accords with a vaccine-like effect of trastuzumab [15,17,42-44].

4.2. *Tumor-infiltrating lymphocytes*

Breast cancers may contain variable numbers of lymphocytes, referred to as tumor infiltrating lymphocytes (TILs). TILs are distributed in both stromal [stromal (s)TILs] and intratumoral [intratumoral (it)TILs] compartments and are usually assessed by trained pathologists and scored by semi-quantitative systems [49]. The composition and functional status of the immune infiltrate could vary widely between pts, stages of disease, and tumor types. The presence of CD8+ cytotoxic T cells, Th1 cells, and NK cells is associated with efficient anti-tumor immune response, whereas immunosuppressive effects are consistent with the presence of tumor-infiltrating FOXP3+ regulatory T (Treg) cells [49]. Response to trastuzumab has been associated with strong tumor infiltration of lymphoid cells [19,20].

Several studies evaluated the predictive and prognostic role of TILs in HER2-positive BC pts treated with anti-HER2-based neoadjuvant chemotherapy (NACT). Higher baseline TIL levels were associated with increased pCR rates and improved long-term outcomes, irrespective of anti-HER2 agents and chemotherapy regimens used [50-53]. Notably, a single study reported that high TILs in post-NACT residual disease may play a poor prognostic role in HER2-positive BC [54].

The predictive value of TILs was also evaluated from pretreatment biopsies of pts receiving neoadjuvant, chemotherapy-sparing, lapatinib plus trastuzumab treatment. A 60% threshold was used to define lymphocyte-predominant breast cancer (LPBC). LPBC was marginally associated with higher pCR rate than non-LPBC (50% vs. 19%, *P*=0.057). Quantitative assessment of the immune infiltrate by multiplexed immunofluorescence identified an immune profile characterized by high CD4+, CD8+, CD20+ (s)TILs, and high CD20+ (it)TILs, which was independently associated with a higher pCR rate (*P*=0.03) [55].

In the adjuvant setting, the FinHER trial suggested a positive correlation between high baseline TILs and trastuzumab benefit [51]. Conversely, in pts enrolled in the N9831 trial, high level of sTILs were associated with lack of trastuzumab efficacy [53]. In the same trial, increased expression of a subset of immune function genes significantly predicted benefit from adjuvant trastuzumab [53]. More recently, the association of TILs with improved distant disease-free survival (DDFS) was confirmed in pts enrolled in the ShortHER adjuvant trial which compared 9 weeks versus 1-year trastuzumab in addition to chemotherapy [56]. In the metastatic setting, no significant association was observed between TILs and PFS after first-line treatment with docetaxel plus trastuzumab, with or without pertuzumab [4,13,57]. However, in the same trial, higher TIL values were significantly associated with improved OS [57].

5. Strategies to enhance adaptive immune response to anti-HER2 mAbs

5.1. *Anti-programmed cell death protein-1/ligand-1 antibodies*

Inhibitory immune checkpoints are molecules involved in the modulation of the immune response through the induction of anergy or apoptosis of immune cells [14,15]. One of the major pathways involves the programmed cell death protein-1 (PD-1), which is expressed on activated T cells, NK cells, B cells, macrophages and several subsets of DCs, and its ligands, PD-L1 and PD-L2 [14,15,17,42]. Cancer cells upregulate PD-L1/ PD-L2 to escape from host immune surveillance

[15,17,42]. Several checkpoint inhibitors, in particular anti-PD-1 and anti-PD-L1 mAbs, have been approved to treat a wide spectrum of tumors [58].

Anti-HER2 mAbs may induce PD-L1 upregulation [59]. Gene expression profiling and immunohistochemistry (IHC) analysis of a series of HER2-positive BCs showed that trastuzumab-sensitive tumors expressed significantly higher levels of chemokines involved in immune cell recruitment, with higher infiltration of T cells and monocytes, and higher levels of PD-1 ligands than tumors that do not benefit from trastuzumab [60]. Similarly, a PD-1-associated gene expression signature significantly correlated with improved survival in pts with HER2-positive BCs treated with neoadjuvant trastuzumab [61]. Interestingly, in the NeoSphere trial, higher expression of PD-1/PD-L1 was associated with lower probability of pCR in the pertuzumab and trastuzumab plus docetaxel (THP) arm [62]. These findings justify the attempts of combining anti-HER2 therapies with either anti-PD-1 or anti-PD-L1 mAbs (Table 1). In a phase Ib/II study of the anti-PD-1 pembrolizumab in combination with trastuzumab in 58 pts with trastuzumab-resistant HER2-positive MBC, the objective response rate (ORR) was 15%, in the PD-L1-positive (≥1% tumor or stroma) population. The ORR was 39% in PD-L1-positive pts with more than 5% of TILs. No responses were observed in the PD-L1-negative cohort [63]. Serious adverse events (SAEs) occurred in 29 (50%) of pts. The most commonly occurring SAEs were dyspnea (n=3 [5%]), pneumonitis (n=3 [5%]), pericardial effusion (n=2 [3%]), and upper respiratory infection (n=2 [3%]). There was one treatment-related death due to the autoimmune Lambert-Eaton myasthenic syndrome [63]. Durvalumab and atezolizumab are anti-PD-L1 mAbs with genetically modified Fc regions to avoid ADCC, which is of particular importance for anti-PD-L1 antibodies as activated T cells readily express PD-L1 [64]. Several trials are evaluating the efficacy and tolerability of these drugs in combination with anti-HER2 mAbs (Table 1). In a phase I study of durvalumab and trastuzumab in HER2-positive MBC, no dose-limiting toxicities and no objective responses were reported, but 29% of pts experienced a stable disease. All pts were PD-L1 negative and showed minimal CD8+ T cell infiltration on tumor biopsies [65].

T-DM1 is a HER2-targeting antibody–drug conjugate (ADC) in which molecules of DM1, a cytotoxic microtubule polymerization inhibitor, are bound via a stable thioether linker to trastuzumab [5]. In a randomized, double-blind, placebo-controlled, phase II study of T-DM1 plus atezolizumab in 202 pts with previously treated HER2-positive MBC [66], the addition of atezolizumab to T-DM1 did not demonstrate a meaningful PFS benefit in the intention-to-treat (ITT) population, whereas subgroup analysis suggested both PFS and OS benefit for the combination therapy in PD-L1-positive (tumor-infiltrating immune cells [IC] ≥ 1%) pts. A numerically higher rate of SAEs leading to discontinuation of study treatment in the atezolizumab arm was also observed [66].

[Fam-] trastuzumab deruxtecan (DS-8201a) is a novel HER2-targeting ADC with a topoisomerase I inhibitor exatecan derivative (DX-8951 derivative, DXd). In an immunocompetent mouse model, DS-8201a increased tumor-infiltrating DCs, CD8+ T cells, and enhanced PD-L1 and MHC class I expression on tumor cells [67]. Furthermore, combination therapy with DS-8201a and an anti-PD-1 antibody was more effective than either monotherapy [67] (Table 1). The antitumor effect of DS-8201a in combination with an anti-CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4) antibody was also evaluated [68]. The roles of anti-CTLA-4 antibodies are distinct from those of anti-PD-1 antibodies; anti-CTLA-4 antibodies restore T-cell priming, whilst anti-PD-1 antibodies restore T-cell effector function [43,44]. In an immunocompetent mouse model, [Fam-] trastuzumab deruxtecan in combination with an anti-CTLA-4 antibody increased tumor-infiltrating CD4+/CD8+ T cells and induced more potent antitumor activity than that by monotherapy with either agent [68]. DESTINY-Breast01 is a two-part, open-label, single-group, multicenter, phase II study that evaluated trastuzumab deruxtecan in heavily pretreated pts with HER2-positive MBC who had received previous treatment with T-DM1. The primary endpoint was the ORR, and a response to therapy was reported in 112 out of 184 patients (60.9%; 95% CI, 53.4 to 68.0). The median response duration was 14.8 months (95% CI, 13.8 to 16.9), and the median duration of PFS was 16.4 months (95% CI, 12.7 to not reached). In addition to nausea and myelosuppression, interstitial

lung disease was observed in a subgroup of patients and requires attention to pulmonary symptoms and careful monitoring [69].

5.2. *Immune stimulatory agonists*

Numerous co-stimulatory receptors are involved in the induction of T-cell proliferation and effector functions [42]. Therefore, co-stimulatory receptor agonists may be used to upregulate immune response [70]. Among those, urelumab and utomilumab are mAbs specific for 4-1BB (CD137), which is a member of the TNF receptor superfamily [71]. 4-1BB is expressed by activated T lymphocytes, APCs and NK cells, and its engagement enhances CD8+ T cell cytotoxic activity and ADCC [70,71]. A phase II study is currently investigating utomilumab in combination with the anti-PD-L1 mAb avelumab, trastuzumab and vinorelbine in HER2-positive MBC (Table 1). Toll-like receptors (TLRs) are usually expressed on macrophages and DCs. After recognizing structurally conserved molecules derived from bacteria and viruses, TLRs trigger potent innate and adaptive immune responses [14]. TLR2, TLR3, TLR8, and TLR9 agonists have been shown to potentiate NK cell-mediated ADCC and antitumor function of anti-HER2 mAbs in preclinical models [15,72]. TLR ligands are being tested with trastuzumab in several clinical trials (Table 1).

5.3. *Bifunctional antibodies*

Bispecific antibodies (BsAbs) are artificial proteins consisting of two Fab arms contained within a single molecule that target two different antigens [73,74]. BsAbs can be divided in two major classes: IgG-like and non-IgG-like. The first one maintains the traditional mAb structure of two Fabs and one Fc region (trifunctional antibody) [73]. The non-IgG-like category lacks the Fc fragment and include chemically linked Fabs, consisting of only the Fab regions, and various types of bivalent and trivalent single-chain variable fragments (scFvs) or fusion proteins mimicking the

variable domains of two antibodies [73-75]. Bispecific T Cell Engagers (BiTEs) are non-IgG-like BsAbs that simultaneously binds to T cells via the CD3 receptor and tumor cell via a tumor-specific molecule [75].

Several BsAbs are under clinical investigations in HER2-positive BC [76-89] (Table 1). ZW25 is an IgG-like HER2-targeted BsAb that simultaneously binds two HER2 epitopes: extracellular domain (ECD) 4 (trastuzumab binding domain) and ECD2 (pertuzumab-binding domain). In a phase I basket trial, researchers enrolled 42 heavily pretreated pts with HER2-positive cancers, including 20 with BC, 13 with gastroesophageal, 5 with colorectal, and 4 with other malignancies. Of 33 evaluable pts, 12 (36%) had an objective response to the drug and six (18%) had stable disease for a disease control rate of 55%. Diarrhea, infusion reactions, and nausea were the most common side effects, and most were classified as grade 1 or 2; no grade 4 or 5 side effects were observed [77,78]. A bispecific tribody (BsTb) [(HER2)2xCD16], which comprises two HER2-specific scFvs fused to a Fab directed to the FcγRIIIA (CD16) antigen, efficiently enhanced the *in vitro* cytotoxic activity of NK cells and γδ T cells in comparison with trastuzumab [79]. A BsAb, Her2(Per)-S-Fab has recently been developed by linking the pertuzumab Fab to an anti-FcγRIIIA single domain antibody showing potent cytotoxicity against HER2-positive tumor cells [80]. Ertumaxomab is a full-length trifunctional antibody that targets HER2, CD3, and the FcγRs I, IIA and III. Phase I/II clinical trials evaluated safety and antitumor efficacy of ertumaxomab in HER2-positive tumors (also pts with HER2 score of 1+ by IHC were eligible) [74]. Most pts experienced treatment-related adverse events (AEs), which were however mild and reversible. The reported ORRs in pts with HER2-positive MBC ranged from 21 to 33% [74,81,82]. An IgG-scFv BsAb with bivalent binding to HER2, monovalent binding to CD3, and its Fc function silenced to reduce the risk of cytokine release syndrome (CRS) was recently presented [86]. Interestingly, this BsAb showed relative insensitivity to the PD-1/PD-L1 axis, with superior antitumor activity to trastuzumab both in vitro and in vivo [86]. MM-111 is a dual BsAb consisting of anti-HER2 and anti-HER3 scFvs, which has been built to prevent HER3-mediated resistance to currently existing anti-HER2 therapies [74]. In a

multi-arm phase I trial, the combination of MM-111 with trastuzumab or lapatinib was feasible with standard doses for the HER2-directed therapies. There were no additional adverse events with MM-111 and the overall clinical benefit rate (CBR) was 55% [87]. A phase II trial is currently running [73,88]. MCLA-128 is a full length IgG-like BsAb also targeting HER2 and HER3. A phase 2 study utilizing MCLA-128 in combination with trastuzumab/chemotherapy is ongoing for the treatment of HER2-positive disease (Table 1). BsPD-L1xrErbB2 is a mouse IgG2a BsAb targeting rat HER2 and mouse PD-L1. In mouse tumor models of HER2-positive mammary cancer, BsPD-L1xrErbB2 successfully reduced tumor growth and increased tumor rejection rate. The enhanced antitumor effect of BsPD-L1xrErbB2 was dependent on CD8+ T lymphocytes and IFN-γ, as depletion of CD8+ T lymphocytes and neutralization of IFN-γ completely abolished the antitumor activity of the BsAb [89]. Activated T cells armed with anti-HER2 bispecific antibody (HER2Bi-aATCs) are generated from PBMCs, expanded with anti-CD3 mAb and IL-2, and armed with a CD3xHER2 BsAb [74]. In a phase I clinical trial of HER2Bi-aATCs in combination with IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in 23 pts with MBC, no dose-limiting toxicity was observed, 59.1% evaluable pts had stable disease (SD) or better, and median OS for all pts was 36.2 months (57.4 months for the HER2 3+ group, 27.4 months for the HER2 0–2+ group) [90].

5.4. *Vaccines*

The activation of the adaptive immune system induced by anti-HER2 mAbs may lead to the generation and maintenance of immunological memory, which could prevent disease recurrence and progression [13,44,57]. Strategies to improve adaptive immunity and immune memory with anti-HER2 therapies include their combination with peptide-based vaccines (Table 2). A multicenter, randomized, placebo-controlled phase II trial of trastuzumab plus the HER2 peptide nelipepimut-S (E75) versus trastuzumab alone is currently ongoing in the adjuvant setting for pts with high-risk

HER2-positive BC. No concerns with the combination of nelipepimut-S plus trastuzumab have been reported [91]. The combination of trastuzumab with a HER2-derived, MHC class II-restricted peptide vaccine was safe and generated robust and persistent tumor-specific T cell immunity in pts with HER2 positive MBC [70,92]. Similarly, the combination of lapatinib plus dHER2, a recombinant protein consisting of ECD and a portion of intracellular domain (ICD) of HER2, was well tolerated with promising long-term survival in pts with HER2-positive MBC refractory to trastuzumab. Anti-HER2-specific antibodies and HER2-specific T cells were induced in 100% and 8% of pts, respectively [93].

To improve immunogenicity of HER2 vaccines, DCs can be loaded ex vivo (pulsed) with synthetic peptides based on the HER2 sequence and then administered to pts [44,94]. Those HER2 DC vaccines have been being tested in combination with trastuzumab and pertuzumab, or trastuzumab and vinorelbine (Table 2).

GM-CSF-secreting BC vaccines provide a different approach to enhance immunogenicity of tumor-associated antigens (TAAs). Either allogeneic or autologous irradiated BC cells are transfected with the GM-CSF gene. Upon repeated intradermal administration of the vaccine, injected BC cells secrete GM-CSF, which stimulates tumor-specific T-cell response through Fc-mediated activation of DCs [48,94]. The combination of cyclophosphamide, allogeneic HER2-positive GM-CSF-secreting BC vaccine, and weekly trastuzumab in 20 pts with HER2-positive MBC was safe, with CBR at 1 year of 40% [95]. Results from a randomized trial of cyclophosphamide and allogeneic GM-CSF-secreting BC vaccine ± trastuzumab are pending (Table 2).

5.5. *Chimeric antigen receptor T-cell therapy*

T cells may be modified to express chimeric antigen receptor (CAR), which comprises an scFv directly fused to a transmembrane domain and signaling domains important for T-cell activation [96]. The engineered CAR is genetically encoded in the T-cell genome following viral vector or plasmid transduction [97]. CAR structure has evolved significantly from the initial composition

involving only the CD3ζ signaling domain (first-generation CAR). Since then, in an effort to increase T-cell persistence and proliferation, costimulatory endodomains were added, giving rise to second- (*e.g.*, CD3ζ plus 41BB- or CD28-signaling domains) and third-generation (*e.g.*, CD3ζ plus 4-1BB- and CD28-signaling domains) CARs [96,97]. Fourth generation CARs are engineered with an inducible expression component such as a cytokine like IL-12 [97]. New generation of CARs may also contain a self-withdrawal mechanism, for instance caspase-9 gene as a suicide gene that can be activated to rapidly withdraw CAR-T cells once the antitumor effect is achieved [96-102].

Anti-CD19 CAR-T cells demonstrated remarkable success in treating acute lymphoblastic leukemia and B-cell lymphomas [96]. However, CAR-T cell therapies were associated with severe or even fatal cytokine release syndrome and neurological events, which were mitigated in most pts with supportive measures and cytokine blockade [96-100]. Peculiar obstacles for activity of CAR-T cells in solid tumors include: antigen specificity, T cell trafficking to tumor sites and penetration, immunosuppressive tumor microenvironment, and tumor heterogeneity [97]. A strategy to increase the on-target specificity and efficacy of CAR-T cells involves the use of T cells specific for antigens associated with chronic viral infection (e.g. cytomegalovirus [CMV]) [102]. These cytotoxic T lymphocytes (CTLs) can recognize both tumor antigen and virus-infected cells through their chimeric and native receptors, and may survive longer than T cells without virus specificity [96-102] (Table 2). Novel CAR-T cells have also been developed to directly bind to tumor-specific mAbs. In particular, an innovative construct has been designed containing the high-affinity FcγRIIIa-V158 variant with a CD8 hinge, transmembrane domains, along with the signaling domains 4-1BB and CD3ζ (antibody-coupled T cell receptor [ACTR]) [96,101]. ACTR-T cells can be efficiently directed against HER2-positive tumors by co-administering anti-HER2 mAbs, such as trastuzumab. Toxicity of these constructs can also be controlled by adjusting the amount of the infused targeting mAb [101]. Based on these findings, a clinical trial using FcγRIIIa-V158 ACTR-T cells plus trastuzumab is currently underway. Ongoing phase 1/2 studies evaluating HER2-targeted CAR-T cells in pts with various HER2-positive solid cancers are shown in Table 2.

5.6. *Subcutaneous route of administration of mAbs*

Subcutaneous (SC) trastuzumab has efficacy and safety profiles similar to those of intravenous (IV) trastuzumab in HER2-positive early BC [103]. Interestingly, in the phase III randomized Hannah trial, SC trastuzumab was more immunogenic than IV trastuzumab: 6.8% of the pts in the SC group developed non-neutralizing anti-trastuzumab antibodies in comparison with an immunogenicity rate of 3.4% observed in the IV group [103]. The higher presence of antidrug antibodies with SC trastuzumab did not increase but rather reduced the risk of infusion-related reactions (IRRs) [103,104]. These findings may be explained by the occurrence of specific immunogenic responses, including IgE-to-IgG class switching, after SC trastuzumab administration [105].

Unlike the IV route, SC administration of trastuzumab does not provide a direct drug absorption into the intravascular compartment [103]. After SC administration, trastuzumab undergoes several steps through the peripheral lymphatic system and central lymph nodes and only then is poured into the blood stream [106]. It is interesting to note that HER2-positive BCs metastasize via the lymph nodes and efficient lymphatic transfer of trastuzumab may enhance anticancer activity against lymph-metastasizing cells [104]. Furthermore, due to its extensive direct absorption to lymphatic system, SC trastuzumab experiences an "early contact" with CD8+ and CD4+ T cells in lymph nodes [107,108]. As previously reported, opsonization of HER2-expressing cancer cells with trastuzumab results in enhanced uptake of HER2 by DCs, thus favoring the induction of a specific, and clinically relevant, T-cell adaptive response [17,42-45]. Therefore, by modifying the modality of administration of trastuzumab, it would be possible to interfere with different pathways of the immune system, and to exert a beneficial immunomodulation in HER2-positive BC.

Based on these considerations, a phase II multicenter, open-label, neoadjuvant, randomized study is being conducted to evaluate variations of host immune response parameters to either SC or IV trastuzumab given in combination with pertuzumab and chemotherapy as neoadjuvant treatment of pts with T2-4d primary HER2-positive BC (ImmunHER trial) (Table 2).

6. Conclusions

Several immune mechanisms may be used to enhance the *in vivo* activity of anti-HER2 mAbs. Innate immune responses generally occur through binding of the Fc fragment of mAbs to the FcγRs, which are principally expressed on NK cells and monocytes/macrophages. Importantly, the *FcγRIIIa*-158V/F polymorphism has been associated with differential anti-HER2 mAbs binding affinities and ADCC. According to these findings, in a phase III clinical trial for pts with previously treated HER2-positive MBC, margetuximab (an Fc-optimized anti-HER2 mAb) demonstrated superior PFS over trastuzumab, particularly in carriers of the low-affinity *FcγRIIIa*-158F allele [41]. Other novel approaches which include tucatinib (HER2-specific TKI) in combination with trastuzumab and capecitabine [29], and trastuzumab deruxtecan (HER2-targeting ADC) [69] are going to transform the treatment landscape of trastuzumab-refractory, HER2-positive MBC.

Increasing evidence suggests a significant contribution of the adaptive immunity to the mechanism of action of anti-HER2 mAbs. Anti-HER2 combination therapies with PD-1/PD-L1 inhibitors, bi(tri)functional antibodies, CAR-T cells, co-stimulatory receptor agonists, and vaccines are promising strategies for improving clinical efficacy of therapeutic mAbs through the induction of a HER2-specific immune response. Drug distribution through the lymphatic system is a characteristic feature of the SC route of administration of mAbs. The efficient transfer of SC trastuzumab to lymph nodes may enhance anticancer drug activity through early activation of HER2-specific immunity. A multicenter phase II randomized study is currently evaluating different pathways of immune response to either SC or IV trastuzumab given in combination with pertuzumab and chemotherapy as neoadjuvant treatment of pts with operable or locally advanced HER2-positive BC.

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with the publication "Role of Innate and Adaptive Immunity in the Efficacy of anti-HER2 Monoclonal Antibodies for HER2-positive Breast Cancer" and there has been no significant financial support for this work that could have influenced its outcome.

Outside the submitted work, Dr. Musolino reports grants, personal fees and non-financial support from Roche, personal fees and non-financial support from Lilly, personal fees from Pfizer, grants, personal fees and non-financial support from EISAI, personal fees from Macrogenics, grants from AstraZeneca. Dr. Frassoldati reports personal fees from roche, personal fees from Novartis, personal fees from Pfizer. The other authors have no conflict of interests to disclose.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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Acknowledgements:

None.

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Table 1. Strategies to enhance immune response to anti-HER2 mAbs

Procedure	Therapeutic agents	Molecular/immune cell targets	Immune response	Notes
Dual anti-HER2 Therapy	Pertuzumab plus trastuzumab	HER2, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Approved in combination with CT in early and advanced setting of HER2+ BC [13,57]
	Lapatinib plus trastuzumab	HER2, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Approved in advanced setting of HER2+ BC [27-28]
	Tucatinib plus trastuzumab and capecitabine	HER2, FcγRs/NK cells, monocytes, **MØ**, DCs (putative, based on reference lapatinib)	ADCC, enhanced HER2 uptake by DCs, TCMC (putative, based on reference lapatinib)	Phase II trial in pretreated HER2+, MBC. Superior PFS and OS over trastuzumab plus capecitabine [69]
Anti-HER2 mAbs plus cytokines	Trastuzumab plus IL-2, IL-15, or IL-21	HER2, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Negative/conflicting results in HER2+ BC [32-36]
Anti-HER2 mAb Fc engineering	Margetuximab	HER2, FcγRIIIa, FcγRIIb/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase III trial in pretreated HER2+, MBC. Superior PFS over trastuzumab (HR=0.76, *P*=0.033) [40,41]
Anti-HER2 plus anti-PD-1/PD-L1 mAbs	Trastuzumab plus pembrolizumab	HER2, FcγRs, PD-1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase Ib/II trial. ORR: 15% in pretreated HER2+, PD-L1+ MBC [63]
	Carboplatin plus trastuzumab ± pembrolizumab	HER2, FcγRs, PD-1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing randomized phase II trial in locally recurrent HER2+ BC [NCT03095352]
	T-DM1 plus pembrolizumab	HER2, FcγRs, PD-1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing phase I trials in pretreated HER2+, MBC [NCT02318901; NCT03032107]
	Trastuzumab Deruxtecan (ADC) plus nivolumab	HER2, FcγRs, PD-1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing phase I trial in in pretreated HER2+ MBC and urothelial cancer [NCT03523572]

	Trastuzumab plus durvalumab	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I trial. SDR: 29% in PD-L1-negative MBC [65]
	Atezolizumab plus trastuzumab and pertuzumab (**w/ and w/o** docetaxel) or atezolizumab plus T-DM1	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I trial in HER2+ MBC: active, not recruiting [NCT02605915]
	High-dose trastuzumab plus pertuzumab and atezolizumab	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing phase II trial in HER2+ MBC with CNS metastases [**NCT03417544**]
	Trastuzumab plus pertuzumab and atezolizumab with paclitaxel	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing phase II trial in HER2+ MBC [NCT03125928]
	Trastuzumab plus pertuzumab, carboplatin and paclitaxel ± atezolizumab	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing randomized phase III neoadjuvant trial in HER2+ BC [NCT03595592]
	T-DM1 ± atezolizumab	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Randomized phase II trial in pretreated HER2+ MBC. PFS events: 51% (atezolizumab) vs. 57% (placebo). Higher rates of SAEs with atezolizumab arm [66]
Anti-HER2 mAbs plus immune stimulatory agonists	Utomilimumab plus avelumab, trastuzumab and vinorelbine	CD137, PD-L1, HER2,FcγRs/ NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing randomized phase II trial in HER2+ MBC [NCT03414658]
	Trastuzumab plus agatolimod	TLR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I/II trials discontinued or completed. No data available [NCT00824733; NCT00031278; NCT00043394]
Bifunctional antibodies	MDX-210	HER2, FcγRI/ monocytes, **MØ**	ADCP	Fab anti-FcγRI x Fab anti-HER2. Phase I trial in pretreated HER2+ breast/ovarian cancers: PR: 10% [76]

	ZW25	HER2-ECD4, HER2-ECD2, FcγRs/ NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	IgG-like BsAb. Phase I trial in pretreated HER2+ tumors. DCR: 55% [77,78]
	Tribody [(HER2)2xCD16]	HER2, FcγRIIIa/ NK cells, γδ T cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	scFvs anti-HER2 x Fab anti-FcγRIIIa. Greater *in vitro* antitumor activity vs. trastuzumab [79]
	HER2bsFab	HER2-ECD4, FcγRIIIa/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Fab anti-FcγRIIIa x Fab anti-HER2. Preclinical antitumor activity [80]
	Her2(Per)-S-Fab	HER2-ECD2, FcγRIIIa/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Single-domain anti-FcγRIIIa x Fab anti-HER2. Potent preclinical antitumor activity [80]
	Ertumaxomab	HER2, CD3, FcγR (I, IIA, III)/ NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Full-length trifunctional antibody. Phase I trials in pretreated HER2+ tumors. ORR (HER2+ MBC): 21-33%. Mild AEs [81,82]
	HER2-S-Fab	HER2-ECD4, FcγRIIIa/ NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Single-domain anti-FcγRIIIa x Fab anti-HER2. Preclinical antitumor activity [83]
	GBR1302	HER2, CD3/T cells	TCMC	HER2 x CD3 BITE. Ongoing phase I trial in pretreated HER2+ tumors. Most common AEs: IRR/CRS [84]
	p95HER2xCD3 BITE	p95HER2, CD3/ T cells	TCMC	*In vitro* activity against p95HER2+ BCs with no "toxic" effect on normal cells [85]
	IgG-scFv BsAb	HER2, CD3/T cells	TCMC	Fc silenced to reduce CRS. Greater preclinical antitumor activity vs. trastuzumab [86]

	MM-111	HER2, HER3/−	−	scFv anti-HER2 x scFv anti-HER3. Phase I trial of MM-111 + SoC in pretreated HER2+ tumors. CBR: 55%. No additional AEs with MM-111 [87,88]
	MCLA-128	HER2, HER3, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Full length IgG-like BsAb. Ongoing phase II trial of MCL-128 with trastuzumab/CT in HER2+ tumors and with ET in ER+/low HER2 BC [NCT03321981]
	BsPD-L1xrErbB2	HER2, FcγRs, PD-L1/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Mouse IgG2a BsAb. *In vivo* activity in mouse tumor models of HER2+ mammary cancer. Anti-tumor effect dependent on CD8+ T lymphocytes and IFN-γ [89]
	HER2Bi-aATCs	HER2,CD3/T cells	TCMC	ATCs armed with CD3xHER2 BsAb. Phase I trial in pretreated MBC. CBR: 59.1% [90]

FcγR, Fc gamma receptor; NK, natural killer, **MØ, macrophages;** DC, dendritic cell; ADCC, antibody-dependent cell-mediated cytotoxicity; TCMC, T cell-mediated cytotoxicity; BC, breast cancer; Fc, fragment crystallizable; MBC, metastatic breast cancer; ORR, overall response rate; ADC, antibody-drug conjugate; SDR: stable disease rate; **w/, with; w/o, without;** PFS, progression-free survival; T-DM1, Trastuzumab emtansine; SAE, serious adverse event; ECD, extracellular domain; BsAb, bispecific antibody; DCR: disease control rate (percentage of patients who have achieved complete response, partial response and stable disease to a therapeutic intervention); Fab, fragment antigen-binding; CD16; FcγRIIIa; scFv, single-chain variable fragment; AE, adverse event; BiTE, bispecific T cell engager; IRR, infusion-related reaction; CRS, cytokine release syndrome; p95HER2, truncated form of HER2; SoC, standard of care; CBR, clinical benefit rate (same definition of DCR); HER2Bi-aATCs, activated T cells armed with anti-HER2 bispecific antibody.

Table 2 (Cont.). Strategies to enhance immune response to anti-HER2 mAbs

Procedure	Therapeutic agents	Molecular/immune cell targets	Immune response	Notes
Anti-HER2 mAbs plus HER2-derived, peptide vaccines	Trastuzumab ± MHC class I A2/A3 (HLA-A2/A3)-restricted HER2 vaccine Nelipepimut-S (E75)	HLA-A2/A3, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing randomized phase II trial in adjuvant setting: active, not recruiting [91]
	HER2 ICD vaccine plus trastuzumab (or trastuzumab and pertuzumab) ± polysaccharide-K	MHC I-II, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Randomized Phase I/II trial in advanced HER2+ breast/ovarian cancers: active, not recruiting [NCT01922921]
	MHC class II-restricted HER2 vaccine plus trastuzumab	MHC II, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I/II trial in HER2+ MBC: minimal toxicity and prolonged T cell response [92]
	MHC class I A2 (HLA-A2)-restricted HER2 vaccine plus trastuzumab	HLA-A2, TCR HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I/II trial in advanced HER2+ breast/ovarian cancers: active, not recruiting [NCT00194714]
	dHER2 plus lapatinib	MHC I/II, TCR/DCs, T cells	TCMC	Phase I trial in pretreated HER2+ MBC: induction of anti-HER2-specific Abs (100%) and HER2-specific T cells (8%) [93]
	HER2 pulsed DC vaccine plus trastuzumab and pertuzumab	MHC I-II, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase I/II trial in DCIS: active, not recruiting [NCT02336984]
	HER2 pulsed DC vaccine plus trastuzumab and vinorelbine	MHC I-II, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Phase II trial in HER2+ MBC: completed. ORR not still reported [NCT00266110]
	HER2+ GM-CSF-secreting BC vaccine + low-dose cyclophosphamide ± trastuzumab	MHC I-II, TCR, HER2, FcγRs/NK cells, monocytes, **MØ**, DCs, T cells	ADCC, enhanced HER2 uptake by DCs, TCMC	Randomized phase II trial in HER2+ MBC: immunologic response as determined by DTH observed in 75% of pts [NCT00847171]

CAR-T therapy	HER2.CD28.4-1BB.CD3ζ-CAR	HER2/CTLs	TCMC	Phase I/II trial in pretreated HER2+ tumors: terminated after occurrence of fatal on-target off-tumor toxicity [99]
	HER2.CD28.CD3ζ-CAR	HER2/CTLs	TCMC	Phase I/II trial in advanced HER2+ sarcomas. SDR: 23%. No significant toxicities (4-1BB not incorporated) [100]
	HER2.CD28.CMV-CAR	HER2, CMV-Ag/CMV-CTLs	TCMC	Phase I trial in HER2+ GBM. No significant toxicities. CTLs could persist for up to 3 months; CBR: 38% [102]
	HER2.CD28.TGF-β DNR.EBV-CAR	HER2, TGF-β, CMV-Ag/TGF-β resistant EBV-CTLs	TCMC	Phase I trial in HER2+ tumors. Active, not recruiting [NCT00889954]
	HER2.CD28-CD3ζ-CAR	HER2/CTLs	TCMC	Phase I trial in HER2+ GBM. Intracranial injection of CAR-T. Recruiting [NCT02442297]
	HER2-CAR	HER2/CTLs	TCMC	Phase I/II trial in pretreated HER2+ tumors. Recruiting [NCT02713984]
	HER2.4-1BB.CD3ζ-CAR	HER2/CTLs	TCMC	Phase I/II trial in pretreated HER2+ tumors. Recruiting [NCT01935843]
	HER2.CD28-CD3ζ-CAR	HER2/CTLs	TCMC	Phase I/II trial in HER2-positive BC. Completed, pending results [NCT02547961]
	FcγRIIIa-V158.CD8α.4-1BB.CD3ζ ACTR plus trastuzumab	Fc, HER2, FcγRs/CTLs, NK cells, monocytes, **MØ**, DCs	TCMC, ADCC, enhanced HER2 uptake by DCs	Phase I trial in pretreated HER2+ tumors. Active, recruiting [NCT03680560]

Subcutaneous administration of mAbs	Pertuzumab plus either IV or SC trastuzumab	HER2, FcγRs/NK cells, monocytes, **MØ**, DCs	ADCC, enhanced HER2 uptake by DCs, TCMC	Ongoing randomized phase II neoadjuvant trial in HER2+ BC [NCT03144947]

MHC, major histocompatibility complex; HLA, human leukocyte antigen; TCR, T cell receptor; ICD, intracellular domain; dHER2, truncated recombinant HER2 peptide; Ab, antibody; DCIS, ductal carcinoma in situ; GM-CSF, granulocyte-macrophage colony-stimulating factor; CAR, chimeric antigen receptor; CTL, cytotoxic T lymphocyte; CMV, cytomegalovirus; Ag, antigen; GBM, glioblastoma; TGF, **transforming growth factor** beta; DNR, dominant-negative receptor; EBV, Epstein-Barr virus; ACTR, **antibody**-coupled T cell receptor.