Breast Cancer: Targets and Therapy

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REVIEW

Can Patients with HER2-Low Breast Cancer Benefit from Anti-HER2 Therapies? A Review

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Abstract: Breast cancer (BC) poses a severe threat to the health of women worldwide. Currently, different therapeutic regimens are used for BC according to the pathological classification of HER2-positive or HER2-negative. Clinical reports of HER2-low expression indicate that the condition is HER2-negative, which was ineligible for HER2-targeted therapy. In contrast to HER2-zero tumors, however, HER2-low BC is a heterogeneous disease with unique genetic characteristics, prognoses, and different therapeutic responses. Clinical efficacy has been demonstrated by numerous potent and innovative anti-HER2 medications, particularly antibody–drug conjugates (ADCs). Certain ADCs, including T-DXd, have demonstrated good efficacy in some trials either used alone or in conjunction with other medications. To enhance outcomes in individuals with HER2-low BC, immunotherapy and other treatments are frequently combined with HER2-targeted therapy. There are also alternative strategies that target both HER2 and HER3 or other antigenic sites. We hope more individuals with HER2-low BC will benefit from more precise treatment regimens in the future. This article provides a review of existing research and clinical trials.

Keywords: HER2-low breast cancer, monoclonal antibodies, antibody–drug conjugates, trastuzumab deruxtecan, immunotherapy

Introduction

Breast cancer (BC) has become the most commonly diagnosed tumor in women worldwide.[1] Human epidermal growth factor receptor 2 (HER2)-positive BC accounted for about 15% of all cases and was considered a poor prognostic predictor in the past. However, the survival outcomes of these patients have improved considerably and were similar to HER2-negative with the evolution of anti-HER2 agents.[2–6] In the past, HER2-low BC could not gain clinical benefit from conventional anti-HER2 agents like trastuzumab.[7] However, with the introduction of novel anti-HER2 compounds, BC with low levels of HER2 expression and no detectable Erb-B2 receptor tyrosine kinase 2 (ERBB2) gene amplification might also derive benefit. The efficacy was found when anti-HER2 agents were combined with other therapies such as immunotherapy.

According to the updated 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, the HER2 status is assessed to evaluate HER2 protein expression levels by IHC (immunohistochemistry) and HER2 gene status by ISH (in situ hybridization).[8] HER2-low is defined as IHC1+ or IHC2+ without gene amplification, accounting for about 55%.[9,10] The application of the new guidelines leads to an increase in the negativity rate on HER2 testing for the reclassification of Group 2 and Group 4 cases.[11] The refinement of future diagnostics may be needed to assess HER2 status more accurately.

Based on the expression of hormone receptors (HR) and the status of HER2, BC is classified into four intrinsic molecular subtypes: luminal A (HR-positive and HER2-negative), luminal B (HR-positive and HER2-positive), HER2 enriched (HR-negative and HER2-positive), and triple-negative BC (TNBC, HR-negative and HER2-negative).[12] The

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four subtypes of tumors have different prognoses, and TNBC is generally considered to be the worst.[13] The possibility of improving the treatment of HER2-low BC has great clinical significance. Also, different treatment approaches for the four subtypes, respectively. In clinical practice, HER2-low BC was reported as HER2-negative, which was classified as TNBC or luminal-like. However, HER2-low BC is a heterogeneous disease, which showed distinctive molecular features.[9,14] Compared with HER2-zero tumors, HER2-low BC has a higher rate of HR-positive (64–88%),[9,15] a higher disease-free survival (DFS) and overall survival (OS),[16] and a limited immune response.[17] This implies that they may have differences in response to therapy, and different therapeutic strategies are needed. Even the HR-positive BC, there are about 50% of these tumors present either inherent or acquired resistance to hormonal therapy.[18] Thus, the HER2-low, luminal B BC with endocrine-resistant may also benefit from anti-HER2 therapies.

The NSABP B-47 trial showed that the addition of trastuzumab to adjuvant chemotherapy did not improve the outcomes of patients with HER2-low BC.[7] Pertuzumab failed to improve the outcomes either.[19] It should be noted that this study used pertuzumab monotherapy, which was ineffective in HER2-positive tumors. HER2-low BC was thought not eligible for anti-HER2 monoclonal antibodies.[20] A range of 100,0000–500,000 HER2 receptor molecules is present on the membrane of score 1+ and 2+ BC cells.[21] So, it still makes sense that anti-HER2 agents could have clinical applications on HER2-low BC mechanistically. Novel and more potent anti-HER2 agents may give the tumor tissue a sufficient chemotherapeutic agent. Antibody–drug conjugates (ADCs) are effective and show clinical efficacy when combined with other treatments such as immunotherapy. We need to reconceptualize the significance of HER2-low and explore anti-HER2 treatments that apply to these patients.

HER2-Targeted Therapies

Monoclonal Antibodies

Although the efficacy of trastuzumab and pertuzumab in HER2-low BC was not found,[7,19] there are still novel monoclonal antibodies being tested for treating HER2-low BC. Margetuximab is a second-generation monoclonal antibody which binds to the same epitope on HER2 as trastuzumab but has higher activity against HER2-positive cancer cells than trastuzumab. Based on the Phase III SOPHIA trial (NCT02492711) results, margetuximab was approved by the FDA in December 2020 to treat HER2+ MBC when combined with chemotherapy.[22] In mice, margetuximab showed activity against HER2-low expressing tumor cells.[23]

Although margetuximab demonstrated an acceptable safety profile in Phase I, II, and III studies,[24] in a Phase II study, it has not shown sufficient clinical efficacy. In 22 showing 2+ by IHC without amplification patients whose efficacy results were available, no responses were achieved, and only 6 disease stabilizations were observed. Notably, when combined with pembrolizumab (an anti-PD-1 monoclonal antibody), it showed clinical efficacy in HER2-low gastro-oesophageal adenocarcinoma.[25] This suggests that the therapeutic effects of margetuximab may be displayed when combined with other treatments.

Antibody–Drug Conjugates

ADCs combine the targeted specificity of monoclonal antibodies with the antitumor's ability of cytotoxic drugs to improve the therapeutic index. An ADC is composed of three elements, a monoclonal antibody to hit the specific molecular target of tumor cells and internalize the entire ADC complex via endocytosis, a cytotoxic agent (also called "payload") to kill tumor cells, and a chemical linker to attach the cytotoxic agent to the antibody.[26] A factor influencing the therapeutic index is the number of molecules of payload linked to each antibody, which is called the drug-to-antibody ratio (DAR). The treatment may be less effective if the DAR is lower, while it may be more difficult to tolerate adverse effects if the DAR is higher.[27] If the released payload is permeable, it can enter and kill the neighboring cells, not only the antigen-positive cells. The phenomenon is called the "bystander effect", which is also one of the reasons for its efficacy to treat HER2-low BC.[28] Table 1 summarizes the ADCs which are useful for the treatment of HER2-low BC.

T-DM1 was the first ADC approved by the US Food and Drug Administration (FDA) for the treatment of HER2-positive metastatic breast cancer (MBC) in 2013. The payload is DM1, which connect with trastuzumab via a non-cleavable linker, and the DAR is 3.5.[29] Retrospective analyses found there was no benefit that can be derived from

Table 1 The ADCs and Their Trials for the Treatment of HER2-Low BC

Drug	Study (NCT Number)	Phase	Number	Population and Setting	Experimental Arm	Control Arm	Efficacy	Adverse Events Grade≥3
T-DXd	-, NCT02564900[36]	I	292 (HER2-low: 54)	HER2-low ABC; Disease is refractory to or intolerable with standard treatment, or for which no standard treatment is available	T-DXd	-	cORR: 37.0% cDCR: 87.0% DOR: 10.4 months TTR: 2.7 months PFS: 11.1 months	63.0%: decreases in neutrophil, platelet, WBC counts, anemia, hypokalemia, AST increase, decreased appetite, diarrhea, interstitial lung disease, pneumonitis
	DEBBRAH, NCT04420598[41]	II	41 (HER2-low: Unknown)	HER2-low ABC with progressing BM after local treatment	T-DXd	-	ORR: Unupdated CBR: Unupdated TTR: Unupdated DOR: Unupdated OS: Unupdated	Unupdated
	DESTINY-Breast 04, NCT03734029[38]	III	557 (373; 184)	HER2-low MBC; Prior 1 to 2 lines of chemotherapy/ adjuvant in the metastatic setting	T-DXd	TPC (capecitabine, eribulin, gemcitabine, paclitaxel, or nab-paclitaxel)	mPFS: HR (+): 10.1 vs 5.4 months; HR (-): 8.5 vs 2.9 months mOS: HR (+): 23.9 vs 17.5 months; HR (-): 18.2 vs 8.3 months	52.6%: interstitial lung disease, pneumonitis, others are unknown
	DESTINY-Breast06, NCT04494425[42]	III	850	HER2-low MBC; Prior ≥2 lines of endocrine therapies or an endocrine therapy combined with a CDK4/6 inhibitor.	T-DXd	TPC (paclitaxel, nab-paclitaxel or capecitabine)	OS: Ongoing PFS: Ongoing ORR: Ongoing DOR: Ongoing	Ongoing
	DESTINY-Breast08, NCT04556773[43]	Ib	182	Module 1 (HR+ or HR-): HR-, only 1 prior line of chemotherapy for mBC; HR+, only 1 prior line of endocrine therapy but no prior chemotherapy for mBC; Module 2 (HR-): no prior chemotherapy for mBC; Module 3 (HR-): only 1 prior line of chemotherapy for mBC; Module 4 and 5 (HR+): only 1 prior line of endocrine therapy but no prior chemotherapy for mBC	Module 1: T-DXd + capecitabine Module 2: T-DXd + durvalumab + paclitaxel Module 3: T-DXd + capivasertib Module 4: T-DXd + anastrozole Module 5: T-DXd + fulvestrant	-	ORR: Ongoing PFS: Ongoing DOR: Ongoing OS: Ongoing	Ongoing
	TALENT, NCT04553770[44]	II	88	HR+, HER2-low BC; Previously untreated operable invasive carcinoma of the breast greater than 2.0 cm	A: T-DXd B: T-DXd + anastrozole	-	pCR: Ongoing COR: Ongoing	Ongoing
SYD985	-, NCT02277717[48]	I	99 (HER2+: 50, HER2-low and HR+: 32, HER2-low and HR-: 17)	HER2+ or HER2-low, HR± ABC/ MBC; Rogressed on standard therapy or no standard therapy exists	SYD985	-	ORR (HER2+): 33% ORR (HER2-low and HR+): 27% ORR (HER2-low and HR-): 40%	10%: neutropenia, conjunctivitis

(*Continued*)

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Table 1 (Continued).

Drug	Study (NCT Number)	Phase	Number	Population and Setting	Experimental Arm	Control Arm	Efficacy	Adverse Events Grade≥3
MRG002	-, NCT04742153[50]	II	66	HER2-low ABC/ MBC; Prior at least first-line standard treatment for recurrent or metastatic breast cancer	MRG002	-	ORR: 34.7% ORR (HER2 IHC 1+): 34.1% ORR (HER2 IHC 2+): 37.5% ORR (TNBC): 37.5% DCR: 75.5% DCR (TNBC): 62.5%	-: neutrophil count decreased
A66	-, NCT03602079[51]	I–II	49 (HER2-low BC: unknown)	HER2 expression (≥ 1+ determined by IHC) MBC;-	A66	-	Ongoing	Ongoing
PF-06804103	-, NCT03284723[53]	I	95 (HER2-low BC: unknown)	HER2-low (1+) TNBC;-	PF-06804103	PF-06804103 + palbociclib +letrozole	DOR: Ongoing PFS: Ongoing TTP: Ongoing	Ongoing
RC48-ADC	C001 CANCER, NCT02881138; C003 CANCER, NCT03052634[54]	I/Ib	118 (HER2+: 70, HER2-low: 48)	HER2+/HER2-low MBC; Prior ≥2 lines of chemotherapy for MBC	RC48-ADC	-	ORR (HER2-low): 39.6% mPFS (HER2-low): 5.7 months ORR (IHC2+/FISH-): 42.9% mPFS (IHC2+/FISH-): 6.6 months ORR (IHC1+): 30.8% mPFS (IHC1+): 5.5 months	41.5%: neutrophil count decreased, GGT increased, fatigue
	-, NCT04400695z	III	366	HER2-low MBC; Prior anthracycline and 1 or 2 systemic chemotherapy after relapse / metastasis	RC48-ADC	TPC (paclitaxel injection, docetaxel injection, vinorelbine tartrate injection, capecitabine tablets)	PFS: Ongoing ORR: Ongoing DOR: Ongoing DCR: Ongoing TTP: Ongoing OS: Ongoing	Ongoing

Abbreviations: (A) (M) BC, (advanced) (metastatic) breast cancer; TNBC, triple-negative breast cancer; BM, brain metastases; (c)ORR, (confirmed) overall response rate; DCR, disease control rate; DOR, duration of response; TTR, time to response; (m) PFS, (median) progression-free survival; WBC, white blood cells; AST, aspartate transaminase; CBR, clinical benefit rate; (m) OS, (median) overall survival; TPC, the physician's choice; pCR, pathologic complete response; COR, clinical objective response; GTT, gamma-glutamyl transferase.

T-DM1 in the group of HER2-low BC by reviewing HER2 status. In the phase II trial of TDM4258g, HER2-low BC had a poorer clinical efficacy than HER2-positive with an insufficient progression-free survival (PFS) (2.6 vs 8.2 months) and a lower objective response rate (ORR) (4.8% vs 33.8%).[30] Similar results were also shown in the TDM4374g trial.[31] The lack of bystander effects limits its utility in tumors with HER2-low.

Trastuzumab deruxtecan (T-DXd) is the second ADC approved by the FDA. It is composed of humanized HER2-targeted antibody and DXd (derivative of exatecan), conjugated through enzyme-cleavable linker making it stable in plasma.[32,33] The payload is cell membrane permeable, so it has bystander effect to kill tumor cells in proximity regardless of their HER2 expression status.[34] At the same time, DXd is a topoisomerase I inhibitor, which is different from the mechanism of chemotherapy drugs commonly used in the treatment of BC, resulting in a reduced risk of cross-drug resistance. Besides, T-DXd also has a higher DAR than T-DM1 (8 vs 3.5), allowing it to deliver more payload molecules to targeted tumor cells.

Based on the results of DESTINY-Breast01 (NCT03248492), T-DXd has been approved for posterior line therapy in patients with HER2-positive BC by the FDA with a confirmed ORR of 61.4% (95% CI 54.0 to 68.5) and a median duration of response (mDOR) of 20.8 months (95% CI 15.0 to NE).[35] A Phase I trial (NCT02564900) has reported the efficacy of T-DXd on HER2-low MBC. Preliminary antitumor activity has been demonstrated with the confirmed ORR of 37.0% (20/54; 95% CI, 24.3% to 51.3%) and the DOR was 10.4 months (95% CI, 8.8 months to not evaluable).[36] Another study showed that ORR and DCR of HER2-low BC patients were marginally lower than HER2-positive BC ones who prior treated with T-DM1 alone or in combination with pertuzumab.[37] DESTINY-Breast04 (NCT03734029) is the first phase III trial to test the efficacy of T-DXd in HER2-low MBC patients. Compared with the physician's choice arm, superior PFS and OS were observed in the T-DXd group (mPFS: 10.1 vs 5.4 months; OS: 23.9 vs 17.5 months). Fewer grade ≥3 treatment-emergent adverse events occurred (52.6% vs 67.4%).[38] Benefit also showed the improvement in health-related quality of life.[39] T-DXd was well tolerated and had significant activity effective in patients with HER2-low BC. T-DXd also demonstrated evidence of central nervous system activity in HER2-positive and HER2-low orthotopic patient-derived xenografts models of MBC with brain metastases, which are resistant to T-DM1.[40] Unlike T-DM1, T-DXd is effective in patients with low-HER2 BC. The DEBBRAH trial assessed the efficacy and safety of T-DXd in HER2-positive and HER2-low ABC patients with a history of brain metastases and/or leptomeningeal carcinomatosis. The trail has been completed, but the data are not reported.[41] Another three trials evaluating the efficacy of T-DXd in treating HER2-low BC are ongoing (NCT04494425, NCT04556773, NCT04553770),[42–44] and one is evaluating the clinical activity of neoadjuvant T-DXd and T-DXd/endocrine therapy in patients with HR+/HER2-low early BC.[45] The publication of the results of these studies may further expand the therapeutic scope of T-DXd, which is effective in both HER2-low and HER2-positive BC.

Trastuzumab duocarmazine (SYD985) is a novel ADC composed of trastuzumab, duocarmycin, and a cleavable linker.[46] Despite it has a lower DAR of 2.8, SYD985 has been shown significantly more potent than T-DM1 in HER2-low BC. Dokter et al predicted that its membrane-permeable nature and the cleavable linker in SYD985 make it has great bystander effects.[47] It has shown efficacy on HER2-low MBC in a phase I trial (NCT02277717), and the ORR in HR-positive and triple-negative BC were 27% (N=9) and 40% (N=7) respectively, compared with 33% (N=16) in HER2-positive BC.[48] That indicates SYD985 might be another treatment option in future for the treatment of HER2-low BC.

MRG002 refers to a novel ADC conjugating monomethyl auristatin E derivative (MMAE) to humanized monoclonal antibody of MAB802 via a protease cleavable valine-citrulline linker with an average DAR of 3.8.[49] At the 2022 AACO Annual Meeting, the results of a phase II (NCT04742153) clinical trial were released. This trial tested MRG002 in 66 patients with HER2-low MBC. The trial showed promising efficacy and a favorable safety profile with an ORR of 34.7% and a DCR of 75.5%, and the most common treatment related adverse events were grade 1 or 2. ORR was similar in the HER2 IHC1+ and IHC2+ subgroups (34.1% VS 37.5%). TNBC patients also benefited from MAB802 with ORR (37.5%) and DCR (62.5%).[50] MRG002 is a highly promising ADC for the treatment of HER2-low BC.

A66 is also an ADC target HER2, which adopts a stable protease-cleavable valine citrulline linker and monomethyl auristatin F derivative. In Phase I–II, the first-in-human study for A166 (NCT03602079), the efficacy of HER2-low BC was evaluated in Phase II, cohort 3 experimental group. The relevant results will be published shortly.[51] PF-06804103 is an ADC with anti-HER2 immunoglobulin G1, which is conjugated with a cleavable linker to the cytotoxic agent

auristatin microtubule inhibitor Aur0101.[52] A site-specific ADC PF-06804103 was tested in patients with HER2-positive and HER2-negative BC in the first-in-human trial (NCT03284723).[53] In this study, HER2-negative performed status of 0 or 1. It also explored the combination of PF-06804103, palbociclib and letrozole. The study has been completed but the results are not yet available. RC48-ADC was generated by conjugating MMAE to a humanized anti-HER2 antibody via a protease cleavable valine-citrulline linker. A pooled analysis of two studies (C001 CANCER, NCT02881138; C003 CANCER, NCT03052634) reported the ORR of HER2-low BC was 39.6% and mPFS was 5.7 months. Patients with IHC2+/FISH- BC had better outcomes than IHC1+ ones.[54] BC with different HER2 status responds differently to RC48-ADC. Meanwhile, a phase III trial (NCT04400695) is ongoing to compare RC48-ADC with the physician's choice in HER2-low BC.

HER2-Targeted Bispecific Antibodies (bsAbs)

bsAbs have two different antigen-binding sites to address different antigens or epitopes, promoting immune cell recruitment and activation.[55] ZW25 (zanidatamab) is a novel HER2-targeted bsAb, targeting HER2 domains ECD2 and ECD4, for which antitumor activity, synergy and additivity with multiple chemotherapeutic agents have been exhibited in HER2-low to -high expressing models.[56] A phase I clinical trial (NCT02892123) is investigating ZW25 in HER2-expressing tumors, of which cohort 4 and 7 in part 1, cohort 1 in part 2 and part 3 involves HER2 IHC2+/FISH- BC. The study is still ongoing with no results about HER2-low BC published. Combining a novel auristatin payload with ZW25, an ADC ZW49 is designed. ZW49 has demonstrated antitumor activity in HER2-low BC cell lines and patient-derived xenograft (PDX) models.[57] However, there are no relevant clinical trials evaluating efficacy in the HER2-low BC population.

HER2/HER3-Targeted Therapies

ErBb family includes four distinct receptors, HER1 (EGFR or ErbB1), HER2, HER3, and HER4. HER2 overexpression is associated with the activation of HER3, and some models show that HER2-driven tumors do not develop or grow if HER3 expression is absent.[58–60] Treatments show efficacy in HER2-low BC when targeting both HER2 and HER3. The HER2/HER3 heterodimer induces phosphorylation of the ER and endocrine resistance.[61] Preclinical data showed that the triple combination of anti-HER2 (pertuzumab), anti-HER3 (lumretuzumab), and anti-estrogen therapy (fulvestrant) lead to long-lasting responses in ER+/HER2-low/HER3+ human BC HBCx-19 xenograft model.[62] A study (NCT01918254) evaluated the clinical activity and safety of lumretuzumab, pertuzumab and paclitaxel in HER3-positive, HER2-low MBC. Chronic diarrhea is the major side effect; thus, the initial antitumor activity could not be confirmed.[63] Mitigating the toxic effects of combination therapies is a key issue in achieving effectiveness.

The bsAbs targeting HER2/HER3 are undergoing clinical development. MCLA-128 (zenocutuzumab) is a humanized bsAb targeting HER2 and HER3. The addition of MCLA-128 to estrogen therapy demonstrated to a better antitumor effect than estrogen therapy alone in HER2-low BC xenograft models.[64] It also showed clinical activity in patients with ER-positive, HER2-low BC after estrogen therapy and CDK4/6i failure according to a phase II trial (NCT03321981). The disease control rate (DCR) was 45% with 2 patients having an unconfirmed partial response and 19 patients keeping the disease stable. It also showed a favorable safety profile with no grade 5 adverse events or diarrhea requiring treatment discontinuation observed.[65] Bispecific humanized IgG1 antibody is safer than the combination therapies when targeting HER2/HER3.

HER2/Other Antigenic Sites-Targeted Therapies

Some therapeutic approaches block both HER2 and other antigenic sites. Evorpacept (ALX148) is a high-affinity CD47-blocking fusion protein that enhances the activity of other antitumor therapies. The safety and antitumor activity of HER2-targeted bsAbs ZX25 in combination with ALX148 is being assessed in a phase Ib/II study (NCT05027139).[66] SAR443216 is a trispecific antibody with binding sites for HER2, CD3 and CD28. It is a HER2-targeted T cell engager that can activate CD4 and CD8 T-cells and has the activity of T cell-dependent cellular cytotoxicity (TDCC) against HER2-expressing tumor.[67] Two hundred patients with HER2-expressing (various levels of HER2 expression) solid

tumors including BC participate in a trial (NCT05013554). The preliminary clinical activity of SAR443216 after intravenous and subcutaneous administration will be assessed.

Immunotherapy

Immune Checkpoint Inhibitor

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of immunogenic cancers by enabling the priming and infiltration of T-cells into the tumor microenvironment, promoting cytotoxic signaling pathways and affecting tumor cytolysis by specifically recognizing, binding to cancer cells and killing them.[68] BC is a moderately immunogenic cancer,[69] in which TNBC and HER2-positive BC have the highest immunogenic potential.[70] However, intrinsic resistance is a big problem to the use of ICIs, which occurs in 60–85% of the patients with TNBC.[71] Some cytotoxic drugs can induce immunogenic cell death in tumor cells, promote tumor antigen release and activate the immune system.[72] HER2-targeted drugs can kill tumor cells by the immune-mediated mechanism of antibody-dependent cellular cytotoxicity (ADCC). Therefore, ICIs are often combined with monoclonal antibodies and ADCs. The combination of trastuzumab with ICIs could overcome trastuzumab resistance in a study.[73] Six of 40 programmed cell death 1 ligand 1 (PD-L1) -positive-patients achieved an objective response, but no objective responders among the PD-L1-negative patients.[74] The payload DXd of T-DXd is a topoisomerase inhibitor, which has gained attention as an immunomodulator.[75] Mechanically, T-DXd is an ideal partner for ICIs. In preclinical studies, T-DXd increased the efficacy of ICIs by increasing immune cell infiltration and upregulating PD-L1 expression when combined with anti-PD-L1 antibody.[76]

Programmed cell death protein 1 (PD-1) and its ligand PD-L1 have been evaluated as putative markers of response to immunotherapy with PD-1/PD-L1 blockade in BC.[77] Anti-PD-1 or anti-PD-L1 therapies have been investigated in combination with HER2-targeted therapies. T-DXd combined with a PD-1 inhibition was more effective than monotherapy with either agent in vitro studies. Nivolumab is the first PD-1 blocking antibody approved for clinical use, and there are many ongoing trials to assess the safety and efficacy of nivolumab combined with other therapies for BC. The combination of T-DXd and nivolumab was tested in a phase Ib trial (NCT03523572). Cohort 2 involved 16 patients with HER2-low MBC who had exhausted any clinically meaningful treatments. After 12.7 months of follow-up, the confirmed ORR (cORR) was 50% and the median PFS was 7.0 months comparing the HER2-positive cohort with a cORR of 65.6% and median PFS of 11.6 months.[78] Combining nivolumab may result in an improved ORR compared to T-DXd alone. Pembrolizumab is another PD-1 inhibition showing efficacy in this subgroup when combined with T-DXd. In the dose extension of part 2 of a trial (NCT04042701), patients with HER2-low BC were treated with pembrolizumab and T-DXd.[79] The estimated completion date of the trial is in May 2023 and ORR is the primary outcome measure.

PD-L1 binds to PD-1 and CD80 receptors, resulting in the inhibition of T-cell function, which can be blocked by durvalumab.[80] Randomized phase Ib/II study BEGONIA reported its initial results, in which cORR was 100% (4/4) and median DOR was not reached.[81] Additionally, DESTINY-Breast08 is also comparing the combination of T-DXd, durvalumab and paclitaxel with others (T-DXd combines with endocrine therapy, chemotherapy, and immunotherapy). No deaths or cases of interstitial lung disease/pneumonitis were reported so far.[82] T-DM1 in combination with atezolizumab (a PD-L1 inhibitor) was effective only in PD-L1-positive BC in the trial KATE2 (NCT02924883), which suggested that the benefit of the combination of PD-L1 inhibitors may be limited to PD-L1-positive patients.[83] It may also be helpful to assess the patient's PD-L1 status to better evaluate the efficacy of T-DXd in combination with immunotherapy. The combination trials with ICIs to treat HER2-low BC are listed in Table 2.

Apart from these, immune-stimulating antibody conjugates (ISACs) are also under development in HER2-positive BC and TNBC.[84] An ISAC comprises a tumor-targeting monoclonal antibody conjugated to an immune agonist, exerting durable antitumor immunity.[85] They are well-tolerated in vivo. For instance, BDC-1001 a novel ISAC and it did not induce interstitial lung disease, cytokine release syndrome, or thrombocytopenia in non-human primate studies.[86]

HER2-Derived Vaccines

Unlike trastuzumab and pertuzumab, which are passive immunotherapy, HER2-derived vaccines are active immunotherapy. Cancer vaccines are designed to elicit or enhance antitumor immune responses by activating immune cells to induce

Table 2 Trials of ICIs in Combination with Other Drugs

Study (NCT Number)	Phase	Target	Number	Population and Setting	Experimental Arm	Control Arm	Efficacy	Adverse Events Grade≥3
-, NCT03523572[78]	Ib	PD-1	48 (HER2+: 32, HER2-low: 16)	HER2-low MBC; Prior received all clinically meaningful treatments	T-DXd + nivolumab	-	cORR (HER2+): 65.6% cORR (HER2-low): 50% mPFS (HER2+): 11.6 months mPFS (HER2-low): 7.0 months mDOR (HER2+): NE mDOR (HER2-low): 5.5 momths	50%: nausea, drug-related interstitial lung disease
-, NCT04042701[79]	Ib	PD-1	115 (HER2-low BC: unknown)	HER2-low MBC; Prior received all clinically meaningful treatments	T-DXd + pembrolizumab	-	ORR: Ongoing DOR: Ongoing DCR: Ongoing PFS: Ongoing TTR: Ongoing OS: Ongoing	Ongoing
BEGONIA, NCT03742102[81]	Ib/II	PD-L1	210 (HER2-low BC: unknown)	Advanced/unresectable or metastatic TNBC with HER2 low; Unknown	T-DXd + durvalumab + paclitaxel	-	cORR: 100% (4/4)	Ongoing
DESTINY-Breast08, NCT04556773[43]	Ib	PD-L1	182	As listed in Table 1	As listed in Table 1	As listed in Table 1	As listed in Table 1	As listed in Table 1

Abbreviations: (c) ORR, (confirmed) overall response rate; (m) PFS, (median) progression-free survival; (m) DOR, (median) duration of response; DCR, disease control rate; PFS, progression-free survival; TTR, time to response; OS, overall survival.

a therapeutic effect.[87] HER2 is a tumor-associated antigen identified as an appropriate vaccine source in BC.[88] HER2-derived vaccines have been studied extensively in HER2-positive BC, and several trials are also conducted in HER-low BC recently.

E75 (Nelipepimut-S, NPS, KIFGLSAFL) vaccine is one of the most widely researched BC vaccines against HER2. In an early clinical study, patients with HER2-low specifically IHC 1(+) BC benefited more from E75 vaccine than those with higher levels of HER2 expression.[89] However, the phase III trial PRESENT (NCT01479244) terminated due to failure to show a significant difference in DFS in 758 patients with HER2-low, node-positive BC.[90] A phase IIb trial (NCT01570036) enrolled 275 disease-free patients after standard therapy completion. Treated with the combination of E75, granulocyte-macrophage colony-stimulating factor and trastuzumab, the estimated DFS of HER2-low patients did not significantly differ from the control group (HR, 0.62; 95% CI, 0.31–1.25; P= 0.18).[91] However, the combination improved 36-month DFS among patients with TNBC (HR, 0.25; 95% CI, 0.08–0.78, p = 0.01) and HLA-A24 (HR, 0.41; 95% CI, 0.16–1.04; P= 0.05) positivity according to a subgroup analysis.[92] There may be a synergistic effect between E75 and HER2-targeted therapy, and further investigation is warranted to be confirmed.

AE37 is another HER2-related peptide vaccine to treat BC which can induce CD8 and CD4 cells.

A trial (NCT00524277) enrolled 298 patients with disease-free node-positive and high-risk node-negative BC expressing any degree of HER2. After receiving AE37 and GM-CSF, 5-year DFS was 77.2% versus 65.7% of the control group in planned subset analyses of HER2-low patients (P=0.21). Specifically, patients with TNBC are more likely to have clinical benefits in DFS (77.7% vs 49.0%, P = 0.12).[93]

The above two vaccines with a tolerable and favorable safety profile. There are some other vaccines tested in patients with HER2-low BC. Cornerstone-001 (NCT05163223) is a phase II trial to assess the efficacy and safety of pNGVL3-hICD (AST-301) plasmid-based vaccine in patients with HER2-low and HR-negative BC with residual disease after neoadjuvant treatment. The trial started on 28 February 2022 and is expected to be completed in December 2025. ETBX-021 is another HER2-targeted vaccine that is being evaluated in phase I clinical trial (NCT02751528) with locally advanced or metastatic HER2-low BC.

CDK4/6 Inhibitors

CDK4/6 is downstream of HER2 and driving resistance to HER2-targeted therapies.[94] CDK4/6 inhibitors not only induce tumor cell cycle arrest but also promote antitumor immunity by enhancing tumor antigen presentation and impacting immunosuppressive cells such as CD4 T-cells.[95,96] The phase II trial of NA-PHER2 evaluated the effects of Ki67 and apoptosis after neoadjuvant pertuzumab, trastuzumab, fulvestrant and palbociclib (a CDK4/6 inhibitor). In cohort C, 23 women with ER-positive and HER2-low BC participated. More than 90% tumors showed a drop of Ki67 below 10% at week 2 and complete cell cycle arrest was achieved in 65%. The ORR before surgery was 78.3%, but no patient achieved a pathologic complete response.[97] HER2-low status does not affect survival outcomes of patients with MBC,[98] and CDK4/6 inhibitors may play an important role in the treatment of HER2-low BC when combined with HER2-targeted therapies.

Others

EZH1 and EZH2 are two markers of aggressive BC and are associated with invasion and cancer progression.[99,100] Valemetostat (DS-3201) is a novel and potent EZH1/2 dual inhibitor. A study (NCT05633979) is designed to test the efficacy and safety of the combination of valemetostat and T-DXd in HER2 low/ultra-low/null (IHC2+/ISH-, IHC1+; IHC0 with detectable faint/barely perceptible incomplete staining in ≤10% tumor cells; IHC0 without any observed tumor cell staining) MBC patients. The study is estimated to start on 23 May 2023. Some tyrosine kinase inhibitors are pan-HER kinase inhibitors already widely used in HER2-positive MBC.[101] Lapatinib (a tyrosine kinase inhibitor) increased HER2 levels and potentiated ADCC in preclinical models.[102] Adding endocrine therapy may improve the efficacy of HER2- and HER3-targeted agents according to a study.[62] Likewise, HER2-targeted agents play a role in the resistance to endocrine therapy,[103] and the HER2-negative patients gained greater benefit from aromatase inhibitors following the TRANS-AIOG meta-analysis.[104]

Discussion

IHC/ISH is the gold standard to define HER2 expression. However, some factors remarkably affect the analytical reliability.[105] Although HER2-low expression has not been formally defined, HER2-low BC is different from HER2-negative cases for which the former can benefit from many anti-HER2 therapies. HER2-low BC with IHC1+ and 2+ may respond differently to HER2-targeted therapies.[54] So more precise identification is needed by harmonizing testing strategy and technique. HER2 gene–protein assay (HER2 GPA) can be used for assessing HER2 status of HER2-positive, equivocal, and -negative BC by combining HER2 ISH and HER2 IHC assays.[106] Other techniques also facilitate the determination of HER2 status such as automated image analysis and digital PCR.[107,108]

Approximately half of BC was HER2-low[109]; in some cases, HER2-negative eventually converted to HER2-low phenotype.[110] A study showed that after neoadjuvant therapy, HER2-low converted from 14.8% of the primary tumors to 8.9% of residual disease.[110] Patients with ER-positive HER2-low residual disease have a high risk of relapse compared to ER-positive HER2-negative cases, who may need HER2-targeted therapies.[110] A similar evolution of HER2-low expression can also be observed from primary to recurrent.[111] HR-positive HER2-negative BC faces the problem of drug resistance to endocrine therapy and TNBC has limited treatment options. A large population of patients have BC, which is a highly unmet medical need.

ADCs play an important role in the treatment of this subtype and T-DXd may be firstly approved because of the bystander effect and its cell membrane permeable payload. HER2-targeted therapies often show clinical efficacy when combined with other treatments, such as immunotherapy.[79] There are also strategies that target both HER2 and HER3 or other antigenic sites.[64] These treatments are usually well tolerated. In many cases, serious adverse events occur after using different drugs, such as the combination of lumretuzumab, pertuzumab and paclitaxel.[63] The design of the conjugates better limits the adverse effects. More and more studies will be conducted to explore the efficacy of HER2-targeted therapies for HER2-low BC in the future, and more patients will benefit from these treatments.

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