CURRENT OPINION

HER2-positive breast cancer: cotargeting to overcome treatment resistance

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Purpose of review
The introduction in clinical practice of anti-HER2 agents changed the prognosis of patients with HER2-positive (HER2+) breast cancer in both metastatic and early setting. Although the incomparable results obtained in the last years with the approval of new drugs targeting HER2, not all patients derive benefit from these treatments, experiencing primary or secondary resistance. The aim of this article is to review the data about cotargeting HER2 with different pathways (or epitopes of receptors) involved in its oncogenic signaling, as a mechanism to overcome resistance to anti-HER2 agents.

Recent findings
Concordantly to the knowledge of the HER2+ breast cancer heterogeneity as well as new drugs, novel predictive biomarkers of response to anti-HER2 treatments are always raised helping to define target to overcome resistance. Cotargeting HER2 and hormone receptors is the most well known mechanism to improve benefit in HER2+/HR+ breast cancer. Additional HER2-cotargeting, such as, with PI3K pathway, as well as different HERs receptors or immune-checkpoints revealed promising results.

Summary
HER2+ breast cancer is an heterogenous disease. Cotargeting HER2 with other signaling pathways involved in its mechanism of resistance may improve patient outcomes. Research efforts will continue to investigate novel targets and combinations to create more effective treatment regimes.

Keywords
anti-HER2 agents, breast cancer, cotargeting, HER2-positive, resistance

INTRODUCTION

Approximately 20% of breast cancers overexpress the human epidermal growth factor receptor 2 (HER2) defining the HER2-positive (HER2+) subtype [1]. HER2+ breast cancer were historically associated with poor prognosis. The introduction of HER2-targeted therapies, including monoclonal antibodies (mAb, trastuzumab, pertuzumab margetuximab), tyrosine kinase inhibitors (TKIs, lapatinib, neratinib tucatinib), and antibody-drug conjugates (ADC, trastuzumab emtansine (TDM1) or deruxtecan (Tdxd)) overturned the patients' outcomes with HER2+ disease [2,3]. Nonetheless, de-novo or acquired resistance to anti-HER2 therapies represents a major hindrance in the treatment of this cancer type, leading to early recurrence in up to 25% of patients with early breast cancer (EBC) and progression in metastatic breast cancer (MBC) [4,5]. Understanding mechanisms involved in resistance to HER2-targeted therapies is crucial for developing strategies to overcome it, improving the patients' outcomes. This review provides an overview of the current knowledge on the mechanisms of resistance to HER2-agents, focusing on the opportunities offered by cotargeting approaches to improve treatment efficacy in both the early and metastatic settings.

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KEY POINTS

- HER2-positive breast cancer is a heterogenous disease.
- Response to anti-HER2 agents is affected by various elements that can induce to primary or secondary resistance.
- Cotargeting HER2 with pathway of signaling involved in its resistance may be effective to restore the sensitivity to HER2-agents.
- The use of novel anti-HER2 based combination strategies is continuedly under investigation.

OFF-TARGET RESISTANCE TO ANTI-HER2 AGENTS AND RATIONALE FOR COTARGETING

HER2 signaling

Binding between growth factors and the HER2-tyrosine kinase receptor (RTK) induces HER2 oncogenic signaling by HER2 homo or heterodimerization with other receptors of the human epidermal growth factor (EGFR) family (HER1/EGFR, HER3, and HER4) (Fig. 1), activating the downstream PI3K/AKT and MAP kinase pathways.

EGFR upregulation activates HER2-signaling and its overexpression has been observed in both preclinical and clinical HER2+ breast cancer resistant to trastuzumab [6–9].

HER3 and HER2 overexpression are strictly related and associated with inferior outcomes in HER2+ breast cancer [10–13]. HER3/ERBB3 is the main partner involved in HER2 heterodimerization, inducing a stronger HER2-signaling, particularly activating PI3K/AKT-pathway [12,14,15].

The role of HER4/ERBB4 is controversial, with limited data suggesting reduced anti-HER2 sensitivity in the context of ERBB4 overexpression in preclinical models [10,16,17].

Anti-HER2 agents inhibit HER2 oncogenic signaling in different ways. Trastuzumab-based agents inhibit HER2-signaling upfront, blocking the extracellular subdomain IV of the HER2 receptor. Pertuzumab, binding the subdomain II of the HER2 receptor, avoids heterodimerization especially with HER3. All HER2-TKIs block the intracellular ATP-binding pocket of HER2 or panHER- RTK. Thus, resistance to anti-HER2 agents can include different mechanisms involving altered HER2-expression (heterogeneity and stability of the target), HER2-homo/heterodimerization, disrupted downstream pathways or collateral RTK signaling, and alterations in tumor microenvironment (especially mAbs and ADCs that can elicit immune-mediated effects). One mechanism of action of trastuzumab is the stimulation of antibody-dependent cell cytotoxicity (ADCC), leading to INF and TGFβ release, which induces high PDL1-expression in tumor cells mediating trastuzumab-resistance [18,19]. Notably, different from other anti-HER2 agents, the efficacy of ADCs targeting HER2 seems not to be influenced mostly by altered downstream pathways but rather from tumor-heterogeneity including target expression, deregulation of tracking proteins or lysosomes [20]. These different ways involved in altered HER2 signaling and resistance to anti-HER2 agents create the rationale to potential new treatment combinations. ADCC mechanism by trastuzumab induces the cross presentation of HER2 specific epitopes by dendritic cells explaining a possible role in the combination of active immunotherapy, such as vaccines, with HER2-mAb [21]. Preliminary data demonstrated the safety and synergy of action between HER2-derivated vaccine (peptide, allogeneic breast cancer and dendritic cells vaccines) and trastuzumab [22]. Moreover, the use of sequential or combined ADC with same or different targeting or payloads can help to overcome anti-HER2 resistance [23].

Hormone receptors cross-talk

Approximately 50% of HER2+ breast cancer cells co-express hormone receptors (HR), named also triple-positive breast cancer [24]. Crosstalk between hormone receptors and HER2 signaling entails resistance to both endocrine therapy and anti-HER2 agents. Hormone receptors can activate HER2-signaling both via G-protein interactions or by activating downstream HER2-mediators [25,26]. Moreover, upregulation of hormone receptors has been described in HER2+ tumor cells following exposure to anti-HER2-agents to drive resistance [27]. This concept is further validated by clinical trials, with triple-positive breast cancer showing lower response to anti-HER2 regimes in the context of high HRs expression (either by immunohistochemistry or mRNA)l [28–30]. Many studies have described the biology behind the triple-positive breast cancer subtype influenced by various factors (e.g., intrinsic molecular subtypes, immune, or tumor gene signatures expressions) determining distinct behavior [31,32].

Downstream pathways

PI3K/AKT/mTOR

The PI3K/AKT/mTOR pathway is involved in the downstream signaling of HER2, and its hyperactivation determines HER2-independent activation of

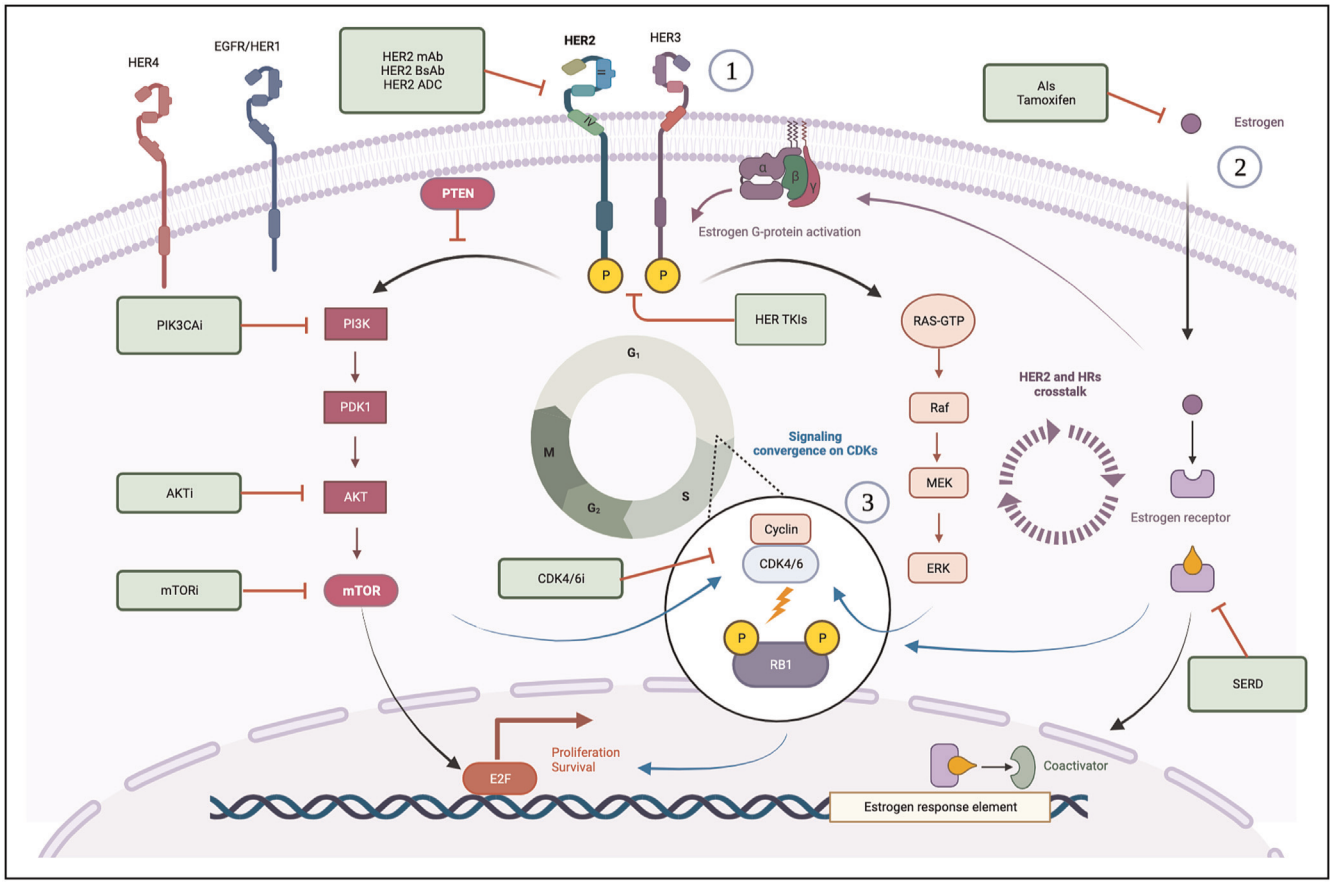

FIGURE 1. Cotargeting strategies in HER2-positive breast tumors. Keys. (1) HER2 is activated through homo or heterodimerization, leading to activation of the PI3K/AKT and MAPKK pathways. (2) Hormone receptors play a pivotal role in HER2 signaling either by activating G-proteins to activate upstream HER2 signaling or by influencing downstream mediators. (3) Intracellular pathways converge to activate cell cycle mediators such as cyclins and CDKs. These pathways offer compelling reasons for selective targeting strategies. AIs, aromatase inhibitors; AKTi, AKT inhibitors; CDK4/6, cyclin-dependent kinase; E2F, elongation factor 2; HER TKIs, human epidermal growth factor receptor tyrosine kinase inhibitor; HER2 ADC, HER2 antibody drug conjugate; HER2 BsAb, HER2 bispecific antibodies; HER2 mAB, HER2 mAbs; HRs, hormone receptors; mTORi, mTOR inhibitors; PIK3CAi, PIK3CA inhibitors; RB1, retinoblastoma 1; SERD, selective estrogen receptor degrader.

the mTOR pathway, mediating anti-HER2 therapy resistance [33,34]. Alteration of the PI3K/AKT/mTOR pathway occurs in approximately 40% of HER2+BC, mostly with mutations in the α subunit of phosphatidylinositol 3-kinase (PI3K) enzyme (*PIK3CA*-gene), as well as loss of phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), a regulator of this pathway [35,36]. In HER2+ EBC, PIK3CA mutation is associated with reduced response to anti-HER2 therapy, translating in lower pathologic complete rate (pCR), with controversial results in long-term outcomes often driven by HRs co-expression [37–41]. In the metastatic setting, data are more consistent, with PIK3CA-mutations associated with lower progression-free survival (PFS) following treatment with lapatinib or anti-HER2 mAbs-based regimens, but not with TDM1 [42,43]. Deregulation of this pathway can derive also from the activation of non-HER2 RTK, such as insulin-like growth factor I receptor (IGF-1R), often overexpressed in solid tumor [44,45].

In contrast to PIK3CA, conflicting data are available regarding the role of PTEN loss in anti-HER2 sensitivity in both early and advanced settings [42,46,47].

Cyclin-dependent kinase

HER2-signaling converges to activate the cell cycle. Upon activation of HER2, increased levels of cyclin D1 actives cyclin-dependent kinases (CDKs) to phosphorylate retinoblastoma (Rb), resulting in the release of elongation transcription factors (E2F) to promote cell cycle progression. CDK4/6 have been implicated in resistance to HER2-targeted therapies, and their inhibition combined with HER2-targeting agents demonstrated to re-sensitize HER2+ tumor cells to anti-HER2 regimens in preclinical models [48–52]. Other CDKs, such as CDK2, CDK7, and

CDK12 may drive resistance to anti-HER2 therapies, with preliminary data suggesting that their inhibition may lead to higher HER2-sensitivity [53,54].

COTARGETING STRATEGIES IN THE HER2+ EARLY BREAST CANCER

The use of dual HER2 blocking with pertuzumab and trastuzumab, targeting two different epitopes of HER2 receptor, led to improved outcomes in HER2+ breast cancer. Other efforts were made to design clinical trial investigating novel combination regimes.

HER2 and PD(L1) cotargeting

Modulation of the tumor microenvironment is a pursued strategy to overcome HER2 resistance. Few data are available regarding the use of immunotherapy in HER2+ EBC. A single-arm study with neoadjuvant pertuzumab, trastuzumab, docetaxel, and atezolizumab achieved a pCR-rate of 61% among 67 women, with modest toxicity [55▪▪]. The phase III IMpassion 050 trial, which randomized 454 patients to receive trastuzumab, pertuzumab, and dose-dense chemotherapy, with or without atezolizumab was prematurely interrupted for futility results. No difference in pCR in either the intention-to-treat (ITT) (62.7 vs. 62.4%; $P = 0.9551$) or in PD-L1-positive population (72.5 vs. 64.2%; $P = 0.1846$) was observed. With a median follow-up of 15.7 months, event-free survival (EFS) events occurred in 5.3 and 3.1% of patients receiving atezolizumab or not, respectively [56▪▪]. The phase II Keyriched-1 trial investigated four cycles of neoadjuvant chemo-free regimen of pembrolizumab, trastuzumab, and pertuzumab in 48 HER2+ tumors, selected by PAM50 HER2-enriched subtype. Forty-six patients achieved a pCR, higher among HR- tumors (58.5 vs. 38.5%) [57].

HER2 and PI3K/AKT/mTOR cotargeting

Following encouraging preclinical results with the use of PI3K-inhibitors (PI3Ki) and trastuzumab in HER2+ tumors, clinical trials have been conducted with this combination [58]. The randomized phase II NeoPHOEBE trial investigated the addition of buparlisib, a pan-PI3K inhibitor, to trastuzumab for 6 weeks, followed by the addition of chemotherapy to each treatment arm. The trial was prematurely interrupted for unacceptable toxicity and clinical futility, as no pCR differences were observed (32 vs. 40% with and without buparlisib, respectively; $P = 0.811$) [59]. Notably, a significant reduction in Ki67% with a trend in response was observed after the first 6 weeks of treatment, opening a specific setting in which PI3Ki could be effective [59]. The addition of the mTOR-inhibitor, everolimus, to trastuzumab in the neoadjuvant setting did not improve pCR in the RADHER trial ($P = 0.727$), but the presence of PI3KCA-mutations was associated with trastuzumab resistance [60▪].

In the adaptive clinical trial platform I-SPY2, the pan-AKT inhibitor, MK-2206, was tested in combination with neoadjuvant trastuzumab and paclitaxel. Among 34 HER2+ EBCs, the addition of MK-2206 improved pCR from 29 to 48% [61].

HER2 and CDK4/6 cotargeting

In the early setting, CDK4/6-inhibitors (CDK4/6i) and antiHER2-agents have been mainly tested among triple-positive breast cancer.

The single-arm phase II NA-PHER2 trial investigated a neoadjuvant regimen with pertuzumab, trastuzumab, fulvestrant, and palbociclib in HER2+/HR+ EBC. Twenty-seven percent of 30 enrolled patients achieved pCR, along with a significant decrease in Ki67% expression at 2 weeks of treatment [62]. Differently, neoadjuvant combination of palbociclib, letrozole ,and trastuzumab was prematurely interrupted due to futility (7.7% pCR among 27 HER2+/HR+ EBC) [63].

Altogether, these data suggest potential novel cotargeting options for selected patients, although need for further data from the trials is undergoing (Table 1).

COTARGETING STRATEGIES IN THE HER2+ METASTATIC BREAST CANCER

HER2 and hormone receptors cotargeting

The role of hormone receptors in establishing crosstalks to drive resistance to anti-HER2 agents provides the rationale for combining endocrine therapies (ET) and anti-HER2 therapies. In the TAnDEM, eLecTRa, and EGF3008 trials, the addition of trastuzumab or lapatinib to aromatase inhibitors, respectively, resulted in a well tolerated and effective treatment [64–66]. The randomized SYSUCC-002 trial demonstrated the noninferiority of ET with trastuzumab compared with trastuzumab and chemotherapy as first-line treatment (median PFS 19.2 vs. 14.8 months, respectively; $P < 0.001$) [67]. Following the results of the CLEOPATRA trial with a double-anti-HER2 blockade, the phase II PERTAIN and phase III ALTERNATIVE trials tested the addition of pertuzumab or lapatinib, respectively, to aromatase inhibitor and a trastuzumab-based regimen. In both studies, aromatase inhibitor and

Table 1. Ongoing studies with cotargeting in HER2-positive breast cancer (early and advanced)

Agent	Target	Trial	Enrollment target	Phase	Setting	Regimens	Primary endpoints
Immunotherapy							
Atezolizumab	PD-L1	NCT04873362 (ASTEFANIA)	1700	III	Adjuvant HER2+ eBC with residual disease	Exp: Atezolizumab + T-DM1 Comp: T-DM1	IDFS
		NCT03595592 APTneo	650	III	Perioperative HER2+ eBC	Exp: neoadj atezolizumab + trastuzumab + pertuzumab + chemotherapy, followed by adj atezolizumab + trastuzumab + pertuzumab Comp: neoadj trastuzumab + pertuzumab + chemotherapy, followed by adj trastuzumab + pertuzumab	EFS
		NCT04740918 (KATE3)	96	III	2–3L HER2+ mBC PD-L1+	Exp: Atezolizumab + T-DM1 Comp: T-DM1	PFS; OS
		NCT03125928	16	II	1–2L HER2+ mBC	Atezolizumab + trastuzumab + pertuzumab + paclitaxel	AEs; ORR
		NCT04759248 ATREZZO	55	II	Pretreated HER2+ mBC with prior T-DM1	Atezolizumab + trastuzumab + vinorelbine	ORR
Durvalumab	PD-L1	NCT04538742 (DESTINY-Breast07)	245	I-II	Part 1: ≥2L HER2+ mBC Part 2: 1L HER2+ mBC	Module 1: durvalumab + T-DXd Module 4: durvalumab + T-DXd + paclitaxel	AEs; SAEs
Pembrolizumab	PD1	NCT04042701	115	I	HER2+ mBCs who received prior T-DM1; HER2+/HER2^{mut} NSCLC	Pembrolizumab + T-DXd	DLTs; ORR
		NCT04789096 TUGETHER	50	II	Pretreated HER2+ mBC	Arm 1: pembrolizumab and trastuzumab (PD-L1+) Arm2: pembrolizumab, trastuzumab and capecitabine (PD-L1-)	ORR
		NCT03747120	174	II	Neoadjuvant HER2+ eBC	Arm 1: trastuzumab, pertuzumab, paclitaxel Arm 2: pembrolizumab, trastuzumab, pertuzumab, paclitaxel Arm 3: pembrolizumab, trastuzumab, paclitaxel	pCR
PI3K/AKT/mTOR							
Alpelisib	PIK3CA	NCT05063786 (ALPHABET)	144 HER2+/ HR-156 HER2+/ HR+	III	2–5 l mBC PIK3CA^{mut} (prior T-DM1 mandatory)	Exp: Alpelisib + trastuzumab (plus fulvestrant in HR+) Comp: Trastuzumab + chemotherapy (either vinorelbine, capecitabine, eribulin)	PFS
		NCT04208178 (EPIK-B2)	511	III	1L MBC PIK3CA^{mut} maintenance setting after induction chemotherapy	Exp: Alpelisib + Trastuzumab + Pertuzumab Comp: Trastuzumab + Pertuzumab	PFS
		NCT05230810	40	I-II	≥3L HER2+ MBC	Alpelisib + Tucatinib (plus Fulvestrant in HR+)	MTD; AEs; PFS (in phase II)
Copanlisib	PIK3CA, PIK3CG	NCT04108858	12	I/II	Pretreated mBC with PIK3CA^{mut} or PTEN^{mut}	Copanlisib + trastuzumab + pertuzumab	
Taselisib	PIK3CA^{mut} specific	NCT02390427	68	I	Pretreated HER2+ mBC	Arm 1: Taselisib + T-DM1 Arm 2: Taselisib + T-DM1 and pertuzumab Arm 3: Taselisib + pertuzumab and trastuzumab Arm 4: Taselisib + with pertuzumab, trastuzumab and paclitaxel	MTD
MEN1611	PIK3CA, PIK3CB, PIK3CG	NCT03767335 (B-PRECISE-01)	62	I	>2L MBC HER2+ PIK3CA^{mut}	MEN1611 + trastuzumab (plus Fulvestrant in HR+)	MTD

Table 1 (*Continued*)

Agent	Target	Trial	Enrollment target	Phase	Setting	Regimens	Primary endpoints
Capivasertib	PIK3CA	NCT01226316	285	I	AKT1^{mut} or PIK3CA^{mut} or PTEN^{mut}	Capivasertib	AEs
GDC-0084	panPI3K, mTOR	NCT03765983	47	II	Pretreated HER2+ mBC with brain mets	GDC-0084 + trastuzumab	ORR in CNS
Ipatasertib	AKT	NCT04253561 (IPATHER)	15	I	1L HER2+ mBC maintenance setting	Ipatasertib + trastuzumab + pertuzumab	RP2D
CDK4/6 inhibitors							
Palbociclib	CDK4/6	NCT02448420 (PATRICIA II)	102	II	≥2L mBC Cohort C1 and C2: HER2+/ HR+ Luminal A and Luminal B BC	Arm 1: Trastuzumab + Palbociclib + ET Arm 2: TPC	6-month PFS; PFS
		NCT02947685 (PATINA)	496	III	1L HER2+ mBC maintenance setting	Induction chemotherapy: Trastuzumab + Pertuzumab + Taxane Maintenance regimen: Exp: Palbociclib + Trastuzumab + Pertuzumab Comp: ET vs. Trastuzumab + Pertuzumab + ET	PFS
		NCT03304080	36	I-II	1L HER2+ mBC	Palbociclib, Trastuzumab, Pertuzumab, Anastrozole	PFS
		NCT03054363	42	I-II	1L or 2L MBC	Tucatinib + Palbociclib + Letrozole	AEs (phase I); PFS (phase II)
		NCT03709082	NR	III	Pretreated HER2+/HR+ mBC	Palbociclib + Letrozolo + T-DM1	ORR
		NCT04334330	34	II	Pretreated HER2+/HR+ mBC with brain mets	Palbociclib, trastuzumab, pyrotinib and fulvestrant	ORR in CNS
		NCT05076695 NeoTPPF	37	II	Neoadjuvant HER2+/HR+ eBC	Palbociclib, trastuzumab, fulvestrant, pyrotinib	pCR
		NCT05429684	120	III	Pretreted HER2+ mBC	Module E: palbociclib + trastuzumab + letrozole	ORR
		NCT03644186	144	II	Neoadjuvant postmenopausal HER2+/HR+ eBC	Palbociclib+letrozolo+trastuzumab+ pertuzumab vs. paclitaxel +trastuzumab+pertuzumab	interaction of gene signature of functional loss of Retinoblastoma with pCR
Abemaciclib	CDK4/6	NCT02057133	198	I	Part H: HER2+ MBC who received at least one line of chemotherapy	Abemaciclib + Trastuzumab + Pertuzumab + ET	AEs
Ribociclib	CDK4/6	NCT03913234	95	I-II	1L HER2+/HR+ mBC	Ribociclib + Trastuzumab + Letrozole	PFS
		NCT05319873	18	I-II	Phase I: pretreated HER2+ mBC Phase II: neoadjuvant (HR+/HER2+ arm A and B)	Exp: Ribociclib + Trastuzumab + Tucatinib + Fulvestrant Comp: Trastuzumab + Pertuzumab + Carboplatin + Docetaxel for phase II)	MTD (phase I); pCR (phase II)
		NCT02344472 (Detect V / CHEVENDO)	270	III	1–3L HER2+ mBC	Arm 1: Induction chemotherapy + Trastuzumab + Pertuzumab, followed by maintenance Ribociclib + Trastuzumab + Pertuzumab + ET Arm 2: Ribociclib + trastuzumab + pertuzumab	AEs
Dalpiciclib	CDK4/6	NCT03772353	79	I-II	2L MBC	Arm 1: Pyrotinib + Dalpiciclib + Letrozole Arm 2: Pyrotinib + Dalpiciclib + Fulvestrant	AEs
Bispecific antibodies							
Zanidatamab (ZW25)	HER2/HER2	NCT04276493	71	I/II	1L mBC (cohort 1)	Zw25 + docetaxel	DLT, AEs
		NCT02892123	279	I	Pretreated HER2+ mBC (part 1 and 2) 2–3L mBC (part 3)	Zw25 alone (part 1 and 2) or + paclitaxel (part 3)	DLT; AEs

Table 1 (*Continued*)

Agent	Target	Trial	Enrollment target	Phase	Setting	Regimens	Primary endpoints
		NCT05035836	20	II	Neoadjuvant HER2+/HR+ eBC	Zw25 + trastuzumab + letrozole	pCR
		NCT01042379 (I-SPY)	NR	II	Neoadjuvant HER2+ eBC	Zw25 followed by SOC	pCR
Zanidatamab zovodotin (ZW49)	HER2/HER2	NCT03821233	174	I	Pretreated metastatic HER2+ tumors	ZW49	DLT; AEs
KN026	HER2/HER2	NCT04165993	68	II	Pretreated mBC	KN026 alone or + docetaxel or + KN046	ORR; DOR
		NCT04521179	30	II	Pretreated mBC	KN026 + KN046	ORR; DOR
		NCT03847168	22	I	Pretreated mBC + mGC	KN026	DLT
		NCT04040699	48	I	Pretreated HER2+ solid tumors	KN026 + KN046	DLT; ORR; DOR
		NCT03619681	63	I	Pretreated mBC and mGC	KN026	DLT
		NCT04778982	36	II	Pretreated HER2+/HR+ mBC	KN026 + palbociclib + fulvestrant	DLT; ORR
Runimotamab	HER2/CD3	NCT03448042	537	I	Pretreated metastatic HER2+ tumors	Runimotamab + trastuzumab	AEs

AEs, adverse events; BC, breast cancer; CNS, central nervous system; DLTs, dose-limiting toxicities; DOR, duration of response; eBC, early breast cancer; ET, endocrine therapy; IDFS, invasive disease-free survival; L, line; mBC, metastatic breast cancer; Mets, metastasis; mGC, metastatic gastric cancer; MTD, maximum tolerated dose; NR, not reported; NSCLC, nonsmall cell lung cancer; ORR, overall response rate; PD-L1, programmed death ligand 1; PFS, progression-free survival; RP2D, recommended phase 2 dose; SAEs, serious adverse events; T-DM1, trastuzumab emtansine.

double-anti-HER2 blockade resulted in superior PFS benefits than single anti-HER2 agent plus aromatase inhibitor [66,68].

HER2 and CDK4/6 cotargeting

Following the signals of activity demonstrated by abemaciclib in a phase I trial in HER2+/HR+ breast cancer subgroup [69], the phase II randomized monarcHER was designed. Among 237 HER2+/HR+ MBC, the addition of abemaciclib to trastuzumab and fulvestrant (arm A), as at least third-line treatment, resulted in PFS benefits compared with trastuzumab and chemotherapy (arm C) (8.3 vs. 5.7 months, $P = 0.051$) [70]. In a sub-analysis, arm B (abemaciclib and trastuzumab without ET) did not yield superior benefits compared with arm C, underlining the rationale for cotargeting hormone receptors and HER2 in triple-positive diseases [70].

In a phase Ib trial, ribociclib was tested with trastuzumab in 13 heavily pretreated HER2+ patients, showing no objective responses [71].

The SOLTI-1303 PATRICIA trial enrolled patients with HER2+ MBC to receive palbociclib and trastuzumab (arm A), with the addition of ET (cohort B2) or not (cohort B1) if HR+. The addition of palbociclib resulted in longer 6-month PFS among HER2+/HR+ diseases than HER2+/HR- tumors (Arm A: 33%; B1: 42.8%; B2: 46.4%) [72]. In the phase Ib LORDSHIP trial, the addition of dalpiciclib to pyrotinib and letrozole in HER2+/HR+ MBC resulted in overall response rate (ORR) of 86 and 50% in the first-line and second-line settings, respectively, with high toxicity [73]. Consistently in monarcHER and PATRICIA trials, luminal tumors defined by PAM50-subtyping correlated with higher CDK4–6i benefit [74,75]. In the phase III DETECT V trial, 153 HER2+/HR+ MBCs in the first-line to third-line setting were randomized to receive trastuzumab and pertuzumab and either chemotherapy (arm A) or ET (arm B), with maintenance treatment with trastuzumab, pertuzumab, and ET. Following a trial amendment, ribociclib was added in the maintenance (arm A) and upfront setting (arm B). At the interim analysis, including 33 patients after the amendment, no differences were observed in long-term outcomes. Notably, study interruptions occurred more frequently in the chemotherapy arm (72.2 vs. 43.9%, $P = 0.001$) [76].

A pivotal phase Ib study investigating the use of palbociclib, letrozole and tucatinib in HER2+/HR+ MBC with or without brain metastasis showed encouraging activity (central nervous system- PFS: 8 months) [77,78]. Preliminary results were observed with the combination of palbociclib and TDM1 as well [79]. Larger prospective clinical trials are

ongoing to validate alternative approaches in triple-positive MBC (Table 1).

HER2 and PI3K/AKT/mTOR cotargeting

Data about cotargeting HER2 with PI3K are more extensive in HER2+ MBC. The addition of everolimus to trastuzumab and chemotherapy did not result in PFS benefits in the phase III BOLERO-1 and BOLERO-3 trials; yet, dysregulation of the PI3K/AKT pathway predicted everolimus-benefits [hazard ratio 0.67; 95% confidence interval (95% CI) 0.48–0.93) [80–82]. The addition of buparlisib to trastuzumab in pretreated HER2+ breast cancer unselected for PI3K/AKT alterations resulted in disappointing results, leading to premature interruption of the trial for futility results [83]. In contrast, the addition of buparlisib to lapatinib in a similar population resulted in a disease control-rate (DCR) of 79%, with a clinical benefit rate (CBR) of 29%, observed more frequently among PIK3CA-mutated and HR- breast cancer [84]. Stable disease for 16 weeks was observed in 50% of the 12 patients treated with trastuzumab and panPIK3i; copanlisib is in the phase Ib PantHER trial [85].

Several trials tested the selective PIK3CA-inhibitor alpelisib in HER2+ MBC. In part 1 of the EPIK-B2 trial, alpelisib and trastuzumab and pertuzumab as first-line maintenance therapy following induction with taxane and double HER2-blokade demonstrated preliminary positive results opening the second part of the trial restricting to PIK3CA-mutated HER2+tumors [86].

In a phase I trial, alpelisib was tested in combination with TDM1 in trastuzumab-resistant patients unselected for PIK3CA-mutation, demonstrating substantial response (43% ORR) and clinical benefit (60% CBR in pretreated-TDM1), although at cost of high toxicity [87]. Preliminary results about MEN1611 (panPI3Ki sparing the delta isoform) combined with trastuzumab with or without fulvestrant (if HR+), derived from the B-PRECISE01 trial, enrolling HER2+/PIK3CA-mutated MBC at more than third line. Among 41 patients, median OS and PFS was 22 and 5.6 m, similar in the triple-positive BC and shorter in HER2+/HR- group (OS 11.9 and PFS 3.9 m) with no unexpected toxic events [88]. Notably, a phase I study investigated the combination of trastuzumab, alpelisib, and LJM716 (anti-HER3) with promising antitumor activity but limited tolerability [89].

HER2 and PD(L)1 cotargeting

The phase Ib JAVELIN trial investigated the use of the anti-PD-L1 avelumab as a single agent among 26 highly pretreated HER2+ tumors unselected for PD-L1, with no clinical response [90]. Similarly, the use of trastuzumab and durvalumab (anti-PD-L1) did not show activity in pretreated HER2+ MBC [91]. In the phase Ib/II PANACEA trial, an ORR of 15% was observed with pembrolizumab and trastuzumab only among 40 PD-L1-positive tumors [92]. In the phase II KATE2 trial, atezolizumab added to TDM1 in 202 pretreated patients provided a numerically superior PFS in the PD-L1-positive population (8.5 vs. 4.1 months, $P = 0.099$) but not among PD-L1-negative (6.8 vs. 8.2 months) [93].

A phase Ib trial investigated the addition of atezolizumab to TDM1 or pertuzumab, trastuzumab, and docetaxel, resulting in an ORR of 30% (7/20) and 100% (6/6) in the two arms, respectively [94].

Differently, the addition of pembrolizumab to TDM1 in 20 HER2+ MBC (pretreated with trastuzumab and a taxane) resulted in an ORR of 29 and 33% among PD-L1 at least 1% and less than 1% tumors, respectively [95]. Nivolumab was tested in combination with Tdxd in a phase I trial. Among 32 heavily pretreated HER2+ patients, an ORR of 59.4% was observed, with a mPFS of 8.6 m [96].

HER2 and HER3 cotargeting

Cotargeting HER2 with other EGRF receptors may overcome resistance by blocking hetero-dimerization and downstream pathways. In this context, a new class of agents, namely bispecific antibodies, targeting two epitopes of the same or different antigens, have been introduced.

Patritumab, an anti-HER3 mAb, was tested in a phase I trial of 18 HER2+ patients pretreated with at least one line of therapy. In combination with trastuzumab and paclitaxel, patritumab resulted in an ORR of 37% and mPFS of 9 m [97].

In a phase I/II trial, the bispecific mAb zenocutuzumab, targeting HER2 and HER3, yielded a CBR of 70% among 10 heavily pretreated HER2+ MBC as a single agent [98]. In a phase II study, zenocutuzumab in combination with trastuzumab and vinorelbine resulted in a DCR of 77% in 30 heavily pretreated HER2+ MBC [99,100].

HER2 cotargeting with bispecific antibodies

With the same rationale to combine pertuzumab and trastuzumab, bispecific anti-HER2 antibodies have been designed. Zanidatamab, BsAb-targeting subdomain II and IV of HER2, is the most investigated agent of this subclass. In a phase Ib/II trial, among 22 HER2+BC, zanidatamab alone or in combination with docetaxel elicited an ORR of 85 and

89%, respectively, as a first-line treatment, with a 6-month PFS of 90.9% [101]. Zanidatamab was further tested among 36 triple-positive patients pretreated with trastuzumab, pertuzumab, and TDM1, achieving an ORR of 33% and a mPFS of 9.6 m [102]. Another BsAb with a similar specificity, KN026, was tested in a phase I trial among 63 heavily pretreated patients, resulting in an ORR of 28.1% [103]. In the first-line setting among 55 HER2+ MBC, KN026 with docetaxel, showed an ORR of 76.4%, mPFS, and a 18-month OS-rate of 19.3 m and 88.3%, respectively [104].

FUTURE PROSPECTIVE AND CONCLUSION

Novel HER2-targeting therapies have yielded unprecedented results in HER2+ tumors, ultimately leading to better survival outcomes. The emergence of off-target resistance mechanisms has become increasingly recognized and remains a significant challenge for the treatment of HER2+ breast cancer. The increasing knowledge about the heterogeneity of HER2+ breast cancer as well as the discovery of new predictive/prognostic biomarkers may help to define new targets to overcome resistance.

By addressing off-target resistance, cotargeting strategies may potentially delay or prevent resistance to anti-HER2 treatments to ultimately improve patient outcomes. In some cases, a molecular-selected population, such as by specific mutations or intrinsic molecular subtypes, may refine patient selection.

New combinations and agents, such as bispecific antibodies, are currently under investigation for cotargeting purposes. Further research is needed to identify optimal biomarkers, develop more effective cotargeting strategies, and improve patient selection criteria for existing therapies.

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Conflicts of interest

G. Curigliano received honoraria for speaker, consultancy, or advisory rule from AstraZeneca, Roche, Pfizer, Novartis, Seattle Genetics, Lilly, Ellipses Pharma, Foundation Medicine, Daiichi Sankyo, and Samsung; all are to be intended outside the present work. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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