Cancer Treatment Reviews 129 (2024) 102786

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Tumour Review

Long road towards effective HER3 targeting in breast cancer

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ARTICLE INFO

Keywords:
HER3
Breast cancer
Brain metastases
Treatment resistance
Patritumab-deruxtecan
Anti-HER3 drugs

ABSTRACT

Breast cancer is a heterogeneous disease, encompassing multiple different subtypes. Thanks to the increasing knowledge of the diverse biological features of each subtype, most patients receive personalized treatment based on known biomarkers. However, the role of some biomarkers in breast cancer evolution is still unknown, and their potential use as a therapeutic target is still underexplored. HER3 is a member of the human epidermal growth factors receptor family, overexpressed in 50%-70% of breast cancers. HER3 plays a key role in cancer progression, metastasis development, and drug resistance across all the breast cancer subtypes. Owing to its critical role in cancer progression, many HER3-targeting therapies have been developed over the past decade with conflicting findings. Next-generation antibody-drug conjugates have recently shown promising results in solid tumors expressing HER3, including breast cancer. In this review, we discuss the HER3 role in the pathogenesis of breast cancer and its relevance across all subtypes. We also explore the new anti-HER3 treatment strategies, calling into question the significance of HER3 detection as crucial information in breast cancer treatment.

Introduction

The epidermal growth factor receptor 3 (HER3) is a transmembrane receptor that belongs to the human epidermal receptor (HER) family and is overexpressed in several cancer types, such as melanoma, cervical, ovarian, colorectal, gastric, and breast cancer [1], which renders it a promising therapeutic target [2]. Its overexpression stimulates downstream signalling pathways that enhance cell cycle progression, angiogenesis, survival, invasion, and metastasis [3,4]. In breast cancer, HER3 is overexpressed in about 50–70 % of all subtypes [5], with the HR+/HER2- subtype showing the highest expression (followed in order by HR+/HER2+ and HR-/HER2+ subtypes) [6]. It has long been assumed that HER3 upregulation is associated with acquired resistance to chemotherapy, endocrine therapy, and HER2- and PI3K/AKT/mTOR targeting therapy [7,8,9]. Hence, targeting HER3 appears to be a promising approach in breast cancer, especially following exposure to therapeutic agents [10]. However, several anti-HER3 therapies, mostly based on mono- and bispecific antibodies, have been integrated into preclinical and clinical development with unsatisfactory results [11,12,13,14,15] and only the anti HER3 antibody drug conjugates seem to have the most promising results [16,17,18,19,20]. In this review, we thoroughly examine the role of HER3 in the pathogenesis of breast cancer, with a focus on its relevance across all subtypes and in developing resistance to a spectrum of breast cancer therapies under selective treatment pressure. Last, we highlight the current landscape of anti-HER3 treatments and discuss future perspectives in therapeutic strategies and, thus, in HER3 assessment.

HER3 unique characteristics

HER3 structure

HER3, first identified by Kraus et al. [21], is a member of the human epidermal growth factor receptors (HER) family, which is composed of four homologous receptors tyrosine kinases (RTKs): EGFR (ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Members of this family play essential roles in cell proliferation, survival, migration, and differentiation. HER3 is composed of an extracellular domain (ECD), an intracellular kinase domain (KD), and an intracellular c-terminal tail [22]. Ligands bind the ECD, leading to a conformational rearrangement

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https://doi.org/10.1016/j.ctrv.2024.102786
Received 21 March 2024; Received in revised form 25 May 2024; Accepted 9 June 2024
Available online 13 June 2024

in the dimerization domain that constitutes the dimer interface with another receptor [23]. While EGFR, HER3, and HER4 have ligands, HER2 does not have a ligand and is always in a constitutively active conformation with its dimerization arm opening even without ligand binding [23]. The EGFR family members induce their activation through both homodimerization and heterodimerization. EGFR or HER4 mostly form homodimers, while HER3 mostly heterodimerizes with HER2, and the HER2-HER3 heterodimer is the most active signaling dimer in this family [24,25]. Indeed, HER3 KD is an allosteric activator of HER2 kinase, leading to greater HER2 autophosphorylation than HER2 homodimers [26]. HER3 has unique features in the ECD domain. The neuregulin (NRG), also called the heregulin (HRG) is the unique ligand of HER3 [27] that activate HER3-ECD [28]. Without ligand, HER3 keeps in a tethered conformation (Fig. 1). NRG does not promote HER3 homodimerization at the cell surface, making it an obligate partner for heterodimerization [29]. To HER2, the most privileged heterodimerization partner is HER3 among all members of HER family [30].

HER3 pathways

Dimerization induces transactivation of the tyrosine kinase domain [31], leading to the recruitment of signaling molecules, and the activation of intracellular signaling pathways [32]. Members of the EGFR family all have powerful intrinsic kinase activity, with the exception of the HER3 member. Unlike the others, HER3 has only modest intrinsic kinase activity, which makes it unable to efficiently phosphorylate peptide substrates and trigger a signal through homodimerization. Therefore, it can activate signalling pathways upon ligand binding through heterodimerization with the other EGFR family members. The c-terminal tail of HER3 contains 14 phosphotyrosines: six sites bind the SH2 domain to interact with subunits of PI3K[33]. HER3 is a potent activator for PI3K/AKT signalling [33], and this evolution of HER3 ECD and KD into structures lacking the function of homodimerization, protects the PI3K signaling pathway from premature activation[31]. Depending on heterodimerization with other RTKs, HER3 can also induce activation of different signaling pathways such as MEK/MAPK, PLCγ/PKC, Jak/Stat, and Src kinase, which have a crucial role in cellular growth, proliferation, and survival [34,35,36,37,38] (Fig. 2).

Overexpression and gene alterations

HER3 expression in breast cancer

HER3 protein is overexpressed in about 50–70 % of breast cancers [3] and promotes carcinogenesis in all breast cancer subtypes. It should be noted that differences in expression are mainly related to the different methods and antibodies targeting HER3. The most used was RTJ1, a mouse monoclonal antibody directed against HER3 kinase. Luthala et al. compared 4 different anti-HER3 antibodies (DAK-H3-IC and RTJ1, rabbit monoclonal antibody clone SP71, and rabbit polyclonal antibody SAB4500793) to explain the differences found in the staining results in breast cancer studies. DAK-H3-IC targets the intracellular HER3 domain and appears to be the most suitable for detecting cell membrane and cytoplasmatic HER3 expression in breast cancer tissue samples [39]. Another variation among studies arises from the immunohistochemistry (IHC) scoring, as some studies considered 1+ as positive, along with 2+ and 3+. In addition, conflicting results have been reported when HER3 mRNA and HER3 protein expression by IHC were compared. In luminal breast cancer, which present the highest mRNA HER3 levels [6], correlation between protein expression and mRNA level is still unclear. A study evaluating the concordance between protein and mRNA expression across HER family receptors showed that HER3 mRNA correlates with HER3 IHC protein expression in ER-positive breast cancer [40]. Conversely, the recent SOLTI TOT HER3 study underlined the weak correlation between HER3 mRNA and HER3 protein expression in

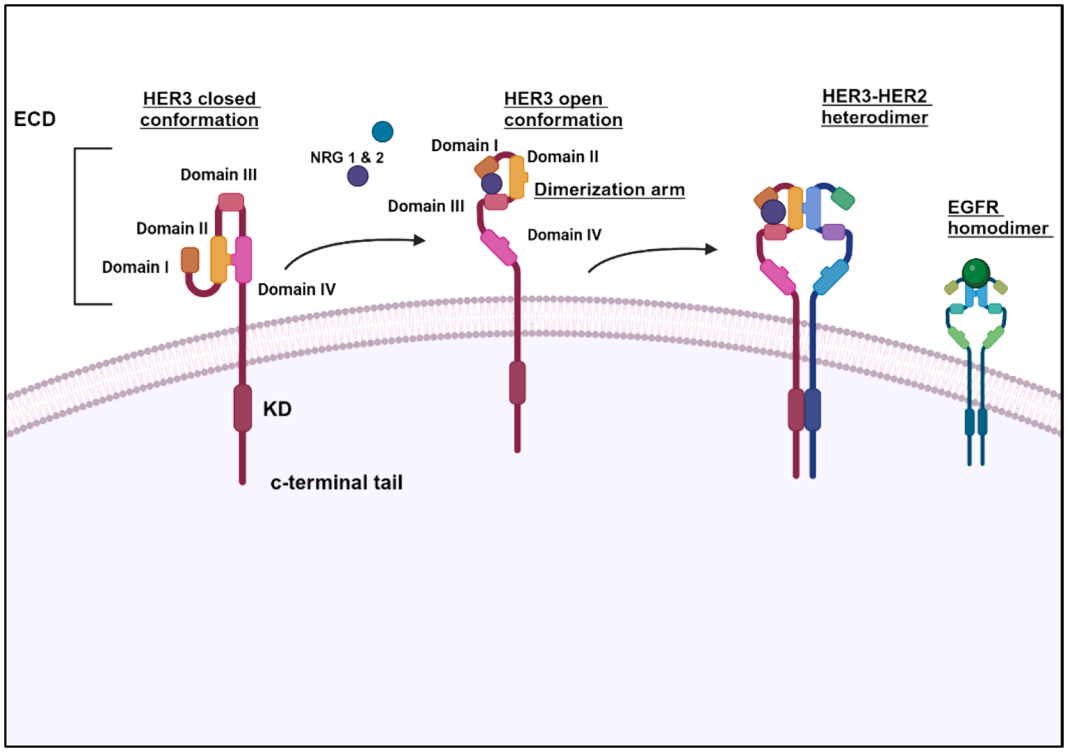

Fig. 1. HER3 structure and fuction: HER3 comprises an extracellular region (ECD), a transmembrane region, and an intracellular region (kinase domain, KD, and c-terminal tail).The ECD region, in turn, presents four parts: in the close conformation, domain II and domain IV are joined to prevent dimerization. When HER3 ligands (NRG 1 or NRG2) bind domains I and III, the ECD region undergoes a conformational change that exposes the dimerization arm, suitable for heterodimerization with other RTK. When a heterodimer is active, kinase domains form a dimer that allosterically activates the other, activating signaling pathways. HER3-HER2 is the most active heterodimer in the EGFR family, while EGFR and HER4 mostly form homodimer.

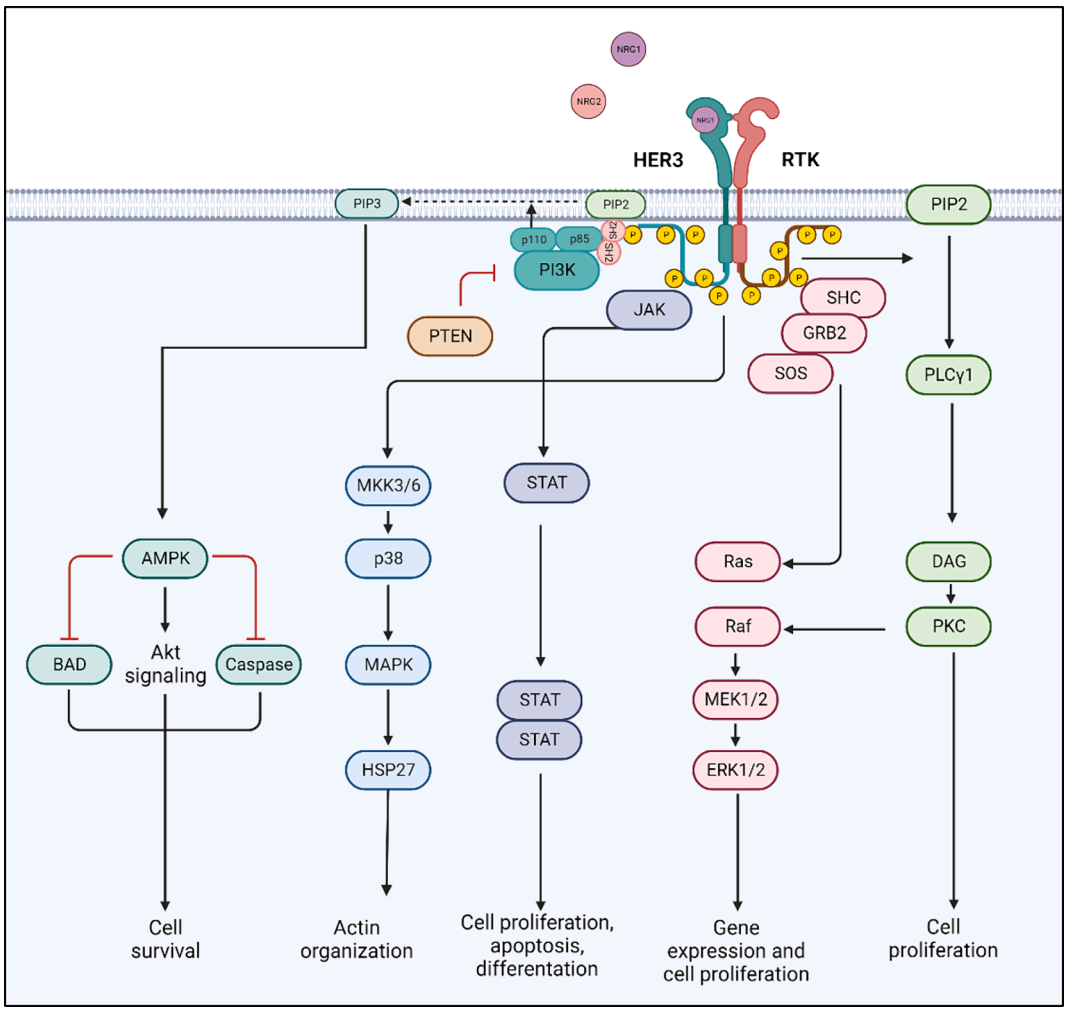

Fig. 2. HER3 signaling: The HER3 c-terminal tail contains 14 phosphotyrosines, 6 of which bind SH2 domains on the PI3K p85 regulatory subunit. SH2-phosphotyrosine interactions bring PI3K close to the plasma membrane, where the p110 PI3K subunit can convert PIP2 into PIP, activating the PI3K/Akt signaling pathway responsible for cell survival. HER3 is a direct activator of this pathway, and its particularities in ECD and KD domains prevent premature PI3K activation. HER3 can activate, through heterodimerization with other RTK, other signaling pathways such as MEK/MAPK, PLCγ/PKC, Jak/Stat, and Src kinase, which have a crucial role in cellular growth, proliferation, and survival.

patients with early ER + breast cancers undergoing preoperative treatment with patritumab-deruxtecan [16,41]. In an extensive analysis of The Cancer Genome Atlas (TCGA) and the Cancer Cell Line Encyclopedia (CCLE), all TNBC expressed HER3 mRNA but some with a lower corresponding protein expression [42]. Although HER3 expression in basal-like/triple-negative breast cancer is relatively low, it has been associated with worse survival outcomes, as the NRG1β/HER3/HER2 axis supports tumor cell dissemination by promoting anchorage-independent cell growth [43].

HER3 gene alterations

HER3 gene alterations have been reported in 5.6 % of breast cancers (cBioportal data). HER3 amplifications are infrequent, with a higher prevalence observed in lobular breast carcinomas, occurring in over 7 % of cases (cBioportal data). HER3–activating somatic mutations have been described in 2 % of all breast cancers. The most common mutations are E928G (kinase domain), V104L, G284R, and T355I (Extracellular domain) [30] (cBioportal data, Fig. 3). These activating mutations make HER3 receptor activation independent of ligand stimulation in breast cancer cell models, by increasing HER2-HER3 heterodimerization, and have been associated with resistance to lapatinib [44]. The ECD is the region primarily impacted by HER3 mutations, causing a shift in the equilibrium between tethered and untethered HER3 ECD toward an untethered conformation in a ligand-independent manner. Although KD of HER has modest activity, kinase domain mutation may alter the conformation of HER3 so that it becomes more permissive to form HER3/HER2 dimers in a ligand-independent way. Interestingly, it has been shown that the oncogenic activity of HER3 mutations depends on the expression of HER2; HER3mut alone can promote neither anchorage-independent growth nor downstream signaling [42].

HER3 oncogenesis and signaling

HER3 plays a key role in the development and progression of all breast cancer subtypes. Indeed, it has been demonstrated that HER3 drives the growth and survival of several ER-positive breast cancer cell lines using its signaling pathway [35]. In mouse mammary HER2 amplified breast cancer tissue, HER3 expression contributes to cancer formation and takes part in the transition of ductal in situ carcinomas to malignant adenocarcinoma, while the loss of HER3 decreases the growth of HER2-overexpressing tumors and improves tumor response to the HER2 tyrosine kinase inhibition [45]. HER3 upregulation was found also in triple negative breast cancer (TNBC) clinical samples and cell lines, in which the genetic silencing led to suppression in tumor growth [46]. Nevertheless, the complete understanding of the mechanisms responsible for abnormal HER3 protein expression remains elusive.

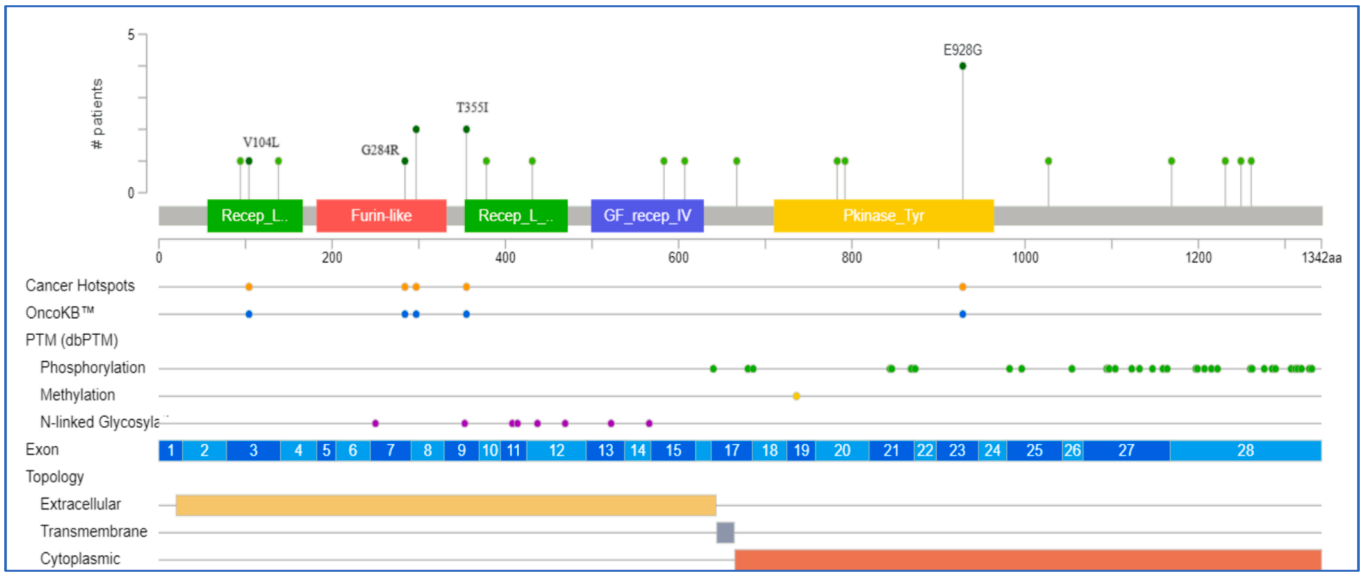

Fig. 3. ErbB3 gene mutation focus: cBioportal ErbB3 gene mutations in breast cancer: The most common HER3 gene mutations in breast cancer are highlighted. E928G, V104L, G284R, and T355I are missense mutations type. E928G is located in the tyrosine kinase domain, and V104L, G284R, and T355I are in the extracellular ligand-binding domain.

Given that HER3 gene amplifications and mutations are rare in breast carcinogenesis, it has been assumed that increased HER3 expression might result from alterations in downstream signaling mechanisms that regulate HER3 membrane trafficking and degradation [39,47]. The E3 ubiquitin ligase neuregulin receptor degradation protein-1 (NRDP-1, also known as FLRF and RNF41) regulates steady-state HER3 levels by marking the receptor for proteolytic degradation. Overexpression of NRDP1 suppresses cellular HER3 levels in human breast cancer cells, inhibiting cell growth motility and attenuating signal transduction pathways. In contrast, NRDP1 knockdown enhances HER3 levels and cellular proliferation in primary breast tumors [48]. NRDP1 protein levels are suppressed in 57 % of breast cancers [49,50]. Neural precursor cells said developmentally downregulated 4 (NEDD4) is also a ubiquitin E3 ligase of human HER3. NEDD4 interacts with the c-terminal tail of HER3 in a neuregulin-1-independent manner. Short hairpin RNA (ShRNA) knockdown of NEDD4 increases HER3, Akt, and ERK1/2 phosphorylation, enhancing HER3 signaling and cancer cell proliferation [51]. In breast cancer, also transmembrane mucin 4 (MUC4) promotes the recruitment of HER3 and HER2 at the plasma membrane, ultimately increasing their signaling functions. MUC4 increases expression of EGFR and HER3 in triple-negative breast cancer, whose activation induces Erk1/2, PKC, and FAK to drive cell proliferation, motility, and invasiveness in vitro, and increased tumor growth and metastasis in vivo [52]. HER3 is one of the most potent activators of the PI3K-AKT pathway, whose deregulation promotes cancer survival via alterations in various cellular processes, such as proliferation, growth, apoptosis, and cytoskeletal rearrangement [53]. While HER2 and EGFR can activate this pathway through the adaptor proteins GRB2 (growth factor receptor-bound 2) and GAB1 (GRB2-associated binding protein 1) HER3 c-terminal tail has six tyrosine residues whose bind directly the phosphotyrosine residues on the receptor and SRC-homology 2 (SH2) domains on the PI3K p85 regulatory subunit [53,54]. There is a tight interaction between the HER3 and PI3K-Akt pathways. The upregulation of PI3K/AKT signaling is thought to be involved in developing resistance to several chemotherapy regimens, as exhibited in breast cancer cell lines simultaneously expressing HER2 and HER3 [7]. Prior preclinical studies have revealed that inhibition of Akt induces HER3 phosphorylation and expression, and reactivation of Akt phosphorylation is associated with subsequent HER3 decrease [55].

Metastasis development

HER3 expression also plays a key role in developing metastases in specific organs, such as bone and brain. It has been reported that NGR1 triggers a HER3-ROR1 (Receptor Tyrosine Kinase-Like Orphan Receptor 1) –long non-coding RNA (lncRNA) axis that leads osteoclast differentiation and resorption, responsible for breast cancer bone metastases [56]. In addition, HER3 has a unique role in cancer cell migration to the brain. Preclinical evidence showed that in co-expressed HER2-HER3 breast cancer, neuregulin-1, which is exceptionally high in the brain being secreted by microglial and neuronal cells, binds HER3 and lead to the heterodimer activation and signaling that can promote the matrix metalloproteinase–dependent *trans*-endothelial migration of cancer cells through the blood–brain barrier, contributing to the establishment of brain metastasis [57]. Consistently, a retrospective study of 44 breast cancer patients with brain metastasis evaluated the HER3 expression by immunohistochemistry. The results showed that HER3 2+/3+ was expressed in 91% of brain metastasis, higher than in primary tumors (59 %) and HER3 was observed in brain metastases for 75 % for TNBC, 89% for HR + HER2-, 100% for HR + HER2+, and 100 % for HR-HER2+ [58].

Resistance to breast cancer treatments

HER3 plays a critical role in driving resistance to therapy across the different breast cancer subtypes [5]. In HER2-positive breast cancer, HER3 is known to confer resistance to HER2-target therapies such as trastuzumab and lapatinib. The activation of downstream signaling pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt signaling, is a major determinant of trastuzumab resistance [59,60]; It has been demonstrated that in trastuzumab-resistant breast cancer cell lines coexpressing HER2, HER3, and IGF1R (Insulin-like growth factor 1 receptor), HER3 forms a heterotrimer with HER2 and IGF1R. This heterodimerization induces the activation of PI3K/AKT and SRC pathways [36]. Likewise, prolonged exposure to lapatinib leads to acquired resistance, characterized by the activation of HER3:EGFR dimerization rather than HER2:HER3 signaling. This resistance is facilitated by an autocrine feedback loop mechanism involving membrane-bound NRG-1, promoting an EGFR-HER3-PI3K-PDK1 signaling axis, despite lapatinib targeting of HER2 [61]. Elevated expression of HER3 also induces paclitaxel resistance in HER2-overexpressing breast cancer cells by

upregulating Survivin, a key apoptosis inhibitor, by activating PI-3K/Akt signaling pathway [62,63]. In luminal breast cancer, HER3 expression contributes to hormonal therapy resistance. There is a bidirectional crosstalk between HERs (human epidermal growth factor receptors) and ERs (estrogen receptors), where the HER2:HER3 heterodimer induces phosphorylation of the ER independently of estrogen. This independent phosphorylation subsequently reduces the effectiveness of endocrine therapies [15] Fulvestrant inhibits cell growth in ER-positive breast cancer by significantly reducing Estrogen-Receptor protein expression. However, it induces the expression of HER3 protein and enhances NRG-1β sensitivity, increasing the magnitude of its response [64]. Fulvestrant-resistant human breast cancer lines showed an increased EGFR, HER2, and HER3 expression, with high upregulation of HER3. Consistently, the downregulation of HER3 appeared to restore the activity of tamoxifen in human breast cancer cell lines [65]. In vitro, TNBC cell lines that express both HER3 and EGFR, treated with an AKT inhibitor or a PI3K inhibitor, showed an increased activation and phosphorylation of HER3 as an acquired drug resistance. Simultaneous inhibition of the PI3K-AKT pathway and both EGFR and HER3 phosphorylation substantially decreased cell proliferation compared with inhibition of the PI3K-AKT pathway alone [66]. In TNBC pretreated tumor samples, high HER3-EGFR heterodimerization was associated with worse 10-year breast-cancer specific survival (BCSS) (83.1% vs 69.2%, log-rank p = 0.017) and distant-metastases free survival DMFS (80.8% vs 70.4%, log-rank p = 0.05) after adjuvant chemotherapy. This dimer led to EGFR- and PARP1-signaling activation and could result in therapeutic resistance [67] These findings suggest that developing new target combinations for HER3, EGFR, PARP-1, and PI3K-Akt pathways should be investigated in this clinical setting.

Current and future HER3-therapies

In the initial development of therapies, mainly monoclonal antibodies and TKIs, targeting the EGFR family members, HER3 was neglected due to its impaired kinase activity. Lately, the technological advances in drug development and the understanding of HER3's role in cancer progression and treatment resistance across different breast cancer subtypes have paved the way to novel strategies against HER3. (Fig. 4) (Table 1 and 2).

Monoclonal antibodies

Lumretuzumab, an anti-HER3 humanized glycoengineered IgG1 monoclonal antibody binding the subdomain I of the HER3 ECD, was evaluated in patients with HER3-positive (any membrane staining assessed by Ventana IHC assay) /HER2-low (as defined by IHC 1+ to 2+ and in-situ hybridization negative) metastatic breast cancer, together with paclitaxel and pertuzumab in a phase Ib trial. This trial included the dose-escalation part (lumretuzumab dose 1000 mg IV every 21 days) and the two extension phase cohorts: Cohort 1 (lumretuzumab dose 1000 mg IV every 21 days) and Cohort 2 (lumretuzumab 2000 mg IV every 21 days). Despite the initial antitumor activity observed with the combination (ORR in the Cohort 2 was 30%, 55% in pts in first line who

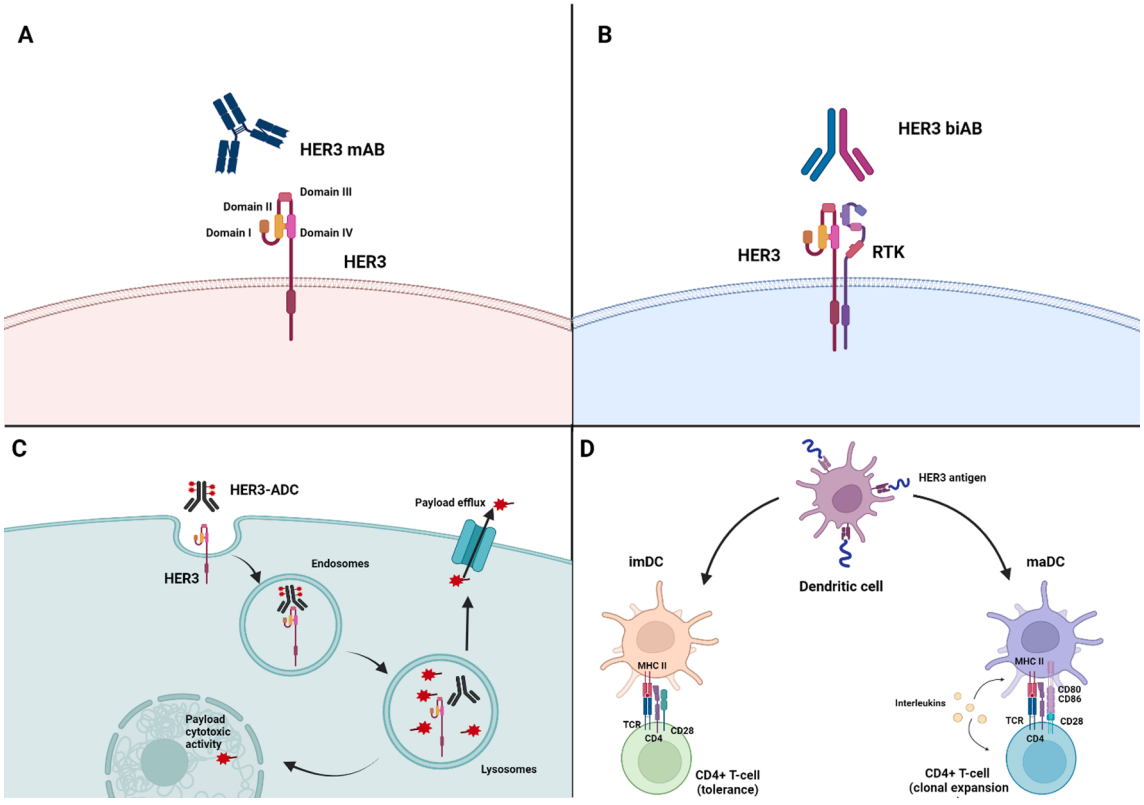

Fig. 4. Anti-HER3 therapies. A: HER3 monoclonal antibodies target the extracellular domain (ECD) to prevent neuregulin (NRG) binding, dimerization with other receptor tyrosine kinases (RTKs), and the subsequent activation of signalling pathways involved in cancer cell proliferation and survival. B: HER3 bispecific antibodies have two distinct binding domains that can simultaneously target two antigens or two epitopes of the same antigen. An example is a bispecific antibody that can bind both HER3 and another RTK. C: HER3 antibody-drug conjugates consist of a HER3 antibody linked to a cytotoxic payload through a peptide-cleavable linker. After binding to HER3, the ADC is internalized, and within lysosomes, the payload is released. This payload exerts cytotoxic effects on the cancer cell and can also affect neighbouring cancer cells through its efflux into the extracellular space, known as the bystander effect. D: A dendritic cell vaccine presents the HER3 antigen to T-cells in two ways. First, dendritic cells (DCs) can present the antigen to T-cell receptors via peptide–MHC complexes (pMHC), leading to T-cell activation. Additionally, HER3 antigens can strongly activate DCs through co-stimulation of Toll-like receptors (TLRs), resulting in the upregulation of co-stimulatory molecules such as CD80 or CD86 on the DC surface. This intensifies and prolongs the TCR-driven activation of antigen-specific T-cells. Moreover, cytokines like IL-1β, IL-12, IL-6, IFN-γ, and TNF-α, released by both DCs and T-cells, further shape the antigen-induced T-cell response.

Table 1
Anti-HER3 therapies and trials in breast cancer. AEs: adverse events, CBR: clinical benefit rate, DTL: dose-limiting toxicity, mBC: metastatic breast cancer, MTD: maximum tolerated dose, PFS: progression-free survival, pCR: pathological complete response, ORR: objective response rate, ORR-IC: intra-cranial objective response rate, OS: overall survival. RDE: the recommended dose for expansion.

Anti-HER3 therapies in Breast Cancer								
Drug type	Name	Trial	Phase	Trial Status	Study population	Drugs	Primary endpoint	Results
Monoclonal Antibodies	Lumretuzumab	NCT01918254	Ib	Completed	HER3+/HER2low mBC	Lumretuzumab + Pertuzumab + Paclitaxel	Percentage of patients with DLTs, AE's and HAHAs to lumretuzumab, Pharmacokinetics	Diarrhea G3 DLT for Cohort 1. Most frequent AEs: diarrhea, nausea, hypokalaemia and weigh loss.
	Seribantumab (MM-121)	NCT01151046	II	Completed	Postmenopausal HR+/HER2- locally advanced or mBC	Exemestane +/- Seribantumab	PFS	mPFS 15.9 weeks vs. 10.7 weeks (placebo + exemestane), HR 0.772 (95 % CI [0.496—1.201]), p = 0.249)
		NCT03241810	II	Terminated	NRG+, ER/PR + HER2-negative mBC	Fulvestrant +/- Seribantumab	PFS	Primary endopoint analysis not done for small sample size available at the premature study closure.
		NCT01421472	II	Completed	Neoadjuvant HR+/HER2- or TNBC	Paclitaxel +/- Seribantumab	pCR	pCR HR+/HER2- group: 10 % (7/66 pts) vs 3.3 % (1/30 pts) in the control arm; pCR TNBC group:: 41 % (23/56 pts) vs 48.3 % (12/29 pts) in the control arm.
	Elgemtumab (LJM716)	NCT02167854	I	Completed	PIK3CA-m HER2 + mBC	Elgemtumab + Trastuzumab + Alpelisib (BYL719)	MTD of BYL719, RDE	MTD arm A: alpelisib 250 mg daily; MTD arm B: 350 mg 4 days on, three days off. Dose expansion was not pursued based on toxicity profile (diarrhea), and the RDE was not formally declared.
	Patritumab (U3-1287)	NCT01512199	I/II	Terminated	HER2 + mBC newly diagnosed	Patritumab + Trastuzumab + Paclitaxel	MTD, PFS (only II phase)	Terminated, no results posted.
Bispecific Antibodies	Zenocutuzumab (MCLA-128)	NCT03321981	II	Active, not recruiting	HER2 + mBC and HR+/HER2 low mBC	Zenocutuzumab + Trastuzumab +/- Vinorelbina, Zenocutuzumab + ET	CBR at 24 weeks	HR+/HER2low mBC CBR was 45 % (90 % CI 32–59; 2 pts PR and 19 SD); HER2 + mBC CBR: DCR was 77 % (90 %CI: 60–89; 1 CR and 4 PR)
	MM-111	NCT01097460	I	Completed	HER2+/NGR + mBC	MM-111 + Trastuzumab	Incidence of AE's	100 % pts AEs, most frequent: anemia, diarreha, fatigue, decreased appetite, headache, insomnia, dyspnoea
	BL-B01D1	NCT05470348	I	Recruiting	mBC	BL-B01D1	DLT, RP2D, MTD	No results still available
		NCT06042894	II	Not yet recruiting	HER2- mBC	BL-B01D1 + SI-B003	ORR, RP2D	No results still available
Dendritic cell (DC) vaccines	Anti-HER2/3 DC Vaccine	NCT04348747	II	Recruiting	TNBC or HER2 + mBC with BM	Anti-HER2/3 DC Vaccine + Pembrolizumab	CNS-RR	No results still available

had not received chemotherapy for mBC), a significant number of patients experienced grade 3 diarrhea (100% in the cohort 1, 50% in the cohort 2 and 30.2 % in the cohort 3) and hypokalemia (100% in the cohort 1, 65% in the cohort 2 and 38.5% in the cohort 3), even with loperamide prophylaxis and dose adjustments. Consequently, the development of lumretuzumab in this context has been discontinued [68]. Seribantumab (MM-121) is an anti-HER3 human monoclonal antibody IgG2 that competes with NRG for binding to HER3. It blocks HER3 dimerization and induces its internalization and degradation. In a phase II clinical trial, seribantumab or placebo was evaluated in combination with exemestane in postmenopausal women with ER/PR+ HER2-negative mBC. Adding seribantumab to exemestane did not significantly prolong progression-free survival (PFS) in this unselected population (mPFS 15.9 weeks vs. 10.7 weeks of placebo + exemestane, HR 0.772, 95% CI [0.496—1.201], p = 0.249) [69]. Conversely, seribantumab was tested also in combination with fulvestrant to evaluate the PFS in patients with NRG+, ER/PR+ HER2-negative mBC (seribantumab administered at 3000 mg intravenously IV on day 1 and 15 of each 28-day cycle), but the study was premature closed [70]. Seribantumab activity was also explored in the neoadjuvant setting in patients with HR+/HER2- and TNBC, combined with paclitaxel, followed by doxorubicin and cyclophosphamide and surgery. The primary endpoint of this study was pCR rate. Although a potential benefit was observed in the HR+ group (pCR rate 10.6% vs 3.3%), this was not the case in the TNBC group (41.1% vs 48.3%) [71]. Seribantumab is still being evaluated in an ongoing, active, but not recruiting phase II trial which included recurrent, locally-advanced or metastatic solid tumors, which harbor the NRG1 gene fusion (NCT04383210). No results have been published yet.

Elgemtumab (LJM716) is a fully human IgG1 mAb that binds to an

Table 2

HER3 Antibody-drug conjugates under clinical development in breast cancer. AEs: adverse events, CBR: clinical benefit rate, DTL: dose-limiting toxicity, mBC: metastatic breast cancer, MTD: maximum tolerated dose, PFS: progression-free survival, pCR: pathological complete response, ORR: objective response rate, ORR-IC: intra-cranial objective response rate, OS: overall survival. RDE: the recommended dose for expansion.

HER3 Antibody-drug conjugates in Breast cancer							
Name	Trial	Phase	Trial Status	Study population	Drugs	Primary endpoint	Results
Patritumab − Deruxtecan	NCT04610528	I	Active, not recruiting	Neoadjuvant HR+/HER2-negative BC	Patritumab − Deruxtecan monotherapy	Change in CelTIL score	CelTIL score change median increase from baseline of 3.5 (interquartile range, 3.8 to 12.7; P ¼ 0.003). Increase in CelTIL score among responders compared with non-responders (mean difference, þ11.9 versus þ1.9)
	NCT05569811	II	Active, not recruiting	Neoadjuvant HR+/HER2- BC, ki67 > 20 %, high genomic risk	Patritumab − Deruxtecan +/- Letrozole	pCR (ypT0/is ypN0) at surgery	No results still available
	NCT04965766	II	Recruiting	HR+/HER2- mBC	Patritumab − Deruxtecan monotherapy	ORR	The 3 m-RR was 28.6 % [95 %CI: 18.4–41.5]. Analysis from 56 pts 16 PR 30 SD 10 PD.
	NCT04699630	II	Recruiting	mBC	Patritumab − Deruxtecan monotherapy	ORR, 6 m-PFS, association with HER3 expression ORR and 6 m-PFS.	Part A ORR: 35 % (95 % Cis 23.1, 48.1). Pts with ≥ 75 % HER3 expression ORR of 33 %, pts with HER3 25–74 % expression had an ORR of 46 %, pts with HER3 < 25 % expression, limiting efficacy assessment. The PFS6months was 60 % for all pts, 50 % for pts with HER3 ≥ 75 %, and 70 % for pts with HER3 25–74 %.
	NCT02980341	I/II	Active, not recruiting	HER3-positive mBC	Patritumab − Deruxtecan monotherapy	Number of patients with AEs and tumor response by RECIST v 1.1	HR+/HER2−, HER3 high and low ORR: 30.1 %; TNBC/HER3 high ORR:22.6 %; HER2+/HER3 high ORR 42.9 %. 71.4 % of pts had grade ≥ 3 TEAEs; the most common (≥15 %) were: decreased neutrophil count (39.6 %), decreased platelet count (30.8 %), anemia (18.7 %), and decreased white blood cell count (18.1 %)
	NCT05865990	II	Recruiting	mBC and mNSCLC with brain mts and solid tumors with leptomeningeal mts	Patritumab − Deruxtecan monotherapy	ORR-IC, OS	No results still available
DB-1310	NCT05785741	I/Iia	Recruiting	Advanced/metastatic solid tumors, including HER2-positive breast cancer	DB-1310	safety, tolerability, pharmacokinetics, ORR	No results still available
BL-B01D1	NCT05470348	I	Recruiting	Unresectable locally advanced BC or mBC	BL-B01D1	DLT, RP2D, MTD	No results still available
SHR-A2009	NCT06222879	I	Not yet recruiting	Unresectable locally advanced BC or mBC	SHR-A1811 (anti-HER2 ADC): HRS-8080 (SERD); SHR-A2009 (anti-HER3 ADC); SHR-1316 (anti-PD-L1 Ab)	DLT, RP2D, MTD, ORR, incidence of AEs and SAEs.	No results still available

epitope located between domains II and IV of the ECD of HER3, keeping it in a closed conformation and preventing the signaling activation. In a phase I clinical trial, elgemtumab (dose fixed at 20 mg/Kg weekly every 28 days) was given in combination with trastuzumab and alpelisib in patients with PIK3CA-mutant HER2-positive (HER2 +) metastatic breast cancer (mBC). The best response was stable disease (SD) in 31% of patients, with no complete response or partial response observed. The median PFS for patients treated was 1.64 months (95% CI: 1.64–1.81 months). Due to the modest activity and the high proportion of gastrointestinal toxicity (diarrhea 52%), with 59% of patients requiring dose adjustment or interruption, this combination was discontinued [72].

Patritumab (U3-1287) is a fully human IgG1 mAb that inhibits ligand binding to HER3 and induces receptor internalization and degradation. In cell lines derived from a variety of tumors (including breast cancer), patritumab caused inhibition in cell proliferation [27]. In a phase I study was evaluated in combination with trastuzumab and paclitaxel in HER2+ mBC (patients received patritumab 9 mg/kg or 18 mg/kg; it showed an overall response rate of 38.9% and a median progression-free survival of 274 days [73].

Despite the encouraging preclinical data, anti-HER3 monoclonal antibodies (MoAbs) failed to show significant efficacy in breast cancer as single agents or in combination.

Bispecific antibodies

Bispecific antibodies (BsAbs) are recombinant molecules with two distinct binding domains that simultaneously target two antigens or two epitopes of the same antigen. HER3 BsAbs were engineered to bind both HER3 and EGFR or HER2 or IGF-1. Bispecific antibodies showed promising results, but still depending on NRG-1 levels [74].

Zenocutuzumab (MCLA-128) is a humanized bispecific IgG1 antibody that inhibits HER3 from interacting with NRG1 and targets HER2 blocking HER2/HER3 dimerization. It also presents ADCC activity [75]. A phase I trial demonstrated a well-tolerated safety profile (infusion related reactions were the most common AEs, followed by diarrhea, rash and fatigue) and antitumor activity in heavily pretreated MBC patients with documented NRG1 fusion, progressing on HER2 therapies (8 mBC patients: 1 had a confirmed PR, 7 had SD; the clinical benefit rate, CBR, was 70%) [74]. In combination with trastuzumab and vinorelbine, zenocutuzumab (750 mg, 2 h IV) was active in heavily pretreated patients with HER2-positive MBC, having received up to 5 anti-HER2 lines, including trastuzumab, pertuzumab,[13]. Zenocutuzumab (750 mg, 2 h IV, flat dose, q3w) in combination with endocrine therapy also showed preliminary antitumor activity in patients with HR+, HER2 low (IHC 1+/IHC 2+ with negative FISH) MBC, who had progressed on a CDK4/6i and multiple prior lines of endocrine therapy and chemotherapy [14].

MM-111 is a HER2/HER3 bispecific antibody, which, through the anti-HER2 arm, recognizes and targets the HER2+ tumor cells, and the anti-HER3 arm blocks NRG binding. In a phase I study, 16 patients with HER2+/HRG + mBC were enrolled to receive MM-111 plus trastuzumab. The main adverse events were anemia (25%), diarreha (43.75%), fatigue (50%), decreased appetite (25%), headache (25%), insomnia (25%), dyspnoea (37.5 %) (NCT01097460).

Antibody-drug conjugates

The targeting of HER3 has found new strength in recent years thanks to the development of the new ADC technology (TAB. 2). The most promising results have been observed with Patritumab-deruxtecan (HER3-DXd). A peptide-cleavable linker links Patritumab to topoisomerase I inhibitor payload, an exatecan derivative [11]. The phase I/II U31402-A-J101 trial was the first study to evaluate HER3-DXd in heavily pretreated patients with HER3-expressing metastatic breast cancer, of any subtype: either HR+ or HER2-positive or triple negative. The dose-escalation and dose-finding parts included patients with HER3-high breast cancer (2+ or 3+ at IHC) while the dose-expansion part included patients with HER3-high– or low (either membrane positivity of $\geq$ 75% or and HER3-low was set at $\geq$ 25% to $<$ 75% at 10x magnification, respectively) HR+ or HER2-positive breast cancer or HER3-high TNBC. Durable antitumor activity was obtained across a wide range of HER3 expression and throughout different BC subsets: HR-positive/HER2-negative BC (ORR 30.1%; median progression-free survival [mPFS], 7.4 months), TNBC (ORR, 22.6%; mPFS, 5.5 months), and HER2-positive breast cancer (ORR, 42.9%; mPFS, 11.0 months) [17,18]. Median OS 14.6 months for HR+/HER2- (11.3 to 19.5) 14.6 months (11.2 to 17.2) for TNBC 19.5 months (12.2 to NE) for HER2-positive. The safety profile showed that 130 pts (71.4%) had grade $\geq$ 3 TEAEs and the most common grade 3 or 4 TEAEs were: decreased neutrophil count (grade 3, 26.9%; grade 4, 12.6%), decreased platelet count (grade 3, 12.1 %; grade 4, 18.7%), anemia (grade 3, 18.1%; grade 4, 0.5 %), and decreased WBC count (grade 3, 15.9 %; grade 4, 2.2%) [18]. Recently, the phase II BRE345 study confirmed the clinical activity of HER3-DXd (5.6 mg/kg Q3W) in patients with HER2-negative metastatic breast cancer, with an ORR of 35% and overall clinical benefit rate (CBR) of 43%, regardless of HER3 membrane expression, yet very few patients presented tumors with HER3 membrane expression $<$ 25 % (4 patients out of 60) [76]. In the advanced breast cancer setting, the ongoing ICARUS-BREAST01 multi-center, single-arm, phase II study is evaluating clinical activity and biomarkers of response and resistance to HER3-DXd in patients with HR+ and HER2- breast cancer who progressed on CDK 4/6-inhibitors and were mostly unselected for HER3 expression (HER3-expression pre-screening population was removed by amendment). The first results confirmed the HER3-DXd manageable safety profile (fatigue 89.3%, nausea 76.8%; grade $\geq$ 3 AEs was fatigue 14.0% and 1 G1 ILD 1.8%) and reported a three months-RR of 28.6% (95 %CI: 18.4–41.5), so underpinning its clinical activity in this setting. (NCT04965766) [19]. The SOLTI TOT-HER3 study evaluated the biological and clinical activity of a single dose of HER3-DXd (6.4 mg/kg or 5.6 mg/kg) in untreated patients with early HR+/HER2-negative or triple-negative breast cancer patients. The primary objective was to assess change from baseline in a combined score based on tumor cellularity and tumor-infiltrating lymphocytes (CelTIL score). In patients with HR+/HER2- breast cancer, a clinical overall response rate of 45% was observed, with a trend toward an increase in CelTIL score among responders compared with non-responders. Change in CelTIL score was independent of baseline HERB3 mRNA and HER3 protein levels[16]. Interestingly, in patients with TNBC, a single dose of HER3-DXd was associated with increased CelTIL score and clinical response and with preliminary evidence of PAM50 switch towards less aggressive biological subtype [77]. VALENTINE trial (NCT05569811) is an ongoing, non-comparative, three-arm, randomized 1:2:2 open-label, multicenter, study that evaluates the clinical benefit and biological effects of HER3-DXd with/without letrozole as a neoadjuvant treatment regimen in primary operable HR+/HER2-negative breast cancer patients, with ki67 $\geq$ 20 % and/or high genomic risk. The primary endpoint is the rate of pCR (ypT0/is ypN0) at surgery. In this phase II study, the experimental arm was Patritumab-Deruxtecan +/- letrozole, and the active comparator arm was the chemotherapy. Results are not available yet. Baseline, on-treatment, and surgical specimens will be collected for molecular characterization and evaluation of response (HER3 gene expression, HER3 IHC; CelTIL change, PAM50 subtypes)[20]. Finally, the TUXEDO-3 (NCT05865990) is a new ongoing phase II, single-arm, multicohort trial which is evaluating the efficacy of HER3-DXd in patients with metastatic breast cancer (cohort 1) or advanced non-small cell lung cancer (cohort 2) with active brain metastases after at least one line of systemic therapy in the advanced setting and patients with advanced solid tumors with leptomeningeal metastasis (cohort 3). The dual primary endpoints are the IC-ORR (intracranial-ORR) in cohorts 1 and 2 and the OS at three months in cohort 3. BL-B01D1 is a first-in-class bispecific antibody-drug conjugate with an EGFRxHER3 bispecific antibody linked to a novel topoisomerase-I inhibitor payload via a cleavable linker. In the first-in-human phase I study (NCT05470348), BL-B01D1 at the tested doses of 2.5, 3.0, and 3.5 mg/kg D1D8Q3W, or 5.0 and 6.0 mg/kg D1Q3W, demonstrated promising activity in heavily

pretreated patients with solid tumors, especially in EGFRm NSCLC: ORR 63.2% (46.0–78.2) in EGFRmut NSCLC and 44.0 % (30.0–58.7) in EGFRwt NSCLC [78]. The most common TRAEs (>10 %, all grade/≥ G3) were anemia (59%/25%), leukopenia (59%/28%), neutropenia (51%/32%), thrombocytopenia (48%/23%). Early signs of clinical activity were also observed in heavily pretreated patients with metastatic breast cancer of any subtype: ORR was 31.4% (16.9–49.3) in TNBC, 44.7% (28.6–61.7) in HR+/HER2-negative and 39.1% (19.7–61.5) in HER2-positive [79]. DB-1310 is a new ADC composed of a novel humanized IgG1 HER3 monoclonal antibody linked to DNA topoisomerase I inhibitor via a cleavable linker. DB-1310 exhibited antitumor activity in vivo and in vitro lung and breast cancer models [80]. These data led to the development of an ongoing phase 1/2a, non-randomized first-in-human study to assess the safety, tolerability, pharmacokinetics, and preliminary antitumor activity of DB-1310 in subjects with advanced/metastatic solid tumors (NCT05785741), including HER2-positive breast cancer.

Cancer vaccines and cell therapies

Dendritic cell (DC) vaccines are based on isolated DCs loaded with tumor antigen ex vivo and administered as a cellular vaccine, capable of inducing protective and therapeutic anti-tumor immunity[81]. An ongoing phase IIa trial (NCT04348747) is exploring how dendritic cell vaccines against Her2/Her3 and pembrolizumab work for the treatment of triple-negative breast cancer or HER2+ breast cancer with brain metastasis. The primary endpoint is the overall central nervous system (CNS) response. New therapeutic strategies are emerging that can inhibit HER3 indirectly, by enhancing immune response. A recombinant adenoviral vector expressing full-length human HER3 (Ad-HER3-FL) is a cancer vaccine that, in vivo and in vitro breast cancer models, stimulates the production of HER3-specific T cells and can generate polyclonal antibodies with multiple functions, including antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity. The combination of Ad-HER3-FL with an anti-PD1, showed enhanced response compared to the vaccine alone [82]. Clinical studies are not yet available, but it is reasonable to think about combination trials destined to HER2-positive breast cancers showing an HER3-mediated resistance and above TNBC patients where checkpoint blockade is clinically active but with a limited treatment response rate [83].

Future directions and challenges

Although its expression has a controversial prognostic value, HER3 has a crucial role among all three breast cancer subtypes in cancer progression, metastases development, and escape from anti-tumor treatments. At the same time, HER3-targeting by monoclonal antibodies failed to show substantial clinical impact. HER3-monoclonal antibodies should be able to inhibit dimerization and, therefore, its activating pathways, but HER3 is unable to homodimerize and lacks potent kinase activity, suggesting that HER3 could be used as a cancer cell driver instead of a cancer cell target. Only recently, with the introduction of mono- or bispecific ADCs, which have been showing promising activity, research on HER3 has reinvigorated the exploration of new drugs. Like TROP2 and HER2 [84,85], HER3-ADCs exert antitumor activity by modulating the signals emanating from HER3 pathways and through Fc-mediated effector functions. However, through internalization and the action of lysosomes, they release the payload, which is responsible for its action in the targeted cancer cell as well as for the bystander effect, exerting its activity in the neighbouring cells regardless of their expression of HER3. However, many questions remain unaddressed. First, whether or not HER3 membrane expression is predictive of the efficacy of HER3-targeting therapies and particularly of HER3-targeting ADCs. HER3-DXd demonstrated significant clinical and biologic activity across a broad range of HER3 membrane expression, both in metastatic and early breast cancer [17,20,76]. Hamilton et al. demonstrated that across patients with different HER3 expression levels, the ORR and CBR values were similar, indicating that HER3 does not appear to be predictive of HER3-Dxd efficacy. Patients with ≥75 % HER3 expression had an ORR of 33%, a CBR of 50%, and a 6-month PFS of 50%. Patients with 25–74% HER3 expression had an ORR of 46%, a CBR of 54%, and a 6-month PFS of 70% [76]. In the SOLTI-1805 TOT-HER3 study, the first analysis showed that changes in the CelTIL score after Patritumab-deruxtecan exposure were not correlated with HER3 expression, either in IHC or mRNA [16]. Nevertheless, it is hard to draw any conclusion on the activity of HER3-DXd in HER3-low or HER3-negative tumors, because on the one hand, no antitumor activity of HER3-DXd was observed in HER3-low expressing tumor xenografts [11], on the other, in most of the studies with HER3-DXd, only a few of patients presented HER3-low or HER3-neg tumors. Second, no validated assays exist so far for measuring HER3 membrane expression and different methods have been applied across several studies and HER3-targeting treatments. In addition, in contrast to HER2, which is mainly found in the plasma membrane, HER3 is primarily located within intracellular compartments[86], and present a rapid internalization, mostly clathrin-mediated [87]. Therefore, it is still unclear whether both cell surface and intracellular expression of HER3 affect treatment activity and, ultimately, if a minimal threshold can be established for predicting the efficacy of HER3-targeting therapies. Third, HER3 expression is highly dynamic and can change over time with exposure to prior endocrine therapy or many TKIs. Therefore, it is still unclear which time point, in the natural history of metastatic breast cancer, is optimal to assess HER3 expression and whether the dynamic changes of HER3 expression could be better monitored by less invasive technologies such as circulating tumor cells (CTCs) and molecular imaging, which are also able to capture intratumor heterogeneity [88,89]. Given that the most promising anti-HER3 therapy currently is ADC, the question of sequencing ADCs arises. The primary resistance mechanisms to ADCs include internalization and trafficking, drug efflux, payload and tumor microenvironment resistance, and antigen modulation. For example, in a preclinical model, long-term exposure to an ADC targeting HER2 reduces HER2 receptor expression in breast cancer cell lines [90]. Switching targets and using an anti-HER3 ADC in the treatment sequence would be the best choice in this resistance scenario. Conversely, in cases of resistance to payload targets or mutations in drug-metabolizing enzymes, using an ADC with the same payload, such as deruxtecan, may lead to poor outcomes due to cross-resistance. More data are needed to address these questions. Currently, two trials are evaluating the efficacy of patritumab-deruxtecan after ADCs: a phase Ib/II trial of patritumab-deruxtecan monotherapy and combination therapy in patients with inoperable advanced breast cancer post-progression on T-DXd (ICARUS-BREAST 02, NCT06298084), and a phase II trial of patritumab-deruxtecan in locally advanced or metastatic breast cancer, including HR+, HER2-, and mTNBC patients (part B) who have received trastuzumab deruxtecan, sacituzumab govitecan, and/or datopotamab deruxtecan (NCT04699630).

Conclusions

In this review, we examined the distinctive characteristics and the pivotal oncogenic function of HER3 within various breast cancer subtypes. Additionally, we elucidated its involvement in promoting resistance to various treatments for breast cancer. Despite the great rationale, nowadays, no treatment explicitly targeting HER3 has been approved for clinical use in breast cancer. Still, the promising results of patritumab–deruxtecan are paving the way to new therapeutic directions, and the development of new immune-modulating technologies is laying the groundwork for new treatment strategies. These future therapeutic approaches may likely render the detection of HER3 indispensable throughout the breast cancer diagnosis and therapeutic journey, influencing the treatment algorithms.

Financial support.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.
None.
Authors contribution.

- Paper concept and design: Barbara Pistilli and Francesca Papa;
- Review of the literature: Francesca Papa;
- First draft: Francesca Papa, Thomas Grinda;
- Manuscript editing and critical review: All authors;
- Approval of the final draft: All authors.
- **Key points.**
- • HER3 is overexpressed in all three breast cancer subtypes and plays a central role in resistance to breast cancer treatments.
- • HER3 is a potent activator of PI3K/Akt signalling.
- • While targeting of HER3 has failed in the past, currently multiple HER3-directed antibodies and ADCs have been entered the clinical development with promising outcomes.

References

[1] A. Ocana, F. Vera-Badillo, B. Seruga, A. Templeton, A. Pandiella, e E. Amir, «HER3 overexpression and survival in solid tumors: A meta-analysis», *Journal of the National Cancer Institute*, vol. 105, fasc. 4. pp. 266–273, febbraio 2013. doi: 10.1093/jnci/djs501.

[2] N. Jiang, N. F. Saba, e Z. G. Chen, «Advances in Targeting HER3 as an Anticancer Therapy», *Chemotherapy Research and Practice*, vol. 2012, pp. 1–9, nov. 2012, doi: 10.1155/2012/817304.

[3] Maennling AE, et al. Molecular targeting therapy against egfr family in breast cancer: Progress and future potentials. Cancers 2019;11:dic. https://doi.org/10.3390/cancers11121826.

[4] M. J. Wieduwilt e M. M. Moasser, «The epidermal growth factor receptor family: Biology driving targeted therapeutics», *Cellular and Molecular Life Sciences*, vol. 65, fasc. 10, pp. 1566–1584, mag. 2008, doi: 10.1007/s00018-008-7440-8.

[5] H. Lyu, A. Han, E. Polsdofer, S. Liu, e B. Liu, «Understanding the biology of HER3 receptor as a therapeutic target in human cancer», *Acta Pharmaceutica Sinica B*, vol. 8, fasc. 4. Chinese Academy of Medical Sciences, pp. 503–510, luglio 2018. doi: 10.1016/j.apsb.2018.05.010.

[6] T. Pascual *et al.*, «2O ERBB3 mRNA expression in breast cancer (BC): A SOLTI biomarker discovery analysis», *Annals of Oncology*, vol. 31, pp. S15–S16, mag. 2020, doi: 10.1016/j.annonc.2020.03.138.

[7] L. Gandullo-Sánchez, A. Ocaña, e A. Pandiella, «HER3 in cancer: from the bench to the bedside», *Journal of Experimental and Clinical Cancer Research*, vol. 41, fasc. 1. BioMed Central Ltd, dicembre 2022. doi: 10.1186/s13046-022-02515-x.

[8] R. Naidu, M. Yadav, S. Nair, e M. Kutty, «Expression of c-erbB3 protein in primary breast carcinomas», *Br J Cancer*, vol. 78, fasc. 10, pp. 1385–1390, nov. 1998, doi: 10.1038/bjc.1998.689.

[9] R. Montaser e H. Coley, «Crosstalk between ERα and Receptor Tyrosine Kinase Signalling and Implications for the Development of Anti-Endocrine Resistance», *Cancers*, vol. 10, fasc. 6, p. 209, giu. 2018, doi: 10.3390/cancers10060209.

[10] L. Guo *et al.*, «Breast cancer heterogeneity and its implication in personalized precision therapy», *Experimental Hematology & Oncology*, vol. 12, fasc. 1, p. 3, gen. 2023, doi: 10.1186/s40164-022-00363-1.

[11] S. Koganemaru *et al.*, «U3-1402, a Novel HER3-Targeting Antibody–Drug Conjugate, for the Treatment of Colorectal Cancer», *Molecular Cancer Therapeutics*, vol. 18, fasc. 11, pp. 2043–2050, nov. 2019, doi: 10.1158/1535-7163.MCT-19-0452.

[12] K. Gala e S. Chandarlapaty. Molecular pathways: HER3 Targeted therapy. Clin Cancer Res 2014;20:1410–6. https://doi.org/10.1158/1078-0432.CCR-13-1549.

[13] E. P. Hamilton *et al.*, «Clinical activity of MCLA-128 (zenocutuzumab), trastuzumab, and vinorelbine in HER2 amplified metastatic breast cancer (MBC) patients (pts) who had progressed on anti-HER2 ADCs.», *Journal of Clinical Oncology*, vol. 38, fasc. 15_suppl, pp. 3093–3093, mag. 2020, doi: 10.1200/JCO.2020.38.15_suppl.3093.

[14] B. Pistilli *et al.*, «Clinical activity of MCLA-128 (zenocutuzumab) in combination with endocrine therapy (ET) in ER+/HER2-low, non-amplified metastatic breast cancer (MBC) patients (pts) with ET-resistant disease who had progressed on a CDK4/6 inhibitor (CDK4/6i).», *JCO*, vol. 38, fasc. 15_suppl, pp. 1037–1037, mag. 2020, doi: 10.1200/JCO.2020.38.15_suppl.1037.

[15] S. Thrane, A. E. Lykkesfeldt, M. S. Larsen, B. S. Sorensen, e C. W. Yde, «Estrogen receptor α is the major driving factor for growth in tamoxifen-resistant breast cancer and supported by HER/ERK signaling», *Breast Cancer Res Treat*, vol. 139, fasc. 1, pp. 71–80, mag. 2013, doi: 10.1007/s10549-013-2485-2.

[16] Oliveira M, et al. Patritumab deruxtecan in untreated hormone receptor-positive/HER2-negative early breast cancer: final results from part A of the window-of-opportunity SOLTI TOT-HER3 pre-operative study. *Ann Oncol*, ago 2023. https://doi.org/10.1016/j.annonc.2023.05.004.

[17] I. E. Krop *et al.*, «Results from the phase 1/2 study of patritumab deruxtecan, a HER3-directed antibody-drug conjugate (ADC), in patients with HER3-expressing metastatic breast cancer (MBC).», *Journal of Clinical Oncology*, vol. 40, fasc. 16_suppl, pp. 1002–1002, giu. 2022, doi: 10.1200/JCO.2022.40.16_suppl.1002.

[18] I. E. Krop *et al.*, «Patritumab Deruxtecan (HER3-DXd), a Human Epidermal Growth Factor Receptor 3–Directed Antibody-Drug Conjugate, in Patients With Previously Treated Human Epidermal Growth Factor Receptor 3–Expressing Metastatic Breast Cancer: A Multicenter, Phase I/II Trial», *JCO*, p. JCO.23.00882, ott. 2023, doi: 10.1200/JCO.23.00882.

[19] B. Pistilli *et al.*, «189O A phase II study of patritumab deruxtecan (HER3-DXd), in patients (pts) with advanced breast cancer (ABC), with biomarker analysis to characterize response to therapy (ICARUS-BREAST01)», *ESMO Open*, vol. 8, fasc. 1, p. 101378, mag. 2023, doi: 10.1016/j.esmoop.2023.101378.

[20] M. Oliveira *et al.*, «155TiP A randomised phase II trial of neoadjuvant multi-agent chemotherapy (CHT) OR patritumab deruxtecan (HER3-DXd; U3-1402) +/- endocrine therapy (ET) for high-risk hormone receptor-positive (HR+/HER2-) early breast cancer (EBC): SOLTI-2103 VALENTINE trial», *ESMO Open*, vol. 8, fasc. 1, p. 101494, mag. 2023, doi: 10.1016/j.esmoop.2023.101494.

[21] M. H. Kraus, W. Issing, T. Miki, N. C. Popescu, e S. A. Aaronson, «Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors.», *Proceedings of the National Academy of Sciences*, vol. 86, fasc. 23, pp. 9193–9197, dic. 1989, doi: 10.1073/pnas.86.23.9193.

[22] A. W. Burgess, «EGFR family: Structure physiology signalling and therapeutic targets †», *Growth Factors*, vol. 26, fasc. 5, pp. 263–274, gen. 2008, doi: 10.1080/08977190802312844.

[23] J. Baselga e S. M. Swain, «Novel anticancer targets: Revisiting ERBB2 and discovering ERBB3», *Nature Reviews Cancer*, vol. 9, fasc. 7. pp. 463–475, luglio 2009. doi: 10.1038/nrc2656.

[24] E. Tzahar *et al.*, «A Hierarchical Network of Interreceptor Interactions Determines Signal Transduction by Neu Differentiation Factor/Neuregulin and Epidermal Growth Factor», *Molecular and Cellular Biology*, vol. 16, fasc. 10, pp. 5276–5287, ott. 1996, doi: 10.1128/MCB.16.10.5276.

[25] R. Pinkas-Kramarski *et al.*, «Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions», *The EMBO Journal*, vol. 15, fasc. 10. pp. 2452–2467, 1996.

[26] M. R. Campbell *et al.*, «Extensive conformational and physical plasticity protects HER2-HER3 tumorigenic signaling», *Cell Reports*, vol. 38, fasc. 5, p. 110285, feb. 2022, doi: 10.1016/j.celrep.2021.110285.

[27] X. Liu, S. Liu, H. Lyu, A. I. Riker, Y. Zhang, e B. Liu, «Development of Effective Therapeutics Targeting HER3 for Cancer Treatment», *Biol Proced Online*, vol. 21, fasc. 1, p. 5, dic. 2019, doi: 10.1186/s12575-019-0093-1.

[28] J. C. Montero, R. Rodríguez-Barrueco, A. Ocaña, E. Díaz-Rodríguez, A. Esparís-Ogando, e A. Pandiella, «Neuregulins and Cancer», *Clinical Cancer Research*, vol. 14, fasc. 11, pp. 3237–3241, giu. 2008, doi: 10.1158/1078-0432.CCR-07-5133.

[29] M. B. Berger, J. M. Mendrola, e M. A. Lemmon, «ErbB3/HER3 does not homodimerize upon neuregulin binding at the cell surface», *FEBS Letters*, vol. 569, fasc. 1–3, pp. 332–336, lug. 2004, doi: 10.1016/j.febslet.2004.06.014.

[30] M. K. Kilroy *et al.*, «HER3 Alterations in Cancer and Potential Clinical Implications», *Cancers*, vol. 14, fasc. 24, p. 6174, dic. 2022, doi: 10.3390/cancers14246174.

[31] D. N. Amin, M. R. Campbell, e M. M. Moasser, «The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics», *Seminars in Cell and Developmental Biology*, vol. 21, fasc. 9. Elsevier Ltd, pp. 944–950, 2010. doi: 10.1016/j.semcdb.2010.08.007.

[32] Q. Sheng e J. Liu. The therapeutic potential of targeting the EGFR family in epithelial ovarian cancer. Br J Cancer Apr 2011;104:1241–5. https://doi.org/10.1038/bjc.2011.62.

[33] Fedi P, Pierce JH, di Fiore PP, e M. H. Kraus. Efficient coupling with phosphatidylinositol 3-kinase, but not phospholipase CY or GTPase-activating protein, distinguishes ErbB-3 signaling from that of other ErbB/EGFR family members. Mol Cell Biol 1994;14:492–500. https://doi.org/10.1128/mcb.14.1.492-500.1994.

[34] Suenaga A, et al. Novel Mechanism of Interaction of p85 Subunit of Phosphatidylinositol 3-Kinase and ErbB3 Receptor-derived Phosphotyrosyl Peptides. J Biol Chem 2005;280:1321–6. https://doi.org/10.1074/jbc.M410436200.

[35] J. Liu e J. A. Kern. Neuregulin-1 Activates the JAK-STAT Pathway and Regulates Lung Epithelial Cell Proliferation. Am J Respir Cell Mol Biol 2002;27:306–13. https://doi.org/10.1165/rcmb.4850.

[36] Huang X, et al. Heterotrimerization of the Growth Factor Receptors erbB2, erbB3, and Insulin-like Growth Factor-I Receptor in Breast Cancer Cells Resistant to Herceptin. Cancer Res Feb 2010;70:1204–14. https://doi.org/10.1158/0008-5472.CAN-09-3321.

[37] Olayioye MA. NEW EMBO MEMBERS' REVIEW: The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J Aug. 2000,;19:3159–67. https://doi.org/10.1093/emboj/19.13.3159.

[38] J. A. Engelman *et al.*, «ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines», *Proceedings of the National Academy of Sciences*, vol. 102, fasc. 10, pp. 3788–3793, mar. 2005, doi: 10.1073/pnas.0409773102.

[39] Luhtala S, Staff S, Barok M, Tanner M, e J. Isola. Comparison of Antibodies for Immunohistochemistry-based Detection of HER3 in Breast Cancer. Appl Immunohistochem Mol Morphol Mar. 2018;26:212–9. https://doi.org/10.1097/PAI.0000000000000406.

[40] Kurozumi S, et al. Comparing protein and mRNA expressions of the human epidermal growth factor receptor family in estrogen receptor-positive breast cancer. Med Mol Morphol 2019;vol. 52:90–8. https://doi.org/10.1007/s00795-018-0206-y.

[41] Prat A, Falato C, Pare Brunet L, et al. Patritumab deruxtecan (HER3-DXd) in early-stage HR+/HER2- breast cancer: final results of the SOLTI TOT-HER3 window of opportunity trial. Ann Oncol 2022;33. https://doi.org/10.1016/annonc/annonc890».

[42] K. Inaki *et al.*, «Pan-cancer gene expression analysis of tissue microarray using EdgeSeq oncology biomarker panel and a cross-comparison with HER2 and HER3 immunohistochemical analysis», *PLoS ONE*, vol. 17, fasc. 9, p. e0274140, set. 2022, doi: 10.1371/journal.pone.0274140.

[43] Miano C, et al. NRG1/ERBB3/ERBB2 Axis Triggers Anchorage-Independent Growth of Basal-like/Triple-Negative Breast Cancer Cells. Cancers Apr. 2022;vol. 14. https://doi.org/10.3390/cancers14071603.

[44] Mishra R, et al. Activating HER3 mutations in breast cancer. Oncotarget 2018;vol. 9:27773–88. https://doi.org/10.18632/oncotarget.25576.

[45] Vaught DB, et al. HER3 Is Required for HER2-Induced Preneoplastic Changes to the Breast Epithelium and Tumor Formation. Cancer Res 2012;vol. 72:2672–82. https://doi.org/10.1158/0008-5472.CAN-11-3594.

[46] Lyu H, et al. HER3 functions as an effective therapeutic target in triple negative breast cancer to potentiate the antitumor activity of gefitinib and paclitaxel. Cancer Cell Int 2023;23:204. https://doi.org/10.1186/s12935-023-03055-w.

[47] Amin DN, Sergina N, Lim L, Goga A, e M. M. Moasser. HER3 signalling is regulated through a multitude of redundant mechanisms in HER2-driven tumour cells. Biochem J Nov. 2012;vol. 447:417–25. https://doi.org/10.1042/BJ20120724.

[48] Yen L, et al. Loss of Nrdp1 enhances ErbB2/ErbB3-dependent breast tumor cell growth. Cancer Res 2006;66:11279–86. https://doi.org/10.1158/0008-5472.CAN-06-2319.

[49] Ingalla EQ, et al. Post-transcriptional mechanisms contribute to the suppression of the ErbB3 negative regulator protein Nrdp1 in mammary tumors. J Biol Chem 2010;vol. 285:28691–7. https://doi.org/10.1074/jbc.M110.127977.

[50] S. Sannino e J. L. Brodsky. Targeting protein quality control pathways in breast cancer. BMC Biol 2017;vol. 15:109. https://doi.org/10.1186/s12915-017-0449-4.

[51] Huang Z, et al. The E3 ubiquitin ligase NEDD4 negatively regulates HER3/ErbB3 level and signaling. Oncogene Feb. 2015;vol. 34:1105–15. https://doi.org/10.1038/onc.2014.56.

[52] C. A. Dreyer, K. V. Vorst, S. Free, A. Rowson-Hodel, e K. L. Carraway, «The role of membrane mucin MUC4 in breast cancer metastasis», *Endocrine-Related Cancer*, vol. 29, fasc. 1. BioScientifica Ltd., pp. R17–R32, novembre 2022. doi: 10.1530/ERC-21-0083.

[53] I. Vivanco e C. L. Sawyers. The phosphatidylinositol 3-Kinase–AKT pathway in human cancer. Nat Rev Cancer 2002;vol. 2:489–501. https://doi.org/10.1038/nrc839.

[54] Lee JY, Chiu Y-H, Asara J, e L. C. Cantley. Inhibition of PI3K binding to activators by serine phosphorylation of PI3K regulatory subunit p85α Src homology-2 domains. Proc Natl Acad Sci USA 2011;vol. 108:14157–62. https://doi.org/10.1073/pnas.1107747108.

[55] Chandarlapaty S, et al. AKT Inhibition Relieves Feedback Suppression of Receptor Tyrosine Kinase Expression and Activity. Cancer Cell 2011;vol. 19:58–71. https://doi.org/10.1016/j.ccr.2010.10.031.

[56] Li C, et al. A ROR1-HER3-lncRNA signalling axis modulates the Hippo-YAP pathway to regulate bone metastasis. Nat Cell Biol 2017;vol. 19:106–19. https://doi.org/10.1038/ncb3464.

[57] Kodack DP, et al. The brain microenvironment mediates resistance in luminal breast cancer to PI3K inhibition through HER3 activation. Sci Transl Med 2017;vol. 9. https://doi.org/10.1126/scitranslmed.aal4682.

[58] Kusuhara S, et al. 264P Increased membrane HER3 expression in brain metastases compared to primary tumors in breast cancer. Ann Oncol 2022,;33:S658. https://doi.org/10.1016/j.annonc.2022.07.303.

[59] B. H. Park e N. E. Davidson. PI3 Kinase Activation and Response to Trastuzumab Therapy: What's neu with Herceptin Resistance? Cancer Cell 2007;vol. 12:297–9. https://doi.org/10.1016/j.ccr.2007.10.004.

[60] Berns K, et al. A Functional Genetic Approach Identifies the PI3K Pathway as a Major Determinant of Trastuzumab Resistance in Breast Cancer. Cancer Cell 2007;vol. 12:395–402. https://doi.org/10.1016/j.ccr.2007.08.030.

[61] Xia W, et al. An heregulin-EGFR-HER3 autocrine signaling axis can mediate acquired lapatinib resistance in HER2+ breast cancer models. Breast Cancer Res 2013;vol. 15. https://doi.org/10.1186/bcr3480.

[62] Wang S, Huang X, Lee CK, e B. Liu. Elevated expression of erbB3 confers paclitaxel resistance in erbB2-overexpressing breast cancer cells via upregulation of Survivin. Oncogene 2010;vol. 29:4225–36. https://doi.org/10.1038/onc.2010.180.

[63] Mita AC, Mita MM, Nawrocki ST, e F. J. Giles. Survivin: Key Regulator of Mitosis and Apoptosis and Novel Target for Cancer Therapeutics. Clin Cancer Res 2008;14:5000–5. https://doi.org/10.1158/1078-0432.CCR-08-0746.

[64] Hutcheson IR, et al. Fulvestrant-induced expression of ErbB3 and ErbB4 receptors sensitizes oestrogen receptor-positive breast cancer cells to heregulin β1. Breast Cancer Res 2011;13. https://doi.org/10.1186/bcr2848.

[65] Liu B, Ordonez-Ercan D, Fan Z, Edgerton SM, Yang XH, e A. D. Thor. Downregulation of erbB3 abrogates erbB2-mediated tamoxifen resistance in breast cancer cells. Int J Cancer 2007;vol. 120:1874–82. https://doi.org/10.1002/ijc.22423.

[66] Tao JJ, et al. Antagonism of EGFR and HER3 enhances the response to inhibitors of the PI3K-Akt pathway in triple-negative breast cancer. Sci Signal 2014;vol. 7. https://doi.org/10.1126/scisignal.2005125.

[67] Ogden A, et al. Combined HER3-EGFR score in triple-negative breast cancer provides prognostic and predictive significance superior to individual biomarkers. Sci Rep 2020;vol. 10. https://doi.org/10.1038/s41598-020-59514-1.

[68] Schneeweiss A, et al. Phase Ib study evaluating safety and clinical activity of the anti-HER3 antibody lumretuzumab combined with the anti-HER2 antibody pertuzumab and paclitaxel in HER3-positive, HER2-low metastatic breast cancer. Invest New Drugs 2018;vol. 36:848–59. https://doi.org/10.1007/s10637-018-0562-4.

[69] Finn G, et al. Abstract A14: A randomized trial of exemestane +/- seribantumab (MM-121) in postmenopausal women with locally advanced or metastatic ER/PR+ HER2- breast cancer: Final analysis and extended subgroup analysis. Clin Cancer Res 2017;23:A14–. https://doi.org/10.1158/1557-3265.PMCCAVULN16-A14.

[70] Kaufman PA, et al. Abstract OT3-06-01: SHERBOC: A double-blind, placebo-controlled, phase 2 trial of seribantumab (MM-121) plus fulvestrant in postmenopausal women with hormone receptor-positive, heregulin positive, HER2 negative metastatic breast cancer whose disease progressed after prior systemic therapy. Cancer Res 2018;78. https://doi.org/10.1158/1538-7445.SABCS17-OT3-06-01.

[71] Holmes FA, et al. Abstract P3–11-03: A randomized, phase 2 trial of preoperative MM-121 with paclitaxel in triple negative (TN) and hormone receptor (HR) positive, HER2-negative breast cancer. Cancer Res 2015;vol. 75. https://doi.org/10.1158/1538-7445.SABCS14-P3-11-03.

[72] Jhaveri K, et al. A Phase I Study of Alpelisib in Combination with Trastuzumab and LJM716 in Patients with *PIK3CA* -Mutated HER2-Positive Metastatic Breast Cancer. Clin Cancer Res 2021;vol. 27:3867–75. https://doi.org/10.1158/1078-0432.CCR-21-0047.

[73] Mukai H, et al. Patritumab plus trastuzumab and paclitaxel in human epidermal growth factor receptor 2-overexpressing metastatic breast cancer. Cancer Sci 2016;vol. 107:1465–70. https://doi.org/10.1111/cas.13017.

[74] Alsina M, et al. First-in-human phase 1/2 study of MCLA-128, a full length IgG1 bispecific antibody targeting HER2 and HER3: Final phase 1 data and preliminary activity in HER2+ metastatic breast cancer (MBC). J Clin Oncol 2017;vol. 35:2522. https://doi.org/10.1200/JCO.2017.35.15_suppl.2522.

[75] Schram AM, et al. Zenocutuzumab, a HER2xHER3 Bispecific Antibody, Is Effective Therapy for Tumors Driven by NRG1 Gene Rearrangements. Cancer Discov 2022;vol. 12:1233–47. https://doi.org/10.1158/2159-8290.CD-21-1119.

[76] Hamilton EP, et al. A phase 2 study of HER3-DXd in patients (pts) with metastatic breast cancer (MBC). JCO 2023;vol. 41:1004. https://doi.org/10.1200/JCO.2023.41.16_suppl.1004.

[77] Oliveira, et al. ESMO BREAST 2023; Annals of Oncology 2023;8(1suppl_4):101220. https://doi.org/10.1016/esmoop/esmoop101220».

[78] Zhang L, et al. 1316MO BL-B01D1, a first-in-class EGFRxHER3 bispecific antibody-drug conjugate, in patients with non-small cell lung cancer: Updated results from first-in-human phase I study. Ann Oncol 2023;34:S758. https://doi.org/10.1016/j.annonc.2023.09.2350.

[79] «PS08-07 BL-B01D1, a first-in-class EGFRxHER3 bispecific antibody-drug conjugate, in patients with Locally Advanced or Metastatic Breast Cancer and other Solid Tumor: Results from a phase 1 study.. Jiong Wu (1) Jian Zhang (2) Yiqun Du (2) Wen Zou (3) Muran Ding (4) Hui Yang (4) Sa Xiao (4) Hongwei Wang (5) Hai Zhu (6) Martin Olivo (7) Yi Zhu (5)».

[80] Li X, et al. Abstract 1884: DB-1310, a novel Her3 targeting antibody-drug conjugate, exhibits therapeutic efficacy for solid tumors. Cancer Res 2023;vol. 83:1884. https://doi.org/10.1158/1538-7445.AM2023-1884.

[81] Timmerman JM, Md e R. Levy, Md. Dendritic Cell Vaccines for Cancer Immunotherapy. Annu Rev Med feb. 1999,;vol. 50:507–29. https://doi.org/10.1146/annurev.med.50.1.507.

[82] Osada T, et al. Vaccination targeting human HER3 alters the phenotype of infiltrating T cells and responses to immune checkpoint inhibition. OncoImmunology 2017;vol. 6. https://doi.org/10.1080/2162402X.2017.1315495.

[83] Zheng Y, Li S, Tang H, Meng X, e Q. Zheng. Molecular mechanisms of immunotherapy resistance in triple-negative breast cancer. Front Immunol 2023;14. https://doi.org/10.3389/fimmu.2023.1153990.

[84] Rassy E, Rached L, e B. Pistilli. Antibody drug conjugates targeting HER2: Clinical development in metastatic breast cancer. Breast dic. 2022,;66:217–26. https://doi.org/10.1016/j.breast.2022.10.016.

[85] Bardia A, et al. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. N Engl J Med 2021;vol. 384:1529–41. https://doi.org/10.1056/NEJMoa2028485.

[86] Offterdinger M, Schöfer C, Weipoltshammer K, e T. W. Grunt. c-erbB-3. J Cell Biol 2002;vol. 157:929–40. https://doi.org/10.1083/jcb.200109033.

[87] Fosdahl AM, et al. «ErbB3 interacts with Hrs and is sorted to lysosomes for degradation», *Biochimica et Biophysica Acta (BBA) - Molecular*. Cell Res 2017;vol. 1864:2241–52. https://doi.org/10.1016/j.bbamcr.2017.08.011.

[88] Rinne SS, et al. HER3 PET Imaging: 68Ga-Labeled Affibody Molecules Provide Superior HER3 Contrast to 89Zr-Labeled Antibody and Antibody-Fragment-Based Tracers. Cancers 2021;vol. 13:4791. https://doi.org/10.3390/cancers13194791.
[89] Pistilli, et al. ESMO Breast 2023; Annals of Oncology 2023;8(1suppl_4):101223. https://doi.org/10.1016/esmoop/esmoop101223».
[90] Irie H, et al. Acquired resistance to trastuzumab/pertuzumab or to T-DM1 in vivo can be overcome by HER2 kinase inhibition with TAS0728. Cancer Sci 2020;vol. 111:2123–31. https://doi.org/10.1111/cas.14407.