Targeting HER2-positive breast cancer: advances and future directions

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Abstract

The long-sought discovery of HER2 as an actionable and highly sensitive therapeutic target was a major breakthrough for the treatment of highly aggressive HER2-positive breast cancer, leading to approval of the first HER2-targeted drug — the monoclonal antibody trastuzumab — almost 25 years ago. Since then, progress has been swift and the impressive clinical activity across multiple trials with monoclonal antibodies, tyrosine kinase inhibitors and antibody–drug conjugates that target HER2 has spawned extensive efforts to develop newer platforms and more targeted therapies. This Review discusses the current standards of care for HER2-positive breast cancer, mechanisms of resistance to HER2-targeted therapy and new therapeutic approaches and agents, including strategies to harness the immune system.

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Introduction

Innovations in pathology, molecular biology and drug development have enabled HER2-positive breast cancer (BC), a historically aggressive subtype, to become one with impressive outcomes. The field was energized in 1976 when the first tyrosine kinase, epidermal growth factor receptor (EGFR), was discovered followed by the identification of growth factor receptors, such as HER2, and the demonstration that the EGFR (EGFR) formed complexes after EGF binding was postulated to be the initial step for cell growth1. Subsequently, in 1986 Cohen and Rita Levi-Montalcini were jointly awarded the Nobel Prize in Physiology or Medicine for the discovery of growth factors (see Related links: https://www.nobelprize.org/prizes/medicine/1986/cohen/lecture/). The discovery and description of oncogenes, genes that transformed cells into tumour cells, was begun in the mid-1970s by Harold Varmus and Michael Bishop who also received the Nobel Prize in Physiology or Medicine for their discovery that retroviruses obtained cellular genes from the host (that is, oncogenes; see Related links: https://www.nobelprize.org/prizes/medicine/1989/varmuss/lecture/; https://www.nobelprize.org/prizes/medicine/1989/press-release/). The intersection of two scientific fields generated knowledge that there was homology between oncogenes and growth factor receptors. ERBB, consisting of two parts, v-erbA and v-erbB, was initially described in 1935 in an avian erythroblastosis retrovirus3. v-erbB was discovered to be transforming, hence an oncogene, while v-erbA was not4,5. Eventually, erythroblastoid leukaemia viral oncogene homologue 2 (v-erbB2) was found to be closely homologous to EGFR6. Moreover, it was thought that EGFR was acquired from the c-erbB2 oncogene. Mouse and non-mouse cell lines were reported to be transformed by neuroblastoma, glioma and carcinoma DNA (later named neu) from malignant rat or mouse cell lines7-10. A 185,000-dalton protein was found to induce the transformation by neu; the neu gene was homologous to erb-B, and p185 was related to EGFR11,12. The sequence for EGFR published in 1984 established that it was similar to v-erbB2 (refs.13,14)11. Sequencing studies revealed that the tyrosine kinase receptor named HER2 had extensive homology to neu, and both were located on chromosome 17 (ref.15). HER2 and neu had different sequences but were closely related to the EGFR gene, located on chromosome 7 (ref.16). Thus, HER2 and neu were determined to be homologous with ERBB2 but different from EGFR (HER1). Eventually two other members of the HER family were described: HER3 on chromosome 12 and HER4 on chromosome 2 (refs.17,18). The tyrosine-binding domains of all but HER3 (which has no catalytic tyrosine kinase activity) are similar. A monoclonal antibody (mAb) to p185 in neu-transformed cell lines was subsequently shown to revert some of the characteristics to a non-transformed phenotype and inhibit tumour growth in mice19-21. This work spearheaded the concept of HER2-targeted therapy.

Evaluating research determined that only gene amplification with resultant overexpression of protein HER2 was needed for cellular transformation22,23. Overexpression of HER2 was found to occur in human breast tumours, and HER2 signalling and transforming functions leading to growth were associated with a poor prognosis24-26. This work led the way to the development of a mAb to target the HER2 receptor in human breast cancer: a murine mAb to HER2, 4D5, generated to p18527,28 that decreased cell proliferation, spurred the development of a humanized mAb to HER2, huMAB4D5-B, eventually named trastuzumab or Herceptin29,30 (Fig. 1). Phase I and II clinical studies demonstrating activity of trastuzumab were followed by a phase III registrational study that led to the approval of trastuzumab in 1998 by the FDA for patients with HER2+ metastatic breast cancer (MBC)31,32.
This Review summarizes the successful therapeutic approaches approved for treatment of HER2+ BC, which are essential to understanding ensuing developments, exploring the potential areas of drug resistance that are the foundation for future drug development. A survey of the landscape of platforms being used to maximize HER2-targeted therapeutic efficacy are enumerated, which includes mAbs, tyrosine kinase inhibitors (TKIs), ADCs, bispecific antibodies, immune system targeting agents, cellular therapy and targeted protein degraders. As HER2 is such a sensitive target, continued investigation to advance therapeutic benefit will undoubtedly lead to improvements in survival.

**Current standard of care for HER2+ BC**

As the understanding of HER2 biology has evolved (Box 1), so has the development of agents that target HER2. HER receptors contain an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. Ligand binding to the HER proteins results in homodimerization or heterodimerization of these receptors, leading to activation of downstream signalling pathways that promote cell division and growth and inhibit apoptosis. There is no known ligand for HER2, but it is a preferred dimerization partner for the other HER proteins, especially HER3 (ref.13). HER2 overexpression or amplification leads to ligand-independent dimerization and abnormal signalling in addition to increased signalling through ligand-dependent heterodimerization (Box 2). The efficacy of HER2-targeted agents is most prominent in these ‘HER2-positive’ tumours.

The definition of HER2 positivity according to American Society of Clinical Oncology–College of American Pathologists (ASCO–CAP) guidelines, includes tumours that have 3+ positive staining by immunohistochemistry (IHC) in ≥10% of tumour cells, or HER2 gene amplification detected by fluorescence in situ hybridization (FISH)15,16 (Box 2). Recent research has identified a subset of patients with ‘HER2-low’ (HER2<30%) BC that is responsive to novel HER2-targeted ADCs17. HER2<30% is defined as HER2 IHC 1+ by itself or 2+ in the absence of HER2 gene amplification by IHC (in situ hybridization). The cut-off for the level of HER2 expression by IHC is only a crude estimation of those who may actually benefit from anti-HER2 therapies. With the introduction of the new HER2<30% definition, additional diagnostic tools may need to be considered.

Since the initial approval of trastuzumab for HER2+ BC, multiple agents exhibiting various mechanisms of action and safety profiles have been approved for the treatment of early-stage and metastatic disease (Fig. 1 and Box 3). Below, agents that have been approved by regulatory agencies are briefly described and the advantages and limitations of each strategy are summarized.

**Monoclonal antibodies**

Trastuzumab was the first humanized mAb developed to HER2 that achieved remarkable success in the treatment of HER2+ BC. Trastuzumab binds to the extracellular domain (ECD) of HER2, suppresses intracellular HER2 signalling pathways, inhibits cell cycle arrest and mediates antibody-dependent cell-mediated cytotoxicity (ADCC)18. Preclinical data demonstrating synergy between cytotoxic agents and trastuzumab paved the way for clinical trial designs with chemotherapy combinations for treatment of HER2-overexpressing metastatic breast cancer (MBC) and subsequently in the adjuvant setting (Supplementary Table 1)19,20. All these trials demonstrated favourable outcomes with trastuzumab and chemotherapy, leading to swift approvals from the regulatory agencies in the metastatic and curative settings (Fig. 1).

With these successes, trastuzumab became firmly established as the treatment of choice for patients with HER2+ BC, and HER2 drug development that would revolutionize the outcome for patients facing this disease commenced.

However, although trastuzumab improves responses and outcomes, a substantial number of patients develop therapeutic resistance and disease relapse21. Early studies showed that antibodies targeting...
Box 2

**HER2 diagnostics**

**ERBB2**, the gene that encodes HER2, is located on chromosome 17q21. HER2 acts as an oncogene, and its amplification results in overexpression of the HER2 protein, a transmembrane receptor kinase. This abnormal expression leads to a cascade of constitutive activation of downstream signalling pathways that promote uncontrolled tumour cell proliferation. HER2 expression is associated with poor prognosis, including early recurrence and metastatic disease in breast cancer. HER2 overexpression is also predictive of response to several HER2-targeted therapies, including monoclonal antibodies (mAbs) such as trastuzumab and pertuzumab, tyrosine kinase inhibitors (TKIs) — lapatinib, tucatinib — as well as antibody–drug conjugates (ADCs) such as ado-trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan. Given the significance of HER2 status for prognosis and clinical decision-making for treatment, accurate assessment of this biomarker is crucial.

Standard methods of HER2 testing include fluorescence in situ hybridization (FISH)/ISH, which quantifies the HER2 gene copy number, and immunohistochemistry (IHC), which measures HER2 expression on the cell surface. IHC quantifies the HER2 expressed on the cell surface using membranous staining, whereas FISH reports the level of amplification of the HER2 gene. Typically, equivocal results with IHC are confirmed by FISH. The results using current testing methods are not unambiguous, complicating clinical decisions regarding use of anti-HER2 therapy. Standards regarding the test used and timing differ from institution to institution.

There are two FDA-approved HER2 IHC tests: HercepTes (Dako) and Pathway (Ventana Medical Systems). These tests use IHC staining of the HER2 protein with a pathologist scoring the extent of staining as 0, 1+, 2+ or 3+. The reliability of the results from IHC is influenced by pre-analytical variables, including sample handling, fixation and storage, as well as staining. The subjective nature of the pathologist’s interpretation also gives rise to variability in the IHC results, as does the lack of reproducibility across laboratories. A big challenge in assessment of HER2 is with samples scored as IHC 2+; the variation across labs for IHC 2+ was fivefold higher than in samples scored as 3+ in both breast and gastric cancers.

The FDA-approved FISH assays include PathVysion (Abbott), INFORM (Ventana Medical Systems) and PharmDx (Dako). Dual probe assays report the ratio of HER2 to CEP17 (centromere 17), whereas single-probe assays give a direct HER2 copy number. CEP17 serves as an internal control, something that is lacking in IHC. Although rare, polysomy of chromosome 17 may lead to false negative results for HER2 amplification. Some drawbacks with FISH are that it is more expensive, technically more challenging, and time consuming. However, FISH is associated with less inter-observer variability, is considered quite accurate and produces equivocal results in only ~5% of cases.

The American Society of Clinical Oncology–College of American Pathologists (ASCO–CAP) guidelines have continued to evolve to improve the accuracy and clinical utility of HER2 testing by providing specific algorithms. In parallel with existing techniques, novel methods to quantify HER2 levels or standardize procedures are being investigated, including quantitative assays to measure HER2 protein expression at the single cell level, automated scoring of HER2 FISH, microRNA signatures in primary tumour tissue as a prognostic/predictive tool, using a mass spectrometry system to measure HER2 at attomols mm⁻² (refs. 262–264). Furthermore, gene expression-based tools for prognostic and predictive purposes are also under evaluation in HER2 breast cancer, although none of these has yet reached mainstream use.

Recent research has defined a ‘HER2-low’ phenotype based on IHC (HER2 IHC 1+ or 2+ and ISH negative), which defines a group that responds to trastuzumab deruxtecan. This agent has just received FDA approval in this patient population. A recent analysis using current standard HER2 assays coupled with CAP data from 1,400 labs worldwide revealed poor agreement in evaluation of HER2 0 and 1+ cases; similar results were seen with a separate Yale cohort. These inaccuracies in the real world underscore the urgency to develop more sensitive HER2 diagnostic assays to ensure that eligible patients are not deprived of effective therapies.
SOPHIA study, adding another drug to the HER2 armamentarium (Supplementary Table 1).

Trastuzumab biosimilars. Unlike novel synthetic HER2-targeting antibodies, trastuzumab biosimilars are biologic agents created from living cells and have similar pharmacokinetic and pharmacodynamic properties to the original product. They may have minor differences in clinically inactive components from the original biologic medication, but there are no clinically meaningful differences between the two with respect to safety, purity and potency (see Related links: https://www.fda.gov/media/82647/download). There are currently five trastuzumab biosimilars approved by the FDA for US markets, with additional agents in development (see Related links: https://www.fda.gov/drugs/biosimilars/biosimilar-productinformation). Trastuzumab-dkst (MYL1401O) was the first trastuzumab biosimilar to receive FDA approval (in 2017) for patients with HER2-overexpressing BC and gastrointestinal (GI) cancers. Subsequent approvals included trastuzumab-pkrb (CT-P6) for HER2+ BC (December 2018); trastuzumab-dttb (SB3) (January 2019), trastuzumab-qyyp (PF-05280014; March 2019) and trastuzumab-anns (ABP980) for HER2+ breast and GI tumours (June 2019). Although clinical guidelines support biosimilars, their adoption in clinical practice has been slow, hampered by physician and commercial payer awareness, perceptions and preferences.

Formulations of monoclonal antibodies. In an effort to conserve resources and reduce the burden of intravenous (i.v.) infusions to patients, a subcutaneous version of trastuzumab (trastuzumab–hyaluronidase-oysk) was developed and validated in clinical trials and approved in the EU in 2013 and in the USA in 2019. Pertuzumab–trastuzumab–hyaluronidase-zzxf, a subcutaneous formulation of a fixed dose formulation of pertuzumab and trastuzumab demonstrated safety and non-inferiority in pathological complete response (pCR) versus corresponding i.v. versions in patients with EBC and was granted FDA approval in the adjuvant and metastatic settings in 2020.

Tyrosine kinase inhibitors

TKIs are small molecules that target the intracellular catalytic kinase domain of HER2, competing with ATP, blocking phosphorylation and activating downstream signalling cascades. Lapatinib, an oral, 4-anilinoquinazoline TKI derivative, is a reversible inhibitor of EGFR.
upregulation by blocking the crosstalk between HER2 and IGF1R 45. resistance mediated by insulin-like growth factor 1 receptor (IGF1R) (also known as HER1) and HER2, with activity in HER2-driven tumours. Several trials were conducted with lapatinib in HER2+ EBC, the approval of these combinations and offering an alternative to HER2- in hormone receptor-positive (HR+)/HER2+ MBC, leading to regulatory lapatinib–letrozole doublet was more efficacious than letrozole alone to capecitabine alone in trastuzumab-treated HER2 + MBC, and the data supported the clinical development of lapatinib in trastuzumab-HER2+ BC cell lines remained sensitive to lapatinib47. These and other In vitro studies showed that pTEN-deficient, trastuzumab-resistant Cell lines and xenografts expressing the p95 variant that lack the trastuzumab-binding ECD were susceptible to lapatinib, presumably because it targets the intracellular kinase domain of HER2 (ref. 44). In vitro studies showed that pTEN-deficient, trastuzumab-resistant HER2+ BC cell lines remained sensitive to lapatinib47. These and other data supported the clinical development of lapatinib in trastuzumab-resistant BC48. Lapatinib in combination with capecitabine was superior to capecitabine alone in trastuzumab-treated HER2+ MBC, and the lapatinib–letrozole doublet was more efficacious than letrozole alone in hormone receptor-positive (HR+)/HER2+ MBC, leading to regulatory approval of these combinations and offering an alternative to HER2-targeting antibody combinations for these patients49–51 (Supplementary Table 1). Several trials were conducted with lapatinib in HER2 EBC, the details of which are succinctly summarized in a recent review52.

A significant sanctuary for recurrent HER2+ disease is the central nervous system (CNS), and up to 50% of patients with HER2+ BC develop brain metastases53–55. Moreover, the blood–tumour barrier (BTB), which evolves from the blood–brain barrier (BBB), regulates drug distribution to brain metastases, posing a clinical challenge56. It has also been suggested that the large size of the HER2 antibodies (for example, trastuzumab) prevents penetration of the BBB and efficacy in the CNS. Lapatinib, by virtue of its small size and potent anti-HER2 activity, found a niche in this arena. Lapatinib monotherapy and combination therapy demonstrated lower rates of CNS progression and responses in the CNS in HER2+ MBC, including in patients with previously untreated brain metastases57–59. These data provided the impetus for further refinements in next-generation TKIs to tackle the growing problem of brain metastases associated with HER2+ MBC.

In contrast to lapatinib, neratinib (HKI-272) is an irreversible pan-HER TKI that targets EGFR, HER2 and HER4 (ref. 59). Neratinib inhibits growth in trastuzumab-resistant cell lines and is synergistic with trastuzumab60,61. A unique feature of neratinib is its activity in cell lines (also known as HER1) and HER2, with activity in HER2-driven tumours that are insensitive to trastuzumab64. Lapatinib overcomes trastuzumab resistance mediated by insulin-like growth factor 1 receptor (IGF1R) upregulation by blocking the crosstalk between HER2 and IGF1R45. Cell lines and xenografts expressing the p9562 variant that lack the trastuzumab-binding ECD were susceptible to lapatinib, presumably because it targets the intracellular kinase domain of HER2 (ref. 44). In vitro studies showed that pTEN-deficient, trastuzumab-resistant HER2+ BC cell lines remained sensitive to lapatinib47. These and other data supported the clinical development of lapatinib in trastuzumab-resistant BC48. Lapatinib in combination with capecitabine was superior to capecitabine alone in trastuzumab-treated HER2+ MBC, and the lapatinib–letrozole doublet was more efficacious than letrozole alone in hormone receptor-positive (HR+)/HER2+ MBC, leading to regulatory approval of these combinations and offering an alternative to HER2-targeting antibody combinations for these patients49–51 (Supplementary Table 1). Several trials were conducted with lapatinib in HER2 EBC, the details of which are succinctly summarized in a recent review52.

Toxicities with HER2-targeted therapies

Although highly effective in disease control and improving survival, approved anti-HER2 therapies are not without potential adverse events (AEs), some of which require careful monitoring.

**Cardiotoxicity.** Cardiac dysfunction with trastuzumab is an AE of concern in the metastatic breast cancer (MBC) and early breast cancer settings, particularly when given in combination with anthracyclines63–66. Dual HER2-targeted therapy with pertuzumab and trastuzumab in HER2+ MBC has not been shown to exacerbate cardiotoxicity or lead to increased cardiac events after long-term follow-up in the adjuvant setting67,68.

- Trastuzumab-induced cardiotoxicity is usually asymptomatic, not related to cumulative dose and largely reversible.
- Numerous measurements of left ventricular ejection fraction (LVEF) during trastuzumab therapy may lead to false positive elevations69.
- Increased rates of cardiotoxicity have not been observed in long-term follow-up70,71.
- The FDA recommends baseline LVEF evaluation before initiating trastuzumab.
- Cardiac monitoring strategies have been developed by the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO)72–74.

**Gastrointestinal toxicity.** Gastrointestinal toxicities and rash are frequently observed with tyrosine kinase inhibitors (TKIs), primarily due to epidermal growth factor receptor (EGFR) targeting.

- Diarrhoea is more frequent and severe with neratinib and pyrotinib than other TKIs (especially relevant in the adjuvant setting where adherence to therapy may be compromised).
- The addition of budesonide or colestipol to loperamide prophylaxis may decrease neratinib discontinuation due to diarrhoea75.

**Liver toxicity.** Elevated liver enzymes are commonly reported with ado-trastuzumab emtansine (T-DM1) and tucatinib76–78.

- Dose interruptions and adjustments are primary management.
- Careful monitoring is recommended, and concurrent treatment with strong or moderate CYP3A inhibitors should be avoided.

**Thrombocytopenia.** Thrombocytopenia has occurred with T-DM1 and is attributed to the DMT-induced impairment of megakaryocyte differentiation79.

- Mitigation strategies include dose interruptions and dose modifications.

**Interstitial lung disease.** Interstitial lung disease (ILD) has been attributed to trastuzumab deruxtecan (T-DXd) and was first reported in 13.6% of patients in the DESTINY-Breast01 trial80. One patient had a grade 3 event, and four deaths were attributed to ILD.

- Most ILD events were grade 1/2 and occurred in the first 12 months, with declining risk thereafter81.
- Increased awareness coupled with guidelines for interrupting therapy and prompt treatment improved ILD (no grade 4/5 events and <1% grade 3 events)82,83,84.

The unique AE profiles of anti-HER2 therapies enable customized treatment based on patients’ comorbidities. Because some AEs may be exacerbated in combination with chemotherapy, awareness and careful monitoring with implementation of mitigation strategies in the event of an AE will enable maximal treatment benefit with minimized toxicity.
with somatic HER2 mutations in the absence of HER2 amplification, suggesting that it can overcome possible resistance to other anti-HER2 therapies. The co-occurrence of somatic HER2 mutations and HER2 amplification has also been observed, and a prevalence of 7.1% was reported in patients with HER2+ MBC, all of whom had poor response to dual anti-HER2 antibody therapy. Durable tumour shrinkage was seen with neratinib, but not with lapatinib or trastuzumab, in animal models with concomitant HER2 mutation and amplification, and similar efficacy with neratinib was also evident in the clinical setting.

Treatment with neratinib after standard adjuvant trastuzumab improved invasive disease-free survival (iDFS) rates in HER2+ BC, especially in patients with HR+ tumours. Similarly to lapatinib, the neratinib–capecitabine combination improved outcomes in HER2+ MBC, earning a nod from the FDA as third-line therapy. Given the rapid development of newer HER2-targeted therapies, including novel TKIs with less toxicity and higher efficacy, the role of the neratinib–capecitabine combination appears limited.

Pyrotinib, another oral, irreversible pan-HER TKI that targets EGFR, HER2 and HER4, has been approved by the Chinese regulatory authority in combination with capecitabine in HER2+ MBC treated with prior trastuzumab and taxane (Supplementary Table 1). Pyrotinib is similar to the other pan-HER2 TKIs, with diarrhoea as its most common toxicity. Several trials with pyrotinib are ongoing in breast and other cancers, but it is unclear whether its use in HER2+ MBC will expand to other countries.

Tucatinib is a potent, oral, HER2-specific TKI with >1,000-fold greater potency for HER2 than EGFR. Tucatinib demonstrated CNS penetration in intracranial xenograft models and was superior to lapatinib in preclinical studies. The pivotal Her2CLIMB trial demonstrated superiority of the tucatinib plus capecitabine–trastuzumab combination in extending progression-free survival (PFS) and overall survival (OS) in HER2+ MBC previously treated with anti-HER2 therapies (Supplementary Table 1). The innovative design of the trial allowed patients with stable as well as active brain metastases, and Her2CLIMB is the first randomized trial to demonstrate clinically meaningful benefits in patients with HER2+ brain metastases, marking an important milestone in the treatment of HER2+ BC. Tucatinib was FDA approved in April 2020 for the treatment of HER2+ MBC, including patients with CNS metastases. Ongoing trials with tucatinib include a maintenance trial in first-line HER2+ MBC with the goal of delaying CNS progression (NCT05132582), an adjuvant study for patients with residual disease following neoadjuvant chemotherapy (NCT04457596) and the neoadjuvant T-SPY 2 trial (NCT01042379) (see Related links).

Antibody-drug conjugates
ADCS were designed to channel the cytotoxic effects of chemotherapy to specific tumour cells. ADCs contain a tumour-targeting antibody covalently bound to a cytotoxic drug (payload) via a synthetic linker. The ADC is directed to cancer cells expressing the target (for example, HER2) on the cell surface, followed by internalization of the ADC and release of the cytotoxic payload, resulting in tumour cell death. Cleavable linkers in ADCs enable release of the cytotoxic payload from the target cell to the extracellular space, leading to destruction of surrounding cancer cells that may not have high target protein expression. This bystander effect further enhances the efficacy of ADCs against tumour cells.

Ado-trastuzumab emtansine (T-DM1), the first anti-HER2 ADC to be developed, is composed of trastuzumab connected via a stable linker to DM1, a maytansine derivative with a drug-to-antibody ratio (DAR) of ~3.5. T-DM1 caused mitotic disruption and apoptosis in HER2-overexpressing cell lines regardless of their sensitivity to trastuzumab and lapatinib. T-DM1 prolonged PFS and OS in patients with HER2+ MBC compared with current standard of care in large, randomized trials, validating HER2 overexpression as a target in trastuzumab-resistant BC and showcasing the efficacy of ADCs in this setting. These results led to FDA approval of T-DM1 for HER2+ MBC (Fig. 1 and Supplementary Table 1). Although the attempt to replace trastuzumab with T-DM1 as part of the dual anti-HER2 (neo)adjuvant regimens was not successful, T-DM1 reduced the risk of recurrence in patients with HER2+ EBC without upfront pCR after neoadjuvant chemotherapy (Fig. 1 and Supplementary Table 1). T-DM1 also demonstrated activity in a subset of patients with HER2+ MBC and brain metastases in the KAMILLA study.

T-DXd is a HER2 ADC comprising a humanized HER2 antibody with the same sequence as trastuzumab conjugated to deruxtecan (DXd). T-DXd has a DAR of 8 and exhibits the enhanced features of DXd. The novel DXd ADC technology consists of a cleavable tetrapeptide-based linker, a self-immolative amino methylene spacer and a novel topoisomerase inhibitor payload derivative of exatecan (DS-8951). The linker of DXd is selectively cleaved by cathepsins, which are upregulated in tumours, releasing the payload preferentially inside cancer cells. This feature, coupled with the short half-life of DXd in vivo, limits systemic exposure of the cytotoxic agent, with the goal of reducing toxicity. The high membrane permeability of DXd enables local bystander effects, leading to the death of tumour cells in the tumour microenvironment (TME).

Single-agent T-DXd demonstrated impressive antitumour activity in refractory HER2+ MBC and an unprecedented improvement in PFS when compared head to head with T-DM1 in second-line HER2+ MBC leading to its FDA approval in these patient populations (Fig. 1 and Supplementary Table 1). T-DXd has also shown encouraging activity in BC metastases, in DESTINY-Breast01 (ref. 87). DEBBRAH is evaluating T-DXd in patients with HER2+ or HER2++ MBC with brain metastases and/or leptomeningeal metastases; preliminary data are promising.

A unique feature of T-DXd is its ability to target HER2low MBC as evidenced by activity in this subset of patients in a phase I trial. This remarkable efficacy appears multifactorial based on enhanced features of T-DXd compared with T-DM1, the ability to deliver a higher dose and the bystander effect tackling intratumour HER2 heterogeneity. T-DXd demonstrated statistically significant and clinically meaningful improvements in PFS and OS versus treatment of physician’s choice (TPC) chemotherapy in the phase III DESTINY-Breast04 trial in HER2++ MBC. These results are likely to redefine nomenclature around HER2 expression and what is considered actionable in terms of HER2 expression. The FDA recently approved the use of T-DXd for patients with HER2low MBC on the basis of data from the DESTINY-Breast04 study (see Related links: https://www.fda.gov/news-events/press-announcements/fda-approves-first-targeted-therapy-her2-low-breast-cancer).

Recent results from the DAISY trial showed that T-DXd antitumour activity was associated with level of HER2 expression in HER2+ MBC. Interestingly, a high percentage of HER2+ BC 0 cells in the tumour and their spatial distribution relative to HER2-overexpressing cells were associated with a decreased response to T-DXd. This highlights the need to develop more sensitive methods for detection of HER2 expression and novel technologies to assess heterogeneous HER2 expression within the tumour.
Mechanisms of HER2-targeted resistance

Resistance to anti-HER2 therapies may occur via multiple mechanisms, some of which appear to be shared between different agents. A common reason for trastuzumab treatment failure is incomplete inhibition of the HER family of receptors, which can be overcome by dual HER2-targeted therapy or ADCs with potent payloads that have activity even with lower HER2 expression. Effective inhibition of HER2 may also be thwarted by the emergence of HER2-activating mutations. Other resistance mechanisms include generation of p95HER2, a truncated form of HER2 that lacks the ECD that is recognized by anti-HER2 antibodies and Δ16HER2, a splice variant lacking the ECD encoded by exon 16, which leads to stabilization of homodimers and constitutive activation of downstream signalling. Figure 2 depicts selected mechanisms of HER2-targeted resistance.

**Fig. 2 | Select mechanisms of HER2-targeted resistance.** a, Mutations and/or alterations in the HER family of receptors that lead to activation of downstream signalling pathways. (1) Mutations in HER2 leading to P13K–AKT and RAS–MAPK pathway activation. (2) Co-occurring mutations in HER2 and HER3 leading to PI3K–AKT pathway activation. b, Loss of HER2 extracellular domain in cells overexpressing p95HER2 receptor. Masking of the trastuzumab-binding site on HER2 owing to overexpression of mucin 4 (MUC4) and CD44–polymeric hyaluronan complex. (3) p95HER2 overexpression. (4) MUC4 overexpression and CD44–polymeric hyaluronan complex. c, Activation of compensatory pathways. (5) Mutations in HER2 promote MEK–ERK signalling, which activates CDK2 kinase. (6) PIK3CA mutations lead to PI3K–AKT pathway activation. (7) Cyclin D1 gene overexpression leads to resistance to anti-HER2 therapies. d, Heterogeneous expression of the HER2 receptor in tumours leads to decreased sensitivity to HER2-targeted therapies that are dependent on overexpression of HER2. ER, oestrogen receptor.
**HER family alterations**

*HER2* mutations can drive BC growth even in the absence of HER2 overexpression or amplification. The frequency of *HER2* mutations in BC is ~3%. *HER2(L755S)* is the most common alteration associated with lapatinib resistance in MBC treated with prior trastuzumab. This activating *HER2* mutation has also conferred resistance to dual blockade by trastuzumab and pertuzumab and reduced sensitivity to T-DM1. Second-generation TKIs (for example, afatinib and neratinib) can overcome treatment resistance in BC models, suggesting that they may be therapeutic alternatives for treatment of patients with HER2+ tumours harbouring the *HER2(L755S)* mutation. Neratinib was shown to be active against HER2 mutant/HER2-non-amplified MBC in the SUMMIT basket trial.

*HER2* mutations frequently co-occur with *HER3* mutations, and cancers with both of these mutations respond poorly to neratinib. The *HER3* kinase domain mutation has been shown to enhance the affinity of HER2/HER3 and reduce binding of HER2 to neratinib. Co-expression of *HER2* and *HER3* mutations leads to enhanced downstream PI3K–AKT pathways and resistance to neratinib. Thus, combined anti-HER2 and PI3K inhibition with a PI3Kα inhibitor such as alpelisib may be a promising strategy to overcome HER2 resistance due to co-occurring *HER2* and *HER3* mutations.

The generation of tucatinib-resistant BC cell lines revealed significant phosphorylation of HER2 receptors and reactivation of downstream signalling pathways, unlike the partial reactivation of HER2 signalling seen in lapatinib- and neratinib-resistant models. Acquired resistance to tucatinib was dependent on amplified EGFR signalling and could be overcome by a combination of gefitinib and tucatinib or pan-HER TKIs, such as neratinib, pyrotinib or poziotinib. Using organoids derived from xenograft tumours of HER2+ BC resistant models, neratinib-resistant models were shown to be cross-resistant to other single-agent HER2 TKIs, but the presence of co-occurring *HER2* and *PIK3CA* mutations suggested susceptibility to combination with a PI3K or AKT inhibitor.

**Loss or masking of HER2 epitope**

The presence of p95HER2 has been associated with poor outcomes in patients with HER2+ BC, and patients with MBC overexpressing p95HER2 had lower response rates to trastuzumab than those expressing full-length HER2 (ref. 4). Chemotherapy sensitized p95HER2/611CTF xenografts to trastuzumab, presumably via HER2 stabilization induced by chemotherapy. Lapatinib inhibited p95HER2 phosphorylation in cell lines, reducing downstream activation of AKT and MAPK and inhibiting cell growth. Retrospective analyses from clinical trials with lapatinib noted that the presence of p95HER2 had no influence on lapatinib efficacy. Similarly, in the CHER-LOB neoadjuvant study, which randomized patients to receive trastuzumab, lapatinib or their combination, p95HER2 expression was not predictive of outcome nor did it predict for sensitivity to either anti-HER2 agent. Therefore, the role of p95HER2 as a biomarker of resistance or sensitivity remains to be confirmed.

Overexpression of mucin 4 (MUC4) and the CD44–hyaluronan polymer complex interferes with trastuzumab binding by masking the HER2 epitope and activating HER2. Increased MUC4 expression in oestrogen receptor-positive (ER+)/HER2+ tumours leads to reduced trastuzumab-binding sites. Soluble TNF upregulated MUC4 expression, resulting in trastuzumab resistance, and combining a soluble TNF inhibitor with trastuzumab prevented tumour growth in preclinical models. Recent data also show that MUC4 expression results in an immunosuppressive TME in HER2+ BC and emphasize the role of tumour-infiltrating macrophages in mounting an antitumour response. INB03, a second-generation TNF inhibitor that increases antitumour macrophage phagocytosis and increases lymphocyte function in the TME, is being considered for evaluation in clinical trials.

**Activation of compensatory pathways**

The activation of compensatory signalling pathways to overcome effects of trastuzumab treatment has been a subject of extensive exploration. Nearly 25–50% of BC harbour *PIK3CA* mutations, with enrichment in HR (~35%) and HER2 (~25%) subtypes. *PIK3CA* mutations are associated with reduced pCR rates to neoadjuvant anti-HER2 therapies and decreased efficacy with trastuzumab or pertuzumab in HER2+ MBC. Loss of PTEN (a key tumour suppressor), which leads to hyperactivation of the PI3K pathway, has also been observed in trastuzumab-resistant tumours. Development of PI3K and mTOR inhibitors offered hope of a therapeutic option, but early trials with buparlisib (a pan-PI3K inhibitor) in the neoadjuvant setting did not yield the desired outcomes. The BOLERO-1 and BOLERO-3 trials evaluated the addition of the mTOR inhibitor everolimus to trastuzumab plus chemotherapy in first-line and trastuzumab-resistant HER2+ MBC settings, respectively. Biomarker analysis from the pooled study populations indicated PI3K–AKT–mTOR pathway aberrations in approximately 40% of tumours, with everolimus treatment leading to a consistent benefit in these patients versus patients with tumours not exhibiting the aberrations. Although these results suggested proof of concept, the triplet combinations led to increased toxicity. The advent of isoform-specific PI3K inhibitors such as alpelisib has led to a maintenance study of triplet alpelisib–trastuzumab-pertuzumab in patients with PIK3CA-mutant HER2+ MBC (NCT04208178; see Related links).

Bidirectional crosstalk exists between the HER2 and ER pathways, and preclinical studies have demonstrated a role for ER signalling in promoting resistance to anti-HER2 therapies. Clinical evidence also shows that ER pathway activation drives an escape from HER2 inhibition, and concomitant inhibition of both ER and HER2 signalling may be necessary as demonstrated in trials of ER+/HER2+ BC. The cyclin D1–CDK4/6 axis also has a role in resistance to anti-HER2 therapies. CDK4/6 inhibitors appear to be synergistic with trastuzumab and/or lapatinib in inhibiting growth of HER2+ cell lines. This was evidenced in the clinical setting in which dual inhibition of CDK4/6 and HER2 led to improved outcomes in monarchHER and PATRICIA trials in heavily pretreated HR+/HER2+ MBC. The PATINA trial is evaluating whether addition of palbociclib to front-line trastuzumab, pertuzumab and taxane plus endocrine therapy (ET) in HR+/HER2+ MBC will delay the onset of therapeutic resistance (NCT02947685; see Related links). Preclinical and clinical data corroborate cyclin D1-mediated resistance to HER2-targeted therapies, and CDK4/6 inhibitors can overcome this resistance. Cyclin D1 overexpression correlated with lower pCR rates in patients receiving neoadjuvant trastuzumab plus chemotherapy. In the Na-PHER2 study, trastuzumab and pertuzumab with fulvestrant and palbociclib neo-adjuvantly to patients with ER+/HER2+ BC, led to a significant Ki67 reduction after 2 weeks of therapy, clinical complete response (CR) in 50% of patients and a 27% pCR rate (breast and axilla). These data suggest that a combination blocking HER2 and ER and CDK4/6–cyclin D1 activation offers a chemotherapy-free alternative for treatment of HER2+ BC. An ongoing trial is evaluating trastuzumab plus palbociclib with or without letrozole in HER2+ and ER+ MBC (NCT02448420; see Related links).

Genomic sequencing analysis of 733 HER2-amplified primary and metastatic breast tumours revealed significant enrichment of...
mutations that activate RAS–MAPK signalling in advanced tumours treated with prior anti-HER2 therapies. These mutations, including *NF1* and *HER2* activating mutations, contribute to resistance to trastuzumab and neratinib. The resistant tumours were highly sensitive to MEK–ERK inhibition, with susceptibility due to MEK-dependent activation of CDK2 kinase, thus offering the possibility of overcoming HER2 resistance with MEK–ERK inhibitors.

**HER2 heterogeneity**

HER2 heterogeneity — the variable expression of HER2 across the tumour — is another potential source of resistance to HER2-targeted therapies. HER2 heterogeneity, defined as HER2 positivity by FISH in 5–50% of tumour cells, or an area of tumour that tested HER2-negative (HER2*) in multiple core biopsies, was found in 10% of patients in a phase II trial of neoadjuvant T-DM1 plus pertuzumab. A significant association was found between HER2 heterogeneity and lack of pCR following dual HER2-targeted therapy; none of the patients with HER2 heterogeneity achieved a pCR, whereas 55% of patients not classified as HER2 heterogeneous had a pCR. T-DXd demonstrated significant activity in HER2+ MBC, significantly improving PFS and OS, and this attribute may also enable T-DXd to overcome resistance due to heterogeneous expression of HER2, which could potentially become even more important in the early setting for patients with tumour heterogeneity.

**Host and tumour immunity**

ADCC is a key mechanism mediating the antitumour activity of trastuzumab, and it can be hampered by an immunosuppressive TME. NK cells have an important role in tumour immunity, and their activity is regulated by careful modulation of inhibitory and activating receptor signalling. Tumour cells expressing high levels of HLA class I molecules can inhibit NK cells through the engagement of killer cell immunoglobulin-like receptors (KIRs). HLA-G was shown to desensitize BC cells to trastuzumab by blocking the immune receptor KIR2DL4, and blocking this HLA-G–KIR2DL4 signalling made HER2+ BC susceptible to trastuzumab treatment in vivo. Moreover, trastuzumab increased the production of TGFβ and interferon-γ (IFN-γ) by BC cells and NK cells, respectively. TGFβ induced PD1 expression on NK cells, and PD1 blockade significantly increased cytotoxicity of NK cells. Accordingly, combined blockade of HLA-G and PD1/L1/PD1 may be necessary for effective treatment of trastuzumab-resistant BC.

Trastuzumab can also engage Fcγ receptors on macrophages to promote antibody-dependent cellular phagocytosis (ADCP), which contributes to its antitumour efficacy. Magrolimab, a humanized mAb that targets CD47 was studied to combat trastuzumab resistance by activating ADCP. CD47 is a protein that acts as a ‘don’t eat me’ signal via its interaction with signal regulatory protein-α (SIRPα) on macrophages to inhibit phagocytosis. CD47 has been shown to be upregulated in HER2+ BC. The combination of magrolimab and trastuzumab was found to eliminate HER2+ BC cells with increased efficacy due to enhancement of ADCP by macrophages, even in HER2+ BC resistant to ADCC. This offers a novel therapeutic approach to treat trastuzumab-sensitive or trastuzumab-resistant HER2+ BC, provided the trastuzumab-binding epitope on HER2 is accessible.

**Other potential mechanisms of resistance**

Several other mechanisms of anti-HER2 therapy resistance have recently been elucidated. A study modelling resistance of HER2* PIK3CA-mutant BC using two patient-derived xenografts, one resistant to paclitaxel and T-DM1 and the other insensitive to T-DM1 and pertuzumab, demonstrated that alveolar epithelial and fibroblastic reticular as well as lymphatic vessel endothelial hyaluronan receptor 1-positive (Lyve1+) macrophages may be putative drivers of therapeutic resistance. These intriguing findings require further studies comparing data from transcriptome and exome profiling from trials with anti-HER2 therapies. Preclinical studies hint that abnormal transit of T-DM1 through the endosomal maturation pathway may be responsible for resistance to T-DM1 treatment, but this has not been validated or studied in clinical trials.

Three novel markers, RAC1, CDK12 and VTCN1, have been found to correlate with response to lapatinib, neratinib and trastuzumab in a study that compared the TKI anti-proliferative effects using a 115-cancer cell line panel to identify novel markers of TKI response and/or resistance markers. Prior data have implicated these genes in resistance to anti-HER2 therapies or immunotherapy. Hence, combinations of HER2 TKIs and CDK12 and RAC1 inhibitors may offer a therapeutic strategy in high CDK12- or RAC1-expressing HER2+ BC.

**Next-generation therapies for HER2+ BC**

The ever-changing face of cancer and its ability to evade existing therapies have underscored the need for continued development of therapeutics based on existing and/or novel platforms and for uncovering new vulnerabilities in resistant tumours. Antibodies targeting alternative HER2 domains or other HER family members have been explored with the goal of achieving a more complete blockade of HER2 and dampening the effects on downstream signalling pathways. Novel ADCs carrying different payloads to avoid cross-resistance to existing therapies or new linkers to offset off-target toxicity are also being actively pursued.

**Monoclonal antibodies**

Disruption of HER2–HER3 dimerization is important for HER2-driven signalling and is targeted effectively by pertuzumab. The positive results from pertuzumab trials supported a strategy of targeting HER3, which has a crucial role in HER2-mediated tumorigenesis. HER3 is unique compared with other HER family members as it is defined by the absence of a functional kinase domain and thus any catalytic activity. HER3 is the preferred dimerization partner for HER2, and HER2–HER3 dimerization leads to oncogenic activation of the PI3K signalling pathway, mediating resistance to HER2-targeted therapy. Several HER3-targeted antibodies have been evaluated in the past decade, mainly targeting the ECD of HER3 (for example, seribantumab and patritumab) and some with modifications to improve ADCC (for example, lumretuzumab, TrasGex) or trap HER3 in an inactive conformation (elgemutumab). Although these drugs have shown some promising preclinical activity, most are no longer in clinical development for HER2+ BC given the high bar of efficacy set by standard-of-care anti-HER2 therapies.

**Antibody–drug conjugates**

ADCs have successfully combined the antitumoural features of cytotoxins and HER2 antibodies into a single pharmacological entity that has greater efficacy than the sum of its parts. In addition to the ability of ADCs to hone in on the cells expressing the target protein and cause tumour cell lysis, the membrane-permeable payload can diffuse into the surrounding tumour milieu, inducing the bystander effect. This...
some of these are discussed in further detail below.

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...expression142. In the phase III TULIP study of SYD985 versus physician’s...a bystander effect that allows activity in tumour cells with low HER2...a protease-cleavable linker, and subsequent release of the payload into the TME by diffusion, promotes...comprises the humanized anti-HER2 antibody hertuzumab coupled...to the cytotoxic agent monomethyl auristatin E (MMAE). Disitamab targets different epitopes of the HER2 recep...The protease-cleavable linker, and subsequent release of the payload into the TME by diffusion, promotes...the development of linkers for ADCs has been a very impor...these ADCs is warranted in this high-risk patient population17.

Given the success of T-DM1 and T-DXd, there are more than a dozen HER2-targeted ADCs now in clinical development, with the aim of improving therapeutic index and efficacy. These ADCs differ from the approved agents in cytotoxic payload, DAR, linker or the HER2 epitope targeted. The development of linkers for ADCs has been a very important area of investigation and has been recently reviewed144. Several ADCs currently in development for HER2+ BC are listed in Table 1, and some of these are discussed in further detail below.

Table 1 | Select HER2-targeted antibody–drug conjugates in development

| Drug name (company) | Linker type | Payload | Payload MOA | DAR | Clinical trial ID | Clinical trial data | Reference |
|---------------------|-------------|---------|-------------|-----|-----------------|---------------------|-----------|
| Trastuzumab duocarmycin (Synthony/Byondis B.V.) | Cleavable | Duocarmycin (vc-seco-DUBA) | DNA alkylator | 2.8 | NCT04602117 (phase I), NCT03262935 (phase III) | Phase III trial SYD985 vs TPC: median PFS 7 vs 4.9 mo; HR 0.64, P=0.002 | Saura et al.145 |
| Disitamab vedotin (RC48-ADC) (RemGen Co./Seagen) | Cleavable | MMAE | Microtubule inhibitor | 4 | NCT02881190 (phase I), NCT03500380 (phase II), NCT04400695 (phase III) | Phase I trial in HER2+ cancers: ORR 15%; DCR 45% | Xu et al.296 |
| A166 (Klaus Pharma/ Sichuan Kelun-Biotech Biopharmaceutical Co. Ltd) | Cleavable | Duo-S | Microtubule inhibitor | 2.8 | CTR20181301 NCT03602079 (phase I) | Phase I trial in advanced solid tumours: ORR 59-71% based on the dose, DCR -85% | Hu et al.277 |
| ALT-P7 (Alteogen, Inc.) | Cleavable | MMAE | Microtubule inhibitor | 2 | NCT03281824 (phase I) | Phase I trial in HER2+ MBC: DCR 72%, CBR 32% | Park et al.298 |
| ARX788 (Ambryx) | Non-cleavable | AS269- synthetic dolastatin | Microtubule inhibitor | 2 | CTR20171162 (phase I), NCT04829604 (phase II) | Phase I trials in HER2+ MBC: ORR 66%, DCR 100% | Hurvitz et al.279 |
| BB-1701 (Bliss Biopharmaceutical) | Cleavable | Eribulin | Microtubule inhibitor | 4 | NCT04257110 (phase I) | Not applicable | Not applicable |
| DB-1303 (Duality Bio, Inc.) | Cleavable | DXd derivative | Topoisomerase 1 inhibitor | 8 | NCT05150691 (phase I) | Not applicable | Not applicable |
| DX126-262 (Hangzhou DAC) | Unknown | Tubulysin | Microtubule inhibitor | NR | CTR20191224 (phase I) | Not applicable | Zhang et al.270 |
| FS-1502/IKS014 (Shanghai Fuson Pharmaceutical Industrial Development Co. Ltd) | Unknown | MMAE | Microtubule inhibitor | NR | NCT03944499 (phase I) | Not applicable | Fasching21 |
| Zanidatamab zovodotin (ZW49) (Zymeworks, Inc.) | Cleavable | Auristatin based | Microtubule inhibitor | 2 | NCT03821233 (phase I) | Phase I trial in advanced solid tumours. ORR 13%; DCR 50%; CBR 25%; MTD not reached | Jhaveri et al.282 |

CBR, clinical benefit rate; DAR, drug-to-antibody ratio; DCR, disease control rate; DXd, deruxtecan; HR, hazard ratio; MBC, metastatic breast cancer; MMAE, monomethyl auristatin E; MOA, mechanism of action; MTD, maximum tolerated dose; NR, not reported; ORR, overall response rate; PFS, progression-free survival; TPC, treatment of physician’s choice.

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|---------------------|-------------|---------|-------------|-----|-----------------|---------------------|-----------|
| Trastuzumab duocarmycin (SYD985). Trastuzumab duocarmycin (SYD985) is a HER2-targeted ADC based on trastuzumab with a cleavable linker duocarmycin (vc-seco-DUBA) payload. The novel payload is an active toxin (DUBA) that alkylates DNA, causing DNA damage in both dividing and non-dividing cells. The protease-cleavable linker, and subsequent release of the payload into the TME by diffusion, promotes a bystander effect that allows activity in tumour cells with low HER2 expression145. In the phase III TULIP study of SYD985 versus physician’s choice of chemotherapy plus anti-HER2 therapy in patients with HER2+ MBC who had received two or more lines of MBC therapy, SYD985 was associated with a significant improvement in PFS143,144. Ocular toxicity was the most common adverse event (AE) reported, and as for other HER2 ADCs, interstitial lung disease (ILD)/pneumonitis was also observed in a small percentage of patients. A biologics licence application for SYD985 has been recently submitted to the FDA (see Related links: https://go.nature.com/3VZlL4a), and it remains to be seen how it may integrate into the standard of care with T-DXd and tucatinib. |

ARX788. ARX788 is a next-generation HER2 ADC created using site-specific oxime conjugation technology and a non-cleavable linker designed for homogeneity and chemical stability147. It also employs a highly hydrophilic payload (AS269, synthetic dolastatin) with limited cell permeability, unlike other ADCs that use highly permeable payloads to elicit a bystander killing effect. Preclinical data with ARX788 demonstrated activity in HER2+ tumours, T-DMi-resistant BCs, and HER2+ve tumours148. Promising efficacy data and low systemic toxicity (due to the stable linker) were reported from a phase I trial, and a phase II randomized trial is ongoing (NCT04829604; see Related links149). |

Disitamab vedotin (RC48-ADC). Disitamab vedotin (RC48-ADC) comprises the humanized anti-HER2 antibody hertuzumab coupled via a cleavable linker to the cytotoxic agent monomethyl auristatin E (MMAE). Disitamab targets different epitopes of the HER2 receptor and has better molecular affinity for HER2 than trastuzumab147. |
Table 2 | Select HER2-targeted TKIs in development

| Drug name (company) | Description | Clinical trial ID | Clinical trial data | Reference |
|---------------------|-------------|-------------------|---------------------|-----------|
| Epertinib (S-222611) (Shionogi & Co. Ltd) | Reversible HER1, HER2, HER4 inhibitor; penetrates CNS | EudraCT number: 2013-003894-87 | Phase I/II trial: ORR >50% (epertinib with trastuzumab or chemotherapy); reduction in CNS lesions in select patients | Macpherson et al.123 |
| Poziotinib (Spectrum Pharmaceuticals) | Irreversible pan-HER inhibitor | NCT02659514 (MBC) NCT03066206 (EGFR exon 20 mutant NSCLC) | Phase II trial in HER2+ MBC: ORR 26% or 27%; DCR 50% or 70% based on dose | Brufsky et al.208 |
| DZD1516 (Dizal Pharmaceuticals) | Selective HER2 TKI; penetrates BBB | NCT04509596 | Phase I trial: most common AEs were anaemia, haemoglobin decrease and headache; efficacy data not available | Zhang et al.204 |
| BDTX-189 (Black Diamond Therapeutics) | Irreversible allosteric EGFR/HER2 inhibitor | NCT04209465 | Phase I trial in solid tumours with HER2 mutations/ amplification: RP2D 800 mg; toxicity: predominantly GI AEs (grade 1/2) and skin disorders, low grade and infrequent. Responses in non-breast tumour types | Schram et al.195 |

AE, adverse event; BBB, blood–brain barrier; CNS, central nervous system; DCR, disease control rate; EGFR, epidermal growth factor receptor; GI, gastrointestinal; MBC, metastatic breast cancer; NSCLC, non-small-cell lung cancer; ORR, overall response rate; RP2D, recommended phase II dose; TKI, tyrosine kinase inhibitor.

Zanidatamab zovodotin (ZW49). Zanidatamab zovodotin (ZW49) is a bispecific HER2-targeted ADC combining the unique design of zanidatamab (ZW25) (binds ECDs II and IV of HER2) with a cytotoxic and cleavable linker. The bifunctional antibody demonstrated lysosomal trafficking and superior internalization relative to a HER2-targeted monospecific ADC149. Preclinical data indicated efficacy in lysosomal trafficking and superior internalization relative to a HER2-targeted ADC combining the unique design of ZW49. Preclinical data demonstrated good activity in HER2-overexpressing cancer cells and a robust bystander effect targeting neighbouring tumour cells147. Multiple clinical trials evaluating disitamab vedotin are ongoing in solid tumours including MBC and gastric cancer. Disitamab vedotin has already received regulatory approval in China for treatment of HER2+ gastric cancer and urothelial cancer145,146.

ALTA-ADC. In an effort to improve the potency of HER2-targeting ADCs, a trastuzumab-based ADC with lower affinity for HER2 at acidic endosomal pH was developed140. Engineering the ADC to confer endosomal dissociation from its target is expected to enable payload entry into lysosomes and recycling of unbound target. This engineered HER2 ADC variant (referred to as an ALTA-ADC) demonstrated increased lysosomal delivery and cytotoxicity even on tumour cells with intermediate levels of HER2 expression, and higher efficacy in xenograft models in mice compared with T-DMI. Furthermore, the ability of the ALTA-ADCs to achieve a therapeutic effect at lower doses may help to overcome the dose-limiting toxicities for other tumour targets.

Targeted thorium-227 conjugates. Targeted α-therapy aims to deliver α-particle-emitting radionuclides selectively to cancer cells in the TME. These α-particles are highly cytotoxic, inducing difficult-to-repair, clustered double strand DNA breaks, leading to cell death141. Targeted thorium-227 conjugates (TTCs) have been generated using efficient chelators that connect the α-particle-emitting radionuclide to an antibody to a target expressed on tumours151. Exposure of cancer cells to TTCs releases markers of danger-associated molecular patterns (DAMPs), which are upregulated by dying cells to alert the immune system and to initiate immunogenic cell death152. The synthetic lethal effect of the combination of TTCs with other DNA-damaging agents was demonstrated using colorectal cancer xenografts153. Combination of a HER2 TTC with olaparib resulted in complete growth inhibition in a human DLD-1 BRCA2−/− xenograft. A first-in-human (FIH) study has been initiated with a HER2 TTC in patients with metastatic breast or gastric cancer (NCT04147819; see Related links).

Tyrosine kinase inhibitors

Targeting the intracellular kinase domain of HER2 using small-molecule inhibitors continues to be investigated with next-generation TKIs. This class of compounds is attractive owing to their unique ability to cross the BBB and BTB although some compounds pose a challenge owing to promiscuous activity and EGFR side effects of rash and diarrhoea. Selected new TKIs in development are listed in Table 2.

DZD1516 was designed as an oral, reversible and selective HER2 kinase that has full BBB penetration. It demonstrated tumour regressions in xenograft mouse models including subcutaneous, brain metastasis and leptomeningeal metastasis models. Phase I pharmacokinetic data supported once-daily dosing. DZD1516 was also well tolerated, with most AEs being grade 1 events144. Interestingly, diarrhoea was not noted as an AE in this study, distinguishing it from most of the other HER2 TKIs studied.

Oncogenic mutations in HER2 have been identified in multiple solid tumours including BC, and most of these occur at allosteric sites outside the ATP-binding site of HER2. BDXT-189 is an oral, ATP-competitive, irreversible, small-molecule inhibitor of EGFR/HER2 alterations and HER2 wild type, designed to spare EGFR wild type to minimize toxicity155. BDXT-189 demonstrated potent, sustained inactivation of multiple HER2+ cancers that have progressed following standard therapies for other tumour targets.

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HER2 had better and a median PFS of 6.8 months. Translational research suggested patients with HER2+ MBC showed a 28% objective response rate (ORR) and endocytose. Results of a FIH trial of KN026 in heavily pretreated epitopes on HER2 (domains II and IV) leading to dual HER2 signal block-KN026.

Further development of zenocutuzumab in HER2+ BC is uncertain; how-
zumab in combination with trastuzumab and vinorelbine demonstrated dimerization and activation of downstream signalling. Zenocutuzumab (MCLA-128). Another bispecific, humanized IgG1 antibody that is under investigation is zenocutuzumab (MCLA-128), which acts via two independent mechanisms of action: inhibition of HER2–HER3 signalling and elimination of tumour cells via ADC. MCLA-128 functions via a ‘dock and block’ mechanism whereby one arm of the antibody binds HER2 domain I and optimally positions the anti-HER3 arm to block the ligand–HER3 receptor interaction, preventing HER2–HER3 dimerization and activation of downstream signalling. Zenocutuzumab in combination with trastuzumab and vinorelbine demonstrated a 35% clinical benefit rate at 6 months in patients with HER2+ MBC who had progressed on prior anti-HER2 therapy including T-DM1 (ref. 164). Further development of zenocutuzumab in HER2+ BC is uncertain; however, its ability to bind HER2 and block NRG1 fusion protein binding and subsequent HER2–HER3 dimerization is being actively explored in NRG1 fusion-positive cancers.

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Bispecific antibodies

Advances in antibody biology and engineering have led to the development of bispecific antibodies that contain two binding sites directed against two separate antigens or conversely, can target two separate epitopes on the same antigen. Great diversity is possible with this format as bispecifics can also target an antigen with one binding site, and the other site can be an immune target that could elicit a synergistic effect. Key examples of bispecific antibodies currently in development are discussed below.

Zanidatamab (ZW25). Zanidatamab (ZW25) is a humanized, bispecific, IgG1 antibody directed against the ECD IV and the dimerization domain (ECD II) of HER2, the same domains as are targeted by trastuzumab and pertuzumab, respectively. Unlike trastuzumab, where each receptor can be bound by only one mAb, zanidatamab promotes receptor clustering whereby each HER2 receptor can be targeted by two zanidatamab antibodies. Hence, treatment with zanidatamab leads to enhanced HER2 internalization, downregulation and potent effector-function-mediated cytotoxicity. Zanidatamab showed promising antitumour activity as monotherapy or in combination with chemotherapy in patients with advanced HER2-expressing cancers that had progressed on anti-HER2 therapies. Zanidatamab in combination with palbociclib and fulvestrant is currently under evaluation in HR+/HER2+ MBC (NCT04224272; see Related links), and zanidatamab in combination with ALX148 (a CD47 blocker) is being investigated in HER2-low and HER2-high BC (NCT05027139; see Related links). A neoadjuvant pilot trial with single-agent zanidatamab is also being planned in HER2+ EBC (NCT05035836; see Related links).

Zenocutuzumab (MCLA-128). Another bispecific, humanized IgG1 antibody that is under investigation is zenocutuzumab (MCLA-128), which acts via two independent mechanisms of action: inhibition of HER2–HER3 signalling and elimination of tumour cells via ADC. MCLA-128 functions via a ‘dock and block’ mechanism whereby one arm of the antibody binds HER2 domain I and optimally positions the anti-HER3 arm to block the ligand–HER3 receptor interaction, preventing HER2–HER3 dimerization and activation of downstream signalling. Zenocutuzumab in combination with trastuzumab and vinorelbine demonstrated a 35% clinical benefit rate at 6 months in patients with HER2+ MBC who had progressed on prior anti-HER2 therapy including T-DM1 (ref. 164). Further development of zenocutuzumab in HER2+ BC is uncertain; however, its ability to bind HER2 and block NRG1 fusion protein binding and subsequent HER2–HER3 dimerization is being actively explored in NRG1 fusion-positive cancers (NCT02912949; see Related links).

KN026. KN026 is a bispecific antibody that targets two distinct epitopes on HER2 (domains II and IV) leading to dual HER2 signal block-
ade, presumably by causing HER2 to aggregate on the cell surface and endocytose. Results of a FIH trial of KN026 in heavily pretreated patients with HER2+ MBC showed a 28% objective response rate (ORR) and a median PFS of 6.8 months. Translational research suggested that patients with co-amplification of CDK12 and HER2 had better responses to KN026 than patients without the co-amplification. On the basis of these results, additional trials are ongoing or planned with KN026 in HER2+ BC (NCT04521179, NCT04881929, NCT04778982; see Related links).

Targeted protein degraders

Targeted protein degradation is being explored as an alternative strategy in cancer, whereby the natural protein degradation system is co-opted for therapeutic purposes. Recently developed novel molecules called proteolysis-targeting chimeras (PROTACs) are hetero-
bifunctional molecules with two ligands joined by a linker. One ligand binds to the ‘protein of interest’ (POI) and the second ligand binds to an E3 ubiquitin ligase. Simultaneous binding of the PROTAC to the POI and ligase induces ubiquitylation of the POI and its degradation by the ubiquitin–proteasome system, followed by regeneration of the PROTAC to tackle another copy of the target. PROTACs are unique because they exhibit a catalyst-type mechanism of action, unlike classic inhibitors which have a one-to-one relationship with the target protein. Two PROTACs, one targeting ER (ARV-471) and the other targeting the androgen receptor (AR) (ARV-110) have demonstrated clinical efficacy; however, these are not tissue specific because they use E3 ligases that have a broad expression profile. In an effort to optimize the therapeutic window and potentially minimize side effects of broad-spectrum PROTACs, an antibody–PROTAC conjugate that specifically targets HER2-expressing cells was developed. This trastuzumab–PROTAC conjugate cages E3 ligase-directed degrader activity with an antibody linker that can be hydrolysed after antibody–PROTAC internalization, releasing the active PROTAC that induces catalytic protein degradation. Studies of a trastuzumab–BRD4 degrader conjugate demonstrated that it selectively targets BRD4 for degradation only in HER2-overexpressing BC cell lines, but not in HER2-negative cell lines. This novel antibody–PROTAC strategy combines the catalytic potency of PROTACs with the tissue specificity of ADCs, enabling the development of new molecules that can target degradation of specific molecules in selected tissues.

An emerging technology aims to selectively degrade HER2-expressing cells by coupling targeted protein degraders to a HER2-specific antibody, generating an antibody neodegrader conjugate (AndDC). Conjugating the protein degrader to the HER2 antibody directs the degrader specifically to the cytosol of the target cells. ORM-5029 is designed to deliver catalytic GSPT1 protein degrader (Smol0006) to HER2-expressing tumours via antibody targeting (pertuzumab). Once the antibody and degrader enter the HER2+ tumour cell by endo-
cytosis, the antibody is degraded in the lysosome, freeing the degrader, which binds to GSPT1 in the cytosol. The natural protein deg-
radation system of the cell is then harnessed to destroy GSPT1, leading to cancer cell death. ORM-5029 displayed in vitro and in vivo efficacy that was comparable to that of other GSPT1 degraders and approved ADCs.

Harnessing the immune system

In addition to directly targeting the oncogenic driver HER2 and inhibiting its function, exploiting the innate and adaptive immune system to tackle proliferating cancer cells is an area of promise and active investigation in HER2+ BC. Some of these strategies are outlined below and in Table 3 and Fig. 3.

Combinations with checkpoint inhibitors

Immune checkpoint inhibitors (CPIs) have transformed the treatment landscape of solid tumours (for example, melanoma and lung cancer) by inducing the immune system to attack cancer cells, resulting in durable
tumour regression and prolonged survival. However, success with these agents in BC has been limited compared with other tumour histologies. There have been no trials in metastatic disease showing benefit outside of the 30–40% of metastatic TNBCs that express PDL1 in the first-line setting. CPI benefits in neoadjuvant TNBC have been broader, irrespective of PDL1 status. Disappointingly, the efficacy of CPI in HER2+ BC has been modest despite high levels of PDL1 expression. Levels of tumour-infiltrating lymphocytes (TILs) in primary HER2+ breast tumours are on par with that in TNBC, indicating a potential for leveraging the immune system11. Furthermore, the immune-mediated ADCC mechanism of trastuzumab and pertuzumab suggests that combination immunotherapies may be effective167,168. Combinations of HER2-targeted agents with CPI have demonstrated preliminary antitumour activity in phase I trials, and T-DXd is being investigated in combination with pembrolizumab (NCT04042701; see Related links)169,170. In the KATE2 randomized phase II trial of T-DM1 with atezolizumab or placebo in previously treated HER2+ MBC, the addition of atezolizumab did not improve PFS relative to T-DM1 alone171. However, this was a later-line setting, and we have learned from TNBC that both setting and line may have profound implications for the activity of immunotherapy in BC. On the basis of the trend to improvement in the PDL1 subset in KATE2, the phase III KATE3 trial is enrolling patients with PDL1+HER2+ MBC to receive T-DMI plus atezolizumab or placebo (NCT04740918; see Related links). Further investigations of anti-HER2 therapies in combination with CPI in earlier lines of treatment are ongoing (NCT03199885, NCT04538742; see Related links). The underlying complexity of tumour–immune system interactions may limit the efficacy of targeting a single immune checkpoint in isolation. Hence, combination strategies that simultaneously hinder multiple checkpoints or those that invoke lasting immunological memory may be more effective and may also counter the development of resistance.

Radiation therapy (RT), a common modality for BC treatment, has an immunostimulatory effect by altering the TME, exposing tumour antigen and inducing anti-inflammatory responses 172. Hence, addition of RT to CPI may enhance responses (Fig. 3). Results from a trial of whole-brain radiation therapy (WBRT) and concurrent CTLA4-mediated immune modulation with tremelimumab plus or minus trastuzumab in patients with BC with brain metastases, demonstrated a 12-week non-CNS disease control rate (primary end point) of 10% in patients with HER2− MBC and 33% in patients with HER2+ MBC173. Tremelimumab plus durvalumab (anti-PD1 antibody) with brain RT has also been evaluated in MBC including in HER2+ disease (NCT02563925; see Related links). Most ongoing trials exploring RT plus CPI are restricted to patients with HER2+ or TNBC disease, although there is one trial

### Table 3 | Immune bispecific agents under evaluation for HER2+ BC

| Therapeutic agent (institution/company) | Tumour-associated antigen | Immune target | Phase/clinical trial ID | Reference |
|---------------------------------------|---------------------------|---------------|-------------------------|-----------|
| HER2/CD3 BsAb (Memorial Sloan Kettering Cancer Center) | HER2 | CD3 | Not applicable | Lopez-Albaitero et al.120 |
| M-802 (Huazhong University of Science and Technology) | HER2 | CD3 | Not applicable | Yu et al.16 |
| p95HER2+CD3 BsAb T cell (Vall d’Hebron Institute of Oncology) | p95 | CD3 | Not applicable | Ruiz et al.206 |
| Triobody [(HER2) 2+CD16] (University Hospital Schleswig-Holstein, Christian-Albrechts University) | HER2 | CD16 | Not applicable | Oberg et al.227 |
| BsPDL1×ErB2 (QIMR Berghofer Medical Research Institute) | HER2 | PDL1 | Not applicable | Mittal et al.238 |
| Runimotamab/BTRC4017A (Genentech, Inc.) | HER2 | CD3 | Phase I/NCT03448042 | Not applicable |
| IBI315 (Innovent Biologics (Suzhou) Co. Ltd) | HER2 | PDL1 | Phase I/NCT04162327 | Not applicable |
| PRS-343 (cinrebufus afia) (Pieris Pharmaceuticals, Inc.) | HER2 | CD137 | Phase I/NCT03650348 | Ku et al.220 |
| SAR-443216 (Sanofi) | HER2 | CD3/CD28 | Phase I/NCT05013554 | Shu et al.219 |
| BDC-1001 (Bolt Biotherapeutics, Inc.) | HER2 | TLR7/8 | Phase I/NCT04278144 | Sharma et al.220 |
| NJH395 (Novartis Pharmaceuticals) | HER2 | TLR7 | Phase I/NCT03696771 | Janku et al.235 |
| SBT6050 (Silverback Therapeutics) | HER2 | TLR8 | Phase I/NCT04460456 | Klemper et al.232 |
| HER2Bi–aATCs/HER2BATs (University of Virginia) | HER2 | CD3+ activated T cells | Phase I/I/NCT03272334 | Lum et al.181 |
| TAC01-HER2 (Triumvirum Immunologics, Inc.) | HER2 | CD3 and CD4 co-receptor domain | Phase I/I/NCT04727151 | NCT04727151 |
| DF-1001 (Dragonfly Therapeutics) | HER2 | NK cells | Phase I/I/NCT04143711 | NCT04143711 |
| ACE1702 (Acepodia Biotech, Inc.) | HER2 | NK cells | Phase I/I/NCT04319757 | NCT04319757 |
| BPX-603 (Bellicum Pharmaceuticals) | HER2 | Dual switch CAR-T cells | Phase I/NCT04650451 | NCT04650451 |
| MT-5111 (Molecular Templates, Inc.) | HER2 scFV | De-immunized Shiga-like toxin-A subunit | Phase I/NCT04029922 | Wainberg et al.234 |

ATC, activated T cell; BsAb, bispecific antibody; CAR-T cell, chimeric antigen receptor–T cell; NK, natural killer; TLR, Toll-like receptor.
that aims to combine pembrolizumab with single-fraction radiation boost to enhance efficacy in operable BC including HER2\(^*\) disease (NCT04454528; see Related links).

**Bispecific engagers**

The poor activity of immunotherapy in BC is attributed to the immunosuppressive TME after chemotherapy, potentially due to loss of the MHC class I molecules on metastatic tumours leading to reduced or delayed recovery of the T cell repertoire clones that target BC-specific antigens\(^{174}\). Novel bispecific antibody (BsAb) formats can overcome this barrier by simultaneously binding a tumour-specific antigen and an immune cell to cause tumour cell death. HER2 is a common antigen targeted by these BsAbs. Bispecific T cell engagers (BiTEs) redirect T cells to target HER2-expressing tumour cells using a BsAb directed against CD3 and HER2 (Fig. 4). The advantage of the BiTE approach is that T cell activation is independent of antigen specificity, and a large fraction of the T cells are activated. Bispecific killer cell engagers (BiKEs) bind to CD16 on natural killer (NK)/monocytic cells and HER2 on tumour cells to eradicate HER2-expressing cancer cells.

An early candidate, 2B1, was a murine-derived HER2 with a Fc\(^+\) bispecific antibody that activated NK cells against HER2-expressing tumour cells. Although 2B1 did not demonstrate antitumour activity in a phase I trial, there was evidence of immune activation in 10 of 20 patients\(^{175}\). These findings contributed to a better understanding of the mechanism by which antibody-induced ADCC may exert antitumour activity.

Innovations led to the ‘knobs-in-hole’ technology, which involves replacing a smaller amino acid with a larger amino acid (T336Y) in the CH3 region of an antibody chain to form a ‘knobs’ structure and at the same time substituting a larger amino acid in the other chain with a smaller amino acid to form a ‘hole’ structure (Y407T). This technology, which enables Fc heterodimerization, results in a BsAb that is more stable, has favourable pharmacokinetics and can be produced with high quality and reproducibility\(^{176}\). M-802 and ruminatamab (BTRC401TA) are examples of bispecifics that use this technology that are under evaluation in clinical trials (Table 3 and Fig. 4; NCT04501770, NCT03448042; see Related links). An alternative approach is to use antibodies directed against specific receptors expressed on T cells or NK cells (for example, CD3\(^{177}\), CD3\(^{179}\)) in combination with HER2\(^{178}\) targeted therapies to enhance antitumour activity. Monalizumab is an antibody that blocks the interaction between the inhibitory checkpoint receptor NKG2A expressed on NK cells and CD8\(^+\) T cells and HLA-E, which is overexpressed on malignant tumours\(^{178}\). Blocking this inhibitory interaction between NKG2A and HLA-E overcomes tumour resistance to NK cells. Since monalizumab can act simultaneously on both NK cells and T cells as well as tumour-infiltrating immune cells, it can enhance the cytotoxic potential of therapeutic antibodies such as trastuzumab and as such, is being investigated in the phase II MIMOSA trial in patients with HER2\(^*\) MBC (NCT04307329; see Related links). CD137 is a costimulatory immune receptor expressed on activated T and B cells and NK cells and a potential target for cancer immunotherapy as it is expressed on TILs. Upon activation, it promotes increased proliferation, cytokine production and cell lysis by T and NK cells\(^{179,180}\).

Other bispecific antibodies

A different approach combining anti-HER2 and PDL1 antibodies into one BsAb (IBI315) was employed to redirect anti-PDL1 response to HER2-expressing tumour cells. Results of a phase I dose-escalation trial in patients with advanced solid tumours treated with various dose levels of IBI315 showed a 20% ORR (see Innovent press release in Related links: https://go.nature.com/3F91Ioe). Theoretically, this may be a very clean way to use immunotherapy: effectively, targeting just the HER2-expressing cancer cells without as many off-target effects.

PRS-343 (cirebafusp alfa) (Table 3) is a novel bispecific fusion protein that combines a 4-1BB (CD137)-targeting anticalin protein and a modified version of trastuzumab. The HER2-targeting moiety localizes the drug to the HER2-expressing cells in the TME and facilitates 4-1BB cross-linking, which ameliorates T cell exhaustion and enables T cell expansion and eventual tumour regression. In a phase I study in patients with HER2 expressing solid tumours, PRS-343 demonstrated activity as monotherapy and in combination with atezolizumab\(^{180}\).

Another BsAb innovation is the generation of patient-derived activated T cells (ATCs) armed with an anti-CD3–anti-HER2 bispecific antibody (HER2Bi), meaning these T cells are coated with the BsAb. Essentially, polyclonal T cell populations expanded and activated ex vivo can be armed with a HER2–CD3 bispecific antibody to specifically recognize and kill HER2-expressing tumour cells in vivo. Arming ATCs with HER2Bi redirects the non-MHC-restricted cytotoxicity of ATCs to a HER2-specific target. A phase I trial that evaluated HER2Bi–aATCs administered with IL-2 and granulocyte–macrophage colony-stimulating factor (GM-CSF) in 23 patients with MBC demonstrated preliminary efficacy, and no dose-limiting toxicities (DLTs) were observed\(^{181}\).

**Immune-stimulating antibody conjugates**

Immune-stimulating antibody conjugates (ISACs) covalently attach immune stimulants to tumour-specific antibodies such as trastuzumab and can trigger target tumour-dependent activation of the innate and adaptive immune systems to eradicate tumours\(^{182}\) (Fig. 3).

Toll-like receptors (TLRs) are important components that initiate innate immunity. These receptors also bridge innate and adaptive immunity\(^{183}\). BDC-1001 is an example of an ISAC that consists of a trastuzumab biosimilar conjugated to a TLR7/8 agonist with a non-cleavable linker. BDC-1001 employs a three-pronged approach — direct antitumour effects mediated by trastuzumab, localized phagocytosis and elimination of HER2-expressing tumour cells by the immune-stimulating TLR7/8 molecules that activate the myeloid antigen-presenting cells (APCs), that is, macrophages and DCs. This results in cytotoxicity and subsequent processing and presentation of neoantigens that stimulates T cell-mediated durable immunity. The advantage with this construct is that TLR7/8 activation occurs only after internalization into the APCs, thus mitigating the risk of nonspecific immune activation. In essence, these ISACs convert the TME from ‘cold’ to ‘hot’ by localized stimulation of the immune system. Additional agents conjugating trastuzumab or biosimilar to TLR7/8 that are in development are listed in Table 3.

**Engineered toxin bodies**

Engineered toxin bodies (ETBs) are novel immunotoxins that combine the specificity of antibodies with the potent mechanism of bacterial toxin cell destruction. MT-511 (Table 3), an example of a de-immunized ETB fused to a HER2 antibody, has a novel mechanism of action that induces direct cell kill via enzymatic and permanent ribosome destruction, thus potentially bypassing resistance mechanisms that exist for HER2 TKIs, ADCs or antibody modalities (Fig. 3). Furthermore, MT-511 binds to an epitope on HER2 that is distinct from that of trastuzumab and pertuzumab, enabling combination with existing HER2-targeting agents\(^{184}\). Preliminary results of a phase I trial of MT-511 in
a Immune stimulating antibody conjugate

Innate Immune response

1. TAA → Tumour cell
2. TLR-conjugated ISAC → Myeloid APC
3. Activated myeloid cell

Adaptive immune response

4. T cell
5. Activated T cell

Activated myeloid cell with MHC tumour peptide

b Chimeric antigen receptor–macrophage (CAR-M)

Targeted phagocytosis

1. Antigen → CAR-M
2. CAR-M

Activation of the TME

3. Dendritic cell
4. Cytokine release

Antigen presentation

Cytokines
TAA

C

Radiotherapy

1. Radiotherapy
2. CTLA4
3. CTLA4 antibody
4. CXCL16 (chemokine)
5. Tumour lysis

Radiation + checkpoint inhibitor

d Engineered toxin body

1. HER2 on tumour cell
2. HER2-targeted scFV
3. De-immunized Shiga-like toxin
4. Cytosol
5. Endosome
6. Golgi body
7. ER
8. Nucleus

Engineered toxin body:
HER2-expressing solid tumours showed stable disease as best response, no DLTs and maximum tolerated dose not yet reached. An expansion cohort for patients with MBC is ongoing.

**CAR-M and CAR-NK therapies**

Chimeric antigen receptors (CARs), engineered molecules that combine the specificity of antibodies with the downstream signalling of T cells, represent a key class of cellular immunotherapies. Although FDA-approved CAR-T therapies are available for haematological malignancies, their application to solid tumours has been hindered by the need for active trafficking and penetration of T cells into the TME. As such, alternative immune cells such as human macrophages have been genetically engineered with CARs (CAR-M) to redirect their phagocytic activity against solid tumours (Fig. 3). A single infusion of these CAR-Ms was able to shrink tumours and improve OS in solid tumour xenograft mouse models. Furthermore, the CAR-Ms converted bystander immunosuppressive M2 macrophages to pro-inflammatory M1 macrophages, upregulated the APC machinery and reprogrammed the TME by activating immature DCs and recruiting activated CD8+ T cells to the tumour sites, thus amplifying the antitumour response.

CT-008 is a cell product comprising autologous peripheral blood monocyte-derived macrophages that are transduced with an adenoviral vector containing an anti-HER2 CAR-M and locked into a pro-inflammatory M1 phenotype. This agent is being investigated in an ongoing FIH trial in patients with HER2-overexpressing solid tumours, and early data demonstrate good tolerability. Correlative analyses demonstrated evidence of broad reprogramming of the TME as well as intratumoural T cell expansion and activation with altered peripheral T cell repertoire.

CAR-engineered NK cells are superior to CAR-T cells; they appear to be safer because they do not induce cytokine release syndrome (CRS) and they engage multiple mechanisms to promote cytotoxicity. They also lend themselves to “off-the-shelf” manufacturing, that is, they can be generated from peripheral blood cells or from stem cell sources or NK-92 cell lines instead of using a patient’s own immune cells. The CAR-NK cells represent receptors against tumour-associated antigens (TAAs) and redirect the effector NK cells to target specific tumours. CAT-179 is an off-the-shelf CAR-NK-T cell therapy in development, with an optimized CAR that targets HER2, an IL-15 cytokine that enables enhanced and sustained NK cell activity and a TME switch to counteract the immunosuppressive TGFβ signal in the TME.

**Cancer vaccines targeting HER2**

Cancer vaccines are designed to kindle the patient’s own immune system to identify and kill tumour cells by stimulating CD8+ and CD4+ T cell responses to tumour-specific antigens. Several platforms have been used to develop cancer vaccines, including HER2-specific vaccines.

These range from simple peptide vaccines to the more complex autologous or allogeneic cell-based vaccines summarized in Table 4.

A key consideration regarding cancer vaccines is the treatment setting. It is well recognized that disease burden and the immunosuppressive TME in metastatic disease may limit T cell activity. Vaccines may be more successful in a setting of very low disease burden, such as post-operative EBC as well as the pre-malignant ductal carcinoma in situ (DCIS) setting. Furthermore, combinations with CPIs or chemotherapy and targeted agents may enhance vaccine efficacy in the (neo)adjuvant setting and potentially help to overcome the suppressive TME in the advanced disease setting.

**Peptide vaccines.** These may be the most common vaccine design and have several advantages over other vaccine types: production is very easy, more cost-effective and easier to administer with relatively few side effects. Peptide vaccines using various domains of the HER2 protein have been evaluated in patients with BC. E75 is a nine-amino-acid immunogenic peptide derived from the extracellular domain of HER2 and is expressed in 60–75% of the population. Nelipepimut-S/NeuVax is an MHC class I vaccine that consists of E75 combined with an immunoadjuvant GM-CSF. A disadvantage is that by being more specific, their T cell immunological responses may be more limited in terms of cell type and may not stimulate T helper cells and B cells sufficiently.

**Protein-based vaccines.** These offer an advantage over peptide vaccines as they contain both HLA class I and II epitopes and are not subject to HLA restrictions. Moreover, they can significantly activate T cells, leading to an enhanced immune response and better T cell activation. Although this approach has not been explored extensively, early trials using a fragment from the intracellular domain (ICD) of HER2 was shown to be well tolerated, and patients developed HER2ICD-specific T cell immunity. A phase I trial evaluating dHER2 vaccine, a recombinant protein comprising the ECD and a fragment of the ICD combined with the adjuvant AS15, combined with lapatinib in 12 patients with metastatic breast cancer demonstrate anti-HER2 antibody induction in all patients. HER2-specific T cells were detected in one patient, and importantly, there were no signals of cardiotoxicity.

**Cell-based vaccines.** These can be derived from the patient’s own tumour cells to generate autologous vaccines or alternatively, allogeneic tumour cell lines can be used to develop cell-based vaccines. Allogeneic vaccines offer an alternative because they can be obtained from established cancer cell lines that express specific TAAs. Initial data with an allogeneic HER2–GM-CSF vaccine given with cyclophosphamide and weekly trastuzumab appear promising, warranting further studies (Table 4). One disadvantage of autologous vaccines is variability in the vaccine and a lengthy manufacturing process.
**Dendritic cells.** DCs are potent modulators of the immune response and are specialized APCs that can stimulate naïve T lymphocytes while generating memory T lymphocytes. The DC vaccine platform allows for antigen processing by the patient’s own immune system, eliciting immunological responses against multiple epitopes on the target, versus a single epitope approach with an antibody or a small-molecule inhibitor. Preliminary studies using DC vaccines have been conducted in patients with invasive BC and even DCIS; the latter hinting at the potential for possible development of a vaccine for prevention of BC\(^1\). Although DC vaccines are a promising individualized strategy, the vaccine manufacturing process is complex and will need to be streamlined to make it more accessible. Exploration of allogeneic or artificial APCs should be pursued to overcome this limitation.

**Recombinant DNA- or virus-based vaccines.** These can be used to deliver tumour-specific antigens and generate antigen-specific cellular and humoral immune responses. DNA-based vaccines are an easy and practical approach given their simplicity, safety and cost effectiveness. Multiple preclinical studies have attested to the efficacy of this strategy. Clinical trials with plasmid-based vaccines are underway in patients with HER2\(^{+}\) BC with the goal of evaluating the immunogenicity of these vaccines (Table 4). The natural immunogenic nature of viruses is an inherent advantage. Unlike DNA-based vaccines, TAAs are presented along with viral antigens, potentially leading to enhanced and long-lasting immune T cell responses\(^2\). Preliminary results from a phase I trial with a novel alphaviral vector encoding a self-amplifying replicon RNA encoding HER2 segment (VRP-HER2) have been encouraging\(^3\). Subsequent preclinical studies have shown that only vaccines targeting true oncogenic drivers of HER2 elicited significant antitumour responses, and long-term tumour control was observed only in combination with a CPI. This has led to a phase II study evaluating a combination of VRP-HER2 with pembrolizumab (Table 4; NCT03632941; see Related links).

Despite being inherently well tolerated and inducing immunogenicity and long-term survival in select patients, cancer vaccines have not demonstrated efficacy in randomized phase II or III trials\(^4\). Novel delivery vehicles such as lipid nanoparticles, virus-like particles, polymeric and non-degradable particles are being explored for cancer vaccines\(^5\). Previous iterations of vaccines have focused on using TAAs over neoantigens; the latter arise from mutations in the tumour cells owing to their inherent genetic instability, whereas the former are non-mutated antigens, which may explain the lacklustre efficacy observed with vaccine therapies. Neoantigens are able to induce T cell responses comparable to that found in antiviral T cells. New bioinformatic tools for neoantigen prediction have been developed with promising results, which may spur further development of personalized, neoantigen-based therapeutic cancer vaccines including for BC\(^6\). The success of the coronavirus disease 2019 (COVID-19) mRNA vaccines has breathed new life into exploring this technology for cancer, and trials with mRNA-based vaccines against solid tumours are in development.

**Conclusions and future directions**

The remarkable journey of discovery of HER2 as an oncogene, biomarker and target for treatment of a very aggressive form of BC has led to an unprecedented improvement in survival. This success results from the exquisite response of the HER2 receptor to HER2-targeted therapy, to an unprecedented improvement in survival. This success results from the capability of this strategy. Clinical trials with plasmid-based vaccines are underway in patients with HER2\(^{+}\) BC with the goal of evaluating the immunogenicity of these vaccines (Table 4). The natural immunogenic nature of viruses is an inherent advantage. Unlike DNA-based vaccines, TAAs are presented along with viral antigens, potentially leading to enhanced and long-lasting immune T cell responses\(^2\). Preliminary results from a phase I trial with a novel alphaviral vector encoding a self-amplifying replicon RNA encoding HER2 segment (VRP-HER2) have been encouraging\(^3\). Subsequent preclinical studies have shown that only vaccines targeting true oncogenic drivers of HER2 elicited significant antitumour responses, and long-term tumour control was observed only in combination with a CPI. This has led to a phase II study evaluating a combination of VRP-HER2 with pembrolizumab (Table 4; NCT03632941; see Related links).

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**Recent drug constructs such as the ADCs and upcoming bispecifics will continue to improve our ability to safely target HER2\(^{+}\) cells, while**

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**Fig. 4 | Structure and mechanism of action of a HER2–CD3 bispecific antibody.** a, Structure of HER2–CD3 bispecific antibody with ‘knobs-in-hole’ technology. b, Mechanism of action of a HER2–CD3 bispecific antibody. Step 1: binding of HER2–CD3 bispecific antibody to HER2 on tumour cell and CD3 on T cell. Step 2: activation of T cell causing release of cytokines (TNF and interferon-γ (IFNγ)). Step 3: tumour cell lysis.
### Table 4 | Vaccines targeting HER2+ breast cancer

| Name of vaccine (institution/company) | Description | Clinical trial ID | Trial design/patient population | Published clinical trial data | Reference |
|--------------------------------------|-------------|------------------|-------------------------------|-------------------------------|-----------|
| **Peptide/protein-based vaccines**    |             |                  |                               |                               |           |
| Nelipepimut-S/ NeuVa (Galena Biopharma) | MHC class I vaccine derived from the HER2 ECD | NCT01479244 | PRESENT phase III: NeuVax vs GM-CSF in node+ BC with low to intermediate HER2 expression | No difference in DFS in patients treated with Neuvax + GM-CSF vs GM-CSF alone | Mittendorf et al.203 |
| AE37 (NuGenerex Immuno-Oncology; Norwell, Inc.) | MHC class II peptide derived from the intracellular domain of HER2 | NCT00524277 | Phase II: AE37 + GM-CSF vs GM-CSF in BC with any HER2 expression | No differences in recurrences rates or DFS between the two arms | Mittendorf et al.202 |
| GP2 (NuGenerex Immuno-Oncology; Norwell, Inc.) | HER2-derived HLA-A2- and HLA-A3-restricted epitope (GP2 + GM-CSF) | NCT00524277 | Phase II: GP2 + GM-CSF vs GM-CSF in high-risk BC with any level of HER2 | No difference in DFS between the two arms | Mittendorf et al.233 |
| GLSI-100 (Greenwich LifeSciences, Inc.) | HER2-derived HLA-A2- and HLA-A3-restricted epitope | NCT05232916 | Phase III: HER2/neu peptide GLSI-100 vs placebo in HER2+ BC | Not applicable | NCT05232916 |
| TPIV100 (NCI) | HER2 multiple epitope-based vaccine | NCT04197687 | Phase II randomized: T-DM1 + GM-CSF + TPIV100 or placebo in HER2+ BC with residual disease after NAC | Not applicable | NCT04197687 |
| MVF-HER-2 (597-626) - MVF-HER-2 (266-296) (Ohio State University Comprehensive Cancer Center) | Two chimeric peptides co-synthesized with B cell epitopes derived from HER2 ECDs 2 and 4 | NCT01376505 | Phase I: advanced solid tumours including HER2+ MBC | 2 PRs (6%); vaccine-generated sustained humoral response | Bekaii-Saab et al.234 |
| dHER2 (GlaxoSmithKline) | Recombinant protein comprising the ECD and a fragment of the ICD combined with the adjuvant AS15 | NCT00952692 | Phase I/II: dHER2 + lapatinib in trastuzumab-refractory HER2+ MBC | Anti-HER2 antibodies detected in all patients; OS at 300 days was 92% | Hamilton et al.194 |
| **Whole cell-based vaccines**         |             |                  |                               |                               |           |
| HER2+, GM-CSF secreting vaccine (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins) | Allogeneic, HER2+ GM-CSF-secreting breast tumour vaccine | NCT00399529 | Phase II: MBC (including HER2+ MBC) treated with vaccine + chemotherapy | HER2-specific T helper-dependent immunity and antibody responses detected to vaccine | Emens et al.215 |
| HER2+, GM-CSF secreting vaccine (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins) | Allogeneic, HER2+ GM-CSF-secreting breast tumour vaccine | NCT00938384 | Phase I: cyclophosphamide + vaccine + weekly trastuzumab for HER2+ MBC | PFS 7 mo; OS 42 mo; HER2-specific immunity was detected | Chen et al.195 |
| **Dendritic cell-based vaccines**     |             |                  |                               |                               |           |
| HER2-dendritic cell vaccine (Duke University) | DCs loaded with HER2 ICD | NCT00005956 | Pilot: HER2 ICD DC vaccine vs DC vaccine containing tetanus/CMV control for patients with stage II-IV HER2+ BC post surgery | >5-year follow-up 6/7 patients had circulating anti-HER2 ICD antibodies and all alive and disease free | Morse et al.206 |
| DCT vaccine (Abramson Cancer Center of the University of Pennsylvania) | HER2 ECD and ICD peptide-pulsed DC vaccine | NCT02061332 | Phase I/II: HER2 DCIS or IBC vaccinated intratumourally, in nodes or both | pCR higher in DCIS vs invasive BC (28.6% vs 8.6%); immune responses detected in nodes were associated with pCR | Lowenfeld et al.206 |
| **DNA-based vaccines**                |             |                  |                               |                               |           |
| pNGVL3-hICD (University of Washington) | HER2 ICD DNA plasmid-based vaccine | NCT00436254 | Phase I: stage III/IV HER2+ BC in remission or stable bone-only disease treated with intradermal plasmid-based vaccine | Intermediate dose (100 μg) was immunogenic and associated with persistence of immunity at 60 weeks; PFS and OS not reached | Disis et al.207 |
| WOKVAC (University of Washington) | Plasmid-based DNA with three epitopes: IGFBP2, HER2 and IGF1R | NCT04329065 | Phase II: vaccine + paclitaxel + trastuzumab + pertuzumab as neoadjuvant therapy for HER2+ BC | Not applicable | NCT04329065 |
limiting AEs for our patients. Building on the past accomplishments in treatment with HER2-targeted therapy, the investigation of these newer concepts and use of these methods will ultimately lead to continued advances.

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References

1. Carpenter, G., King, L. Jr & Cohen, S. Epidermal growth factor stimulates phosphorylation in membrane preparations in vitro. Nature 276, 409–410 (1978).

2. Schechter, A. L. et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 312, 513–516 (1984).

3. Slamon, D. J. et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235, 177–182 (1987). This work demonstrated that HER2 amplification is a prognostic factor and predictive outcomes in breast cancer.

4. Giordano, S. H. et al. Systemic therapy for advanced human epidermal growth factor receptor 2-positive breast cancer: ASCO guideline update. J. Clin. Oncol. 40, 2612–2635 (2022).

5. von Minckwitz, G. et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N. Engl. J. Med. 377, 122–131 (2017).

6. Tripathy, D. et al. De novo versus recurrent HER2-positive metastatic breast cancer: patient characteristics, treatment, and survival from the SystHERs registry. Oncologist 25, e214–e222 (2020).

7. Kostova, V., Déos, P., Starck, J.-B. & Kotschy, A. The chemistry behind ADCs. Pharmaceuticals 14, 442 (2021).

8. Fu, Z., Li, S., Han, S., Shi, C. & Zhang, Y. Antibody drug conjugate: the “biological missile” for targeted cancer therapy. Signal. Transduc. Target. Ther. 71, 93 (2022).

9. Roussos Torres, E. T. & Emans, L. A. Emerging combination immunotherapy strategies for breast cancer: dual immune checkpoint modulation, antibody-drug conjugates and bispecific antibodies. Breast Cancer Res. Treat. 199, 291–302 (2022).

10. Kichinsky, M. et al. Human chimeric antigen receptor macroglyphosphates for cancer immunotherapy. Nat. Biotechnol. 38, 947–953 (2020).

11. Savas, P. et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat. Rev. Clin. Oncol. 13, 228–241 (2016).

12. Diss, M. L. & Ceci, D. L. Breast cancer vaccines for treatment and prevention. Breast Cancer Res. Treat. 191, 481–489 (2022).

13. Tebbutt, N., Pedersen, M. W. & Johns, T. G. Targeting the ERBB family in cancer: couples therapy. Nat. Rev. Cancer 13, 663e73 (2013).

14. Baselga, J. & Swain, S. M. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. Nat. Rev. Cancer 9, 463–475 (2009).

15. Wolff, A. C. et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. 31, 3997–4013 (2013).

16. Wolff, A. C. et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. J. Clin. Oncol. 36, 2105–2122 (2018). Important guidelines for interpretation of HER2 testing results from IHC and FISH.

17. Modi, S. et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N. Engl. J. Med. 387, 9–20 (2022).

18. A phase III trial that demonstrated the efficacy of the anti-HER2 ADC, trastuzumab deruxtecan, in HER2-low metastatic breast cancer.

19. Baselga, J. Treatment of HER2-overexpressing breast cancer. Ann. Oncol. 21, vii36–vii40 (2010).

20. Baselga, J., Norton, L., Albanel, J., Kim, Y. M. & Mendelsohn, J. Recombinant human anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res. 58, 2825–2831 (1998).

21. Pegram, M. et al. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancer. Oncogene 18, 2241–2251 (1999).

22. Pieters, R. J. et al. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene 9, 1829–1838 (1994).

23. Pieters, R. J., Pegram, M. D., Finn, R. S., Maneval, D. A. & Slamon, D. J. Remission of human breast cancer xenografts on therapy with monoclonal antibody to HER-2 receptor and DNA reactive drugs. Oncogene 17, 2235–2249 (1998).

24. Piccart-Gebhart, M. J. et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N. Engl. J. Med. 353, 1659–1672 (2005).

25. Romond, E. H. et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N. Engl. J. Med. 353, 1673–1684 (2005).

26. Slamon, D. et al. Adjuvant trastuzumab in HER2-positive breast cancer. N. Engl. J. Med. 365, 1273–1283 (2011).

27. Moja, L. et al. Trastuzumab containing regimens for early breast cancer. Cochrane Database Syst. Rev. 2012, CD006343 (2012). A review of 12,000 patients with breast cancer enrolled in randomized controlled trials evaluating trastuzumab alone or in combination with chemotherapy versus no treatment or standard chemotherapy alone as adjuvant therapy for breast cancer.

28. Nata, R. & Esteve, F. J. Herceptin: mechanisms of action and resistance. Cancer Lett. 232, 123–138 (2006).

29. Debib, J. A., Link, V. C. & Greene, M. I. Monoclonal antibodies react with distinct domains of the neu oncoprotein-encoded p85S molecule exert synergistic anti-tumor effects in vivo. Oncogene 2, 273–277 (1988).

30. Ishii, K., Mori, N. & Yamashiro, H. Pertuzumab in the treatment of HER2-positive breast cancer: 671 an evidence-based review of its safety, efficacy, and place in therapy. Core Evid. 14, 51–70 (2019).

31. Agus, D. B. et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell 2, 127–137 (2002).

32. Lee-Hoeflich, S. T. et al. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. Cancer Res. 68, 5878–5887 (2008).
Review article

32. Nahta, R., Hung, M. C. & Esteve, F. J. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res. 64, 2343–2346 (2004).
33. Scheuer, W. et al. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER-2 positive human xenograft tumor models. Cancer Res. 69, 9330–9336 (2009).
34. Mamidi, S., Cinci, M., Hasmann, M., Fehring, V. & Kirschkorn, M. Lipopolys mediated silencing of membrane regulator CD46, CD55 and CD59 enhances complement-dependent anti-tumor activity of trastuzumab and pertuzumab. Mol. Oncol. 7, 580–594 (2013).
35. Swain, S. M. et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N. Engl. J. Med. 372, 724–734 (2015).
36. The first randomized phase III trial to demonstrate superior activity of dual HER2 therapy of pertuzumab and trastuzumab in HER2* metastatic breast cancer.
37. Gianni, L. et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early HER2-positive breast cancer (Neosphere): a randomized phase II clinical safety study (TRYPHENA). Ann. Oncol. 24, 2278–2284 (2013).
38. Musolino, A. et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu positive metastatic breast cancer. J. Clin. Oncol. 26, 1789–1796 (2008).
39. Nordstrom, J. L. et al. Anti-tumor activity and toxicokinetics analysis of MGAH2, an anti-HER2 monoclonal antibody with enhanced Fcγ receptor binding properties. Breast Cancer Res. 13, R23 (2011).
40. Liu, L., Yang, Y. & Burns, R. Margetuximab mediates greater Fc-dependent anti-tumor activities than trastuzumab or pertuzumab in vitro. Cancer Res. 79 (Suppl. 13). Abstr. 15839 (2019).
41. Rupel, S. F. et al. Efficacy of margetuximab vs trastuzumab in patients with pretreated ERBB2-positive advanced breast cancer: a phase 3 randomized clinical trial. JAMA Oncol. 7, 573–584 (2021).
42. Jackisch, C. et al. Subcutaneous vs intravenous trastuzumab for patients with ERBB2-positive early breast cancer: final analysis of the Hannah phase 3 randomized clinical trial. JAMA Oncol. 5, e190339 (2019).
43. Tan, A. R. et al. Fixed-dose combination of pertuzumab and trastuzumab for subcutaneous injection plus chemotherapy in HER2-positive early breast cancer (FechDeviCa): a randomised, open-label, multicentre, non-inferiority, phase 3 study. Lancet Oncol. 22, 85–97 (2021).
44. Konerec, G. E. et al. Activity of the dual kinase inhibitor lapatinib (GW576582) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res. 66, 1639–1649 (2006).
45. Nahta, R., Yuan, L. X., Du, Y. & Esteve, F. J. Lapatinib induces apoptosis in trastuzumab-resistant breast cancer cells: effects on insulin-like growth factor I signaling. Mol. Cancer Ther. 6, 667–674 (2007).
46. Limberg, M. et al. Expression of p95HER2, a truncated form of the HER2 receptor, and selective activity in preclinical modes of HER2-driven cancer. Cancer Res. 70, (Suppl. 16), Abstr. 1962 (2020).
47. Stringer, E. M. et al. Pharmacokinetic (PK) analyses in CSF and plasma from TBCR049, an ongoing trial to assess the safety and efficacy of the combination of tucatinib, trastuzumab and capecitabine for the treatment of leptomeningeal metastasis (LM) in HER2 positive breast cancer. J. Clin. Oncol. 39, 1044 (2021).
48. O’Brien, N. A. et al. The small molecule inhibitor of HER2, tucatinib, has potent and highly selective activity in preclinical modes of HER2-driven cancer. Cancer Res. 79 (Suppl. 4). Abstr. P6-17-11 (2019).
49. Murthy, R. K. et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N. Engl. J. Med. 380, 609–619 (2020).
50. The first randomized trial in HER2* MBC that enrolled patients with active breast metastases and demonstrated improved outcomes with tucatinib + trastuzumab + capecitabine in this patient population.
51. Von Minckwitz, G. et al. Tucatinib + trastuzumab + capecitabine in patients with locally advanced unresectable or HER2-positive metastatic breast cancer (HER2CLIMB). Outcome by hormone receptor status. Cancer Res. 81 (Suppl. 4). Abstr. PD14-01 (2021).
52. Bekkering, A., Goetsch, L., Dammann, O., & Brown, M. P. Strategies and challenges for the next generation of antibody-drug conjugates. Nat. Rev. Drug. Discov. 16, 315–337 (2017).
53. Staudacher, A. H. & Brown, M. P. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? Br. J. Cancer 117, 1736–1742 (2017).
54. Lewis Phillips, G. D. et al. Targeting HER2-positive breast cancer with trastuzumab-DT1, an antibody-cytotoxid drug conjugate. Cancer Res. 68, 9280–9290 (2008).
55. Juntilla, T. T., Li, G., Parsons, K., Phillips, G. L. & Slivkowsky, M. K. Trastuzumab-DT1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. Breast Cancer Res. Treat. 128, 347–356 (2011).
56. von Minckwitz, G. et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N. Engl. J. Med. 380, 617–628 (2019).
57. Hurvitiz, S. A. et al. Neoadjuvant trastuzumab, pertuzumab, and chemotherapy versus trastuzumab-emtansine plus pertuzumab in patients with HER2-positive breast cancer (Kristine): a randomised, open-label, multicentre, phase 3 trial. Lancet Oncol. 19, 115–126 (2018).
58. Krop, I. E. et al. Trastuzumab emtansine plus pertuzumab versus taxane plus pertuzumab plus trastuzumab after anthracycline for high-risk human epidermal growth factor receptor 2-positive early breast cancer: the phase III KAITLIN study. J. Clin. Oncol. 40, 438–448 (2022).
59. Montemurro, F. et al. Trastuzumab emtansine (T-DM1) in patients with HER2-positive metastatic breast cancer: exploratory final analysis of cohort 1 from KAIMILLA, a single-arm phase IIb clinical trial. Ann. Oncol. 31, 1350–1358 (2020).
60. Verre, A., Agatsuma, T. & Soria, J. C. The art of innovation: clinical development of trastuzumab deruxtecan and refedlemin how antibody-drug conjugates target HER-2+ cancer. Oncotarget 9, 1034–1038 (2018).
61. Tamura, K. et al. Trastuzumab deruxtecan (DS-8201a) in patients with advanced HER2-positive breast cancer previously treated with trastuzumab emtansine: a dose-expansion, phase 1 study. Lancet Oncol. 20, 816–826 (2019).
Kancha, R. K. et al. Differential sensitivity of ERBB2 kinase domain mutations towards Wagle, N. et al. Whole exome sequencing (WES) of HER2+ metastatic breast cancer. Nature Reviews Drug Discovery | Volume 22 | February 2023 | 101–126

Cortés, J. et al. Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. N. Engl. J. Med. 386, 1143–1154 (2022).

Swain, S. M. et al. Multidisciplinary clinical guidance on trastuzumab deruxtecan (T-DXd)-related interstitial lung disease/pneumonitis — focus on proactive monitoring, diagnosis, and management. Cancer Treat. Rev. 106, 103378 (2021).

Jerusalem, G. H. M. et al. Trastuzumab deruxtecan (T-DXd) in patients with HER2+ metastatic breast cancer with brain metastases: a subgroup analysis of the DESTINY-Breast01 trial. J. Clin. Oncol. 39, S26 (2021).

Pérez-García, J. M. et al. Trastuzumab deruxtecan in patients with central nervous system involvement from HER2-positive breast cancer: the DEBBRAH trial. Neuro- oncrol. https://doi.org/10.1038/s41388-022-00164-2 (2022).

Mosele, M. F. et al. Unraveling the mechanism of action and resistance to trastuzumab deruxtecan (T-DXd): biomarker analyses from patients from DAISY trial. Ann. Oncol. 33, S123 (2022).

Kancha, R. K. et al. Differential sensitivity of ERBB2 kinase domain mutations towards lapatinib. PLoS ONE 6, e26760 (2011).

Wagle, N. et al. Whole exome sequencing (WES) of HER2+ metastatic breast cancer (MBC) from patients with or without prior trastuzumab (T): a correlative analysis of TBCRC003. Cancer Res. 75 (Suppl. 9), Abstr. P3-5 (2015).

Xu, X. et al. HER2 reactivation through acquisition of the HER2.1.75S mutation as a mechanism of acquired resistance to HER2-targeted therapy in HER2(+) breast cancer. Curr. Cancer Res. 2013, S13–S14 (2015).

Hymas, D. M. et al. HER kinase inhibition in patients with HER2- and HER2+ metastatic breast cancers. Nature 554, 189–194 (2018).

Results from the SUMMIT basket trial which demonstrated the clinical activity of HER2 and HER2 mutations.

Harland, A. B. et al. Co-occurring gain-of-function mutations in HER2 and HER2 modulate HER2/HER2 activation, oncogenesis, and HER2 inhibitor sensitivity. Cancer Cell 39, 1099–1114.e8 (2021).

Veeraraghavan, K. et al. Acquired resistance to tucatinib is associated with EGFR amplification in HER2+ breast cancer (BC) models and can be overcome by a more complete blockade of HER receptor signaling. Cancer Res. 82 (Suppl. 4), Abstr. PD06-06 (2022).

Bose, S. et al. Resistance to next generation tyrosine kinase inhibitors (TKIs) in HER2-positive breast cancer (BC): role of HER and PIK3CA mutations and development of new treatment strategies and study models. Cancer Res. 82 (Suppl. 4), Abstr. P4-01-01 (2022).

Derakhshani, A. et al. Overcoming trastuzumab resistance in HER2+ positive breast cancer using combination therapy. J. Cell Physiol. 235, 3142–3156 (2020).

De Melo Gagliato, J., Dardim, D. L. F., Marchesi, M. S. P. & Hortobagyi, G. N. Mechanisms of resistance and sensitivity to anti-HER2 therapies in HER2+ breast cancer. Oncotarget 7, 64431–64446 (2016).

Siciliano, M. et al. Clinical benefit of lapatinib-based therapy in patients with human epidermal growth factor receptor 2-positive breast tumors expressing the truncated p95HER2 receptor. Clin. Cancer Res. 16, 2688–2695 (2010).

Guarnieri, V. et al. Prospective biomarker analysis of the randomized CHER-LOB study evaluating the dual anti-HER2 treatment with trastuzumab and lapatinib plus chemotherapy as neoadjuvant therapy for HER2 positive breast cancer. Oncologist 2020, 1001–1010 (2015).

Miranda, F., Prazeres, H., Mendes, F., Martins, D. & Schmitt, F. Resistance to endocrine therapy in HR- and/or HER2+ breast cancer: the most promising predictive biomarkers. Mol. Biol. Rep. 49, 717–733 (2022).

Schiliacci, R. et al. Neutralizing soluble tumor necrosis factor alpha overcomes trastuzumab-resistant breast cancer immune evasion by downregulating mucin 4, improving NK cell function and decreasing myeloid-derived suppressor cells in tumor microenvironment. Cancer Res. 79 (Suppl. 4), Abstr. P6-20-14 (2019).

Schiliacci, R. et al. Mucin 4 expression in high risk breast cancer: predicting and overcoming resistance to immunotherapy. Cancer Res. 82 (Suppl. 4), Abstr. PS15-33 (2022).

Loibl, S. et al. PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. Ann. Oncol. 27, 1519–1525 (2016).

Baselga, J. et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J. Clin. Oncol. 32, 3753–3761 (2014).

Chandraratnap, S. et al. Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. Clin. Cancer Res. 18, 6784–6791 (2012).

Rimawi, M. F., De Angelis, C. & Schiff, R. Resistance to anti-HER2 therapies in breast cancer. Am. Soc. Clin. Oncol. Ed. Book 35, e157–e164 (2015).

Loibl, S. et al. Neoadjuvant buparlisib plus trastuzumab and paclitaxel for women with HER2+ primary breast cancer: a randomised, double blind, placebo-controlled phase II trial (NeoPHOEBE). Eur. J. Cancer 53, 133–145 (2017).

André, F. et al. Molecular alterations and everolimus efficacy in human epidermal growth factor receptor 2-overexpressing metastatic breast cancers: combined exploratory biomarker analysis from the BOLERO-1 and BOLERO-3 J. Clin. Oncol. 34, 215–224 (2016).

Xia, W. et al. A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. Natl. Acad. Sci. USA 103, 7795–7800 (2006).
138. Im, S. A. et al. A phase 1 dose-escalation study of anti-HER3 monoclonal antibody LM716 in combination with trastuzumab in patients with HER2-overexpressing metastatic breast or gastric cancer. J. Clin. Oncol. 32, 2519 (2014).

139. Fielder, W. et al. Phase I study of TrastEsX, a glycooptimized anti-HER2 monoclonal antibody, in patients with HER2-positive solid tumours. ESMO Open 3, e000381 (2018).

140. Bartsch, R. et al. Trastuzumab deruxtecan (T-DXd) in HER2-positive breast cancer patients (pts) with active brain metastases: primary outcome analysis from the TUXEDO-1 trial. Ann. Oncol. 33, S198 (2022).

141. Su, Z. et al. Antibody drug conjugates: recent advances in linker chemistry. Acta Pharmacutaica Sin. 81, 8899–3907 (2021).

142. van der Lee, M. et al. The preclinical profile of the diacycromycin-based HER2-targeting ADC SCD985 predicts for clinical benefit in low HER2-expressing breast cancers. Mol. Cancer Ther. 14, 692–703 (2015).

143. Banerji, U. et al. Trastuzumab duocarmazine in locally advanced and metastatic gastric cancers. Lancet Oncol. 23, 1218–1227 (2022).

144. Deeks, E. D. Disitamab vedotin: first approval. Nat. Rev. Drug. Discov. 21, 1335–1344 (2022).

145. Zhang, J. et al. Preclinical and early clinical safety and pharmacokinetics data of antibody-drug conjugate (AnDC) for HER2-expressing breast cancer. Cancer Res. 82 (Suppl. 4), Abstr. P3-13-42 (2022).

146. Stagg, J. et al. Anti-EB6b 2 mAb therapy requires type I and II interferons and synergizes with anti-POD1 or anti-CD137 mAb therapy. Proc. Natl Acad. Sci. USA 108, 7142–7247 (2011).

147. Varchetts, S. et al. Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer. Cancer Res. 67, 11991–11999 (2007).

148. Loi, S. et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase Ib/2 trial. Lancet Oncol. 20, 371–382 (2019).

149. Hamilton, E. et al. Trastuzumab deruxtecan (T-DXd)- DS-8201) with nivolumab in patients with HER2-expressing, advanced breast cancer: a 2-part, phase 1b, multicenter, open-label study. Cancer Res. 81 (Suppl. 4), P03-07 (2021).

150. Emens, L. A. et al. Trastuzumab emtansine plus atezolizumab versus trastuzumab emtansine plus placebo in previously treated, HER2-positive advanced breast cancer (KATE2): a phase 2, multicentre, randomized, double-blind trial. Lancet Oncol. 21, 1283–1295 (2020).

151. Nguyen, A. T., Shiao, S. L. & McArthur, H. L. Advances in combining radiation and immunotherapy in breast cancer. Clin. Breast Cancer 21, 143–152 (2021).

152. Page, D. B. et al. Brain radiotherapy, tremelimumab-mediated CTLA-4-directed blockade +/- trastuzumab in patients with breast cancer brain metastases. NPJ Breast Cancer 8, 50 (2022).

153. Masseoudiene, M. et al. T-cell bispecific antibodies in node-positive breast cancer: novel therapeutic avenue for MHC class I loss variants. Ann. Oncol. 30, 934–944 (2019).

154. Bedard, P. L. et al. Zanidatamab (ZW25), a HER2-targeted bispecific antibody, in patients with HER2-positive, advanced breast cancer: results from a phase II trial of 2B1 bispecific murine monoclonal antibody in metastatic breast cancer (E1814A), a trial coordinated by the eastern cooperative oncology group. J. Immunother. 30, 455–467 (2007).

155. Su, Z. et al. Antibody-drug conjugates: recent advances in linker chemistry. Acta Pharmacutaica Sin. 81, 8899–3907 (2021).

156. Hamilton, E. et al. Trastuzumab deruxtecan (T-DXd)- DS-8201) with nivolumab in patients with HER2-expressing, advanced breast cancer: a 2-part, phase 1b, multicenter, open-label study. Cancer Res. 81 (Suppl. 4), P03-07 (2021).

157. Elamin, Y. Y. et al. Poziotinib for patients with HER2 exon 20 mutant non–small-cell lung cancer: results from a phase II trial. J. Clin. Oncol. 39, 81–88 (2021).

158. Weisser, N. E. et al. The bispecific antibody zanidatamab’s (ZW25’s) unique mechanisms of action for HER2-expressing solid tumors. Cancer Res. 82 (Suppl. 4), Abstr. P2-13-42 (2022).

159. Ku, G. et al. A phase I dose escalation study of PRS-343, a HER2/4-1BB bispecific molecule, in patients with HER2-positive malignancies. Ann. Oncol. 31, S462–S463 (2020).

160. Hamilton, E. et al. Zanidatamab (ZW25), a HER2-targeted bispecific antibody, in patients with HER2-positive, advanced breast cancer: results from a phase II trial of 2B1 bispecific murine monoclonal antibody in metastatic breast cancer (E1814A), a trial coordinated by the eastern cooperative oncology group. J. Immunother. 30, 455–467 (2007).

161. Su, Z. et al. Antibody-drug conjugates: recent advances in linker chemistry. Acta Pharmacutaica Sin. 81, 8899–3907 (2021).

162. Masseoudiene, M. et al. T-cell bispecific antibodies in node-positive breast cancer: novel therapeutic avenue for MHC class I loss variants. Ann. Oncol. 30, 934–944 (2019).

163. Bedard, P. L. et al. Zanidatamab (ZW25), a HER2-targeted bispecific antibody, in patients with HER2-positive metastatic breast cancer. Cancer Res. 82 (Suppl. 4), Abstr. P2-13-42 (2022).

164. Schram, A. M. et al. Safety and preliminary efficacy from the phase 1 portion of Masterkey-OI: a first-in-human dose-escalation study to determine the recommended phase 2 dose (RP2D), pharmacokinetics (PK) and preliminary antitumor activity of BDXT-189, an inhibitor of allotropic ERβ mutations, in patients (pts) with advanced solid malignancies. J. Clin. Oncol. 39, 3086 (2021).

165. Connell, C. M. & Doherty, G. J. Activating HER2 mutations as emerging targets in multiple solid cancers. ESMO Open 2, e000279 (2017).

166. Elmam, Y. Y. et al. Pozizoinib for patients with HER2 exon 20 mutant non–small-cell lung cancer: results from a phase II trial. J. Clin. Oncol. 40, 702–709 (2022).

167. Weisser, N. E. et al. The bispecific antibody zanidatamab's (ZW25's) unique mechanisms of action and durable anti-tumor activity in HER2-expressing cancers. Cancer Res. 81, 1005 (2021).

168. Meric-Bernstam, F. et al. Safety, anti-tumor activity, and biomarker results of the HER2-targeted bispecific antibody ZW25 in HER2-expressing solid tumors. Ann. Oncol. 30, V593–V593 (2019).

169. Bedard, P. L. et al. Zanidatamab (ZW25), a HER2-targeted bispecific antibody, in combination with chemotherapy (chemo) for HER2-positive breast cancer (BC): results from a phase I study. Cancer Res. 82 (Suppl. 4), Abstr. P2-13-07 (2017).

170. Geuens, G. et al. Unbiased combinatorial screening identifies a bispecific IgG1 that potently inhibits HER3 signaling via HER2-guided ligand blockade. Cancer Cell 33, 922–936 (2018).

171. Hamilton, E. P. et al. Clinical activity of MCLA-128 (cencutzumab), trastuzumab, and vinorelbine in HER2-exposed metastatic breast cancer (MBC) patients (pts) who had progressed on anti-HER2 ADCS. J. Clin. Oncol. 38, 3093 (2020).

172. Zhang, J. et al. First-in-human HER2-targeted bispecific antibody KN026 for the treatment of patients with HER2-positive metastatic breast cancer: results from a phase I study. Cancer Res. 82 (Suppl. 4), Abstr. P2-13-07 (2017).

173. Békési, M., Langley, D. R. & Crews, C. M. PROTAC targeted protein degraders: the past is prologue. Nat. Rev. Drug. Discov. 21, 180–202 (2022).

174. Maneiro, M. et al. Antibody–PROTAC conjugates enable HER2-dependent targeted promoted degradation of HER2-kinase. ACS Chem. Biol. 15, 1302–1312 (2020).

175. Palacio, J. et al. ORM-5209: a first-in-class targeted protein degradation therapy using antibody neodegrader conjugate (AnDeo) for HER2-expressing breast cancer. Cancer Res. 82 (Suppl. 4), Abstr. P3933 (2022).
Review article

258. Slamon, D. J. et al. Use of chemotherapy plus a monochlonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med. 344, 783–792 (2001). The first publication to demonstrate the efficacy of trastuzumab + chemotherapy in HER2-overexpressing metastatic breast cancer.

259. Baselga, J. Phase I and II clinical trials of trastuzumab. Ann. Oncol. 12, S49–S55 (2001).

260. Payne, S. J., Bowen, R. L., Jones, J. L. & Wells, C. A. Predictive markers in breast cancer—the present. Histopathology 52, 82–90 (2008).

261. Chia, S.-H. et al. Human epidermal growth factor receptor 2 overexpression as a prognostic factor in a large tissue microarray series of node-negative breast cancers. J. Clin. Oncol. 26, 5697–5704 (2008).

262. Fritzsche, F. R. et al. Tissue pretreatment with formic acid might lower HercepTest scores in breast cancer. Diagn. Mol. Pathol. 15, 237–242 (2006).

263. Siddiqua, S. & Rimm, D. L. Pre-analytic variables and phoshopho-specific antibodies: the Achilles heel of immunohistochemistry. Breast Cancer Res. 12, 110 (2010).

264. Press, M. F. et al. Diagnostic evaluation of HER2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin. Cancer Res. 11, 6598–6607 (2005).

265. Schnitt, S. J. & Jacobs, T. W. Current status of HER2 testing: caught between a rock and a hard place. Am. J. Clin. Pathol. 116, 806–810 (2001).

266. Choritz, H., Busche, G. & Kreppe, H. Quality assessment of HER2 testing by monitoring of positivity rates. Virchows Arch. 459, 283–289 (2011).

267. Yeh, I.-T. et al. Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event. Mod. Pathol. 22, 1169–1175 (2009).

268. Tse, C.-H. et al. Determining true HER2 gene status in breast cancers by polyclonality versus alternative chromosome 17 reference genes: implications for anti-HER2 targeted therapy. Clin. Cancer Res. 29, 4168–4174 (2011).

269. Ballinger, T. J., Sanders, M. E. & Abramson, V. G. Current HER2 testing recommendations and clinical relevance as a predictor of response to targeted therapy. Clin. Breast Cancer 15, 171–180 (2015).

270. Onsum, M. D. et al. Single-cell quantitative HER2 measurement identifies heterogeneity and distinct subgroups within traditionally defined HER2-positive patients. Am. J. Pathol. 183, 1446–1460 (2013).

271. Gu, J., Bowen, R. L., Jones, J. L. & Wells, C. A. Predictive markers in breast cancer: the present. Histopathology 52, 82–90 (2008).

272. Fritzsche, F. R. et al. Tissue pretreatment with formic acid might lower HercepTest scores in breast cancer. Diagn. Mol. Pathol. 15, 237–242 (2006).

273. Siddiqua, S. & Rimm, D. L. Pre-analytic variables and phospho-specific antibodies: the Achilles heel of immunohistochemistry. Breast Cancer Res. 12, 110 (2010).

274. Press, M. F. et al. Diagnostic evaluation of HER2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin. Cancer Res. 11, 6598–6607 (2005).

275. Schnitt, S. J. & Jacobs, T. W. Current status of HER2 testing: caught between a rock and a hard place. Am. J. Clin. Pathol. 116, 806–810 (2001).

276. Choritz, H., Busche, G. & Kreppe, H. Quality assessment of HER2 testing by monitoring of positivity rates. Virchows Arch. 459, 283–289 (2011).

277. Yeh, I.-T. et al. Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event. Mod. Pathol. 22, 1169–1175 (2009).

278. Tse, C.-H. et al. Determining true HER2 gene status in breast cancers by polyclonality versus alternative chromosome 17 reference genes: implications for anti-HER2 targeted therapy. Clin. Cancer Res. 29, 4168–4174 (2011).

279. Ballinger, T. J., Sanders, M. E. & Abramson, V. G. Current HER2 testing recommendations and clinical relevance as a predictor of response to targeted therapy. Clin. Breast Cancer 15, 171–180 (2015).

280. Onsum, M. D. et al. Single-cell quantitative HER2 measurement identifies heterogeneity and distinct subgroups within traditionally defined HER2-positive patients. Am. J. Pathol. 183, 1446–1460 (2013).

281. Gu, J., Bowen, R. L., Jones, J. L. & Wells, C. A. Predictive markers in breast cancer: the present. Histopathology 52, 82–90 (2008).

282. Fritzsche, F. R. et al. Tissue pretreatment with formic acid might lower HercepTest scores in breast cancer. Diagn. Mol. Pathol. 15, 237–242 (2006).

283. Siddiqua, S. & Rimm, D. L. Pre-analytic variables and phospho-specific antibodies: the Achilles heel of immunohistochemistry. Breast Cancer Res. 12, 110 (2010).

284. Press, M. F. et al. Diagnostic evaluation of HER2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin. Cancer Res. 11, 6598–6607 (2005).

285. Schnitt, S. J. & Jacobs, T. W. Current status of HER2 testing: caught between a rock and a hard place. Am. J. Clin. Pathol. 116, 806–810 (2001).

286. Choritz, H., Busche, G. & Kreppe, H. Quality assessment of HER2 testing by monitoring of positivity rates. Virchows Arch. 459, 283–289 (2011).

287. Yeh, I.-T. et al. Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event. Mod. Pathol. 22, 1169–1175 (2009).

288. Tse, C.-H. et al. Determining true HER2 gene status in breast cancers by polyclonality versus alternative chromosome 17 reference genes: implications for anti-HER2 targeted therapy. Clin. Cancer Res. 29, 4168–4174 (2011).

289. Ballinger, T. J., Sanders, M. E. & Abramson, V. G. Current HER2 testing recommendations and clinical relevance as a predictor of response to targeted therapy. Clin. Breast Cancer 15, 171–180 (2015).

290. Onsum, M. D. et al. Single-cell quantitative HER2 measurement identifies heterogeneity and distinct subgroups within traditionally defined HER2-positive patients. Am. J. Pathol. 183, 1446–1460 (2013).

Additional information

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Byondis B. V. FDA Approves Byondis’ Biologics License Application for [V-1] Trastuzumab Ducarmazine (SYD985) in HER2-Positive Metastatic Breast Cancer. Cision PR Newswire: https://go.nature.com/3VZlL4a (2022).
Review article

Cohen, S. Epidermal growth factor. Nobel lecture (1986): https://www.nobelprize.org/prizes/medicine/1986/cohen/lecture/.

Innovent Biologics. Innovent Releases Preliminary Results of the Phase Ia Dose-Escalation study of IBI315 (Anti-Her2/PD-1 Bispecific Antibody) in Patients with Advanced Solid Tumors at CSCO Annual Meeting 2021. Cision PR Newswire: https://go.nature.com/3f91toe (2021).

Levi-Montalcini, R. The nerve growth factor: thirty-five years later. Nobel lecture (1986): https://www.nobelprize.org/prizes/medicine/1986/levi-montalcini/lecture/.

US Food and Drug Administration. https://www.fda.gov/drugs/biosimilars/biosimilar-productinformation (2022).

US National Library of Medicine. ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT01042379 (2022).

US National Library of Medicine. ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT01953926 (2022).

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US National Library of Medicine. ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT04778982 (2022).

US National Library of Medicine. ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT04042701 (2022).

US National Library of Medicine. ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT04147819?term=NCT04147819&draw=2&rank=1 (2022).

Varmus, H.E. Retroviruses and oncogenes I. Nobel lecture (1989): https://www.nobelprize.org/prizes/medicine/1989/varmus/lecture/.

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