nature reviews drug discovery

https://doi.org/10.1038/s41573-022-00579-0

Review article

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Targeting HER2-positive breast cancer: advances and future directions

Sandra M. Swain [1] ✉, Mythili Shastry [2] & Erika Hamilton [2,3]

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.

Sections

[1]Department of Medicine, Georgetown Lombardi Comprehensive Cancer Center and MedStar Health, Washington, DC, USA. [2]Sarah Cannon Research Institute, Nashville, TN, USA. [3]Tennessee Oncology, Nashville, TN, USA. ✉e-mail: sandra.swain@georgetown.edu

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 1978 when the first tyrosine kinase, epidermal growth factor receptor (EGFR), was discovered followed by the identification of the *neu* or *HER2* (also known as *ERBB2*) gene in 1984[1,2]. The discovery that amplification or overexpression of HER2 was associated with extremely poor survival in BC ultimately led to the development of a monoclonal antibody (mAb) to HER2, trastuzumab[3] (Box 1).

Until this point, triple-negative and HER2-overexpressing disease were widely regarded as the most aggressive BC histologies, with unfavourable prognoses. Advanced BC was considered incurable, and treatment was purely palliative. However, newer and novel therapeutic strategies have led to markedly improved survival outcomes. The dependence of the tumour on HER2, coupled with effective HER2-targeted drugs such as trastuzumab, pertuzumab and most recently, tucatinib and trastuzumab deruxtecan (T-DXd), have contributed to these survival improvements in patients with HER2-positive (HER2$^+$) BC[4]. Currently, survival rates exceed 90% in HER2$^+$ early breast cancer (EBC) treated with chemotherapy and dual antibody therapy[5]. More than half of patients with metastatic HER2$^+$ disease are diagnosed de novo, further demonstrating that most patients presenting with early disease are cured[6].

However, despite this success, metastatic HER2$^+$ tumours inevitably develop resistance, leading to disease progression. As such, the goal of therapy in HER2$^+$ BC is to expand the number of patients cured in the early setting and prevent recurrence. In those HER2$^+$ cancers that do present with de novo stage IV disease or ultimately recur, development of novel therapies is needed as these tumours continue to be dependent on HER2 signalling. Therefore, extensive research is ongoing in the preclinical, translational and clinical arenas to develop original and more potent therapies for this exceptionally sensitive target, HER2.

Advances in targeting HER2 include further exploitation of antibody–drug conjugates (ADCs), altering the linkers, payload or antibody scaffold to optimize efficacy[7,8]. Another approach is the development of bispecific antibodies, which use binding of two different HER2 epitopes to maximize efficacy[9]. As immunotherapy has shown benefit in triple-negative breast cancer (TNBC), attempts to mobilize the immune system in HER2$^+$ disease are also ongoing. Immunotherapy is being approached from various angles including administering

Box 1

History of HER2 receptor biology

In 1962, Stanley Cohen discovered the protein responsible for incisor and eyelid opening in mice, termed epidermal growth factor (EGF), thus beginning the journey from bench to bedside for HER2-targeted therapy[237,238]. Demonstration that the EGF receptor (EGFR) formed complexes after EGF binding was postulated to be the initial step for cell growth[1]. 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 description and discovery 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/varmus/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 retrovirus[239]. v-*erbB* was discovered to be transforming, hence an oncogene, while v-*erb*A was not[240]. Eventually, erythroblastic leukaemia viral oncogene homologue 2 (v-erbB2) was found to be closely homologous to EGFR[241]. 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 lines[242,243]. 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 EGFR[2,244,245]. The sequence for EGFR published in 1984 established that it was similar to v-*erbB2* (refs.[241,246]).

Sequencing studies revealed that the tyrosine kinase receptor named HER2 had extensive homology to *neu*, and both were located on chromosome 17 (ref.[247]). HER2 and *neu* had different sequences but were closely related to the *EGFR* gene, located on chromosome 7 (ref.[247]). 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.[248,249]). 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 mice[250–252]. This work spearheaded the concept of HER2-targeted therapy.

Ensuing research determined that only gene amplification with resultant overexpression of protein HER2 was needed for cellular transformation[253,254]. 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 prognosis[3,255]. 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, m4D5, generated to p185^{HER2} that decreased cell proliferation, spurred the development of a humanized mAb to HER2, humAb4D5-8, eventually named trastuzumab or Herceptin[256,257] (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)[258,259].

Glossary

Antibody dependent cell-mediated cytotoxicity
(ADCC). An immune mechanism through which Fc receptor-bearing cytotoxic effector cells can recognize and kill antibody-coated target cells that express tumour- or pathogen-derived antigens on their surface.

Antibody-dependent cellular phagocytosis
(ADCP). An immunological mechanism of elimination whereby tumour cells are targeted with monoclonal antibodies to promote their clearance from the body by phagocytic immune cells such as macrophages.

Biosimilar
A biologic product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product

Complement-mediated cytotoxicity
(CDC). The mechanism by which antibody-coated target cells recruit and activate components of the complement cascade, leading to the formation of a membrane attack complex (MAC) on the cell surface and subsequent cell lysis.

De novo stage IV disease
Cancer that is already metastatic or stage IV at the time of diagnosis

Epitope
A portion of a protein or antigen that is recognized by the immune system (specifically by antibodies).

Fc domain
The fragment crystallizable or the constant fragment region of the antibody, which is the tail region of the antibody that interacts with the cell surface receptors (Fc receptors) and some proteins of the complement system. This property enables antibodies to activate the immune system.

Natural killer (NK) cells
A type of immune cell that has granules (small particles) with enzymes that can kill tumour cells or cells infected with a virus. A NK cell is a type of white blood cell.

Proteolysis-targeting chimeras
(PROTACs). Protein degraders that use the cell's own waste disposal machinery to eliminate a target protein. PROTACs can simultaneously bind to a target protein and an E3 ligase protein, which then ubiquitylates the target, marking it for proteasomal degradation.

T cells
A type of white blood cell. T cells or T lymphocytes are part of the immune system and develop from stem cells in the bone marrow. They help protect the body from infection and may help fight cancer.

Theranostics
A combination of the terms therapeutics and diagnostics. It is used to describe the combination of using one radioactive drug to identify (diagnose) and a second radioactive drug to deliver therapy to treat the main tumour and any metastatic tumours.

Tumour-infiltrating lymphocytes
(TILs). A type of immune cell that has moved from the blood into a tumour. TILs can recognize and kill cancer cells.

checkpoint inhibitors, linking effector T cells to HER2 antibodies, cellular therapy and vaccines[9–12].

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[13]. There is no known ligand for HER2, but it is a preferred dimerization partner for the other HER proteins, especially HER3 (ref.[14]). HER2 overexpression or amplification leads to ligand-independent dimerization and abnormal signalling in addition to increased signalling through ligand-dependent heterodimerization[13]. 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^{low}) BC that is responsive to novel HER2-targeted ADCs[17]. HER2^{low} is defined as HER2 IHC 1+ by itself or 2+ in the absence of *HER2* gene amplification by ISH (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^{low} 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)[19–26]. 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 relapse[27]. 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[3,260,261]. 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[262,263]. 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[264,265]. 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[266].

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[267,268]. 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[269].

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[15,16]. 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.[270–273]). 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[274,275].

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[17,276]. 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[277]. 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.

multiple domains in HER2 can exert synergistic antitumour effects[28]. Consequently, a second humanized anti-HER2 mAb, pertuzumab, was developed. Unlike trastuzumab, which binds to ECD IV of HER2, pertuzumab binds to ECD II, preventing HER2 heterodimerization with HER1, HER3 and HER4, blocking downstream tumour signalling[29]. Trastuzumab is more effective at inhibiting cell growth in the absence of HER3 ligand[30,31]. These complementary mechanisms of action and the effect of the two agents on immune system-mediated antitumour activity via ADCC and/or complement-mediated cytotoxicity (CDC), suggested that combination therapy could be synergistic[32–34].

Trials that combined these two mAbs with chemotherapy as treatment for $HER2^+$ BC in the metastatic, adjuvant and neoadjuvant settings demonstrated better outcomes than trastuzumab and chemotherapy combinations, leading to FDA approval of pertuzumab in these settings[5,35–37] (Supplementary Table 1). These trials changed the course of disease for patients with $HER2^+$ BC and improved survival.

The efficacy of trastuzumab, in part, is dependent on ADCC mediated through its Fc domain. Patients whose immune effector cells (natural killer (NK) cells or dendritic cells (DCs)) can bind more tightly to the Fc domain have stronger responses to trastuzumab[38,39]. A novel anti-HER2 IgG1 mAb, margetuximab, has an engineered Fc domain to increase the affinity for the activating Fcγ receptor (CD16A) and to decrease the affinity for the inhibitory Fcγ receptor (CD32B) expressed on immune effector cells. The optimized Fc domain confers enhanced ADCC activity against $HER2^+$ tumours and more potently stimulates ADCC than trastuzumab or pertuzumab in vitro[40]. The FDA granted regulatory approval for margetuximab plus chemotherapy for the treatment of $HER2^+$ MBC on the basis of the results from the

SOPHIA study, adding another drug to the HER2 armamentarium[41] (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[42]. Pertuzumab–trastuzumab–hyaluronidase-zzxf, a subcutaneous formulation of a fixed dose formulation of trastuzumab and pertuzumab 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[43].

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

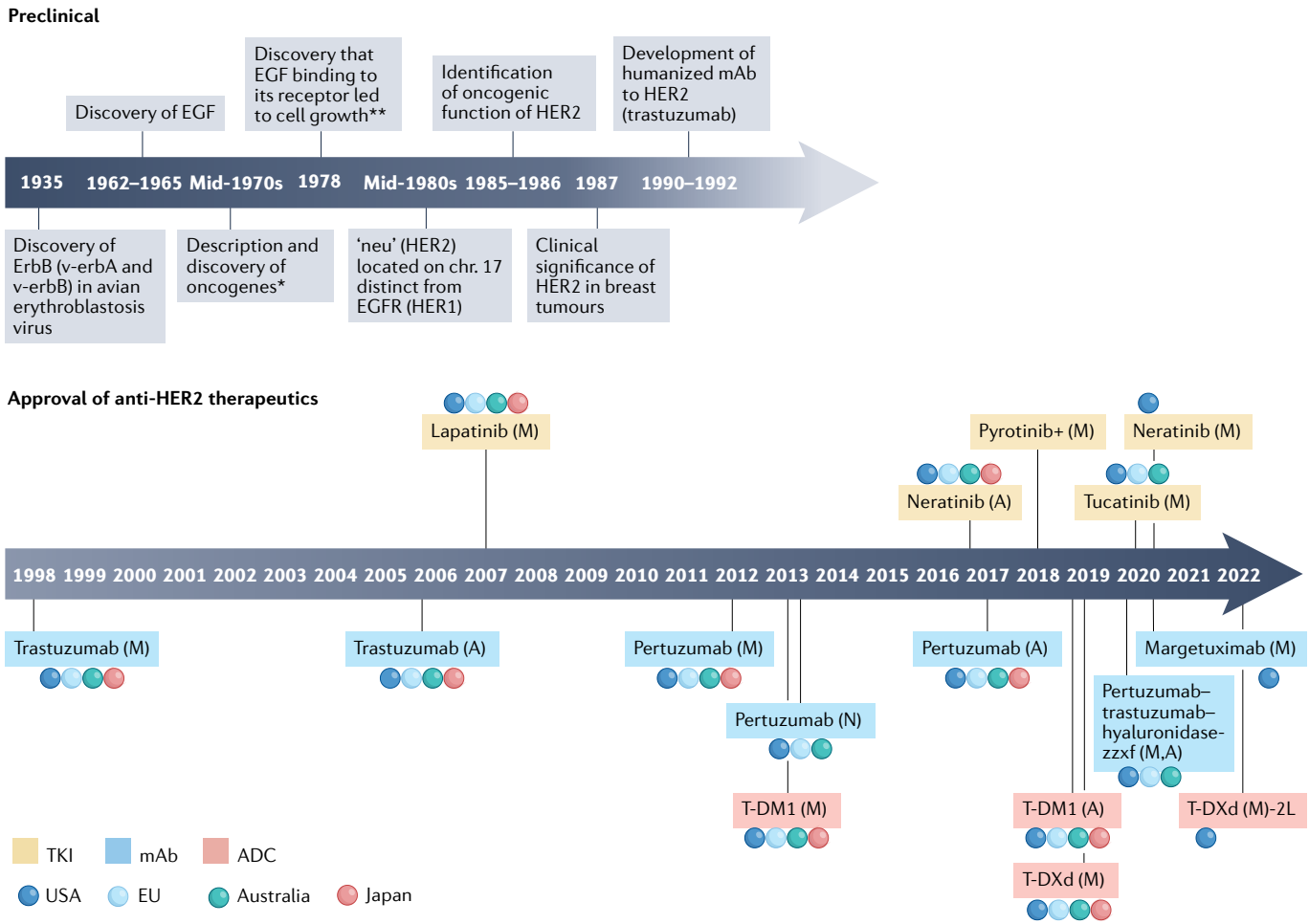

Fig. 1 | Evolution of HER2 as a biomarker and target for treatment for breast cancer. Timeline of preclinical discovery milestones for HER2 biology and regulatory approval for anti-HER2 therapies. A, adjuvant setting; M, metastatic setting; N, neoadjuvant setting; +, approved in China only; *, M. Bishop and H. Varmus awarded Nobel Prize in 1989 for this discovery; **, S. Cohen and R. Levi-Montalcini awarded Nobel Prize in 1986 for discovery of growth factors and their receptors.

Box 3

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 anthracyclines[258,278]. 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 setting[279,280].

- 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 elevations[281].
- Increased rates of cardiotoxicity have not been observed in long-term follow-up[278,282].
- 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)[283,284].

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 diarrhoea[285].
- Starting with a lower dose of neratinib and working up to the 240 mg daily dose over 2 weeks may improve tolerability.
- The higher specificity of tucatinib for HER2 over EGFR has led to less severe gastrointestinal effects, rash and skin toxicity[72].

Liver toxicity. Elevated liver enzymes are commonly reported with ado-trastuzumab emtansine (T-DM1) and tucatinib[72,286,287].

- 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 DM1-induced impairment of megakaryocyte differentiation[288].

- 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 trial[84]. 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 thereafter[289].
- Increased awareness coupled with guidelines for interrupting therapy and prompt treatment improved ILD (no grade 4/5 events and <1% grade 3 events)[84,85,290].

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.

(also known as HER1) and HER2, with activity in HER2-driven tumours that are insensitive to trastuzumab[44]. Lapatinib overcomes trastuzumab resistance mediated by insulin-like growth factor 1 receptor (IGF1R) upregulation by blocking the crosstalk between HER2 and IGF1R[45]. Cell lines and xenografts expressing the p95^{HER2} variant that lack the trastuzumab-binding ECD were susceptible to lapatinib, presumably because it targets the intracellular kinase domain of HER2 (ref.[46]). In vitro studies showed that pTEN-deficient, trastuzumab-resistant HER2$^+$ BC cell lines remained sensitive to lapatinib[47]. These and other data supported the clinical development of lapatinib in trastuzumab-resistant BC[48]. 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 patients[49–51] (Supplementary Table 1). Several trials were conducted with lapatinib in HER2$^+$ EBC, the details of which are succinctly summarized in a recent review[52].

A significant sanctuary for recurrent HER2$^+$ disease is the central nervous system (CNS), and up to 50% of patients with HER2$^+$ BC develop brain metastases[53–55]. Moreover, the blood–tumour barrier (BTB), which evolves from the blood–brain barrier (BBB), regulates drug distribution to brain metastases, posing a clinical challenge[56]. 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 metastases[49,57,58]. 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 trastuzumab[60,61]. A unique feature of neratinib is its activity in cell lines

with somatic *HER2* mutations in the absence of HER2 amplification, suggesting that it can overcome possible resistance to other anti-HER2 therapies[61]. 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[62]. 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[62].

Treatment with neratinib after standard adjuvant trastuzumab improved invasive disease-free survival (iDFS) rates in HER2$^+$ BC, especially in patients with HR$^+$ tumours[63,64] (Supplementary Table 1). Similarly to lapatinib, the neratinib–capecitabine combination improved outcomes in HER2$^+$ MBC, earning a nod from the FDA as third-line therapy[65] (Supplementary Table 1). 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[66] (Supplementary Table 1). Pyrotinib is similar to the other pan-HER2 TKIs, with diarrhoea as its most common toxicity[66]. 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[67]. Tucatinib demonstrated CNS penetration in intracranial xenograft models and was superior to lapatinib in preclinical studies[68–71]. 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[72,73] (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 I-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[74]. 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[75].

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[76,77]. 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[78–80] (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[81].

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)[82]. 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 its 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)[82].

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[83–86] (Fig. 1 and Supplementary Table 1). T-DXd has also shown encouraging activity in BC brain metastases, in DESTINY-Breast01 (ref.[87]). DEBBRAH is evaluating T-DXd in patients with HER2$^+$ or HER2^{low} MBC with brain metastases and/or leptomeningeal metastases; preliminary data are promising[88].

A unique feature of T-DXd is its ability to target HER2^{low} MBC as evidenced by activity in this subset of patients in a phase I trial[83]. 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^{low} MBC[17]. 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 HER2^{low} 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[89]. Interestingly, a high percentage of HER2 IHC 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 p95^{HER2}, 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.

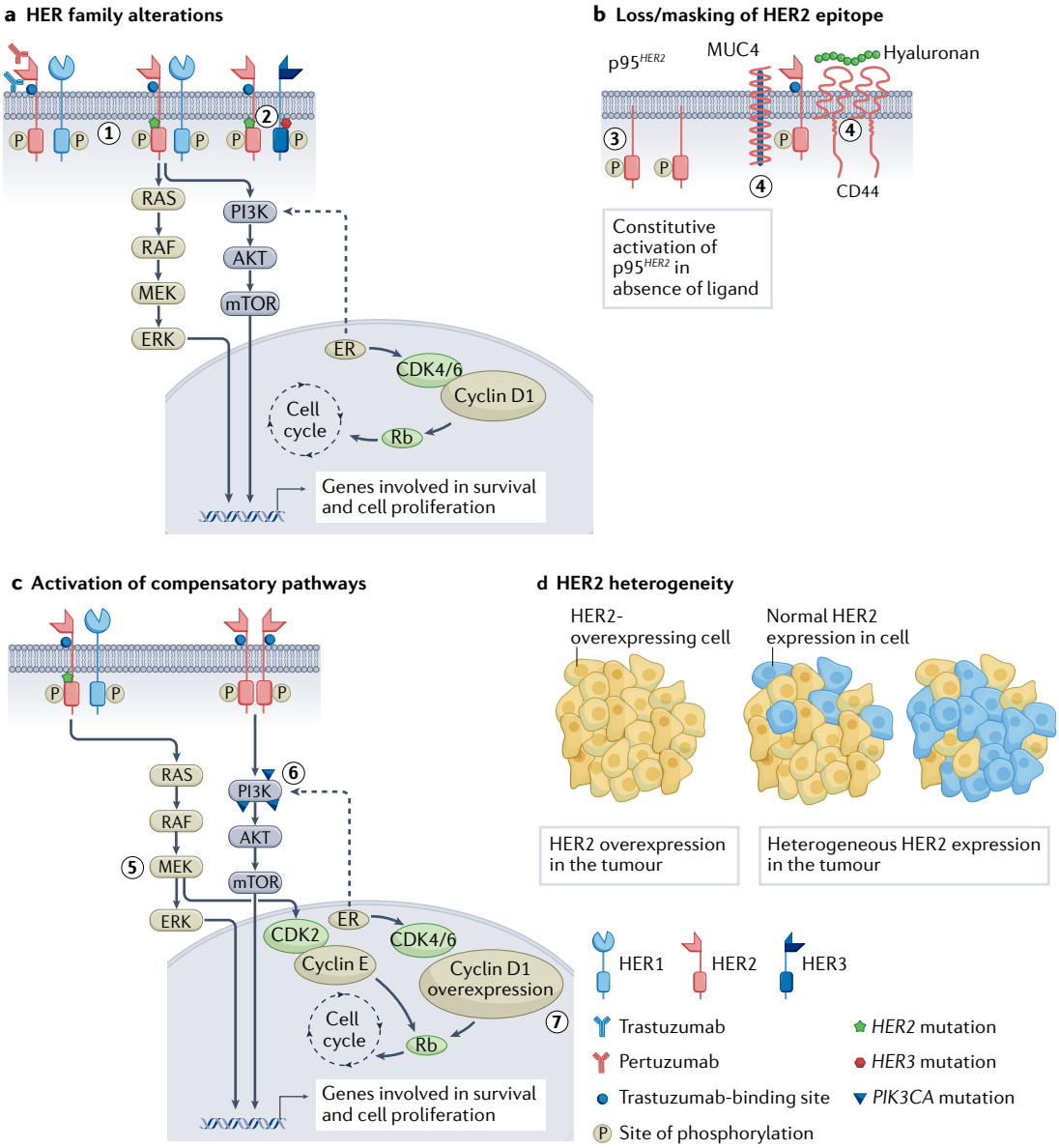

Fig. 2 | Select mechanisms of HER2-targeted resistance. a, Mutations and/or alterations in the HER family of receptors that lead to activation of downstream signalling pathways. (1) Mutations in *HER2* leading to P13K–AKT and RAS–MAPK pathway activation. (2) Co-occuring mutations in *HER2* and *HER3* leading to PI3K–AKT pathway activation. **b**, Loss of HER2 extracellular domain in cells overexpressing p95^{HER2} receptor. Masking of the trastuzumab-binding site on HER2 owing to overexpression of mucin 4 (MUC4) and CD44–polymeric hyaluronan complex. (3) p95^{HER2} 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 P13K–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[90,91]. 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[92]. Neratinib was shown to be active against HER2 mutant/HER2-non-amplified MBC in the SUMMIT basket trial[93].

HER2 mutations frequently co-occur with *HER3* mutations, and cancers with both of these mutations respond poorly to neratinib. The *HER3*^{E928G} kinase domain mutation has been shown to enhance the affinity of HER2/HER3 and reduce binding of HER2 to neratinib[94]. 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 HER signalling seen in lapatinib- and neratinib-resistant models[95]. 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[96].

Loss or masking of HER2 epitope

The presence of p95^{HER2} has been associated with poor outcomes in patients with HER2$^+$ EBC, and patients with MBC overexpressing p95^{HER2} had lower response rates to trastuzumab than those expressing full-length HER2 (ref.[97]). Chemotherapy sensitized p95^{HER2}/611CTF xenografts to trastuzumab, presumably via HER2 stabilization induced by chemotherapy[98]. Lapatinib inhibited p95^{HER2} phosphorylation in cell lines, reducing downstream activation of AKT and MAPK and inhibiting cell growth[46]. Retrospective analyses from clinical trials with lapatinib noted that the presence of p95^{HER2} had no influence on lapatinib efficacy[99]. Similarly, in the CHER-LOB neoadjuvant study, which randomized patients to receive trastuzumab, lapatinib or their combination, p95^{HER2} expression was not predictive of outcome nor did it predict for sensitivity to either anti-HER2 agent[100]. Therefore, the role of p95^{HER2} 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[101]. Soluble TNF upregulated MUC4 expression, resulting in trastuzumab resistance, and combining a soluble TNF inhibitor with trastuzumab prevented tumour growth in preclinical models[102]. 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[103]. 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 BCs 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[104,105]. Loss of PTEN (a key tumour suppressor), which leads to hyperactivation of the PI3K pathway, has also been observed in trastuzumab-resistant tumours[106,107]. 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[108]. 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[109]. 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[110,111]. Clinical evidence also shows that ER pathway activation offers 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[51,112–114]. The cyclin D1–CDK4/6 axis also has a role in resistance to anti-HER2 therapies[115]. CDK4/6 inhibitors appear to be synergistic with trastuzumab and/or lapatinib in inhibiting growth of HER2$^+$ cell lines[116,117]. This was evidenced in the clinical setting in which dual inhibition of CDK4/6 and HER2 led to improved outcomes in monarcHER and PATRICIA trials in heavily pretreated HR$^+$/HER2$^+$ MBC[118,119]. 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[117]. In the Na-PHER2 study, trastuzumab and pertuzumab given with fulvestrant and palbociclib neoadjuvantly 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)[120]. These data suggest that a combination blocking HER2 and ER and CDK4/6–cyclin D1 activation offers a chemotherapy-free alternative for treatment of HR$^+$/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[121]. These mutations, including *NF1* and *HER2* activating mutations, contribute to resistance to tucatinib 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[122]. 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 $HER2^{low}$ 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[17].

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 antitumour immunity, and their activity is regulated by careful modulation of inhibitory and activating receptor signalling[123]. 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 binding to the NK cell receptor KIR2DL4, and blocking this HLA-G–KIR2DL4 signalling made $HER2^+$ BC susceptible to trastuzumab treatment in vivo[123]. 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 PDL1/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[124]. 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[125]. 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[124]. 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[126]. 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[127].

Three novel markers, RAC1, CDK12 and VTCN1, have been found to correlate with response to lapatinib, neratinib and tucatinib 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[128]. Prior data have implicated these genes in resistance to anti-HER2 therapies or immunotherapy[129–131]. 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[132]. 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[133]. 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 (elgemtumab). Although these drugs have shown some promising preliminary 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[133–139]. Now, there is greater focus on novel ADCs that target HER2 or HER3. Additionally, bispecific antibodies that target multiple epitopes of HER2, or HER2 and HER3 together in one molecule, are under clinical investigation and are discussed in the next section.

Antibody–drug conjugates

ADCs have successfully combined the antitumoural features of cytotoxics and HER2 antibodies into a single pharmacological entity that has greater efficacy than the sum of its parts[7,8]. 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

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 (Synthon/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 Manich et al.[144]
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.[216]
A166 (Kluss Pharma/ Sichuan Kelun-Biotech Biopharmaceutical Co. Ltd)	Cleavable	Duo-5	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.[217]
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.[218]
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.[219]
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.[220]
FS-1502/IKS014 (Shanghai Fosun Pharmaceutical Industrial Development Co, Ltd)	Unknown	MMAE	Microtubule inhibitor	NR	NCT03944499 (phase I)	Not applicable	Fasching[221]
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.[222]

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.

feature enables activity against tumours with low or heterogeneous target expression, thus expanding the pool of susceptible tumour cells. Furthermore, the ADCC of the Fc fragment of the antibody may also contribute to antitumour efficacy. HER2 ADCs can also retain trastuzumab-mediated activity such as inhibiting the HER2 dimerization and suppression of downstream signalling. There are encouraging data against HER2$^+$ brain metastases with these HER2 ADCs[81,87,140]. Coupled with their demonstrated activity in HER2^{low} MBC, further evaluation of these ADCs is warranted in this high-risk patient population[17].

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 reviewed[141]. Several ADCs currently in development for HER2$^+$ BC are listed in Table 1, and some of these are discussed in further detail below.

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 expression[142]. 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 PFS[143,144]. 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 stability[145]. 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-DM1-resistant BCs, and HER2^{low} tumours[145]. 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 links)[146].

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 trastuzumab[147].

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.[223]
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.[224]
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.[154]
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.[155]

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.

The linker in disitamab vedotin uses random coupling of cysteine, which is more homogeneous than lysine. Furthermore, this agent has better endocytosis, which is independent of V-ATPase activity, and has no lysosomal resistance[147]. Preclinical data demonstrated good activity in HER2-overexpressing cancer cells and a robust bystander effect targeting neighbouring tumour cells[147]. 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 cancer[147,148].

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 biparatopic antibody of ZW49 demonstrated lysosomal trafficking and superior internalization relative to a HER2-targeted monospecific ADC[149]. Preclinical data indicated efficacy in HER2^{low}- and HER2^{high}-expressing models and also in a brain metastasis model[149]. ZW49 is being evaluated in a phase I trial in patients with metastatic HER2-expressing cancers that have progressed following standard therapies, including HER2-targeted agents (NCT03821233; see Related links).

ALTA-ADC. In an effort to improve the potency of HER2-targeting ADCs, a pertuzumab-based ADC with lower affinity for HER2 at acidic endosomal pH was developed[150]. 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-DM1. 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 death[151]. 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 tumours[151]. 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 death[152]. The synthetic lethal effect of the combination of TTCs with other DNA-damaging agents was demonstrated using colorectal cancer xenografts[153]. 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 events[154]. 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. BDTX-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 toxicity[155]. BDTX-189 demonstrated potent, sustained inactivation of multiple allosteric ERBB mutants in vivo. A phase I trial in advanced solid tumours harbouring specific allosteric *HER2* or *HER3* mutations or other EGFR/HER2 alterations is ongoing. Early data show activity with BDTX-189 in HER2-amplified tumours and in patients with non-small-cell lung cancer (NSCLC) with *EGFR* and *HER2* exon 20 insertions[155].

In general, it appears that the focus of newer HER2 TKIs has shifted from HER2$^+$ BC to solid tumours harbouring *HER2* point mutations or

alterations given that the frequency of these alterations varies from 1% to 2% in BC and up to 5–10% in other cancers (for example, stomach and bladder cancers)[156]. Preliminary activity of poziotinib in *HER2* exon 20 mutant NSCLC has been reported, and there is an ongoing basket trial with neratinib in solid tumours with *HER2* mutations (NCT01953926; see Related links)[157].

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[158]. 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[159,160]. 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 $HER2^{high}$ and $HER2^{low}$ 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 ADCC. 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[161]. 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.[162]). 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 blockade, 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[163]. 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 heterobifunctional 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[164]. 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[165]. 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[165]. 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 (AnDC). 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 (SMol006) to HER2-expressing tumours via antibody targeting (pertuzumab). Once the antibody and degrader enter the $HER2^+$ tumour cell by endocytosis, the antibody is degraded in the lysosome, releasing the free degrader, which binds to GSPT1 in the cytosol. The natural protein degradation 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[166].

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

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

Therapeutic agent (institution/company)	Tumour-associated antigen	Immune target	Phase/clinical trial ID	Reference
In vitro data only				
HER2/CD3 BsAb (Memorial Sloan Kettering Cancer Center)	HER2	CD3	Not applicable	Lopez-Albaitero et al.[225]
M-802 (Huazhong University of Science and Technology)	HER2	CD3	Not applicable	Yu et al.[176]
p95HER2×CD3 BsAb T cell (Vall d'Hebron Institute of Oncology)	p95^{HER2}	CD3	Not applicable	Ruiz et al.[226]
Tribody [(HER2) 2×CD16] (University Hospital Schleswig-Holstein, Christian-Albrechts University)	HER2	CD16	Not applicable	Oberg et al.[227]
BsPDL1×rErbB2 (QIMR Berghofer Medical Research Institute)	HER2	PDL1	Not applicable	Mittal et al.[228]
In clinical trials				
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 (cinrebafusp alfa) (Pieris Pharmaceuticals, Inc.)	HER2	CD137	Phase I/NCT03650348	Ku et al.[180]
SAR-443216 (Sanofi)	HER2	CD3/CD28	Phase I/NCT05013554	Sha et al.[229]
BDC-1001 (Bolt Biotherapeutics, Inc.)	HER2	TLR7/8	Phase I/NCT04278144	Sharma et al.[230]
NJH395 (Novartis Pharmaceuticals)	HER2	TLR7	Phase I/NCT03696771	Janku et al.[231]
SBT6050 (Silverback Therapeutics)	HER2	TLR8	Phase I/NCT04460456 Phase I/II/NCT05091528	Klemper et al.[232]
HER2Bi–aATCs/HER2BATs (University of Virginia)	HER2	CD3$^+$ activated T cells	Phase I/II/NCT03272334	Lum et al.[181]
TAC01-HER2 (Triumvira Immunologics, Inc.)	HER2	CD3 and CD4 co-receptor domain	Phase I/II/NCT04727151	NCT04727151
DF-1001 (Dragonfly Therapeutics)	HER2	NK cells	Phase I/II/NCT04143711	NCT04143711
ACE1702 (Acepodia Biotech, Inc.)	HER2	NK cells	Phase 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 Ib/NCT04029922	Wainberg et al.[184]

ATC, activated T cell; BsAb, bispecific antibody; CAR-T cell, chimeric antigen receptor–T cell; NK, natural killer; TLR, Toll-like receptor.

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 system[11]. Furthermore, the immune-mediated ADCC mechanism of trastuzumab and pertuzumab suggests that combination immunotherapies may be effective[167,168]. 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 alone[171]. 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-DM1 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$^+$ MBC[173]. 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

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 Fcγ 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 runimotamab (BTRC4017A) 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, CD137, NKG2A) in combination with HER2-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[177]. 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[178,179].

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/3f91toe). 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 (cinrebafusp 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-5111 (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-5111 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-5111 in

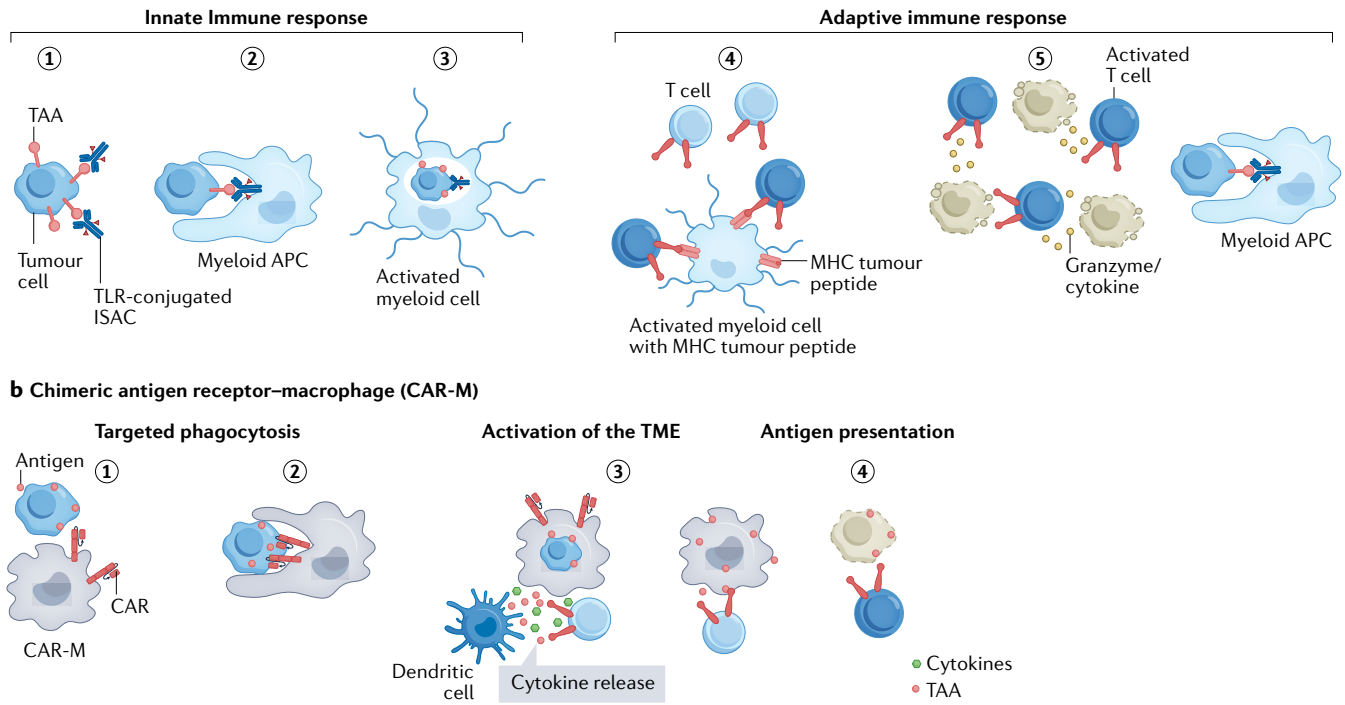
a Immune stimulating antibody conjugate
Innate Immune response
Adaptive immune response
TAA
Tumour cell
TLR-conjugated ISAC
Myeloid APC
Activated myeloid cell
T cell
MHC tumour peptide
Activated myeloid cell with MHC tumour peptide
Activated T cell
Granzyme/ cytokine
Myeloid APC
b Chimeric antigen receptor–macrophage (CAR-M)
Targeted phagocytosis
Activation of the TME
Antigen presentation
Antigen
CAR
CAR-M
Dendritic cell
Cytokine release
Cytokines
TAA
c Radiation + checkpoint inhibitor
CTLA4
CTLA4 antibody
Radiotherapy
CXCL16 (chemokine)
Tumour lysis
d Engineered toxin body
HER2 on tumour cell
Endosome
Cytosol
Golgi body
ER
Nucleus
Engineered toxin body:
De-immunized Shiga-like toxin
HER2-targeted scFV

Fig. 3 | Immune strategies that target HER2$^+$ MBC. a, Immune-stimulating antibody conjugate (ISAC). (1) ISAC binds to cognate tumour-associated antigen (TAA). (2) Fc receptor-dependent phagocytosis of the tumour cell by myeloid antigen-presenting cell (APC). (3) Toll-like receptor (TLR)-mediated activation of the myeloid cells leads to chemokine and/or cytokine secretion and enhanced antigen presentation. (4) T cell priming by expression of major histocompatibility complex (MHC)–tumour peptide on myeloid cells and expansion of activated T cells. (5) Chemokines attract immune effector cells. Increased myeloid APC phagocytosis. Migration of activated T cells to the tumour and killing of tumour cells. **b**, Chimeric antigen receptor–macrophage (CAR-M). (1) Targeting of CAR-M to tumour cell expressing the antigen leads to its activation. (2) Phagocytosis of the tumour cell by CAR-M. (3) CAR-M activates the tumour microenvironment (TME) and primes T cells. (4) Primed T cells induce antitumour immune response. **c**, Radiation plus checkpoint inhibitor. (1) Exposure to radiation results in release of chemokines from tumour cells. (2) Migration of CXC chemokine receptor type 6 (CXCR6)-expressing T cells attracted by chemokines to tumour. (3) Addition of anti-CTLA4 antibody helps to neutralize the CTLA4-mediated inhibition of T cell activation. Activated T cells cause tumour cell lysis. **d**, (1) HER2-engineered toxin body (ETB) binds to HER2 receptor, followed by (2) forced internalization, (3) intracellular self-routing and (4) ribosome inactivation.

HER2-expressing solid tumours showed stable disease as best response, no DLTs and maximum tolerated dose not yet reached[185]. 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[186] (Fig. 3). A single infusion of these CAR-Ms was able to shrink tumours and improve OS in solid tumour xenograft mouse models[10]. 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-0508 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[187]. 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[188]. 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[188]. 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 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[189]. 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 allogenic 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[12,190]. 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[191].

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[192]. 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 hence 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 HER2 ICD-specific T cell immunity[193]. 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 trastuzumab-refractory HER2$^+$ MBC demonstrated anti-HER2 antibody induction in all patients. HER2-specific T cells were detected in one patient, and importantly, there were no signals of cardiotoxicity[194].

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[195] (Table 4). One disadvantage of autologous vaccines is variability in the vaccine and a lengthy manufacturing process.

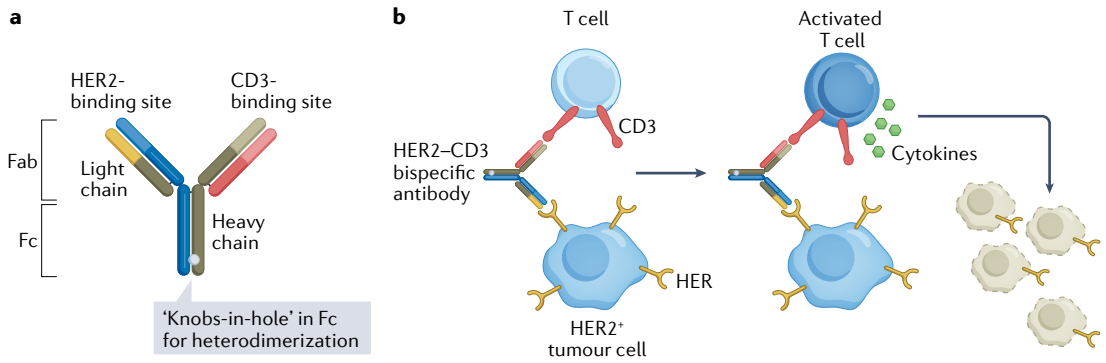

Fig. 4 | Structure and mechanism of action of a HER2–CD3 bispecific antibody. a, Structure of HER2–CD3 bispecific antibody with 'knobs-in-hole' technology. **b**, Mechanism of action of a HER2–CD3 bispecific antibody. Step 1: binding of HER2–CD3 bispecific antibody to HER2 on tumour cell and CD3 on T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

Dendritic cells. DCs are potent modulators of the immune response and are specialized APCs that can stimulate naive 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[196–199]. 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$^+$ EBC 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[200]. 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[201]. 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[202,203]. Novel delivery vehicles such as lipid nanoparticles, virus-like particles, polymeric and non-degradable particles are being explored for cancer vaccines[191,204]. 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[205,206]. 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, which is maintained even after multiple lines of treatment. The huge interest in further development and drug discovery for this focused group of BCs is evidenced by the 1,922 clinical trials for HER2$^+$ BC currently listed in Clinical Trials.gov (see Related links: https://clinicaltrials.gov/ct2/results?cond=HER2-positive+Breast+Cancer&term=&cntry=&state=&city=&dist=). This success and continued enthusiasm truly represent an enormous effort and cooperation between many basic and clinical scientists to improve the lives of patients. The bridging of drug development from academia to industry and vice versa is a crucial element of the process.

Several pathways have been successfully targeted, and there are various mechanisms of resistance that can be explored to potentially cure HER2$^+$ MBC. The recent update of the hallmarks of cancer adding 'enabling characteristics' serves to remind us of the constant discoveries in the area of cancer research and the dynamics of thought regarding the carcinogenic process[207]. There are many fascinating fields of research that are being explored, such as harnessing the microbiome and continued exploration of the role of the gut in immunotherapy as one example[208]. In addition, many tools have been discovered and flourished to enhance drug development, such as artificial intelligence, CRISPR–Cas9, single-cell sequencing, spatial transcriptomics, spatial proteomics and theranostics; the list keeps growing[209–215]. Recent drug constructs such as the ADCs and upcoming bispecifics will continue to improve our ability to safely target HER2$^+$ cells, while

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	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 (GP2 + GM-CSF)	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 cosynthesized 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.[235]
HER2$^+$, GM-CSF secreting vaccine (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins)	Allogeneic, HER2$^+$ GM-CSF-secreting breast tumour vaccine	NCT00093834	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.[196]
DC1 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.[198]
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.[236]
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

Table 4 (continued) | Vaccines targeting HER2⁺ breast cancer

Name of vaccine (institution/company)	Description	Clinical trial ID	Trial design/patient population	Published clinical trial data	Reference
DNA-based vaccines (continued)					
WOKVAC (H. Lee Moffitt Cancer Center and Research Institute)	Plasmid-based DNA with 3 epitopes: IGFBP2, HER2 and IGF1R	NCT03384914	Phase II: WOKVAC vs DC1 vaccine in HER2⁺ stage I–III BC with residual disease post NAC	Not applicable	NCT03384914
Virus-based vaccines					
VRP-HER2 (Duke University)	Alphaviral vector encoding the ECD and transmembrane domains of HER2 (VRP-HER2)	NCT01526473	Phase I: VRP-HER2 monotherapy or combination with anti-HER2 therapy in HER2⁺ MBC	One PR and two SDs reported; HER2-specific CD8⁺ T cells detected; median OS 50.2 mo with vaccine only and 32.7 mo with combination	Crosby et al.[201]
VRP-HER2 (Duke University)	Alphaviral vector encoding the ECD and transmembrane domains of HER2 (VRP-HER2)	NCT03632941	Phase II: VRP-HER2±pembrolizumab vs pembrolizumab alone in HER2⁺ MBC	Not applicable	NCT03632941

BC, breast cancer; CMV, cytomegalovirus; DC, dendritic cell; DCIS, ductal carcinoma in situ; DFS, disease-free survival; ECD, extracellular domain; GM-CSF, granulocyte–macrophage colony-stimulating factor; IBC, inflammatory breast cancer; ICD, intracellular domain; IGF1R, insulin-like growth factor 1 receptor; IGFBP2, insulin-like growth factor-binding protein 2; MBC, metastatic breast cancer; MHC, major histocompatibility complex; mo, months; NAC, neoadjuvant chemotherapy; ORR, objective response rate; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival; PR, partial response; SD, stable disease; T-DM1, ado-trastuzumab emtansine.

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.

Published online: 7 November 2022

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Acknowledgements
The authors thank C. Gilmore, of Phillips Gilmore Oncology Communications, Inc., for editorial assistance with manuscript preparation. Financial support was provided by Breast Cancer Research Foundation.

Author contributions
All authors contributed to the design, research and writing of the manuscript.

Competing interests
M.S. reports no conflict of interest. E.H.'s institution has received research funding from the following: AbbVie, Acerta Pharma, Accutar Biotechnology, ADC Therapeutics, AKESOBIO Australia, Amgen, Aravive, ArQule, Artios, AtlasMedx, Bliss BioPharmaceuticals, Cascadian Therapeutics, Clovis, Compugen, Cullen-Florentine, Curis, Dana Farber Cancer Institute, Duality Biologics, eFFECTOR Therapeutics, Ellipses Pharma, Elucida Oncology, EMD Serono, Fochon, FujiFilm, G1 Therapeutics, H3 Biomedicine, Harpoon, Hutchinson MediPharma, Immunogen, Immunomedics, Incyte, Infinity Pharmaceuticals, InventisBio, Jacobio, Karyopharm, Leap Therapeutics, Lycera, Mabspace Biosciences, Macrogenics, MedImmune, Merus, Millennium, Molecular Templates, Myraid Genetic Laboratories, Nucana, Olema, OncoMed, Onconova Therapeutics, ORIC Pharmaceuticals, Orinove, PharmaMar, Pieris Pharmaceuticals, Pionyr Immunotherapeutics, Plexxikon, Radius Health, Regeneron, Repertoire Immune Medicine, Rgenix, Sermonix Pharmaceuticals, Shattuck Labs, StemCentRx, Sutro, Syndax, Syros, Taiho, TapImmune, Tesaro, Tolmar, Torque Therapeutics, Treadwell Therapeutics, Verastem, Vincerx Pharma, Zenith Epigenetics and Zymeworks. E.H.'s institution has received consulting fees from the following: Arcus, Eisai, Greenwich Lifesciences, H3 Biomedicine, iTeos, Janssen, Loxo, Orum Therapeutics, Propella Therapeutics and Puma Biotechnology; and her institution has received research funding and consulting fees from the following: Arvinas, Black Diamond, Boehringer Ingelheim, CytomX, Dantari, Deciphera, Lilly, Merck, Mersana, Novartis, Pfizer, Relay Therapeutics, Roche/Genentech, SeaGen, Silverback. S.M.S. reports grants or contracts from Genentech/Roche, Kailos, Genetics, BCRF; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Genentech/Roche, Daiichi Sankyo; support for attending meetings and/or travel from Genentech/Roche (travel 11/2019), Daiichi Sankyo (travel 9/2022) and Sanofi (travel 9/2022); participation on a Data Safety Monitoring Board for AstraZeneca; participation in an advisory board for AstraZeneca, Daiichi Sankyo, Exact Sciences, Biotheranostics, Natera, Merck, Silverback Therapeutics, Athenex, Lilly, Aventis; and participation in a Scientific Advisory Board for Inivata. S.M.S. reports leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid: NSABP Vice Chairman; CCF, ASCO Director; and third party writing support from Genentech/Roche and AstraZeneca.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41573-022-00579-0.

Correspondence should be addressed to Sandra M. Swain.

Peer review information *Nature Reviews Drug Discovery* thanks Jamuna Veeraraghavan, who co-reviewed with Rachel Schiff, Alvin Wong and the other, anonymous reviewer for their contribution to the peer review of this work.

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