Evolving novel anti-HER2 strategies

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The approval of trastuzumab for use in metastatic breast cancer marked a breakthrough in the understanding of the biology of the disease. However, like most cancer therapies, the disease finds a way to advance despite the treatments developed to eradicate it. Although trastuzumab has had a large effect on the treatment of early and advanced-stage disease, a substantial proportion of patients with HER2-positive breast cancer still progress after receiving the drug. Potential mechanisms of resistance to trastuzumab include bypass mechanisms, mutations of the HER2 target, masking of HER2 proteins, inhibition of insulin-like growth factor, and phosphatase and tensin homologue (PTEN) deficiency. Many therapies are being developed to target these mechanisms in patients with HER2-positive, trastuzumab-resistant breast cancer. Additionally, treatment strategies other than trastuzumab with unique mechanisms of action are being assessed in this specific group of patients. In this review, we discuss the emerging data assessing therapeutic approaches in the management of trastuzumab-resistant HER2-positive disease.

Introduction

Targeted therapy has been used for more than 100 years in the treatment of breast cancer. In 1896, Beatson reported a treatment response after oophorectomy in a premenopausal patient. After ovarian ablation, other targeted agents were developed, such as tamoxifen and aromatase inhibitors. Trastuzumab, a monoclonal antibody targeting the HER2 protein, was introduced in 1998. Around 25% of patients with breast cancer have HER2-positive disease, with positivity assessed by immunohistochemistry, which detects overexpression of the HER2 protein, or fluorescence in-situ hybridisation, which detects amplification of the HER2 gene. Patients with 3+ staining from immunohistochemistry (from a HER2 which detects amplification of the gene. Patients the HER2 protein, or fluorescence in-situ hybridisation, immunohistochemistry, which detects overexpression of 1998. Around 25% of patients with breast cancer have 25% of patients with HER2-positive disease, which positivity assessed by immunohistochemistry, which detects overexpression of the HER2 protein, or fluorescence in-situ hybridisation, which detects amplification of the HER2 gene. Patients with 3+ staining from immunohistochemistry (from a possible 1+, 2+, or 3+) and patients with a positive result from in-situ hybridisation are considered to benefit most from trastuzumab. Patients with HER2-positive disease have a higher risk of recurrence and death than those with HER2-negative disease. The approval of trastuzumab broadened the scope of targeted therapy and marked the first of many steps toward improved understanding of the biology of breast cancer.

Mechanism of action of trastuzumab

Trastuzumab is a recombinant, humanised monoclonal antibody directed against the extracellular domain of the HER2 protein, which is expressed on the surface of epithelial cells in many healthy tissues, including the breast. Trastuzumab’s mechanisms of action are numerous and complex (figure 1). One mechanism of action is via antibody-dependent cellular cytotoxicity; the activation of natural killer cells initiates lysis of cancer cells that are bound to trastuzumab. Trastuzumab also inhibits the formation of p95, a truncated membrane-bound fragment that results from cleavage of the extracellular domain of HER2 and has in-vitro kinase activity. Additionally, trastuzumab inhibits the phosphoinositide 3-kinase (PI3K) pathway, which is activated by overexpression of HER2. PI3K causes translocation of AKT, resulting in its phosphorylation and activation. Once activated, AKT can phosphorylate many sites, leading to cell proliferation. Activated AKT is negatively regulated by phosphatase and tensin homologue (PTEN). Trastuzumab inhibits the PI3K pathway, reducing PTEN phosphorylation and AKT dephosphorylation, therefore increasing cell death. Preclinical studies identified another mechanism of action as the reduction of microvessel density, normalisation of vasculature, or both, which improved tumour response; this occurred only in response to combinations of trastuzumab and chemotherapy.

Trastuzumab’s many mechanisms of action give rise to various mechanisms of resistance (table 1). Although trastuzumab targets HER2, cross-talk among the other extracellular HER proteins (HER1 and HER3) can result in incomplete inhibition and lateral activation, promoting

Figure 1: Mechanism of action of current therapies for HER2-expressing breast cancer

Constitutively active HER2 receptors on the surface of HER2-expressing breast-cancer cells dimerise with other HER receptors, activating downstream signalling pathways that mediate tumorigenic cell proliferation, survival, and invasion. Trastuzumab prevents constitutive activation of HER2, induces internalisation and degradation of the protein, and stimulates the immune system to recognise HER2-overexpressing cells. Lapatinib binds to HER2 and HER1 and inhibits tumorigenic receptor signalling.
trastuzumab and lapatinib—mechanisms of action and of resistance

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<td>PI3K-phosphoinositide 3-kinase. PTEN-phosphatase and tensin homologue. TKI=tyrosine-kinase inhibitor.</td>
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Table 1: Trastuzumab and lapatinib—mechanisms of action and of resistance

cellular proliferation—ie, the bypass mechanism of resistance.10 Development of a mutation in the HER2 target, leading to trastuzumab failure, is another mode of resistance.11

Levels of MUC-4, a membrane-associated mucin, are increased in trastuzumab-resistant cells. MUC-4 can mask the membrane proteins, thus decreasing trastuzumab's ability to bind to the appropriate target.7

Early progression of HER2-positive breast cancer has been associated with high expression of insulin-like growth factor-1 receptor, and lower response rates have been reported in PTEN-deficient tumours.4,12,13 These resistance patterns are potential targets for new drug development to overcome trastuzumab resistance.

Downregulation of HER2 expression can occur after treatment with trastuzumab. In the neoadjuvant setting, patients who did not achieve a pathological complete response after trastuzumab were assessed for HER2 status. About a third of the HER2-positive patients who did not achieve a complete response had converted to HER2-negative disease.9 This result highlights an area of research to better understand trastuzumab resistance and the biology of breast cancer in these particular patients.

Strategies for trastuzumab-resistant disease

Role of trastuzumab after initial progression

In retrospective analyses, continuing trastuzumab alone or in combination with other cytostatic drugs is feasible and safe in patients progressing on trastuzumab therapy. Randomised studies are investigating whether continuing trastuzumab therapy after disease progression (in combination with another chemotherapeutic drug) provides better results than stopping trastuzumab.11 After 24 weeks, O’Shaughnessy and colleagues6 found that trastuzumab plus lapatinib significantly improved progression-free survival (12.0 weeks vs 8.4 weeks; p=0.029) and clinical benefit rates (25-2% vs 13-2%, p=0.020) compared with lapatinib alone in patients who had progressed on prior trastuzumab. Response rates and overall survival were similar.6 These data show that different mechanisms of action of trastuzumab, such as antibody-dependent cell-mediated cytotoxicity, can be a target to overcome trastuzumab resistance.9

New drugs for trastuzumab resistance

Lapatinib is the only therapy other than trastuzumab approved for HER2-positive breast cancer. The US Food and Drug Administration approved lapatinib in 2007 for use in patients with HER2-positive metastatic breast cancer, in whom combined anthracycline, taxane, and trastuzumab therapy had failed. Lapatinib is a small-molecule, dual tyrosine-kinase inhibitor (TKI) of HER1 and HER2.2 The drug works by competing with ATP for binding sites on intracellular portions of HER1 and HER2, and targets the downstream ERK1–2 pathway, which regulates cell proliferation, and PI3K–AKT, which regulates cell survival. In preclinical trials, lapatinib did not show cross-resistance with trastuzumab, making it a candidate for studies in trastuzumab-resistant breast cancers.9 Phase 1 data showed responses in heavily pretreated patients with HER1-positive or HER2-positive solid tumours (or both).9,30 Phase 2 trials showed that lapatinib had efficacy and was well tolerated.13 Response rates as high as 38% were seen when lapatinib was given as first-line therapy in metastatic cancers.7

Synergism between lapatinib and capecitabine in early phase 1 trials led to a phase 3 trial in patients with HER2-positive, locally advanced, or metastatic breast cancer refractory to anthracyclines, taxanes, and trastuzumab.24,25 The study was stopped early when results from a planned interim analysis showed the superiority of the combination therapy.

The combination of lapatinib and trastuzumab was found to be clinically active in a phase 1 dose-escalation study.29 In a phase 3 trial, progression-free survival and clinical benefit rates were significantly improved by the combination of lapatinib with trastuzumab compared with lapatinib alone.30 3000 participants are enrolled in the TEACH (Tykerb Evaluation After Chemotherapy) trial20 of adjuvant lapatinib therapy, and the global ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization) trial is open for enrolment.27 Lapatinib is being assessed in the neoadjuvant setting by the CHERLOB (Preoperative Chemotherapy plus Lapatinib or Trastuzumab or Both in HER2-positive Operable Breast Cancer) trial.26 As additional data become available, the role of lapatinib in different treatment settings (neoadjuvant, adjuvant, and metastatic) will be better defined.

New therapies for metastatic breast cancer

Breast cancer continues to adapt to the treatments available, and clinicians must alter treatment regimens to overcome the disease. Various compounds are being tested to overcome trastuzumab resistance (figure 2). Some of the newer agents are approved for other disease states (TKIs, vascular endothelial growth-factor receptor [VEGFR] inhibitors, and mammalian target of rapamycin [mTOR] inhibitors) or are similar to approved drugs, but are being assessed in the specific population of patients with HER2-positive, trastuzumab-resistant breast cancer (table 2).
TKIs
Targeting the epidermal growth-factor receptor (EGFR) family is a main strategy for drug development in the treatment of metastatic breast cancer. One approach is to inhibit the cross-talk among different EGFRs by inhibiting multiple receptors at once. Neratinib (HKI-272) is an irreversible pan-ERBB TKI that targets HER1, HER2, and HER4. This drug could overcome the bypass mechanism of resistance in patients previously exposed to trastuzumab by inhibiting the lateral activation of other HER proteins. In preclinical studies, neratinib overcame mutation-acquired resistance in patients with lung cancer. In a phase 1 trial, eight of 25 patients with breast cancer who were given escalating doses of neratinib showed a partial response, and one patient showed disease stabilisation for longer than 24 weeks. The dose-limiting toxic effect was grade 3 diarrhoea; other common toxic effects included nausea, vomiting, fatigue, and anorexia. A phase 2 trial showed a benefit with neratinib in advanced HER2-positive breast cancer. Of the 136 individuals enrolled, objective responses were observed in 51% who had received no prior trastuzumab and 26% of those who had received prior trastuzumab. Dose reductions were required in 27% of patients because of diarrhoea.

VEGF inhibitors
Drugs that can alter the vasculature are a possible approach to overcome resistance to trastuzumab. A direct link between HER2 and VEGF expression has been identified in preclinical trials and is a logical target for investigating the simultaneous inhibition of both pathways. Patients with increased HER2 and VEGF expression have worse outcomes. Bevacizumab is being assessed in combination with trastuzumab to overcome this potential mechanism of resistance (bypass mechanism), and phase 2 trials have shown its feasibility and activity. Another drug in development with activity against VEGFR is pazopanib; however, it is a multitargeted inhibitor of VEGFR, PDGFR, and c-KIT. Phase 1 data showed partial responses and stabilisation of disease in patients with solid tumours. A phase 2 trial compared the combination of lapatinib and pazopanib with lapatinib alone as first-line therapy in HER2-positive metastatic breast cancer. This combination was used to exploit the blockade of both EGFR and VEGF pathways. Response rates of 44% (14 patients) were seen with combination therapy and 30% (nine patients) with lapatinib alone. The most common toxic effects of the combination treatment were diarrhoea, rash, nausea, and increased liver enzymes. One patient had an asymptomatic decline in left ventricular ejection fraction and was removed from the study. This was the first phase 2 trial investigating lapatinib with pazopanib, and further studies are warranted to better understand the potential for this combination to reduce recurrence rates and overcome trastuzumab resistance by blocking multiple downstream pathways.

Sunitinib inhibits multiple TKIs (VEGFR, PDGFR, KIT, RET, FLT3, and CSF-1R), and is currently approved for use in metastatic renal-cell carcinoma and gastrointestinal stromal tumours. Like pazopanib, sunitinib inhibits cross-talk between the HER2 and VEGF pathways. This oral therapy has been studied in combination with trastuzumab in patients with HER2-advanced metastatic breast cancer, or combined with docetaxel and trastuzumab in first-line therapy for HER2-positive advanced breast cancer. In an ongoing phase 2 trial, 49 patients who received previous trastuzumab (with or without lapatinib) were given sunitinib in combination with trastuzumab; overall response rate was 26% (95% CI 13.5–41.2) with a clinical benefit rate of 35% (21–50.9). Grade 1 and 2 toxicities consisted of diarrhoea, asthenia, and hypertension and grade 3 toxicities included neutropenia and asthenia. When combined with docetaxel and trastuzumab, toxic effects with sunitinib were manageable, with fatigue and neutropenia the most common. Of the 11 patients evaluable for antitumour activity, one showed a complete response and seven had a partial response. Results are encouraging with the use of sunitinib, and with its ease of administration this therapy might provide another alternative for patients with trastuzumab resistance.

mTOR inhibitors
Loss of PTEN activity has been associated with trastuzumab resistance. The mTOR kinases alter and regulate PTEN and are important mediators of the PI3K–AKT pathway. This pathway, once activated, leads to cell proliferation and is negatively regulated by PTEN. Rapamycin was the first mTOR inhibitor, but other analogues (temsirolimus and everolimus) have been developed for the treatment of solid tumours. Everolimus inhibits the mTOR kinases, and is currently approved for use in metastatic renal-cell carcinoma, gastrointestinal stromal tumours, and lung cancer. Everolimus was assessed in combination with trastuzumab to overcome resistance to this therapy. In a phase 3 trial, 803 patients were given trastuzumab with or without everolimus; overall response rate was 11% (95% CI 7.3–15.0) with a clinical benefit rate of 22% (16.3–27.9). Grade 3 toxicities were more frequent in patients receiving everolimus compared to trastuzumab alone; however, the risk of grade 4 toxicities was similar in the two groups. The risk of death was lower in patients receiving everolimus, and this study supports further investigation of this combination to reduce recurrence rates in patients with advanced breast cancer.

Figure 2: Therapies being developed for HER2-overexpressing breast cancer
Pertuzumab binds to HER2 to prevent receptor dimerisation. 2B1 and MDX-H210 bind to HER2 and immune effector cells, sequestering the immune system. Trastuzumab-DX1 sequesters the cytotoxic drug, maytansine, to HER2-overexpressing cells via binding of trastuzumab. Perifosine disrupts the cell membrane and interferes with membrane localisation, phosphorylation, and activation of AKT.
Mechanism of action | Phase of clinical development
--- | ---
Vaccines

E75  
Activate cytotoxic T lymphocytes that identify HER2 cancer cells, leading to cell death  
1, 2
gp2  
1  
AE37, li-Key  
Direct antigenic epitope charging of MHC class II molecules on the cell surface  
2
New compounds

T–DM1  
Trastuzumab conjugated with maytansine to improve potency  
1, 2, 3
KU-0059436 (Ku)  
PARP inhibitor  
1, 2
Pertuzumab  
Inhibits heterodimerisation of HER2 and other EGFRs  
1, 2, 3
Ertuxmoxan  
Bi specific monoclonal antibody that blocks HER2 and CD2  
2
Neratinib  
Inhibits heterodimerisation of HER2 and HER3  
1, 2
Tanespimycin  
HSP90 inhibitor  
1, 2
Alvespimycin  
HSP90 inhibitor  
1, 2
Temsirrolimus  
mTOR inhibitor  
1, 2, 3
Everolimus  
mTOR inhibitor  
1, 2
Pazopanib  
mTOR inhibitor  
1, 2
Anastrozole plus trastuzumab  
Aromatase inhibitor plus HER2 inhibition  
2, 3
Signal-transduction inhibition

MHC=major histocompatibility complex. T–DM1=trastuzumab conjugated with maytansine. PARP=poly-ADP-ribose polymerase I. EGFR=epidermal growth-factor receptor. HSP90=heat shock protein 90. mTOR=mammalian target of rapamycin. VEGFR=vascular epidermal growth-factor receptor. PDGFR=platelet-derived growth factor receptor.

Table 2: Agents currently being assessed in HER2-resistant breast cancer

and everolimus) are being investigated because of rapamycin’s instability and poor solubility. Temsirolimus and everolimus are currently approved for use in patients with metastatic renal-cell carcinoma. Preclinical data showed an association between mTOR inhibition and activation of the PI3K–AKT pathway, and early trials showed a clinical response to temsirolimus. Phase 2 and 3 trials are underway. In 98 patients with locally advanced disease who were previously treated with an anthracycline and taxane, the clinical benefit rate (partial response or stable disease) was 37%.36 A partial or taxane, the clinical benefit rate (partial response or stable disease who were previously treated with an anthracycline and everolimus showed activity in trastuzumab-resistant disease.40 Of seven evaluable patients, three achieved a partial response with daily therapy, and two achieved a partial response and one had a minor regression with weekly therapy. When combined with vinorelbine and trastuzumab, everolimus showed activity in trastuzumab-resistant disease.46 Of nine evaluable patients, one had a partial response and seven had stable disease. Clinical trials are ongoing to find optimum doses and treatment combinations (chemotherapy, hormone therapy, or trastuzumab) for mTOR inhibitors.

**Novel HER2-targeted agents**

**Trastuzumab–DM1**

One strategy for overcoming resistance to a drug is to improve its potency. A novel antibody–drug conjugate of trastuzumab and maytansine DM1 (T–DM1) has been formulated to allow trastuzumab to be non-toxic until it reaches its target site. Maytansine is a potent inhibitor of microtubule assembly, which showed in-vitro cytotoxicity of more than 1000 times higher than any other chemotherapeutic agents; however, this result did not extrapolate to clinical benefit in humans. In T–DM1, trastuzumab is conjugated with DM1 via an MCC (mutated in colorectal cancer protein) linker, which stabilises the bond and helps maintain efficacy and decrease toxicity.48 In a phase 1 study, 24 patients were given T–DM1 every 3 weeks. Six partial responses have been observed with doses of 2·4 or 3·6 mg/kg, and five patients had stable disease up to 260 days. In the same study, 28 patients were given the drug weekly and 15 responses were observed. The most frequent dose-limiting toxicity was grade 3–4 thrombocytopenia. No cardiac toxicity was noted on either dosing schedule. Although efficacy was not the primary endpoint of this trial, it led to phase 2 studies.49 In a phase 2 study, T–DM1 was given every 3 weeks in patients with metastatic, HER2-positive breast cancer who had progressed on prior trastuzumab.50 107 patients were assessed for efficacy; 41 patients showed a partial response and one a complete response. Grade 3–4 toxicities consisted of thrombocytopenia and hypokalaemia. With a median follow up of 9·5 months, the objective response rate (complete + partial response) was 25% and the clinical benefit rate (complete + partial + stable disease) was 34·8%.51 Phase 2 trials continue and a phase 3 trial has...
begun. This novel formulation of a drug conjugate with trastuzumab could lead to other biological therapy–cytotoxic agent combinations for use in HER2-positive, trastuzumab-resistant breast cancer.

**Pertuzumab**

Pertuzumab is a monoclonal antibody that targets the extracellular domain of HER2 and is considered a HER-dimerisation inhibitor. Pertuzumab blocks the ability of HER2 to heterodimerise with other members of the EGFR family, thus preventing signal transduction. The epitope recognised by pertuzumab might be different than the epitope trastuzumab binds to on the extracellular portion of HER2. Previous preclinical data showed synergy between trastuzumab and pertuzumab, which could overcome the bypass mechanism of resistance in trastuzumab-resistant patients. In phase 1 trials, pertuzumab was well tolerated and antitumour activity was identified. Pertuzumab also showed activity in phase 2 trials in trastuzumab-refractory metastatic breast cancer, and results suggested that the drug could reverse trastuzumab resistance. 16 of 66 patients had a partial response and 17 had stable disease for longer than 6 months when given a combination of trastuzumab and pertuzumab. Diarrhoea, fatigue, nausea, vomiting, and rash were the most common side-effects. A phase 3 trial is currently comparing pertuzumab, trastuzumab, and docetaxel to placebo, trastuzumab, and docetaxel in previously untreated patients with HER2-positive metastatic breast cancer.

**Ertumaxomab**

Resistance to trastuzumab could also be overcome by formulating drugs with a dual mechanism of action. Ertumaxomab is a monoclonal, trifunctional, bispecific antibody that binds both HER2 and CD3. Binding forms a complex of T cells, HER2-positive tumour cells, and macrophages or dendritic cells, which leads to phagocytosis of the tumour cells. In in-vitro studies, ertumaxomab killed HER2-positive cells of many different cell lines and tumour types. A phase 1 trial noted a response in 15 patients with HER2-positive metastatic breast cancer. Patients who had previous trastuzumab therapy were eligible if treatment was completed more than 3 months before enrolment. The most common toxic effects were fever, rigors, headache, nausea, and vomiting; and grade 3–4 toxic effects consisted of lymphocytopenia and raised liver-enzyme concentrations. One patient had a worsening of congestive heart failure. On the basis of this data, phase 2 trials are currently underway. Ertumaxomab induced cytotoxicity in cell lines with low expression of HER2, suggesting another group of patients in whom this drug might be beneficial.

**Vaccines**

Immunotherapy is a unique approach to the treatment of HER2-positive breast cancer. Stimulating an immune response causes the body to attack and eliminate tumour cells. Although vaccinations have been assessed in the metastatic setting in various cancer types, a lack of immunogenicity has lead to decreased interest. Most current studies of cancer vaccines are in the adjuvant setting to help prevent recurrence. Autologous dendritic cells pulsed with tumour-specific antigens that stimulate a cytotoxic response are one route of administration. Vaccination with a synthetic peptide offers a defined immune response to individuals with the appropriate genetic sequence. HER2 is a proto-oncogene and its protein product has been recognised as a tumour-associated antigen and a source of immunogenic peptides. These peptides activate cytotoxic T lymphocytes (CTL) that identify HER2-expressing cancer cells and destroy them. Numerous HER2-derived peptides have been studied for efficacy in breast cancer, including E75, AE37, and GP2. E75 induced an immune response in preclinical studies and was safe when given in combination with an immunoadjuvant such as granulocyte-macrophage colony-stimulating factor (GM-CSF). The addition of immunoadjuvant therapy to induce peptide-specific immunity is simpler than other methods, such as infusing autologous dendritic cells or embedding E75 into longer peptides to bind HLAAs. HER2-derived vaccines with E75, AE37, or GP2 peptides have been assessed at varying dose schedules. Data support use of the E75 vaccine in HLA-A2-positive patients, since E75 binds primarily to HLA-A2. E75 can also bind to HLA-A3, and so patients with this serotype are eligible to receive the vaccine and GM-CSF. In one trial, 101 patients who were positive for both HLA-A2 and HLA-A3 were vaccinated and 85 patients were observed. Toxic effects were minimal, and dose reductions were necessary in 18.7% of patients because of local and systemic reactions to GM-CSF. Immune response to the vaccine was assessed by E75-specific CTL. At a median follow-up of 18 months, the recurrence rates were 5.6% (five patients) in the vaccinated group and 14.2% (11 patients) in the observation group (p=0.04). At 26 months, however, recurrence was not significantly better for the vaccinated group (8.3%; eight patients) than for the observation group (14.8%; 12 patients; p=0.15). One reason for the absence of benefit at the extended follow-up interval could be diminishing immunity over time; around 50% residual immunity is apparent at 6 months. Booster programmes have been initiated, and a recent study used E75 booster vaccinations every 6 months after completion of initial vaccine therapy. There was a slight increase in grade 1 and 2 toxic effects between booster injections. The number of E75-specific CTLs increased with the use of a late booster (>6 months after vaccine), whereas the number stayed constant with an early booster (6 months after vaccine). Data collection is ongoing to establish the best use of booster therapy to enhance and prolong immunity.

Other vaccines that increase antigen-specific stimulation of T cells have been assessed, such as the invariant protein product has been recognised as a tumour-associated antigen and a source of immunogenic peptides. These peptides activate cytotoxic T lymphocytes (CTL) that identify HER2-expressing cancer cells and destroy them. Numerous HER2-derived peptides have been studied for efficacy in breast cancer, including E75, AE37, and GP2. E75 induced an immune response in preclinical studies and was safe when given in combination with an immunoadjuvant such as granulocyte-macrophage colony-stimulating factor (GM-CSF). The addition of immunoadjuvant therapy to induce peptide-specific immunity is simpler than other methods, such as infusing autologous dendritic cells or embedding E75 into longer peptides to bind HLAAs. HER2-derived vaccines with E75, AE37, or GP2 peptides have been assessed at varying dose schedules. Data support use of the E75 vaccine in HLA-A2-positive patients, since E75 binds primarily to HLA-A2. E75 can also bind to HLA-A3, and so patients with this serotype are eligible to receive the vaccine and GM-CSF. In one trial, 101 patients who were positive for both HLA-A2 and HLA-A3 were vaccinated and 85 patients were observed. Toxic effects were minimal, and dose reductions were necessary in 18.7% of patients because of local and systemic reactions to GM-CSF. Immune response to the vaccine was assessed by E75-specific CTL. At a median follow-up of 18 months, the recurrence rates were 5.6% (five patients) in the vaccinated group and 14.2% (11 patients) in the observation group (p=0.04). At 26 months, however, recurrence was not significantly better for the vaccinated group (8.3%; eight patients) than for the observation group (14.8%; 12 patients; p=0.15). One reason for the absence of benefit at the extended follow-up interval could be diminishing immunity over time; around 50% residual immunity is apparent at 6 months. Booster programmes have been initiated, and a recent study used E75 booster vaccinations every 6 months after completion of initial vaccine therapy. There was a slight increase in grade 1 and 2 toxic effects between booster injections. The number of E75-specific CTLs increased with the use of a late booster (>6 months after vaccine), whereas the number stayed constant with an early booster (6 months after vaccine). Data collection is ongoing to establish the best use of booster therapy to enhance and prolong immunity.

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Use of HER2-specific vaccines has been assessed in patients with metastatic breast cancer, to see if the addition of a vaccine could induce prolonged immune responses. In a phase 1–2 study,26 21 patients received a HER2-neu vaccine in addition to trastuzumab, and 14 patients received all six of the planned vaccinations. 19 participants (90%) developed new or improved immunity. This trial was not designed to answer the clinical endpoint of progression-free and overall survival, but it did show that greater immunity was generated in individuals who survived. Patients had substantial pre-existing immunity, so a more robust response could be generated by giving both therapies together. Therapy was well tolerated with only three grade 4 toxicities: injection-site reaction, fainting, and ulceration. Three patients had an asymptomatic drop in left ventricular ejection fraction. This combination is a potential therapy for patients with HER2-positive breast cancer and might prevent trastuzumab resistance if immunity is increased and prolonged.

Endocrine therapy
Combining endocrine therapy with signal-transduction inhibitors is a possible means of overcoming endocrine resistance. This is a novel approach that utilises
trastuzumab therapy, rather than overcoming resistance to it. In preclinical studies, the interaction between HER2 and oestrogen-receptor expression was associated with increased tumour growth and was thought to lead to endocrine resistance. Leary and colleagues tested this hypothesis by treating an endocrine-resistant cell line with lapatinib and tamoxifen, and found that the most effective suppression of oestrogen-receptor activity was in cells treated with the combination of drugs. This result, and the knowledge that around half of HER2-positive tumours are also oestrogen-receptor positive, provided strong rationale to investigate combinations of EGFR or HER2 inhibitors and hormone therapy. The Trastuzumab and Anastrozole directed against ER-positive HER2 positive mammary carcinoma (TAnDEM) study compared weekly trastuzumab plus anastrozole with anastrozole alone as first-line therapy in 207 postmenopausal women with HER2-positive and hormone-receptor-positive metastatic breast cancer.\(^7\) Combination therapy was associated with significant improvements in progression-free survival (4.8 vs 2.4 months; \(p=0.016\)), clinical benefit rate (42.7% vs 27.9%; \(p=0.026\)), and time to progression (4.8 vs 2.4 months; \(p=0.0007\). Overall response rates were also higher in the combination therapy group (20.3% vs 6.8%; \(p=0.018\). Combinations of other aromatase inhibitors and TKIs are being investigated in phase 2 and 3 trials.

The EGF30008 trial is the largest so far to investigate blocking both HER2 and oestrogen receptor. 1286 patients with hormone-positive, untreated metastatic breast cancer were given lapatinib plus letrozole or letrozole plus placebo.\(^7\) In patients with HER2-positive disease, progression-free survival was significantly longer with lapatinib plus letrozole (8.2 months) than with letrozole alone (3.0 months; HR 0.71; 95% CI 0.53–0.96). Overall response rates were also higher with the combination (27.9% vs 14.8%), and the therapy was well tolerated. However, there are some conflicting reports on the benefit of this combined approach in enhancing or overcoming endocrine resistance and more data is needed.

**Future directions for HER2 resistance**

One mechanism of trastuzumab resistance is a loss of HER2 expression. A new class of agents has been found to work in patients with HER2-neu-negative breast cancer. Poly (ADP-ribose) polymerase 1 (PARP-1) is an enzyme that repairs DNA breaks. Enhanced PARP-1 expression has been noted in many tumour-cell lines and might confer greater resistance to chemotherapy drugs targeting DNA. In preclinical studies, inhibitors of PARP-1 showed greater activity when given in combination with chemotherapy and even restored sensitivity in previously resistant tumours.\(^8\) Overexpression of PARP-1 has also been identified in oestrogen-receptor, progesterone-receptor, and HER2-negative breast cancers (triple negative). The first phase 1 study of KU-0059436 (Ku), a small-molecule PARP-1 inhibitor, was done in patients with advanced solid tumours.\(^8\) A Ku dose of 40 mg daily inhibited PARP by more than 50%. Response rates as high as 40% have been identified in patients with hereditary breast cancer-associated ovarian cancer (ie, with BRCA1/2 mutation).\(^9\) Phase 2 studies are underway to assess the use of this therapy in specific subpopulations of patients, such as those with hereditary breast and ovarian cancer, or in combination with chemotherapy. A novel PARP-1 inhibitor, BSI-201, was assessed in combination with gemcitabine and carboplatin in patients with triple-negative breast cancer. An analysis of 86 patients showed significantly higher clinical benefit rates (62% vs 21%; \(p=0.0002\)), progression-free survival (6.9 vs 3.3 months, \(p=0.0005\)), and overall survival (9.2 vs 5.7 months, \(p=0.005\) for the combination compared with chemotherapy alone.\(^9\) With loss of HER2 expression as a possible mechanism of resistance, PARP-1 inhibitors are a promising treatment option for patients with triple-negative breast cancers.

**Conclusion**

Our understanding of the biology of breast cancer is increasing steadily, and we know that HER2-positive disease is associated with poorer outcomes. Two drugs have been approved for the treatment of HER2-positive breast cancer: trastuzumab and lapatinib. Trastuzumab prolongs progression-free and overall survival, but disease still recurs in many patients. Various resistance mechanisms are likely, such as bypass mechanisms, mutation of the HER2 target, masking of the membrane protein, and increased expression of insulin-like growth factors. This review highlights several new agents that show encouraging results for patients faced with this therapeutic dilemma. For many of the drug entities currently being studied for use in breast cancer, very little or no clinical data regarding efficacy has been published. Their mechanisms of action (figure 2) suggest a theoretical benefit, but future clinical trials will define their role in the treatment of trastuzumab-resistant breast cancer. In the ever-changing world of breast cancer therapy, these approaches to treatment may one day prevent resistance and also cure disease.

**Contributors**

Both authors contributed equally to the conception, writing, literature search, and approval of this paper.

**Conflicts of interest**

AUB has done protocol research for AstraZeneca, Roche, Lilly, Genentech, Pfizer, and Taiho, and has received honoraria from AstraZeneca, Genentech, and Amgen. KLJ declared no conflicts of interest.

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**Search strategy and selection criteria**

References for this review were identified by searches of Medline, Ovid, PubMed, and references from relevant articles using the search terms “HER2”, “breast cancer”, “trastuzumab”, and “resistance”. Abstracts and reports from meetings were included when they related directly to previously published work. Only papers published in English between January, 1997, and September, 2009, were included.
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