New drugs for breast cancer

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Recently there have been significant advances in rational drug design for the treatment of breast cancer, especially in the area of targeted drug therapy. These include drugs which target the HER2 receptor and angiogenesis and the novel class of drug the PARP inhibitors. Some of these agents, for example, trastuzumab used in the treatment of HER2 positive breast cancer are already established as the standard of care. However, the duration of adjuvant trastuzumab, whether to continue it beyond progression in metastatic disease and the mechanism for developing trastuzumab resistance, remain to be determined. There is also much still to be learnt regarding other targeted therapies; the efficacy of different agents, the optimal duration of use and combination of therapies. Many of these agents are already in clinical trials, the results of which are likely to change clinical practice.

Keywords: targeted therapy/breast cancer/HER2/angiogenesis/PARP inhibitors

Accepted: August 7, 2010

Introduction

Over the last two decades, breast cancer research has lead to significant progress in the understanding of this disease. Increasingly it is recognized that breast cancer encompasses a range of diseases with different behaviors, which require different treatments. Gene expression studies have made a significant contribution to our understanding with the identification of different intrinsic subtypes. These include the luminal A subtype, which are mostly oestrogen receptor positive and histologically low grade; the luminal B subtype, which are also mostly oestrogen receptor positive but frequently high histological grade; the HER2-positive subtype characterized by amplification and overexpression of the HER2 gene and the basal-like subtype, which are mostly oestrogen receptor, progesterone receptor and HER2 negative and therefore at present lack any specific therapeutic target. This area is the focus of intensive current research.
The non-surgical management of breast cancer depends on chemotherapy and endocrine therapy with not all patients requiring both modalities. However, with the greater understanding of the underlying biology considerable advances have been made in the development of targeted therapies resulting in a wider range of therapeutic options. This review will focus on these new targeted therapies. Agents which target the HER2 receptor, angiogenesis and the recently described PARP inhibitors will be discussed. While many of these new biological and targeted therapies remain under development and/or entering clinical trials, some have already become the standard of care.

### HER2 targeted therapy

Overexpression of HER2 occurs in approximately 25% of breast cancers and is predominantly due to amplification of the HER2 gene. Overexpression is correlated with a more aggressive phenotype and worse prognosis.\(^1\) HER2 (also known as ErbB2) is a member of the epidermal growth factor receptor family of tyrosine kinases (TK), which in humans consists of four members: HER1, 2, 3 and 4 (also called epidermal growth factor receptors EGFR or ERB1, 2, 3 and 4, respectively). Each one is a transmembrane TK receptor with partial homology that normally regulates cell growth and survival, as well as adhesion, migration, differentiation and other cellular responses.\(^2\) Each receptor consists of an extracellular-binding domain, a transmembrane lipophilic segment and (except for HER3) a functional intracellular TK domain. The TK domains are activated by both homo-and hetero-dimerization, generally induced by ligand binding, although no natural ligand for HER2 has been identified to date. Dimerization in the absence of ligand can occur by the extracellular domain of HER2 adopting a fixed conformation resembling a ligand-activated state,\(^3\) and by receptor overexpression and mutation. Once activated, the signal transduction cascades of these receptors promote cellular proliferation, through the RAS-MAPK pathway and survival by inhibiting cell death through the phosphatidylinositol 3'-kinase-AKT-mammalian target of rapamycin (mTOR) pathway.\(^4\) Clinically overexpression is detected using immunohistochemistry (IHC), with an antibody directed against the HER2 receptor or by FISH (fluorescence *in situ* hybridization), which uses a fluorescently labelled DNA probe to the *ERBB2* gene to detect gene copy number. A scoring system from 0 to 3+ is employed for IHC, where 3+ is positive, 0/1 is negative and 2+ is indeterminate, requiring FISH confirmation of gene amplification.
Trastuzumab

The advent of trastuzumab (Herceptin™), a recombinant humanized monoclonal antibody (mAb) targeting the extracellular domain of the HER2 receptor, has changed the treatment paradigm for HER2-positive breast cancer. Trastuzumab consists of two antigen-specific sites that bind to the juxtamembrane portion of the extracellular domain of the HER2 receptor and prevents the activation of its intracellular TK. The remainder of the antibody is human IgG with a conserved Fc portion. Several possible mechanisms by which trastuzumab might decrease signaling include prevention of the HER2-receptor dimerization, increased endocytic destruction of the receptor, inhibition of shedding of the extracellular domain and immune activation.5

In the metastatic setting, a pivotal randomized Phase III trial compared chemotherapy with or without trastuzumab in 469 HER2-positive patients. Chemotherapy naïve patients received either doxorubicin or epirubicin in combination with weekly trastuzumab, whereas patients who had received an anthracycline in the adjuvant setting received paclitaxel with weekly trastuzumab. Trastuzumab plus chemotherapy was associated with a significant improvement in median time to disease progression (7.4 months versus 4.6 months, \( P < 0.001 \)), response rates (50% versus 32%, \( P < 0.001 \)) and median survival (25.1 months versus 20.3 months \( P = 0.01 \)) compared with chemotherapy alone.6

However, cardiotoxicity was a clinically significant and unexpected side effect, seen in 27% of patients receiving anthracycline plus trastuzumab, compared with 8% with anthracycline alone, 13% with paclitaxel and trastuzumab and 1% with paclitaxel alone. This led to the recommendation that concomitant anthracyclines and trastuzumab should be avoided. The mechanism of cardiotoxicity remains to be elucidated, but animal studies suggest signaling through the HER2 pathway may be important for maintenance of normal cardiac function.7

Following the outcome of the pivotal study, trastuzumab has been tested in the adjuvant setting in four large multicentre trials: the National Surgical Adjuvant Breast and Bowel Project trial [B-31], the North Central Cancer Treatment Group trial [N9831], the Trastuzumab Adjuvant trial [HERA] and the Breast Cancer International Research Group trial 006 [BCIRG-006]. Altogether these trials involved over 12 000 women. Results are available from three of these studies (HERA8,9 NSABP B-3110 and N983110) with to date, the fourth only published in the abstract form (BCRIG00611,12). The results from the first three trials were so significant that the respective data and safety monitoring committees stopped the trials after the
interim analyses and trastuzumab was offered to all patients in the control groups.

Both NSABP B-31 and the NCCTG N9831 included node-positive and N9831 also included high-risk node-negative patients. B-31 compared four cycles of doxorubicin and cyclophosphamide followed by paclitaxel with and without weekly trastuzumab starting on day 1 of paclitaxel and continued for 12 months. The N9831 trial compared three regimens, four cycles of doxorubicin and cyclophosphamide followed by 12 cycles of weekly paclitaxel. Trastuzumab was either given concurrently starting on day 1 of paclitaxel or on completion of chemotherapy for a total of 12 months. The control arms of both trials together with the concurrent chemotherapy trastuzumab arms differed in only minor details, therefore an NCI and FDA approved joint analysis was undertaken (however this was not a preplanned analysis). It should be noted that the sequential treatment arm in N9831 was excluded from this interim analysis and has just reported in the abstract form. The interim analysis resulted in a significant difference between groups resulting in early trial stoppage as discussed above. A total of 2043 patients were enrolled in B-31 with a median follow-up of 2.4 years. There were 1633 patients in the analysis arms of N9831 with a median follow-up of 1.5 years. In the combined analysis there were 133 events in the trastuzumab arm and 261 in the control group. The hazard ratio (HR) for a first event in the trastuzumab group compared with control was 0.48 (95% CI: 0.39 to 0.59; \( P < 0.0001 \)). The percentage of patients alive and disease free at 3 years were 75.4 and 87.1% in the control and trastuzumab groups, respectively (absolute difference: 11.8%; 95% CI: 8.1–15.4%). The absolute survival rate at 3 years was 94.3% in the trastuzumab group compared with 91.7% in the control group (absolute difference: 2.5%; 95% CI: 0.1–5.0%). The 3 year cumulative incidence in congestive cardiac failure or death from cardiac causes was 4.1 and 2.9% in the B-31 trial and N9831 trial, respectively.

The HERA trial had a three-way randomization to observation following standard institution defined (neo-)adjuvant treatment or to trastuzumab given on a three weekly schedule for 12 or 24 months in patients with HER2-positive node-positive or high-risk node-negative breast cancer. Randomization was performed within 7 weeks of day 1 of the last cycle of chemotherapy or within 6 weeks from the end of definitive surgery or radiotherapy whichever was last. The rationale for the prolonged treatment group was several fold; a major peak in the rate of relapse occurs 18–24 months after surgery, effective treatment of HER2-positive breast cancer may need prolonged attenuation of HER2 activity and using an analogy with tamoxifen, also a targeted therapy, it has proven to be more effective with therapy up to 5 years.
After median follow-up of 2 years, the 12 month trastuzumab arm versus observation was updated, but with insufficient events for a comparison of 24 versus 12 months. There were 1703 and 1698 women in the 12 month treatment and observation arms, respectively. There were 218 events in the trastuzumab arm and 321 in the observation group. The unadjusted HR for the risk of an event in the trastuzumab group compared with the observation group was 0.64 (95% CI: 0.54–0.76; \(P < 0.0001\) log rank test), which corresponds to an absolute DFS benefit of 6.3% (80.6 versus 74.3%) at 3 years. Severe cardiotoxicity was only seen in 0.6% of women receiving trastuzumab.

The Breast Cancer International Research Group (BCIRG) 006 trial has reported the third interim safety and efficacy analysis. In this Phase III trial the control arm was four cycles of doxorubicin and cyclophosphamide (AC) followed by 4 cycles of docetaxel (T) compared with two experimental arms AC followed by T plus trastuzumab (H) weekly during chemotherapy and then subsequently three weekly for 12 months (ACTH) or six cycles of docetaxel (T) plus carboplatin (C) plus trastuzumab (H) for 12 months (TCH). This third arm was designed to obviate the need for anthracyclines thereby potentially eliminating cardiotoxicity. A total of 3222 patients were randomized and at a median of 65 months follow-up, there were 185 events in the ACTH arm, 214 events in the TCH arm and 257 in the control arm. The HR for the risk of an event in the ACTH arm was 0.64 (95% CI: 0.53–0.78; \(P = 0.001\)) and 0.75 (95% CI: 0.63–0.90 \(P = 0.04\)) for TCH compared with the observation group, which corresponds to an absolute DFS improvement of 9% for ACTH (84 versus 75%) and 6% for TCH (81 versus 75%). Although there was no statistically significant difference between the two trastuzumab containing arms, there was a trend towards the TCH arm doing less well, but the incidence of cardiotoxicity was greater in the ACTH compared with the control and TCH arms.

In contrast, the FNCLCC-PACS 04 trial has shown no significant advantage for adjuvant trastuzumab (Table 1). This trial randomized 528 HER2-positive patients to 1 year of trastuzumab or observation following completion of adjuvant chemotherapy (either six cycles of F(100)C or six cycles of epirubicin and docetaxel). After 47-month median follow-up, the trastuzumab arm was associated with a non-significant 14% reduction in the risk of relapse (HR 0.86; 95% CI: 0.61–1.22; \(P = 0.41\)) but with no difference in the 3 year DFS, which was 78% (95% CI: 72.3–82.5) and 81% (95% CI: 75.3–85.4) in the observation and trastuzumab arms, respectively. Possible explanations for this negative result may be the small sample size and/or the sequential versus concurrent administration of trastuzumab.
### Table 1 Summary of the adjuvant trastuzumab trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Median follow-up (months)</th>
<th>Treatment regimes per arm</th>
<th>Number of patients/arm</th>
<th>DFS (%)</th>
<th>HR</th>
<th>P-value</th>
<th>OS (%)</th>
<th>HR</th>
<th>P-value</th>
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<tr>
<td>HERA&lt;sup&gt;9&lt;/sup&gt;</td>
<td>24</td>
<td>CT → observation</td>
<td>1698</td>
<td>74</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td>90</td>
<td>0.66</td>
<td>0.11</td>
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<td></td>
<td></td>
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<td>CT → H × 24 months</td>
<td>1689</td>
<td></td>
<td></td>
<td>Arm not yet reported</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NSABP B-31 and N-9831&lt;sup&gt;10&lt;/sup&gt;</td>
<td>24 (28.8 months in B-31, 18 months in N9831)</td>
<td>AC → P</td>
<td>1679</td>
<td>75.4</td>
<td></td>
<td></td>
<td>91.7</td>
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<tr>
<td></td>
<td></td>
<td>AC → P + H</td>
<td>1672</td>
<td>87.1</td>
<td>0.48</td>
<td>&lt;0.001</td>
<td>94.3</td>
<td>0.67</td>
<td>0.02</td>
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<td>BCIRG 006&lt;sup&gt;11&lt;/sup&gt;</td>
<td>65, third analysis</td>
<td>AC → T</td>
<td>1073</td>
<td>75</td>
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<td></td>
<td>92</td>
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<tr>
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<td></td>
<td>AC → T + H → H</td>
<td>1074</td>
<td>84</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td>91</td>
<td>0.63</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
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<td>TCH</td>
<td>1075</td>
<td>81</td>
<td>0.75</td>
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<td>87</td>
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<td>FinHER&lt;sup&gt;19&lt;/sup&gt;</td>
<td>62</td>
<td>V or T → FE(60)C</td>
<td>116</td>
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<td>82.3</td>
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<td>V or T + H → FE(60)C</td>
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<td>83.3</td>
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<td>91.3</td>
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<td>PACS-O4&lt;sup&gt;14&lt;/sup&gt;</td>
<td>47</td>
<td>FE(100)C or ED → Observation</td>
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<td></td>
<td>96</td>
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<tr>
<td></td>
<td></td>
<td>FE(100)C or ED → H</td>
<td>260</td>
<td>81</td>
<td>0.86</td>
<td>0.41</td>
<td>95</td>
<td>1.27</td>
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</tr>
</tbody>
</table>

CT, chemotherapy institution choice; H, herceptin; AC, doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m²; P, paclitaxel 175 mg/m²; T, docetaxel 100 mg/m²; TC, docetaxel 75 mg/m² carboplatin AUC6, V, vinorelbine 25 mg/m²; FE(60)C, 5-fluorouracil 600 mg/m² epirubicin 60 mg/m² cyclophosphamide 600 mg/m²; FE(100)C, 5-fluorouracil 500 mg/m² epirubicin 100 mg/m² cyclophosphamide 500 mg/m²; ED, epirubicin 75 mg/m² docetaxel 75 mg/m².
Following publication of the B-31, N9831 and HERA trials, which had all demonstrated an approximate 50 and 33% improvement in DFS and OS, respectively, by the addition of trastuzumab, regardless of the chemotherapy regimen or sequence of its delivery, trastuzumab was FDA approved for the adjuvant treatment of HER2-positive disease. However, there remain significant unanswered questions. Firstly, should trastuzumab be given sequentially or concurrently with cytotoxic chemotherapy? While there are basic data both in vitro and in vivo to suggest that it enhances the proapoptotic activity of chemotherapy,\textsuperscript{15–17} the mechanism is not fully understood. The comparative analysis of the treatment arms in the N9831 trial was designed to address this question. Recently reported three were 174 events in the sequential arm compared with 138 in the concurrent arm. Although there is a strong trend for a reduction in risk towards concurrent versus sequential treatment, this did not reach preplanned statistical significance.\textsuperscript{13} Conversely the relative risk reduction in HERA using sequential treatment, is of a similar magnitude to that seen in the US trials, and furthermore, while in HERA 95% of patients received trastuzumab within 6 months of chemotherapy, many had a delay of several months from chemotherapy and an analysis of the effect of timing of trastuzumab may provide further information regarding this issue.

Secondly what is the optimal duration of treatment with trastuzumab? The results of the extended duration of trastuzumab from the HERA trial are still awaited. An intriguing trial is the FinHER study,\textsuperscript{18} which examined the use of just 9 weeks of trastuzumab. A total of 1010 women with node-positive or node-negative breast cancer with a tumor >20 mm, were randomized to receive either taxotere or vinorelbine followed by FEC (5-fluorouracil, epirubicin, cyclophosphamide). Patients with ERBB2 amplification (23% of patients) underwent a second randomization to receive 9 weekly cycles of trastuzumab concomitantly with either taxotere or vinorelbine, or no trastuzumab. After a median follow-up of 62 months the results from the subgroup of patients with HER2-positive disease showed that patients treated with just 9 weeks of trastuzumab had a non-significant improvement in distant DFS compared with chemotherapy alone (HR 0.65; 95% CI: 0.38–1.12; $P = 0.12$).\textsuperscript{19} The question of trastuzumab duration is currently being addressed in clinical trials.

Despite its success in the treatment of both early and advanced stage HER2-positive breast cancer, a proportion of patients who receive trastuzumab containing adjuvant therapy will relapse and nearly all patients receiving trastuzumab for metastatic disease will progress. A number of potential mechanisms underlying trastuzumab resistance have been proposed either affecting the receptor or its downstream signaling pathways. These include inhibition of trastuzumab interaction...
with its target protein; shedding of the extracellular receptor domain leaving behind the truncated form (p95), which retains kinase activity; cross-talk or heterodimerization between HER2 and IGF-1R or other HER family members, and decreased levels of PTEN expression and/or activation of the PI3K/AKT signaling proteins. Ultimately, a clearer and better understanding of the molecular mechanisms of resistance is needed as it will allow development of further treatment strategies for HER2-positive breast cancer.

**Lapatinib**

Lapatinib is an orally active reversible tyrosine kinase inhibitor (TKI), which inhibits the TK of HER1 and 2. A pivotal randomized Phase III study compared capecitabine plus lapatinib with capecitabine alone in 324 women with progressive, HER2 positive, locally advanced or metastatic breast cancer who had been treated with an anthracycline, a taxane and trastuzumab. An increased time to progression was seen in the combination arm (8.4 months compared with 4.4 months, HR 0.49 95% CI: 0.34–0.71, \( P < 0.001 \)). Lapatinib appeared to be generally well tolerated with diarrhoea, skin rash, fatigue and nausea as the main toxicities. Compared to trastuzumab there is less cardiotoxicity. A retrospective review of cardiac safety evaluated 3689 patients who had received lapatinib in 44 clinical trials and found an overall decrease in LVEF (defined as >20% fall relative to baseline and below the institution’s lower limit of normal) in 1.6% of patients and only 0.2% were symptomatic.

One-third of women with HER2-positive breast cancer develop central nervous system (CNS) metastases. Although trastuzumab reduces the risk of distant relapse, the CNS remains a site of initial and subsequent relapse. These and other data suggest that trastuzumab has limited penetration across the blood–brain barrier and this coupled with prolonged control of what was previously rapidly lethal systemic disease, leads to ‘unmasking’ of brain metastases. It has been postulated that lapatinib may have activity in CNS disease, although a small Phase II study has shown disappointing results, with just one of 39 patients demonstrating a partial CNS response. This is only a small study and the comparative incidence of brain metastases with trastuzumab and lapatinib is being addressed in a number of ongoing trials both in the adjuvant and metastatic setting.

Based on preclinical data indicating synergistic activity between lapatinib and trastuzumab and non-cross resistance, this combination has been examined in clinical trials. A Phase III trial involving 296 heavily pretreated, trastuzumab-refractory metastatic breast cancer patients...
randomized to treatment with lapatinib alone or with trastuzumab has been reported. Combination therapy significantly improved progression-free survival (PFS) (12 versus 8.4 weeks, HR 0.77 95% CI: 0.6–1.0  \( P = 0.029 \)) compared with lapatinib alone, with a non-significant trend toward improved median OS (51.6 versus 39 weeks \( P = 0.106 \)). Grade 1/2 diarrhoea was higher in the combination group (53 versus 41%) but acneiform rash was higher in the lapatinib alone arm (likely due to higher dose). An asymptomatic decline in LVEF was seen in 5% of patients in the combination arm compared with 2% in the lapatinib only arm. Whether the combination of two anti-HER2 targeted therapies with chemotherapy will prove beneficial in early stage disease is currently being tested in two accruing clinical trials. The four-arm randomized Phase III ALTTO (Adjuvant Lapatinib and/or trastuzumab Treatment Options) trial, is comparing the combination arm with single agent trastuzumab, single agent lapatinib as well as the drugs given sequentially. The similar NeoALTTO (Neoadjuvant Lapatinib and/or trastuzumab Treatment Optimization) trial is examining the combination together with single agent lapatinib and trastuzumab in the neoadjuvant setting.

**Other HER2 targeted therapy**

**Pertuzumab**

Pertuzumab is a humanized monoclonal antibody directed against the extracellular dimerization domain of the HER2 receptor, and works by hindering dimerization. The different and potentially complementary mechanism of action is the rationale for combining pertuzumab and trastuzumab. A Phase II trial involving trastuzumab-refractory, HER2-positive metastatic breast cancer demonstrated an objective response rate in 6 of 33 evaluable patients when trastuzumab was combined with pertuzumab. \(^{30}\) This combination has already moved into Phase III trials including the CLEOPATRA (clinical evaluation of pertuzumab and trastuzumab) trial, which is a randomized placebo-controlled trial comparing standard first-line therapy with trastuzumab in combination with docetaxel with or without the addition of pertuzumab in metastatic or locally advanced HER2-positive breast cancer.

**Neratinib**

Neratinib is an oral irreversible TKI of HER1 and 2. Preliminary Phase I data from 25 HER2 or EGFR-positive advanced breast cancer
patients demonstrated a partial response in eight patients. In a Phase II clinical trial of 33 evaluable patients the objective response rate was 27% (95% CI: 13–46%) and the median PFS was 19 weeks (95% CI: 13–46%). There are several ongoing Phase I/II trials investigating this TKI in combination with trastuzumab, or chemotherapy and a randomized placebo-controlled Phase III trial using it in an extended adjuvant trial following trastuzumab is also underway.

**Trastuzumab-DM1**

Trastuzumab-DM1 (T-DM1) is an antibody–drug conjugate that uses trastuzumab to specifically deliver the antimicrotubule agent DM1 to HER2-positive cells. It is hypothesized that after binding to HER2, T-DM1 undergoes receptor-mediated internalization with intracellular release of DM1, thus resulting in enhanced cell killing with reduced toxicity. A Phase I study in 24 patients with HER2-positive metastatic breast cancer who had previously progressed on trastuzumab-based therapy showed clinical activity at the maximum tolerated dose (MTD) of 3.6 mg/kg. The clinical benefit rate (an objective response plus stable disease at 6 months) in 15 patients treated at the MTD was 73%, including five objective responses. Adverse events included elevated hepatic transaminases, thrombocytopenia, anaemia and fatigue, but there were no reports of cardiac toxicity. A Phase II study in 112 patients who had previously received a median of three prior chemotherapy agents and who had progressed on HER2 targeted therapy for metastatic breast cancer showed an objective response of 39.2% after a median follow-up of 4.4 months. The most common Grade 3–4 adverse event was thrombocytopenia (7.1%). Phase III trials are now ongoing. Emilia is a Phase III randomized controlled open-label study comparing the efficacy and safety of T-DM1 versus capecitabine and lapatinib in patients with HER2-positive locally advanced or metastatic breast cancer who have had prior trastuzumab-based therapy.

**mTOR inhibitors**

mTOR is a serine-threonine kinase which is a downstream component of the PTEN/PI3K pathway. The mTOR pathway plays a central role in the regulation of cell growth, proliferation and survival. Furthermore as discussed above, loss of expression of PTEN and/or activation of the PI3K/AKT signaling proteins may be possible mechanisms of trastuzumab resistance. Two mTOR inhibitors are currently under clinical development, with the oral agent everolimus looking the
most promising. In preclinical models it enhanced growth inhibition by trastuzumab in PTEN-deficient cells overcoming resistance to trastuzu-
mab.\textsuperscript{35} It has been evaluated with combinations of trastuzumab and various chemotherapy and endocrine agents in Phase I studies. Two of these studies included everolimus on a daily or weekly schedule com-
bined with weekly trastuzumab and paclitaxel\textsuperscript{36} or vinorelbine,\textsuperscript{37} in patients with trastuzumab-refractory HER2-positive metastatic disease. The most common dose-limiting toxicities were neutropenia and stomatitis. Responses were very encouraging; in the vinorelbine study, 8 responses (1 complete and 7 partial responses) with a further 27 patients showing disease stabilization among 44 evaluable patients was seen. Phase III trials are underway in combination with chemotherapy and trastuzumab.

Although temsirolimus has been shown to have a synergistic effect with endocrine therapy,\textsuperscript{38} a large randomized, placebo-controlled, double-blind Phase III trial in combination with letrozole as first-line hormonal therapy in metastatic disease was closed early following a planned interim analysis due to a lack of efficacy.\textsuperscript{39}

\textbf{Anti-angiogenic therapy}

Angiogenesis is recognized as a key process in the progression and metastasis of breast cancer\textsuperscript{40} and multiple angiogenic factors are commonly expressed by invasive breast cancers.\textsuperscript{41} Vascular endothelial growth factor (VEGF) is one of the most important and is a key regulator of physiological angiogenesis but is also implicated in pathological tumor angiogenesis. It exists as several splice variants with the 121-amino-acid isoform predominating.\textsuperscript{41} Differences in function among the various VEGF isoforms are not well defined but VEGF-A is more potent in inducing vasodilatation and pathological angiogen-

Bevacizumab, is a humanized monoclonal antibody against all iso-
forms of VEGF-A. It has been assessed in a number of clinical trials in the metastatic setting in combination with chemotherapy, but has shown limited efficacy (Table 2). The first reported trial using it in combination with capecitabine in patients previously treated with anthracyclines and taxanes showed an increased objective response rate (19.8 versus 9.1\%, \textit{P} = 0.001) but did not improve PFS (4.9 versus 4.2 months, HR = 0.98, \textit{P} = 0.857).\textsuperscript{45}

Three subsequent randomized Phase III trials using bevacizumab as first-line treatment for locally recurrent or metastatic breast cancer
**Table 2** Summary of bevacizumab trials in metastatic breast cancer.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Median follow-up (months)</th>
<th>Regime</th>
<th>Bevacizumab dose</th>
<th>Number of patients</th>
<th>PFS (mo)</th>
<th>HR</th>
<th>P-value</th>
<th>Response rate (%)</th>
<th>P-value</th>
<th>OS</th>
<th>HR</th>
<th>P-value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Capecitabine</td>
<td>15 mg/kg q3w</td>
<td>230</td>
<td>4.2</td>
<td>4.8</td>
<td>0.98</td>
<td>0.857</td>
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<td>15.1</td>
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<td>Capecitabine + bevacizumab</td>
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<td>232</td>
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<td>Docetaxel + bevacizumab</td>
<td>7.5 mg q3w</td>
<td>248</td>
<td>8.7</td>
<td>6.9</td>
<td>0.0035</td>
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<td></td>
<td>15 mg/kg q3w</td>
<td>247</td>
<td>8.8</td>
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<td>63</td>
<td>0.0001</td>
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<td>AVADO</td>
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<tr>
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<td>Capecitabine</td>
<td>15 mg/kg q3w</td>
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<td>5.7</td>
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<td>21.2</td>
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<tr>
<td></td>
<td></td>
<td>Capecitabine + bevacizumab</td>
<td></td>
<td>409</td>
<td>8.6</td>
<td>6.9</td>
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<td>RIBBON-1</td>
<td>19.2</td>
<td>Anthracyclines/taxanes</td>
<td></td>
<td>207</td>
<td>8</td>
<td></td>
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<td></td>
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<td>23.8</td>
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<tr>
<td></td>
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<td>Anthracycline/taxane + bevacizum</td>
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<td>415</td>
<td>9.2</td>
<td>6.4</td>
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<td>37.9</td>
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L. Tookman and R. Roylance

British Medical Bulletin 2010; 96
have demonstrated a benefit. The E2100 trial used paclitaxel 90 mg/m\(^2\) given D1, D8, D15 every 4 weeks with bevacizumab 10 mg/kg every 2 weeks\(^{46}\) and the AVADO (Avastin and Docetaxel in metastatic breast cancer) study used docetaxel in combination with bevacizumab at 7.5 or 15 mg/kg every 3 weeks.\(^{47,48}\) Both studies showed that the combination arms significantly prolonged PFS compared with single agent chemotherapy; 11.8 months versus 5.9 months; \(P < 0.001\) in E2100 and 8.8 months (15 mg/kg bevacizumab) versus 8.0 months \(P = 0.0001\) in AVADO. There was no difference between the different doses of bevacizumab in this study. There was no difference in OS in either study. A possible explanation for the greater improvement in PFS observed in the combination arm in E2100 compared with the AVADO study may be the metronomic dosing of paclitaxel, which may yield antiangiogenic effects in addition to bevacizumab.

RIBBON-1 evaluated the benefit of bevacizumab with investigator choice chemotherapy of capecitabine, a taxane (either Nab-paclitaxel or docetaxel) or an anthracycline-based regimen.\(^{49}\) After a median follow-up of 15.6 months in the capecitabine cohort, PFS was significantly improved in the combination arm (8.6 months versus 5.7 months, HR 0.69, \(P = 0.0097\)). Similarly for the taxane and anthracycline cohort with a median follow-up of 19.2 months, there was a significantly improved PFS in the combination arm (9.2 months versus 8 months HR 0.64, \(P = 0.0054\)).

Adverse side effects in all these trials seen more frequently in the bevacizumab arm included Grade 3 or 4 hypertension and proteinuria. What is urgently required though are markers predictive of response because although VEGF levels are increased there has been no correlation yet found between levels of VEFR and those women who respond.

Due to the promising results seen in metastatic disease, bevacizumab is now being trialed in the (neo)-adjuvant setting. The Beatrice (bevacizumab adjuvant therapy in triple-negative breast cancer) trial is investigating the addition of bevacizumab to local institution choice of adjuvant chemotherapy regimes in patients with triple-negative breast cancer (i.e. oestrogen, progesterone and HER2 negative). Following completion of chemotherapy, the bevacizumab is continued for a total duration of 1 year and has just successfully completed accrual. Artemis (Avastin randomized trial with neoadjuvant chemotherapy for patients with early breast cancer) is examining the role of adding bevacuzimab to docetaxel given three weekly for three cycles followed by FE(100)C chemotherapy given three weekly for three cycles in the neoadjuvant setting, with patients receiving four cycles of bevacuzimab.

The combination of HER2-targeted therapies and angiogenesis inhibitors has been investigated, as HER2 overexpression is associated
with an increase in VEGF levels in primary breast cancers and preclinical studies have demonstrated cross-talk between VEGF and HER2 pathways\textsuperscript{50} and a synergistic effect between bevacizumab and trastuzumab.\textsuperscript{51} In a Phase II trial combining trastuzumab and bevacizumab in patients with HER2-positive metastatic breast cancer of 37 evaluable patients, the overall response rate was 54.1\text%.\textsuperscript{52} Early data from a 50-patient single-arm study evaluating lapatinib with bevacizumab\textsuperscript{53} in heavily pretreated HER2-positive metastatic breast cancer patients showed signs of clinical activity with a 12-week PFS rate of 62\% and negligible evidence of cardiotoxicity. This is also being evaluated in the BETH adjuvant trial which aims to assess the efficacy and safety of adding bevacizumab to chemotherapy (docetaxel and carboplatin or 5-fluorouracil, epirubicin, cyclophosphamide) plus trastuzumab in patients with node-positive or high-risk node-negative, HER2-positive breast cancer.

**VEGF receptor TKIs**

In addition to those already discussed, a number of other TKIs are currently under investigation including sunitinib, sorafenib, vatalanib, pazopanib, cediranib, motesanib and axitinib. All these agents are multi-targeted, inhibiting numerous other receptor TK in addition to VEGFR. To date most data are available for sunitinib, an oral multi-targeted TKI of VEGFR and platelet-derived growth factor receptor. However, despite promising Phase II data, a randomized Phase III trial comparing sunitib with capecitabine in metastatic breast cancer has closed early due to lack of efficacy.\textsuperscript{54}

**PARP inhibitors**

Early studies of a new class of drug, which inhibits PARP (poly adenosine-diphosphate (ADP)-ribose), have created some very interesting data. The PARP family of proteins, of which there may be as many as 18 members are characterized by the enzymatic property of PARP. This reaction uses NAD\textsuperscript{+} as a substrate and catalyses the addition of long, branching chains of poly (ADP-ribose) polymers to target proteins. The activity of the PARP superfamily are responsible for a range of cellular processes but PARP-1 is responsible for at least 80\% of total cellular PARP activity and together with PARP-2, constitutes the DNA damage response arm of the PARP family.

DNA damage can be either single- or double-strand breaks (SSB or DSB, respectively) and can occur as a result of normal cell functioning.
It is repaired through a number of different pathways. Although PARP-1 binds to both SSB and DSB, its role in SSB via base excision repair (BER), is most clearly defined. BER plays an important role in repairing damaged bases that occur as a consequence of normal cell function but also as a result of damage induced by alkylating agents and ionizing radiation. When PARP is inhibited, unrepaired SSBs are converted into DSB during DNA replication, which are normally repaired by the error-free homologous recombination pathway.

BRCA1 and 2 dysfunction has been shown to sensitize cells to the inhibition of PARP enzymatic activity, resulting in chromosomal instability, cell cycle arrest and subsequent apoptosis. BRCA1 and 2 are important for DSB repair by homologous recombination. Cells carrying heterozygous loss-of-function BRCA mutations can lose the remaining wild-type allele, resulting in deficient homologous recombination DNA repair, which causes genetic aberrations that drive carcinogenesis. The result is a tumor that carries a DNA defect that is not shared by the normal tissues of the patient, which can be exploited by PARP inhibitors. Thus, in tumor cells with deficient homologous recombination repair, following PARP inhibition unrepaired SSB will cause accumulation of DSBs which cannot be repaired. In normal tissues, with one functioning BRCA gene, homologous repair pathways will be intact and so DSBs can be repaired and have a sensitivity to PARP inhibitors similar to that of the wild-type cells. This demonstrates synthetic lethality where there is a potent and lethal synergy between two otherwise non-lethal events and is a novel approach to cancer therapy.

A Phase I trial of olaparib, an oral PARP inhibitor has shown both promising efficacy and an acceptable side effect profile. Of the 60 treated patients, 22 were BRCA1 or 2 mutation carriers, and objective tumor response was only seen in mutation carriers. Nine BRCA carriers (with breast, ovarian or prostate cancer) had a response according to RECIST, with the response sustained for more than 76 weeks in one patient. Adverse side effects were largely Grade 1 or 2 and included nausea (32%), fatigue (30%) and anorexia (12%) and significantly BRCA mutation carriers did not have an increased risk of side effects. Preliminary data from a randomized Phase II trial of the PARP inhibitor, BSI-201 in combination with gemcitabine plus carboplatin in patients with triple-negative metastatic breast cancer, which shares molecular and pathological features with BRCA1-related cancers, has also shown very promising efficacy. Analyses of the first 86 of a planned 120 patients showed that with the addition of BSI-201, there were improved clinical benefit rate (52 versus 12% \(P = 0.0012\)), median PFS (211 versus 87 days HR 0.30 (0.15–0.59) \(P = 0.0003\) and median OS (254 days versus 169 days HR 0.24 (0.09–0.61) \(P = 0.0012\)). A Phase III trial is ongoing.
Conclusion

This review has discussed a number of new targeted therapies. Some have already become the standard of care, while others remain in various stages of clinical development. It is likely that the outcome of these trials will result in altered patient management just as the introduction of trastuzumab has altered significantly the management of HER2-positive breast cancer. There remain however many unanswered questions with regard to timing of these therapies, their combination with chemotherapy and how to manage resistance which requires further investigation. Another significant issue is cost, and how these expensive therapeutic options will be funded. Within the UK only trastuzumab is currently NICE approved and therefore widely available within the National Health Service. The development of further targeted therapies means funding will be a major determinant to access of these potentially effective but expensive treatment options.

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