

## HER2-family signalling mechanisms, clinical implications and targeting in breast cancer.

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2 **HER2-family signalling mechanisms, clinical implications and**  
3 **targeting in breast cancer**

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**Abstract** Approximately 20 % of human breast cancers (BC) overexpress HER2 protein, and HER2-positivity is associated with a worse prognosis. Although HER2-targeted therapies have significantly improved outcomes for HER2-positive BC patients, resistance to trastuzumab-based therapy remains a clinical problem. In order to better understand resistance to HER2-targeted therapies in HER2-positive BC, it is necessary to examine HER family signalling as a whole. An extensive literature search was carried out to critically assess the current knowledge of HER family signalling in HER2-positive BC and response to HER2-targeted therapy. Known mechanisms of trastuzumab resistance include reduced receptor-antibody binding (MUC4, p95HER2), increased signalling through alternative HER family receptor tyrosine kinases (RTK), altered intracellular signalling involving loss of PTEN, reduced p27kip1, or increased PI3 K/AKT activity and altered signalling via non-HER family RTKs such as IGF1R. Emerging strategies to circumvent resistance to

HER2-targeted therapies in HER2-positive BC include co-targeting HER2/PI3 K, pan-HER family inhibition, and novel therapies such as T-DM1. There is evidence that immunity plays a key role in the efficacy of HER-targeted therapy, and efforts are being made to exploit the immune system in order to improve the efficacy of current anti-HER therapies. With our rapidly expanding understanding of HER2 signalling mechanisms along with the repertoire of HER family and other targeted therapies, it is likely that the near future holds further dramatic improvements to the prognosis of women with HER2-positive BC.

**Keywords** Trastuzumab · HER2 · Breast cancer · PI3 K

### Introduction

BC is the second most common cancer in the world, and the fifth highest cause of cancer mortality worldwide [1]. 20 % of human BC's overexpress HER2, and HER2-positivity is associated with a significantly worse prognosis. HER2 first became targetable in patients with trastuzumab (Herceptin, <sup>TM</sup> Genentech/Roche), a monoclonal antibody that has significantly improved outcomes for patients with HER2-positive BC, but the efficacy of trastuzumab is limited in some patients by acquired and *de novo* resistance [2].

### HER family signalling

There are 20 known RTK families: since members of over half of these have been found to be mutated or overexpressed in diseases marked by abnormal proliferation, RTK's have been considered potential targets for cancer therapy. HER2, a type 1 transmembrane protein RTK, and

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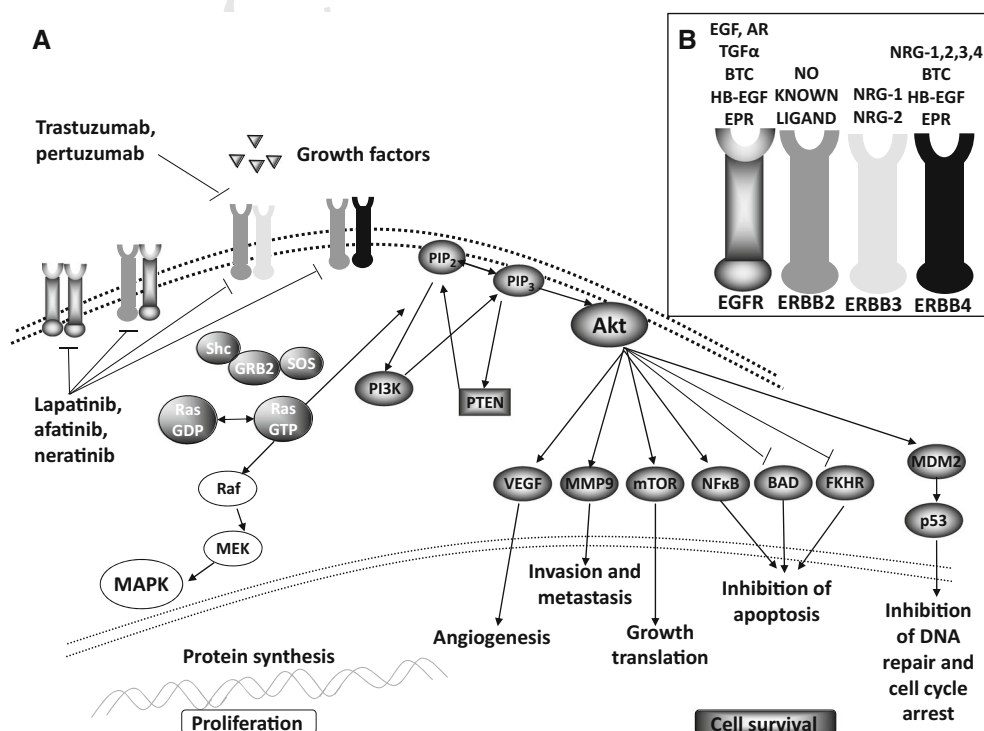
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57 an oncogenic driver of the growth of HER2-positive BC, is  
 58 associated with a shorter time to relapse and decreased  
 59 overall survival (OS). A meta-analysis in 2003 found that  
 60 of 81 studies spanning sixteen years of research and  
 61 incorporating 27,161 patients [3], HER2 overexpression  
 62 predicted a worse BC outcome. In contrast to the other  
 63 known HER family members, those being epidermal  
 64 growth factor receptor (EGFR/HER1), HER3 and HER4,  
 65 no ligand has yet been identified for HER2 (Fig. 1). When  
 66 overexpressed, HER2 exists in a constitutively open con-  
 67 formation, leaving it intrinsically capable of interacting  
 68 with available RTK binding partners even in the absence of  
 69 ligand [4]. HER family ligands induce quantitative differ-  
 70 ences in receptor phosphorylation but quantitatively similar  
 71 physiological responses, suggesting that the identity of  
 72 activated receptors, rather than the number of activated  
 73 receptors, determines the cellular response [5]. Coordi-  
 74 nated overexpression of EGFR and HER2 frequently  
 75 occurs in HER2-positive BC. Expression profiling has  
 76 identified at least two subgroups within HER2-positive  
 77 primary breast tumours. Many of the differently expressed  
 78 genes track with oestrogen receptor (ER) status, suggesting  
 79 that HER2+/ER+ and HER2+/ER- represent two distinct  
 80 entities [6].

81 HER2 dimerization is mediated by the formation of  
 82 disulphide bonds between cysteine residues in the juxta-  
 83 membrane region, and disrupting these disulphide bonds  
 84 disrupts the ability of HER2 to transform cells [7].  
 85 Phosphorylated tyrosine residues on the receptor molecule

serve as recognition and docking sites for SH2-containing  
 86 proteins. These serve as linker molecules, recruiting  
 87 components of downstream signalling pathways, such as  
 88 the phosphoinositide-3-kinase (PI3 K) pathway, through  
 89 which the activated RTK exerts its biological effect(s)  
 90 (Fig. 1). HER family signalling is governed by a strict  
 91 hierarchy, with HER2 the preferred dimerization partner  
 92 of all other HER family members [8]. Cells transformed  
 93 by HER2 display increased tyrosine phosphorylation of  
 94 both HER2 and other proteins [9], and a recent study  
 95 identified a subset of patients which were classed as  
 96 HER2-negative by FISH analysis yet displayed HER2  
 97 activation that was coincident with EGFR and HER3  
 98 activation ( $n = 415$ ) [10]. HER2/HER3 heterodimers have  
 99 been proposed to be the main oncogenic unit in HER2-  
 100 positive BC, with HER3 coupling activated HER2 to the  
 101 downstream PI3 K and other pathways [11]. There is a  
 102 correlation between simultaneous high HER2 and high  
 103 HER3 levels and reduced sensitivity to trastuzumab [12].  
 104 Further synergistic targeting of HER2 and HER3 was  
 105 demonstrated to achieve higher therapeutic efficacy [13],  
 106 and the HER3 ligand neuregulin confers resistance to  
 107 chemotherapy and has recently been implicated as a  
 108 potential mechanism of resistance to T-DM1 [14]. In  
 109 contrast, some studies suggest a tumour suppressor role  
 110 for HER4 in HER2-positive BC, although this is likely to  
 111 be isoform specific and context specific [15]. A recent  
 112 study suggested that the localisation of HER4 may play a  
 113 role in its activity, with nuclear, but not cytoplasmic  
 114

**Fig. 1** Overview of HER family signalling in HER2-positive breast cancer including known HER family ligands, the potential dimerization partners of the HER family members and components of the downstream MAPK and PI3 K pathways, and the targeted therapies that are currently in testing or in use to treat HER2-positive breast cancer



115 HER4 associated with poorer survival and trastuzumab  
116 resistance [16].

117 Current HER2-targeted therapies

118 *First generation HER2-targeted agents*

119 The first indication that HER2-targeted therapy could attain  
120 high specificity and avoid off-target toxicity came when  
121 murine antibodies against HER2 were shown to selectively  
122 inhibit growth of *neu*-transformed cells, but not ras-trans-  
123 formed cells [17]. Subsequently, a humanised mAb against  
124 HER2 inhibited proliferation of HER2-amplified cells  
125 in vitro, and enhanced the antitumour effect of paclitaxel  
126 and doxorubicin in xenograft models of HER2-positive BC  
127 [18]. That trastuzumab significantly improves outcomes for  
128 HER2-positive BC patients is now well established  
129 (Table 1), although its mechanism of action remains  
130 incompletely defined.

131 Potential mechanisms include inhibition of HER2  
132 dimerization [4], inhibition of cleavage of the ectodomain  
133 of activated HER2 [19], induction of p27<sup>KIP1</sup> [20], inhibi-  
134 tion of PI3 K signalling, downregulation of HER2 leading  
135 to enhanced apoptosis mediated by tumour necrosis factor  
136 alpha-related apoptosis inducing ligand [21], and antibody-  
137 dependent cell-mediated cytotoxicity [22]. Trastuzumab-  
138 mediated internalisation and degradation of HER2 may  
139 inhibit receptor signalling, although some studies report  
140 that receptor levels are unaffected by trastuzumab treat-  
141 ment [23]. Despite its benefits, trastuzumab is limited in  
142 some patients by *de novo* and acquired resistance, and  
143 because it cannot cross the blood-brain barrier. Approxi-  
144 mately 35 % of metastatic HER2-positive BC patients  
145 treated with trastuzumab go on to develop brain metastases  
146 [24].

147 Lapatinib (Tykerb,<sup>TM</sup> GlaxoSmithKline) is an orally  
148 bioavailable small molecule tyrosine kinase inhibitor (TKI)  
149 targeted to EGFR and HER2. Pre-clinical [25] and clinical  
150 [26] evidence shows that lapatinib is effective against  
151 trastuzumab-resistant HER2-positive BC, and it is cur-  
152 rently used as subsequent therapy for patients with disease  
153 that has progressed on trastuzumab. Lapatinib inhibits  
154 HER2 phosphorylation more strongly than trastuzumab,  
155 and unlike trastuzumab, it inhibits extracellular signal-  
156 related kinase (Erk) 1 and 2 as well as PI3 K in vivo [27,  
157 28]. Lapatinib inhibited tumour growth in p95HER2-  
158 overexpressing pre-clinical mouse models and has shown  
159 clinical benefit in patients refractory to trastuzumab whose  
160 tumours overexpressed p95HER2 ( $n = 537$ ) [26]. It  
161 inhibits the development of brain metastases in vivo [24/  
162 and has modest activity against HER2-positive brain  
163 metastases clinically ( $n = 242$ ) [29]. Trastuzumab and la-  
164 patinib have complementary mechanisms of action, and the

165 combination of both [30, 31] confers an OS benefit in  
166 patients with heavily pretreated, trastuzumab-resistant  
167 HER2-positive metastatic BC compared to lapatinib  
168 monotherapy [32]. However, the success of lapatinib has  
169 been hit by a number of recent disappointing clinical trial  
170 results including the adjuvant study ALTO [33], a number  
171 of neoadjuvant studies, and the NCIC CTG first-line met-  
172 astatic study [34] (Table 1). These studies, along with the  
173 success of pertuzumab and T-DM1, mean that lapatinib's  
174 place in the clinic remains in patients with HER2-positive  
175 metastatic BC, who have received at least 1–2 prior lines of  
176 therapy for metastatic disease.

177 *Second generation HER2-targeted agents*

178 Pertuzumab (Omnitarg<sup>TM</sup>, Genentech) is a humanised  
179 monoclonal antibody which binds to HER2's extracellular  
180 domain II, which is involved in dimerization [4]. This is in  
181 contrast to trastuzumab, which binds to domain IV. Pert-  
182 uzumab thus blocks HER2/HER3 interaction, diminishes  
183 ligand-activated HER2 signalling in BC cell lines, and  
184 inhibits the growth of high- and low-HER2-expressing  
185 HER2-positive breast xenografts *in vivo* [35, 36]. The  
186 combination of trastuzumab and pertuzumab in vivo results  
187 in an additive increase in ADCC and marked regression of  
188 metastatic HER2-positive BC in treated animals [37]. In  
189 clinical trials, pertuzumab significantly improved patient  
190 outcomes when added to trastuzumab and docetaxel in  
191 first-line metastatic HER2-positive BC and in the neoad-  
192 juvant setting (Table 1). Other trials with pertuzumab are  
193 ongoing (Table 3).

194 Trastuzumab-emtansine (T-DM1, Genentech) is an  
195 antibody-drug conjugate (ADC) which links trastuzumab to  
196 a highly cytotoxic maytansinoid agent, emtansine, which  
197 binds tubulin and arrests mitosis at metaphase [38]. Fol-  
198 lowing the binding of T-DM1 to HER2, receptor-mediated  
199 internalisation transports it to the cytoplasm, where lyso-  
200 somal degradation releases and activates the cytotoxic  
201 agent [39]. In addition to the anti-mitotic properties of  
202 emtansine, T-DM1 retains the mechanisms of action of  
203 trastuzumab including initiation of ADCC, inhibition of  
204 HER2 shedding and downregulation of PI3 K/AKT path-  
205 way activity, and is effective in models of lapatinib-  
206 resistance in vitro [40]. TDM-1 is now in clinical use in the  
207 second-line setting in metastatic HER2-positive BC based  
208 on the results of the EMILIA study [41] (Table 1).

209 *The role of immunology in HER2-targeted therapy*

210 There is compelling pre-clinical evidence of the impor-  
211 tance of the immune response in the efficacy of  
212 trastuzumab in HER2-positive disease, and from a clinical  
213 perspective, data point to HER2-positive and triple

**Table 1** Significant clinical trials of HER2-targeted therapies completed to date in HER2-positive BC

Clinical trial	Details	Sample size	Findings
<b>Trastuzumab-based trials</b>			
H0648 g [73]	Adjuvant chemotherapy ± trastuzumab	469	Relative risk of death reduced by 20 % and longer time to disease progression with trastuzumab (7.4 vs 4.6 months, $P < 0.001$ )
HERA [74]	Adjuvant chemotherapy ± trastuzumab	4,482	24 % reduction of recurrence with trastuzumab ( $P < 0.0001$ )
NCCTG N9831/NSABP B-31 [75]	Adjuvant chemotherapy ± trastuzumab	4,405	48 % relative reduction in disease-free survival (DFS) ( $P < 0.001$ ) and 39 % relative reduction in overall survival (OS) events ( $P < 0.001$ ) with trastuzumab
BCIRG 006 [76]	Adjuvant chemotherapy ± 1 year of trastuzumab	3,222	One year of trastuzumab significantly improved DFS (8 vs 75 %, $P < 0.001$ ) and OS (92 vs 87 %, $P < 0.001$ )
NOAH [77]	Neoadjuvant chemotherapy ± neoadjuvant trastuzumab	235	Almost double the rate of pCR to therapy, significantly improved event-free survival with trastuzumab (71 vs 56 %, $P = 0.013$ )
<b>Lapatinib-based trials</b>			
NCT00078572 [78]	Capecitabine ± lapatinib in HER2+ metastatic BC patients with disease that had progressed on trastuzumab	399	51 % reduced risk of progression and improved DFS (8.4 months vs 4.4 months, $P < 0.001$ ) without a significant OS improvement with lapatinib
NSABP B-41 [79]	Neoadjuvant lapatinib plus chemotherapy vs neoadjuvant trastuzumab plus chemotherapy	519	Similar pCR rates between trastuzumab (52.5 %) and lapatinib (53.2 %), and a non-significant increase in pCR ( $P = 0.095$ ) with both trastuzumab and lapatinib compared to the use of either alone
CHER-LOB [50]	Neoadjuvant chemotherapy plus trastuzumab, lapatinib, or both	121	Significant relative increase in pCR (80 %, $P = 0.019$ ) with both trastuzumab and lapatinib compared to either alone
ALTT0 [33]	One year of trastuzumab alone, one year of lapatinib alone, their sequence or combination in the adjuvant treatment of HER2-positive early BC	8,381	Nonsignificant reduction in DFS with both lapatinib and trastuzumab compared to trastuzumab alone (88 % vs 86 %, $P = 0.048$ )
NeoALTT0 [80]	Neoadjuvant trastuzumab, lapatinib or a combination of both	455	Significantly improved pCR ( $P < 0.01$ ) to the combination of trastuzumab and lapatinib (51.3 %) compared to that of trastuzumab alone (29.5 %)
NCIC CTG [34]	Lapatinib + chemotherapy vs trastuzumab + chemotherapy as first-line treatment for women with metastatic HER2-positive BC	636	Significantly reduced PFS ( $P = 0.01$ ) with lapatinib + chemotherapy compared to trastuzumab + chemotherapy (8.8 months compared to 11.4 months)
<b>Pertuzumab-based trials</b>			
CLEOPATRA [81]	Trastuzumab and docetaxel ± pertuzumab in first-line treatment of HER2-positive metastatic BC patients	808	Prolonged DFS, significantly improved OS (17.2 vs 23.6 %, $P = 0.005$ ) and 34 % reduced risk of death with pertuzumab
NeoSphere [77]	Neoadjuvant trastuzumab and docetaxel ± pertuzumab in women with locally advanced, inflammatory or early HER2-positive BC	417	Significantly higher pCR with pertuzumab (45.8 vs 29.0 %, $P = 0.014$ ). Further, 17 % of patients given trastuzumab and pertuzumab without chemotherapy achieved pCR

Table 1 continued

Clinical trial	Details	Sample size	Findings
T-DM1-based trials EMILIA [44]	T-DM1 vs capecitabine and lapatinib in patients with advanced HER2-positive BC who had previously been treated with trastuzumab	991	Significantly improved PFS (9.6 months vs 6.4 months, $P < 0.001$ ) and increased OS (30.9 months vs 25.1 months, $P < 0.001$ ) with T-DM1
Neratinib-based trials Phase II multicentre trial [82]	Efficacy and safety of neratinib in patients with advanced HER2-positive BC	136	Neratinib was well-tolerated, with ORR of 24 % (prior trastuzumab treatment) and 56 % (trastuzumab-naïve)
Phase I/II study of Neratinib [83]	Neratinib in combination with trastuzumab in patients with advanced solid tumours	33	Neratinib in combination with trastuzumab is well tolerated and has a 27 % ORR
Afatimib-based trials Phase II Afatinib trial [70]	Safety and efficacy of afatinib monotherapy in patients who had progressed on trastuzumab treatment	52	Afatimib monotherapy induced PR and maintenance of stable disease

*DFS* disease-free survival, *ORR* objective response rate, *OS* overall survival, *pCR* pathologic complete response, *PR* partial response, *PFS* progression-free survival

negative as the breast cancer subtypes with the most consistent association between immune infiltration and good prognosis [42]. The interaction of monoclonal antibody therapies with Fc $\gamma$  receptors expressed on effector immune cells is the basis of ADCC, [43] and the association between tumour-infiltrating lymphocytes and benefit from trastuzumab and chemotherapy has been observed in The FinHER and GeparQuattro trials [44, 45]. Prospective analysis of BIG 02-98 showed increasing stromal lymphocyte infiltration (10 % increments) was related to benefit from adjuvant anthracycline-only chemotherapy in HER2-positive disease [46] suggesting lymphocyte predominant BC status may have repercussions for anticipated response to classical chemotherapies as well as newer targeted therapies. Cytotoxic drugs may also alter the immune response directly and these effects may play a major role in the efficacy of chemotherapy [47].

Efforts have been made to improve the effector function of mAb therapies as a strategy to enhance their efficacy. Afucosylated trastuzumab has shown enhanced ADCC function and efficacy in vitro and in vivo pre-clinical tests [48]. Margetuximab (MGAH22) is an Fc-optimised anti-HER2 antibody proteolytic cleavage has been shown to reduce the ADCC function of trastuzumab in a pre-clinical study and could be the basis for reduced trastuzumab efficacy in matrix metalloprotease-rich tumours [49]. Protease resistant antibodies maintaining effector function are being developed [50].

An IgE-homologue of trastuzumab (containing an epsilon in the place of the gamma-1 heavy chain constant region) has been shown to initiate monocyte-mediated ADCC against HER2-positive breast cancer cells [51]. Trastuzumab IgE also induced mast cell degranulation which is capable of triggering a potent antitumour immune response in vivo with pre-clinical studies point to improved efficacy compared to IgG1 equivalents providing support for clinical evaluation [52].

CD137, a member of the tumour necrosis factor (TNF) receptor family, is upregulated on human natural killer cells following exposure to trastuzumab-treated HER2-positive tumour cells [53]. In vitro and in vivo studies have shown that the ADCC response to monoclonal antibody therapies including trastuzumab is augmented through stimulation of the CD137 receptor on NK cells with an agonistic antibody therapy [53–55]. Anti-CD137 agonistic antibodies are currently in Phase I and II clinical trials [42].

#### Adaptive immune response

Murine models have been used to exhibit the importance of Fc $\gamma$  receptors and T cells in an effective response to trastuzumab in vivo, providing the basis of a link between NK cell induced trastuzumab-mediated ADCC and the

**Table 2** Mechanisms of Resistance HER2-targeted therapies in HER2-positive BC

Biomarker	Mechanism	Known to mediate resistance to	Shown in vitro	Shown in vivo	Clinical studies	Possible targeting strategies
PIK3CA mutation	HER2-independent activation of the PI3 K pathway downstream from HER2	Trastuzumab/Lapatinib	Yes [2]	Yes [51]	Yes [2, 46, 54, 56]	Cotarget PI3 K/HER2
PTEN loss	HER2-independent activation of the PI3 K pathway downstream of HER2	Trastuzumab/Lapatinib	Yes [51, 84]	Yes [51]	Yes [46]	Cotarget PI3 K/HER2
p95HER2	Lacks extracellular antibody binding domain but retains full kinase activity	Trastuzumab	Yes [85]	Yes [85, 86]	Yes [12] [49, 87]	Lapatinib/novel TKI's
MUC4	Masks trastuzumab binding site	Trastuzumab	Yes [45]	Yes [45]	No	Lapatinib/novel TKI's
MET receptor	Upregulates AKT and abrogates p27 induction in response to trastuzumab	Trastuzumab	Yes [88]	No	Yes [89]	MET inhibition
IGF1R	Heterodimerizes with HER2 to activate downstream signalling	Trastuzumab	Yes [47]	No	Yes [47]	Co-target IGF1R/HER2
Inhibition/loss of P27Kip1	Impairs anti-HER2 antibody induced cell cycle arrest, thereby increasing proliferation	Trastuzumab	Yes [20]	No	Yes [90]	None currently available

*IGF1R* insulin-like growth factor-1 receptor, *MUC4* mucin-4, *PTEN* phosphatase and tensin deleted in chromosome 10

**Table 3** Important ongoing clinical trials with novel HER2-targeted therapies in HER2-positive BC

Trial	Setting	Sample size	Aims/arms/investigation	Results expected
Pertuzumab-based trials				
Pherexa	HER2-positive BC patients who progressed following trastuzumab	450	Trastuzumab and capecitabine ± pertuzumab	June 2017
Aphinity	Early stage HER2-positive BC	3,806 (estimated enrolment)	Adjuvant chemotherapy and trastuzumab ± pertuzumab	December 2023
T-DM1-based trials				
Marianne	Metastatic HER2-positive BC	1,095	Combination pertuzumab and T-DM1	April 2016
Katherine	HER2-positive BC with residual tumour in breast/lymph nodes following preoperative therapy	1,484 (estimated enrolment)	Adjuvant trastuzumab vs adjuvant T-DM1	March 2023
Neratinib-based trials				
ExteNET	Early stage HER2-positive BC	2,842	Neratinib after adjuvant trastuzumab on overall survival	Completed, not yet reported
NALA	Metastatic HER2-positive BC	600 (estimated enrolment)	Neratinib plus capecitabine vs lapatinib plus capecitabine	May 2018
Afatinib-based trials				
Lux-Breast 1	HER2-positive metastatic BC patients who have progressed on trastuzumab	508 (estimated enrolment)	Afatinib plus vinorelbine vs. trastuzumab plus vinorelbine	June 2014
Lux-Breast 3	HER2-positive BC patients with brain metastasis	120	Vinorelbine ±/− Afatinib	September 2014

270 adaptive immune response [56]. A more recent study has  
271 shown that tumour cells from patients expressing a breast  
272 cancer stem cell-related marker (ALDH1) evade direct NK

cell cytotoxicity through downregulation of the NKG2D  
ligands, MICA and MICB resulting in increased metastases  
[57]. Increases in NK2GD and DNAM1 ligands in response

276 to taxane treatment have been shown to increase trastuzumab-mediated ADCC in HER2-positive cell line models [58]. This may provide further indications for the importance of trastuzumab alone and in combination with chemotherapy in the treatment of HER2-expressing breast cancer.

282 Monoclonal antibody therapies like trastuzumab, pertuzumab, TDM1 and anti-PD-1/PD-L1 could be considered passive immunotherapies. The exclusive localisation of HER2 overexpression in tumours also makes HER2 an attractive target for active immunotherapies. Some patients are capable of producing a specific anti-HER2 response involving cellular and humoral immunity [59, 60]. Peptide-based vaccines aim to elicit an immune response using epitopes from tumour-associated antigens. E75, consisting of HER2 amino acids 369–377, is the most extensively studied peptide-based vaccine in the clinic. Phase I studies in the metastatic setting showed that the combination of E75 with an adjuvant was safe and generated cytotoxic T lymphocyte responses [61]. Combined analysis of two trials combining escalating E75 doses and GM-CSF in the adjuvant setting found that DFS was 94 versus 79.4 % in the vaccine group and control group, respectively, at 24 months, [62] and a trend towards reduced recurrence was observed in optimally dosed patients. [63] Protein-based vaccines utilise entire or truncated forms of HER2 in order to take advantage of HLA-I and HLA-II class epitopes within HER2 and therefore potentially activate a CD4+ T cell response. One clinical study has reported limited tumour regression (2/42 patients) [64, 65]. DNA vaccines and whole cell (autologous or allogeneic) vaccines are designed to interact with antigen presenting cells (APCs) with subsequent activation of T cells. These have been shown to produce a pronounced immune response which included antibody production with no dose limiting toxicity in the metastatic setting ( $n = 28$ ) [66]. Dendritic cells (DC) are potent APCs, expressing HLA-CLASS I and II, T cell co-stimulatory factors and producing T cell stimulating cytokines [67]. Dendritic cell vaccines are in the preliminary phase of development. Clinical studies examining combinations of active and passive immunotherapies are ongoing with the hope that these combinations will produce increased immunological responses [68].

### 320 Mechanisms of resistance to HER2-targeted therapies

321 Many potential mechanisms of trastuzumab resistance in HER2 positive BC have been proposed (Table 2); these include reduced receptor-antibody binding due to increased HER2 masking [69]; increased signalling through alternative HER family RTKs [12]; altered intracellular signalling involving loss of PTEN, reduced p27kip1, or increased

PI3 K/AKT activity (e.g. by PIK3CA mutations) [70]; and altered signalling via non-HER family RTKs [71, 72]. P95HER2, which lacks an extracellular domain but retains kinase activity, has been proposed as a mechanism of resistance [73]. However, it was not shown to have a significant association with pCR clinically, [74] and difficulties in developing a robust clinical assay for p95HER2 have prevented its introduction as a clinically relevant biomarker.

Clinical studies provide strong evidence that the PI3 K pathway is involved in trastuzumab resistance, reflecting in vitro observations that the PI3 K pathway is involved in both trastuzumab and lapatinib resistance [75]. Pre-clinical studies have demonstrated that AKT can be activated independently of HER2 [2]. Such HER2-independent PI3 K pathway activation may result from aberrant RTK signalling upstream of PI3 K, PTEN loss or PIK3CA mutations and lead to less dependency on HER family signalling for tumorigenesis [75], indicating that HER2 inhibition without co-inhibition of the PI3 K pathway may not be sufficient to inhibit tumour growth in some HER2-positive BC's. Patients with PI3 K pathway activation in their HER2-positive BC have shorter OS and a worse response to trastuzumab [70, 76]. Although some reports are conflicting in this regard [77], [78], PIK3CA mutations have been shown to predict resistance to HER2-targeted therapy-based regimens in primary HER2-positive BC [2, 79], with one study suggesting that this effect is restricted to cancers that are HER2+/ER+ [80].

### Targeting the PI3 K pathway

Pre-clinical data consistently suggest that targeting PI3 K pathway signalling nodes downstream from HER2 (e.g. mammalian target of rapamycin (mTOR) or PI3 K itself) in addition to targeting HER2 will overcome resistance of HER2-amplified BC to HER2-targeted therapies in some cases [81].

mTOR, a serine/threonine kinase, is a downstream component of the PI3 K pathway. The mTOR inhibitor everolimus (Afinitor,™ Novartis) improves the antitumour efficacy of trastuzumab [82]. However, the added efficacy of everolimus in combination with trastuzumab and vinorelbine in the metastatic setting was disappointing in the phase 3 clinical trial BOLERO-3 [82]. mTOR may thus not be not an optimal target for inhibiting the PI3 K pathway as mTOR is only one downstream target of PI3 K. Furthermore, targeting mTOR leads to feedback loop-induced AKT activation, shown to significantly decrease the antitumour efficacy of mTOR inhibition [83].

Therefore newer inhibitors of PI3 K and AKT are being investigated in combination with HER2-targeted therapies in HER2-positive BC. Examples include copanlisib, a pan-



378 class 1 PI3 K inhibitor, GDC-0941, and dual PI3 K/mTOR  
 379 inhibitors GDC0980 and NVP-BEZ235 [84]. Such com-  
 380 pounds show clear in vitro and in vivo efficacy [85] and are  
 381 in early clinical trials in HER2-positive breast and other  
 382 cancers, [86] both alone and in combination with trast-  
 383 uzumab[87]. The combination of the PI3 K inhibitor  
 384 buparlisib (BKM120) and trastuzumab was recently shown  
 385 to be well tolerated with preliminary signs of clinical  
 386 activity in HER2-positive BC patients with trastuzumab-  
 387 resistant disease [88]. In this trial, pharmacodynamic  
 388 studies showed inhibition of both the PI3 K and MAPK  
 389 pathways.

### 390 Novel HER2-targeted therapies

391 Some early phase clinical trials (Table 1) suggest encour-  
 392 aging efficacy for the novel HER2-directed TKI's neratinib  
 393 and afatinib in HER2-positive BC. Neratinib is an irre-  
 394 versible TKI against EGFR and HER2. It potently inhibits  
 395 HER2 and EGFR kinase activity, MAPK and AKT phos-  
 396 phorylation, and enhances p27 induction in vitro, and  
 397 inhibits the growth of HER2-positive tumours in vivo [89].  
 398 Unlike trastuzumab, it decreases phosphorylation of EGFR,  
 399 HER2, HER4 and ERK, and the addition of neratinib to  
 400 trastuzumab overcomes trastuzumab resistance in vitro  
 401 [90]. The combination of neratinib and vinorelbine has  
 402 shown significant antitumour effects with no synergistic  
 403 toxicity [91].

404 Somatic, including activating, HER2 mutations have  
 405 recently been found to be present at a low frequency in  
 406 HER2-negative BC [92], suggesting that HER2-targeted  
 407 therapy may benefit some patients who are HER2-negative  
 408 but bear HER2 somatic mutations. Several of those muta-  
 409 tions were associated with resistance to lapatinib; one  
 410 mutation increased the phosphorylation of EGFR and  
 411 HER3, suggesting that HER2 signalling could be activated  
 412 by HER2 somatic mutations as well as by HER2 gene  
 413 overexpression. Neratinib potently inhibited the growth of  
 414 cells bearing these HER2 mutations, including those  
 415 associated with lapatinib resistance.

416 Afatinib is a TKI which irreversibly binds EGFR and  
 417 HER2. Afatinib inhibits ligand-dependent phosphorylation of  
 418 HER3 [93], and demonstrates antitumour activity in patients  
 419 with HER2-positive BC who have progressed on trastuzumab  
 420 [94]. It may also have potential to treat some patients with  
 421 triple-negative BC, due to its anti-EGFR activity [95]. Afati-  
 422 nib monotherapy may have a higher overall response rate  
 423 compared to both trastuzumab and lapatinib monotherapy in  
 424 treatment naïve patients with HER2-positive, locally  
 425 advanced BC [96]. Table 3 lists some ongoing clinical trials  
 426 with neratinib and afatinib in HER2-positive BC.

### Conclusion

427  
 428 The HER family is a group of related RTKs that signal  
 429 cooperatively to mediate oncogenic effects. One member,  
 430 HER2, is overexpressed by gene amplification in approxi-  
 431 mately 20 % of human BC. Although the established  
 432 HER2-targeted therapies trastuzumab and lapatinib have  
 433 had some success, resistance remains a clinical problem.  
 434 Emerging strategies to circumvent this resistance include  
 435 co-targeting the PI3 K pathway and HER family, pan-HER  
 436 family inhibition, and novel therapies such as T-DM1. **AQ5**

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### References

- 442 1. GLOBOCAN 2012: Estimated cancer incidence, mortality and  
 443 prevalence worldwide in 2012 444
- 445 2. Berns K, Horlings HM, Hennessy BT et al (2007) A functional  
 446 genetic approach identifies the PI3 K pathway as a major deter-  
 447 minant of trastuzumab resistance in breast cancer. *Cancer Cell*  
 448 12:395–402 449
- 450 3. Ross JS, Fletcher JA, Linette GP et al (2003) The Her-2/neu gene  
 451 and protein in breast cancer 2003: biomarker and target of ther-  
 452 apy. *Oncologist* 8:307–325 453
- 454 4. Cho HS, Mason K, Ramyar KX et al (2003) Structure of the  
 455 extracellular region of HER2 alone and in complex with the  
 456 Herceptin Fab. *Nature* 421:756–760 457
- 458 5. Riese DJ, Kim ED, Elenius K et al (1996) The epidermal growth  
 459 factor receptor couples transforming growth factor- $\alpha$ , hepa-  
 460 rin-binding epidermal growth factor-like factor, and amphiregulin  
 461 to Neu, ErbB-3, and ErbB-4. *J Biol Chem* 271:20047–20052 462
- 463 6. Cancer Genome Atlas N (2012) Comprehensive molecular por-  
 464 traits of human breast tumours. *Nature* 490:61–70 465
- 466 7. Siegel PM, Muller WJ (1996) Mutations affecting conserved  
 467 cysteine residues within the extracellular domain of Neu promote  
 468 receptor dimerization and activation. *Proc Natl Acad Sci USA*  
 469 93:8878–8883 470
- 471 8. Graus-Porta D, Beerli RR, Daly JM, Hynes NE (1997) ErbB-2,  
 472 the preferred heterodimerization partner of all ErbB receptors, is  
 473 a mediator of lateral signaling. *EMBO J* 16:1647–1655 474
- 475 9. Stern DF, Kamps MP, Cao H (1988) Oncogenic activation of  
 476 p185neu stimulates tyrosine phosphorylation in vivo. *Mol Cell*  
 477 Biol 8:3969–3973 478
- 479 10. Wulfkuhle JD, Berg D, Wolff C et al (2012) Molecular analysis  
 480 of HER2 signaling in human breast cancer by functional protein  
 481 pathway activation mapping. *Clin Cancer Res* 18:6426–6435 482
- 483 11. Holbro T, Beerli RR, Maurer F et al (2003) The ErbB2/ErbB3  
 484 heterodimer functions as an oncogenic unit: ErbB2 requires  
 485 ErbB3 to drive breast tumor cell proliferation. *Proc Natl Acad Sci*  
 486 USA 100:8933–8938 487
- 488 12. Lipton A, Goodman L, Leitzel K et al (2013) HER3, p95HER2,  
 489 and HER2 protein expression levels define multiple subtypes of  
 490 HER2-positive metastatic breast cancer. *Breast Cancer Res Treat*  
 491 141:43–53 492

- 482 13. Garner AP, Bialucha CU, Sprague ER et al (2013) An antibody  
483 that locks HER3 in the inactive conformation inhibits tumor  
484 growth driven by HER2 or neuregulin. *Cancer Res* 73:6024–6035
- 485 14. Phillips GD, Fields CT, Li G et al (2014) Dual targeting of  
486 HER2-positive cancer with trastuzumab emtansine and pertu-  
487 zumab: critical role for neuregulin blockade in antitumor  
488 response to combination therapy. *Clin Cancer Res* 20:456–468
- 489 15. Sassen A, Rochon J, Wild P et al (2008) Cytogenetic analysis of  
490 HER1/EGFR, HER2, HER3 and HER4 in 278 breast cancer  
491 patients. *Breast Cancer Res* 10:R2
- 492 16. Nafi S, Generali D, Kramer-Marek G et al (2014) Nuclear HER4  
493 mediates acquired resistance to trastuzumab and is associated  
494 with poor outcome in HER2 positive breast cancer. *Oncotarget*  
495 2014
- 496 17. Drebin JA, Link VC, Weinberg RA, Greene MI (1986) Inhibition  
497 of tumor growth by a monoclonal antibody reactive with an  
498 oncogene-encoded tumor antigen. *Proc Natl Acad Sci USA*  
499 83:9129–9133
- 500 18. Baselga J, Norton L, Albanell J et al (1998) Recombinant  
501 humanized anti-HER2 antibody (Herceptin) enhances the anti-  
502 tumor activity of paclitaxel and doxorubicin against HER2/neu  
503 overexpressing human breast cancer xenografts. *Cancer Res*  
504 58:2825–2831
- 505 19. Molina MA, Codony-Servat J, Albanell J et al (2001) Trastu-  
506 zumab (Herceptin), a humanized anti-Her2 receptor monoclonal  
507 antibody, inhibits basal and activated Her2 ectodomain cleavage  
508 in breast cancer cells. *Cancer Res* 61:4744–4749
- 509 20. Le XF, Claret FX, Lammayot A et al (2003) The role of cyclin-  
510 dependent kinase inhibitor p27Kip1 in anti-HER2 antibody-  
511 induced G1 cell cycle arrest and tumor growth inhibition. *J Biol*  
512 *Chem* 278:23441–23450
- 513 21. Cuello M, Ettenberg SA, Clark AS et al (2001) Down-regulation  
514 of the erbB-2 receptor by trastuzumab (Herceptin) enhances  
515 tumor necrosis factor-related apoptosis-inducing ligand-mediated  
516 apoptosis in breast and ovarian cancer cell lines that overexpress  
517 erbB-2. *Cancer Res* 61:4892–4900
- 518 22. Collins DM, O'Donovan N, McGowan PM et al (2012) Trastu-  
519 zumab induces antibody-dependent cell-mediated cytotoxicity  
520 (ADCC) in HER-2-non-amplified breast cancer cell lines. *Ann*  
521 *Oncol* 23:1788–1795
- 522 23. Gennari R, Menard S, Fagnoni F et al (2004) Pilot study of the  
523 mechanism of action of preoperative trastuzumab in patients with  
524 primary operable breast tumors overexpressing HER2. *Clin*  
525 *Cancer Res* 10:5650–5655
- 526 24. Gril B, Palmieri D, Bronder JL et al (2008) Effect of lapatinib  
527 on the outgrowth of metastatic breast cancer cells to the brain. *J Natl*  
528 *Cancer Inst* 100:1092–1103
- 529 25. Nahta R, Yuan LX, Du Y, Esteva FJ (2007) Lapatinib induces  
530 apoptosis in trastuzumab-resistant breast cancer cells: effects on  
531 insulin-like growth factor I signaling. *Mol Cancer Ther* 6:667–  
532 674
- 533 26. Scaltriti M, Chandarlapaty S, Prudkin L et al (2010) Clinical  
534 benefit of lapatinib-based therapy in patients with human epi-  
535 dermal growth factor receptor 2-positive breast tumors  
536 coexpressing the truncated p95HER2 receptor. *Clin Cancer Res*  
537 16:2688–2695
- 538 27. Xia W, Mullin RJ, Keith BR et al (2002) Anti-tumor activity of  
539 GW572016: a dual tyrosine kinase inhibitor blocks EGF activa-  
540 tion of EGFR/erbB2 and downstream Erk1/2 and AKT pathways.  
541 *Oncogene* 21:6255–6263
- 542 28. Gilmer TM (2011) Lapatinib: functional genomics study leads to  
543 insights into mechanism of action. *Mol Cancer Ther* 10:2025
- 544 29. Lin NU, Dieras V, Paul D et al (2009) Multicenter phase II study  
545 of lapatinib in patients with brain metastases from HER2-positive  
546 breast cancer. *Clin Cancer Res* 15:1452–1459
- 547 30. Xia W, Gerard CM, Liu L et al (2005) Combining lapatinib  
548 (GW572016), a small molecule inhibitor of ErbB1 and ErbB2  
549 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances  
550 apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene*  
551 24:6213–6221
- 552 31. Scaltriti M, Verma C, Guzman M et al (2009) Lapatinib, a HER2  
553 tyrosine kinase inhibitor, induces stabilization and accumulation  
554 of HER2 and potentiates trastuzumab-dependent cell cytotoxicity.  
555 *Oncogene* 28:803–814
- 556 32. Blackwell KL, Burstein HJ, Storniolo AM et al (2012) Overall  
557 survival benefit with lapatinib in combination with trastuzumab  
558 for patients with human epidermal growth factor receptor 2-  
559 positive metastatic breast cancer: final results from the  
560 EGF104900 Study. *J Clin Oncol* 30:2585–2592
- 561 33. Piccart-Gebhart MJHA, Baselga J et al (2014) First results from  
562 the phase III ALTTO trial (BIG 2-06; NCCTG [Alliance] N063D)  
563 comparing one year of anti-HER2 therapy with lapatinib  
564 alone (L), trastuzumab alone (T), their sequence (T → L), or their  
565 combination (T+L) in the adjuvant treatment of HER2-positive  
566 early breast cancer (EBC). *JCO* 32:5s
- 567 34. Gelmon KA, Boyle F, Kaufman B, et al (2012) Open-label phase  
568 III randomized controlled trial comparing taxane-based chemo-  
569 therapy (Tax) with lapatinib (L) or trastuzumab (T) as first-line  
570 therapy for women with HER2+ metastatic breast cancer: interim  
571 analysis (IA) of NCIC CTG MA.31/GSK EGF 108919. *JCO* 30  
572 572
- 573 35. Agus DB, Akita RW, Fox WD et al (2002) Targeting ligand-  
574 activated ErbB2 signaling inhibits breast and prostate tumor  
575 growth. *Cancer Cell* 2:127–137
- 576 36. Lee-Hoeflich ST, Crocker L, Yao E et al (2008) A central role for  
577 HER3 in HER2-amplified breast cancer: implications for targeted  
578 therapy. *Cancer Res* 68:5878–5887
- 579 37. Scheuer W, Friess T, Burtscher H et al (2009) Strongly enhanced  
580 antitumor activity of trastuzumab and pertuzumab combination  
581 treatment on HER2-positive human xenograft tumor models.  
582 *Cancer Res* 69:9330–9336
- 583 38. Cassady JM, Chan KK, Floss HG, Leistner E (2004) Recent  
584 developments in the maytansinoid antitumor agents. *Chem Pharm*  
585 *(Tokyo)* 52:1–26
- 586 39. Erickson HK, Park PU, Widdison WC et al (2006) Antibody-  
587 maytansinoid conjugates are activated in targeted cancer cells by  
588 lysosomal degradation and linker-dependent intracellular pro-  
589 cessing. *Cancer Res* 66:4426–4433
- 590 40. Junttila TT, Li G, Parsons K et al (2011) Trastuzumab-DM1 (T-  
591 DM1) retains all the mechanisms of action of trastuzumab and  
592 efficiently inhibits growth of lapatinib insensitive breast cancer.  
593 *Breast Cancer Res Treat* 128:347–356
- 594 41. Verma S, Miles D, Gianni L et al (2012) Trastuzumab emtansine  
595 for HER2-positive advanced breast cancer. *N Engl J Med*  
596 367:1783–1791
- 597 42. Bianchini G, Gianni L (2014) The immune system and response  
598 to HER2-targeted treatment in breast cancer. *Lancet Oncol* 15:  
599 e58–e68
- 600 43. Nimmerjahn F, Ravetch JV (2008) Fcγ receptors as regu-  
601 lators of immune responses. *Nat Rev Immunol* 8:34–47
- 602 44. Loi SMS, Lambrechts D (2012) Tumor PIK3CA mutations,  
603 lymphocyte infiltration, and recurrence-free survival in early  
604 breast cancer (BC): results from the FinHER trial. *Proc Am Soc*  
605 *Clin Oncol* 30:282–290
- 606 45. Loi S, Michiel S, Salgado R, Sirtaine N, Jose V, Fumagalli D,  
607 Brown DN, Kellokumpu-Lehtinen P-L, Bono P, Kataja V, Des-  
608 medt C, Piccart-Gebhart MJ, Loibl S, Untch M, Denkert C,  
609 Smyth MJ, Joensuu H, Sotiriou C (2013) Tumor infiltrating  
610 lymphocytes (TILs) indicate trastuzumab benefit in early-stage  
611 HER2-positive breast cancer (HER2+ BC). *Cancer Res* 73: Abstr  
S1-05

- 612 46. Loi S, Sirtaine N, Piette F et al (2013) Prognostic and predictive  
613 value of tumor-infiltrating lymphocytes in a phase III randomized  
614 adjuvant breast cancer trial in node-positive breast cancer comparing  
615 the addition of docetaxel to doxorubicin with doxorubicin-based  
616 chemotherapy: BIG 02-98. *J Clin Oncol* 31:860–867
- 617 47. Zitvogel L, Kepp O, Kroemer G (2011) Immune parameters  
618 affecting the efficacy of chemotherapeutic regimens. *Nat Rev*  
619 *Clin Oncol* 8:151–160
- 620 48. Junttila TT, Parsons K, Olsson C et al (2010) Superior in vivo  
621 efficacy of afucosylated trastuzumab in the treatment of HER2-  
622 amplified breast cancer. *Cancer Res* 70:4481–4489
- 623 49. Fan X, Brezski RJ, Fa M et al (2012) A single proteolytic  
624 cleavage within the lower hinge of trastuzumab reduces immune  
625 effector function and in vivo efficacy. *Breast Cancer Res* 14:R116
- 626 50. Kinder M, Greenplate AR, Grugan KD et al (2013) Engineered  
627 protease-resistant antibodies with selectable cell-killing functions.  
628 *J Biol Chem* 288:30843–30854
- 629 51. Stagg J, Loi S, Divisekera U et al (2011) Anti-ErbB-2 mAb  
630 therapy requires type I and II interferons and synergizes with  
631 anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci USA*  
632 108:7142–7147
- 633 52. Park S, Jiang Z, Mortenson ED et al (2010) The therapeutic effect  
634 of anti-HER2/neu antibody depends on both innate and adaptive  
635 immunity. *Cancer Cell* 18:160–170
- 636 53. Kohrt HE, Houot R, Goldstein MJ et al (2011) CD137 stimulation  
637 enhances the antilymphoma activity of anti-CD20 antibodies.  
638 *Blood* 117:2423–2432
- 639 54. Kohrt HE, Houot R, Weiskopf K et al (2012) Stimulation of  
640 natural killer cells with a CD137-specific antibody enhances  
641 trastuzumab efficacy in xenotransplant models of breast cancer. *J*  
642 *Clin Invest* 122:1066–1075
- 643 55. Stagg J, Loi S, Divisekera U et al (2011) Anti-ErbB-2 mAb  
644 therapy requires type I and II interferons and synergizes with  
645 anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci USA*  
646 108:7142–7147
- 647 56. Park S, Jiang Z, Mortenson ED et al (2010) The therapeutic effect  
648 of anti-HER2/neu antibody depends on both innate and adaptive  
649 immunity. *Cancer Cell* 18:160–170
- 650 57. Wang B, Wang Q, Wang Z et al (2014) Metastatic consequences  
651 of immune escape from NK cell cytotoxicity by human breast  
652 cancer stem cells. *Cancer Res* 74:5746–5757
- 653 58. Tagliabue E, Sfondrini L, Regondi V et al (2012) Chemotherapy  
654 can enhance trastuzumab-mediated ADCC. *Cancer Res* 72: P5-  
655 18-10
- 656 59. Disis ML, Calenoff E, McLaughlin G et al (1994) Existent T-cell  
657 and antibody immunity to HER-2/neu protein in patients with  
658 breast cancer. *Cancer Res* 54:16–20
- 659 60. Peoples GE, Goedegebuure PS, Smith PS et al (1995) Breast and  
660 ovarian cancer-specific cytotoxic T lymphocytes recognize the  
661 same HER2/neu-derived peptide. *Proc Natl Acad Sci USA*  
662 92:432436
- 663 61. Salazar L, Goodell V, O'Meara M et al (2009) Persistent  
664 immunity and survival after immunization with a HER2/neu  
665 (HER2) vaccine. *ASCO Meet Abstr* 27:3010
- 666 62. Mittendorf EA, Clifton GT, Holmes JP et al (2012) Clinical trial  
667 results of the HER-2/neu (E75) vaccine to prevent breast cancer  
668 recurrence in high-risk patients: from US Military Cancer Institute  
669 Clinical Trials Group Study I-01 and I-02. *Cancer* 118:2594–  
670 2602
- 671 63. Holmes JP, Gates JD, Benavides LC et al (2008) Optimal dose  
672 and schedule of an HER-2/neu (E75) peptide vaccine to prevent  
673 breast cancer recurrence: from US Military Cancer Institute  
674 Clinical Trials Group Study I-01 and I-02. *Cancer* 113:1666–  
675 1675
- 676 64. Disis ML, Schiffman K, Guthrie K et al (2004) Effect of dose on  
677 immune response in patients vaccinated with an her-2/neu  
intracellular domain protein-based vaccine. *J Clin Oncol* 22:1916–1925
- 678 65. Limentani S, Dorval T, White S et al (2005) Phase I dose-escalation  
680 trial of a recombinant HER2 vaccine in patients with Stage  
681 II/III HER2+ breast cancer. *ASCO Meet Abstr* 23:2520
- 682 66. Emens LA, Asquith JM, Leatherman JM et al (2009) Timed sequential  
683 treatment with cyclophosphamide, doxorubicin, and an allogeneic  
684 granulocyte-macrophage colony-stimulating factor-secreting breast  
685 tumor vaccine: a chemotherapy dose-ranging factorial study of safety  
686 and immune activation. *J Clin Oncol* 27:5911–5918
- 687 67. Palucka K, Banchereau J (2012) Cancer immunotherapy via  
688 dendritic cells. *Nat Rev Cancer* 12:265–277
- 689 68. Milani A, Sangiolo D, Montemurro F et al (2013) Active  
690 immunotherapy in HER2 overexpressing breast cancer: current  
691 status and future perspectives. *Ann Oncol* 24:1740–1748
- 692 69. Nagy P, Friedlander E, Tanner M et al (2005) Decreased accessibility  
693 and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant,  
694 MUC4-expressing breast cancer cell line. *Cancer Res* 65:473–482
- 695 70. Esteva FJ, Guo H, Zhang S et al (2010) PTEN, PIK3CA, p-AKT,  
696 and p-p70S6 K status: association with trastuzumab response and  
697 survival in patients with HER2-positive metastatic breast cancer.  
698 *Am J Pathol* 177:1647–1656
- 699 71. Browne BC, Eustace AJ, Kennedy S et al (2012) Evaluation of  
700 IGF1R and phosphorylated IGF1R as targets in HER2-positive  
701 breast cancer cell lines and tumours. *Breast Cancer Res Treat*  
702 136:717–727
- 703 72. Zhang S, Huang WC, Li P et al (2011) Combating trastuzumab  
704 resistance by targeting SRC, a common node downstream of  
705 multiple resistance pathways. *Nat Med* 17:461–469
- 706 73. Saez R, Molina MA, Ramsey EE et al (2006) p95HER-2 predicts  
707 worse outcome in patients with HER-2-positive breast cancer.  
708 *Clin Cancer Res* 12:424–431
- 709 74. Guarneri V, Frassoldati A, Bottini A et al (2012) Preoperative  
710 chemotherapy plus trastuzumab, lapatinib, or both in human  
711 epidermal growth factor receptor 2-positive operable breast  
712 cancer: results of the randomized phase II CHER-LOB study. *J*  
713 *Clin Oncol* 30:1989–1995
- 714 75. Gayle SSAS, O'Regan RM, Nahta R (2012) Pharmacologic  
715 inhibition of mTOR improves lapatinib sensitivity in HER2-  
716 overexpressing breast cancer cells with primary trastuzumab  
717 resistance. *Anti-Cancer Agents Med Chem* 12:151–162
- 718 76. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A et al (2008) An  
719 integrative genomic and proteomic analysis of PIK3CA, PTEN,  
720 and AKT mutations in breast cancer. *Cancer Res* 68:6084–6091
- 721 77. Zardavas D FD, Borwn DM, et al (2013) Abstract P2-11-02:  
722 Understanding the biology and prognosis of PIK3CA gene  
723 mutations in primary breast cancer using gene expression  
724 profiling: A pooled analysis. *Cancer Res* 73
- 725 78. Loi S, Michiels S, Lambrechts D et al (2013) Somatic mutation  
726 profiling and associations with prognosis and trastuzumab benefit  
727 in early breast cancer. *J Natl Cancer Inst* 105:960–967
- 728 79. Hennessy BT, Smith DL, Ram PT et al (2005) Exploiting the  
729 PI3 K/AKT pathway for cancer drug discovery. *Nat Rev Drug*  
730 *Discov* 4:988–1004
- 731 80. S Loibl CD, A Schneeweis, et al (2013) Abstract S4-06: PIK3CA  
732 mutation predicts resistance to anti-HER2/chemotherapy in  
733 primary HER2-positive/hormone-receptor-positive breast cancer—  
734 prospective analysis of 737 participants of the GeparSixto and  
735 GeparQuinto studies. *Cancer Res* 73
- 736 81. Garcia-Garcia C, Ibrahim YH, Serra V et al (2012) Dual  
737 mTORC1/2 and HER2 blockade results in antitumor activity in  
738 preclinical models of breast cancer resistant to anti-HER2  
739 therapy. *Clin Cancer Res* 18:2603–2612
- 740 82. Andre F, O'Regan R, Ozguroglu M et al (2014) Everolimus for  
741 women with trastuzumab-resistant, HER2-positive, advanced  
742 breast cancer: results of the randomized phase III BOL-40007  
743 study. *J Clin Oncol* 32:1097–1105

- 744 breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol* 15:580–591
- 745
- 746 83. Sun SY, Rosenberg LM, Wang X et al (2005) Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res* 65:7052–7058
- 747
- 748
- 749 84. Serra V, Markman B, Scaltriti M et al (2008) NVP-BEZ235, a dual PI3 K/mTOR inhibitor, prevents PI3 K signaling and inhibits the growth of cancer cells with activating PI3 K mutations. *Cancer Res* 68:8022–8030
- 750
- 751
- 752
- 753 85. Liu N, Rowley BR, Bull CO et al (2013) BAY 80-6946 is a highly selective intravenous PI3 K inhibitor with potent p110alpha and p110delta activities in tumor cell lines and xenograft models. *Mol Cancer Ther* 12:2319–2330
- 754
- 755
- 756 86. Dreyling M, Morschhauser F, Bron D, Bouabdallah K, Vitolo U, Linton K, Van Den Neste E, Mappa S, Giurescu M, Childs BH, Zinzani PL (2013) Preliminary results of a Phase II study of single agent bay 80-6946, a novel PI3 K inhibitor, in patients with relapsed/refractory, indolent or aggressive lymphoma. *Blood* 122:87
- 757
- 758
- 759 87. Junttila TT, Akita RW, Parsons K et al (2009) Ligand-independent HER2/HER3/PI3 K complex is disrupted by trastuzumab and is effectively inhibited by the PI3 K inhibitor GDC-0941. *Cancer Cell* 15:429–440
- 760
- 761
- 762 88. Saura C, Bendell J, Jerusalem G et al (2014) Phase Ib study of buparlisib plus Trastuzumab in patients with HER2-positive advanced or metastatic breast cancer that has progressed on trastuzumab-based therapy. *Clin Cancer Res* 20:1935–1945
- 763
- 764
- 765 89. Rabindran SK, Discafani CM, Rosfjord EC et al (2004) Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res* 64:3958–3965
- 766
- 767
- 768
- 769
- 770
- 771
- 772
- 773
90. Canonici A, Gijssen M, Mullooly M et al (2013) Neratinib overcomes trastuzumab resistance in HER2 amplified breast cancer. *Oncotarget* 4:1592–1605
- 774
- 775
- 776
- 777 91. Awada A, Dirix L, Manso Sanchez L et al (2013) Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. *Ann Oncol* 24:109–116
- 778
- 779
- 780 92. Bose R, Kavuri SM, Searleman AC et al (2013) Activating HER2 mutations in HER2 gene amplification negative breast cancer. *Cancer Discov* 3:224–237
- 781
- 782
- 783 93. Li D, Ambrogio L, Shimamura T et al (2008) BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene* 27:4702–4711
- 784
- 785
- 786 94. Lin NU, Winer EP, Wheatley D et al (2012) A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. *Breast Cancer Res Treat* 133:1057–1065
- 787
- 788
- 789 95. Schuler M, Awada A, Harter P et al (2012) A phase II trial to assess efficacy and safety of afatinib in extensively pretreated patients with HER2-negative metastatic breast cancer. *Breast Cancer Res Treat* 134:1149–1159
- 790
- 791
- 792 96. Rimawi MF, Aleixo S, Rozas AA, et al (2012) A neoadjuvant, randomized, open-label phase II trial of afatinib (A) versus trastuzumab (T) versus lapatinib (L) in patients (pts) with locally advanced HER2-positive breast cancer (BC). *JCO* 30
- 793
- 794
- 795
- 796
- 797
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During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	Please confirm the affiliation 1, 2 and 3 inserted city name are correct and amend if necessary.	
AQ2	Please check the clarity of the sentence "HER2 dimerization...cells." and amend if necessary.	
AQ3	Please confirm and confirm the symbol in the sentence "with Fc receptors expressed".	
AQ4	Kindly check whether the reference [42], [45], [46], [47], [48], [49], [50], [53], [54], [55], [56], [62], [68], [67], [68], [86], [96] and [57] are correct.	
AQ5	Please check journal titles for references [61] and [65].	