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

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REVIEW

HER2-family signalling mechanisms, clinical implications and 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

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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, ™ 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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an oncogenic driver of the growth of HER2-positive BC, is associated with a shorter time to relapse and decreased overall survival (OS). A meta-analysis in 2003 found that of 81 studies spanning sixteen years of research and incorporating 27,161 patients [3], HER2 overexpression predicted a worse BC outcome. In contrast to the other known HER family members, those being epidermal growth factor receptor (EGFR/HER1), HER3 and HER4, no ligand has yet been identified for HER2 (Fig. 1). When overexpressed, HER2 exists in a constitutively open conformation, leaving it intrinsically capable of interacting with available RTK binding partners even in the absence of ligand [4]. HER family ligands induce quantitative differences in receptor phosphorylation but quantitatively similar physiological responses, suggesting that the identity of activated receptors, rather than the number of activated receptors, determines the cellular response [5]. Coordinated overexpression of EGFR and HER2 frequently occurs in HER2-positive BC. Expression profiling has identified at least two subgroups within HER2-positive primary breast tumours. Many of the differently expressed genes track with oestrogen receptor (ER) status, suggesting that HER2+/ER+ and HER2+/ER− represent two distinct entities [6].

HER2 dimerization is mediated by the formation of disulphide bonds between cysteine residues in the juxtamembrane region, and disrupting these disulphide bonds disrupts the ability of HER2 to transform cells [7]. Phosphorylated tyrosine residues on the receptor molecule serve as recognition and docking sites for SH2-containing proteins. These serve as linker molecules, recruiting components of downstream signalling pathways, such as the phosphoinositide-3-kinase (PI3 K) pathway, through which the activated RTK exerts its biological effect(s) (Fig. 1). HER family signalling is governed by a strict hierarchy, with HER2 the preferred dimerization partner of all other HER family members [8]. Cells transformed by HER2 display increased tyrosine phosphorylation of both HER2 and other proteins [9], and a recent study identified a subset of patients which were classed as HER2-negative by FISH analysis yet displayed HER2 activation that was coincident with EGFR and HER3 activation ($n = 415$) [10]. HER2/HER3 heterodimers have been proposed to be the main oncogenic unit in HER2-positive BC, with HER3 coupling activated HER2 to the downstream PI3 K and other pathways [11]. There is a correlation between simultaneous high HER2 and high HER3 levels and reduced sensitivity to trastuzumab [12]. Further synergistic targeting of HER2 and HER3 was demonstrated to achieve higher therapeutic efficacy [13], and the HER3 ligand neuregulin confers resistance to chemotherapy and has recently been implicated as a potential mechanism of resistance to T-DM1 [14]. In contrast, some studies suggest a tumour suppressor role for HER4 in HER2-positive BC, although this is likely to be isoform specific and context specific [15]. A recent study suggested that the localisation of HER4 may play a role in its activity, with nuclear, but not cytoplasmic

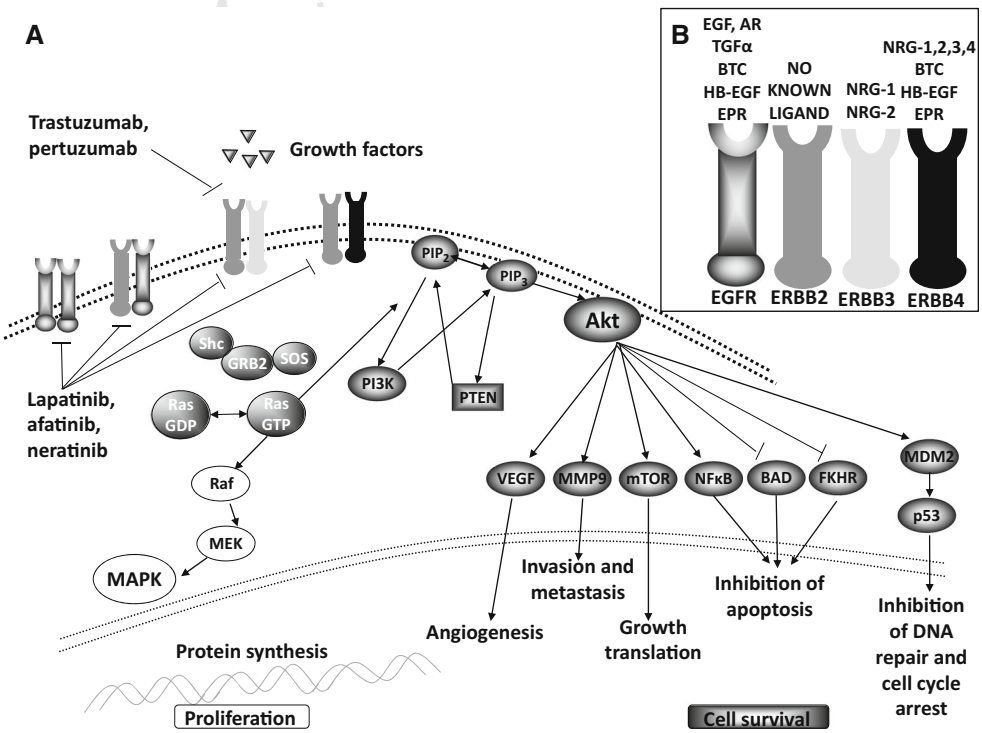

Fig. 1 Overview of HER family signalling in HER2-positive breast cancer including known HER family ligands, the potential dimerization partners of the HER family members and components of the downstream MAPK and PI3 K pathways, and the targeted therapies that are currently in testing or in use to treat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

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

Current HER2-targeted therapies

First generation HER2-targeted agents

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

Potential mechanisms include inhibition of HER2 dimerization [4], inhibition of cleavage of the ectodomain of activated HER2 [19], induction of $p27^{KIP1}$ [20], inhibition of PI3 K signalling, downregulation of HER2 leading to enhanced apoptosis mediated by tumour necrosis factor alpha-related apoptosis inducing ligand [21], and antibody-dependent cell-mediated cytotoxicity [22]. Trastuzumab-mediated internalisation and degradation of HER2 may inhibit receptor signalling, although some studies report that receptor levels are unaffected by trastuzumab treatment [23]. Despite its benefits, trastuzumab is limited in some patients by *de novo* and acquired resistance, and because it cannot cross the blood-brain barrier. Approximately 35 % of metastatic HER2-positive BC patients treated with trastuzumab go on to develop brain metastases [24].

Lapatinib (Tykerb,™ GlaxoSmithKline) is an orally bioavailable small molecule tyrosine kinase inhibitor (TKI) targeted to EGFR and HER2. Pre-clinical [25] and clinical [26] evidence shows that lapatinib is effective against trastuzumab-resistant HER2-positive BC, and it is currently used as subsequent therapy for patients with disease that has progressed on trastuzumab. Lapatinib inhibits HER2 phosphorylation more strongly than trastuzumab, and unlike trastuzumab, it inhibits extracellular signal-related kinase (Erk) 1 and 2 as well as PI3 K in vivo [27, 28]. Lapatinib inhibited tumour growth in p95HER2-overexpressing pre-clinical mouse models and has shown clinical benefit in patients refractory to trastuzumab whose tumours overexpressed p95HER2 ($n = 537$) [26]. It inhibits the development of brain metastases in vivo [24] and has modest activity against HER2-positive brain metastases clinically ($n = 242$) [29]. Trastuzumab and lapatinib have complementary mechanisms of action, and the combination of both [30, 31] confers an OS benefit in patients with heavily pretreated, trastuzumab-resistant HER2-positive metastatic BC compared to lapatinib monotherapy [32]. However, the success of lapatinib has been hit by a number of recent disappointing clinical trial results including the adjuvant study ALTTO [33], a number of neoadjuvant studies, and the NCIC CTG first-line metastatic study [34] (Table 1). These studies, along with the success of pertuzumab and T-DM1, mean that lapatinib's place in the clinic remains in patients with HER2-positive metastatic BC, who have received at least 1–2 prior lines of therapy for metastatic disease.

Second generation HER2-targeted agents

Pertuzumab (Omnitarg ™, Genentech) is a humanised monoclonal antibody which binds to HER2's extracellular domain II, which is involved in dimerization [4]. This is in contrast to trastuzumab, which binds to domain IV. Pertuzumab thus blocks HER2/HER3 interaction, diminishes ligand-activated HER2 signalling in BC cell lines, and inhibits the growth of high- and low-HER2-expressing HER2-positive breast xenografts *in vivo* [35, 36]. The combination of trastuzumab and pertuzumab in vivo results in an additive increase in ADCC and marked regression of metastatic HER2-positive BC in treated animals [37]. In clinical trials, pertuzumab significantly improved patient outcomes when added to trastuzumab and docetaxel in first-line metastatic HER2-positive BC and in the neoadjuvant setting (Table 1). Other trials with pertuzumab are ongoing (Table 3).

Trastuzumab-emtansine (T-DM1, Genentech) is an antibody-drug conjugate (ADC) which links trastuzumab to a highly cytotoxic maytansinoid agent, emtansine, which binds tubulin and arrests mitosis at metaphase [38]. Following the binding of T-DM1 to HER2, receptor-mediated internalisation transports it to the cytoplasm, where lysosomal degradation releases and activates the cytotoxic agent [39]. In addition to the anti-mitotic properties of emtansine, T-DM1 retains the mechanisms of action of trastuzumab including initiation of ADCC, inhibition of HER2 shedding and downregulation of PI3 K/AKT pathway activity, and is effective in models of lapatinib-resistance in vitro [40]. TDM-1 is now in clinical use in the second-line setting in metastatic HER2-positive BC based on the results of the EMILIA study [41] (Table 1).

The role of immunology in HER2-targeted therapy

There is compelling pre-clinical evidence of the importance of the immune response in the efficacy of trastuzumab in HER2-positive disease, and from a clinical perspective, data point to HER2-positive and triple

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

Clinical trial	Details	Sample size	Findings
Trastuzumab-based trials			
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, $P < 001$)
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 %, $P = 0.013$)
Lapatinib-based trials			
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
ALTTO [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 %, $P = 0.048$)
NeoALTTO [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	Singnificantly reduced PFS ($P = 0.01$) with lapatinib + chemotherapy compared to trastuzumab + chemotherapy (8.8 months compared to 11.4 months)
Pertuzumab-based trials			
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

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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
Afatinib-based trials			
Phase II Afatinib trial [70]	Safety and efficacy of afatinib monotherapy in patients who had progressed on trastuzumab treatment	52	Afatinib 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 Fcγ 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 Fcγ 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

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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 vinolrebine vs. trastuzumab plus vinolrebine	June 2014
Lux-Breast 3	HER2-positive BC patients with brain metastasis	120	Vinorelbine ±/− Afatinib	September 2014

adaptive immune response [56]. A more recent study has shown that tumour cells from patients expressing a breast 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

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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.

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].

Mechanisms of resistance to HER2-targeted therapies

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 tumourigenesis [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-

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

Novel HER2-targeted therapies

Some early phase clinical trials (Table 1) suggest encouraging efficacy for the novel HER2-directed TKI's neratinib and afatinib in HER2-positive BC. Neratinib is an irreversible TKI against EGFR and HER2. It potently inhibits HER2 and EGFR kinase activity, MAPK and AKT phosphorylation, and enhances p27 induction in vitro, and inhibits the growth of HER2-positive tumours in vivo [89]. Unlike trastuzumab, it decreases phosphorylation of EGFR, HER2, HER4 and ERK, and the addition of neratinib to trastuzumab overcomes trastuzumab resistance in vitro [90]. The combination of neratinib and vinorelbine has shown significant antitumour effects with no synergistic toxicity [91].

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

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

Conclusion

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

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