

# Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer

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Trastuzumab is a humanized mAb directed against the extracellular domain of the tyrosine kinase receptor HER2. Trastuzumab has shown clinical activity in HER2-overexpressing breast cancers and, at present, is currently approved for patients whose tumours have this abnormality, in both the metastatic and the adjuvant setting. Several issues about its optimal use, however, are still unresolved. One of the reasons for these uncertainties lies in the absence of conclusive data about its mechanism of action and possible primary or acquired resistance mechanisms. Therefore, clinical questions such as how to optimize patient selection, how to prevent resistance to trastuzumab, or what is the optimal management of those patients whose tumours progress during treatment still await convincing answers. This review summarises the current knowledge on the preclinical and clinical evidence about the mechanism of action of trastuzumab and on the mechanisms underlying the development of resistance and also briefly discusses their possible clinical implications.

**Key words:** breast neoplasms, HER2, mechanism of action, resistance, trastuzumab

## Introduction

HER2 is a transmembrane tyrosine kinase receptor which belongs to the family of the EGFR (epidermal growth factor receptor), which is overexpressed in 25%–30% of human breast cancers [1]. Its unique feature, which differentiates it from the other members of the family, is the absence of a known ligand. Its activity is subsequent to homo- or heterodimerisation with the other family members [2] (Figure 1).

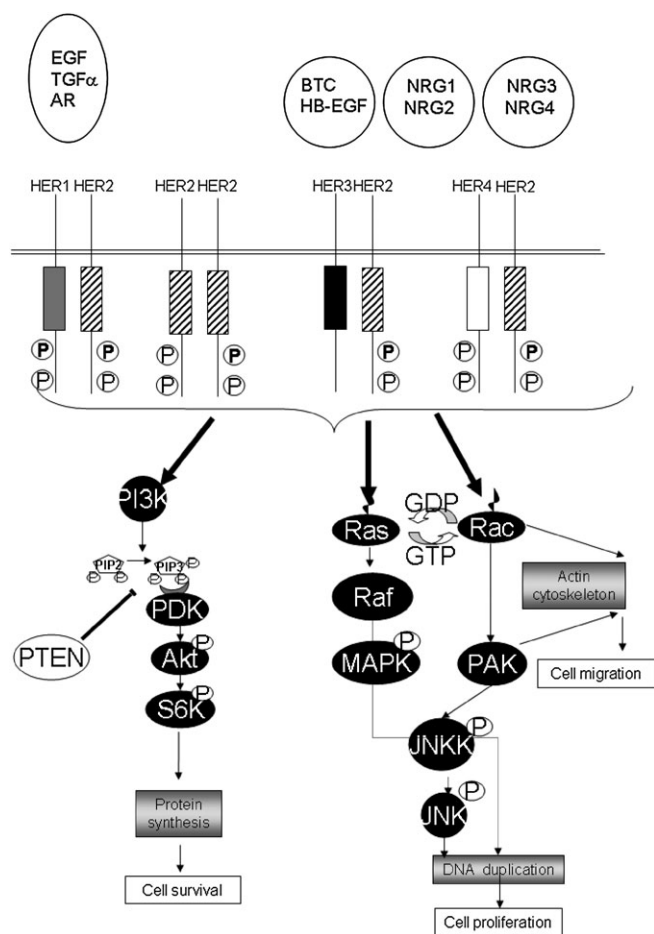
HER2 has several features of the ideal target for breast cancer treatment:

- HER2 levels correlate strongly with carcinogenesis, as demonstrated by gain [3] and loss of function experiments [4, 5]. Furthermore, HER2 overexpression is an adverse prognostic factor in women with breast cancer [6].
- The level of HER2 in human cancer cells with membrane overexpression is much higher than in normal adult tissues, which are therefore potentially less sensitive to the toxicity of HER2-targeting drugs.
- HER2 overexpression is found in both the primary tumour and in metastatic sites, indicating that anti-HER2 therapy may be effective at all disease sites.

Research has therefore focussed on HER2 inhibitors as potential anticancer agents. Trastuzumab is the first of such agents which was registered for use in patients with HER2-overexpressing breast cancer. Trastuzumab is a humanised mAb of the immunoglobulin G1 type directed against the extracellular portion of HER2. Phase II and III trials in metastatic disease showed that trastuzumab has relevant clinical activity against HER2-positive breast cancer. As a single agent, overall response rates (complete plus partial responses) ranging from 15% to 30% have been reported [7]. In combination with nonanthracycline conventional agents, such as taxanes or vinorelbine, response rates range from 50% to 80% [8]. Most importantly, one phase III and one phase II randomised trial demonstrated a survival advantage when a combination of trastuzumab plus chemotherapy was compared with the same chemotherapy alone [9, 10]. Benefit brought by the addition of trastuzumab to chemotherapy does not lead to increased toxicity to patients, except for cardiotoxicity in the form of left ventricular ejection fraction depression. The encouraging results of trastuzumab in patients with metastatic disease prompted the evaluation of this agent in patients with HER2-positive early breast cancer. Four large randomised trials with short follow-up have been recently reported, showing that the addition of trastuzumab to chemotherapy halves the risk of relapse and, in one joint analysis of two North-American trials, prolongs survival [11–13]. At present, therefore, 1 year of trastuzumab has become part of the adjuvant treatment of women with HER2-positive early breast cancer.

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**Figure 1.** A simplified illustration of key pathways involved in downstream signalling after HER2 activation. HER2 forms homodimers or heterodimers with the other members of the family. Heterodimerisation is induced by the ligands of the other members of the epidermal growth factor receptor (EGFR)-family (AR, amphiregulin; BTC, betacellulin; EPR, epiregulin; HB-EGF, heparin-binding EGF; NRGs, neuregulins; TGF- $\alpha$ , transforming growth factor- $\alpha$ ). Phosphatidylinositol 3-kinase (PI3K) signalling pathway begins with PI3K activation. PI3K activity phosphorylates and converts the lipid second messenger phosphatidylinositol (4,5) biphosphate (PIP<sub>2</sub>) into phosphatidylinositol (3,4,5) triphosphate (PIP<sub>3</sub>), which recruits and activates phosphatidylinositol-dependent kinase 1 (PDK). PDK, in turn, phosphorylates AKT, which inhibits the activities of the transcription factors (which are mediators of apoptosis and cell cycle arrest), resulting in cell survival. The tumour-suppressor phosphatase with tensin homologue (PTEN) negatively regulates PI3K signalling by dephosphorylating PIP<sub>3</sub>, converting it back to PIP<sub>2</sub>. RAF-MEK-MAPK and PAK-JNKK-JNK, two cascades of serine/threonine kinases which regulate the activity of a number of transcription factors downstream the GTPases RAS and RAC.

Despite this escalation, considering that ~10 years have elapsed since the initial evidence of its clinical activity in patients, it is surprising to note that relevant questions concerning the mechanism of action of trastuzumab *in vivo* are still uncertain (Table 1).

This review summarises the current knowledge on the preclinical and clinical evidence about the mechanism of action of trastuzumab and on the mechanisms underlying the development of resistance and also briefly discusses their

**Table 1.** Proposed mechanisms of trastuzumab action and resistance

Mechanisms of action
Inhibition of HER2 shedding
Inhibition of PI3K-AKT pathway
Attenuation of cell signalling
ADCC
Inhibition of tumour angiogenesis
Mechanisms of resistance
Increased cell signalling
PTEN loss
Increased AKT activity
Alternative cell signalling mediated by EGFR family pathways
TGF- $\alpha$ overexpression
Neuregulin overexpression
Alternative cell signalling mediated by different pathways
VEGF overexpression
IGF1R overexpression

ADCC, Antigen-dependent cellular cytotoxicity; PTEN, phosphatase and tensin homologue; EGFR, epidermal growth factor receptor; TGF- $\alpha$ ; transforming growth factor- $\alpha$ ; VEGF, vascular endothelial growth factor; IGF1R, insulin-like growth factor 1 receptor.

possible clinical implications in HER2-overexpressing breast cancer patients.

**molecular markers needed for eligibility to trastuzumab treatment**

Currently, the two most common methods to assess the HER2 status in the clinical setting are immunohistochemistry (IHC) and FISH [14–16].

IHC identifies HER2 overexpression on the cell membrane. Results are usually expressed using a semiquantitative scoring system ranging from 0+ (no expression) to 3+ (high expression) on the basis of the comparison with cell lines of known HER2 receptor density. Despite being economically convenient and largely available, IHC suffers from interobserver variability [17].

FISH detects HER2 gene amplification and is more specific and sensitive than IHC [14]. FISH offers quantitative results on the number of gene copies but not on the real amount of HER2 expressed on the cell membrane and therefore reachable by trastuzumab. Clinical trials, however, have shown that FISH more accurately predicts response to trastuzumab than IHC, because those patients whose tumours overexpress HER2 in the absence of gene amplification are less likely to respond to trastuzumab-based therapy [18]. In general, IHC and FISH demonstrate a concordance rate of ~90% [19]. Much of the discordance is due to tumours scored as 2+ by IHC, which are found to have amplification of the HER2 oncogene in up to 25% of cases. Therefore, recent algorithms indicate IHC as initial test, followed by FISH for those tumours scoring 2+ at IHC [15].

**mechanisms of action**

Although trastuzumab is currently widely used for the treatment of HER2-overexpressing breast cancer, its mechanism of action is not fully understood.

## immune-mediated response

*In vivo* breast cancer models and clinical trials have demonstrated that trastuzumab has not only cytostatic but also cytotoxic properties. At least in part, these properties may be due to the activation of antibody-dependent cellular cytotoxicity (ADCC). This immunological effect has been demonstrated in numerous breast cancer cell lines [20–22]. ADCC is mainly due to the activation of natural killer cells (NK), expressing the Fc gamma receptor, which can be bound by the Fc domain of trastuzumab. This event activates the lysis of cancer cells bound to trastuzumab. Mice bearing BT474 HER2-overexpressing xenografts demonstrated a tumour regression rate of 96% when treated with trastuzumab. In contrast, in mice lacking the Fc receptor (FcR<sup>-/-</sup>) much of the therapeutic effect of trastuzumab was lost, with only 29% tumour growth inhibition observed [22]. ADCC as a possible mechanism of action of trastuzumab in patients has also been shown by Gennari et al. [23]. The investigators selected 11 patients with HER2-positive breast tumours receiving trastuzumab at a standard dose for 4 weeks before breast surgery. Tumour biopsy and peripheral blood mononucleated cells were collected from patients for biological studies before and after systemic treatment. Substantial infiltration of lymphoid cells into the tumour was observed in all cases. Patients with complete remission or partial remission were found to have a higher *in situ* infiltration of leucocytes and a higher capability to mediate *in vitro* antibody and ADCC. Although it may be argued that a tumour biopsy *per se* can induce immune response because of mechanic injury, these data support the hypothesis of an ADCC activity of trastuzumab. Repka et al. [24] treated 10 HER2-overexpressing metastatic breast cancer patients with interleukin 2 for 7 weeks and trastuzumab for 6 weeks. In this small cohort of patients, no correlation was observed between the clinical response and NK cell expansion or the level of ADCC activity. It should however be noted that patients with advanced metastatic breast cancer may not be the optimal population to study as their immune system might have been impaired by the disease and by previous treatments. Additional studies are needed for a better understanding of the importance of ADCC in mediating the response to trastuzumab.

## inhibition of HER2 shedding

When overexpressed, HER2 undergoes proteolytic cleavage which results in the release of the extracellular domain and in the production of a truncated membrane-bound fragment (p95) [25].

In HER2-overexpressing breast cancer cell lines (SKBR3 and BT474), Molina et al. [26] demonstrated that trastuzumab can block the shedding of the extracellular domain of HER2 by inhibiting metalloproteinase activity. Indeed, the authors detected a phosphorylated truncated receptor in 14 out of 24 human breast cancer specimens that were analysed. Once cleaved, the extracellular domain of HER2 is likely to be released in serum. Several clinical studies show that a decline in serum HER2 extracellular domain during trastuzumab treatment predicts tumour response and improves progression-free survival [27, 28], which indirectly supports the hypothesis that trastuzumab may act by inhibiting HER2 cleavage.

## induction of HER2

Whether trastuzumab induces HER2 downregulation and subsequent degradation in HER2-overexpressing breast cancer cells is currently subject of discussion [29–31]. In the above-mentioned study conducted by Gennari et al. [23], no significant downregulation of HER2 was seen in breast cancer patients who achieved tumour response to 4 weeks of trastuzumab given before surgery. The neoplastic tissue analysed for HER2 downregulation, however, might also be composed by the neoplastic cells that do not respond to treatment.

## inhibition of the PI3K pathway

PI3K (Phosphoinositide 3-kinase) is activated by survival factors or transforming events such as HER2 overexpression/activation (Figure 1). Activated PI3K generates phosphoinositides causing translocation of AKT to the plasma membrane, where it is phosphorylated and activated. Activated AKT can then phosphorylate numerous targets. Activated AKT negatively regulated by the antagonising action of phosphatase and tensin homologue (PTEN) on PI3K (Figure 1). A proposed mechanism of action of trastuzumab is the reduction of the signalling from these pathways, thus promoting apoptosis and the arrest of proliferation [32]. Diminished receptor signalling may result from trastuzumab-mediated internalisation and degradation of the HER2 receptor, but, as discussed above, it is unclear whether trastuzumab actually down-regulates HER2. An alternative mechanism by which trastuzumab may block PI3K signalling was described by Nagata et al. [33], who demonstrated that trastuzumab specifically inhibits PI3K signalling by reducing PTEN tyrosine phosphorylation and by increasing PTEN membrane localisation and phosphatase activity. This in turn leads to rapid AKT dephosphorylation and inhibition of cell proliferation.

## inhibition of angiogenesis

Overexpression of HER2 in breast cancer cells is closely associated with increased angiogenesis [34]. In mice, treatment with trastuzumab induces normalisation and regression of the vasculature in an experimental human HER2-overexpressing breast tumour model. Izumi et al. [35], however, observed a reduction in vascular endothelial growth factor (VEGF) production by cancer cells only *in vitro*, indicating that a VEGF independent antiangiogenic mechanism might act *in vivo*, which is probably due to the modulation of different regulators of the complex machinery of angiogenesis.

Furthermore, Klos et al. [36] treated severe combined immunodeficiency mice bearing HER2-overexpressing breast cancer xenografts with trastuzumab alone, paclitaxel alone, or with the association of both drugs. Interestingly, the best tumour response was observed in mice treated with paclitaxel and trastuzumab and there was a nice concordance between tumour response and a reduction of microvessel density. These observations may reflect more efficient drug delivery consequent to trastuzumab-induced normalisation of tumour vasculature.

## G1 arrest

Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle, with a concomitant reduction in proliferation. Cell cycle arrest is accompanied by reduced expression of proteins involved in the sequestration of the cyclin-dependent kinase (cdk) inhibitor p27<sup>kip1</sup>, including cyclin D1. This results in the release of p27<sup>kip1</sup>, allowing it to bind and inhibit cyclin E/cdk2 complexes [37].

## development of resistance

The uncertainties in defining the ways trastuzumab exerts its effect on cancer cells are mirrored by the difficulties in explaining the ways cells are resistant *ab initio* or become resistant during treatment. Nevertheless several hypotheses have been raised.

### PTEN loss

Nagata et al. [33] suggested an important role of decreased expression of the PTEN protein. The authors demonstrated that, in sensitive cells, trastuzumab causes a disruption of the binding of Src to HER2, allowing PTEN to inhibit AKT and induce growth arrest. When PTEN levels are low, however, AKT remains active and trastuzumab efficacy is impaired. The authors validated their *in vitro* results with a retrospective analysis in neoplastic tissues derived from HER2-positive breast cancer patients treated on a trastuzumab-based regimen. Patients with PTEN-deficient tumours derived a limited benefit from treatment, compared with patients with intact PTEN activity. Unfortunately, the fact that these patients received a combination of trastuzumab and a conventional cytotoxic agent (mainly docetaxel) does not allow definitive conclusions to be drawn on the loss of PTEN and resistance to trastuzumab as a single agent.

### activation of alternative pathways

*insulin-like growth factor-I receptor.* The insulin-like growth factor-I receptor (IGF-IR) is a transmembrane tyrosine kinase receptor frequently expressed in human breast cancers. Its downstream effectors promote proliferation and metastatisation. The IGF signalling pathway seems to be deeply involved in breast cancer, as its ligands and receptors are frequently overexpressed, induce cell proliferation, and promote metastatisation. Lu et al. [38] engineered the human breast cancer cell line SKBR3 to overexpress IGF-IR in order to assess the efficacy of trastuzumab. Unlike the parental counterpart, IGF-IR-positive SKBR3 cells were insensitive to trastuzumab-induced inhibition in the presence of IGF-I. Interestingly, these findings were also confirmed using another cell line model where HER2 overexpression was exogenously induced (MCF7-HER2/IGF1R). The investigators observed that trastuzumab-induced p27<sup>Kip1</sup> expression in parental SKBR3 but not in SKBR3/IGF-IR cells. Similar biological results were obtained in their experiments by Nahta et al. [39]. In their cell line model (SKBR3 cells resistant to trastuzumab), the authors observed the presence of IGF1R/HER2 heterodimers. In the presence of heterodimers, trastuzumab was completely unable to block cell proliferation. These results point to IGF-IR as

a possible mediator of trastuzumab resistance and a possible therapeutic target in patients with trastuzumab-resistant disease.

### expression of ligands of the EGFR family

Of the four members of the EGFR family, trastuzumab is the only one with no known natural ligand. Heterodimerisation of HER2 with the other receptors, however, can be induced by ligands of HER1, HER3, and HER4. In the presence of an excess of ligands, HER2 or both, the resulting heterodimers drive cells towards proliferation and inhibition of apoptosis [2] and, possibly, interfere with trastuzumab [40]. Among these ligands, transforming growth factor- $\alpha$  (TGF- $\alpha$ ) plays a potentially important role in resistance to trastuzumab. We studied the possible role of induction of TGF- $\alpha$  expression as a mediator of acquired resistance to trastuzumab [30]. For three HER2-overexpressing advanced breast cancer patients responding to trastuzumab-based combinations, we obtained tumour material from the same metastasis before treatment and upon tumour regrowth during therapy. In all three cases, tumours that did not stain for TGF- $\alpha$  before treatment acquired immunopositivity when, after an initial reduction, started regrowing.

This clinical observation prompted us to build an *in vitro* model to study the phenomenon. For this purpose, we engineered an HER2-positive, trastuzumab sensitive, breast cancer cell line, to overexpress TGF- $\alpha$ . We observed that, in TGF $\alpha$  positive cells, trastuzumab could not induce HER2 downregulation and was less efficient to inhibit cell growth, in comparison to parental cells. Our data indicate that resistance to trastuzumab may be associated with a TGF- $\alpha$ -related escape mechanism to HER2 inhibition [30].

### receptor masking or epitope inaccessibility

Nagy et al. [41], investigated the properties of HER2 in a trastuzumab-resistant cell line, JIMT1, established from a breast cancer patient showing HER2 gene amplification. The authors observed that the expression of MUC4, a membrane-associated mucin which according to reports contributes to the masking of membrane proteins, was higher in the resistant clone (JIMT1) than in trastuzumab-sensitive lines, and its level was inversely correlated with the trastuzumab binding capacity of single cells. Knockdown of MUC4 expression by RNA interference increased the binding of trastuzumab.

On the basis of the observation that trastuzumab binds a HER2 epitope which is different from that employed by IHC methods, Bussolati et al. [42] retrospectively tested a biotinylated trastuzumab (BioTHER) in breast cancer and other cancer specimens. HER2 amplification was confirmed by FISH in 42 of 164 specimens from primary breast cancers. BioTHER staining was found in half of the FISH-positive tumours (21) and in none of the FISH-negative tumours. The analysis was then extended to tumour specimens from 54 HER2-amplified (FISH positive) advanced breast cancer patients who underwent treatment with trastuzumab-based combinations. Also in these specimens, about a half was found to be BioTHER positive. Interestingly, the efficacy of trastuzumab-based therapy was significantly higher in patients whose tumours stained positively

for BiothHER, despite all tumours being FISH positive. These results seem to indicate that lack of accessibility of the epitope to trastuzumab may limit the activity of this antibody *in vivo*, although prospective validation is needed.

## possible strategies to overcome resistance

### EGFR family inhibitors associated with trastuzumab

Because HER2 and EGFR coexpression occurs in ~30% of breast cancers, blockage of both receptors is a rational strategy which may improve response rates to trastuzumab. Such a combination may also be considered for trastuzumab-resistant tumours, in which compensatory signalling by EGFR may inhibit the response to trastuzumab.

### Lapatinib (GW572016)

Lapatinib is a small molecule dual reversible inhibitor of the tyrosine kinase activity of EGFR and HER2. Preclinical studies have shown its efficacy in both HER2-overexpressing and in normally expressing breast cancers by efficiently blocking the signal transduction downstream EGFR and HER2 [43].

Preclinical synergy with trastuzumab has also been shown [44].

A phase II study reported an overall response rate of 33% after 12 weeks of treatment with single-agent lapatinib in 40 women with HER2-positive metastatic breast cancer who had not previously received trastuzumab and/or chemotherapy for advanced or metastatic disease [45]. Intriguing and clinically relevant results, however, have been obtained using this compound in women with HER2-positive advanced breast cancer meeting, at least clinically, the definition of 'trastuzumab-resistant' disease [46–49]. Single-agent lapatinib was evaluated in a multicentre, international phase II trial in patients with relapsed or refractory inflammatory breast cancer [43]. The study accrued a total of 58 patients who were divided in two cohorts according to patterns of HER1 and HER2 expression. Cohort A included patients with HER2-positive disease (either 3+ at IHC or 2+/FISH positive), whereas cohort B included patients with HER1-positive/HER2-negative disease. Lapatinib was administered orally at a daily dose of 1500 mg until disease progression or unacceptable toxicity. In 36 patients assessable for response, investigators reported a 62% partial response rate in cohort A (24 patients) and an 8% response rate in cohort B. Very interestingly, the prevalence of expression of IGF-IR, which is one of the potential mediators of resistance to trastuzumab, was ~84% in cohort A, where 75% of the patients had been previously treated with trastuzumab and were either refractory or had become resistant to treatment. A large randomized study compared lapatinib plus chemotherapy with chemotherapy alone in women with HER2-positive (either a 3+ IHC, or 2+ by IHC, and HER2 gene-amplification, detected by FISH) refractory advanced or metastatic breast cancer has recently been reported [48]. This study was planned to enrol a total of 528 women with measurable disease according to the Response Evaluation in Solid Tumors (RECIST) criteria, who had previously been treated with anthracycline and taxanes in either the adjuvant or the metastatic setting, and whose disease

was progressing on trastuzumab treatment. Patients were randomized to receive single-agent capecitabine (2500 mg/m<sup>2</sup> administered orally for 14 consecutive days, with 1 week's rest) or capecitabine (2000 mg/m<sup>2</sup> administered orally for 14 consecutive days, with 1 week's rest) and lapatinib (1250 mg/day continuously). The study was closed after the first interim analysis of efficacy and safety triggered by the occurrence of 114 breast cancer events in 321 patients. The addition of lapatinib to capecitabine reduced the hazard of relapse (hazard ratio-HR 0.51,  $P = 0.00016$ ) and determined a trend towards higher response rate (22.5% versus 14.3%,  $P = 0.113$ ). Both treatment arms were well tolerated, as testified by similar discontinuation rates in the absence of tumour progression (14% versus 11% in the combined and capecitabine alone arms, respectively). Interestingly, the authors reported a numerical advantage for the combined treatment in terms of less central nervous system (CNS) progression in patients receiving lapatinib (4/160 versus 11/161 cases). This seems to confirm previous findings that small tyrosine kinase inhibitors may cross the blood–brain barrier and reduce the risk of CNS metastasis. This hypothesis was explored in a study conducted in 39 HER2-positive advanced breast cancer with CNS metastatic disease (at least 1 lesion >10 mm in major diameter) which was progressing after whole-brain radiation therapy or stereotactic radiosurgery [49]. Lapatinib was administered at a dose of 750 mg twice a day, in 4-week cycles. Patients were heavily pretreated and all had received prior trastuzumab for metastatic disease. Despite a very low response rate in CNS metastases (5%), there was evidence of some clinical benefit worthy of further investigation.

On the basis of potential synergy due to a different mechanism of action and receptor site activity [50], the association of lapatinib and trastuzumab has been recently evaluated in advanced breast cancer. Storniolo's team first undertook a dose-escalation study in 53 women with advanced or metastatic HER2-overexpressing breast cancer. Lapatinib was administered orally in escalating doses (750–1500 mg/day) in combination with standard weekly dosing of trastuzumab (4 mg/kg loading dose followed by 2 mg/kg weekly). This association showed interesting clinical activity, as the patients were heavily pretreated and had progressed on prior treatments, including trastuzumab. One complete and 5 partial responses were reported in 27 evaluable patients. The dose of lapatinib suggested for further studies in association with weekly trastuzumab was 1000 mg/day. On these premises, a phase III trial combining lapatinib and trastuzumab versus lapatinib alone given at progression only to HER2-overexpressing breast cancer developing resistance to trastuzumab is now underway.

### Gefitinib (ZD1839)

Preclinical models have shown a synergy between trastuzumab and gefitinib (an EGFR tyrosine kinase inhibitor) producing complete remissions in HER2-overexpressing breast cancer xenografts [51, 52]. On the basis of this rationale, Arteaga et al. conducted a phase II study where women with HER2-positive advanced breast cancer not previously treated with trastuzumab received a combination of weekly trastuzumab and gefitinib at the dose of 250 mg/day. Sadly, the study was closed at the first interim analysis because of low levels of activity, indicating, by

indirect comparison with trials with trastuzumab alone, that this combination might be antagonistic [53]. Lack of selection of patients on the basis of EGFR status or suboptimal dosing of gefitinib are potential caveats of this study, which seems to contradict the preclinical observations [54].

### Pertuzumab (2C4)

Pertuzumab is a mAb directed against a portion of the extracellular domain of HER2 that sterically blocks the ability of HER2 to heterodimerize with other members of the family. This event impairs HER2/HER3 signalling and HER2/EGFR heterodimers both in HER2 overexpressing and in cells that express normal levels of HER2 [55]. Recent work by Cho et al. [56] suggests that the extracellular portion of HER2 bound to trastuzumab is different from that recognized by pertuzumab. Preclinical data were also obtained from the combination of trastuzumab and pertuzumab (2C4, Genentech Inc, South San Francisco, CA, USA) [57]. In an HER2-overexpressing human breast cancer cell line (BT474), the association of trastuzumab and pertuzumab increased apoptosis and cell growth arrest when compared with trastuzumab alone. On the basis of these results, a National Cancer Institute (NCI)-sponsored phase II trial combining trastuzumab and pertuzumab in HER2-overexpressing locally advanced and metastatic breast cancer is currently recruiting patients. The main objectives of the study are response rate, progression-free survival, and duration of response.

### association with agents targeting other pathways

The study of combinations of trastuzumab with inhibitors of downstream signalling is currently under early clinical investigation for breast cancer after promising results in a preclinical setting. Among these compounds, two inhibitors of mTOR (kinase located downstream the PI3K-AKT pathway) seem to be of particular interest: CCI-779 (Wyeth-Ayerst; Madison, NJ) and RAD001 (Novartis, New York, NY) [58]. Potentially interesting results may also derive from the association of trastuzumab with the anti-VEGF mAb bevacizumab. A NCI-sponsored phase I/II trial, preliminary data of which were presented at the San Antonio Breast Cancer Conference 2004 is currently ongoing in HER2-overexpressing metastatic breast cancer patients [59].

### modified anti-HER2 antibodies

To increase the potency of antibody-directed therapy, the specificity of the antigen-binding site has been combined with a wide variety of effector agents, including toxins. Using this approach, HER2-targeted antibodies have been linked with the toxin DM-1 in ongoing preclinical studies. Additionally, recombinant molecules in which the antibody-combining site is fused directly to the toxin have been developed and show strong selectivity for HER2 binding [60, 61]. Recombinant toxins are promising because they can be safely delivered to experimental animals at effective doses and may penetrate tumours more effectively than trastuzumab does alone. One limitation, however, facing the development of toxin targeting is the potential for immune response to the protein [62].

## conclusions

The history of trastuzumab for the treatment of HER2-overexpressing breast cancer is certainly a very successful one. Trastuzumab alone or in combination with chemotherapy prolongs survival in both the metastatic and the adjuvant setting and these results have marked a significant step forward in the treatment of a relevant subset of breast cancer patients. Several research groups utilising different models have provided interesting evidence of the possible mechanisms of action and the development of resistance to trastuzumab.

Nevertheless, at least three unanswered questions still remain:

- Why is it that only an overexpression driven by gene amplification renders HER2-positive breast cancer cells sensitive to the treatment with trastuzumab?
- Is it possible to predict which patients will develop resistance to trastuzumab before starting the treatment?
- What should be done in the presence of HER2-overexpressing breast cancer patients developing resistance during a trastuzumab-based regimen?

There are several reasons to explain why the range of the unresolved questions is so broad. Almost all the preclinical studies on the development of resistance to trastuzumab were conducted in pure models of HER2-amplified breast cancer cells (or xenograft) treated with trastuzumab alone. On the contrary, most of the clinical trials with HER2-overexpressing metastatic breast cancers have been carried out in association with different chemotherapeutic agents. The few phase II studies with trastuzumab alone did not select patients on the exclusive basis of HER2 gene amplification. Indeed, none of these studies had solid biological end points to integrate the clinical efficacy assessments. In our opinion a solid answer to the question 'Why is resistance such a frequent event?' must pass through clinical phase II trials with trastuzumab alone in FISH-positive patients. These studies should be sustained by solid biological and molecular evaluations.

Such an approach is probably the only one that will allow in patients progressing upon trastuzumab to:

- Define a rational approach to overcome resistance or, better still, to prevent its development.
- Decide whether there is a rational in continuing trastuzumab by changing the associated chemotherapeutic agents.

The latter issue represents one example of how the uncertainties that we have summarised in this review impact on clinical decision making. On the basis of preclinical observations indicating that trastuzumab may slow down tumour growth even in the presence of disease progression, many oncologists throughout the world adopt the policy of continuing the administration of trastuzumab in patients who show tumour progression, usually changing the companion chemotherapeutic agent [63]. This behaviour differs from that commonly adopted with chemotherapy or endocrine therapy, where disease progression during treatment is considered a marker of resistance to the agent employed. A few retrospective analyses [64–66] have further supported this policy but, as we have recently pointed out, the biases intrinsic to such methodology make conclusions from these studies not directly applicable to

the clinical practice [67, 68]. Rather, they would make a strong case for the conduction of adequately designed prospective, phase III trials. Sadly, the few initiatives which have been launched in the past have failed in their goals to accrue patients and the issue of the optimal treatment of patients progressing during trastuzumab-based therapy might remain unresolved. At present we are aware of only two active randomized trials, one conducted in Germany ([www.germanbreastgroup.de/herceptin/](http://www.germanbreastgroup.de/herceptin/)) and the other in Italy ([www.studiothor.it](http://www.studiothor.it)), that are evaluating the worth of trastuzumab beyond progression.

With the introduction of trastuzumab in the adjuvant setting, health care systems are faced with a steep increase in the costs of breast cancer treatment. Obviously, elucidating primary and acquired resistance to this antibody to avoid its administration to patients who are less likely to derive a true benefit would bring significant improvements in its cost-benefit ratio.

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