

Role of trastuzumab in the management of HER2-positive metastatic breast cancer

Andrea Milani^{1,2}
Filippo Montemurro¹
Luisa Gioeni¹
Massimo Aglietta^{1,2}
Giorgio Valabrega^{1,2}

¹Oncological Department, Medical Oncology, Institute for Cancer Research and Treatment (IRCC), Candiolo, Torino, Italy; ²University of Turin Medical School, Department of Biomedical Sciences and Human Oncology, Turin, Italy

Abstract: Breast cancer is a major health issue in developed countries. Overexpression of HER2, a member of epidermal growth factor receptor family, occurs in 20%–30% of breast cancers. HER2 drives the cancer cells to develop a more aggressive phenotype, to metastasize to viscera and central nervous system, and to be less sensitive to chemotherapeutic agents. Trastuzumab (Herceptin®) is a monoclonal antibody directed against the extracellular domain of HER2. As single agent or with chemotherapy, trastuzumab improves survival of HER2-positive breast cancers. In the past years, trastuzumab has completely revolutionized the scenario of the treatment of HER2-positive breast cancer, representing one of the most remarkable examples of targeted therapy in oncology. However, issues such as the best chemotherapeutic companion to associate with trastuzumab, cardiac toxicities, and clinical resistance still require tremendous efforts by researchers. Here, we review pharmacology, efficacy studies, and toxicities of trastuzumab in metastatic breast cancer. Moreover, we provide some insights on resistance to therapy. Finally, we briefly discuss trastuzumab's place in the clinical setting.

Keywords: HER2, trastuzumab, breast cancer, cardiotoxicity, resistance

Introduction to HER2-positive metastatic breast cancer management

Breast cancer is a major public health issue in developed countries in terms of morbidity, mortality, and costs.¹ Integrating multiple strategies such as early diagnosis, surgery, radiotherapy, and chemotherapy has resulted in decreased mortality in previous years.² A part of the merit should be given to a better understanding of mechanisms underlying breast cancer development. This, in turn, has resulted in the identification of druggable molecular targets in cancer cells.

Epidermal growth factor receptor 2 (ErbB2/HER2) is a ligandless tyrosine kinase receptor, member of the epidermal growth factor receptor (EGFR) family and is overexpressed in 20%–30% of breast cancers.^{3,4} Its overexpression distinguishes a subgroup of breast cancers characterized by increased aggressiveness, mortality, and high sensitivity to anthracyclines.⁵ HER2 overexpression is primarily associated with amplification of the *HER2/neu*.⁶

Its activation follows dimerization with other tyrosine kinase receptors belonging to the EGFR family (EGFR, HER3, or HER2 itself) or to other families, such as insulin-like growth factor receptor 1 (IGF-1R).^{7,8} Dimerization activates downstream signaling cascades, including mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, which promotes cellular proliferation, survival, migration, invasion, and differentiation.⁸

Correspondence: Giorgio Valabrega
Fondazione del Piemonte per l'Oncologia/
Division of Medical Oncology I, Institute
for Cancer Research and Treatment
(IRCC), Strada Provinciale 142, Km 3.95,
10060 Candiolo, Turin, Italy
Tel +39 011 9933251
Fax +39 011 9933299
Email giorgio.valabrega@ircc.it

Several features render HER2 an optimal target for breast cancer treatment:⁹

1. HER2 overexpression strongly correlates with tumor progression.
2. HER2-overexpressing breast cancer cells become almost totally dependent for their survival on signaling network cascades triggered by HER2.
3. The level of expression of HER2 in normal adult tissue is much lower than in cancer cells that overexpress the protein.

For these reasons, research has focused on developing HER2 inhibitors as potential anticancer agents. The first of such agents registered for clinical use was trastuzumab (Herceptin[®]).

HER2 status assessment

Clinical studies have shown that women who most benefit from trastuzumab have high levels of HER2 expression.¹⁰ Aspects regarding the best way to assess HER2 status have been largely discussed, and clinical implications have been outlined in recent guidelines.¹¹

Currently, HER2 status is assessed by immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), and chromogenic in situ hybridization (CISH).

IHC identifies HER2 overexpression on the cell membrane. Results are usually expressed using a semi-quantitative scoring system ranging from 0+ (no expression) to 3+ (high expression). Tumors that show no (0+) or low levels (1+) of expression are considered HER2-negative; vice-versa tumors that show high levels (3+) of expression should be considered as HER2-positive. This method is economically advantageous and readily available, but suffers from low sensitivity and high interobserver variability.¹²

FISH detects HER2 gene amplification and is more specific and sensitive than IHC.^{6,13} FISH offers quantitative results on the number of HER2 gene copies/centromere. Another FDA-approved method to assess HER2 gene amplification is CISH. CISH is very similar to FISH but utilizes conventional peroxidase or alkaline phosphatase reactions visualized under a standard bright-field microscope. Both gene amplification detected by FISH or CISH and protein expression by IHC are commonly used as initial test to assess HER2 status. Equivocal cases, defined as either IHC 2+ or FISH/CISH ratio of 1.8–2.2 or average *HER2* gene copy number four to six signals/nucleus for test systems without an internal control probe, undergo further testing with the alternative method.

A recent report by the ASCO/CAP demonstrated that, after a rigorous standardization, concordance between HER2 3+ and gene amplification detection is about 98%–98.5%.¹⁴ Phase II and III trials in metastatic disease showed that trastuzumab has relevant clinical activity against HER2-positive metastatic breast cancer. In the next paragraphs, we will summarize pharmacological issues, clinical activity, toxicities, and some biology on resistance to trastuzumab.

Review of mode of action, pharmacology, and pharmacokinetics of trastuzumab in breast cancer

Mechanism of action

Trastuzumab is a humanized IgG1k monoclonal antibody that selectively binds to the extracellular domain (ECD) of the human ErbB2 protein HER2.^{4,15} In vivo, the most relevant mechanism of action is antibody-dependent cellular cytotoxicity (ADCC). In brief, natural killer (NK) cells are able to bind trastuzumab on HER2-positive cancer cells through an Fc receptor. Upon binding, NK cells are able to induce cancer cell death by releasing lytic enzymes.¹⁶ Trastuzumab triggers antibody-dependent cell-mediated cytotoxicity (ADCC) principally by activating Fc γ receptor on NK cells.^{16,17} Unfortunately, clinical trials failed to show clinical benefit derived from association of trastuzumab with immune-modulating agents, such as IL-2, despite NK cell expansion with enhanced in vitro targeted killing of HER2-expressing cells.¹⁸

Musolino et al¹⁹ studied a population of 54 patients with HER2-amplified breast cancer who have received taxanes plus trastuzumab for metastatic disease and evaluated genotypes for the Fc γ RIIIa-158 valine(V)/phenylalanine(F), Fc γ RIIa-131 histidine(H)/arginine(R), and Fc γ RIIb-232 isoleucine(I)/threonine(T) polymorphisms. Interestingly, the authors showed that the Fc γ RIIIa-158 V/V genotype, alone and in combination with the Fc γ RIIa-131 H/H genotype, was significantly associated with better response rate and progression-free survival (PFS) to trastuzumab compared with other Fc γ R genotypes. This study supports the hypothesis that Fc γ R polymorphisms play a role in trastuzumab-mediated ADCC and predict response to trastuzumab.

At ASCO 2009, Tamura et al²⁰ presented preliminary results of a similar study on a population of 19 operable and 36 metastatic patients with HER2-overexpressing breast cancer treated with trastuzumab-containing chemotherapy, showing that Fc γ RIIa-131 H/H genotype was significantly

correlated with pCR ($P = 0.0034$) and OR ($P = 0.037$), whereas Fc γ RIIIa-158V/V genotype had a tendency to be correlated with pCR ($P = 0.067$) and was significantly correlated with OR ($P = 0.037$).

Similarly to trastuzumab, it was shown that Fc γ receptor polymorphisms play a role also in differential response to other antibodies, such as cetuximab in colorectal cancer.^{21,22}

Furthermore, impaired T cell and NK function can possibly contribute to trastuzumab resistance.

Several studies showed that a reduced number or impaired function of NK cells is correlated with shortened response to trastuzumab,^{23,24} and often, this is due to surgical or chemo- and radiotherapy.²⁵

In vitro, trastuzumab induces the following perturbations in cancer cells: HER2 receptor downregulation and degradation and subsequent attenuation of downstream signaling;²⁶ G0 arrest;²⁷ and induction of apoptosis.²⁸ The mechanisms of action of trastuzumab are summarized in Figure 1.

Pharmacology

The pharmacokinetics of trastuzumab has been studied in patients with metastatic breast cancer and subsequently in early-stage breast cancer patients in addition to adjuvant chemotherapy. Short-duration intravenous infusions of 10, 50, 100, 250, and 500 mg of trastuzumab once weekly demonstrated dose-dependent pharmacokinetics. The half-life averages 1.1 (using a one-compartment model at 10 mg) and 23 (using a two-compartment model at 500 mg) days at the 10- and 500-mg dose levels, respectively. At the highest weekly dose studied (500 mg), mean peak serum concentration was 377 μ g/mL.²⁹

In clinical trials, where a loading dose of 4 mg/kg trastuzumab followed by a subsequent weekly dose of 2 mg/kg was used, the mean clearance was 0.225 L/day. Between weeks 16 and 32, trastuzumab serum concentrations reached a steady state with a mean trough and peak concentrations of approximately 79 μ g/mL and 123 μ g/mL, respectively.³⁰

Population pharmacokinetics analysis of data from the initial phase I, II, and III studies suggested a half-life of 28.5 days, which justifies an every 3-week schedule: this long half-life is similar to that of endogenous IgG1 immunoglobulins (23 days).³¹ Moreover, it was shown that trastuzumab administered every three weeks has pharmacokinetics similar to the weekly regimen;³² the washout period is up to 20 weeks (95% confidence interval, 18–24 weeks); steady state pharmacokinetics should be reached by approximately 20 weeks (95% confidence interval, 18–24 weeks).

Trastuzumab's volume of distribution is approximately that of serum volume (44 mL/kg).²⁹

The distribution of trastuzumab does not seem to be altered by age or serum creatinine (up to 2.0 mg/dL), although formal interaction studies have not been performed.³³

Monoclonal antibodies can face added difficulties due to their high molecular weights, target specificity, kinetics of metabolism and internalization, as well as the patterns of antigen expression in combination with target-binding affinity. The high molecular weight (145.5 KDa) and binding affinity of trastuzumab, in combination with microenvironmental factors, may limit its distribution and efficacy.^{34,35} Tumor vascular abnormalities contribute to inefficient drug penetration by creating regions of irregular or intermittent blood flow, slowed interstitial fluid velocity, and often elevated interstitial fluid pressure.^{36,37} Notwithstanding considerable heterogeneity in trastuzumab distribution, the ability of trastuzumab to penetrate through tissues seems to be relatively efficient.³⁵

Trastuzumab and blood–brain barrier

Because of its high molecular weight, trastuzumab is not able to cross an intact blood–brain barrier (BBB). However, the patients with brain metastases (BM) are commonly treated as indicated with at least one of the following treatment modalities: whole brain radiotherapy (WBRT), stereotactic radiotherapy, and metastasectomy.

These treatments for BM could disrupt the BBB and subsequently make it possible to deliver trastuzumab into the central nervous system (CNS).³⁸ In a pilot study, Stemmler et al³⁹ have evaluated the ability of trastuzumab to penetrate the BBB measuring trastuzumab levels in the serum and in cerebrospinal fluid of HER2-positive breast cancer patients with BM. The authors demonstrated that, at different time points, trastuzumab levels are increased in cerebrospinal fluid when BBB is impaired (eg, in the presence of meningeal carcinomatosis or radiotherapy).

Efficacy studies in metastatic breast cancer

Trastuzumab as single agent or in combination with chemotherapy

Phase II trials of single-agent trastuzumab showed overall response rate of 11.6%–15% in women with heavily pretreated HER2-positive metastatic breast cancer^{30,40} and of 35% in the first-line setting³³ and, very interestingly, median duration of response was similar to the one that can be

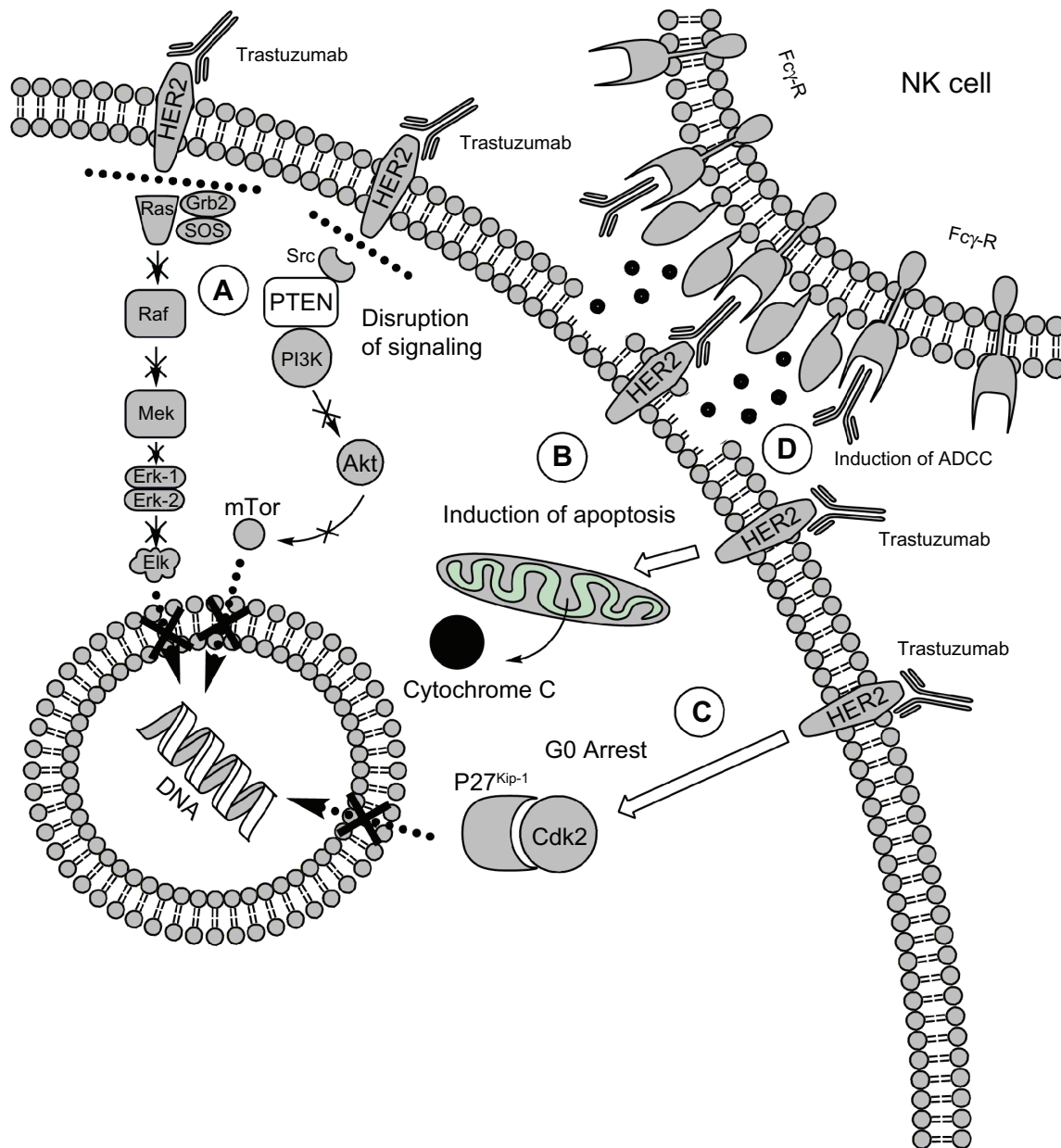


Figure 1 Mechanisms of action of trastuzumab. **A**) In vitro, trastuzumab is able to disrupt signaling through PI3K/Akt and MAPK signaling pathways; causes a disruption of the binding of Src to HER2, allowing PTEN to inhibit Akt **B**) induces apoptosis of target cells and **C**) cell cycle arrest in G0-G1 phase, via modulating the cyclin-dependent kinase (CDK) inhibitor 27^{Kip-1}. **D**) In vivo, trastuzumab binds the Fcγ receptor on NK cells and triggers the antibody-dependent cell-mediated cytotoxicity (ADCC).

Abbreviations: HER2, human epidermal growth factor receptor 2; NK, natural killer; FcγR, fragment crystallizable region gamma receptor; Grb2, growth factor receptor-bound protein 2; SOS, son of sevenless protein; Ras, small GTPase protein; Raf, Mek, and Erk, serine/threonine-protein kinases; Elk, transcription factor; Src, Rous sarcoma tyrosine kinase; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol 3-kinases; Akt, serine/threonine protein kinase; mTor, mammalian target of rapamycin; p27^{Kip-1}, cyclin-dependent kinase inhibitor 1B; Cdk2, cyclin-dependent kinase 2a.

obtained with the association with chemotherapy. The first-line study also showed that dose escalation does not increase overall response rate.

These studies led to the pivotal clinical trial, H0648g, which started in 1995 and enrolled 469 patients with previously untreated, HER2-positive metastatic breast cancer. Patients were randomly assigned to receive chemotherapy (either doxorubicin or epirubicin combined with

cyclophosphamide, in patients without previous exposure to adjuvant anthracyclines, or paclitaxel in patients who had previously received adjuvant anthracyclines) with or without trastuzumab. The primary end point of this study was median time to disease progression, which was 4.6 months in patients who received chemotherapy alone and 7.4 months for those who received chemotherapy plus trastuzumab ($P < 0.001$); trastuzumab was also associated

with an increase in objective response rate (50% versus 32%; $P < 0.001$), longer duration of response (median 9.1 versus 6.1 months; $P < 0.001$), and longer median survival (25.1 versus 20.3 months; $P = 0.046$).¹⁰ A subsequent randomized phase II trial of docetaxel with trastuzumab or docetaxel alone published in 2005 also showed an improved overall response rate (61% versus 34%; $P = 0.0002$), better overall survival (median 31.2 versus 22.7 months; $P = 0.0325$), and longer time to disease progression (median 11.7 versus 6.1 months; $P = 0.0001$) for the association arm.⁴¹ Various non-randomized phase II trials have shown the efficacy and safety of trastuzumab in combination with most other chemotherapies (also including liposomal anthracyclines) for treatment of breast cancer. Currently, it is unclear whether any specific chemotherapeutic drug or class can be particularly effective in combination with trastuzumab (Table 1).^{42–58}

Second-line treatment

Continuation versus discontinuation of trastuzumab after disease progression is controversial among oncologists. Retrospective studies have had conflicting results.^{59,60} The only available randomized phase III trial which apparently supports continuation of trastuzumab in association with capecitabine in patients who have progressed while receiving trastuzumab⁶¹ prematurely closed accrual and, most importantly, was biased by significant unbalances in treatment arms.

Lapatinib is an orally bioavailable, small-molecule dual HER1/HER2 tyrosine kinase inhibitor. In patients whose disease has progressed after prior treatment with an anthracycline, a taxane, and trastuzumab, a randomized, controlled phase III study supports the use of the lapatinib in combination with capecitabine.⁶² The median time to progression for patients who received capecitabine plus lapatinib was 8.4 months compared with 4.4 months in women who received capecitabine monotherapy (HR 0.49; 95% CI 0.34–0.71; $P < 0.001$), and there was a possibility of improved overall response (22% versus 14%; $P = 0.09$). Phase II data also show modest activity of lapatinib either alone or in combination with capecitabine for the treatment of brain metastases in patients with HER2-positive breast cancer.⁶³ Trastuzumab and lapatinib without chemotherapy is another treatment option in women with HER2-positive breast cancer who have disease progression while receiving trastuzumab. In a randomized phase III study, 296 patients with metastatic disease who had progression on trastuzumab treatment were randomly assigned to receive lapatinib plus trastuzumab or lapatinib alone. Patients were heavily

pretreated and had received a median of six prior anticancer regimens and a median of three prior lines of trastuzumab. In the combination group, compared with lapatinib monotherapy, the median progression free survival (PFS) was 12 weeks versus 8.1 weeks and the overall clinical benefit rate was 24.7% versus 12.4% ($P = 0.01$).⁶⁴

Trastuzumab and endocrine therapy

About 40%–50% of HER2-positive tumors coexpress hormone receptors (HR). Coexpression of HER2 in HR-positive tumors is associated with reduced efficacy of endocrine therapy.^{65,66} One of the most credited mechanism that has been proposed to explain endocrine resistance is cross-talk between the estrogen receptor (ER) and HER family pathways.^{67,68} Hyperactive HER2 or EGFR can activate the ER directly, in the absence of its natural ligand. This explains not only resistance to selective estrogen receptor down-modulators, but also to aromatase inhibitors. Combined blocking of both pathways has been tested in two randomized clinical trials in patients with previously untreated HER2-/HR-positive advanced breast cancer.^{69–71}

The trastuzumab in dual HER2-positive, ER-positive metastatic breast cancer (TANDEM) trial randomly assigned women with HER2-positive and hormone-receptor-positive untreated metastatic breast cancer to anastrozole with or without trastuzumab. This study found a significant advantage for both response rate (6.8% versus 20.3%; $P = 0.018$) and PFS (2.4 versus 4.8 months; $P = 0.0016$) with the addition of trastuzumab.⁶⁹

A similar study of the same population of patients randomly assigned women to receive letrozole plus lapatinib or letrozole alone and showed increased PFS (8.2 months versus 3 months; HR 0.71; $P = 0.019$) and higher overall response rate (28% versus 15%; $P = 0.021$).⁷⁰ In conclusion, in postmenopausal women with hormone-receptor positive, HER2-positive, metastatic breast cancer with low disease burden, the combination of anti-HER2-targeted therapy with an aromatase inhibitor is a reasonable option.

Review of the mechanisms of trastuzumab resistance in breast cancer

The outstanding results obtained with the introduction of trastuzumab in the clinical management of HER2-overexpressing breast cancer are limited by primary and acquired resistance to the antibody. Currently, there is no widely accepted definition of resistance. In the clinical setting, resistance is defined as progressive disease.⁷²

Table I Results of the main studies of trastuzumab with chemotherapy in metastatic breast cancer

Author	Study	Drugs	No. of patients	Patient's characteristics	Main results
Slamon et al ¹⁰	Phase III	Taxanes or anthracyclines ± wTrastuzumab	469	Previously untreated HER2+ metastatic BC	mTTP (chemo + trastuzumab versus chemo) 7.4 versus 4.6 months ($P = 0.001$) OS (chemo + trastuzumab versus chemo) 25.1 versus 20.3 months ($P = 0.01$)
Marty et al ⁴¹	R phase II	Docetaxel 100 mg/m ² + q3wTrastuzumab versus docetaxel 100 mg/m ² alone	186	Previously untreated HER2+ metastatic BC	ORR 61% versus 34% ($P = 0.0002$) OS 32 versus 22 months ($P = 0.0325$)
Esteve et al ⁴³	Phase II	Docetaxel 35 mg/m ² /w + wTrastuzumab	30	HER2+ metastatic BC	ORR 63% (95% CI = 44%–80%)
Sledge ⁴⁶	Phase II	Gemcitabine 1200 mg/m ² days 1,8 q21 + paclitaxel 175 mg/m ² day 1 q21 + wTrastuzumab	42	Previously untreated HER2+ metastatic BC	ORR 67% mTTP 9 months
Bianchi et al ⁴⁷	Phase II	Cohort 1: 3 cycles of DT (60/150 mg/m ²) and wTrastuzumab followed by 9 cycles of T (80 mg/m ²) and wTrastuzumab Cohort 2: 3 cycles of DT (60/150 mg/m ²) followed by 9 cycles of T (80 mg/m ²) and wTrastuzumab	Cohort 1: 16 Cohort 2: 16	Previously untreated HER2+ advanced and metastatic BC	ORR (for both cohorts) 87.5% (95% CI = 61.65%–98.45%)
Pegram et al ^{48*}	Phase II	1. Docetaxel 75 mg/m ² q3wks + cisplatin 75 mg/m ² q3wks + wTrastuzumab 2. Docetaxel 75 mg/m ² q3wks + carboplatin AUC6 + wTrastuzumab	1.62 2.62	HER2+ advanced and metastatic BC*	1. ORR 79% (95% CI = 66%–89%) mTTP 9.9 months (95% CI = 8.3–13.1 months) 2. ORR 58% (95% CI = 44%–70%) mTTP 12.7 months (95% CI = 8.6–15.5 months)
Robert et al ⁵¹	Phase III	Arm A: 6 cycles of T (175 mg/m ²) q3wks + carboplatin AUC6 q3wks + wTrastuzumab Arm B: 6 cycles of T(175 mg/m ²) q3wks + wTrastuzumab	196	Previously untreated HER2+ metastatic BC	ORR Arm A 52% (95% CI = 42%–62%) ORR Arm B 36% (95% CI = 26%–46%) ($P = 0.04$) PFS Arm A 10.7 months PFS Arm B 7.1 months HR 0.66 (95% CI = 0.59–0.73) ($P = 0.03$)
Montemurro et al ⁵⁸	Phase II	Docetaxel 75 mg/m ² q3wks + wTrastuzumab	25	Heavily pretreated HER2+ metastatic BC	ORR 70%
Jahanzeb et al ⁴⁴	Phase II	Vinorelbine 30 mg/m ² /w + wTrastuzumab	40	Previously untreated HER2+ metastatic BC	ORR 78% (95% CI = 62%–90%) mTTP 18 months (95% CI = 13.7–34.2 months)
Burstein et al ⁴⁵	Phase II	Vinorelbine 25 mg/m ² /w + wTrastuzumab	54	Previously untreated HER2+ metastatic BC	ORR 68% (95% CI = 54%–80%) mTTP 5.6 months (95% CI = 0.46–16 months)
Schaller et al ⁵²	Phase II	Capecitabine 2500 mg/m ² day 1–14 q21 + wTrastuzumab	27	Heavily pretreated HER2+ metastatic BC	ORR 78% PFS 6.7 months

(Continued)

Table I (Continued)

Author	Study	Drugs	No. of patients	Patient's characteristics	Main results
O'Shaughnessy et al ⁴⁹	Phase II	Gemcitabine 1200 mg/m ² days 1,8 q21 + wTrastuzumab	64	HER2+ metastatic BC	ORR 38% (95% CI = 26%–50%) mTTP 5.8 months
Pegram and Slamon ⁴²	Phase I/II	Cisplatin + wTrastuzumab	37	HER2+ metastatic BC	ORR 24.3% mTTP 8.4 months
Chia et al ⁵⁰	Phase II	PLD 50 mg/m ² q4wks + wTrastuzumab	30	Previously untreated HER2+ metastatic BC	ORR 38% (95% CI = 21.1%–78.9%) PFS 12 months
Andreopoulou et al ⁵³	Phase II	PLD 50 mg/m ² q4wks + wTrastuzumab	12	Heavily pretreated HER2+ metastatic BC	The trial closed after 2.5 years for slow accrual
Cortes et al ⁵⁴	Phase II	Nonpegylated LD 50 mg/m ² q3wks + paclitaxel 80 mg/m ² /w + wTrastuzumab	69	Previously untreated HER2+ metastatic BC	ORR 98.1% (95% CI = 90.1%–99.9%) mTTP 22.1 months (95% CI = 16.4–46.3 months)
Christodoulou et al ⁵⁵	Phase II	PLD 30 mg/m ² q3wks + q3wTrastuzumab	37	HER2+ metastatic BC	ORR 22% PFS 6.5 months (95% CI = 0.8–31.1 months)
Stickeler et al ⁵⁶	Phase II	PLD 40 mg/m ² q4wks + wTrastuzumab	16	Previously untreated HER2+ metastatic BC	CBR 50% PFS 9.67 months
Venturini et al ⁵⁷	Phase II	Nonpegylated LD 50 mg/m ² q3wks + docetaxel 75 mg/m ² q3wks + wTrastuzumab	31	Previously untreated HER2+ metastatic BC	ORR 65.5% mTTP 13 months

Notes: *This study includes two phase II studies: (1) BCIRG101 and (2) UCLA-ORN. In the UCLA-ORN study, patients were previously treated with taxanes.

Abbreviations: wTrastuzumab, trastuzumab loading dose of 4 mg/kg followed by weekly doses of 2 mg/kg; R, randomized; HER2+, HER2 positive; BC, breast cancer; chemo, chemotherapy; mTTP, median time to progression; OS, overall survival; q3wTrastuzumab, trastuzumab loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks; ORR, overall response rate (CR-complete remission + PR-partial remission); w, week; wks, weeks; D, doxorubicin; T, paclitaxel; AUC, area under curve; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; LD, liposomal doxorubicin; CBR, clinical benefit rate (CR + PR + stable disease-SD lasting 6 months or more).

Clinical practice shows that initial response to trastuzumab is almost invariably followed by tumor progression.^{59,73} Moreover, a large percentage of women fail to respond to trastuzumab, showing primary resistance.^{10,74}

At present, neither the mechanism of action nor the mechanisms of resistance to trastuzumab are completely understood. Nevertheless, several hypotheses have been proposed (Figure 2).

HER2 downstream signaling adaptability

HER2 plays a key role as positive regulator of a high complexity signaling network, consisting of distinct semiautonomous functional units that show strong internal connections, high adaptability, and very complex regulation.

This complexity and, at the same time, the flexibility and modularity of the signaling transduction pathway explain why the network can maintain proper function in the face of efficient inhibition of individual component, such as HER2.⁷⁵

An increasing body of evidence sustains the central role of adaptive modulation of PI3K/Akt/mTor signaling pathway in resistance to anti-HER2-targeted agents.

Sergina et al⁷⁶ have provided evidence that a chronic exposure to HER2 inhibitors may result in enhanced positive feedback mechanism controlled by Akt, whereby Akt inhibition leads to HER3 redistribution to the cell membrane and to sustained HER3 phosphorylation, resulting in cellular adaptation with the potential of reducing the efficacy of these drugs.

Nagata et al⁷⁷ suggested an important role of decreased expression of the PTEN (phosphatase and tensin homolog) protein in development of resistance to trastuzumab. In preclinical models, they demonstrated that trastuzumab causes a disruption of the binding of Src to HER2, allowing PTEN to inhibit Akt therefore inducing growth arrest. When PTEN levels are low, however, Akt remains active and trastuzumab efficacy is impaired. A correlation between PTEN loss and resistance to trastuzumab was also shown in a retrospective analysis on HER2 positive BC patients.

Moreover, mutations in PI3KCA gene sequence can impair the ability of PTEN to inhibit Akt, also in presence of normal levels of PTEN, therefore contributing to trastuzumab resistance.⁷⁸

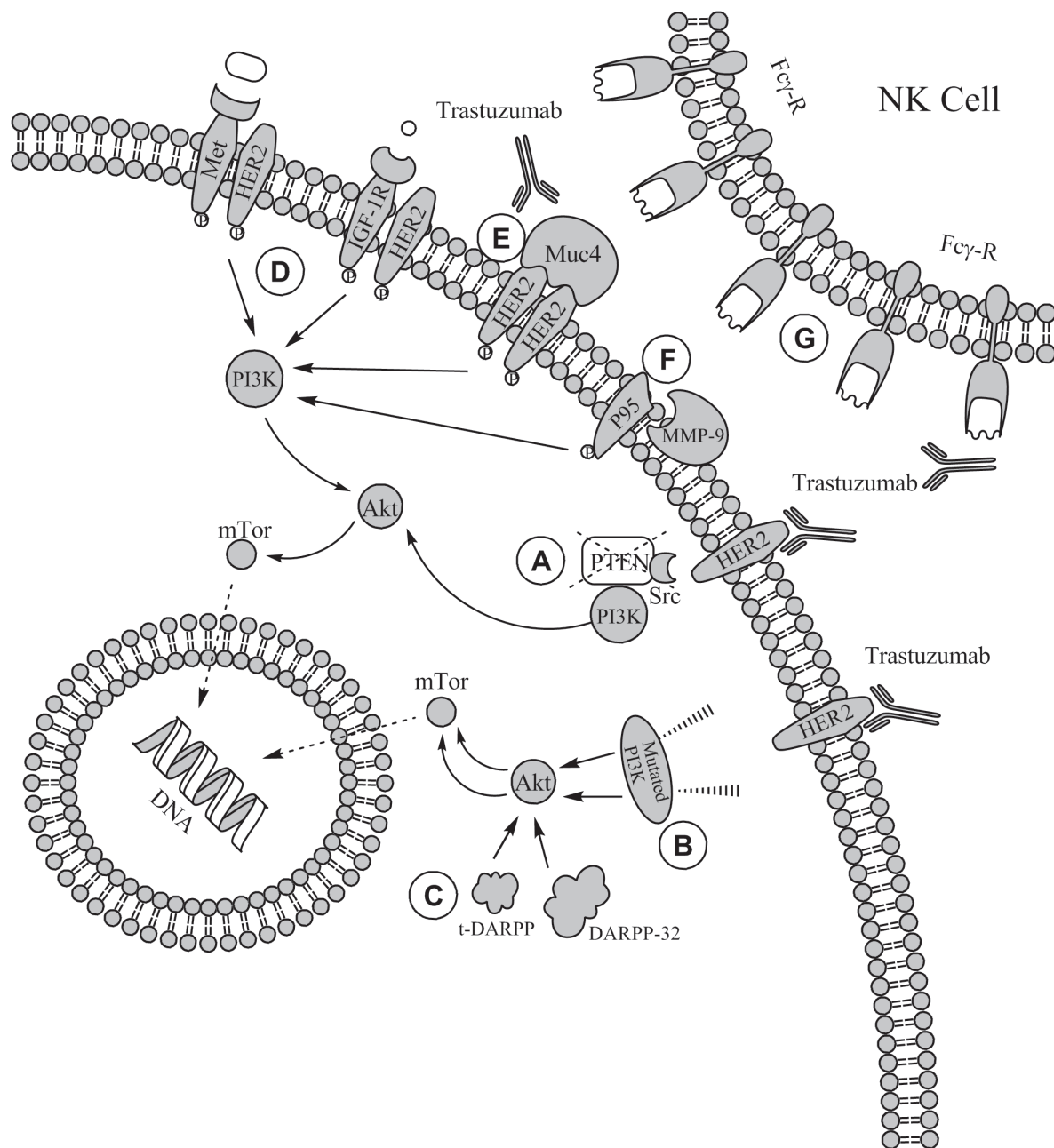


Figure 2 Mechanisms of resistance to trastuzumab. **A)** Loss of PTEN protein can impair efficacy of trastuzumab; **B)** mutation of PI3KCA gene sequence **C)** Overexpression of DARPP-32 and its truncated form t-DARPP, leading to increased phosphorylation of Akt; **D)** HER2 can dimerize with other tyrosine kinase receptors, such as Met or IGF-1R, activating alternative signaling pathways; **E)** binding of HER2 with trastuzumab can be prevented by other cellular surface proteins, such as Muc4, **F)** or by the shedding of the extracellular domain of HER2 mediated by metalloproteinases; **G)** Fcγ-receptor polymorphisms can impair antibody-dependent cell-mediated cytotoxicity (ADCC) in vivo.

Abbreviations: HER2, human epidermal growth factor receptor 2; NK, natural killer; FcγR, fragment crystallizable region gamma receptor; Src, Rous sarcoma tyrosine kinase; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol 3-kinases; Akt, serine/threonine protein kinase; mTor, mammalian target of rapamycin; DARPP-32, dopamine- and cyclic-AMP-regulated phosphoprotein; t-DARPP, truncated form of dopamine- and cyclic-AMP-regulated phosphoprotein; MMP9, matrix metalloproteinase 9; p95, truncated form of HER2; Muc4, mucin 4; IGF-1R, insulin-like growth factor receptor I; Met, hepatocyte growth factor receptor.

More recently, Belkhiri et al⁷⁹ described a possible mechanism of resistance involving both DARPP-32, a neuronally characterized protein that is centrally involved in dopamine-induced signaling pathways in the brain and is best known as a potent inhibitor of phosphatase I in neurosignaling

and its truncated form, known as t-DARPP. Overexpression of both DARPP32 and t-DARPP led to increased phosphorylation of Akt and increased BCL2 protein levels in trastuzumab-resistant cell lines⁷⁹ and in vivo.⁸⁰ Moreover, t-DARPP contributes to trastuzumab resistance by blocking

the antibody's effect on HER2 and maintaining its high levels in these cells via HSP90-mediated stabilization.⁷⁹

Furthermore, a role in developing resistance to trastuzumab is probably played by dysregulation of mechanisms controlling survival and apoptosis.⁸¹ Recently, it has been shown that upregulation of survivin leads to the development of resistance to lapatinib, a dual inhibitor of EGFR and HER2.^{82,83}

Moreover, although downregulation of survivin seems to be essential for trastuzumab-mediated induction of apoptosis,⁸⁴ a failure in its downregulation could contribute to resistance.⁸⁵

Activation of alternative signaling pathways

HER2 is the preferred dimerization partner of the other ErbB family receptors and acts as an 'amplifier' for the signal transduction coming outside the cell.⁷⁵ However, the activation of downstream signaling components can also occur via the formation of complexes that do not contain HER2, such as EGFR homodimers or EGFR/HER3 heterodimers.⁵ Signaling through these alternate complexes might provide a mechanism for bypassing the requirement for HER2-mediated signaling and thereby circumvent HER2-targeted inhibitors.⁸⁶ Moreover, overexpression of EGF family ligands, such as TGF- α , EGF, heregulin, betacellulin, and others, can contribute to trastuzumab resistance.⁸⁷⁻⁹⁰

IGF-1R plays a role in resistance. It was shown that cells overexpressing both HER2 and IGF-1R are insensitive to trastuzumab inhibition.⁹¹ Nahta et al⁹² demonstrated that IGF-1R can heterodimerize with HER2 and can induce its phosphorylation in trastuzumab-resistant cells, resulting in activation of a downstream cascade that involves phosphorylation of PI3K/Akt.

More recently, it was also shown that other tyrosine kinase receptors such as Met receptor (hepatocyte growth factor receptor) can interact with HER2 and IGF-1R and can contribute to resistance through the activation of the PI3K/Akt signaling cascade. Met receptor is overexpressed with HER2 in a subset of aggressive breast cancers⁹³ and trastuzumab treatment induces upregulation of Met.⁹⁴ Moreover, Shattuck et al⁹⁴ have provided evidence that in trastuzumab-resistant cell lines, Met receptor activation protects cells against trastuzumab inhibition; conversely, loss of Met function, either through RNA-interference-mediated depletion or small-molecule-mediated inhibition, restores sensitivity to the antibody.

The nuclear factor NF- κ B, which is a downstream mediator of growth signaling, has been shown to be frequently overexpressed and deregulated in HER2-positive breast cancers.^{95,96} It has been shown by Cardoso et al that the proteasome inhibitor bortezomib, which specifically inhibits the activity of NF- κ B, is able to synergize with trastuzumab in HER2-overexpressing breast cancer cell lines.⁹⁷

Inaccessibility of epitope to trastuzumab

Trastuzumab exerts its effects by binding an epitope on the juxtamembrane extracellular domain (ECD) of HER2.⁹⁸ Steric interference by extracellular molecules can prevent the binding of the antibody to its target and can therefore contribute to resistance.

Overexpression of MUC4, a membrane-associated sialomucin, was associated by Nagy et al⁹⁹ with resistance to trastuzumab in a HER2-overexpressing cancer cell line (JIMT-1) established from a patient showing resistance to the antibody. The authors observed that the expression of MUC4 was higher in the resistant clone (JIMT-1) 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.

Furthermore, overexpression of hyaluronan receptor CD44 and MUC1 (a membrane mucin) truncated forms were shown to have a similar role in trastuzumab-resistant cancer cells.^{100,101}

On the basis of the observation that trastuzumab binds a HER2 epitope which is different from that employed by IHC methods, Bussolati et al¹⁰² retrospectively tested a biotinylated trastuzumab (BioHER) in HER2-amplified (FISH positive) breast cancer specimens. Positivity to BioHER seems to predict more accurately than IHC and FISH the efficacy of trastuzumab-based therapy. These results indicate that lack of accessibility of the epitope to trastuzumab may limit the activity of this antibody in vivo, although further validation is needed.¹⁰³

The association between trastuzumab and HER2 can also be prevented by shedding the ECD of the receptor and producing a truncated form of protein named p95-ErbB2. This truncated form of protein has constitutive kinase activity, but cannot bind trastuzumab; furthermore, its overexpression contributes to resistance.^{98,104}

The major responsible factors for the proteolytic cleavage of HER2 are metalloproteinases,¹⁰⁵ and inhibition of their proteolytic activity can circumvent trastuzumab resistance.¹⁰⁶

Moreover, monitoring serum HER2 levels is a fascinating and relatively cheap strategy to predict response to

trastuzumab treatment. Although several studies have shown a potential utility of the approach,^{107–109} others have failed to demonstrate a clear correlation between serum ECD and response to trastuzumab.¹¹⁰

Safety and tolerability

Although the focus of our critical review is metastatic breast cancer, we will discuss also the available data on cardiac toxicity derived from adjuvant trials.

Cardiac toxicity in metastatic breast cancer

Cardiac toxicity was an unexpected finding during the clinical development of trastuzumab; therefore, early clinical trials in the metastatic setting did not perform baseline or prospective cardiac monitoring, and did not exclude patients with underlying cardiac disease. The first signal of trastuzumab-associated cardiotoxicity was seen in the pivotal phase III trial by Slamon et al, which randomized 469 patients with HER2-overexpressing breast cancer to standard chemotherapy (either an anthracycline-containing regimen or paclitaxel) alone or standard chemotherapy plus concurrent weekly trastuzumab.¹⁰ This led to the formation of an independent Cardiac Review and Evaluation Committee (CREC) to retrospectively review the cardiac toxicity data from seven phase II and III studies in 1219 patients who received trastuzumab as monotherapy or in combination with chemotherapy.¹¹¹ The CREC defined cardiac dysfunction as cardiomyopathy characterized by a decrease in cardiac left-ventricular ejection fraction (LVEF) that was either global or more severe in the septum, by symptoms or associated signs of congestive heart failure (CHF), or a decline in LVEF of $\geq 5\%$ (with symptoms) or 10% (no symptoms) with an absolute LVEF of $\leq 55\%$.

In the phase III trial, data from the CREC analysis demonstrated an unacceptable rate of cardiac toxicity in the group receiving concurrent anthracycline and trastuzumab.¹⁰ Cardiotoxicity (both symptomatic and asymptomatic) was reported in 27% of patients who received trastuzumab plus adriamycin and cyclophosphamide (AC) compared with 8% who received AC without trastuzumab. In contrast, 13% of patients in the paclitaxel plus trastuzumab arm developed cardiotoxicity, while only 1% of patients receiving paclitaxel alone were affected. Among patients with cardiac toxicity, the incidence of New York Heart Association (NYHA) class III or IV CHF was highest among patients who had received an anthracycline-based chemotherapy with trastuzumab (16%) when compared to patients who received

an anthracycline-based regimen without trastuzumab (3%), paclitaxel-based chemotherapy with trastuzumab (2%), and paclitaxel only (1%). Furthermore, the recovery of patients who developed cardiac dysfunction differed between the treatment groups. Of the 34 patients who developed cardiac dysfunction while receiving AC plus trastuzumab, 7 had persistent NYHA class III/IV symptoms after treatment for CHF, compared with none of the 10 patients in the paclitaxel and trastuzumab arm.⁴⁰ Recently, an interesting randomized phase II trial in HER2-positive metastatic breast cancer demonstrated that the association of the less-cardiotoxic anthracycline epirubicin at a low dose (60 mg/sqm) with trastuzumab is able to induce a significant amount of durable responses (57%) and low cardiac toxicity (only 1.7% of dose-limiting cardiac toxicity).¹¹² Moreover, most of the trials associating liposomal anthracyclines with trastuzumab in metastatic disease revealed low incidence of symptomatic cardiac events.^{50,53–57}

The CREC also reviewed a number of trastuzumab monotherapy trials and found a 3%–7% range of cardiac dysfunction. Patients enrolled in these trials were largely unselected and many of the patients who developed cardiac dysfunction had underlying cardiac disease or had received a cumulative anthracycline dose >400 mg/m.^{2,33,40,113} Guarneri et al evaluated the cardiac safety of long-term trastuzumab therapy in patients with advanced disease at a single institution. Cardiac events in this study were defined as an asymptomatic decrease of LVEF $< 50\%$, an absolute 20% LVEF drop from baseline, or signs or symptoms of heart failure. Of 173 evaluable patients who received ≥ 1 year of trastuzumab-based therapy (median length of treatment was 21.3 months), 49 patients (28%) experienced a cardiac event, 19 of whom (10.9%) suffered grade 3 cardiac toxicity. All but 3 of these patients had improvement in LVEF and/or symptoms with the discontinuation of trastuzumab and activation of appropriate medical therapy.¹¹⁴

Cardiac toxicity in the adjuvant setting

The studies with trastuzumab in the adjuvant setting were designed considering, among other factors, the modest cardiotoxicity of this compound. The cardiac exclusion criteria were very strict in some trials and took into account the fact that no information of the long-term cardiac safety of trastuzumab was available at the time of study planning. In all studies, the baseline LVEF measured either by ultrasonography or multiple-gated acquisition scan (MUGA) had to be above the institutional lower normal limit, which

is usually 50%–55%. Furthermore, LVEF was monitored at regular intervals during treatment (every 3 months in most of the trials) in asymptomatic patients. Symptomatic cardiac toxicity occurred at acceptably low rates in all trials. It was slightly more frequent in trials where trastuzumab was administered concomitantly with taxanes, after exposure to anthracyclines, and less frequent in trials adopting the ‘sequential’ strategy. A relevant finding is that, in the two North American trials¹¹⁵ considering patients with symptomatic cardiotoxicity, those unable to start trastuzumab because of LVEF drop after AC and those developing asymptomatic cardiac toxicity, a total of 20% of patients did not receive the planned treatment with trastuzumab. This is much higher than what registered in the HERA trial,¹¹⁶ where only 5.2% of the patients discontinued trastuzumab because of cardiac toxicity.

The cardiac safety data of the BCIRG 006 study confirmed that the omission of anthracyclines resulted in reduced cardiac toxicity in the TCH arm.¹¹⁷

Cardiac safety results in the FinHER suggested that a short-term treatment with trastuzumab before anthracycline exposure might minimize the incidence of symptomatic CHF, which occurred in 0.9% and 1.7% of patients receiving chemotherapy alone or with trastuzumab, respectively.¹¹⁸

The cardiac safety findings of the randomized trials have several practical implications that merit being addressed. The cumulative incidence of cardiac events increased gradually during the scheduled trastuzumab treatment period.^{119–121} However, it remained approximately constant during follow-up after the completion of treatment. Therefore, although regular cardiac monitoring, (every 3 months), by LVEF assessment is generally advised in patients on treatment, its role during patient follow-up needs to be defined.

Another important issue to be addressed is whether the apparently low rates of cardiac events, either symptomatic or asymptomatic, are reproducible in the clinical practice. For example, some reports in patients receiving sequential trastuzumab, including our own, describe an incidence of trastuzumab discontinuation because of either overt cardiotoxicity or of asymptomatic LVEF drops outside clinical trials that is higher than that reported in the HERA trial.^{122,123} Although clinical cardiotoxicity occurs at a rate that is clinically acceptable, as many as 12%–18% of the patients may need trastuzumab discontinuation even if the sequential strategy is employed. The application of algorithms of trastuzumab

discontinuation or prosecution in asymptomatic patients with LVEF drop¹²⁴ and the establishment of trastuzumab-based regimens that minimize the risk of cardiac toxicity are two possible areas of intervention. One example is the TCH regimen used in the BCIRG 006 study, which several healthcare systems have approved for use in patients with contraindications to anthracyclines.

Despite having been reported as reversible by trastuzumab discontinuation and prompt administration of cardiac medications, trastuzumab-related cardiac toxicity may not recover in a significant proportion of patients.¹²⁵ Obviously, this calls for the involvement of the cardiologist in the management of patients who are candidates to trastuzumab to evaluate their cardiac risk profile. At the same time, markers that could help predict patients more likely to develop reversible or irreversible cardiotoxicity are eagerly awaited. Recently, for example, an elevation of troponin I during trastuzumab has been shown to correlate with irreversible trastuzumab-related cardiac toxicity.¹²⁶

The early use of beta-blockers and angiotensin-converting enzyme (ACE) inhibitors to prevent trastuzumab-related cardiotoxicity is another promising approach.¹²⁷ These drugs can act on myocardial remodeling and have a well-established role in patients with trastuzumab-related cardiac toxicity. Preliminary results of a prospective trial have been recently reported by Munoz et al at the 2010 ASCO meeting.¹²⁸ The use of beta-blockers and ACE inhibitors concomitantly with trastuzumab was associated with a smaller decrease in mean LVEF (4.7 versus 10.3 percentage points $P < 0.001$) when compared to untreated patients.

Interestingly, smaller studies in the neoadjuvant setting have shown that the concurrent administration of conventional anthracyclines is associated with a low incidence of cardiac events.^{129,130} At the present time, it is impossible to ascertain whether these low rates of cardiac toxicity are due to the particular clinical setting or just due to accurate selection of patients. Therefore, outside clinical trials, the concomitant administration of trastuzumab and anthracyclines should be avoided.

Conclusions, place in therapy

Trastuzumab has improved survival of HER2-positive advanced breast cancer patients. On the basis of phase II and III trials, trastuzumab in association with chemotherapy is the standard treatment for HER2-overexpressing metastatic breast cancers. However, the optimal chemotherapeutic

companion for trastuzumab is not defined. Probably, the best evidence-based combination is with a taxane (docetaxel or paclitaxel).

Although it is difficult to define an optimal strategy for second-line treatment of HER2-positive breast cancers, the most reasonable approach is the all-oral combination capecitabine-lapatinib. Continuation of trastuzumab beyond disease progression is sustained only by a few retrospective trials^{60,131–134} and the weak and biased randomized phase III trial by von Minckwitz et al,⁶¹ which compared capecitabine with capecitabine and trastuzumab after failure of a trastuzumab-containing frontline treatment. The only concern about trastuzumab treatment in metastatic disease is cardiac toxicity. However, clinical trials clearly indicate that, if the antibody is not used in association with anthracyclines, the rate of cardiac events is low and most of them are reversible. Moreover, not only associations with taxanes, vinorelbine, gemcitabine, capecitabine are safe and active but also with liposomal anthracyclines. However, in the absence of randomized phase III trials, the last category of drugs cannot be currently recommended in association with trastuzumab.

Future perspectives: novel drugs under investigation

At the moment, a number of novel anti-HER2-targeted agents are under evaluation. Because a comprehensive discussion of all these agents is beyond the scope of the review, in the following section, only the most promising drugs will be briefly considered.

Neratinib (HKI-272), an orally administered small molecule that acts as an irreversible inhibitor of the tyrosine kinase domain of EGFR, HER2, and HER4 (pan-HER inhibitor),¹³⁵ showed impressive results in a multinational, multicenter, open-label, phase II trial conducted by Burstein et al, on locally advanced or metastatic breast cancer patients.¹³⁶ Patients enrolled were included in two cohorts according to whether or not they had been previously exposed to trastuzumab. Patients in the latter cohort had to have progressed after at least 6 weeks of trastuzumab given in the metastatic or locally advanced setting, or during or after adjuvant trastuzumab. Treatment with neratinib yielded a response rate of 26% in trastuzumab-treated patients and 55% in trastuzumab-naïve patients. About 59% and 78% of trastuzumab exposed and unexposed patients, respectively, were alive and free from disease progression at 16 weeks from study entry (primary end point). Due to these impressive activity data, neratinib is now being actively investigated in combination

with cytostatic agents such as paclitaxel, vinorelbine, and capecitabine.^{137–139}

Pertuzumab is a fully humanized monoclonal antibody based on the human IgG1(κ) framework sequences, directed against the ECD of HER2.¹⁴⁰ Pertuzumab differs from trastuzumab in the epitope binding regions of the light chain (12 amino acid differences).¹⁴¹ Upon epitope binding, pertuzumab neutralizes the ability of HER2 to dimerize with other HER2 molecules or with other members of the EGFR family.

As single agent, pertuzumab showed disappointing activity in a phase II study.¹⁴² Baselga et al conducted a phase II study with trastuzumab and pertuzumab in HER2-positive advanced breast cancer.¹⁴³ Patients were eligible if they had received ≤ 3 chemotherapy regimens and had developed progression during trastuzumab-based therapy. In 66 enrolled patients, the authors reported a response rate, clinical benefit rate (CBR), and median PFS of 24.4%, 50%, and 5.5 months, respectively.

Trastuzumab-DM1 is a conjugated antibody that uses trastuzumab to deliver the maytansinoid agent DM1 to HER2-positive cells.¹⁴⁴ Once internalized, DM1 is released and binds to tubulin, thereby disrupting microtubule assembly/disassembly dynamics and inhibiting cell division and proliferation of cancer cells that overexpress HER2.¹⁴⁴ In a phase I study in trastuzumab-refractory, HER2-positive advanced breast cancer patients, this agent showed a favorable profile of toxicity at the dose of 3.6 mg/kg administered intravenously every 3 weeks.¹⁴⁵ The authors also reported a response rate and CBR of 21% and 73%, respectively, which make this agent a promising therapeutic opportunity.

Similarly, a phase Ib/II study associating trastuzumab-DM1 and pertuzumab in patients with trastuzumab resistant, HER2-positive advanced breast cancer was presented at the 2010 ASCO meeting.¹⁴⁶ The study included a dose-escalation phase followed by an expansion phase consisting of a formal phase II study in 60 patients. The expansion phase showed that, in the subset of patients with relapsed stage IV disease who could be evaluated for tumor response, this combination yielded a 35.7% ORR, which is an encouraging achievement. More recently, at ESMO 2010 a randomized phase III trial showed that trastuzumab DM1 has similar activity and a significantly lower toxicity in comparison with the association docetaxel and trastuzumab as frontline treatment of HER2 positive metastatic breast cancers.¹⁴⁷

In conclusion, the treatment of HER2-positive metastatic breast cancer is in continuous evolution, and many different targeted therapies are in advanced stages of

development. The future challenge for oncologists will be to optimally integrate all the available treatments to improve the outcome of HER2-positive metastatic breast cancers.

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