Cancer Therapy: Clinical

Clinical Cancer Research

Clinical Benefit of Lapatinib-Based Therapy in Patients with Human Epidermal Growth Factor Receptor 2–Positive Breast Tumors Coexpressing the Truncated p95HER2 Receptor

Maurizio Scaltriti[1,3], Sarat Chandarlapaty[6], Ludmila Prudkin[2,3], Claudia Aura[2,3], José Jimenez[2,3], Pier Davide Angelini[1], Gertrudis Sánchez[2,3], Marta Guzman[1,3], Josep Lluis Parra[1,3], Catherine Ellis[7], Robert Gagnon[7], Maria Koehler[7], Henry Gomez[8], Charles Geyer[9], David Cameron[10], Joaquin Arribas[1,3,4,5], Neal Rosen[6], and José Baselga[1,3,4]

Abstract

Purpose: A subgroup of human epidermal growth factor receptor 2 (HER2)–overexpressing breast tumors coexpresses p95HER2, a truncated HER2 receptor that retains a highly functional HER2 kinase domain but lacks the extracellular domain and results in intrinsic trastuzumab resistance. We hypothesized that lapatinib, a HER2 tyrosine kinase inhibitor, would be active in these tumors. We have studied the correlation between p95HER2 expression and response to lapatinib, both in preclinical models and in the clinical setting.

Experimental Design: Two different p95HER2 animal models were used for preclinical studies. Expression of p95HER2 was analyzed in HER2-overexpressing breast primary tumors from a first-line lapatinib monotherapy study (EGF20009) and a second-line lapatinib in combination with capecitabine study (EGF100151). p95HER2 expression was correlated with overall response rate (complete + partial response), clinical benefit rate (complete response + partial response + stable disease ≥24 wk), and progression-free survival using logistic regression and Cox proportional hazard models.

Results: Lapatinib inhibited tumor growth and the HER2 downstream signaling of p95HER2-expressing tumors. A total of 68 and 156 tumors from studies EGF20009 and EGF100151 were evaluable, respectively, for p95HER2 detection. The percentage of p95HER2-positive patients was 20.5% in the EGF20009 study and 28.5% in the EGF100151 study. In both studies, there was no statistically significant difference in progression-free survival, clinical benefit rate, and overall response rate between p95HER2-positive and p95HER2-negative tumors.

Conclusions: Lapatinib as a monotherapy or in combination with capecitabine seems to be equally effective in patients with p95HER2-positive and p95HER2-negative HER2-positive breast tumors. *Clin Cancer Res; 16(9); 2688–95. ©2010 AACR.*

The human epidermal growth factor receptor (HER) family of receptors is composed of four closely related tyrosine kinase (TK) receptors: HER1, HER2, HER3, and HER4. All HER receptors consist of an NH_2 terminus extracellular ligand-binding domain, a short transmembrane region, and an intracellular TK domain (1–3).

Dimerization of HER receptors, induced by ligand binding or receptor overexpression in the case of HER2, leads to the interaction between the intracellular kinase domain of the receptors and subsequent transphosphorylation of several tyrosine residues located in the COOH-terminal tails (4, 5). These phosphorylation events trigger the recruitment of several adaptor proteins that mediate the activation of downstream signaling pathways. Among them, the phosphoinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin and the mitogen activated protein

Authors' Affiliations: Departments of [1]Medical Oncology and [2]Pathology, Vall d'Hebron University Hospital; [3]Vall d'Hebron Institute of Oncology; [4]Universitat Autonoma de Barcelona; and [5]Institucio Catalana de Recerca i Estudis Avancats, Barcelona, Spain; [6]Program in Molecular Pharmacology and Chemistry and Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York; [7]Medicine Development Center Oncology, GlaxoSmithKline, Collegeville, Pennsylvania; [8]Department of Medical Oncology, Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; [9]Allegheny General Hospital, Pittsburgh, Pennsylvania; and [10]University of Leeds, Leeds, United Kingdom

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: José Baselga, Department of Medical Oncology, Vall d'Hebron University Hospital, Passeig Vall d'Hebron119-129, 08035, Barcelona, Spain. Phone: 34-93-2746085; Fax: 34-93-2746059; E-mail: jbaselga@vhio.net.

doi: 10.1158/1078-0432.CCR-09-3407

Translational Relevance

A subgroup of human epidermal growth factor receptor 2 (HER2)–overexpressing breast tumors coexpresses p95HER2, a truncated form of the HER2 receptor that lacks the extracellular domain but retains a highly functional HER2 kinase domain. We have previously shown that p95HER2-expressing tumors are intrinsically resistant to trastuzumab. In the present study, we have shown that p95HER2-expressing breast tumors are sensitive to the tyrosine kinase inhibitor lapatinib. Most importantly, we provide evidence that breast cancer patients coexpressing both HER2 and p95HER2 seem to respond similarly to lapatinib as patients with tumors expressing only the full-length HER2 receptor. Our work suggests that p95HER2-expressing tumors should be treated preferentially with a tyrosine kinase inhibitor and that further subclassification of HER2-positive tumors based on the presence or absence of p95HER2 will result in a better choice of therapy and improved management of this disease.

kinases (MAPK) pathways promote cell proliferation, transformation, and survival (6, 7).

Overexpression/amplification of HER2 occurs in ~ 20% of human breast cancers and is associated with a more aggressive phenotype and worse prognosis (8, 9). HER2-overexpressing tumors are sensitive to monoclonal antibodies (mAb) and small-molecule TK inhibitors (TKI) that interfere with HER2 function and signaling (10–14). Trastuzumab, a humanized mAb directed against the extracellular domain (ECD) of the receptor, was the first approved therapy for the treatment of HER2-positive breast cancer. Trastuzumab in combination with chemotherapy has shown a robust improvement in disease-free and progression-free survival (PFS) in addition to overall survival in the advanced disease (15, 16) as well as in the early (adjuvant) setting (17–19).

Despite the considerable clinical benefit provided, a large fraction of HER2-positive tumors display primary or acquired resistance to trastuzumab (for review, see ref. 20). Among the potential mechanism of resistance to trastuzumab, the presence of truncated forms of HER2 lacking the trastuzumab-binding ECD [collectively known as p95HER2 or HER2 COOH-terminal fragments (CTF); ref. 14] has been extensively studied.

p95HER2 is expressed in up to 30% of HER2-positive breast cancers and is associated with increased nodal metastasis and shorter disease-free survival when compared with patients that overexpress full-length HER2 (21, 22). These truncated receptor fragments, produced by either proteolytic shedding of the HER2 receptor ECD (23) or, more frequently, by alternative initiation of translation of the HER2 mRNA (24), retain kinase activity and promote mammary tumor progression and metastasis even more aggressively than full-length HER2 (25). Because p95HER2 lacks the extracellular trastuzumab binding domain, we hypothesized that these receptors would not be inhibited by this antibody. This turned out to be the case. We have recently shown that tumor xenografts expressing p95HER2 are refractory to the inhibitory effects of trastuzumab (14, 24). Importantly there was an association between p95HER2 expression and lack of clinical response to trastuzumab in HER2-amplified breast cancer patients (14). On the other hand, p95HER2 retains its kinase activity and, we and others, have shown that p95HER2-expressing

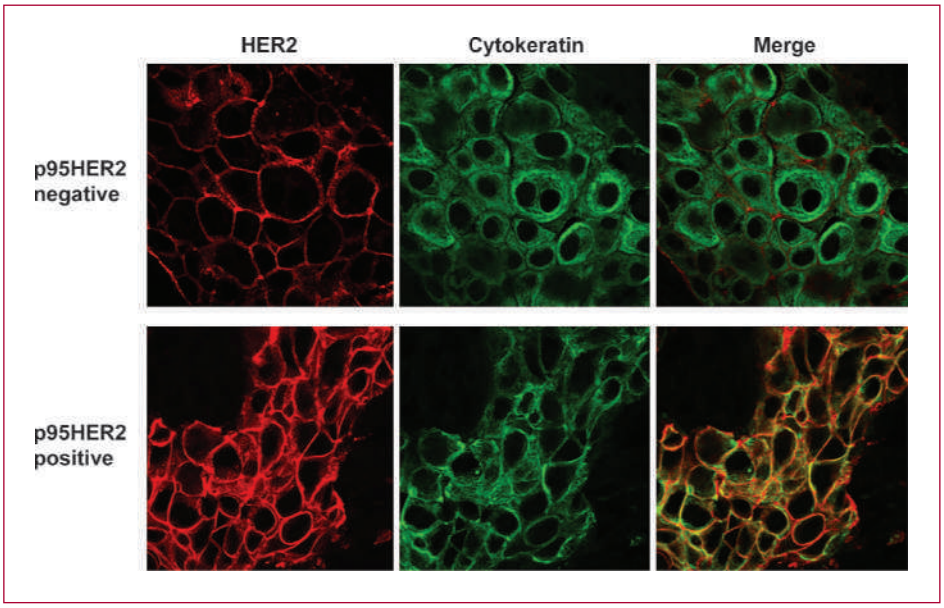

Fig. 1. p95HER2 staining in breast tumors. HER2 ICD staining (red) colocalizes with cytokeratin staining (green) only in p95HER2-positive tumors. Colocalization appears as a yellow-orange signal.

cells are sensitive to the antiproliferative activity of lapatinib (14, 26), a dual HER2 and HER1 TKI (27).

Lapatinib binds to the ATP binding site of both HER1 and HER2, preventing receptor phosphorylation and activation of downstream signaling (14, 28–30).

Lapatinib when given in combination with capecitabine significantly improved time to progression in HER2-positive breast cancer patients that progressed on trastuzumab-based therapy, compared with capecitabine alone (13). Moreover, lapatinib as monotherapy and in combination with paclitaxel has clinical activity as first-line treatment in HER2-positive breast cancer patients (31, 32). Taken together, these observations led to the hypothesis that lapatinib may be active in patients with p95HER2-expressing tumors.

To test this hypothesis, we first studied the antitumor activity of lapatinib in two animal models characterized by p95HER2-positive tumors that are either resistant to trastuzumab or depend on p95HER2 to grow. Subsequently, we analyzed the relationship between p95HER2 expression and response to lapatinib in HER2-positive patients who received lapatinib as monotherapy or in combination with capecitabine.

Materials and Methods

Cell lines and animal studies. Four- to 6-week-old BALB/c *nu/nu* athymic female mice were purchased from the National Cancer Institute Frederick Cancer Center and were maintained in pressurized ventilated caging. All studies were done in compliance with the Institutional Animal Care and Use Committee guidelines.

Fo5 tumors (kindly provided by Gail Lewis Phillips and Mark Sliwkowski: Genentech Inc) were established by s.c. implanting 2 × 2 × 2-mm-sized tumor pieces in the right flanks of the nude mice. Animals with well-established tumors were randomized and treated with 75 mg/kg BID 5× week lapatinib (provided by GlaxoSmithKline).

Tumor xenografts were measured with calipers and tumor volumes were determined using the formula: (length × width^2) × (π/6).

MEFs-3T3 tet-off cell lines, engineered to express the tetracycline-controlled transactivator (33), were obtained from Clontech Laboratories. Cells (1 × 10^6) stably transfected with the pUHD10-3h vector encoding the cDNA of p95HER2 starting at methionine 611 (611-CTF; ref. 25) were injected into the right flanks of 6- to 8-week-old female BALB/c athymic mice purchased from Charles Rivers Laboratories and the expression of 611-CTF was induced by doxycycline removal. Animals with well-established tumors were randomized and treated with 150 mg/kg daily lapatinib or with 10 mg/kg twice weekly trastuzumab (Herceptin; kindly provided by F. Hoffmann-La Roche). Tumor xenografts were measured as described above.

Tumor volumes are plotted as means ± SEM.

Immunoblotting. Tumor lysates were prepared by homogenization in SDS lysis buffer [50 mmol/L Tris-HCl (pH7.4), 2% SDS], boiling for 10 minutes, followed by brief sonication. Lysates were cleared by centrifugation at 14,000 × *g* for 10 minutes and the supernatants were collected. Protein concentration of each sample was determined using the BCA kit (Pierce) as per the manufacturer's instructions. Fifty micrograms of protein lysate were loaded onto 7% or 10% SDS-PAGE minigels for immunoblotting. Transfer was onto nitrocellulose membranes followed by incubation with the following primary antibodies: PI3K-p85 from Upstate Biotechnology and total HER2 (CB11, Biogenex), phospho-HER2, phospho-Akt (pAkt, S473, and T308), and phospho MAPKs (p-MAPK) from Cell Signaling.

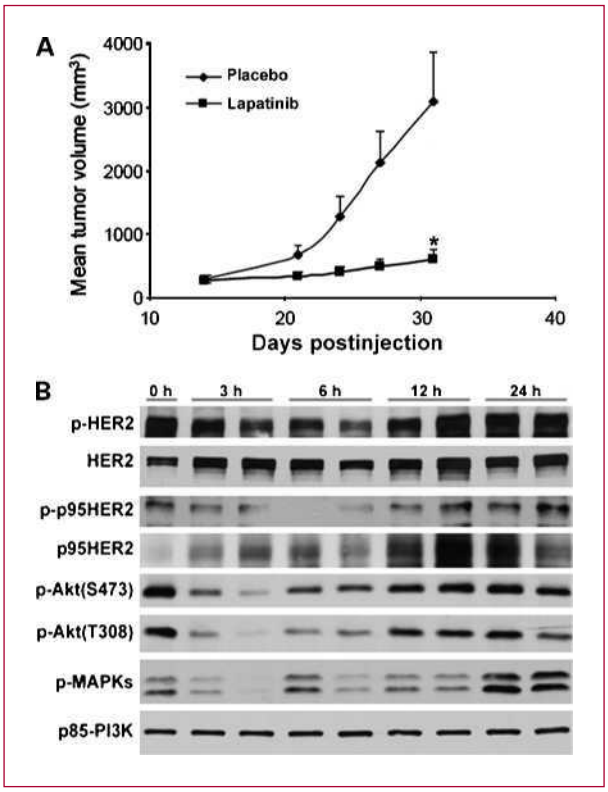

Fig. 2. Antitumor activity of lapatinib on Fo5 tumors. A, mice with established Fo5 tumors (five mice per group) were randomized to receive either placebo or treatment with lapatinib 75 mg/kg BID from Monday to Friday. Treatment was discontinued after day 32. *, $P < 0.01$ placebo versus lapatinib. B, mice with established Fo5 tumors were treated with five doses (75 mg/kg BID ×2.5 d) of lapatinib and tumors were collected at indicated times after the fifth dose. Immunoblotting was done on tumor lysates using the listed antibodies.

Immunohistochemistry. Xenografts samples were prepared as described earlier (34). Primary antibody was p-MAPKs from Cell Signaling and secondary anti-rabbit antibody was from Amersham Biosciences. As a negative control, primary antibody was omitted. Slides were scanned with the ScanScope CS system (Aperio). Quantification of p-MAPKs was obtained by scoring two slides each from five different tumors, from both placebo and lapatinib-treated groups, by a qualified pathologist (LP) and displayed as H score plotted as means ± SEM.

Patients. Women participating in one of two clinical trials provided written informed consent, allowing for biomarker research to be done on tumor tissue obtained at the time of their diagnosis or at surgery. The details for both trials, including efficacy and safety outcome, have been previously reported (13, 31, 35). Briefly, in study EGF20009, 138 women with HER2-positive (fluorescence *in situ* hybridization positive) treatment-naïve, advanced breast cancer randomized in a 1:1 ratio to receive oral lapatinib 1,500 mg once daily or oral lapatinib 500 mg twice daily. In study EGF100151, 399 women with HER2-positive (immunohistochemistry 3+ and/or fluorescence *in situ* hybridization positive) advanced breast cancer previously treated with anthracycline-, taxane-, and trastuzumab-based therapy were randomized in a 1:1 ratio to receive 2,500 mg/m^2 capecitabine (days 1-14 of a 21-d cycle) or 2,000 mg/m^2 capecitabine (days 1-14 of a 21-d cycle) with 1,250 mg lapatinib once daily (continuously). In both clinical studies, efficacy analyses were conducted based on both investigator and blinded independent central review assessment of tumor response and progression using the Response Criteria in Solid Tumor guidelines (36) in the intent-to-treat patient population.

Immunofluorescence detection of p95HER2 in breast tumors. Patients with sufficient tumor tissue available (i.e., at least two slides per section) and with a clear invasive cellular component were included in the evaluation of p95HER2 expression with the aim of obtaining a more robust estimation of the incidence of p95HER2 expression.

The expression of NH_2 terminally truncated HER2 was evaluated by immunofluorescence as previously described and scored by two pathologists (L.P. and C.A.; ref. 14). The investigators at our institution, including the pathologists, were blinded to the study or the therapy arm of the analyzed slides.

To increase the stringency of the analyses, tumors were scored positive for p95HER2 expression if ≥50% of tumoral cells showed any cytoplasmic staining detected with the

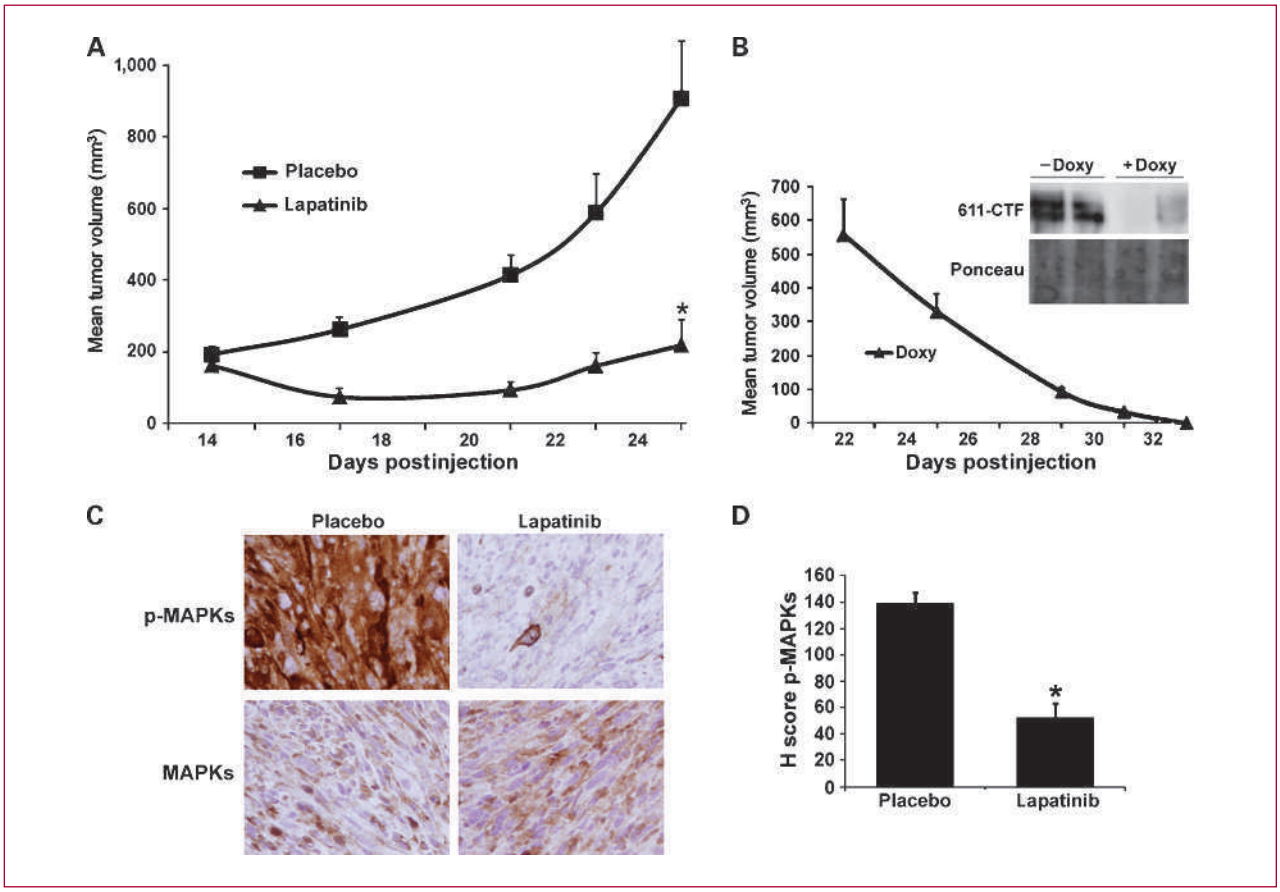

Fig. 3. Lapatinib-specific targeting of p95HER2. A, established xenografts (five mice per group) derived from MEFs expressing 611-CTF were randomized to receive either placebo or treatment with 150 mg/kg lapatinib daily. Treatment was discontinued after day 25 after injection. *, $P < 0.01$ placebo versus lapatinib. B, six mice bearing MEFs 611-CTF xenografts with a volume of ≥500 mm^3 received 1 g/l doxycycline in the drinking water. Complete tumor shrinkage was achieved in 11 days. 611-CTF expression in the absence (–Doxy) or presence (+Doxy; 48 h) of doxycycline is shown. C, representative p-MAPKs staining in 611-CTF MEFs tumors receiving either placebo (control) or lapatinib. Tumors were collected at the end of the experiment shown in A. D, quantification of the p-MAPKs staining (H score) was obtained by scoring two slides each from five different tumors from both placebo and lapatinib-treated groups. Columns, mean; bars, SEM. *, $P < 0.01$ placebo versus lapatinib.

Table 1. Patient demographic and disease history

	EGF20009 *n* = 68	EGF100151 L+C *n* = 76	EGF100151 C *n* = 80
Median (Q1, Q3) age, y	50 (45, 63)	53 (47, 60)	51 (43, 57)
ECOG PS			
0, *n* (%)	23 (34)	49 (64)	45 (56)
≥1, *n* (%)	45 (66)	27 (36)	33 (41)
Visceral metastasis, *n* (%)	42 (62)	62 (82)	60 (75)
No. of metastatic sites			
<3, *n* (%)	30 (44)	44 (58)	46 (57.5)
≥3, *n* (%)	38 (56)	32 (42)	34 (42.5)
Stage			
IIIB or C, *n* (%)	16 (24)	4 (5)	2 (2.5)
IV, N (%)	52 (76)	72 (95)	78 (97.5)

Abbreviations: L, lapatinib; C, capecitabine; *n*, sample number; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

anti-HER2 ICD antibody (recognizing both membrane and cytoplasmic HER2). Cytoplasmic staining was confirmed by colocalization with the anti-cytokeratin antibody, as observed by a yellow (red and green merged) signal (Fig. 1). In addition, HER2 ICD staining was compared with the pure membrane staining observed with the anti–HER2 ECD antibody that does not colocalize with cytokeratin staining. All fluorescence assays were done using a DAKO Autostainer.

Fluorescent signals were analyzed with a FluoView FV1000 Olympus Confocal Microscope and evaluated by two independent pathologists (L.P. and C.A.) blinded to clinical information.

Statistical methods. For nude mice experiments, comparisons between groups were made using a two-tailed Student's *t* test. Differences for which P was <0.05 were considered statistically significant.

Blinded independent central review was the protocol-defined assessment of the primary end point in both studies and thus used in analyses with p95HER2. Overall response rate (ORR; confirmed complete or partial response) and clinical benefit rate (CBR; confirmed complete response or partial response, or stable disease for >6 mo) were compared between p95HER2-positive and p95HER2-negative groups using χ^2 tests. PFS was compared between p95HER2-positive and p95HER2-negative groups using Cox proportional hazards models and Kaplan-Meier analyses. SAS was used for statistical analysis and S-Plus was used for graphical display. In study EGF20009, the data from the two lapatinib monotherapy arms (two dose cohorts) were combined as no significant differences in the clinical outcome parameters were observed and both patient as well as tumor characteristics were balanced (31).

Results

Lapatinib efficacy in mice bearing trastuzumab-resistant p95HER2-positive breast tumors. We recently showed that MCF-7 cells stably expressing p95HER2 and resistant to trastuzumab remained sensitive to the antiproliferative effects of the TKI lapatinib, both *in vitro* and *in vivo* (14). To expand these results in a model that more closely resembles the situation encountered in the clinic, we tested the growth-inhibitory activity of lapatinib in MMTV-HER2 Fo5 mammary tumor transplants, a trastuzumab-refractory breast tumor that coexpresses, as in clinical specimens, both full-length HER2 and p95HER2 (37). These cells were isolated from breast tumors derived from the MMTV-HER2 transgenic mouse lineage 5 that spontaneously became insensitive to trastuzumab therapy (38, 39).

In Fig. 2A, we show that lapatinib (75 mg/kg BID 5× week) markedly reduces tumor growth in mice bearing Fo5 tumors compared with placebo-treated controls. In a pharmacodynamic study, we treated Fo5-bearing mice with five consecutive doses of lapatinib (100 mg/kg BID) and then sacrificed the mice at the indicated times after the final dose (two mice per time point are shown). Immunoblots of lysates of these tumors show that the phosphorylation of p95-HER2 and HER2 is downregulated 3 and 6 hours after the last lapatinib administration (Fig. 2B). The inhibition of HER2 and p95-HER2 phosphorylation is associated with the downregulation of phosphorylated Akt (S473 and T308) and MAPKs (Fig. 2B). p85-PI3K blots serve as loading controls. These results are representative of two independent experiments.

Lapatinib activity in targeting p95HER2-dependent tumors. To assess the exquisite activity of lapatinib in inhibiting p95HER2-dependent tumor growth, we used an experimental model based on mouse embryonic fibroblasts (MEF) stably transfected with the most active fragment of p95HER2 (611-CTF), produced by alternative initiation of translation starting at methionine 611 of the HER2 sequence (25). The expression of this form of p95HER2 is tightly controlled by a tet-off inducible system (see Materials and Methods) and the tumorigenicity of these MEF stable clones stringently depend on the expression/activity of the oncogene. As expected, tumors derived

from MEFs expressing 611-CTF were refractory to trastuzumab (10 mg/kg twice weekly, Supplementary Fig. S1). Lapatinib treatment (150 mg/kg daily) strongly inhibits the tumor growth of MEFs expressing 611-CTF (Fig. 3A).

To confirm that the tumorigenesis of these cells is dependent on 611-CTF expression, we switched off 611-CTF expression (by adding doxycycline) in a separate group of animals with a xenograft volume of ≥500 mm^3. In these mice, doxycycline administration in the drinking water rapidly abolishes 611-CTF expression and, consequently, leads to tumor shrinkage (Fig. 3B).

Inhibition of MAPKs phosphorylation is shown (Fig. 3C and D) as a readout of lapatinib inhibition of 611-CTF signaling (25). These results are representative of two independent experiments.

p95HER2 expression in tumor tissue from studies EGF20009 and EGF100151. Tumor tissue was evaluable for p95HER2 analysis in 68 of 138 patients from study EGF20009 and in 156 of 399 patients from study EGF100151. Although we were able to obtain a higher tumor number (105 and 223 for EGF20009 and EGF100151, respectively), not all tumor sections were evaluable. In EGF20009, the first-line lapatinib single-agent study, 20.5% (14 of 68) had tumors that were p95HER2 positive whereas 79.5% (54 of 68) were negative. In EGF100151, the capecitabine plus lapatinib study, 28.5% (45 of 156) tumors were p95HER2 positive and 71.5% (111 of 156) were negative. Overall, 26% of HER2-positive tumors were positive for p95HER2 expression, an incidence consistent with previous reports (14, 21, 22). In those patients with p95HER2 evaluable results (Table 1), the patient and disease characteristics were generally a representative of the corresponding study populations as a whole (13, 31).

Relationship of p95HER2 status with clinical outcome. To determine whether the presence of p95HER2 affected the efficacy of lapatinib in the clinic, PFS was analyzed in lapatinib-treated patients subgrouped according to the p95HER2 status of their tumor. In patients receiving lapatinib as first-line treatment, no statistically significant difference in PFS was observed between the p95HER2-positive and p95HER2-negative subgroups [hazard ratio, 1.35; 95% confidence interval (95% CI), 0.64, 2.8; P = 0.417; Fig. 4A]. Similarly, PFS as a result of lapatinib plus capecitabine treatment was not significantly different between the two p95HER2 subgroups (hazard ratio, 1.30; 95% CI, 0.63-2.69; P = 0.471; Fig. 4B).

An analysis was conducted to determine whether the presence of p95HER2 had an effect on CBR or ORR as an outcome of lapatinib-based treatment. CBR and ORR were not significantly associated with p95HER2 expression (Table 2). In patients treated with monotherapy lapatinib (EGF20009), the CBR for the p95HER-positive group was 29% (95% CI, 2-56) versus 43% (95% CI, 29-57) for the p95HER2-negative group (P = 0.379). In patients treated with lapatinib plus capecitabine (EGF100151), a CBR of 38% (95% CI, 13-68) versus 40% (95% CI, 25-57) was observed in the p95HER2-positive versus p95HER2-negative groups, respectively (P = 0.999). Additionally, ORR was not significantly different between the two p95HER2 subgroups regardless of whether patients were treated with lapatinib alone or in combination with capecitabine (EGF20009: P = 0.7580; EGF100151: P = 0.999). The ORR and CBR in both p95HER2 populations in each trial are consistent with the tumor response rates reported in the respective overall study populations (31, 32).

Discussion

Resistance to trastuzumab remains a challenge in the therapy of HER2-overexpressing breast cancer and efforts are being directed at identifying potential underlying mechanisms (40). A potential leading cause of trastuzumab resistance is the coexpression in HER2-amplified tumors of p95HER2, a truncated form of the HER2 receptor that lacks the trastuzumab-binding ECD but retains a functional HER2 kinase domain and is highly tumorigenic (25). Given the high HER2 kinase activity of p95HER2, a potential approach

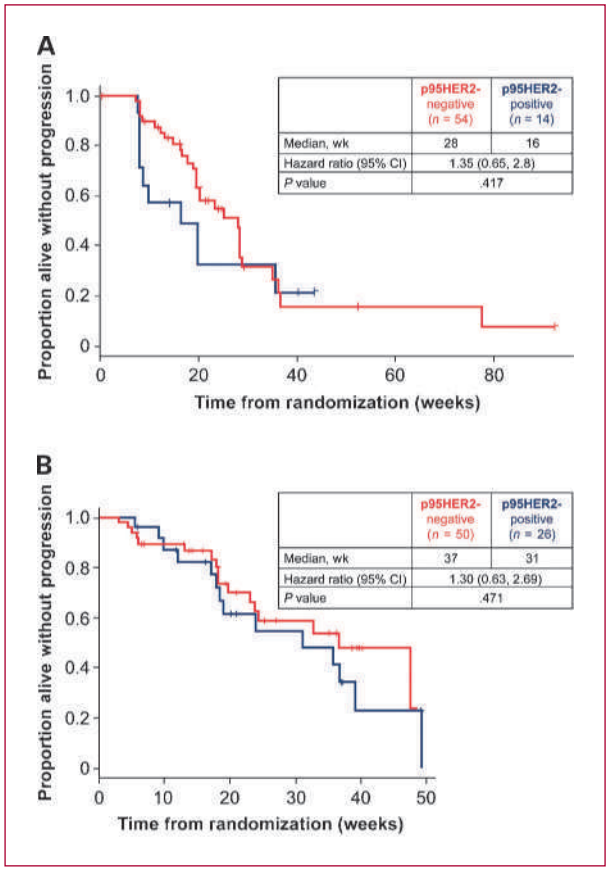

Fig. 4. Association of clinical outcome as a result of lapatinib-based treatment and p95HER2 status in patients with HER2-positive MBC. A, analysis of PFS subgrouped by p95HER2 status in patients treated with lapatinib monotherapy (EGF20009) or (B) with lapatinib in combination with capecitabine (EGF100151). Among patients with HER2-positive tumors exhibiting the coexpression of p95HER2 versus those with HER2-positive tumors that were p95HER2 negative, no significant difference in PFS was observed in either study.

Table 2. Tumor response in lapatinib-treated patients subgrouped by p95HER2 status

	EGF20009 L	EGF100151 L plus C
p95HER2 positive, *n*	14	26
ORR, %	29	31
(95% CI, %)	(5, 53)	(13, 49)
CBR, %	29	38
(95% CI, %)	(2, 56)	(13, 63)
p95HER2 negative, *n*	54	50
ORR, %	35	34
(95% CI, %)	(22, 48)	(21, 47)
CBR, %	43	40
(95% CI, %)	(29, 57)	(25, 57)

to treat these tumors would be with HER2 receptor TKIs (14, 26).

In this study, we have that trastuzumab-refractory breast tumors that coexpress, as in clinical specimens, both full-length HER2 and p95HER2 are sensitive to the TKI lapatinib. We also show that tumors coexpressing p95HER2 seem to respond similarly to lapatinib as tumors expressing only the full-length HER2 receptor; this result needs further validation due to the small sample sizes. In clinical samples from two large lapatinib studies in patients with advanced HER2-positive breast cancer, we observed that the presence of p95HER2 occurs in ~25% of HER2-amplified breast tumors. In these two studies, tumors expressing p95HER2 were sensitive to lapatinib. In fact, both the PFS and CBR in both studies were not statistically different in the p95HER2-positive and p95HER2-negative groups. Taking in consideration that p95HER2 tumors are resistant to trastuzumab (14), our results suggest that lapatinib, as well as other TKIs, may be a preferred therapeutic option for these tumors.

Hence, the presence of p95HER2 could determine the choice of anti-HER2 therapy if the results are further validated. We are prospectively studying the role of p95HER2 in the neoadjuvant study NeoALTTO as well in the adjuvant study ALTTO as both compare the clinical benefit with trastuzumab versus lapatinib versus the two agents given in combination. In addition, we are planning a prospective clinical trial with lapatinib in patients with tumors selected for p95HER2 expression. There is also a need to develop a user friendly and reproducible method of p95HER2 detection. Immunofluorescence detection of p95HER2, as used here, is complex, time demanding, and requires a confocal microscope, which would limit its widespread implementation. We have recently developed a mAb directed against an epitope uniquely present in p95HER2 and not full-length HER2. The mAb performs well in paraffin-embedded tissues (data not shown) and is in the process of being evaluated in clinical samples. If validated, the presence of p95HER2 could be detected through immunohistochemistry, the same method widely used to detect HER2 overexpression.

There are additional questions that deserve to be explored. At this time, we do not know whether p95HER2 is solely responsible for primary resistance to trastuzumab or if, on the contrary, acquired expression can occur as a result of clonal selection in tumors treated with trastuzumab over prolonged periods of time. If this were the case, upfront therapy with a combination of trastuzumab and lapatinib could potentially delay its appearance. We also do not know if p95HER2 coexists with other potential mechanism of resistance to anti-HER2 therapies such as PI3K mutations or PTEN loss of function.

As with other breast cancer subtypes, there will be an increasing need to further classify HER2 tumors into different subsets that may have different clinical outcomes and benefit from anti-HER2 therapies. Although this will undoubtedly add complexity to our daily clinical practice, it will also result in an improved management of patients with HER2-positive breast cancer and a better choice of therapy.

Disclosure of Potential Conflicts of Interest

D. Cameron, N. Rosen, members of speakers' bureau, GlaxoSmithKline; C. Ellis, R. Gagnon, employed by and ownership interest in GlaxoSmithKline; C. Geyer, D. Cameron, advisory board members, GlaxoSmithKline.

Grant Support

Breast Cancer Research Foundation and GlaxoSmithKline.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/30/2009; revised 02/04/2010; accepted 03/05/2010; published OnlineFirst 04/20/2010.

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.
2. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003;21:2787–99.
3. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.
4. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 2000;19:3159–67.
5. Ferguson KM, Berger MB, Mendrola JM, Cho HS, Leahy DJ, Lemmon MA. EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. Mol Cell 2003;11:507–17.
6. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7:505–16.
7. Zhang H, Berezov A, Wang Q, et al. ErbB receptors: from oncogenes to targeted cancer therapies. J Clin Invest 2007;117:2051–8.
8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.

9. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707–12.
10. Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell 2009;15: 429–40.
11. Agus DB, Akita RW, Fox WD, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell 2002;2:127–37.
12. Portera CC, Walshe JM, Rosing DR, et al. Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with [corrected] human epidermal growth factor receptor 2-positive metastatic breast cancer. Clin Cancer Res 2008;14:2710–6.
13. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355: 2733–43.
14. Scaltriti M, Rojo F, Ocana A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007;99:628–38.
15. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
16. Hudis CA. Trastuzumab-mechanism of action and use in clinical practice. N Engl J Med 2007;357:39–51.
17. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659–72.
18. Smith I, Procter M, Gelber RD, et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 2007;369:29–36.
19. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 2006;354:809–20.
20. Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. Nat Clin Pract Oncol 2006;3:269–80.
21. Molina MA, Saez R, Ramsey EE, et al. NH([2])-terminal truncated HER-2 protein but not full-length receptor is associated with nodal metastasis in human breast cancer. Clin Cancer Res 2002;8: 347–53.
22. Saez R, Molina MA, Ramsey EE, et al. p95HER-2 predicts worse outcome in patients with HER-2-positive breast cancer. Clin Cancer Res 2006;12:424–31.
23. Codony-Servat J, Albanell J, Lopez-Talavera JC, Arribas J, Baselga J. Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. Cancer Res 1999;59:1196–201.
24. Anido J, Scaltriti M, Bech Serra JJ, et al. Biosynthesis of tumorigenic HER2 C-terminal fragments by alternative initiation of translation. EMBO J 2006;25:3234–44.
25. Pedersen K, Angelini PD, Laos S, et al. A naturally occurring HER2 carboxy-terminal fragment promotes mammary tumor growth and metastasis. Mol Cell Biol 2009;29:3319–31.
26. Xia W, Liu LH, Ho P, Spector NL. Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. Oncogene 2004;23:646–53.
27. Spector NL, Xia W, Burris H, et al. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. J Clin Oncol 2005;23:2502–12.
28. Chu I, Blackwell K, Chen S, Slingerland J. The dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), cooperates with tamoxifen to inhibit both cell proliferation- and estrogen-dependent gene expression in antiestrogen-resistant breast cancer. Cancer Res 2005;65:18–25.
29. Konecny GE, Pegram MD, Venkatesan N, et al. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res 2006;66: 1630–9.
30. Xia W, Mullin RJ, Keith BR, et al. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. Oncogene 2002;21: 6255–63.
31. Gomez HL, Doval DC, Chavez MA, et al. Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol 2008;26:2999–3005.
32. Di Leo A, Gomez HL, Aziz Z, et al. Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. J Clin Oncol 2008;26:5544–52.
33. Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A 1992;89:5547–51.
34. Serra V, Markman B, Scaltriti M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res 2008;68:8022–30.
35. Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008;112:533–43.
36. Therasse P, Arbuck SG, Eisenhauer EA, et al, European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
37. Chandarlapaty S, Scaltriti M, Angelini P, et al. Inhibitors of HSP90 block p95-2 signaling in trastuzumab-resistant tumors and suppress their growth. Oncogene 2010;29:325–34.
38. Finkle D, Quan ZR, Asghari V, et al. HER2-targeted therapy reduces incidence and progression of midlife mammary tumors in female murine mammary tumor virus huHER2-transgenic mice. Clin Cancer Res 2004;10:2499–511.
39. Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 2008;68:9280–90.
40. Baselga J, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. Nat Rev Cancer 2009;9:463–75.