Biomarker Analysis of the Phase III NALA Study of Neratinib + Capecitabine versus Lapatinib + Capecitabine in Patients with Previously Treated Metastatic Breast Cancer

Cristina Saura[1,2,3], Judit Matito[2], Mafalda Oliveira[1,2,3], Hans Wildiers[4], Adam M. Brufksy[5], Simon H. Waters[6], Sara A. Hurvitz[7], Beverly Moy[8], Sung-Bae Kim[9], William J. Gradishar[10], Geraldo Silva Queiroz[11], Eduardo Cronemberger[12], Gerald J. Wallweber[13], Judith Bebchuk[14], Kiana Keyvanjah[14], Alshad S. Lalani[14], Richard Bryce[14], Ana Vivancos[2,3], Lisa D. Eli[14], and Suzette Delaloge[15]

ABSTRACT

Purpose: Neratinib plus capecitabine (N+C) demonstrated significant progression-free survival (PFS) benefit in NALA (NCT01808573), a randomized phase III trial comparing N+C with lapatinib + capecitabine (L+C) in 621 patients with HER2-positive ($HER2^+$) metastatic breast cancer (MBC) who had received ≥2 prior HER2-directed regimens in the metastatic setting. We evaluated correlations between exploratory biomarkers and PFS.

Patients and Methods: Somatic mutations were evaluated by next-generation sequencing on primary or metastatic samples. HER2 protein expression was evaluated by central IHC, H-score, and VeraTag/HERmark. p95 expression (truncated HER2) was measured by VeraTag. HRs were estimated using unstratified Cox proportional hazards models.

Results: Four hundred and twenty samples had successful sequencing: 34.0% had *PIK3CA* mutations and 5.5% had *HER2 (ERBB2)* mutations. In the combined patient populations, *PIK3CA* mutations trended toward shorter PFS [wild-type vs. mutant, HR = 0.81; 95% confidence interval (CI), 0.64–1.03], whereas *HER2* mutations trended toward longer PFS [HR = 1.69 (95% CI, 0.97–3.29)]. Higher HER2 protein expression was associated with longer PFS [IHC 3+ vs. 2+, HR = 0.67 (0.54–0.82); H-score ≥240 versus <240, HR = 0.77 (0.63–0.93); HERmark positive vs. negative, HR = 0.76 (0.59–0.98)]. Patients whose tumors had higher HER2 protein expression (any method) derived an increased benefit from N+C compared with L+C [IHC 3+, HR = 0.64 (0.51–0.81); H-score ≥ 240, HR = 0.54 (0.41–0.72); HERmark positive, HR = 0.65 (0.50–0.84)], as did patients with high p95 [p95 ≥2.8 relative fluorescence (RF)/mm^2, HR = 0.66 (0.50–0.86) vs. p95 < 2.8 RF/mm^2, HR = 0.91 (0.61–1.36)].

Conclusions: *PIK3CA* mutations were associated with shorter PFS whereas higher *HER2* expression was associated with longer PFS. Higher HER2 protein expression was also associated with a greater benefit for N+C compared with L+C.

Introduction

HER2 overexpression occurs in 15% to 20% of breast cancers (1). Before the approval of trastuzumab, HER2-overexpressing breast cancer was the subtype with the worst prognosis (2). Multiple additional HER2-targeted therapies have subsequently been developed, two of which are the small-molecule tyrosine kinase inhibitors (TKI) neratinib and lapatinib, the focus of comparison in this analysis. Neratinib and lapatinib target the adenosine triphosphate–binding domain of HER proteins. Their distinguishing features include their binding mechanisms, preclinical cytotoxicity, and impact on HER family dimerization and downstream signaling and gene expression (3). Neratinib binds covalently/irreversibly to HER2 (4), whereas lapatinib binds noncovalently/reversibly (5). Neratinib binds EGFR, HER2, and HER4 with high affinity; lapatinib targets only EGFR and HER2 with high affinity (3). Both have demonstrated *in vitro* and *in vivo* inhibition of growth and proliferation in HER2- or EGFR-amplified preclinical breast cancer models, with lower median inhibitory concentration values for neratinib (6). Finally, neratinib, but not lapatinib, inhibits tumors with a wide spectrum of somatic *HER2* mutations (7–9).

Multiple mechanisms of resistance to HER2-targeted therapies have been reported. These include upregulation of alternative receptor tyrosine kinases (10), upregulation of the estrogen receptor (ER) pathway (11), dysregulation of

[1]Vall d'Hebron University Hospital, Barcelona, Spain. [2]Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University Hospital Campus, Barcelona, Spain. [3]SOLTI Innovative Breast Cancer Research, Barcelona, Spain. [4]University Hospitals Leuven, Leuven, Belgium. [5]Magee-Womens Hospital of UPMC, Pittsburgh, Pennsylvania. [6]Velindre Cancer Centre, Cardiff, Wales, United Kingdom. [7]University of California at Los Angeles, Los Angeles, California. [8]Massachusetts General Hospital Cancer Center, Boston, Massachusetts. [9]Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea (South). [10]Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, Illinois. [11]Hospital Araújo Jorge, Goiânia, Goiás, Brazil. [12]Centro Regional Integrado de Oncologia, Fortaleza, Ceará, Brazil. [13]Monogram Biosciences, Inc., Laboratory Corporation of America Holdings, South San Francisco, California. [14]Puma Biotechnology Inc., Los Angeles, California. [15]Gustave Roussy, Villejuif, France.

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

Corresponding Author: Cristina Saura, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University Hospital Campus, CELLEX CENTER, C/ Natzaret, 115-117, Barcelona 08035, Spain. Phone: 349-3489-3000, ext. 2658; E-mail: csaura@vhio.net

Clin Cancer Res 2021;27:5818–27

doi: 10.1158/1078-0432.CCR-21-1584

Translational Relevance

Neratinib is an irreversible pan-HER tyrosine kinase inhibitor (TKI), whereas lapatinib is a reversible, HER2-specific TKI. The phase III NALA trial of neratinib + capecitabine (N+C) versus lapatinib + capecitabine (L+C) in patients with HER2-positive metastatic breast cancer (MBC) who had previously received two or more HER2-targeted therapies in the metastatic setting was the basis for FDA approval of neratinib in that setting. Our retrospective biomarker analysis reports that within this HER2-positive group, those with tumors expressing higher HER2 levels had longer progression-free survival (PFS), and greater PFS benefit from N+C vs. L+C. This predictive effect was more pronounced in patients with hormone receptor–negative disease. *PIK3CA* mutation was associated with shorter PFS, whereas *HER2* mutation was associated with longer PFS.

downstream PIK3K/Akt signaling (12), increased expression of p95, a highly active truncated form of HER2 that lacks the extracellular trastuzumab-binding domain (13), and additional upregulation of HER2 via acquired amplifications and/or somatic mutations (14, 15).

The phase III NALA study compared neratinib (240 mg once a day) plus capecitabine (750 mg/m^2 twice a day on days 1–14 of a 21-day cycle; N+C) with lapatinib (1,250 mg once a day) plus capecitabine (1,000 mg/m^2 twice a day on days 1–14 of a 21-day cycle; L+C) in patients with HER2-positive (HER2$^+$) metastatic breast cancer (MBC) who had received ≥2 prior HER2-directed regimens in the metastatic setting (16). There was a statistically significant benefit in progression-free survival (PFS) favoring N+C (HR = 0.76; 12-month PFS, N+C 29% vs. L+C 15%), translating to a 2.2-month mean PFS improvement without significant benefit in overall survival. Patients treated with N+C had significantly fewer interventions for symptomatic central nervous system disease compared with L+C (cumulative incidence 22.8% vs. 29.2%; $P = 0.043$). Based on these results, the FDA approved N+C for adult patients with advanced or metastatic HER2$^+$ breast cancer who have received ≥2 prior anti-HER2–based regimens in the metastatic setting (17).

The high rate of early events in both arms and the largely indistinguishable PFS curves up until 6 months in NALA suggest that the trial enrolled a group of patients resistant to HER2-directed therapies, capecitabine, or both. In this exploratory analysis, we assessed associations of biomarkers with prognostic or predictive PFS benefit (N+C over L+C) in biomarker-defined subgroups in NALA.

Patients and Methods

Study design

NALA (NCT01808573) was a randomized, international, multicenter, parallel-group, active-controlled phase III trial in patients with HER2$^+$ MBC. Patient characteristics, eligibility criteria, and treatment have been described (16). The protocol was approved by national/institutional ethics committees at participating sites and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. HER2 positivity was centrally confirmed before enrollment as IHC 3+ or IHC 2+/FISH positive (18, 19).

Specimen characteristics

Tumor samples were collected at screening before randomization and classified by study sites as originating from primary or metastatic tumor, and as archival or fresh biopsy. Sites were instructed to send either one formalin-fixed, paraffin-embedded (FFPE) block or 10 unstained, freshly cut 4-μm FFPE slides, and to utilize tissue from the time of metastasis whenever possible.

Assay methods

Biomarkers and assays were selected based on documented evidence of association with response or resistance to HER2-targeted therapies, and, because of limitations in tissue availability, ability to reliably test minimal amounts of tissue. Hormone receptor status was established via central IHC, with Allred total score ≥3 considered ER/progesterone receptor (PR) positive (ER/PR PharmDx Kit, Dako). All assays were performed blinded to study endpoint and treatment assignment. Additional candidate biomarkers that could not be performed due to lack of sufficient tissue included RNA expression, including but not limited to molecular subtype categorization, and retrospective FISH evaluation of all samples.

Somatic mutation analysis

The spectrum of somatic mutations was evaluated by whole-exome next-generation sequencing (NGS; ref. 20). DNA extraction was performed from FFPE slides (1–3 × 4 μm) using Maxwell FFPE Tissue LEV DNA Purification Kit (Promega). All slides were pathologist-evaluated to determine ≥20% of tumor area content. If required, microdissection was performed to enrich for tumor content. DNA quality and concentration were measured with double-stranded DNA (dsDNA) Qubit broad-range assay kit (Invitrogen). A minimum of 4 ng of extracted DNA was input for amplicon-based NGS using VHIO-Card, an in-house developed panel of more than 800 primer pairs targeting hotspot mutations in 55 genes (Supplementary Table S1). PCR products were pooled, and libraries prepared with NEBNext Ultra DNA Library Prep Kit for Illumina (New England BioLabs). Indexed libraries were pooled, loaded onto a HiSeq 2500 System (Illumina), and sequencing performed (2 × 100) at average coverage of more than 1000×.

Somatic mutations identified in *PIK3CA, ESR1, HER2, AKT1*, and *KRAS* were confirmed by droplet digital PCR (ddPCR; QX200 system Bio-Rad Laboratories). These were of special interest because of involvement in HER2 signaling. Custom Taqman SNP genotyping assays for ddPCR were designed and validated with in-house available mutant samples. A black-hole quencher was used with fluorescent dye reporters, fluorescein amidite (FAM) for mutant probes, and 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) for wild-type (WT) probes (Thermo Fisher Scientific).

HER2 protein expression

HER2 IHC and FISH scores were determined according to manufacturer specifications (HercepTest and IQFISH pharmDX, respectively; Dako). To better understand the spectrum of HER2 protein expression, HER2 was retrospectively evaluated by two additional methods: H-score (Targos GMBH) and HERmark (Monogram Biosciences). H-scores were evaluated from original IHC images. The HERmark assay, which quantitatively measures HER2 receptors using a dual-antibody, proximity-based, fluorescence assay (VeraTag technology; refs. 21, 22), was performed on an adjacent section. p95 levels were evaluated using VeraTag technology and categorized as high or

low based on previously established cut-off points (23, 24). VeraTag measurements were expressed in units of relative fluorescence per square millimeter of invasive tumor (RF/mm^2; ref. 21).

Statistical analysis

Efficacy endpoints were assessed in individual biomarker populations, defined as any randomized patient with that biomarker result. The primary analysis methods were log-rank test for hypothesis testing and Cox proportional hazards model to estimate HRs and 95% confidence intervals (CI). Multivariable Cox proportional hazard models were used with the following covariates: treatment, time from diagnosis to randomization, time from metastasis to randomization, Eastern Cooperative Oncology Group (ECOG) performance score, previous lines of HER2 therapy, HER2 IHC, histologic grade at diagnosis, region, ER/PR status, and disease location. Kaplan–Meier methodology was used for time-to-event endpoints. Subpopulation treatment effect pattern plot (STEPP) was used to assess patterns of effect size for continuous HER2 protein variables. Analyses were performed using SAS (version 9.1; SAS Institute Inc. RRID: SCR_008567) and R (RRID:SCR_001905; ref. 25).

Results

Patients and samples

As previously reported (16), 621 patients with HER2-positive MBC were randomized to N+C or L+C. Most samples originated from primary tumors ($n = 393$; 63%), with the remainder classified as metastatic ($n = 184$; 30%) or otherwise uncharacterized ($n = 63$; 10%). Across the 621 available samples, NGS was successfully performed on 420 patients (68%), H-scoring was possible for 615 (99%) of HER2 IHC-stained slides, HERmark score was reported for 526 patients (85%), and VeraTag p95 was reported for 451 patients (73%; Supplementary Fig. S1). Biomarker-expression characteristics of patients with biomarker analysis results were generally comparable across treatment arms (**Table 1**). Biomarker-analysis populations were generally comparable with the intent-to-treat population (Supplementary Table S2). HER2 levels were not

Table 1. Biomarker expression by treatment arm for the intention-to-treat population.

Biomarker	N+C ($n = 307$)	L+C ($n = 314$)	Total ($n = 621$)
HER2 IHC (Targos), *n* (%)			
2+	80 (26.1%)	115 (36.6%)	195 (31.4%)
3+	227 (73.9%)	199 (63.4%)	426 (68.6%)
HERmark, *n* (%)			
Low	56 (18.2%)	70 (22.3%)	126 (20.3%)
Equivocal	33 (10.7%)	30 (9.6%)	63 (10.1%)
High	174 (56.7%)	163 (51.9%)	337 (54.3%)
Missing	44 (14.3%)	51 (16.2%)	95 (15.3%)
HERmark, RF/mm^2			
Number	263	263	526
Mean	91.5	95.0	93.2
SD	126.8	147.4	137.3
Median	39.0	39.1	39.1
Minimum, maximum	1.3, 764	0.9, 1,179	0.9, 1,179
HER2 H-score, *n* (%)			
≥240 (median)	164 (53.4%)	146 (46.5%)	310 (49.9%)
<240 (median)	142 (46.3%)	163 (51.9%)	305 (49.1%)
Missing	1 (0.3%)	5 (1.6%)	6 (1.0%)
HER2 H-score			
Number	306	309	615
Mean	229.5	220.2	224.8
SD	57.4	64.8	61.3
Median	240	230	240
Minimum, maximum	42, 300	35, 300	35, 300
p95, RF/mm^2			
Number	224	227	451
Mean	5.0	6.1	5.6
SD	4.6	7.5	6.2
Median	4.0	4.1	4.0
Minimum, maximum	0.5, 30.5	0.5, 76.1	0.5, 76.1
≥2.8	153 (68.3%)	149 (65.6%)	302 (67.0%)
HER2 mutation			
Mutant	14 (4.6%)	9 (2.9%)	23 (3.7%)
WT	203 (66.1%)	194 (61.8%)	397 (63.9%)
Missing	90 (29.3%)	111 (35.4%)	201 (32.4%)
PIK3CA mutation			
Mutant	77 (25.1%)	66 (21.0%)	143 (23.0%)
WT	140 (45.6%)	137 (43.6%)	277 (44.6%)
Missing	90 (29.3%)	111 (35.4%)	201 (32.4%)

distributed evenly between hormone receptor–negative and –positive cohorts; higher HER2 was more common in patients who were hormone receptor negative (Supplementary Table S3), consistent with prior reports (26).

Somatic mutations

After NGS identification, *PIK3CA, HER2, HER3, AKT1*, and *ESR1* activating mutations were orthogonally confirmed by ddPCR for cases with sufficient material (n = 150/176; 86%); 100% concordance was observed. Mutation prevalence was: *PIK3CA* 34% (143/420); *HER2* 5% (23/420); *HER3* 1% (4/420), *AKT1* 1% (3/420), and *ESR1* 2% (7/420). *PIK3CA* and *HER2* mutations were balanced between treatment arms (**Table 1**); mutation frequencies were consistent with prior MBC datasets (27).

PIK3CA

Most *PIK3CA* mutations were kinase-domain hotspots, e.g., H1047R/L (n = 83; 56%), or helical-domain hotspots, e.g., E545K/A (n = 32; 22%) and E542K (n = 24; 16%); patients could have more than one *PIK3CA* mutation (Supplementary Fig. S2A). Ninety-five of the 143 mutations (66%) were identified from primary tissue, 29 (20%) were from metastases, and 19 (13%) were from otherwise unclassified lymph node tissue. *PIK3CA* mutations were distributed similarly in hormone receptor–positive and hormone receptor–negative disease: they were detected in samples from 58 of 180 patients (32.2%) with hormone receptor–negative disease and 85 of 240 patients (35.4%) with hormone receptor–positive disease. *PIK3CA* mutations were also distributed similarly between lower and higher HER2 expression levels (Supplementary Table S4).

The presence of a *PIK3CA* mutation trended toward shorter PFS, independent of treatment [**Fig. 1A**; WT vs. mutant *PIK3CA*: HR = 0.81 (95% CI, 0.64–1.03); median PFS 5.55 vs. 4.34 months, respectively].

HER2

HER2 gene mutations (n = 23) included kinase-domain hotspots L755S (n = 5; 22%), D769Y (n = 5; 22%), V777 L (n = 5; 22%), G776V (n = 1; 4%), I767M (n = 1; 4%), and L841V (n = 1; 4%); extracellular-domain hotspot S310F/Y (n = 2; 9%); exon 20 insertion G778_P780dup (n = 1; 4%); transmembrane-domain hotspot R678Q (n = 1; 4%); and non–hotspot mutations L841V (n = 1; 4%) and V669 L (n = 1; 4%; Supplementary Fig. S2B). Fifteen of the 23 *HER2* mutations (65.2%) were identified from primary tumor tissue, 5 were from metastases (22%), and 3 were from lymph node tissue (13%). Median PFS was 5.45 months in patients with WT *HER2* tumors and 5.72 months in those with mutant *HER2* tumors [**Fig. 1B**; WT vs. mutant; HR = 1.686 (95% CI, 0.967–3.288)].

HER2 mutations were not predictive of PFS benefit for N+C over L+C (P-value$_{inter}$ = 0.502; **Fig. 1C**). After adjusting for other prognostic factors in a multivariable Cox model, *PIK3CA* mutation maintained a trend toward shorter PFS [HR = 0.82 (95% CI, 0.65–1.05, WT vs. mutant)]. *PIK3CA* mutations were not predictive of PFS benefit for N+C over L+C (P-value$_{inter}$ = 0.995; **Fig. 1C**). Too few *AKT1*, *HER3*, and *ESR1* mutations occurred for meaningful evaluation.

HER2 protein expression

Among 621 patients in NALA, 426 (69%) had IHC 3+, and 195 (31%) had IHC 2+/FISH-positive breast cancer. HER2 protein expression orthogonally measured by H-score and HERmark was well balanced between treatment arms (**Table 1**). Differences were observed in the extent of quantification dependent on methodology (**Fig. 2A**): HERmark reported across a larger dynamic range, particularly at highest HER2 protein levels; H-scores ranged from 35 to 300 (median 240), and HERmark scores ranged from 0.9 to 1,179 RF/mm^2 (median 39 RF/mm^2).

Higher HER2 protein expression was prognostic of longer PFS when both treatment arms were combined, using all three methods (**Fig. 2B**). Higher HER2 protein level also significantly predicted PFS benefit for N+C versus L+C [**Fig. 2C**; IHC 3+: HR = 0.64 (95% CI, 0.51–0.81); H-score ≥240: HR = 0.54 (95% CI, 0.41–0.72); HERmark positive: HR = 0.65 (95% CI, 0.50–0.84); P-value$_{inter}$ < 0.001 for IHC and H-score and P = 0.061 for HERmark]. Evaluation of HER2 protein level as a continuous variable using STEPP analysis of H-score and HERmark revealed that increasing HER2 protein levels was associated, although not statistically significantly, with HRs favoring the neratinib combination (**Fig. 2D**).

p95

High p95 was associated with PFS benefit favoring N+C over L+C [**Fig. 2C**; high p95 (≥2.8 RF/mm^2): HR = 0.66 (95% CI, 0.50–0.86) vs. low p95 (<2.8 RF/mm^2): HR = 0.91 (95% CI, 0.61–1.36); P-value$_{inter}$ = 0.236]. N+C benefit versus L+C was shown in patients with HERmark-positive/p95-high [HR = 0.63 (95% CI, 0.46–0.86)] and HERmark-positive/p95-low [HR = 0.55 (95% CI, 0.29–1.04)] tumors; P-value$_{inter}$ for HERmark/p95 was 0.063 (**Fig. 2C**). This supports the hypothesis that patients with high levels of active HER2, whether full-length or truncated, derive greater benefit from N+C compared with L+C.

Hormone receptor status

In the intent-to-treat population, PFS benefit from N+C versus L+C was seen in the hormone receptor–negative subgroup [HR = 0.42 (95% CI, 0.31–0.57)] but not in the hormone receptor–positive subgroup [HR = 1.08 (95% CI, 0.84–1.40); ref. 16]. A multivariable Cox model indicated a significant interaction between treatment and hormone receptor status (P = 0.0038); the effect of HER2 protein levels was therefore investigated by hormone receptor status (**Fig. 3**). The predictive effect of HER2 level appeared more pronounced in patients with hormone receptor–negative tumors. Patients whose tumors were both hormone receptor–negative and high in HER2 protein expression derived the greatest PFS benefit from N+C compared with L+C [IHC 3+: HR = 0.35 (95% CI, 0.25–0.49); H-score ≥240: HR = 0.32 (95% CI, 0.22–0.46); HERmark-positive: HR = 0.35 (95% CI, 0.24–0.51)].

Discussion

This preplanned, exploratory analysis examined somatic mutations, HER2 protein expression, and hormone receptor status and their association with outcomes in NALA, providing meaningful information on the potential role of biomarkers in clinical outcomes for this patient population.

PIK3CA mutation has been described as a biomarker of resistance to multiple HER2-targeted therapies in MBC (28–30). The trend observed for shorter PFS with *PIK3CA* mutation in NALA is therefore as expected. In contrast, no associations between *PIK3CA* mutations and prognosis have been reported for patients with HER2-positive breast cancer undergoing adjuvant treatment with targeted agents, including neratinib in the extended adjuvant setting (31–33).

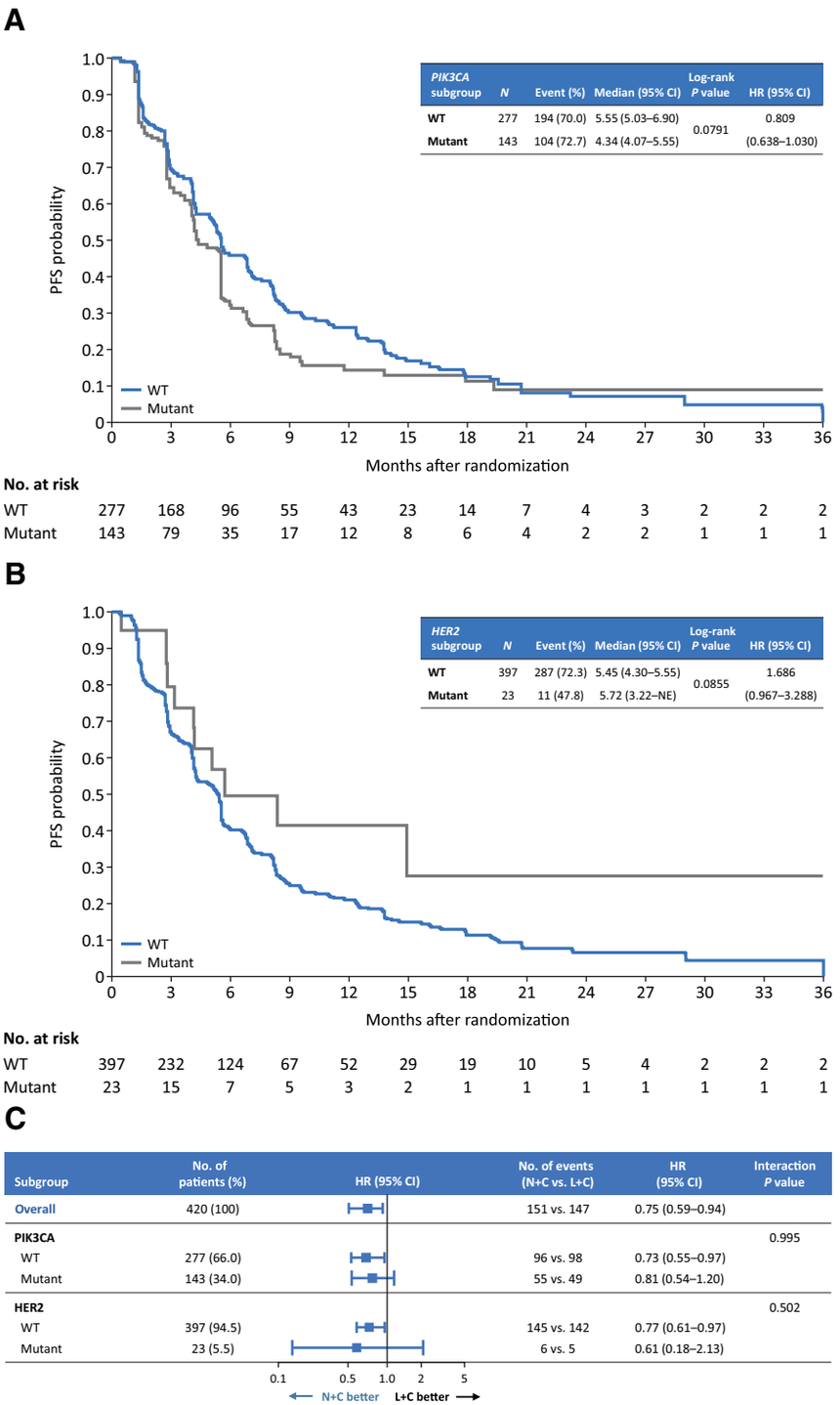
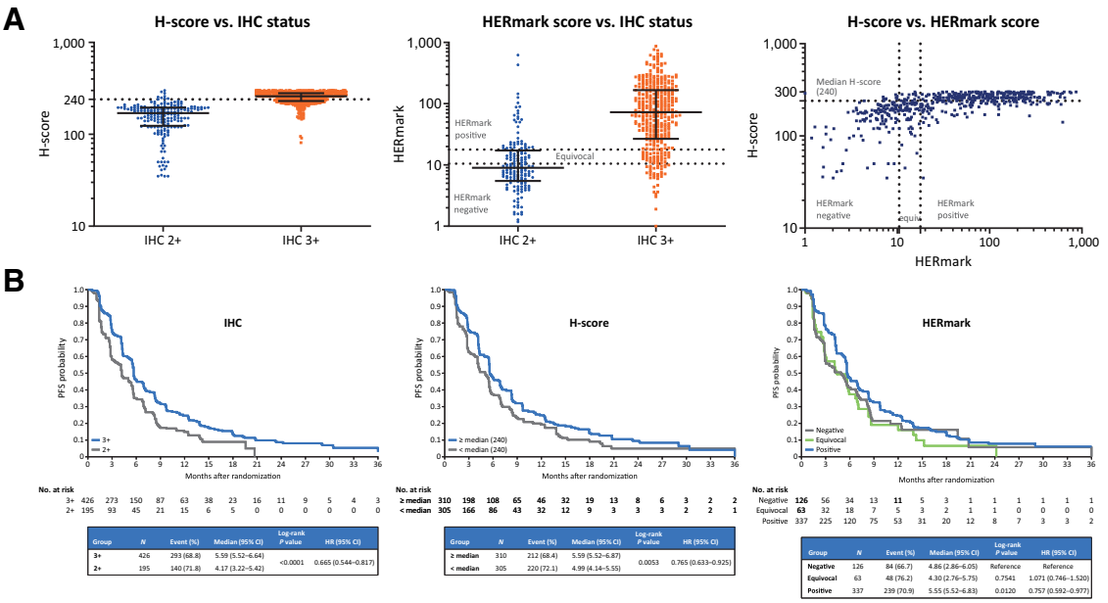


Figure 1.

A, *PIK3CA* mutations were associated with worse PFS outcome, independent of treatment arm. **B,** *PIK3CA* mutations and *HER2* mutations were not predictive of PFS benefit for neratinib versus lapatinib. **C,** *HER2* mutations were associated with a trend toward better PFS outcome, independent of treatment arm. NE, not evaluable.

HER2 somatic mutations have been adversely associated with prognosis in the absence of HER2-targeted therapy (34). In contrast, *HER2* mutations in patients in NALA tended to be associated with better prognosis, although this interpretation is limited by a small sample size ($n = 23$). This may be due to prior exposure to HER2-targeted therapy in NALA, although neither trastuzumab nor pertuzumab—the most common therapies received before study entry—has proven activity against *HER2*-mutant MBC (35). Preclinically, tumors with *HER2* mutations are more sensitive to neratinib than lapatinib (7–9). In patients with MBC whose tumors harbored somatic *HER2* mutations in the absence of *HER2* amplification, the SUMMIT trial (NCT01953926) reported responses to neratinib monotherapy or in combination with fulvestrant (15). *HER2* mutations coincident with amplification are less well characterized, although *HER2* amplification



Group	N	Event (%)	Median (95% CI)	Log-rank P value	HR (95% CI)
3+	426	293 (68.8)	5.59 (5.52–6.64)	<0.0001	0.665 (0.544–0.817)
2+	195	140 (71.8)	4.17 (3.22–5.42)		

Group	N	Event (%)	Median (95% CI)	Log-rank P value	HR (95% CI)
≥ median	310	212 (68.4)	5.59 (5.52–6.87)	0.0053	0.765 (0.633–0.925)
< median	305	220 (72.1)	4.99 (4.14–5.55)		

Group	N	Event (%)	Median (95% CI)	Log-rank P value	HR (95% CI)
Negative	126	84 (66.7)	4.86 (2.86–6.05)	Reference	Reference
Equivocal	63	48 (76.2)	4.30 (2.76–5.75)	0.7541	1.071 (0.746–1.520)
Positive	337	239 (70.9)	5.55 (5.52–6.83)	0.0120	0.757 (0.592–0.977)

C

Subgroup	No. of patients (%)	No. of events (N+C vs. L+C)	HR (95% CI)	Interaction P value
Overall	621 (100)	210 vs. 223	0.76 (0.63–0.93)	
IHC category				<0.001
2+	195 (31.4)	63 vs. 77	1.24 (0.88–1.73)	
3+	426 (68.6)	147 vs. 146	0.64 (0.51–0.81)	
H-score				<0.001
< median	305 (49.1)	106 vs. 114	1.04 (0.80–1.36)	
≥ median	310 (49.9)	104 vs. 108	0.54 (0.41–0.72)	
HERmark				0.061
Negative	126 (20.3)	41 vs. 43	1.21 (0.78–1.88)	
Equivocal	63 (10.1)	25 vs. 23	0.74 (0.41–1.33)	
Positive	337 (54.3)	117 vs. 122	0.65 (0.50–0.84)	
p95				0.236
High	302 (48.6)	108 vs. 112	0.66 (0.50–0.86)	
Low	149 (24.0)	51 vs. 48	0.91 (0.61–1.36)	
HERmark/p95				0.063
Negative/high	48 (7.7)	14 vs. 16	0.75 (0.36–1.56)	
Negative/low	67 (10.8)	22 vs. 23	1.53 (0.84–2.80)	
Positive/high	222 (35.7)	81 vs. 85	0.63 (0.46–0.86)	
Positive/low	63 (10.1)	20 vs. 20	0.55 (0.29–1.04)	

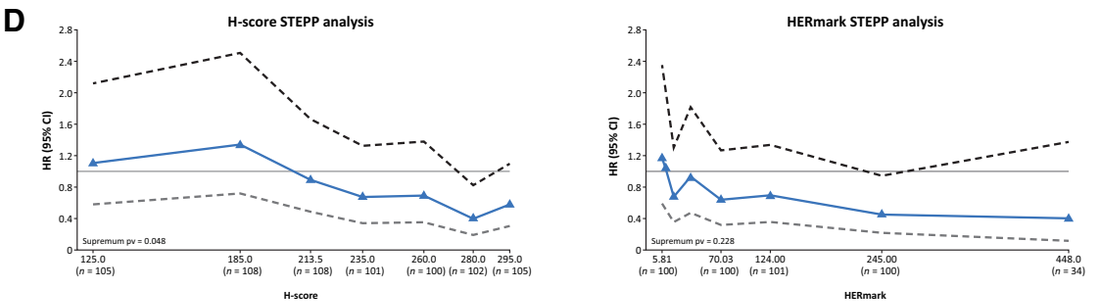


Figure 2.

HER2 protein expression. **A,** Comparison of test methods. All three methods detect HER2 protein expression but differ in the extent of their quantification. HERmark has a wider dynamic range than H-score, especially at the highest levels of protein expression. **B,** Orthogonal methods show association of increased HER2 with better PFS, independent of treatment. **C,** Forest plot of PFS according to HER2 testing methods. HERmark-negative, -equivocal, and -positive categories, and p95 high and low, were based on published cutpoints (22, 23). Higher HER2 protein expression measured by all three methods correlated with better response to neratinib over lapatinib. **D,** STEPP analysis of H-score and HERmark results showing PFS of N+C versus L+C for different HER2 levels. pv, *P* value.

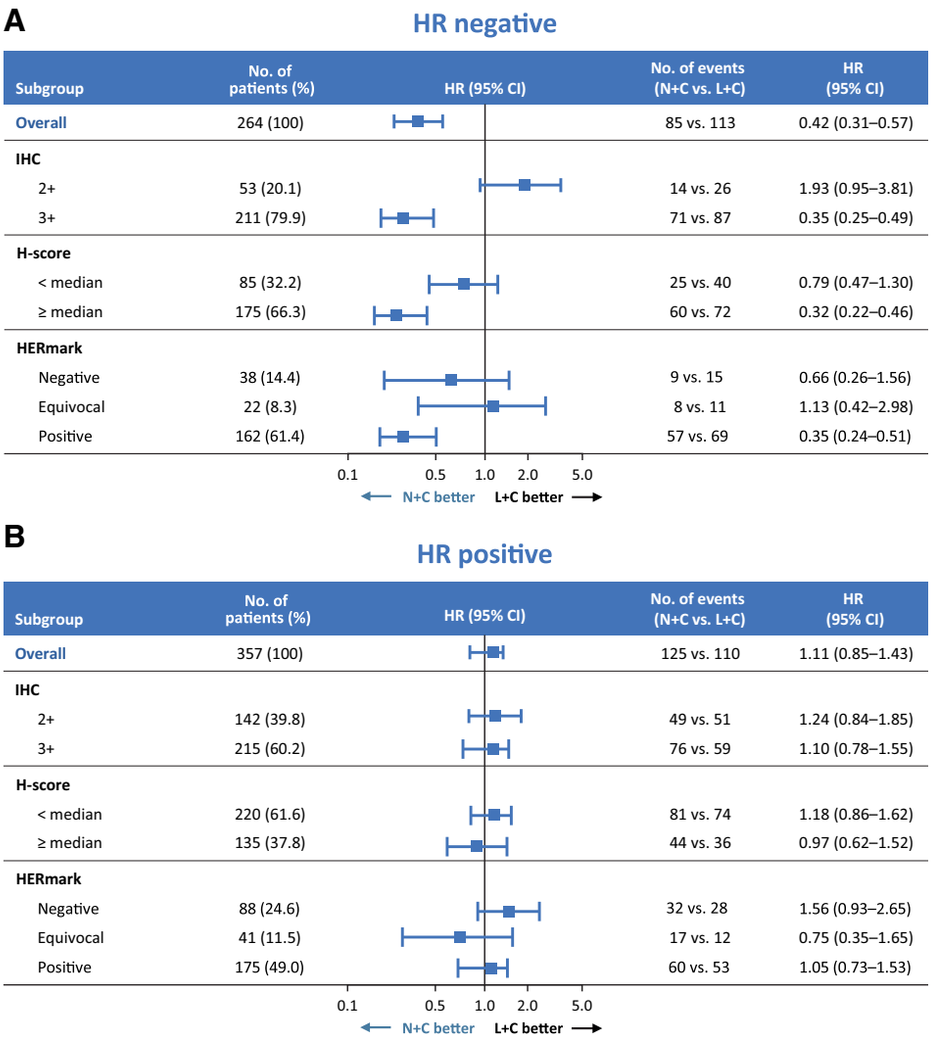


Figure 3.
Biomarker expression according to hormone receptor status: hormone receptor–negative (**A**) and hormone receptor–positive (**B**) tumors.

has been described as an acquired resistance mechanism to neratinib in *HER2*-mutated MBC (15). Notably, *HER2* mutations in NALA were identified in tissues from both primary and metastatic samples, suggesting that *HER2* mutation and amplification can potentially, albeit very rarely, emerge together.

HER2 protein level was a clear response biomarker in NALA. Not only was higher HER2 expression prognostic of PFS, but patients with tumors expressing higher HER2 levels also benefited more from N+C compared with L+C. This association may help explain the late separation of PFS Kaplan–Meier curves in the primary clinical analysis, as tumors with lower HER2 protein level may simply be less sensitive to HER2-targeted therapies. Notably, because of differences in the maximum tolerated dose of capecitabine with neratinib and lapatinib (36), the capecitabine dose was lower in the N+C arm (750 mg/m^2 twice a day) than in L+C (1,000 mg/m^2 twice a day) in NALA; consequently, the capecitabine component in NALA regimens may have been responsible for response to treatment among patients whose tumors were not HER2 driven, including those with low HER2 expression.

Neratinib and lapatinib are both small-molecule TKIs; however, neratinib is considered a more potent inhibitor (3). Our results are consistent with prior phase III clinical trials reporting association of higher HER2 levels with better response to the stronger HER2-targeting regimen in HER2$^+$ MBC: CLEOPATRA, EMILIA, and TH3RESA all reported increased PFS favoring the more potent regimen in patients with greater HER2 mRNA expression (28, 29, 37). In the adjuvant setting, APHINITY reported increased benefit with the stronger regimen in patients with higher HER2 protein levels, as measured by IHC (38). Finally, in the neoadjuvant NeoALTTO trial, the predicted probability of pathologic complete response to lapatinib + trastuzumab versus either agent alone was associated with increasing HER2 levels as measured by HERmark (39). Although beyond the scope of this manuscript, it would be interesting to understand whether the same trend holds for the novel HER2-specific, reversible TKI tucatinib, which has been reported to be less potent than neratinib when comparing IC_{50} values in the context of cell-line models (40). Within the context of HER2 positivity, p95 quantitation did not provide additional predictive or prognostic information, perhaps due to the fact that p95 trended with HERmark score in patients who were HER2 positive, and/or that both neratinib and lapatinib bind to the intracellular domain of HER2.

Negative hormone receptor status in NALA was associated with a trend toward better prognosis and increased response to N+C compared with L+C. The ExteNET extended adjuvant trial, in contrast,

reported an association of neratinib with improved invasive disease-free survival in patients with hormone receptor–positive status (41). Cross-talk between the ER and HER2 signaling pathways, which has been well described (42, 43), may explain this apparent discrepancy. Patients with hormone receptor–positive disease received coincident hormonal therapy in ExteNET; however, consistent with standard of care and with other MBC trials in similar populations, hormonal therapy was not permitted in NALA. Preclinical models of ER-positive/HER2$^+$ breast cancer evaded HER2 blockade with neratinib via upregulation of ER-pathway signaling, and conversely, inhibition with hormonal therapy directed upregulation of HER2 signaling (44). Dual inhibition with endocrine therapy and neratinib may warrant evaluation in patients with hormone receptor–positive, HER2$^+$ MBC.

A primary limitation of this study is the lack of a prerandomization biopsy, which necessitated use of a combination of primary and metastatic samples, resulting in an analysis that may not reflect all the molecular changes acquired due to anticancer therapies administered after biopsy. A second consideration is the limited number of slides that were available for each patient (2–4 slides per patient in general). This restricted the analyses that could be performed, e.g., gene-expression profiling was technically unfeasible for such limited tissue.

In conclusion, meaningful biomarker associations were identified in this analysis of tumor samples from patients included in the NALA study. Patients with MBC with high HER2 protein expression regardless of p95 level may benefit most from N+C. Exploration of alternative therapies in patients with low HER2 may be warranted, and patients with high HER2 and hormone receptor–positive disease, and/or *PIK3CA* mutations, may derive greater benefit from addition of endocrine therapy or PI3K pathway inhibitors.

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the article. The authors may be contacted for further data sharing.

Authors' Disclosures

C. Saura reports personal fees from AstraZeneca, Daiichi-Sankyo, Eisai, Exact Sciences, Exeter Pharma, F. Hoffmann-La Roche Ltd, MediTech, Merck Sharp & Dohme, Novartis, Pfizer, Philips, Pierre Fabre, Puma, Roche Farma, Sanofi-Aventis, SeaGen, and Zymeworks outside the submitted work. M. Oliveira reports grants from PUMA Biotechnology during the conduct of the study. M. Oliveira also reports personal fees from PUMA Biotechnology and iTEOS; grants, personal fees, and non-financial support from Roche and Novartis; grants and personal fees from AstraZeneca, GSK, and SeaGen; personal fees and non-financial support from Pierre Fabre and Eisai; grants from Genentech, Zenith Epigenetics, Immunomedics, and Boehringer Ingelheim; and personal fees from MSD outside the submitted work. H. Wildiers reports grants from Roche, as well as other support from AbbVie, Biocartes, Novartis, The Planning Shop, Ariez Internat, Lilly, Eisai, AstraZeneca, TRM Oncology, Pfizer, Roche, AstraZeneca Ireland, Daiichi-Sankyo, KCE, Novartis, Orion Corporation, PSI Cro AG, Puma Technology, Sirtex, MSD, Immutep Pty, and Congress Care outside the submitted work; in addition, H. Wildiers is President of SIOG, the International Society of Geriatric Oncology. A.M. Brufksy reports personal fees from Puma during the conduct of the study, as well as personal fees from Daiichi, Roche Genentech, Eisai, Lilly, Novartis, Pfizer, AstraZenenca, and Tyme outside the submitted work. S.H. Waters reports personal fees from Novartis Pharmaceuticals Ltd and Roche Products Ltd outside the submitted work. S.A. Hurvitz reports contracted research support (paid to institution) from Ambrx, Amgen, AstraZeneca, Arvinas, Bayer, Daiichi-Sankyo, Dignitana, Genentech/Roche, Gilead, GSK, Immunomedics, Lilly, Macrogenics, Novartis, Pfizer, OBI Pharma, Phoenix Molecular Designs, Ltd. Pieris, PUMA, Radius, Samumend, Sanofi, Seattle Genetics, and Zymeworks, as well as travel reimbursed by Eli Lilly; in addition, S.A. Hurvitz's spouse holds stocks of ROMTech and Ideal Implant. B. Moy reports grants from PUMA Biotechnology during the conduct of the study. S.-B. Kim reports research funding from Novartis, Sanofi-Aventis, and DongKook Pharm Co.; in addition, S.-B. Kim is a consultant in advisory boards of Novartis, AstraZeneca, Lilly, Dae Hwa Pharmaceutical Co. Ltd, ISU Abxis, and Daiichi-Sankyo, and owns stocks of Genopeaks and NeogeneTC. E. Cronemberger reports grants from Puma Biotechnology during the conduct of the study, as well as other support from Roche, AstraZeneca, Daiichi-Sankyo, and Pfizer outside the submitted work. G.J. Wallweber reports personal fees from Laboratory Corporation of America/Monogram Biosciences during the conduct of the study, as well as personal fees from Genentech/Roche outside the submitted work. K. Keyvanjah reports personal fees and other support from Puma Biotechnology Inc during the conduct of the study; in addition, K. Keyvanjah has been employed by QED Therapeutics Inc outside of the submitted work, is a stockholder of Puma Biotechnology and BridgeBio Pharma, and is currently employed by Amgen. A.S. Lalani reports other support from Puma Biotechnology, Inc. during the conduct of the study, as well as other support from Puma Biotechnology, Inc. outside the submitted work; in addition, A.S. Lalani is an employee and shareholder of Puma Biotechnology, Inc. R. Bryce reports other support from Puma Biotechnology during the conduct of the study. A. Vivancos reports grants and personal fees from BMS, as well as personal fees from Bayer, Novartis, AstraZeneca, and Sysmex outside the submitted work. L.D. Eli reports personal fees from Puma Biotechnology during the conduct of the study. S. Delaloge reports grants from Puma, Daiichi, Roche, Seagen, and Pierre Fabre during the conduct of the study; S. Delaloge also reports grants and non-financial support from Pfizer and AstraZeneca, as well as grants from Sanofi, BMS, Besins, Orion, and MSD outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

C. Saura: Conceptualization, supervision, investigation, writing–review and editing. **J. Matito:** Data curation, validation, investigation, methodology, writing–review and editing. **M. Oliveira:** Investigation, methodology, writing–review and editing. **H. Wildiers:** Investigation, writing–review and editing. **A.M. Brufksy:** Investigation, writing–review and editing. **S.H. Waters:** Investigation, writing–review and editing. **S.A. Hurvitz:** Investigation, writing–review and editing. **B. Moy:** Investigation, writing–review and editing. **S.-B. Kim:** Investigation, writing–review and editing. **W.J. Gradishar:** Investigation, writing–review and editing. **G.S. Queiroz:** Investigation, writing–review and editing. **E. Cronemberger:** Investigation, writing–review and editing. **G.J. Wallweber:** Methodology, writing–review and editing. **J. Bebchuk:** Software, formal analysis, methodology, writing–review and editing. **K. Keyvanjah:** Conceptualization, writing–review and editing. **A.S. Lalani:** Conceptualization, resources, supervision, writing–review and editing. **R. Bryce:** Conceptualization, supervision, writing–review and editing. **A. Vivancos:** Supervision, investigation, methodology, writing–review and editing. **L.D. Eli:** Conceptualization, formal analysis, supervision, validation, writing–original draft, project administration, writing–review and editing. **S. Delaloge:** Conceptualization, investigation, writing–review and editing.

Acknowledgments

We thank the Independent Data Monitoring Committee, study investigators, research staff, clinical research organizations, and other vendors, as well as the patients who participated in the NALA trial. We would like to acknowledge statistical programming support provided by Yan Yan (Puma Biotechnology). We thank Jeff Sperinde and Weidong Huang (Monogram Biosciences) for additional support for HERmark and p95 assay design and execution. We thank Sandra Bernard (Targos GMBH) for oversight of clinical sample management. We thank Lee Miller and Deirdre Carman (Miller Medical Communications Ltd) for writing/editorial support.

This work was supported by Puma Biotechnology Inc. (no grant number is applicable). The funders of the study designed the trial, were responsible for data collection, data integrity and analyses, and interpretation of the data with oversight from the authors. The manuscript was written with input from authors, and with review and input from the sponsor. The lead and senior authors were responsible for the final decision regarding manuscript contents and submission. Puma Biotechnology Inc. funded the provision of editorial support provided by Miller Medical Communications.

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 May 4, 2021; revised July 9, 2021; accepted August 6, 2021; published first August 11, 2021.

References

1. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997–4013.
2. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, 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.
3. Collins DM, Conlon NT, Kannan S, Verma CS, Eli LD, Lalani AS, et al. Preclinical characteristics of the irreversible pan-HER kinase inhibitor neratinib compared with lapatinib: implications for the treatment of HER2-positive and HER2-mutated breast cancer. Cancers (Basel) 2019;11:737.
4. Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J, et al. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Cancer Res 2004;64:3958–65.
5. Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH, et al. A unique structure for epidermal growth factor receptor bound to GW572016 (lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res 2004;64:6652–9.
6. Stanley A, Ashrafi GH, Seddon AM, Modjtahedi H. Synergistic effects of various Her inhibitors in combination with IGF-1R, C-MET and Src targeting agents in breast cancer cell lines. Sci Rep 2017;7:3964.
7. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, et al. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013;3:224–37.
8. Yang B, Zhang H, Wang H. Atomistic insights into the lung cancer-associated L755P mutation in HER2 resistance to lapatinib: a molecular dynamics study. J Mol Model 2015;21:24.
9. Zuo W-J, Jiang Y-Z, Wang Y-J, Xu X-E, Hu X, Liu G-Y, et al. Dual characteristics of novel HER2 kinase domain mutations in response to HER2-targeted therapies in human breast cancer. Clin Cancer Res 2016;22:4859–69.
10. Yamaguchi H, Chang SS, Hsu JL, Hung MC. Signaling cross-talk in the resistance to HER family receptor targeted therapy. Oncogene 2014;33:1073–81.
11. Giuliano M, Hu H, Wang Y-C, Fu X, Nardone A, Herrera S, et al. Upregulation of ER signaling as an adaptive mechanism of cell survival in HER2-positive breast tumors treated with anti-HER2 therapy. Clin Cancer Res 2015;21:3995–4003.
12. Eichhorn PJA, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. Cancer Res 2008;68:9221–30.
13. Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, 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.
14. Hanker AB, Brewer MR, Sheehan JH, Koch JP, Sliwoski GR, Nagy R, et al. An acquired HER2(T798I) gatekeeper mutation induces resistance to neratinib in a patient with HER2 mutant-driven breast cancer. Cancer Discov 2017;7:575–85.
15. Smyth LM, Piha-Paul SA, Won HH, Schram AM, Saura C, Loi S, et al. Efficacy and determinants of response to HER kinase inhibition in HER2-mutant metastatic breast cancer. Cancer Discov 2020;10:198–213.
16. Saura C, Oliveira M, Feng Y-H, Dai M-S, Chen S-W, Hurvitz SA, et al. Neratinib plus capecitabine versus lapatinib plus capecitabine in HER2-positive metastatic breast cancer previously treated with ≥ 2 HER2-directed regimens: Phase III NALA trial. J Clin Oncol 2020;38:3138–49.
17. U.S. Food & Drug Administration. FDA approves neratinib for metastatic HER2-positive breast cancer. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-neratinib-metastatic-her2-positive-breast-cancer#:~:text=On%20February%2025%2C%202020%2C%20the,regimens%20in%20the%20metastatic%20setting.
18. Agilent Technologies, Inc. Dako. HERceptTest package insert. 2018.
19. Agilent Technologies, Inc. Dako. HER2 IQFISH pharmDx package insert. 2018.
20. Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med 2015;21:560–2.
21. Larson JS, Goodman LJ, Tan Y, Defazio-Eli L, Paquet AC, Cook JW, et al. Analytical validation of a highly quantitative, sensitive, accurate, and reproducible assay (HERmark) for the measurement of HER2 total protein and HER2 homodimers in FFPE breast cancer tumor specimens. Patholog Res Int 2010;2010:814176.
22. Huang W, Reinholz M, Weidler J, Yolanda L, Paquet A, Whitcomb J, et al. Comparison of central HER2 testing with quantitative total HER2 expression and HER2 homodimer measurements using a novel proximity-based assay. Am J Clin Pathol 2010;134:303–11.
23. Duchnowska R, Sperinde J, Chenna A, Haddad M, Paquet A, Lie Y, et al. Quantitative measurements of tumoral p95HER2 protein expression in metastatic breast cancer patients treated with trastuzumab: independent validation of the p95HER2 clinical cutoff. Clin Cancer Res 13–20:2805;2014.
24. Sperinde J, Huang W, Vehtari A, Chenna A, Kellokumpu-Lehtinen P-L, Winslow J, et al. p95HER2 methionine 611 carboxy-terminal fragment is predictive of trastuzumab adjuvant treatment benefit in the FinHer trial. Clin Cancer Res 2018;24:3046–52.
25. R Core Team. R: A language and environment for statistical computing. Available from: http://www.R-project.org/.
26. Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, Aguilar Z, et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J Natl Cancer Inst 2003;95:142–53.
27. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703–13.
28. Baselga J, Cortés J, Im S-A, Clark E, Ross G, Kiermaier A, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 2014;32:3753–61.
29. Baselga J, Lewis Phillips GD, Verma S, Ro J, Huober J, Guardino AE, et al. Relationship between tumor biomarkers and efficacy in EMILIA, a phase III study of trastuzumab emtansine in HER2-positive metastatic breast cancer. Clin Cancer Res 2016;22:3755–63.
30. Xu B, Guan Z, Shen Z, Tong Z, Jiang Z, Yang J, et al. Association of phosphatase and tensin homolog low and phosphatidylinositol 3-kinase catalytic subunit alpha gene mutations on outcome in human epidermal growth factor receptor 2-positive metastatic breast cancer patients treated with first-line lapatinib plus paclitaxel or paclitaxel alone. Breast Cancer Res 2014;16:405.
31. Chia SKL, Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, et al. PIK3CA alterations and benefit with neratinib: analysis from the randomized, double-blind, placebo-controlled, phase III ExteNET trial. Breast Cancer Res 2019;21:39.
32. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen P-L, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J Natl Cancer Inst 2013;105:960–7.
33. Pogue-Geile KL, Song N, Jeong J-H, Gavin PG, Kim S-R, Blackmon NL, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. J Clin Oncol 2015;33:1340–7.
34. Griffith OL, Spies NC, Anurag M, Griffith M, Luo J, Tu D, et al. The prognostic effects of somatic mutations in ER-positive breast cancer. Nat Commun 2018;9:3476.
35. Gaibar M, Beltrán L, Romero-Lorca A, Fernández-Santander A, Novillo A. Somatic mutations in HER2 and implications for current treatment paradigms in HER2-positive breast cancer. J Oncol 2020;2020:6375956.
36. Saura C, Garcia-Saenz JA, Xu B, Harb W, Moroose R, Pluard T, et al. Safety and efficacy of neratinib in combination with capecitabine in patients with metastatic human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 2014;32:3626–33.
37. Kim S-B, Wildiers H, Krop IE, Smitt M, Yu R, Lysbet de Haas S, et al. Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) vs. treatment of physician's choice in previously treated HER2-positive advanced breast cancer. Int J Cancer 2016;139:2336–42.
38. Krop IE, Paulson J, Campbell C, Kiermaier AC, Andre F, Fumagalli D, et al. Genomic correlates of response to adjuvant trastuzumab (H) and pertuzumab (P) in HER2+ breast cancer (BC): Biomarker analysis of the APHINITY trial. J Clin Oncol 2019;37:1012.

39. Scaltriti M, Nuciforo P, Bradbury I, Sperinde J, Agbor-Tarh D, Campbell C, et al. High HER2 expression correlates with response to the combination of lapatinib and trastuzumab. Clin Cancer Res 2015;21:569–76.
40. Conlon NT, Kooijman JJ, van Gerwen SJC, Mulder WR, Zaman GJR, Diala I, et al. Comparative analysis of drug response and gene profiling of HER2-targeted tyrosine kinase inhibitors. Br J Cancer 2021;124:1249–59.
41. Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18:1688–700.
42. Arpino G, Wiechmann L, Osborne CK, Schiff R. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev 2008;29:217–33.
43. Giuliano M, Trivedi MV, Schiff R. Bidirectional crosstalk between the estrogen receptor and human epidermal growth factor receptor 2 signaling pathways in breast cancer: Molecular basis and clinical implications. Breast Care 2013;8:256–62.
44. Sudhan DR, Schwarz LJ, Guerrero-Zotano A, Formisano L, Nixon MJ, Croessmann S, et al. Extended adjuvant therapy with neratinib plus fulvestrant blocks ER/HER2 crosstalk and maintains complete responses of ER (+)/HER2(+) breast cancers: Implications to the ExteNET trial. Clin Cancer Res 2019;25:771–83.