Neratinib in Combination With Trastuzumab for the Treatment of Patients With Advanced HER2-positive Breast Cancer: A Phase I/II Study

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Abstract

In this international, open-label phase I/II study, neratinib in combination with trastuzumab was well-tolerated and had encouraging antitumor activity in patients with advanced trastuzumab-pretreated human epidermal growth factor receptor 2-positive breast cancer. Durable responses lasting approximately 10 years were achieved in some patients.

Background: Despite the availability of several human epidermal growth factor receptor 2 (HER2)-directed treatments, many HER2-positive (HER2$^+$) breast cancers eventually progress because of primary or acquired resistance. **Patients and Methods:** A 2-part, open-label, multicenter phase I/II study was conducted to determine the recommended dose of neratinib when administered with trastuzumab (part I), and to assess the antitumor activity of this combination in women with locally advanced or metastatic HER2$^+$ breast cancer previously treated with at least 1 prior trastuzumab-based regimen (part II). Patients received oral neratinib (160 or 240 mg/d) once daily plus intravenous trastuzumab 4 mg/kg (loading dose) then 2 mg/kg weekly. Diarrhea prophylaxis was not permitted. The primary endpoint in part II was investigator-assessed 16-week progression-free survival (PFS). **Results:** Forty-five patients received neratinib plus trastuzumab (part I: neratinib 160 mg/d, n = 4; neratinib 240 mg/d, n = 4; part II: neratinib 240 mg/d, n = 37). In part I, there were no dose-limiting toxicities and the recommended neratinib dose was 240 mg/d. In part II, the 16-week PFS rate was 44.8% (90% confidence interval, 28.8%-59.6%), and the median PFS was 15.9 weeks (95% confidence interval, 15.1-31.3 weeks) in 28 evaluable patients. Three patients had durable clinical benefit lasting 9.4 to 9.7 years. Diarrhea was the most common adverse event (grade 3, n = 7 [15.6%]; grade 4, n = 0). No clinically significant cardiac toxicity was seen. **Conclusions:** Neratinib in combination with trastuzumab was well-tolerated and had encouraging antitumor activity in patients with advanced trastuzumab-pretreated HER2$^+$ breast cancer. Durable responses can be achieved in some patients.

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Introduction

Approximately 15% to 20% of breast cancers have gene amplification or overexpression of human epidermal growth factor receptor 2 (HER2),[1,2] which can be treated effectively with HER2-directed targeted agents. Trastuzumab, a humanized monoclonal antibody that binds to the extracellular domain of the HER2 receptor,[3] improves overall survival when given in combination with chemotherapy in both early-stage and advanced HER2-positive (HER2$^+$) breast cancers,[4-6] although many patients eventually relapse, highlighting the need for new targeted therapies. In the

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metastatic setting, trastuzumab is generally given with chemotherapy, and superior, non–chemotherapy-containing combinations would be of significant clinical benefit in terms of minimizing chemotherapy-related toxicity.

Neratinib (Nerlynx; Puma Biotechnology Inc, Los Angeles, CA) is a potent small-molecule irreversible pan-HER tyrosine kinase inhibitor that has a mechanism of action distinct from that of trastuzumab. Neratinib binds to a cysteine residue within the adenosine triphosphate-binding pocket of HER receptor kinases, leading to reduced receptor phosphorylation and inhibition of downstream signal transduction.[7] Neratinib has activity in HER2$^+$ breast cancer cell lines with acquired and innate resistance to trastuzumab,[8] and has demonstrated efficacy in trastuzumab-pretreated patients with HER2$^+$ metastatic breast cancer.[9,10] In the randomized NEfERT-T study, neratinib-paclitaxel showed similar efficacy to trastuzumab-paclitaxel as first-line therapy in HER2$^+$ metastatic breast cancer, but significantly delayed the onset and frequency of central nervous system (CNS) progression.[11] In the Extended Adjuvant Treatment of Breast Cancer with Neratinib (ExteNET) trial, a 12-month course of neratinib after trastuzumab-based adjuvant therapy significantly improved 2-year[12] and 5-year[13] invasive disease-free survival compared with placebo in early-stage HER2$^+$ breast cancer.

Preclinical data suggest that simultaneous use of trastuzumab and a HER-directed tyrosine kinase inhibitor can overcome trastuzumab resistance and enhance antitumor activity in HER2$^+$ breast cancers.[8,14,15] The validity of this approach was demonstrated in the EGF104900 trial, which showed significantly improved progression-free survival (PFS)[16] and overall survival[17] with lapatinib plus trastuzumab versus lapatinib alone in patients with HER2$^+$ metastatic breast cancer who had progressed on prior trastuzumab-containing therapy.[16]

It was hypothesized that the combination of neratinib and trastuzumab may improve clinical outcomes, while avoiding the toxicities typically observed when trastuzumab is combined with chemotherapy. The present study, 3144A1-202-WW, was conducted to determine the recommended dose of neratinib when co-administered with trastuzumab, and to assess the antitumor activity of this combination in patients with advanced HER2$^+$ tumors previously treated with trastuzumab.

Patients and Methods

Study Design

This was a 2-part, open-label, multi-institutional phase I/II study conducted in 11 centers in the United States, Europe, and China. Part I was a dose-escalation study conducted to identify the recommended dose of neratinib for use in combination with trastuzumab. Part II was conducted to confirm the safety of the recommended dose, and to evaluate the efficacy of neratinib plus trastuzumab in an expanded cohort of patients with HER2$^+$ breast cancer.

The study was initiated in April 2007, and the study database closed in July 2012. As 3 patients remained on treatment at this time, the protocol was amended in March 2012 to include an extension phase with the aim of providing ongoing treatment to patients who continued to derive clinical benefit from therapy after the primary study objectives had been completed.

The study was registered (ClinicalTrials.gov identifier: NCT00398567) and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol was approved by an institutional review board or independent ethics committee at each study site. Written informed consent was obtained from all patients prior to enrollment.

Patients

Eligibility criteria were identical for both study parts I and II. Patients aged $\geq$ 18 years with a pathologic diagnosis of breast cancer (stage IIIB, IIIC, or IV) and disease progression following $\geq$ 1 trastuzumab-containing regimen in the (neo)adjuvant or metastatic setting were eligible. There were no stated eligibility criteria relating to the prior use of other HER2-directed agents. Local documentation of *HER2* amplification by fluorescence in situ hybridization or HER2 overexpression by immunohistochemistry (IHC, score 3+) was required; no centralized testing was performed. Other key inclusion criteria were a left ventricular ejection fraction (LVEF) within institutional limits of normal by multigated acquisition scan or echocardiogram, and adequate organ and hematologic function. Patients were excluded if they had extensive visceral disease (including bilateral diffuse lymphangitic involvement of $>$ 50% of the lung, or involvement of more than one-third of the liver confirmed by computed tomography [CT] scan and/or magnetic resonance imaging [MRI]), active CNS metastases (ie, cerebral edema, and/or progressive growth; patients with a history of CNS metastases or cord compression were allowed if they had been definitively treated, were clinically stable, and were off steroids and/or anticonvulsants for $\geq$ 4 weeks before study treatment), or had received $>$ 3 chemotherapy regimens for advanced disease.

Treatment

In parts I and II, patients received oral neratinib 160 or 240 mg once daily continuously, based on findings from a phase I study,[10] and intravenous trastuzumab 4 mg/kg as a loading dose on day 1, then 2 mg/kg weekly thereafter until disease progression, death, or withdrawal from study. Patients entering the treatment extension phase received neratinib 240 mg/d continuously plus trastuzumab 6 mg/kg 3-weekly. Concomitant use of chemotherapy and other anticancer treatments, including endocrine therapy, was prohibited.

In cases of diarrhea, patients were instructed to contact the study site to discuss appropriate treatment, and to treat diarrhea per institutional guidelines at its earliest occurrence. No prophylactic medications were allowed to prevent neratinib-related diarrhea. In cases of grade 2/3 diarrhea lasting $>$ 2 days despite optimal medical therapy, grade 3/4 non-hematologic events, or grade 4 hematologic events, treatment interruption was recommended. If recovery from these events was slow (ie, 1-3 weeks) or in cases of recurrent grade 2/3 diarrhea, neratinib dose was reduced to the next available dose level (ie, 160 mg, then 80 mg).

Assessments

Adverse events were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. An adverse event was considered to be treatment-emergent if onset occurred between or on the date of the first and last dose (plus 28 days). A dose-limiting toxicity was defined as any of the following events at least possibly related to neratinib plus trastuzumab during the first 21 days of treatment: grade 3/4 non-hematologic toxicity; grade $\geq$ 2 diarrhea lasting $>$ 2 days or

associated with fever or dehydration; any grade 4 hematologic toxicity; or symptomatic congestive heart failure.

Radiologic assessments were performed using CT or MRI scans of the chest and abdomen (and CT or MRI scans of other sites or bone x-rays if clinically indicated) at screening, every 8 weeks thereafter, and at the final visit (ie, within 2-6 weeks of the last dose of study treatment). Tumor responses were assessed by investigators and classified using modified Response Evaluation Criteria in Solid Tumors 1.0; there was no central review of tumor assessments. For patients participating in the treatment extension period, procedures were limited to study drug administration and safety monitoring. Tumor assessments to determine disease progression were performed at the investigator's discretion according to standard of care.

Pharmacokinetics

Blood samples were collected from patients participating in part II on day 1 pre-dose, and day 22 pre-dose and 1, 2, 4, 6, 8, and 21 to 24 hours post-dose. One pre-dose sample per month from months 2 to 6 was also collected. Curves of neratinib plasma concentrations versus time were constructed for each patient and analyzed by non-compartmental methods. Parameters included observed maximum concentration (C_{max}) and area under the concentration-time curve truncated at last-reported plasma concentration (AUC_T).

Statistical Considerations

In part I, sample size was determined by clinical rather than statistical considerations, and no formal statistical analysis was performed. In part II, the null hypothesis for a 16-week PFS rate was $\leq$ 15% with an alternative hypothesis of $\geq$ 35%, based on a 16-week PFS rate of 12.9% with lapatinib plus trastuzumab in trastuzumab-refractory patients.[16] The overall probability of accepting neratinib plus trastuzumab for additional study under the null hypothesis was 0.05. Assuming a dropout rate of 15%, 30 patients were required to provide approximately 69% power to detect a 16-week PFS rate of $\geq$ 35%.

The efficacy analysis included only patients enrolled in study part II. Patients were evaluable for efficacy if they completed $\geq$ 1 week of neratinib treatment, $\geq$ 2 doses of trastuzumab, and $\geq$ 1 follow-up tumor assessment. Patients were also evaluable if clinical disease progression occurred before first follow-up tumor assessment. Safety analyses included all patients who received $\geq$ 1 dose of either neratinib or trastuzumab.

The primary endpoint was 16-week PFS, defined as time from the first dose of study drug to first documented recurrence, progression, or death from any cause by investigator review. Secondary endpoints included safety, overall response rate, clinical benefit rate, PFS, duration of response, and pharmacokinetics. Objective response rate was defined as the percentage of patients who had a complete or partial response. Clinical benefit rate was defined as the percentage of patients who had a complete response, partial response, or stable disease lasting $\geq$ 24 weeks. Time-to-event endpoints were analyzed using the Kaplan-Meier method.

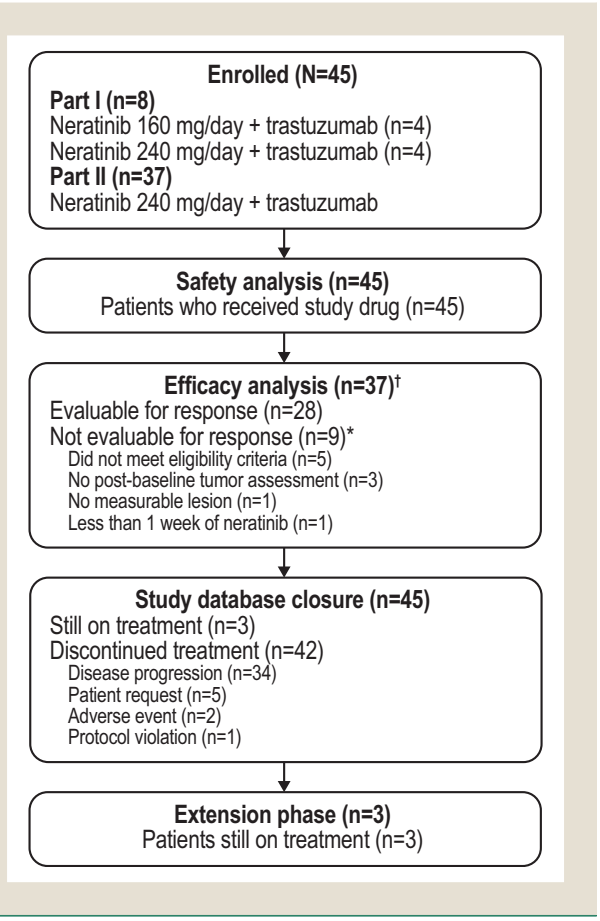
The cutoff date for all analyses was July 23, 2012. Analyses were performed using SAS, version 9.3.

Results

Patients

A total of 45 women were enrolled; 8 patients in part I (neratinib 160 mg/d, n = 4; neratinib 240 mg/d, n = 4) and 37 patients in part II. As of July 2012, 3 patients were still being treated with neratinib plus trastuzumab (Figure 1).

Figure 1 Study Flowchart



Patients may Have Been Excluded for More than 1 Reason. †Patients in Part I Were not Included in the Efficacy Analysis.

Baseline characteristics are presented in Table 1. Most (93.3%) patients had stage IV breast cancer at screening and were heavily pretreated; 38 (84.4%) patients had received 3 or more lines of therapy for metastatic disease. Most (86.7%) patients had received prior trastuzumab for metastatic disease, and 5 (11.1%) patients had received lapatinib; no patients had previously been exposed to trastuzumab emtansine (T-DM1) or pertuzumab.

The median duration of treatment was 16.0 weeks (interquartile range [IQR], 7.4-32.0 weeks) for neratinib and 15.1 weeks (IQR, 7.1-31.0 weeks) for trastuzumab in the total population (see Supplemental Table 1 in the online version). The median relative dose intensity was 100% for both neratinib and trastuzumab.

Safety

No dose-limiting toxicities were observed during part I, and neratinib was well-tolerated at both dose levels. The recommended dose of neratinib for part II was 240 mg/d.

The most common treatment-emergent adverse events with neratinib in combination with trastuzumab were diarrhea, nausea,

Table 1 Patient Baseline Characteristics

	Neratinib Plus Trastuzumab, n (%)[a]			
	Part I		Part II	
Neratinib Dose	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (N = 45)
Median age, y (range)	53.0 (40.0-64.0)	59.5 (47.0-78.0)	50.0 (23.0-79.0)	51.0 (23.0-79.0)
Disease stage at screening				
III (B, C)	0	0	3 (8.1)	3 (6.6)
IV	4 (100)	4 (100)	34 (91.9)	42 (93.3)
ECOG performance status				
0	3 (75.0)	2 (50.0)	21 (56.8)	26 (57.8)
1	1 (25.0)	2 (50.0)	15 (40.5)	18 (40.0)
2	0	0	1 (2.7)	1 (2.2)
Hormone receptor status				
Positive	4 (100)	1 (25.0)	18 (48.6)	23 (51.1)
Negative	0	3 (75.0)	17 (46.0)	20 (44.4)
Unknown	0	0	2 (5.4)	2 (4.4)
Prior trastuzumab (Neo)adjuvant setting				
0	4 (100)	4 (100)	34 (91.9)	42 (93.3)
1-2	0	0	3 (8.1)	3 (6.7)
Metastatic setting				
0	0	0	6 (16.2)	6 (13.3)
1	1 (25.0)	2 (50.0)	14 (37.8)	17 (37.8)
≥ 2	3 (75.0)	2 (50.0)	17 (45.9)	22 (48.9)
Duration of prior trastuzumab, mos				
Median (IQR)	7.0 (4.5-7.5)	6.7 (2.3-28.1)	9.8 (3.3-20.5)	7.5 (3.1-19.8)
Prior lapatinib	0	0	5 (13.5)	5 (11.1)
Duration of prior lapatinib, mos				
Median (IQR)	–	–	3.5 (2.4-7.3)	3.5 (2.4-7.3)
Prior cytotoxics				
(Neo)adjuvant				
0	2 (50.0)	1 (25.0)	6 (16.2)	9 (20.0)
≥ 1	2 (50.0)	3 (75.0)	31 (83.8)	36 (80.0)
Metastatic				
0	0	0	1 (2.7)	1 (2.2)
1	0	1 (25.0)	3 (8.1)	4 (8.9)
≥ 2	4 (100)	3 (75.0)	33 (89.2)	40 (88.9)
Prior taxanes				
0	0	2 (50.0)	3 (8.1)	5 (11.1)
≥ 1	4 (100)	2 (50.0)	34 (91.9)	40 (88.9)
Prior anthracyclines				
0	2 (50.0)	1 (25.0)	6 (16.2)	9 (20.0)
≥ 1	2 (50.0)	3 (75.0)	31 (83.8)	36 (80.0)
Prior taxanes and anthracyclines				
0	2 (50.0)	2 (50.0)	7 (18.9)	11 (24.4)
≥ 1	2 (50.0)	2 (50.0)	30 (81.1)	34 (75.6)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; IQR = interquartile range.
[a]Neratinib at doses shown plus trastuzumab 4 mg/kg loading then 2 mg/kg weekly (parts I and II) or 6 mg/kg every 3 weeks (extension phase).

decreased appetite, vomiting, and asthenia (Table 2). Grade 3 diarrhea was reported in 15.6% of patients; all other grade 3 events were each reported in less than 5% of patients. Two grade 4 adverse events were observed (brain metastasis, n = 1; hyperbilirubinemia, n = 1). Treatment-emergent adverse events led to dose reductions in 5 (11.1%) patients, dose holds in 11 (24.4%) patients, and

Table 2 Treatment-emergent Adverse Events Occurring in at Least 10% of Patients (Safety Population)

Adverse Event	Neratinib Plus Trastuzumab[a] (N = 45), n (%)	
	All-grade	Grade 3
Diarrhea	42 (93.3)	7 (15.6)
Nausea	23 (51.1)	2 (4.4)
Decreased appetite	22 (48.9)	1 (2.2)
Vomiting	18 (40.0)	2 (4.4)
Asthenia	13 (28.9)	0
Rash	10 (22.2)	1 (2.2)
Fatigue	9 (20.0)	1 (2.2)
Weight decreased	9 (20.0)	0
Abdominal pain	9 (20.0)	0
Headache	8 (17.8)	0
Dizziness	5 (11.1)	0
Erythema	5 (11.1)	0
Hemoglobin decreased	5 (11.1)	1 (2.2)
Dysuria	5 (11.1)	0
Dyspnea	5 (11.1)	2 (4.4)
Dry skin	5 (11.1)	0
Pyrexia	5 (11.1)	0
Urinary tract infection	5 (11.1)	0
Aspartate aminotransferase increased	5 (11.1)	2 (4.4)

Note: no grade 4 or 5 events for the toxicities listed in the table were reported.
[a]Neratinib 160 mg/day (n = 4) or 240 mg/day (n = 41) plus trastuzumab 4 mg/kg loading then 2 mg/kg weekly (parts I and II) or 6 mg/kg every 3 weeks (extension phase).

treatment discontinuation in 2 (4.4%) patients. A full summary of treatment-emergent adverse events in study parts I and II is presented in Supplemental Table 2 (in the online version), and treatment-related events are presented in Supplemental Table 3 (in the online version).

Although diarrhea was the most common adverse event, no grade 4 diarrhea events were reported. Dose reductions were required in 2 (4.4%) patients with diarrhea (grade 2), and treatment discontinuation was required in 1 (2.2%) patient with diarrhea (grade 2). Diarrhea (grade 3) led to hospitalization in 1 (2.2%) patient. The median time to onset of grade 1, 2, and 3 diarrhea was 2.0 days (IQR, 2.0-6.0 days), 4.5 days (IQR, 2.0-9.0 days), and 8.0 days (IQR, 3.0-44.0 days), respectively, with a median cumulative duration of 86.5 days (IQR, 23.0-138.0 days), 8.0 days (IQR, 3.0-17.0 days), and 6.0 days (IQR, 3.0-6.0 days), respectively. Grade 3 events were generally observed during the first 2 weeks of treatment (week 1, 6.7% [n = 3]; week 2, 6.8% [n = 3]), with only 2 other isolated grade 3 events observed after this time (see Supplemental Figure 1 in the online version). Medications most commonly used to manage first occurrences of diarrhea were loperamide (n = 23; 51.1%), diphenoxylate/atropine (lomotil) plus loperamide (n = 4; 8.9%), aluminium magnesium silicate (n = 4; 8.9%), and aluminium magnesium silicate plus loperamide (n = 4; 8.9%), and medications most commonly used to manage second occurrences of diarrhea were loperamide (n = 19; 42.2%), diphenoxylate/atropine (lomotil) (n = 8; 17.8%), and aluminium magnesium silicate (n = 7; 15.6%).

Cardiac disorders (all grade 1) were reported in 3 (6.7%) patients (first-degree atrioventricular block, n = 1; palpitations, n = 1; tachycardia, n = 1); 1 event (tachycardia) was considered to be drug-related. In addition, grade 1 decreases in ejection fraction were reported in 3 (6.7%) patients, 1 of which was considered to be drug-related, and grade 1 QT-interval prolongation was reported for 1 patient. A box-plot of LVEF at baseline and worst post-baseline visit is presented in Supplemental Figure 2 (in the online version); LVEF decreased to < 50% (41%) in 1 patient.

Three (6.7%) patients died during the study. Disease progression was the reported cause of death for all 3 patients.

Pharmacokinetics

Complete blood samples for pharmacokinetic analysis were available for 33 patients in study part II. At steady-state, mean neratinib C_{max} and AUC_T were 78.9 ± 34.7 ng/mL and 1110 ± 430 ng•h/mL, respectively (see Supplemental Table 4 in the online version).

Antitumor Activity

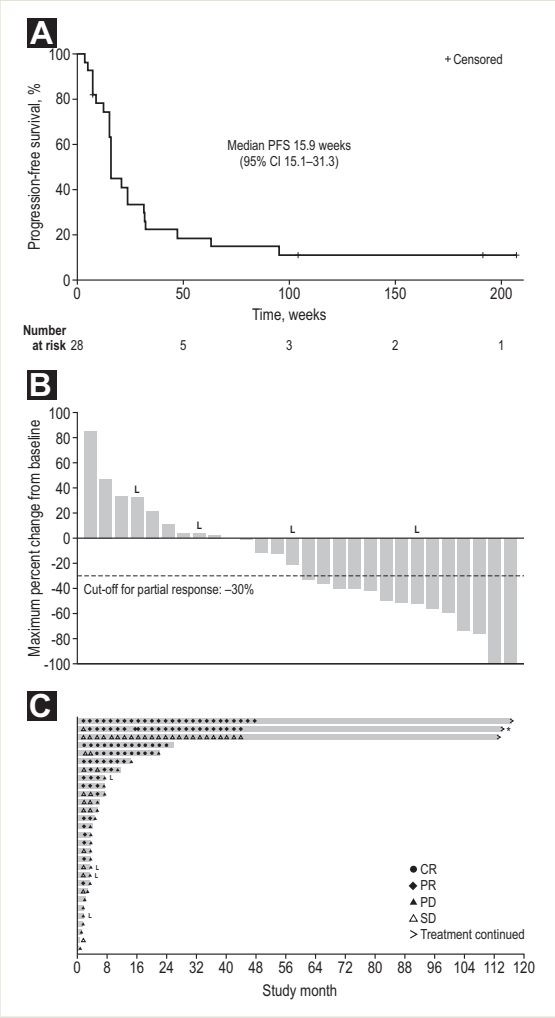
Twenty-eight patients were included in the efficacy analysis (Table 3); 9 patients were excluded for the reasons shown in Figure 1. The 16-week PFS rate, based on investigator assessment, was 44.8% (90% CI, 28.8%-59.6%). The median PFS was 15.9 weeks (95% CI, 15.1-31.3 weeks) (Figure 2A). Overall response rate was 28.6% (95% CI, 13.2%-48.7%), and clinical benefit rate was 35.7% (95% CI, 18.6%–55.9%). The median duration of objective response was 43.6 weeks (95% CI, 13.1 weeks to not

Table 3 Investigator-assessed Efficacy in Part II (Evaluable Population)

	Neratinib 240 mg/day[a] Plus Trastuzumab[b] (n = 28), n (%)
Primary endpoint	
16-week PFS rate, %	44.8
90% CI	28.8-59.6
95% CI	25.9-62.1
Secondary endpoints	
Overall response rate	8 (28.6)
95% CI	13.2-48.7
Clinical benefit rate[c]	10 (35.7)
95% CI	18.6-55.9
Complete response	2 (7.1)
Partial response	6 (21.4)
Stable disease ≥ 24 weeks	2 (7.1)
Stable disease < 24 weeks	12 (42.9)
Progressive disease	6 (21.4)
Median PFS, w (95% CI)	15.9 (15.1-31.3)
Median duration of response, w (95% CI)	43.6 (13.1-NE)

Abbreviations: CI = confidence interval; NE = not estimable; PFS = progression-free survival.
[a]Recommended dose of neratinib from part I.
[b]Trastuzumab 4 mg/kg loading then 2 mg/kg weekly (part II) or 6 mg/kg every 3 weeks (extension phase).
[c]Defined as patients who had a complete response, partial response, or stable disease lasting ≥ 24 weeks.

Figure 2 **Efficacy of Neratinib Plus Trastuzumab (Evaluable Population – Part II). Panels Show Kaplan-Meier Estimates of Progression-free Survival (A), Waterfall Plot of Maximum Percentage Change From Baseline in Target Tumor Size (B), and Swimmer Plot of Duration of Therapy and Responses by Individual Patient (C)**



Abbreviations: CI = confidence interval; CR = complete response; L = lapatinib-pretreated; PD = progressive disease; PFS = progression-free survival; PR = partial response; SD = stable disease. *Patient did not meet inclusion criteria for the efficacy evaluable population (ie, progression following ≥ 1 trastuzumab-containing regimen).

estimable). For the intent-to-treat population, the 16-week investigator-assessed PFS rate was 48.9% (95% CI, 31.1%-64.3%); median PFS was 16.0 weeks (95% CI, 15.1-31.3 weeks). In the 5 patients who had received prior lapatinib therapy, 1 had a partial response lasting for 24.7 weeks, 2 had stable disease lasting < 24 weeks, and 1 had disease progression; the remaining patient was unevaluable for efficacy.

The maximum percentage reduction in target tumor sizes is presented in Figure 2B. Of the 28 patients with target lesions at baseline and ≥ 1 follow-up tumor assessment, 18 (64.3%) patients had reductions in tumor size, 5 patients had either no increase or increases < 20%, and 5 patients had > 20% increases in tumor size.

When analyzed by hormone receptor (HR) status, the 16-week PFS rate in patients with HR-positive tumors (n = 14) was 28.6% (90% CI, 8.8%-52.4%), the median PFS was 15.8 weeks (95% CI, 7.1-20.7 weeks), and the overall response rate was 21.4% (95% CI, 4.6%-50.8%), and in patients with HR-negative tumors (n = 12), the 16-week PFS rate was 64.8% (90% CI, 31.0%-85.2%), the median PFS was 31.3 weeks (95% CI, 7.1-63.1 weeks), and the overall response rate was 41.7% (95% CI, 15.2%-72.3%).

Three patients showed durable clinical benefit with neratinib plus trastuzumab and entered the treatment extension phase (top bars of Figure 2C). All 3 of these patients were heavily pretreated and had received 1 (n = 2) or 2 (n = 1) previous courses of trastuzumab in the metastatic setting with intervals of 0.6, 4.2, and 24.4 months between the last trastuzumab dose and start of study treatment. HER2 testing showed that all patients were 3+ by IHC; 2 patients had documented HR status and were estrogen receptor-positive. At database closure in July 2012, these 3 patients were receiving neratinib 160 (n = 1) or 240 (n = 2) mg/d plus trastuzumab and had documented partial responses (n = 2) or stable disease (n = 1). At last follow-up (June 2017), the 3 patients had received study treatment for 9.4 to 9.7 years and continued to derive ongoing clinical benefit (ie, at least stable disease and no treatment-limiting toxicities) from therapy.

Discussion

Our study showed that neratinib at a dosage of 240 mg/d can be administered safely in combination with standard doses of trastuzumab, and that the combination has antitumor activity in patients previously exposed to trastuzumab with locally advanced or metastatic HER2+ breast cancer. Approximately 45% of patients were alive and progression-free at 16 weeks, with the lower bounds of the 90% CI (and more stringent 95% CI) exceeding the prespecified criterion for further evaluation (15%) in this heavily pretreated population. A notable finding from this study was that 3 patients had durable responses to neratinib plus trastuzumab, and continued to derive ongoing clinical benefit from study treatment, having been on therapy for approximately 10 years.

The combination of neratinib and trastuzumab seeks to address the complex issue of trastuzumab resistance in metastatic HER2-amplified breast cancer by blocking HER-receptor tyrosine kinase activity. Although there is still more to learn about the mechanisms by which neratinib circumvents trastuzumab resistance, it has been shown that neratinib inhibits phosphorylation of HER2 and HER3 and downstream signaling molecules (ERK and Akt) in HER2+ breast cancer cell lines with acquired trastuzumab resistance.[8] Neratinib also prevents trastuzumab-induced upregulation of HER4 in vitro and in vivo, another mechanism by which HER2+ breast cancer cells acquire resistance to trastuzumab.[18] Further, in HER2+ breast cancer cell lines, neratinib sensitivity was not correlated with levels of p95HER2,[8] a truncated HER2 receptor with intrinsic trastuzumab resistance.[19]

The high clinical benefit rate (36%) with neratinib plus trastuzumab is comparable with another study[9] that examined neratinib alone in a less heavily pretreated population of patients with HER2+ breast cancer. This finding is surprising, even given the limitation of a cross-trial comparison of 2 small studies. One hypothesis is that neratinib is such a potent anti-HER2 agent that it does

not gain any synergy with trastuzumab as with the less potent inhibitor, lapatinib. This is supported by the slightly increased activity observed with neratinib plus trastuzumab in our study (median PFS, 15.9 weeks; overall response rate, 29%; clinical benefit rate, 36%) compared with lapatinib plus trastuzumab (median PFS, 12.0 weeks; overall response rate, 10%; clinical benefit rate, 25%) in a similar trastuzumab-refractory patient population (EGF104900 trial).[16] These data are even more impressive given that some of the patients in the present study had received prior treatment with lapatinib and derived benefit from neratinib plus trastuzumab (partial response, n = 1; stable disease, n = 2). Potential differences in the clinical activity of neratinib and lapatinib suggested by these findings may be attributable to their distinct mechanisms of action (ie, neratinib is an irreversible inhibitor of HER1, 2, and 4, whereas lapatinib is a reversible inhibitor of HER1 and 2), and the greater inherent in vitro activity of neratinib in $HER2^+$ cell lines.[8] Neratnib Against Lapatinib in Advanced Breast Cancer (NALA), an ongoing international randomized phase III trial, which is comparing neratinib plus capecitabine to lapatinib plus capecitabine as third-line or later therapy in women with $HER2^+$ metastatic breast cancer (ClinicalTrials.gov identifier: NCT01808573), will help to better understand the relative clinical efficacy of these 2 agents.

At the present time, the neratinib plus trastuzumab combination should be considered as a treatment option only after the optimal first-, second-, and third-line approved regimens of trastuzumab plus pertuzumab, trastuzumab emtansine, and lapatinib and capecitabine have been given. Our study was initiated before the newer HER2-directed agents—trastuzumab emtansine and pertuzumab—were introduced into clinical practice. Although our study does not provide any insight into the efficacy of the neratinib plus trastuzumab combination following treatment with these newer agents, the ongoing phase Ib/II National Adjuvant Breast and Bowel Project (NSABP) FB-10 study, which is evaluating neratinib in combination with trastuzumab emtansine in women with $HER2^+$ breast cancer previously treated with pertuzumab and trastuzumab, is of particular interest.[20] The investigators reported an overall response rate of 60% (12 of 20 evaluable patients) in this patient population, which compares favorably with a rate of 18% with single-agent trastuzumab emtansine in similarly pretreated patients.[21] These findings suggest that the neratinib plus trastuzumab combination has promising activity in a contemporary patient population treated according to current treatment algorithms.

For the future, we suggest that consideration should also be given to trials including neratinib in combination with trastuzumab earlier in the $HER2^+$ breast cancer treatment algorithm. In the neoadjuvant setting, enrollment for a phase Ib/II study at the MD Anderson Cancer Center (Clinicaltrials.gov identifier: NCT03101748) has recently started to investigate the maximum tolerated dose of neratinib in combination with trastuzumab, pertuzumab, and paclitaxel followed by doxorubicin and cyclophosphamide; phase II will evaluate the efficacy of this combination in $HER2^+$ locally advanced inflammatory breast cancer. In addition, a randomized multicenter phase II study (NSABP FB-7) is currently comparing trastuzumab plus paclitaxel, neratinib plus paclitaxel and trastuzumab, neratinib plus paclitaxel in 126 patients with locally advanced $HER2^+$ breast cancer.[22] The pathologic complete response rate (breast and nodes) was higher (50%) with dual-targeted therapy compared with trastuzumab plus paclitaxel (38.1%) and neratinib plus paclitaxel (33.3%); patient follow-up is continuing.[22] In the extended adjuvant setting, where the efficacy of neratinib following trastuzumab-based adjuvant therapy is already established,[12] the benefits of neratinib may be further improved with earlier use of neratinib in combination with adjuvant trastuzumab.

In our study, neratinib in combination with trastuzumab was generally well-tolerated. Grade 3 events generally occurred infrequently, with the exception of diarrhea, which is the dose-limiting toxicity of neratinib. In our study, patients were instructed to treat diarrhea at its earliest occurrence according to institutional guidelines, with dose modifications implemented in those unresponsive to medical therapy. Antidiarrheal prophylaxis was not permitted according to the study protocol. Under these guidelines, grade 3 diarrhea was documented in 16% of patients in our study, which is considerably lower than rates reported in other early studies of neratinib without supporting prophylaxis (30%-53%).[23] It also compares well with more recent studies of neratinib in the metastatic setting, which included antidiarrheal prophylaxis with high-dose loperamide given for the first 1 to 2 cycles of therapy (12%-22%).[20,24,25] The findings from our study appear to highlight the importance of good patient counseling and close observation during the early stages of therapy, and the effectiveness of such measures when applied diligently.

By combining 2 agents that inhibit HER2 by distinct mechanisms, there is the potential for an increased risk of cardiac dysfunction with trastuzumab. Careful cardiac monitoring in our study revealed no clinically significant cardiac toxicities with neratinib plus trastuzumab, and only 1 patient had a decrease in LVEF to less than 50%. This is consistent with previous studies of lapatinib given in combination with trastuzumab, which have also reported low levels of cardiac toxicity.[16,26] Pharmacokinetic analyses suggested no drug-drug interaction between neratinib and trastuzumab, and the observed steady-state exposures of neratinib after administration with trastuzumab were similar to those observed with single-agent neratinib in patients with advanced solid tumors.[10]

Conclusion

In summary, oral neratinib 240 mg/d, an irreversible pan-HER tyrosine kinase inhibitor, given in combination with trastuzumab was safe and well-tolerated and had encouraging antitumor activity in patients with advanced $HER2^+$ breast cancer even after failure of prior trastuzumab, thus warranting further studies of this combination. There was a small subset of patients who derived prolonged clinical benefit from this combination, and the molecular underpinnings of such a response need further investigation.

Clinical Practice Points

- In this international phase I/II trial, the 16-week PFS rate was 45% with neratinib plus trastuzumab in patients with advanced trastuzumab-pretreated $HER2^+$ breast cancer.
- Three patients showed durable clinical benefit (approximately 10 years).
- Diarrhea was the most common toxicity, although antidiarrheal prophylaxis was not permitted by the study protocol.

- Several ongoing trials are looking at the neratinib plus trastuzumab combination in the neoadjuvant and metastatic settings based upon the findings from this study.

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Disclosure

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Supplemental Data

Supplemental figures and tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2018.12.011.

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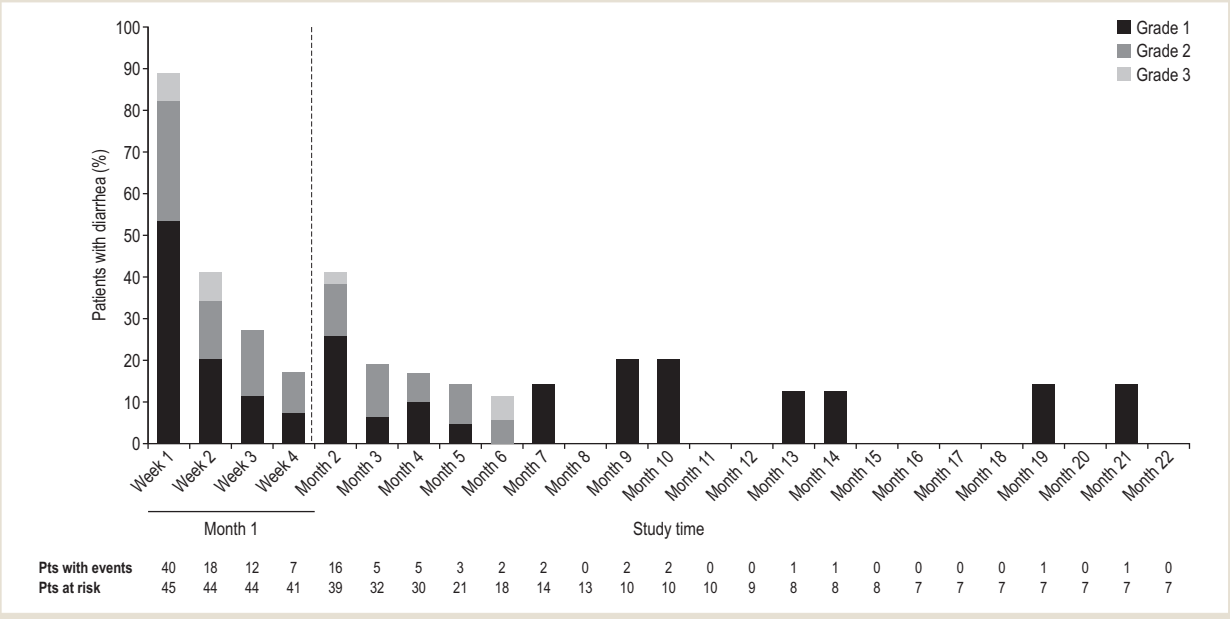
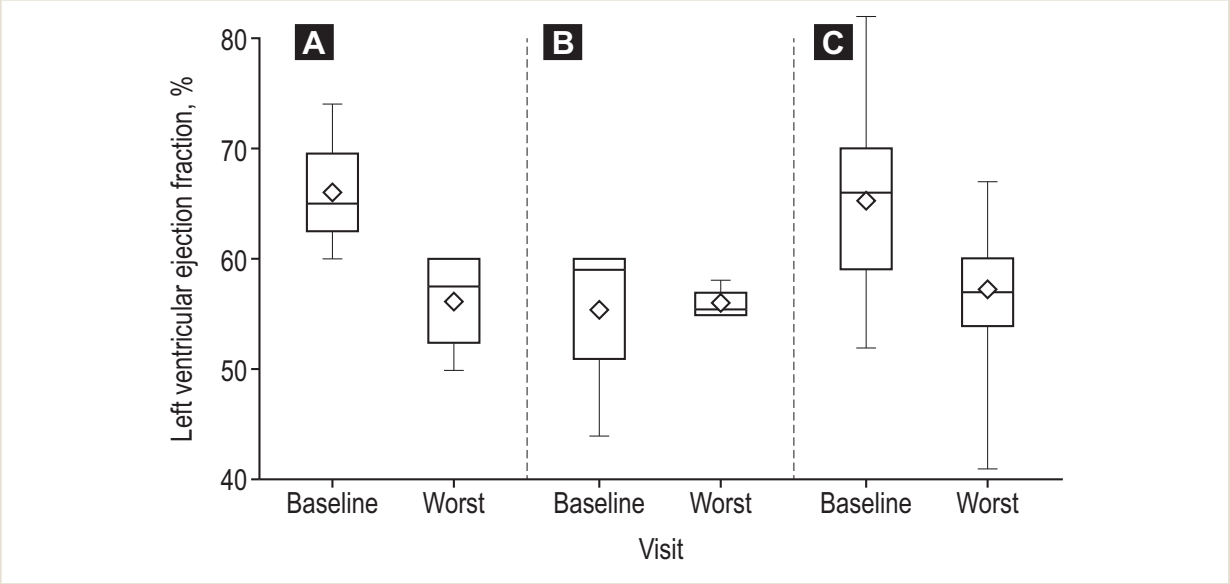
Supplemental Figure 1 Incidence of Diarrhea Events by Grade Over Time (Safety Population)



The Maximum Toxicity Grade is Listed for Patients with Multiple Events in a Particular Time Period. There Were No Reported Occurrences of Grade 4 Events.

Supplemental Figure 2 Boxplot of Left Ventricular Ejection Fraction at Baseline and Worst Post-baseline Visit (Safety Population). Panels Show Neratinib 160 mg/d Plus Trastuzumab (Part I; n = 4) (A), Neratinib 240 mg/d Plus Trastuzumab (Part I; n = 4) (B), and Neratinib 240 mg/d Plus Trastuzumab (part II; n = 37) (C)



For Each Group, the Mean Value is Represented by the '◇', the Median is Shown by the Line Inside the box, the 25% and 75% Distribution are the Bottom and Top of the Box, Respectively, and the Range (Minimum, Maximum) is Represented by the Whiskers.

Supplemental Table 1 Treatment Exposure (Safety Population)

Variable	Neratinib Plus Trastuzumab[a]			
	Part I		Part II	
	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (n = 45)
Total exposure, g				
Neratinib	14.9 (12.4)	43.7 (22.8)	73.2 (111.7)	65.4 (102.8)
Trastuzumab	2.5 (1.8)	3.8 (1.5)	5.7 (7.9)	5.2 (7.3)
Duration of treatment, w				
Neratinib	14.1 (4.2-22.9)	22.4 (16.0-36.2)	16.0 (7.4-32.1)	16.0 (7.4-32.0)
Trastuzumab	13.5 (4.1-21.9)	21.6 (15.6-35.1)	15.1 (7.1-31.1)	15.1 (7.1-31.0)
Dose intensity,[b] mg/w				
Neratinib	1101.4 (977.6-1116.5)	1680.0 (1664.2-1680.0)	1680.0 (1680.0-1680.0)	1680.0 (1668.3-1680.0)
Trastuzumab	185.1 (179.9-226.4)	149.6 (119.0-199.9)	144.7 (126.9-176.7)	145.8 (126.9-180.0)
Relative dose intensity[c]				
Neratinib	1.0 (0.9-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Trastuzumab	1.1 (1.0-1.2)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)
Actions owing to treatment-emergent adverse events, n (%)				
Dose reductions	1 (25)	0	4 (10.8)	5 (11.1)
Dose holds	1 (25)	1 (25)	9 (24.3)	11 (24.4)
Discontinuation	0	0	2 (5.4)	2 (4.4)

Exposure data are presented as median (interquartile range), except for total exposure, which is presented as mean (standard deviation).
[a]Neratinib at doses shown plus trastuzumab 4 mg/kg loading then 2 mg/kg weekly (parts I and II) or 6 mg/kg every 3 weeks (extension phase).
[b]Dose intensity is defined as the total exposure divided by the duration of drug administration in weeks.
[c]Relative dose intensity is defined as the actual total exposure divided by the expected total exposure.

Supplemental Table 2 Treatment-emergent Adverse Events (Safety Population)

	Neratinib Plus Trastuzumab[a]							
	All-Grade Events, n (%)				Grade ≥ 3 Events, n (%)			
	Part I		Part II		Part I		Part II	
	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (N = 45)	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (N = 45)
Diarrhea	4 (100)	4 (100)	34 (91.9)	42 (93.3)	1 (25.0)	2 (50.0)	4 (10.8)	7 (15.6)
Nausea	3 (75.0)	3 (75.0)	17 (45.9)	23 (51.1)	0	0	2 (5.4)	2 (4.4)
Decreased appetite	1 (25.0)	2 (50.0)	19 (51.4)	22 (48.9)	0	0	1 (2.7)	1 (2.2)
Vomiting	2 (50.0)	2 (50.0)	14 (37.8)	18 (40.0)	0	0	2 (5.4)	2 (4.4)
Asthenia	0	0	13 (35.1)	13 (28.9)	0	0	0	0
Rash	1 (25.0)	1 (25.0)	8 (21.6)	10 (22.2)	0	0	1 (2.7)	1 (2.2)
Fatigue	1 (25.0)	2 (50.0)	6 (16.2)	9 (20.0)	0	0	1 (2.7)	1 (2.2)
Weight decreased	0	2 (50.0)	7 (18.9)	9 (20.0)	0	0	0	0
Abdominal pain	1 (25.0)	1 (25.0)	7 (18.9)	9 (20.0)	0	0	0	0
Headache	2 (50.0)	1 (25.0)	5 (13.5)	8 (17.8)	0	0	0	0
Dizziness	0	0	5 (13.5)	5 (11.1)	0	0	0	0
Erythema	2 (50.0)	1 (25.0)	2 (5.4)	5 (11.1)	0	0	0	0
Hemoglobin decreased	0	1 (25.0)	4 (10.8)	5 (11.1)	0	0	1 (2.7)	1 (2.2)
Dysuria	0	1 (25.0)	4 (10.8)	5 (11.1)	0	0	0	0
Dyspnea	1 (25.0)	0	4 (10.8)	5 (11.1)	1 (25.0)	0	1 (2.7)	2 (4.4)
Dry skin	1 (25.0)	0	4 (10.8)	5 (11.1)	0	0	0	0
Pyrexia	1 (25.0)	0	4 (10.8)	5 (11.1)	0	0	0	0
Urinary tract infection	0	2 (50.0)	3 (8.1)	5 (11.1)	0	0	0	0
AST increased	0	0	5 (13.5)	5 (11.1)	0	0	2 (5.4)	2 (4.4)

Abbreviation: AST = aspartate aminotransferase.
Note: All-grade events occurring in ≥ 10% of patients.
[a]Neratinib at doses shown plus trastuzumab 4 mg/kg loading then 2 mg/kg weekly (parts I and II) or 6 mg/kg every 3 weeks (extension phase).

Supplemental Table 3 Treatment-related Treatment-emergent Adverse Events (Safety Population)

	Neratinib Plus Trastuzumab[a]							
	All-grade Events, n (%)				Grade 3 Events, n (%)[b]			
	Part I		Part II		Part I		Part II	
	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (N = 45)	160 mg/d (n = 4)	240 mg/d (n = 4)	240 mg/d (n = 37)	Total (N = 45)
Diarrhea	4 (100)	4 (100)	34 (91.9)	42 (93.3)	1 (25.0)	2 (50.0)	4 (10.8)	7 (15.6)
Nausea	2 (50.0)	3 (75.0)	16 (43.2)	21 (46.7)	0	0	2 (5.4)	2 (4.4)
Decreased appetite	0	1 (25.0)	16 (43.2)	17 (37.8)	0	0	1 (2.7)	1 (2.2)
Vomiting	1 (25.0)	1 (25.0)	10 (27.0)	12 (26.7)	0	0	2 (5.4)	2 (4.4)
Asthenia	0	0	11 (29.7)	22 (24.4)	0	0	0	0
Rash	1 (25.0)	1 (25.0)	6 (16.2)	8 (17.8)	0	0	0	0
Fatigue	0	2 (50.0)	3 (8.1)	5 (11.1)	0	0	0	0
Weight decreased	0	0	5 (13.5)	5 (11.1)	0	0	0	0
Dyspepsia	0	0	3 (8.1)	3 (6.7)	0	0	0	0
Ejection fraction decreased	0	0	3 (8.1)	3 (6.7)	0	0	0	0
Rhinorrhea	0	0	3 (8.1)	3 (6.7)	0	0	0	0

Note: Adverse events occurring in $\geq$ 5% of patients.
[a]Neratinib at doses shown plus trastuzumab 4 mg/kg loading then 2 mg/kg weekly (parts I and II) or 6 mg/kg every 3 weeks (extension phase).
[b]No grade 4 treatment-related adverse events.

Supplemental Table 4 Steady-state Neratinib Pharmacokinetic Parameters

Parameter	Neratinib 240 mg/d Plus Trastuzumab (N = 33)
C_{max} (ng/mL)	78.9 ± 34.7
t_{max} (hr)	4.0 (0.0-24.0)
$t_{1/2}$ (hr)	21.22 ± 10.16[a]
AUC_T (ng•hr/mL)	1110 ± 430
AUC (ng•hr/mL)	2160 ± 1070[a]
λ_z (L/hr)	0.0404 ± 0.0195[a]
CL/F (L/hr)	142 ± 73.7[a]
V_z/F (L)	3850 ± 1980[a]

Abbreviations: AUC = total area under the concentration-time curve; AUC_T = area under the concentration-time curve truncated at the last reported concentration at time T; CL/F = apparent oral dose clearance; C_{max} = peak concentration; λ_z = terminal-phase disposition rate constant; $t_{1/2}$ = terminal-phase elimination half-life; t_{max} = time to peak concentration; V_z/F = apparent volume of distribution.
Note: Values are reported as mean ± standard deviation, except for t1/2 which is reported as median (range).
[a]N = 27.