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ORIGINAL REPORT

PIK3CA Mutations Are Associated With Decreased Benefit to Neoadjuvant Human Epidermal Growth Factor Receptor 2–Targeted Therapies in Breast Cancer

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A B S T R A C T

Purpose
We investigated whether mutations in the gene encoding the phosphatidylinositol 3-kinase (PI3K) catalytic subunit (*PIK3CA*) correlates with response to neoadjuvant human epidermal growth factor receptor 2 (HER2) –targeted therapies in patients with breast cancer.

Patients and Methods
Baseline tissue biopsies were available from patients with HER2-positive early breast cancer who were enrolled onto the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial (NeoALTTO). Activating mutations in *PIK3CA* were identified using mass spectrometry–based genotyping.

Results
PIK3CA mutations were identified in 23% of HER2-positive breast tumors, and these mutations were associated with poorer outcome in all of the treatment arms. Patients treated with a combination of trastuzumab and lapatinib who had wild-type *PIK3CA* obtained a total pathologic complete response (pCR) rate of 53.1%, which decreased to 28.6% in patients with tumors that carried *PIK3CA* activating mutations ($P = .012$).

Conclusion
Activating mutations in *PIK3CA* predicted poor pCR in patients with HER2-positive breast cancer treated with neoadjuvant therapies that target HER2. Consequently, the combination of anti-HER2 agents and PI3K inhibitors is being investigated.

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INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) –positive breast cancer is defined by overexpression and/or amplification of the *HER2* gene (also known as *ERBB2*).[1,2] HER2 is a receptor tyrosine kinase that does not bind ligand directly, but instead mediates signaling in concert with other epidermal growth factor receptor family members (heterodimers) or by forming homodimers. Blocking signaling through the receptor complex, either with monoclonal antibodies that bind the receptor at the cell surface[3-5] or with small-molecule drugs that inhibit kinase activity directly,[6,7] is associated with significant clinical benefit, and these agents have become standard of care for treating patients with HER2-positive breast cancer.

The phosphatidylinositol 3-kinase (PI3K) pathway sits at the nexus of cellular growth and metabolic signaling downstream for HER2 and is frequently aberrantly activated in cancer.[8] The gene encoding the catalytic component p110α, *PIK3CA*, is one of the most frequently mutated genes in breast cancer,[9] emphasizing the importance of this pathway for regulating growth in breast malignancies. Although *PIK3CA* activating mutations are present in all breast cancer subtypes, they are enriched in estrogen receptor (ER) –positive and HER2-positive disease and are less common in basal cancers. Although the role of this pathway in driving cellular transformation is well defined, knowledge regarding the influence of PI3K on the biology of disease and its role in governing therapy response in patients remains limited.

The PI3K pathway is a critical mediator of signaling downstream of receptor tyrosine kinases and has been implicated in mediating resistance to therapies that target HER2. Activating mutations in *PIK3CA* and loss of *PTEN*, which is a key negative regulator of PI3K signaling, drive resistance to both trastuzumab and lapatinib in breast cancer cell lines, and low levels of *PTEN* have been associated with worse outcome in patients.[10-12]

In this study, we assessed the correlation of *PIK3CA* mutation status with therapy response measured by pathologic complete response (pCR), event-free survival (EFS), and overall survival (OS) in 355 patients with HER2-positive breast cancer who had been recruited to the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) trial (Breast International Group 1-06), a randomized, multicenter, open-label, neoadjuvant, phase III trial designed to assess the efficacy of dual inhibition of HER2.[13]

PATIENTS AND METHODS

Patient Population and Samples

NeoALTTO, a phase III parallel-group, open-label, randomized, neoadjuvant study of trastuzumab, lapatinib, or their combination, included patients with newly diagnosed HER2-positive invasive breast cancer amenable to surgery. Full eligibility criteria can be accessed elsewhere.[13] Patients received anti-HER2 therapy for 6 weeks, and paclitaxel was then added to the regimen for a further 12-week period until definitive surgery, for a total period of 18 weeks of anti-HER2 therapy. The study primary end point was the rate of pCR in the breast. Secondary end points included rate of locoregional total pCR, EFS, and OS. Core biopsies were obtained before treatment (baseline), at 2 weeks on treatment, and at the time of surgery. Here, we report the results on baseline biopsies. Recently, EFS and OS were reported at a median clinical follow-up of 3.8 years.[14]

Laboratory Methods (sample processing and genotyping)

Baseline frozen biopsies were embedded in frozen tissue matrix (OCT; Sakura Finetek, Torrance, CA) and cut at the cryostat for tumor cellularity assessment by a pathologist. Genomic DNA was isolated from 20 × 10 μm sections using the DNeasy Kit (Qiagen, Hilden, Germany). The Sequenom (San Diego, CA) mass-spectrometry genotyping system was used to test for hotspot mutations in *PIK3CA* (E542A/K, E545A/K, and H1047R/L), *AKT1* (E17K), *KRAS* (G12A/C/D/V and G13D), and *BRAF* (V600E). All polymerase chain reaction (PCR) primers were designed using the Sequenom online assay design system and are listed in Appendix Table A1 (online only). PCR reactions were prepared with the iPLEX-Gold PCR reaction mix (Sequenom) with 15 ng of input DNA. The PCR, shrimp alkaline phosphatase treatment, and extension reaction were performed according to the manufacturer's protocol (Sequenom). The results were analyzed using MassArrayTyper v 4.0.22.67 (Sequenom) using both automated analysis and visual inspection. Confirmation of kinase mutations was performed by next-generation sequencing on an Illumina Hiseq2000 (Illumina, San Diego, CA) with a paired-end 51 base protocol as described.[15] DNA enrichment was performed with the human kinome DNA capture baits (Agilent, Santa Clara, CA).

Statistical Analysis

The differences in pCR rates by *PIK3CA* mutation status were calculated with a corresponding 95% CI and tested using a χ^2 test of association. A logistic regression model was fitted to pCR and included ER status and treatment arm, as well as *PIK3CA* mutation status. Another model was fitted adding an interaction term for *PIK3CA* and treatment arm to assess whether the proportions of patients with pCR who have the *PIK3CA* mutation are statistically different between the treatment arms. As a hypothesis-generating, exploratory analysis, the treatment effect was further examined by performing χ^2 tests of association on pCR and *PIK3CA* mutation status for each treatment arm separately.

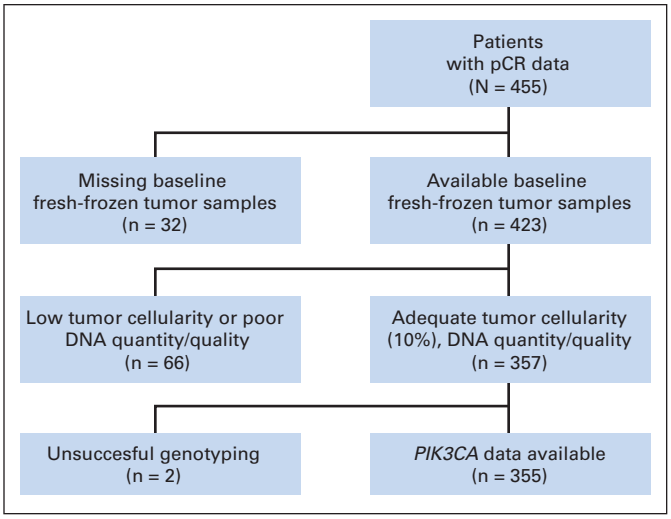

Fig 1. Tumor sample disposition for analysis of *PIK3CA* mutation. pCR, pathologic complete response.

The number and percentage of EFS and OS events by *PIK3CA* mutation status were calculated. Cox regression models were fitted to EFS and OS with treatment arm, hormone receptor status, and *PIK3CA* mutation status. Interaction tests were performed for treatment arm and *PIK3CA* mutation status.

RESULTS

Study Design

To study the effect of *PIK3CA* mutations on the response to HER2-targeted therapies in breast cancer, we used tumor biopsies from the NeoALTTO trial.[13] pCR data were available for 455 patients with HER2-positive breast cancer enrolled onto the NeoALTTO trial, of whom 423 (93%) had baseline fresh-frozen tumor samples; a total of 355 samples (78%) underwent successful *PIK3CA* genotyping (Fig 1).

Somatic Mutation Profiling

For assessment of the impact of *PIK3CA* mutation on treatment outcome, we analyzed DNA from baseline samples. Samples with less than 10% tumor material or that produced low DNA yield were excluded from the analysis, which left a total of 357 samples (78.5%) for genotyping (Fig 1). Genotypes for hotspot mutations in *PIK3CA*, *AKT1*, *KRAS*, and *BRAF* were obtained for 355 samples (78%; Fig 1). As shown in Table 1, this subset of patients is representative of the whole NeoALTTO population.

No activating mutations were detected in *AKT1* or *BRAF*, and only a single activating mutation in *KRAS* (0.3%) was identified in the entire cohort. *PIK3CA* mutations were detected in 80 samples (23%), which included nine in codon 542 (2.5%), 13 in codon 545 (3.7%), and 58 in codon 1047 (16.3%). The overall proportion of helical and kinase domain mutations was consistent with those observed in breast cancers in the Catalogue of Somatic Mutations in Cancer (Appendix Table A2, online only). The *PIK3CA* mutation frequency was similar in each treatment arm (23% in lapatinib arm, n = 124; 19% in trastuzumab arm, n = 112; and 25% in combination arm, n = 119) and was not influenced by ER status (23% in ER-positive patients, n = 169; and 22% in ER-negative patients, n = 186; Appendix Table A3, online only).

Table 1. Patient Clinical Characteristics

Characteristic	NeoALTTO (N = 455)		NeoALTTO *PIK3CA* Subset (n = 355)		NeoALTTO Patients Not in *PIK3CA* Subset (n = 100)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
ER status						
Negative	239	52.5	186	52.4	53	53.0
Positive	216	47.6	169	47.6	47	47.0
pCR						
Yes	138	31.4	108	31.6	30	30.6
No	302	68.6	234	68.4	68	69.4
Missing	15		13		2	
Treatment						
Lapatinib	154	33.8	124	34.9	30	30.0
Trastuzumab	149	32.7	112	31.6	37	37.0
Lapatinib + trastuzumab	152	33.4	119	33.5	33	33.0
Grade						
1	12	2.6	8	2.2	4	4.0
2	172	37.8	127	35.8	45	45.0
3	205	45.1	170	47.9	35	35.0
X	65	14.3	49	13.8	16	16.0
Unknown	1	0.2	1	0.3	0	0.0
Tumor size, cm						
≤ 5	274	60.2	212	59.7	62	62.0
> 5	181	39.8	143	40.3	38	38.0
Age, years						
< 65	403	88.6	321	90.4	82	82.0
≥ 65	52	11.4	34	9.6	18	18.0
N stage						
N0	123	27.0	94	26.5	29	29.0
N1	260	57.1	196	55.2	64	64.0
N2a	44	9.7	40	11.3	4	4.0
N3a	3	0.7	2	0.6	1	1.0
N3b	7	1.5	7	2.0	0	0.0
N3c	12	2.6	11	3.1	1	1.0
Nx	6	1.3	5	1.4	1	1.0

Abbreviations: ER, estrogen receptor; NeoALTTO, Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization; pCR, pathologic complete response.

To verify the Sequenom genotyping result, *PIK3CA* mutation status was assessed in a random selection of 96 samples using next-generation sequencing with capture of the relevant exons of the genes studied here. The presence of the *PIK3CA* mutation was confirmed in 56 (96.6%) of 58 samples. The signal intensity observed in the Sequenom assay was strongly correlated with the mutation frequency observed by sequencing ($r = 0.944$).

PIK3CA *Mutation Status and pCR*

We analyzed the correlation between tumor *PIK3CA* mutation status and locoregional total pCR, as defined by no invasive cancer in the breast and no pathologic involvement of axillary nodes. Patients with *PIK3CA* mutations were less likely to have a pCR. The rate of pCR decreased from 34.5% (92 of 267 patients) in wild-type patients to 21.3% (16 of 75 patients) in patients who carried *PIK3CA* mutations (Fig 2A). The difference was 13.1% (95% CI, 2.2% to 24.0%; $P = .03$ for the χ^2 test of association). Likewise, there was also a lower breast pCR, defined as absence of invasive tumor cells in the breast, in tumors with *PIK3CA* mutations (Fig 2A). Patients with *PIK3CA* mutations had a lower pCR rate independent of ER status (Fig 2B, Appendix Table A4, online only).

For each treatment arm, the pCR rate was lower for patients with *PIK3CA* mutations than for wild-type patients, although this difference was largest in the combination (lapatinib plus trastuzumab) treatment arm (Fig 3, Appendix Table A5, online only). The correlation between breast pCR and total pR is shown in Appendix Table A6 (online only). The logistic regression model is summarized in Table 2, showing that *PIK3CA* status, ER status, and treatment arm are all statistically significant. The interaction test for treatment arm and *PIK3CA* was not statistically significant ($P = .5$), but individual tests by treatment arm found significant differences in the combination arm ($P = .012$), but not for lapatinib only ($P = .51$) or trastuzumab only ($P = .44$). However, the test for interaction to demonstrate a significant value of the mutant to predict a better response to dual inhibition compared with the response to the single agents is underpowered.

PIK3CA *Mutation Status and EFS and OS*

Using a simplified summary of EFS, we note that 15 patients (18.7%) with *PIK3CA* mutations and 64 patients (23.3%) with normal *PIK3CA* had EFS events. The Cox model of EFS, including treatment arm, ER status, and *PIK3CA* status, found that EFS does not seem to be affected by *PIK3CA* status (hazard ratio [HR], 0.78; 95%

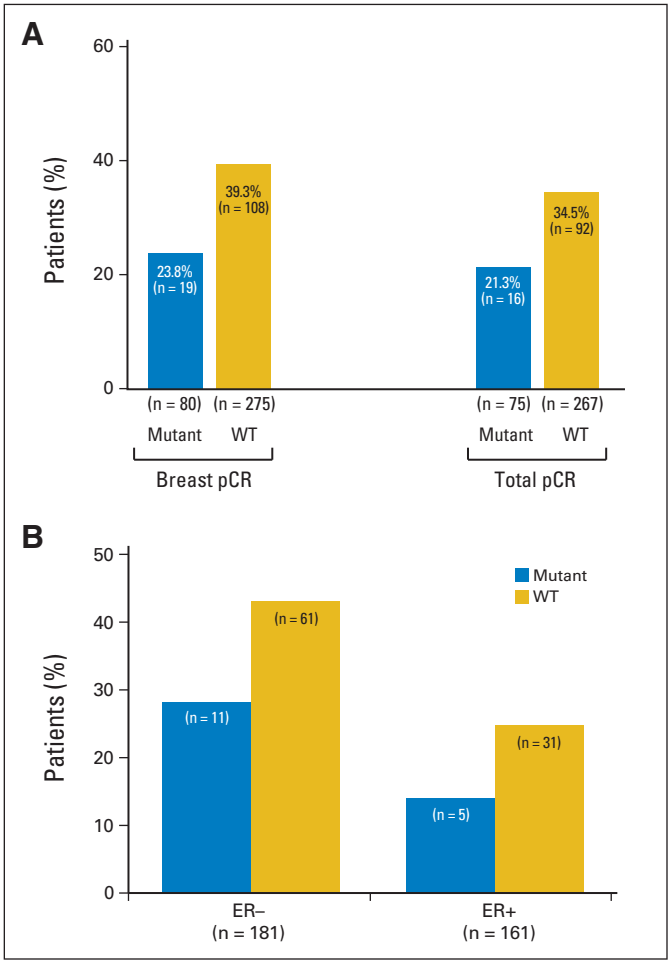

Fig 2. Influence of *PIK3CA* mutation and estrogen receptor (ER) status on pathologic complete response (pCR). (A) The proportion of patients who obtained breast and locoregional total pCR is shown for the entire patient cohort (gold, *PIK3CA* wild type [WT]; blue, *PIK3CA* mutant). (B) Total pCR rates for ER-negative and ER-positive tumors separately (gold, *PIK3CA* WT; blue, *PIK3CA* mutant). Patients were further subdivided based on ER status (negative or positive). In each of the bar graphs, the number of patients is being displayed. The total number of patients with available pCR data in each group is shown.

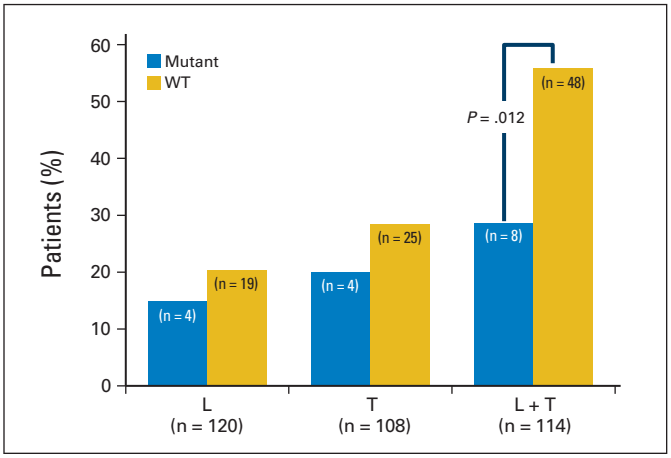

Fig 3. Influence of *PIK3CA* mutation on pathologic complete response (pCR) by treatment arm. The proportion of patients who obtained total pCR is shown for each treatment arm, separated based on the *PIK3CA* mutation status. The total number of patients in each group is shown (gold, *PIK3CA* wild type [WT]; blue, *PIK3CA* mutant). In each of the bar graphs the number of patients is being displayed. The total number of patients with available pCR data in each group is shown. L, lapatinib; T, trastuzumab.

CI, 0.44 to 1.36; $P = .38$). A model testing the interaction term was nonsignificant ($P = .27$).

The analysis of OS events found that five patients (6.2%) with *PIK3CA* mutations had died compared with 35 patients (12.7%) with normal *PIK3CA*. As with EFS, the Cox model for OS found that OS does not seem to be affected by *PIK3CA* status (HR, 0.50; 95% CI, 0.19 to 1.23; $P = .14$). A model fitted to test a possible interaction between treatment arm and *PIK3CA* status was nonsignificant ($P = .97$).

DISCUSSION

In HER2-positive breast cancer, a recent meta-analysis of published neoadjuvant studies has shown a relationship between pCR and long-term outcome regardless of hormone receptor status (EFS: HR, 0.39; OS: HR, 0.34), with a stronger association in the hormone receptor–negative subgroup (EFS: HR, 0.25; OS: HR, 0.19).[16] Therefore, neoadjuvant studies have been proposed to be suited to test biologic hypotheses in early HER-positive breast cancer.

In this study, we find that mutations in *PIK3CA* downstream of HER2 correlate with a lower response to HER2-targeted neoadjuvant therapy in breast cancer as measured by pCR. There is no significant difference for *PIK3CA* mutation status in survival follow-up (EFS and OS).

The finding that activating mutations in components of a signaling pathway downstream of a receptor tyrosine kinase are associated with reduced response to inhibitors of the receptor is not without precedent. Indeed, in colon cancer, the effectiveness of anti–epidermal growth factor receptor antibody drugs is limited by activating mutations in receptor downstream *RAS* genes.[17,18] In the case of the closely related HER2 receptor, in vitro studies with HER2-targeted drugs in HER2-positive breast cancer have already provided preclinical evidence for a role of PI3K mutations in mediating resistance to HER2 therapy.[10,12,19] Our present data may provide clinical support for these preclinical findings by suggesting that in HER2-positive tumors, the presence of *PIK3CA* mutations results in lower clinical benefit from trastuzumab, lapatinib, and especially dual HER2 blockade, as measured by pCR. Our findings are supported by similar reports from clinical trials in the advanced disease setting. In the phase III CLEOPATRA trial, the effects of the anti-HER2 monoclonal antibody pertuzumab, which prevents the formation of HER2-HER3 heterodimers, were studied when added to trastuzumab as first-line treatment for patients with HER2-positive metastatic breast

Table 2. Logistic Regression Model Results for pCR

Variable	Odds Ratio	95% CI	*P*
PIK3CA (mutant *v* WT)	0.45	0.23 to 0.85	.015
ER status (negative *v* positive)	2.44	1.45 to 4.03	< .001
Treatment arm			< .001
L versus T	0.67	0.36 to 1.27	
L + T versus T	2.97	1.66 to 5.33	

Abbreviations: ER, estrogen receptor; L, lapatinib; pCR, pathologic complete response; T, trastuzumab; WT, wild type.

cancer. This study compared the efficacy of pertuzumab, trastuzumab, and docetaxel versus placebo, trastuzumab, and docetaxel. As in NeoALTTO, this study demonstrated the superiority of dual HER2 blockade. The longest progression-free survival (PFS) was observed in patients whose tumors expressed wild-type versus mutated *PIK3CA* in both the placebo, trastuzumab, and docetaxel group (13.8 *v* 8.6 months, respectively) and pertuzumab, trastuzumab, and docetaxel group (21.8 *v* 12.5 months, respectively).[20] Overall, patients with tumors with wild-type *PIK3CA* had a better outcome to therapy than those with *PIK3CA* mutations (HR, 0.63; $P < .001$).[20]

In a recently reported analysis of the neoadjuvant GeparSixto study of chemotherapy and dual HER2 blockade with trastuzumab and lapatinib, patients with *PIK3CA*-mutant tumors also had a significantly lower pCR rate compared with the wild-type population (17.8% *v* 36.8%, respectively; $P = .015$).[21] Similarly, in the EMILIA trial, a phase III study that compared lapatinib and capecitabine versus the antibody-drug conjugate T-DM1, patients with tumors harboring a *PIK3CA* mutation had a shorter PFS with lapatinib when compared with patients with wild-type tumors (4.3 *v* 6.4 months, respectively). Interestingly, for patients treated with T-DM1, an antibody-drug conjugate that contains trastuzumab attached to a potent chemotherapeutic agent, PFS was unaffected by *PIK3CA* mutation status.[22] The only published report that failed to find a correlation between *PIK3CA* status and response to therapy with trastuzumab was the adjuvant FinHER study, in which HER2-positive patients were randomly assigned to receive 9 weeks of trastuzumab or no trastuzumab.[23] However, the study was small, with only a total of 157 patients with HER2-positive disease with available tumor samples and 34 patients (19.3%) with *PIK3CA* mutations. Furthermore they reported on distant disease-free survival despite only 9 weeks therapy with trastuzumab.

Although the number of events was too small to reach statistical significance, we did not observe worse EFS and OS in *PIK3CA*-mutant tumors. In this regard, it may be complex to dissect out the potential effect of HER2 therapy in *PIK3CA*-mutant tumors from the prognostic implications of *PI3KCA* mutations in breast cancer in the absence of a control arm without HER2 therapy. Indeed, *PIK3CA* mutations have been associated with improved outcome, including OS and breast cancer–specific survival.[24,25] Although there are limited data, *PIK3CA* mutations may also confer an improved prognosis in HER2-positive tumors. For example, out of the 760 breast cancer samples from the Cancer Genome Atlas, 104 tumors (14%) had genomic amplification of *HER2*. In this subset of 104 HER2-positive tumors, 26 tumors (25%) had a known activating mutation in *PIK3CA*. The OS of the *PIK3CA*-mutant tumors was better than that of the *PIK3CA* wild-type tumors, although the data were not statistically significant (log-rank $P = .1561$).[26]

In *HER2*-amplified and *PIK3CA*-mutant breast cancer models, the observed resistance against HER2 therapies is reverted in full by the addition of PI3K inhibitors.[10,12] Building from these observations, a number of clinical trials combining anti-HER2 agents with pan-PI3K inhibitors are under way, and clinical activity has already been reported in patients with trastuzumab-resistant disease.[27,28] More recently, a new class of PI3K inhibitors that have a selective effect on the p110α isoform of PI3K, the product of *PIK3CA*, has entered the clinic. These PI3Kα-specific inhibitors are more potent and have been shown to be clinically active in *PIK3CA*-mutant breast tumors.[29,30] Given the strong scientific rationale and the emerging clinical observations that *PIK3CA*-mutant tumors respond less to HER2 therapies, a strong case can be made to study these combinations in the clinic.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Manuscript writing: All authors
Final approval of manuscript: All authors

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Appendix

Table A1. Sequenom Genotyping Primer Sequences

Oligo Sequence (5′ to 3′)	Oligo Name	Identifier	Reaction
ACGTTGGATGTCTTCATGAAGACCTCACAG	*BRAF*-V600E-F	rs113488022	W-1
ACGTTGGATGTTCAAACTGATGGGACCCAC	*BRAF*-V600E-R		W-1
CCCACTCCATCGAGATTTC	*BRAF*-V600E-D		
ACGTTGGATGTAGAGTGTGCGTGGCTCTCA	*AKT1*-E17K-F	rs121434592	W-1
ACGTTGGATGTTCTTGAGGAGGAAGTAGCG	*AKT1*-E17K-R		W-1
CGCCAGGTCTTGATGTACT	*AKT1*-E17K-D		
ACGTTGGATGTCCATTTTTGTTGTCCAGCC	*PIK3CA*-H1047R/L-F	rs121913279	W-1
ACGTTGGATGAACTGAGCAAGAGGCTTTGG	*PIK3CA*-H1047R/L-R		W-1
GAAACAAATGAATGATGCAC	*PIK3CA*-H1047R/L-D		
ACGTTGGATGAGGCCTGCTGAAAATGACTG	*KRAS*-G13D-F	rs112445441	W-1
ACGTTGGATGTAGCTGTATCGTCAAGGCAC	*KRAS*-G13D-R		W-1
GTCAAGGCACTCTTGCCTACG	*KRAS*-G13D-D		
ACGTTGGATGTACACGAGATCCTCTCTCTG	*PIK3CA*-E545A-F	rs121913274	W-1
ACGTTGGATGTAGCACTTACCTGTGACTCC	*PIK3CA*-E545A-R		W-1
TCCATAGAAAATCTTTCTCCTGC	*PIK3CA*-E545A-D		
ACGTTGGATGAGGCCTGCTGAAAATGACTG	*KRAS*-G12C-F	rs121913530	W-2
ACGTTGGATGTAGCTGTATCGTCAAGGCAC	*KRAS*-G12C-R		W-2
ACTCTTGCCTACGCCAC	*KRAS*-G12C-D		
ACGTTGGATGTAGCACTTACCTGTGACTCC	*PIK3CA*-E542A-F	PIK3CA E542A	W-2
ACGTTGGATGGCAATTTCTACACGAGATCC	*PIK3CA*-E542A-R		W-2
ACGAGATCCTCTCTCTG	*PIK3CA*-E542A-D		
ACGTTGGATGTAGCACTTACCTGTGACTCC	*PIK3CA*-E545K-F	rs104886003	W-3
ACGTTGGATGTACACGAGATCCTCTCTCTG	*PIK3CA*-E545K-R		W-3
CCTCTCTCTGAAATCACT	*PIK3CA*-E545K-D		
ACGTTGGATGTAGCTGTATCGTCAAGGCAC	*KRAS*-G12*-F	rs121913529	W-3
ACGTTGGATGAGGCCTGCTGAAAATGACTG	*KRAS*-G12*-R		W-3
CTTGTGGTAGTTGGAGCTG	*KRAS*-G12*-D		
ACGTTGGATGGCAATTTCTACACGAGATCC	*PIK3CA*-E542K-F	rs121913273	W-4
ACGTTGGATGTAGCACTTACCTGTGACTCC	*PIK3CA*-E542K-R		
CTCCTGCTCAGTGATTTC	*PIK3CA*-E542K-D		

NOTE. Sequences are included for the forward, reverse, and detection primers. Genotyping was performed in four separate multiplexed reactions (W-1 to W-4).
Abbreviations: D, detection; F, forward; R, reverse.
*Assay measures multiple amino acid changes at that position.

Table A2. *PIK3CA* Genotyping Results Compared With COSMIC Data Set

	NeoALTTO		COSMIC	
Site	No. of Cancers	%	No. of Cancers	%
PIK3CA E542	9	2.5	227	2.5
PIK3CA E545	13	3.7	437	4.8
PIK3CA H1047	58	16.3	1,423	16.3

Abbreviations: COSMIC, Catalogue of Somatic Mutations in Cancer; NeoALTTO, Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization.

Table A3. *PIK3CA* Genotyping Results by ER Status and Treatment Arm

Patient Group	Total No. of Patients	*PIK3CA* Mutant	
		No. of Patients	%
ER status			
Negative	186	41	22.0
Positive	169	39	23.1
Treatment arm			
Lapatinib	124	29	23.4
Trastuzumab	112	21	18.8
Lapatinib + trastuzumab	119	30	25.2

Abbreviation: ER, estrogen receptor.

Table A4. *PIK3CA* Mutation Status and pCR by Hormone Receptor Status

PIK3CA Status	Total pCR			
	No		Yes	
	No. of Patients	%	No. of Patients	%
ER negative				
Mutant	28	71.8	11	28.2
WT	81	57.0	61	43.0
ER positive				
Mutant	31	86.1	5	13.9
WT	94	75.2	31	24.8

Abbreviations: ER, estrogen receptor; pCR, pathologic complete response; WT, wild type.

Table A5. *PIK3CA* Mutation Status and pCR by Treatment Group

Treatment and *PIK3CA* Status	Total pCR			
	No		Yes	
	No. of Patients	%	No. of Patients	%
Lapatinib				
Mutant	23	85.2	4	14.8
WT	74	79.6	19	20.4
Trastuzumab				
Mutant	16	80.0	4	20.0
WT	63	71.6	25	28.4
Lapatinib + trastuzumab				
Mutant	20	71.4	8	28.6
WT	38	44.2	48	55.8

Abbreviations: pCR, pathologic complete response; WT, wild type.

Table A6. Breast pCR and Total pCR

Total pCR	Breast pCR (No.)	
	pCR	No pCR
pCR	108	0
No pCR	13	221
Missing	6	7

Abbreviation: pCR, pathologic complete response.