

PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab

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Background: This study was conducted to determine the frequency of *PIK3CA* mutations and human epidermal growth factor receptor-2 (HER2) phosphorylation status (pHER2-Tyr^{1221/1222}) and if *PIK3CA*, phosphatase and tensin homolog (PTEN), or pHER2 has an impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab.

Patients and methods: Two hundred and forty HER2-positive early-stage breast cancer patients receiving adjuvant treatment (cyclophosphamide 600 mg/m², epirubicin 60 mg/m², and fluorouracil 600 mg/m²) before administration of 1 year trastuzumab were assessable. PTEN and pHER2 expression were assessed by immunohistochemistry.

PIK3CA mutations (exons 9 and 20) were determined by pyrosequencing.

Results: Five-year overall survival (OS) and invasive disease-free survival were 87.8% and 81.0%, respectively. Twenty-six percent of patients had a *PIK3CA* mutation, 24% were PTEN low, 45% pHER2 high, and 47% patients had increased PI3K pathway activation (PTEN low and/or *PIK3CA* mutation). No significant correlations were observed between the clinicopathological variables and *PIK3CA*, PTEN, and pHER2 status. In both univariate and multivariate analyses, patients with *PIK3CA* mutations or high PI3K pathway activity had a significant worse OS [multivariate: hazard ratio (HR) 2.14, 95% confidence interval (CI) 1.01–4.51, *P* = 0.046; and HR 2.35, 95% CI 1.10–5.04, *P* = 0.03].

Conclusion: Patients with *PIK3CA* mutations or increased PI3K pathway activity had a significantly poorer survival despite adequate treatment with adjuvant chemotherapy and trastuzumab.

Key words: HER2-positive early-stage breast cancer, pHER2, *PIK3CA* mutations, PI3K activity, PTEN, trastuzumab resistance

introduction

Breast cancer is a heterogeneous disease with distinct molecular characteristics that have an impact on disease outcome, predict response to targeted treatment, and may influence resistance mechanisms. Aberrations in members of the phosphatidylinositol 3-kinase (PI3K) pathway are common in breast cancer as overexpression of human epidermal growth factor receptor-2 (HER2), loss of phosphatase and tensin homolog (PTEN), and *PIK3CA* mutations frequently occur [1]. Overexpression of HER2 is identified in ~20% of early-stage invasive breast carcinomas [2] and induces enhanced cell signaling involving particularly the PI3K pathway [3]. HER2

overexpression is associated with a more aggressive phenotype and poor prognosis [4, 5]. Mutations in the *PIK3CA* gene, encoding the p110 α catalytic subunit of PI3K, have been reported in 20%–40% of primary breast cancers [6–14]. These somatic mutations located in hotspots in exons 9 and 20 are functionally activators, resulting in increased phosphorylated Akt and downstream signaling [15–17]. The tumor suppressor gene PTEN is a key negative regulator of the PI3K pathway as it antagonizes the effects of PI3K leading to decreased Akt activity [18].

Trastuzumab, a humanized mAb directed against the extracellular domain of HER2, has shown efficacy in HER2-positive breast cancer both in the adjuvant and advanced disease settings [19–22]. Several large trials showed that the addition of trastuzumab to chemotherapy in early-stage HER2-positive breast cancer significantly improved disease-free survival (DFS) [20, 21, 23–27] and overall survival (OS) [21, 23,

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25, 27]. However, many HER2-positive cancers exhibit *de novo* or develop acquired resistance [19, 28]. Approximately half of the patients with metastatic disease show up-front resistance to trastuzumab-based therapy [22, 29], and the majority of patients develop progressive disease within 1 year of treatment initiation [19, 22, 29]. In the adjuvant setting, some patients develop recurrent breast cancer despite adjuvant trastuzumab, chemotherapy, and endocrine therapy [21, 23, 25].

Potential mechanisms of trastuzumab resistance [30] include increased signaling via the PI3K pathway due to either *PIK3CA* mutations or PTEN loss of function, which eliminates the effects of upstream HER2 inhibition. Data from *in vitro* and *in vivo* studies suggest that mutations in the *PIK3CA* gene [3, 31–33] or loss of PTEN function [31–37] confer resistance to trastuzumab.

The activation status of HER2 as phosphorylated human epidermal growth factor receptor-2 (pHER2) indicates active pathway signaling [38], and may be more biologically relevant than expression of total protein, as it reflects functional activity of the receptor [39–41]. A subset of HER2-positive patients display activation (phosphorylation of Tyr^{1221/1222} or Tyr¹²⁴⁸) of the HER2 receptor (pHER2) when assessed by immunohistochemistry (IHC) [38, 40–43]. pHER2 is associated with poor prognosis [38, 40, 44] and may confer increased sensitivity toward trastuzumab-targeted therapy [42, 43, 45].

The objectives of this study were to determine the frequency of *PIK3CA* mutations and HER2 phosphorylation status (pHER2-Tyr^{1221/1222}) and if *PIK3CA*, PTEN, or pHER2 had an impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab.

patients and methods

Patients enrolled in the Danish Breast Cancer Cooperative Group (DBCG) 05H protocol were included in the study. The DBCG05H protocol preceded administration of trastuzumab as a standard adjuvant treatment recommended by DBCG in 2007 [46]. Patients were eligible if they had undergone intended curative surgery for HER2-positive early-stage breast cancer and had a histologically verified diagnosis of primary invasive breast cancer. Patients had completed a minimum four series of adjuvant chemotherapy before trastuzumab administration. HER2-positive status was defined as immunohistochemical staining for HER2 of 3+ and/or FISH positive (ratio >2). Other requirements were high-risk disease according to DBCG guidelines, age <70 years, performance status of less than 2, and normal cardiac function.

Figure 1 shows the availability of patients and test materials. According to DBCG guidelines, seven cycles of CEF (cyclophosphamide 600 mg/m², epirubicin 60 mg/m², and fluorouracil 600 mg/m²) were recommended before trastuzumab administration. Dates of administration of first trastuzumab treatment ranged from 11 January 2006 to 1 February 2008. Trastuzumab was administered as an initial loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks, totaling 17 series. Thereafter, patients were followed every half year for 4 years and the following 5 years once a year with clinical examination. Patients with hormone receptor-positive disease further received adjuvant antihormonal therapy (tamoxifen and/or aromatase inhibitor) after chemotherapy.

immunohistochemistry

From each formalin-fixed paraffin-embedded (FFPE) tissue block, hematoxylin–eosin (H&E) sections were made to confirm the diagnosis of invasive breast carcinoma. H&E-guided three 2-mm cores were sampled,

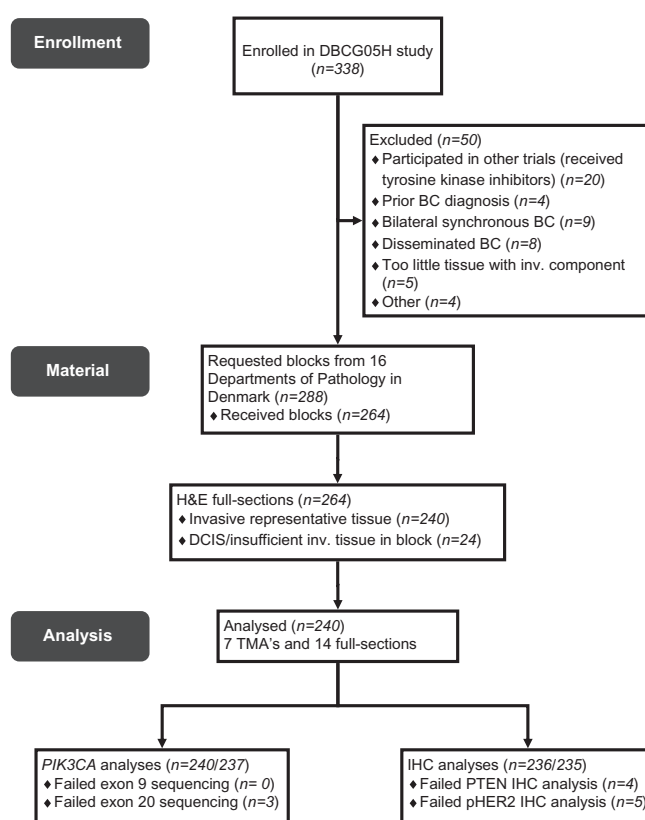


Figure 1. Flow diagram illustrating the patient material available. BC, breast cancer.

one for DNA extraction and two for tissue microarray (TMA) preparation. IHC stainings were manually scored (bright field microscopy) by an experienced breast pathologist (AVL) blinded to clinical outcome and mutation status. TMA and full sections (4 µm) were stained with mouse monoclonal anti-PTEN (clone 6H2.1; Dako, Glostrup, Denmark) and rabbit monoclonal anti-pHER2-Tyr^{1221/1222} (clone 6B12; Cell Signaling Technology, Danvers, MA; for further details, see supplemental data and Table S1, available at *Annals of Oncology* online), based on previously published protocols [38].

PTEN cytoplasmic staining was scored semiquantitatively based on staining intensity (0–3) and percentage of stained cells (0–100) using the histoscore (H-score; range 0–300) as described previously [47]. When scoring PTEN, the expression was compared with surrounding normal tissue (generally present on the 2-mm TMA). PTEN cut-off corresponding to the lower quartile (PTEN low, H-score ≤40) was chosen. pHER2 membrane staining was scored in accordance with the HercepTest™ guidelines, as described previously [38]. Consequently, a positive membrane staining for pHER2 of 2+ and 3+ was considered as pHER2 overexpression [38].

mutational analysis

Genomic DNA was extracted from either 2-mm cores from FFPE tissue blocks or from full sections and DNA purified using the QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. The most common *PIK3CA* mutations in exons 9 and 20 were analyzed by newly developed pyrosequencing assays on a PyroMark Q24 instrument (QiagenVenlo, Netherlands; for further details, see supplemental data, available at *Annals of Oncology* online). A subset of randomly selected samples ($n = 15$) were also sequenced by direct dideoxynucleotide sequencing to validate the pyrosequencing results. Full agreement of the results was observed with the two methods (Figure 2).

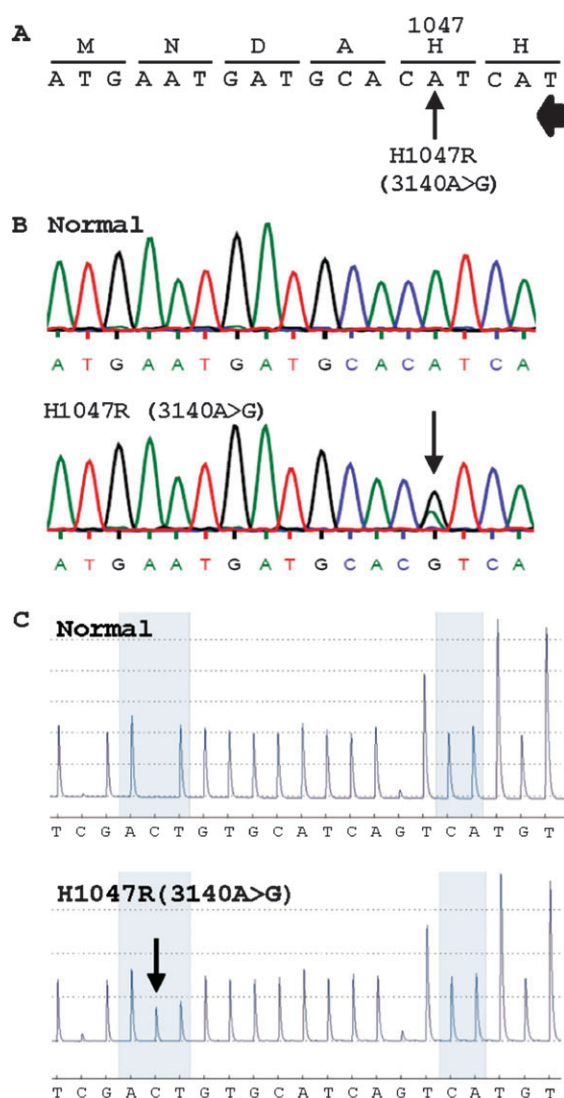


Figure 2. *PIK3CA* mutational analysis exemplified by H1047R (3140A>G) mutation. (A) Nucleotide and amino acid sequence of the *PIK3CA* exon 20 H1047R/L hotspot. Thin arrow indicates the common H1047R (3140A>G) substitution and bold arrow indicates the 3' end of sequencing primer used in the pyrosequencing reaction (in reverse orientation; C). (B and C) Analysis of one wild-type individual and one heterozygous H1047R (3140A>G) mutated individual by dideoxynucleotide sequencing (B) and pyrosequencing (C).

statistical analysis

Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up [48]. Associations between *PIK3CA*, PTEN, and pHER2 status and clinicopathological variables were assessed by χ^2 test. Invasive disease-free survival (IDFS) was defined as the time elapsed from surgery until invasive breast cancer recurrence irrespective of localization, new invasive breast cancer involving the same or the contralateral breast, second primary non-breast invasive cancer, or death of any cause [49]. Recurrence-free survival (RFS) was defined as the time from surgery to invasive breast cancer recurrence irrespective of localization or death of any cause. OS was defined as the time elapsed from surgery until death of any cause [49]. IDFS and OS rates were estimated according to the Kaplan–Meier method, and univariate comparisons between groups were made using the log-rank test. The effects of PTEN, pHER2, *PIK3CA* mutations, and PI3K pathway

activity on IDFS, RFS, and OS were quantified in terms of hazard ratios (HRs), estimated unadjusted and adjusted using the Cox proportional hazards model. The effects of menopausal status, histological type, grade, lymphovascular invasion, and receptor status were not statistically significant, and these factors were not included in the multivariate model. Tumor size (cm) and nodal status (node negative versus one to three positive nodes versus more than three positive nodes) retained significance and were included in the multivariate models. The effects of PTEN, pHER2, *PIK3CA* mutations, and PI3K pathway activity were included one at a time in separate models. The assumption of proportional hazards was assessed by Schoenfeld residuals. All tests were two tailed and $P \leq 0.05$ was considered significant. Statistical analyses were carried out with SAS v. 9.2.

The data used and presented were collected and analyzed by the DBCG Data Center. The study was approved by the Ethical Committee of Region Southern Denmark.

results

patient characteristics

Among the 240 patients assessable for biomarker analysis (Figure 1), 31 had died, and 48 IDFS events had been recorded. IDFS events comprised 39 patients with distant breast cancer recurrence, 2 patients with locoregional breast cancer recurrence, 3 patients diagnosed with a new invasive contralateral breast cancer, 3 patients diagnosed with a second primary non-breast invasive cancer, and 1 patient died as first event.

Five-year OS and IDFS were 87.8% [95% confidence interval (CI) 82.8–91.4] and 81.0% (95% CI 75.4–85.5), respectively. Median estimated potential follow-up was 5.6 years.

All patients had a minimum of four cycles of chemotherapy before trastuzumab administration. Two hundred and fifteen patients received minimum seven cycles of CEF. All patients received EC (epirubicin and cyclophosphamide), and only five patients did not receive fluorouracil. Nine patients received docetaxel-containing therapy (sequentially after EC/CEF). Thirty-nine patients (16%) received <17 series of trastuzumab, of whom 16 had breast cancer recurrence during therapy, 5 stopped treatment due to toxicity, 3 according to patient wish, and 15 for unknown reasons.

Of the 240 patients, 240 (237 for exon 20), 236, and 235 patients were assessable for *PIK3CA*, PTEN, and pHER2 status, respectively. Twenty-one patients [21 of 240 (9%)] had *PIK3CA* exon 9 mutations (11 E542K, 9 E545K, and 1 Q536K), and 40 of 237 (17%) had exon 20 mutations (34 H1047R and 6 H1047L). No patients had double mutations; therefore, a total of 61 (26%) patients had an exon 9 or 20 mutation. Low PTEN IHC expression, dichotomized at the lower quartile, was observed in 57 (24%) patients. pHER2 IHC expression was scored as 0, +1, +2, and +3 in 31%, 24%, 20%, and 24% of the patients, respectively. Dichotomized (high $\geq 2+$) high pHER2 expression was detected in 105 of 235 (45%) patients. The PI3K pathway was considered activated by combining *PIK3CA* mutational status and PTEN expression [high pathway activity: PTEN low and/or *PIK3CA* mutation (exon 9 or 20)]. According to these parameters, 109 (47%) patients had increased PI3K pathway activation.

Table 1 shows baseline characteristics according to *PIK3CA* (wild type versus exon 9 or 20 mutations), PTEN, and pHER2

Table 1. Patient and tumor characteristics by *PIK3CA* mutation, PTEN, and pHER2 status

	Study patients	<i>PIK3CA</i> (N = 237 ^a)		PTEN (N = 236)		pHER2 (N = 235)	
		Mutation	Wildtype	Low	High	Low	High
All	240	61	176	57	179	130	105
Menopausal status							
Premenopausal	143 (60)	36 (59)	107 (61)	32 (56)	108 (60)	77 (59)	63 (60)
Postmenopausal	97 (40)	25 (41)	69 (39)	25 (44)	71 (40)	53 (41)	42 (40)
Positive nodes							
0	77 (32)	16 (26)	61 (35)	19 (33)	55 (31)	33 (25)	40 (38)
1–3	84 (35)	21 (34)	61 (35)	18 (32)	65 (36)	51 (39)	32 (30)
>3	79 (33)	24 (39)	54 (31)	20 (35)	59 (33)	46 (35)	33 (31)
Tumor size, mm							
0–20	118 (49)	25 (41)	92 (52)	24 (42)	90 (50)	54 (42)	60 (57)
21–50	109 (45)	33 (54)	74 (42)	28 (49)	81 (45)	68 (52)	40 (38)
>50	11 (5)	2 (3)	9 (5)	4 (7)	7 (4)	6 (5)	5 (5)
Unknown	2 (1)	1 (2)	1 (1)	1 (2)	1 (1)	2 (2)	0 (0)
Histology							
Ductal carcinoma	231 (96)	58 (95)	171 (97)	55 (96)	172 (96)	125 (96)	101 (96)
Other	9 (4)	3 (5)	5 (3)	2 (4)	7 (4)	5 (4)	4 (4)
Malignancy grade							
I	11 (5)	3 (5)	8 (5)	2 (4)	7 (4)	6 (5)	3 (3)
II	89 (37)	24 (39)	65 (37)	22 (39)	66 (37)	47 (36)	41 (39)
III	128 (53)	31 (51)	95 (54)	31 (54)	97 (54)	72 (55)	55 (52)
Unknown	12 (5)	3 (5)	8 (5)	2 (4)	9 (5)	5 (4)	6 (6)
ER status							
Positive	118 (49)	32 (52)	85 (48)	28 (49)	89 (50)	71 (55)	45 (43)
Negative	122 (51)	29 (48)	91 (52)	29 (51)	90 (50)	59 (45)	60 (57)

In brackets, patient numbers in percent.

^aTwo hundred and thirty-seven patients assessable for both exon 9 and 20 analyses.

ER, estrogen receptor; pHER2, phosphorylated human epidermal growth factor receptor-2; PTEN, phosphatase and tensin homolog.

status. No significant correlations were observed between the clinicopathological variables and the biomarkers.

survival analyses

Kaplan–Meier curves of IDFS and OS according to *PIK3CA*, PTEN, and PI3K pathway activity are presented in Figure 3. Univariate and multivariate HR estimates for OS and IDFS by marker status are presented in Table 2. OS was significantly poorer among patients with a *PIK3CA* mutation (HR 2.39; 95% CI 1.14–5.01; $P = 0.02$) and among patients with a high PI3K pathway activity (HR 2.57; 95% CI 1.20–5.50; $P = 0.01$), while no significant associations were observed with OS for PTEN and pHER2 or IDFS for all markers.

When adjustments were made for other prognostic factors (tumor size and nodal status) in a multivariate analysis, patients with a *PIK3CA* mutation and patients with a high PI3K pathway activity had a significantly higher risk of dying (HR 2.14, 95% CI 1.01–4.51; $P = 0.046$ and HR 2.35, 95% CI 1.10–5.04; $P = 0.03$, respectively). The associations between PTEN and pHER2 and survival remained nonsignificant.

All associations between IDFS and the various biomarkers turned out nonsignificant. As 13% of IDFS events were nonrecurrence related (i.e. second primary non-breast invasive cancer or new contralateral breast cancer), we also analyzed RFS and obtained nonsignificant results (data not shown).

discussion

The addition of trastuzumab to adjuvant chemotherapy in patients with HER2-positive early-stage breast cancer has resulted in substantial improvements in outcome [20, 21, 23–26, 50]. In this report of the DBCG05H trial with assessable samples, 5-year IDFS was 81.0% and OS 87.8%, which is similar to the efficacy data from prior adjuvant trials reporting 5-year DFS of 80%–84% [50] in patients treated with chemotherapy and trastuzumab.

In this subpopulation of HER2-positive breast cancer patients, 26% had *PIK3CA* mutations, which agrees well with previous reports of ~20% in HER2-positive breast carcinoma [33, 35, 51, 52]. In this study, *PIK3CA* mutational status was assessed by pyrosequencing, an increasingly used method for detecting mutations (e.g. analysis of *KRAS* mutational status) in the clinical setting. Pyrosequencing is a sensitive method for analyzing sequence variations in shorter DNA fragments, and since it is only requiring 5%–10% tumor cell content [53], it is useful for analyzing small tumor samples.

Data from preclinical studies link *PIK3CA* mutations to trastuzumab resistance [3, 31, 32]. Our results confirm this finding demonstrating that *PIK3CA* mutations (exon 9 or 20) had a significant effect on OS with an increased risk of death. This is in contrast to other studies that did not find significant associations between *PIK3CA* mutations and outcome after trastuzumab-based therapy [35, 37, 52]. However, a combined

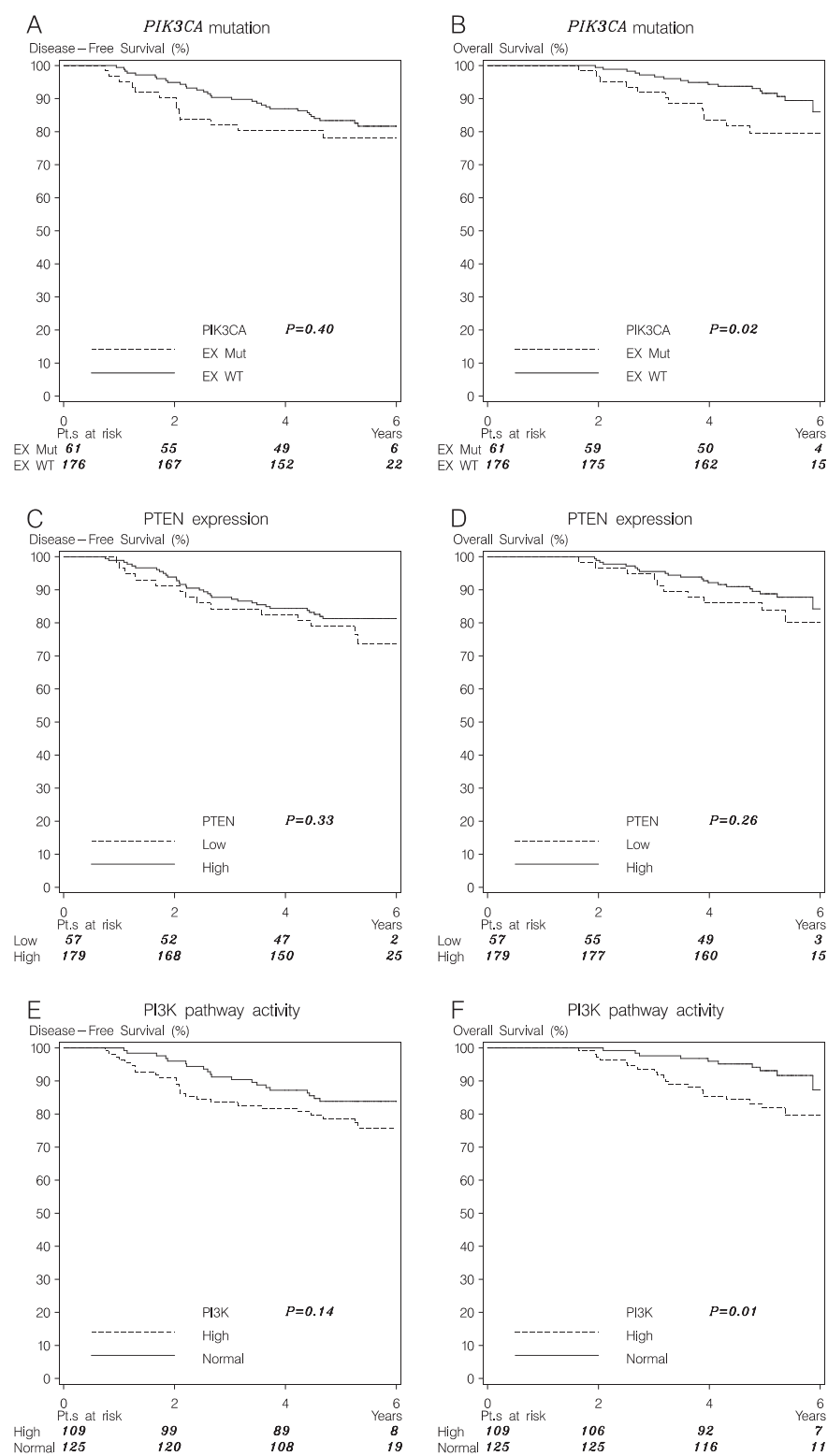


Figure 3. Kaplan–Meier curves of invasive disease-free survival and overall survival in patients with early-stage HER2-positive breast cancer treated with adjuvant trastuzumab. (A and B) *PIK3CA* mutation status [wild type (WT) versus mutation (Mut) (exon 9 or 20)]. (C and D) PTEN expression (low versus high). (E and F) PI3K pathway activity high [PTEN low and/or mutation (exon 9 or 20)] versus normal. PTEN, phosphatase and tensin homolog.

analysis of PI3K pathway activation by PTEN loss and/or *PIK3CA* mutation in HER2-positive breast cancer patients treated with trastuzumab showed a significant worse outcome associated with pathway activation [32, 33, 37, 52]. Our data

agree with this finding as activation of the PI3K pathway predicted a significant poorer survival. With the integrated use of *PIK3CA* mutational status and PTEN expression, patients were classified as having normal ($N = 125$) or high ($N = 109$)

Table 2. Univariate and multivariate HR estimates for OS and IDFS by biomarker status

	OS, univariate		OS, multivariate	
	HR (95% CI)	P	HR (95% CI)	P
PTEN expression				
Low versus high	1.54 (0.73–3.28)	0.26	1.36 (0.64–2.90)	0.43
pHER2 expression				
High versus low	0.77 (0.37–1.58)	0.47	0.79 (0.38–1.66)	0.54
<i>PIK3CA</i> mutational status ^a				
Exon 9 mutation versus WT	3.06 (1.12–8.34)	0.03	2.05 (0.73–5.74)	0.17
Exon 20 mutation versus WT	2.06 (0.85–4.99)	0.11	2.20 (0.90–5.41)	0.08
Exon 9 or 20 mutation versus WT	2.39 (1.14–5.01)	0.02	2.14 (1.01–4.51)	0.046
PI3K pathway activity				
High ^b versus normal	2.57 (1.20–5.50)	0.01	2.35 (1.10–5.04)	0.03
	IDFS, univariate		IDFS, multivariate	
	HR (95% CI)	P	HR (95% CI)	P
PTEN expression				
Low versus high	1.36 (0.73–2.55)	0.33	1.25 (0.67–2.35)	0.49
pHER2 expression				
High versus low	1.19 (0.67–2.10)	0.56	1.34 (0.76–2.39)	0.32
<i>PIK3CA</i> mutational status ^a				
Exon 9 mutation versus WT	1.57 (0.61–4.03)	0.35	1.15 (0.44–3.03)	0.76
Exon 20 mutation versus WT	1.21 (0.55–2.62)	0.64	1.10 (0.51–2.40)	0.81
Exon 9 or 20 mutation versus WT	1.32 (0.69–2.53)	0.40	1.12 (0.59–2.14)	0.73
PI3K pathway activity				
High ^b versus normal	1.55 (0.86–2.79)	0.14	1.36 (0.76–2.46)	0.30

^aN = 237; three patients with unknown exon 20 status were excluded.

^bHigh PI3K pathway activity defined as PTEN low and/or *PIK3CA* mutation (exon 9 or 20). In the multivariate analyses, adjusted for tumor size and nodal status, as these variables were significant in the univariate analyses.

CI, confidence interval; HR, hazard ratio; IDFS, invasive disease-free survival; OS, overall survival; pHER2, phosphorylated human epidermal growth factor receptor-2; PTEN, phosphatase and tensin homolog; WT, wild type.

PI3K pathway activity. Of the 109 patients with high PI3K activity, 44% were selected on PTEN low alone, 48% selected on *PIK3CA* mutation alone, and only 8% had both characteristics present. Including both PTEN and *PIK3CA* status in the same model increased the HRs for both PTEN and *PIK3CA* only slightly (the reference group being patients without any of the characteristics), but a formal test for interaction between the two markers was not possible because of few patients (N = 9) with both characteristics.

PTEN activation contributes to the antiproliferative effect of trastuzumab [36]. Several *in vitro* studies show that PTEN knockdown decreases growth inhibition by trastuzumab [31, 32, 34, 36, 37]. Immunohistochemical analysis of PTEN expression in tissue samples from metastatic breast cancer patients treated with trastuzumab and chemotherapy have demonstrated that low protein level of PTEN predicted resistance to trastuzumab [33, 34, 36]. However, other studies have found no correlation between PTEN expression and trastuzumab response [52, 54] or outcome [32, 54]. In the adjuvant setting, significantly poorer OS in PTEN-low tumors after adjuvant trastuzumab has been demonstrated [35]. We were not able to confirm that PTEN IHC status had a significant impact on any outcome measure.

Methods for evaluation of PTEN protein expression on FFPE tissue are not standardized. Immunohistochemical PTEN reports vary in terms of choice of antibody, methods for tissue

processing, scoring method, and cut-off values [54, 55]. Probably as a consequence of this lack of consistency, low PTEN expression by IHC in HER2-positive breast cancer patients has been reported with frequencies of 15%–65% [32–34, 36, 52, 54] making comparisons between studies difficult. Establishing and standardization of the optimal method to assess PTEN expression is highly required.

The active HER2 receptor was assessed by determining site-specific phosphorylation of intracellular tyrosine residues, pHER2^{1221/1222}, one of the tyrosine residues with ErbB2 downstream interaction partners [56]. Previous studies have shown that pHER2 positivity may correlate with increased trastuzumab sensitivity [42, 43, 45]. In this study, pHER2 status did not have a significant impact on IDFS or OS. Assessment of the expression of phosphorylated proteins by IHC is subject to limitations, especially attributable to potential rapid dephosphorylation before fixation [57, 58]. Also, multiple ErbB2 tyrosine residues exist and as the anti-phospho-ErbB2 antibodies are site specific, not all activated forms of the receptor are recognized.

strengths and limitations of the study

This study is the first to report the implications of PI3K pathway dysregulation on outcome in a uniformly treated early-stage HER2-positive population who received adjuvant trastuzumab and chemotherapy. The uniform administration

of adjuvant trastuzumab and chemotherapy was one of the strengths of the study as this eliminates potential differences in outcome due to administration of different treatment regimens. The small patient number included is, however, a limitation. This may account for why statistical significance was achieved only in OS but not in IDFS. Though nonsignificant, IDFS estimates were in the expected direction according to the hypotheses. Therefore, validation in a larger patient sample is needed. Also, one may speculate that patients with *PIK3CA* mutations or high PI3K signaling were resistant to further anti-HER2 therapy administered after recurrence in the metastatic setting, reflected in reduced OS. Data on treatment administered in the metastatic setting are not available as this information is not registered in the DBCG database.

The maturity of the DBCG05H data with relatively few events occurring is a potential limitation though the HER2-positive subpopulation is characterized by having early recurrences, primarily within 5 years [59]. Controversy exists regarding the prognostic value of *PIK3CA* mutations [7, 10, 13, 47, 60, 61] and this has not been evaluated in a large untreated HER2-positive population. Establishing whether the markers are prognostic, predictive (regarding trastuzumab efficacy), or both could be determined (theoretically) in a randomized study with an untreated HER2-positive population but in reality by assessing the markers in a comparable HER2-positive population not treated with adjuvant trastuzumab [62].

targeting HER2-positive breast cancer

Resistance to trastuzumab is a major challenge as a significant number of patients do not benefit from trastuzumab. Understanding the mechanisms of resistance may lead to novel therapeutic approaches by identifying subsets of patients who would benefit from treatment regimens other than single HER2 inhibition by trastuzumab. Thus, therapy with tyrosine kinase inhibitors targeting HER2 might increase clinical outcome in patients with increased PI3K signaling [37, 63]. This is supported by newly published neoadjuvant data reporting that the odds of achieving pathological complete response was significantly better in patients with increased PI3K activity (PTEN low or *PIK3CA* mutation) treated with lapatinib than in patients treated with trastuzumab [37]. Several novel anti-HER2 agents that may circumvent resistance are under clinical investigation [39, 64]. Development of therapies that target tumor-specific pathway components as PI3K alterations is emerging, and PI3K inhibitors are potentially effective in overcoming trastuzumab resistance caused by *PIK3CA* mutations [3, 31] or PTEN loss [31, 36]. Incorporation of PTEN by IHC and *PIK3CA* genotyping should be considered in the design of future trials in order to determine the predictive value of these biomarkers in HER2-positive breast cancer.

conclusions

The present study of HER2-positive early-stage breast cancer patients demonstrated that patients with *PIK3CA* mutations or increased PI3K pathway activity had a significantly poorer survival despite adequate treatment with adjuvant chemotherapy and trastuzumab. These markers should be considered in future studies of resistance to trastuzumab.

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disclosure

The authors declare no conflict of interest.

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