Somatic Mutation Profiling and Associations With Prognosis and Trastuzumab Benefit in Early Breast Cancer

Sherene Loi*, Stefan Michiels, Diether Lambrechts, Debora Fumagalli, Bart Claes, Pirkko-Liisa Kellokumpu-Lehtinen, Petri Bono, Vesa Kataja, Martine J. Piccart, Heikki Joensuu, Christos Sotiriou

Manuscript received November 7, 2012; revised March 14, 2013; accepted March 18, 2013.

*Present affiliation: Translational Breast Cancer Genomics Lab, Division of Research, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.

Correspondence to: Sherene Loi, MD, PhD, Peter MacCallum Cancer Centre, Department of Medical Oncology, Locked Bag 1, A Beckett St, Melbourne, VIC 8006, Australia (e-mail: sherene.loi@petermac.org).

Background

Certain somatic alterations in breast cancer can define prognosis and response to therapy. This study investigated the frequencies, prognostic effects, and predictive effects of known cancer somatic mutations using a randomized, adjuvant, phase III clinical trial dataset.

Methods

The FinHER trial was a phase III, randomized adjuvant breast cancer trial involving 1010 women. Patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer were further randomized to 9 weeks of trastuzumab or no trastuzumab. Seven hundred five of 1010 tumors had sufficient DNA for genotyping of 70 somatic hotspot mutations in 20 genes using mass spectrometry. Distant disease-free survival (DDFS), overall survival (OS), and interactions with trastuzumab were explored with Kaplan-Meier and Cox regression analyses. All statistical tests were two-sided.

Results

Median follow-up was 62 months. Of 705 tumors, 687 were successfully genotyped. PIK3CA mutations (exons 1, 2, 4, 9, 13, 18, and 20) were present in 25.3% (174 of 687) and TP53 mutations in 10.2% (70 of 687). Fewer other mutations were found: three ERBB2 and single cases of KRAS, ALK, STK11/LKB1, and AKT2. PIK3CA mutations were associated with estrogen receptor positivity (P < .001) and the luminal-A phenotype (P = .04) but were not statistically significantly associated with prognosis (DDFS: hazard ratio [HR] = 0.88, 95% confidence interval [CI] = 0.58 to 1.34, P = .56; OS: HR = 0.603, 95% CI = 0.32 to 1.13, P = .11), although a statistically significant nonproportional prognostic effect was observed for DDFS (P = .002). PIK3CA mutations were not statistically significantly associated with trastuzumab benefit (Pinteraction; DDFS P = .14; OS P = .24).

Conclusions

In this dataset, targeted genotyping revealed only two alterations at a frequency greater than 10%, with other mutations observed infrequently. PIK3CA mutations were associated with a better outcome, however this effect disappeared after 3 years. There were no statistically significant associations with trastuzumab benefit.


Gene expression profiling divides breast cancer into distinct molecular portraits according to the presence of the estrogen receptor (ER) and amplification/overexpression of the ERBB2/HER2/neu oncogene (1). Notably, HER2 amplification/overexpression (HER2-positive) predicts response to anti-HER2 therapy, suggesting that somatic alterations in breast cancer are associated with prognosis and potentially amenable to targeted therapy (2). This has inspired efforts to better understand the spectrum of somatic “driver” mutations and, in particular, targetable mutated kinases.

An abundance of data suggests that genetic aberrations and activation of the phosphatidylinositol 3-kinase (PI3K) pathway are important in determining breast cancer prognosis and the efficacy of standard chemo- and endocrine therapies (3). Furthermore, mutations in the PIK3CA gene, which encodes the p110α catalytic subunit of the class IA PI3K, are frequent in breast cancer (4-7). These mutations have been shown to be oncogenic in mammary epithelial cells by driving constitutive, growth factor–independent PI3K pathway activation (8,9).

Despite being the focus of intense research interest, a clear association between PIK3CA mutations and a poorer prognosis has not been shown. To the contrary, PIK3CA mutations have been associated with statistically significantly better survival when compared with PIK3CA wild-type breast cancers in larger series obtained from single institutions (4,7–10). An association with resistance to endocrine therapy has also not been demonstrated (6,11,12). PIK3CA mutations have also been shown to be associated with trastuzumab resistance in preclinical models overexpressing HER2 (13–15). Clinical validation of this association could have important clinical utility given the emergence of a broadening array of anti-HER2 agents and the concept of dual anti-HER2 therapy.

960 Articles | JNCI

10.1093/jnci/djt121
© The Author 2013. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
24 mutations were also confirmed using Sanger genetic in situ hybridization in one of two reference laboratories. In this study, we further confirmed one sample of each positive PIK3CA mutation, as well as a scattering of other “druggable” mutations. In total, 70 mutations in 20 genes were evaluated (Supplementary Table 1, available online).

The samples were genotyped centrally using the Sequenom MassARRAY Assay Design 3.1 with the default parameters. Multiplex polymerase chain reaction was done in 5-μL volume containing 5–10 ng of DNA. Samples were considered to be of sufficient quality when genotyping could be performed for >75% of the mutations. A total of 687 samples (68.1% of the original FinHER cohort) were successfully genotyped (2.5% [18 of 705] samples were discarded for this reason). Sixteen samples were genotyped in duplicate and were found to have 100% concordance. Details about the assay and independent validation have been previously published: the Sequenom can detect low-frequency mutant alleles to maximize mutation detection in impure samples (≥5% for the PIK3CA hotspot mutations) with sensitivity and specificity of 90% and 99%, respectively, in FFPE-derived DNA, and 100% concordance with other technologies (25,26). In this study, we further confirmed one sample of each positive PIK3CA mutation, as well as 99 sample mutations (exons 1, 2, 4, 9, 13, 18, 20) reported in COSMIC to be occurring in breast cancer in this study, 12% of TP53 mutations (all cancer types), selected ERBB2, PTEN, AKT1/2 mutations, and known EGFR, BRAF, KRAS, MAP3K1, and CDK4 mutations, as well as a scattering of other “druggable” mutations. In total, 70 mutations in 20 genes were evaluated (Supplementary Table 1, available online).

The ethical committee of the Helsinki University Central Hospital also approved the current study. Of the 1010 samples, 935 (92.5%) could be retrieved. All samples were reevaluated by one reference pathologist to ensure tumor was present in the tissue sample. Of these samples, 705 (75.4%) were able to have adequate DNA extracted. DNA was extracted from FFPE tumor tissue using a salt precipitation method (23).

Because few data were available about the mutational landscape of breast cancer at the time of genotyping, we queried the COSMIC (Catalogue of Somatic Mutations in Cancer) database to identify a broad range of genes for hotspot somatic mutation profiling (24). We ultimately covered 94% of reported PIK3CA mutations (exons 1, 2, 4, 9, 13, 18, 20) reported in COSMIC to be occurring in breast cancer in this study, 12% of TP53 mutations (all cancer types), selected ERBB2, PTEN, AKT1/2 mutations, and known EGFR, BRAF, KRAS, MAP3K1, and CDK4 mutations, as well as a scattering of other “druggable” mutations. In total, 70 mutations in 20 genes were evaluated (Supplementary Table 1, available online).

The samples were genotyped centrally using the Sequenom MassARRAY Assay Design 3.1 with the default parameters. Multiplex polymerase chain reaction was done in 5-μL volume containing 5–10 ng of DNA. Samples were considered to be of sufficient quality when genotyping could be performed for >75% of the mutations. A total of 687 samples (68.1% of the original FinHER cohort) were successfully genotyped (2.5% [18 of 705] samples were discarded for this reason). Sixteen samples were genotyped in duplicate and were found to have 100% concordance. Details about the assay and independent validation have been previously published: the Sequenom can detect low-frequency mutant alleles to maximize mutation detection in impure samples (≥5% for the PIK3CA hotspot mutations) with sensitivity and specificity of 90% and 99%, respectively, in FFPE-derived DNA, and 100% concordance with other technologies (25,26). In this study, we further confirmed one sample of each positive PIK3CA mutation, as well as a wild-type sample, using Sanger sequencing (except for the rarer G241A, G3019C, and C473T); another 9 samples (both positive and wild type) were confirmed with the Qiagen Rotor-gene Kit. All of these were found to be 100% concordant with the Sequenom results. ERBB2 mutations were also confirmed using Sanger sequencing (primers TCCTGGAGGGCAGTTAGGATCCAG and AGTCTAGGTTTGCCGGAGTCATATCTC).

Statistics Analysis
In this study, a sample was considered to be wild type for a given gene if no mutation was found. Associations between mutations and clinicopathologic characteristics were investigated with χ² tests for categorical variables. For the survival analyses, the primary end point was DDFS, which was defined as the time period from the date of random treatment assignment to the date of first cancer recurrence outside the ipsilateral locoregional region or to death, whenever death occurred before distant recurrence (21). Relapse-free survival (RFS) was defined as the time from the date of random assignment to the date of the local, distant, or contralateral invasive cancer recurrence or death. Overall survival (OS) was defined as the time period from the date of random assignment to the date of death, whenever death occurred before distant recurrence.
Cox proportional hazards regression models were used to test the prognostic value of PIK3CA mutation status (hazard ratios [HRs] and 95% confidence intervals [CIs]) and its possible interaction with trastuzumab treatment (after adding a trastuzumab main effect and a product interaction term) using the Wald test. The Cox models used a separate baseline hazard for chemotherapy type (docetaxel or vinorelbine). Departures from the proportional hazards assumption were assessed based on the Schoenfeld residuals (27).

All P values were two-sided and a P value of less than .05 was considered statistically significant. The Kaplan–Meier survival curves were calculated (with group differences assessed using the log-rank test). Interaction effects were also displayed using forest plots. No adjustment was planned for multiple testing of the prespecified hypotheses. For this study, breast cancer subtypes were classified using IHC as previously published (28): luminal (ER-positive and/or progesteron receptor [PgR]-positive, HER2-negative), HER2-positive/overexpressing by (chromogenic in situ hybridization), and triple negative: ER-negative, PgR-negative, HER2-negative (luminal). In the three main breast cancer subtypes (luminal and HER2-positive subtypes, defined using IHC, as expected, TP53 mutations in the triple-negative group were statistically significantly associated with smaller tumor size (T1, P = .03), histological grade 1 (P < .001), positive expression of the ER (P < .001), and the luminal-A phenotype (P = .04; Table 1). As expected, TP53 mutations were associated with ER negativity (P = .005), histological grade 3 (P = .007), larger tumor size (P = .009), and four or more positive lymph nodes (P = .003). All three ERBB2 mutant samples were ER-positive and HER2-negative (luminal). In the three main breast cancer subtypes defined using IHC, as expected, PIK3CA mutations were highly frequent in luminal and HER2-positive subtypes (P < .001) and TP53 mutations in the triple-negative group (P = .003; Table 2).

**Results**

**Patient Characteristics**

The patient characteristics of the genotyped series (n = 687) are compared with the original series and those who were not genotyped in Supplementary Table 2 (available online). There were more tumors that were ER-negative, of larger size and higher grade, and from younger patients genotyped compared with those not genotyped. There were no statistically significant differences in survival between groups (DDFS log-rank P = .19, RFS P = .34, OS P = .64).

**Frequency and Associations Between Mutations**

Despite genotyping this cohort for 70 known cancer somatic “driver” mutations in 20 genes, only PIK3CA and TP53 somatic mutations occurred at frequencies >10%.

PIK3CA mutations were successfully genotyped in 100% of samples that passed the quality control criteria. 176 PIK3CA mutations were found (Supplementary Table 3, available online). The vast majority of these were located on the hotspots on the helical and kinase domains (exons 9 and 20, respectively—161 of 176 [91.5%]), with two samples having a double PIK3CA mutation present (A3140G + C473T; T1035A + G1633A). The overall frequency of tumor samples with a PIK3CA mutation was 25.3% (174 of 687). TP53 mutations, with coverage of approximately 12% of known mutations, were present in 10.2% (70 of 687) of samples. Three ERBB2 kinase domain mutations (two *T2264C, C2313T) were present in 0.5% of samples genotyped (3 of 659 [28 of 687 samples could not be assigned]). Mutations that occurred only once were KRAS (G35A), AKT2 (G49A), ALK (G3824A), and STK11/LKB1 (C1062G) (Figure 1).

**Association With Clinicopathological Features and Breast Cancer Subtypes**

PIK3CA mutations were statistically significantly associated with smaller tumor size (T1, P = .03), histological grade 1 (P < .001), positive expression of the ER (P < .001), and the luminal-A phenotype (P = .04; Table 1). As expected, TP53 mutations were associated with ER negativity (P = .005), histological grade 3 (P = .007), larger tumor size (P = .009), and four or more positive lymph nodes (P = .003). All three ERBB2 mutant samples were ER-positive and HER2-negative (luminal). In the three main breast cancer subtypes defined using IHC, as expected, PIK3CA mutations were highly frequent in luminal and HER2-positive subtypes (P < .001) and TP53 mutations in the triple-negative group (P = .003; Table 2).

**Associations With Prognosis**

In the whole cohort that was genotyped, PIK3CA mutations were not statistically significantly associated with prognosis (DDFS: HR = 0.88 [95% CI = 0.58 to 1.34], P = .56; OS: HR = 0.603 [95% CI = 0.32 to 1.13], P = .11; Figure 2). However, we noted that there was a statistically significant nonproportional prognostic effect over time for DDFS (P = .002) and RFS (P = .007) but not for OS.
(\(P = .12\)). An exploratory subdivision of the time axis at three years shows a favorable prognostic effect before three years (DDFS: HR = 0.57 [95% CI = 0.31 to 1.03], \(P = .06\); RFS: HR = 0.55 [95% CI = 0.31 to 0.98], \(P = .04\), respectively, and statistically nonsignificant effect after 3 years: DDFS: HR = 1.69 [95% CI = 0.90 to 3.16], \(P = .10\); RFS: HR = 1.58 [95% CI = 0.86 to 2.88], \(P = .14\)).

No statistically significant differences in patient outcome were observed when \(PIK3CA\) mutations were evaluated separately according to their location (Figure 3). Patients whose tumors contained a \(PIK3CA\) mutation were also not found to have a statistically significantly different survival than those with wild type in any of the breast cancer subtypes (Supplementary Figure 1, available online).

\(TP53\) mutations were not statistically significantly associated with prognosis in the whole genotyped cohort (DDFS log-rank \(P = .36\); RFS \(P = .34\); OS \(P = .11\)). Of the three \(ERBB2\) mutated tumors, one patient had a distant relapse and died of her disease.

### Table 1. Patient and tumor characteristics by \(PIK3CA\) genotype*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole cohort (N = 687)</th>
<th>(PIK3CA) genotype</th>
<th>(TP53) genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WT (n = 511)</td>
<td>Any mt (PIK3CA) (n = 176)</td>
</tr>
<tr>
<td>Age category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50 y</td>
<td>364 (53%)</td>
<td>274 (53.6%)</td>
<td>90 (51.1%)</td>
</tr>
<tr>
<td>&gt;50 y</td>
<td>323 (47%)</td>
<td>237 (46.4%)</td>
<td>86 (48.9%)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>275 (40%)</td>
<td>192 (37.8%)</td>
<td>83 (47.2%)</td>
</tr>
<tr>
<td>T2</td>
<td>364 (53%)</td>
<td>274 (53.9%)</td>
<td>90 (51.1%)</td>
</tr>
<tr>
<td>T3</td>
<td>45 (6.6%)</td>
<td>42 (8.3%)</td>
<td>3 (1.7%)</td>
</tr>
<tr>
<td>Missing</td>
<td>3 (0.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>81 (11.8%)</td>
<td>64 (12.5%)</td>
<td>17 (9.7%)</td>
</tr>
<tr>
<td>1–3</td>
<td>410 (59.7%)</td>
<td>297 (58.1%)</td>
<td>113 (64.2%)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>196 (28.5%)</td>
<td>150 (29.4%)</td>
<td>46 (26.1%)</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>80 (11.6%)</td>
<td>46 (9.3%)</td>
<td>34 (20.2%)</td>
</tr>
<tr>
<td>II</td>
<td>270 (39.3%)</td>
<td>187 (37.8%)</td>
<td>83 (49.4%)</td>
</tr>
<tr>
<td>III</td>
<td>313 (96.5%)</td>
<td>262 (52.9%)</td>
<td>51 (30.4%)</td>
</tr>
<tr>
<td>Missing</td>
<td>23 (3.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>475 (69.1%)</td>
<td>335 (69.7%)</td>
<td>140 (79.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>212 (30.9%)</td>
<td>176 (30.3%)</td>
<td>56 (20.5%)</td>
</tr>
<tr>
<td>HER2 amplification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>157 (22.9%)</td>
<td>123 (24.1%)</td>
<td>34 (19.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>530 (77.1%)</td>
<td>388 (65.9%)</td>
<td>142 (72.8%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>558 (81.2%)</td>
<td>422 (83.6%)</td>
<td>136 (78.6%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>120 (17.5%)</td>
<td>83 (16.4%)</td>
<td>37 (21.4%)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (1.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(defined by IHC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal (ER-positive/HER2-negative)</td>
<td>410 (59.7%)</td>
<td>284 (55.6%)</td>
<td>126 (71.6%)</td>
</tr>
<tr>
<td>HER2-amplified</td>
<td>157 (22.9%)</td>
<td>123 (24.1%)</td>
<td>4 (19.3%)</td>
</tr>
<tr>
<td>Triple negative (ER-negative/PgR-negative/HER2-negative)</td>
<td>120 (17.5%)</td>
<td>104 (20.4%)</td>
<td>16 (9.1%)</td>
</tr>
<tr>
<td>Luminal A/B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki67 IHC &lt;14%</td>
<td>127 (30%)</td>
<td>80 (31.7%)</td>
<td>47 (42.7%)</td>
</tr>
<tr>
<td>Ki67 IHC ≥14%</td>
<td>235 (57.3%)</td>
<td>172 (63.3%)</td>
<td>63 (57.3%)</td>
</tr>
<tr>
<td>NA</td>
<td>48 (11.7%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* \(P\) values were calculated using a two-sided \(\chi^2\) test. ER = estrogen receptor; IHC = immunohistochemistry; mt = mutation; NA = not applicable; WT = wild type.

### Table 2. Frequency of mutations by breast cancer subtype*

<table>
<thead>
<tr>
<th>Subtype</th>
<th>(PIK3CA) mutations, No.</th>
<th>(TP53) mutations, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal (ER-positive/HER2-negative)</td>
<td>126/410 (30.7%)</td>
<td>30/409 (7.3%)</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>34/157 (21.7%)</td>
<td>19/157 (12.1%)</td>
</tr>
<tr>
<td>ER-negative/HER2-negative</td>
<td>16/120 (13.3%)</td>
<td>21/120 (17.5%)</td>
</tr>
</tbody>
</table>

* \(P\) values were calculated using a two-sided \(\chi^2\) test. ER = estrogen receptor.
The primary objective of this study was to investigate the clinical relevance of PIK3CA mutations with regard to prognosis and benefit from adjuvant trastuzumab. While confirming the dominance of PIK3CA and TP53 mutations in breast cancer with few other known mutations being present in breast cancer at a high rate, we showed that PIK3CA mutations, regardless of location, were not statistically significantly associated with prognosis in breast cancer over the entire follow-up period, although, interestingly, there was a statistically significant nonproportional prognostic effect for DDFS and RFS over time. Initially, a better outcome for the mutant genotype’s association with favorable clinicopathological features; however, this effect disappeared after three years. Perhaps this pattern can be explained by the high-risk population studied or reflect a biological tendency for long-term relapse, as endocrine therapy resistance could conceivably develop through enhanced PI3K signaling. In general, however, the prognostic direction in the first three years supports the results from many of the larger cohort series reported in the literature, even though the prognostic association has yielded conflicting reports overall (4–7,29).

The unique advantage and strength of our study was that we could evaluate interactions between PIK3CA mutations and trastuzumab benefit in the context of a randomized clinical trial in which patients with HER2-positive breast cancer received treatment with or without trastuzumab. To our knowledge, this study...
Figure 4. Interaction between PIK3CA genotype and trastuzumab efficacy. A) Kaplan-Meier plots comparing trastuzumab vs no trastuzumab treatment arms for PIK3CA mutated (mt), HER2-positive cohorts. Cumulative proportions of patients surviving distant disease free are shown. B) Kaplan-Meier plots comparing trastuzumab vs no trastuzumab for PIK3CA wild-type (WT), HER2-positive cohorts. Cumulative proportions of patients surviving distant disease free are shown. C) Interaction forest plots indicate Cox regression hazard ratios (HRs) and 95% confidence intervals (CIs) stratified by chemotherapy type given for trastuzumab benefit for distant disease-free survival (DDFS). According to PIK3CA genotype and by overall series. All statistical tests are two-sided.

Also represents the largest breast cancer cohort with clinical outcome data to be genotyped for PIK3CA and multiple other known cancer somatic mutations. Furthermore, we covered greater than 94% of known PIK3CA mutations, rather than limiting to hot-spot areas. Preclinical data suggest that PIK3CA mutations could identify a subgroup of patients with HER2-positive disease resistant to trastuzumab, but our data do not support this. In fact, the PIK3CA mutant compared with wild-type, HER2-positive tumors seemed to derive more benefit from adjuvant trastuzumab, suggesting increased dependency on p110α, although the interaction test is not statistically significant (30). All the patients in this study also received chemotherapy with trastuzumab, which is standard practice, so we cannot discount the possibility that mutations could cause resistance to trastuzumab as a single agent. It has been proposed that scheduling of chemotherapy either before or after administration of trastuzumab could affect clinical outcomes, particularly through immune mechanisms (31). As the generation of antitumor immunity has been proposed as a dominant mechanism for the efficacy of trastuzumab, it is plausible that PIK3CA mutations could alter the immune microenvironment to be either...
or antitumor or protumor (31,33). PI3K signaling per se is known to affect immune signaling, although no data currently exist with regard to specific mutation-related events (34). Therefore, despite PIK3CA mutations being oncogenic activators of the PI3K pathway, overall our data support the notion that PIK3CA mutant tumors when compared with the PIK3CA wild-type tumors are not resistant to standard adjuvant chemotherapy, trastuzumab, and endocrine therapy regimens.

A biological mechanism for these observations is currently unknown. We have speculated previously that PIK3CA mutations are not effective at completely activating the pathway and negative feedback mechanisms may serve to weaken the oncogenic signal (6). Full AKT activation has not been associated with the mutation, and AKT-independent signaling has been proposed through PDK1-SGK3, with SGK3 also implicated with estrogen signaling (7,35–37). Estrogen has also been shown predominantly to repress transcription of many genes, which may also reduce the final signaling output (38,39). High levels of pathway activation could be detrimental for tumor growth (ie, result in senescence), analogous to PTEN deficiency (40). Regardless, it seems that high levels of pathway activation are not associated with PIK3CA mutations per se. We hypothesize that PIK3CA mutations may be more important in breast cancer initiation and malignant transformation whereas other mutations may be required to drive the acquisition of aggressive biological features: it is notable that PIK3CA mutations often coexist with other lesions in the same pathway (30,41–43). It remains to be seen if primary and/or metastatic breast cancer patients with PIK3CA mutations will derive increased benefit from PI3K pathway–targeted drugs, which has been observed in vitro (44–46). Many clinical trials evaluating potential benefit from specific PI3K targeted drugs are currently ongoing.

This study, as well as others using massively parallel sequencing, have confirmed that breast cancers contain a large number of known cancer driver mutations that occur infrequently (42,43,47,48). In this cohort we have identified three ERBB2 as well as single KRAS, ALK, STK11/LKB1, and AKT2 mutations. These are known “driver” mutations, yet it is unknown how these influence outcomes or are amenable to targeted therapies in breast cancer. ERBB2 kinase domain mutations have recently been shown to be important in breast cancer; hence, this mutation could represent a new target for non-HER2-amplified/overexpressing breast cancer (49–52).

To our knowledge, this is the only study thus far to address the relevance of PIK3CA genotype and trastuzumab benefit. We acknowledge several limitations of our study, specifically the low number of events in the HER2-positive subgroup, which does not exclude the possibility that an effect might be seen in a larger series; less than 100% coverage of all reported PIK3CA mutations in breast cancer; and sequencing from one tumor section, given emerging data on intratumoral heterogeneity (53). Next-generation sequencing technologies may give us a more complete picture of the clonal composition and molecular landscape of these tumors. However, it is becoming clear that elucidating the relationship between infrequent but known driver genetic aberrations, prognosis, and drug response will require the genotyping of tumors from many thousands of breast cancer patients. This may also prove challenging for drug development. Nevertheless, our study provides important information from a large randomized clinical trial dataset about the prevalence and relationship between targetable and known somatic driver mutations, trastuzumab efficacy, and prognosis.

References
21. Joensuu H, Bonn P, Kataja V, et al. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab,


Funding
This study was funded by the Breast Cancer Research Foundation. The funding source had no role in the study design, data analysis, data collection, data interpretation or writing of the report. SL, SM, and CS are supported by the Breast Cancer Research Foundation. SL is supported by the Fonds JC Heuson Belgium. This project was also supported by EU FP7 project RESPONSIFY Nr 278659.

Notes
PB previously received payment for a lecture from Roche Finland. The other authors report no conflicts of interest. No medical writing assistance was used for this manuscript. SL conceived the study; SL, SM, HJ, and CS analyzed and interpreted the data; BC, DL, and DF contributed to the sample processing and mutation testing; PK, LB, VK, and HJ contributed samples to the study; SL wrote the manuscript; all authors reviewed and approved the final version of the manuscript for submission; SL, SM, CS, and HJ had full access to all the data in the study; and SL had the final responsibility to submit the manuscript for publication.

Affiliations of authors: Breast Cancer Translational Research Laboratory, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium (SL, SM, DF, CS); Department of Biostatistics and Epidemiology, Institut Gustave Roussy, Villejuif, France (SM); Vesalius Research Centre, KU Leuven and VIB, Leuven, Belgium (DL, BC); Laboratory for Translational Genetics, Department of Oncology, University of Leuven, Leuven, Belgium (DL, BC); Department of Oncology, Tampere University Hospital, Tampere, Finland (PK-L-K); Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland (PB, HJ); Cancer Center, Kuopio University Hospital, Kuopio, Finland (VK); Department of Medicine, Institut Jules Bordet, Brussels, Belgium (MJJP).