Improved classification of breast cancer by analysis of genetic alterations and gene expression profiling
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Mutational analysis of PIK3CA and TP53 and their role in breast cancer prognosis
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Abstract

Introduction: PIK3CA and TP53 mutations are frequent in breast cancer and have previously been found to be associated with specific clinicopathological features. We set out to evaluate the association of PIK3CA and TP53 mutation status with various gene expression signatures and to combine PIK3CA and TP53 mutation status, gene expression signatures and clinicopathological factors to predict outcome in patients with early stage breast cancer.

Material and methods: For 295 breast carcinomas (Stage I and II) clinical, pathological and gene expression data were previously reported [1]. In these tumors, TP53 mutations were identified by DNA sequencing of exons 2-11. PIK3CA mutations were assessed using PCR based amplification of exons 9 and 20. Results: Mutations in PIK3CA and TP53 were found in 30% and 33% of invasive breast carcinomas respectively. Although they were not mutually exclusive. PIK3CA mutations were associated with PR positive tumors and TP53 mutations with ER negative tumors. In the TP53 gene, non-missense mutations (HR: 2.2; 95% CI 1.2-4.3; p-value 0.02) were associated with the highest risk of developing distant metastases and die from breast cancer, compared to wildtype/silent mutation. PIK3CA mutations were not associated with altered prognosis of breast cancer patients. Neither TP53 nor PIK3CA mutation status significantly improved the predictive accuracy of any of the nine well-known, validated prognostic gene expression signatures investigated. Conclusion: TP53 mutations are associated with increased risk of developing distant metastases in patients with early stage breast cancer, while PIK3CA mutations are not predictive of outcome. The prognostic power of gene expression signatures was not be improved by integration with TP53 and/or PIK3CA mutational status.
Introduction

Adjuvant systemic therapy in breast cancer patients consisting of chemotherapy and/or hormonal therapy and/or ‘targeted therapies’ such as trastuzumab, improve outcome for breast cancer patients. Prognostic and predictive markers are needed to improve the selection of patients that will benefit from these treatments [2]. The most important prognostic and predictive factors in breast carcinoma patients are age at diagnosis, tumor size, status of axillary lymph nodes, histological type of the tumor, pathological grade, HER2 status and hormone-receptor status, i.e. estrogen receptor (ER) and progesterone receptor (PR) [3, 4]. These factors reflect the intrinsic biology of breast tumors and are the basis for adjuvant systemic treatment decisions for breast cancer patients. Significant efforts have been made in order to identify novel prognostic and predictive markers in breast cancer, e.g. by studying genomic alterations [5], immunohistochemical phenotypes [6], morphological features [7], gene expression profiles [1], microRNAs (reviewed by Zoon et al. [8]) and long non coding RNAs [9]. Some of the findings from these studies have already been implemented in clinical decision making.

Breast cancer is a heterogeneous disease, caused like many other cancer types by multiple genetic alterations in ‘breast’ cancer cells [10]. The most commonly mutated genes in breast tumors are tumor protein p53 (TP53) and phosphatidylinositol 3-kinase (PI3K) p110α catalytic subunit (PIK3CA), both mutated in approximately 20-30% of breast carcinomas [11-14]. PIK3CA and TP53 mutation status have been described as prognostic factors in breast cancer, but currently available data are insufficient to recommend use of TP53 and/or PIK3CA analysis for the management of patients with breast cancer [15]. Tumors with a TP53 mutation are associated with shorter overall survival, independent of other risk factors [16-18]; especially when combined with the absence of PR expression [18], although this has not been independently validated [19]. There are several reports indicating that TP53 mutations are associated with resistance to specific chemotherapy regimens, most notably anthracyclins, but the results of studies in this research area have been inconclusive [20-24]. Mutations in TP53 are mostly missense mutations, widely spread throughout the gene, but with the highest frequency of base substitutions in six hotspot codons within the DNA binding domain of the molecule: 175, 220, 245, 248, 273, and 282 (reviewed in Robles et al. 2010 [25] and [26]). It has been suggested by Olivier [18] and Alsner [16] that missense mutations affecting DNA binding are particularly deleterious and are associated with worse survival than missense mutations outside the DNA binding motifs of the transcription factor. Non-missense mutations are associated with poor survival than missense mutations, and whether these mutations give a slightly worse or better prognosis than the missense mutations affecting DNA binding is questionable due to studies with different patient cohorts, different treatment and different classification of mutations. Patients with silent mutations show good survival. Translating these results into the clinic remains challenging and currently more than 20 trials involving determination of TP53-status in patients with breast cancer are listed in the National Cancer Institute Clinical trials database. Most of these trials focus on the stratification of patients to different drugs based on TP53 status, assessed by immunohistochemistry and/or TP53 mutational analyses. Other TP53-based therapies include TP53-gene therapy, use of p53 as a vaccine, or use of MDM2 inhibitors to activate TP53 antitumor response. [27, 28].
Conflicting data have been reported for the prognostic role of PIK3CA in breast cancer. Some studies have demonstrated that PIK3CA mutation is a marker of favorable survival in breast cancer [13, 29]; whereas, other authors have observed no association [30] or an unfavorable prognosis for these mutations [31]. In one of the largest studies so far, Saal et al. [32] demonstrated positive associations between PIK3CA mutation and nodal involvement, over-expression of HER2 and hormone receptor expression. More recently, Kalinsky et al. [13] showed significant associations between PIK3CA mutations and older age at diagnosis, hormone receptor positivity, HER2 negativity, lower tumor grade and stage, and lymph node negativity. The majority of PIK3CA mutations occur in three hotspots: E542K, E545K, and H1047R. E542K and E545K are located within exon 9 in the helical domain while H1047R is in exon 20 located in the kinase domain. Barbareschi et al. [31] showed that exon 9 mutations are typical of infiltrating lobular carcinomas and are independently associated with early recurrence and death, whereas exon 20 mutations are associated with improved prognosis. PI3K signaling, including PIK3CA activating mutations, may play a role in both de novo or acquired resistance to therapies that target HER2 [2, 33] [34] [35]. The validity of the PI3K pathway as a predictor of trastuzumab response is currently being assessed as a side study in the “ALTTO” clinical trial [36]. Gene expression profiling studies have resulted in the classification of subgroups of breast carcinomas which are characterized by specific gene expression patterns, i.e. Luminal A, Luminal B, HER2-enriched, Basal-like and Normal Breast-like [the “molecular subtypes”][37-39]. In addition, gene expression profiles have also been associated with clinicopathological parameters such as ER/PR status, PTEN status, PIK3CA exon 20 mutation, TP53 and BRCA1/2 mutation status, and histological grade [1, 30, 40-44]. Many of these signatures have also been shown to be prognostic factors and identify patients at increased risk of developing distant metastases [45, 46], including a 70-gene prognosis signature [1, 44], a 76 gene prognosis signature [47] and a 21-gene classifier for paraffin-embedded tissues (OncotypeDX©; Genomic Health, Redwood, California, USA) using quantitative RT-PCR [48]. Currently, large clinical trials are being conducted in order to confirm the accuracy of the 70-gene and OncotypeDX signatures [49, 50] [51]. Most studies have compared the traditional clinical and pathological risk factors with either a single or combination of these gene expression signatures. Only a limited number of studies [33, 52-55] have attempted to integrate genomic information and clinical risk factors to provide a more detailed assessment of clinical risk and treatment decisions.

The aim of this work is twofold. First we aim to investigate the association of PIK3CA and TP53 mutations with pathological features, clinical outcome and gene expression profiles in 295 breast cancers. Second we aim to evaluate whether PIK3CA or TP53 mutation status, when combined with gene expression signatures, further improves prediction for patients with early stage breast cancer.

**Material and Methods**

**Patients and Tumor Samples**

All tumors were part of a series of breast tumor specimens of 295 consecutive women with stage I and II breast cancer treated at the Netherlands Cancer Institute (NKI) between 1984 and 1995. For all samples, clinical, pathological and gene expression data were previously published
PIK3CA and TP53 mutational analysis

[1, 56]. All patient charts were reviewed and updated until 1st January 2005 [56]. The median follow-up for the updated series is 10.2 years for all patients and 12 years for patients alive at last follow-up (range 0.05–21.7). The study was approved by the medical-ethics committee of the NKI. Clinical information is summarized in Supplementary Tables 1 and 2.

Immunophenotypic analysis

Estrogen Receptor (ER-α), progesterone (PR), HER2, and TP53 status were assessed by analyzing protein expression using immunohistochemistry on tissue microarrays (TMAs) produced by a manual tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). Core tissue biopsies of 600-µm cores were taken from each individual paraffin-embedded tumor and arrayed in triplicate. Serial sections of 3 µm were cut from the tissue microarray blocks, deparaffinized in xylene, and hydrated in 100%, 90%, 70% ethanol, and \( \text{H}_2\text{O} \) respectively. Staining was performed using the Lab Vision Immunohistochemical Autostainer (Lab Vision Corporation, Fremont, CA) with primary antibodies for ER-α, PR, HER2, and TP53 according to standard protocols, Supplementary Table 3. When IHC analysis for ER-α, PR, HER2, TP53 could not be assessed on TMAs, individual paraffin-embedded tumor slides were used.

Immunohistochemical scoring systems

ER-α, PR, HER2, and TP53 were scored by an experienced pathologist (MJV). ER-α and PR were assessed based on the percentage of tumor cells showing positive nuclear staining and were considered positive if nuclear staining was present in ≥10% of the cells. HER2 expression was scored as follows: 0 for no staining or membrane staining in < 10% of the tumor cells; 1+ for a faint partial membrane staining in ≥10% of the tumor cells; 2+ for weak to moderate complete membrane staining in ≥10% of the tumor cells; 3+ for strong complete membrane staining in ≥10%. A 3+ score was considered to be HER2 positive; 0 or 1+ HER2 negative while 2+ scores were evaluated by chromogenic in situ hybridization (CISH) to assess HER2 status [57]. Tumors with HER2 gene amplification (6 or more spots per tumor cell) were scored as HER2 positive and all other tumors as HER2 negative. Samples were scored as positive for TP53 if more than 50% of tumor cells showed protein staining in the nuclei.

Mutational analysis

DNA extraction was done as described previously [58]. TP53 mutations were identified by sequencing the entire coding region of the gene (exons 2-11) using the 3730 DNA analyzer (Applied Biosystems) as described by Zhou et al [59]. SeqScape_ Software v2.5 (Applied Biosystems) was used for alignment to reference sequence (NM_000546) and scoring of mutations. Classification of TP53 mutations were done according to predicted effect of the mutated protein based on a combination of character and location of the mutation. [18] PIK3CA mutations were assessed using PCR based amplification for exons 9 and 20 and sequence analysis as previously described [33]. PCR products were purified over a QIAquick spin column (QIAGEN) and were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and an ABI 3730 automated capillary sequencer.

Prognostic gene expression signatures

For each of the 295 samples, gene expression data have previously been described [1]. Many predictors of breast cancer outcome have been derived or validated using this dataset,
including: a 70-gene prognosis signature [1, 44], the molecular subtypes [37-39], the Core Serum Response Signature (CSR) [60], chromosomal instability signature [61], hypoxia signature (HS) [62], invasiveness gene signature (IGS) [63], recurrence score [48], PTEN loss signature [41], P53 signature [42] and genomic grade index (GGI) [40]. For all signatures, except the TP53 signature where supervised clustering was used, sample assignment was retrieved from the original publication or provided by the author (Supplementary Table 4).

Statistical analysis
We assessed the association of mutation status (TP53 and PIK3CA) with clinicopathological, and molecular variables (breast cancer gene expression signatures) using either the Fisher Exact or chi-square test. p values were corrected for multiple testing using the Holm-Bonferroni method. Univariate and multivariate Cox proportional hazard analyses were performed in order to explore the relationship between clinicopathological variables and both disease free survival (DMFS) and breast cancer specific survival (BCSS). Predictive accuracy was investigated using the method of Schemper and Henderson [64], implemented in the R package Surev [65]. Briefly, multivariate Cox proportional regression models were built in a stepwise fashion by evaluating predictive accuracy instead of the usual likelihood ratio statistics (full details in Supplementary Methods). Standard errors were obtained by bootstrapping 200 samples. In order to directly compare the predictive inaccuracies of various models, only samples without any missing records were used in these analyses. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc, Chicago, IL) and R (www.r-project.org).

Results
The morphological features of 295 breast carcinomas were re-evaluated and showed that 20% of the tumors were grade I, 34% grade II and 46% grade III. Further morphological characteristics are summarized in Supplementary Table 5. Immunophenotypic analysis demonstrated that 75% of tumors stained positive for estrogen receptor (ER), 65% were positive for progesterone receptor (PR); and 21% were positive for HER2 and 21% for TP53 (Supplementary Table 6). Mutation analysis was conducted for the TP53 gene (exons 2-11) and the PIK3CA (exons 9 and 20) in breast tumors (Table 1). PIK3CA was analyzed in 262 patients, whereas TP53 mutations were successfully analyzed in 213 patients. Sixteen patients had mutations in both TP53 and one of the PIK3CA hotspots, although there is no association between tumors with a TP53 mutation and PIK3CA mutation (p =0.11).

PIK3CA mutations were identified in 79 of 262 (30%) primary breast tumors; 30 (38%) mutations were in exon 9 (E545K, E545G and E542K) and 49 (62%) in exon 20 (H1047R and H1047L). For detailed results of PIK3CA mutation analysis see Supplementary Table 7. We then investigated the association between PIK3CA status and clinicopathological parameters. Table 2 shows that PIK3CA mutated tumors were more likely to be ER and PR positive, were associated with the PTEN loss signature and with the good prognosis group defined by the 70 gene prognosis signature. We found PIK3CA mutants to be associated with the Luminal A subtype (41%) and to be least common in Basal-like breast cancers (16%) (p = 0.019). After correcting for multiple testing, tumor PIK3CA mutation status was associated only with PR status. The finding that PIK3CA mutant tumors are associated with PR positivity
status is consistent with previous reports however; we could not validate the previously found associations between PIK3CA mutation and nodal status, HER2 and grade III tumors or Basal-like tumors. In our series, PIK3CA mutations in exon 9 and 20 were not associated with prognosis, although a tendency of mutant PIK3CA to have the best prognosis is seen (Figure 1A-1D).

**TP53 mutations** for 202 out of 215 samples (94%) were successfully analyzed for all exons (exon 2-11). For detailed results of TP53 mutation analysis see Supplementary Table 7. In addition, 11 more samples were included in the further statistical analysis based on a close to complete sequence, whereas two samples were excluded. Included in the analysis were 8 samples with poor sequence quality in one exon only (4%), and 1 sample with poor sequence quality in two exons outside the conserved area with the highest frequency of mutations (exon 4-9). Two samples with a mutation were detected even though all exons were not fully sequenced (1%). Two samples with unsuccessful results (1%) were excluded from the analysis. Three samples were found to have a double mutation (1%). TP53 mutations were identified in 71 of the 213 (33%) primary breast tumors tested. 55 out of 71 mutations were located in exon 5-8 (DNA binding Domain) and 10 in the other exons (2-4 and 9-11). Six mutations were located in the splicing region of introns (8%), mostly frequently in intron 6 (6%).

We investigated the association of TP53 with clinicopathological variables and gene expression signatures in breast carcinomas (Table 2). TP53 mutation was more common in ER and PR negative and IHC TP53 positive tumors (p < 0.001), histological grade 3 tumor

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**Table 1: Mutational status for TP53 and PIK3CA in breast cancer patients**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Assignment</th>
<th>Frequency</th>
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<td>mutation</td>
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<td>E545G</td>
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PIK3CA and TP53 mutational analysis

125

(p < 0.001) and tumors with central necrosis (p < 0.001). Tumor with TP53 mutation were strongly associated with the poor outcome group(s) defined by several prognostic gene expression signatures in breast carcinomas. TP53 mutation were found in 65 out of 135 tumors with a poor prognosis signature in contrast to only 6 out of 78 tumors with a good prognosis profile (p < 0.001).

In 35 out of 45 (77%) basal-like tumors versus only 21 out of 127 (16.5%) luminal tumors a TP53 mutation (p < 0.001) was found. TP53 mutation status was also assessed by immunohistochemical (IHC) detection of mutant p53 accumulation. For 68 out of 71 tumors that contained a mutation in TP53, immunohistochemistry was available. 34 of these 68 tumors express high levels of TP53 immunohistochemically in contrast to only 12 out of 134 TP3-wildtype tumors (p < 0.001). 26 out of 40 (65%) tumors with a missense mutation showed TP53 overexpression and 8 of 28 (28%) tumors with a non-missense mutation showed TP53 overexpression (Fisher Exact p=0.006). The Kappa score of agreement between

Figure 1A: Kaplan-Meier curves for DMFS in 262 breast cancer patients stratified according to wildtype and PIK3CA mutation. Logrank p-value =0.47. Figure 1B: Kaplan-Meier curves for BCSS in 262 breast cancer patients stratified according to wildtype and PIK3CA mutation. Logrank p-value = 0.1. Figure 1C: Kaplan-Meier curves for DMFS in 262 breast cancer patients stratified according to wildtype and PIK3CA mutation in Exon9 or Exon 20. Logrank p-value = 0.54. Figure 1D: Kaplan-Meier curves for BCSS in 262 breast cancer patients stratified according to wildtype and PIK3CA mutation in Exon 9 or Exon 20. Logrank p-value =0.25.
the two methods to detect TP53 mutations was low in our series (0.44), demonstrating that this is not a preferred method to determine the TP53 mutation status. The assignment of patients into good or poor prognosis based on gene expression signatures is largely dependent on the expression levels of genes pertaining to the ‘proliferation cluster’ and ER status. In agreement with this, all prognostic gene expression signatures analyzed here (see material and methods) showed a strong association with histological grade and very strong associations with both ER and PR status (p < 0.005). ER and PR negative and HER2 and TP53 mutated tumors were overrepresented among tumors assigned to a poor prognosis gene expression signature (p < 0.001, respectively).

TP53 mutations were categorized into 4 groups as described previously [18]. Missense mutations affecting amino acids directly involved in DNA binding or zinc binding domains accounted for 27 out of 70 (39%). The remaining missense mutations affecting amino acids outside these domains accounted for 15 out of 71 tumors (21%) and non-missense mutations were found in 29 out of 71 mutations (41%). Tumors with a TP53 mutation had shorter survival times than wild type tumors (BCSS: logrank p= 0.03; DFMS: p=1.5 E-04, Figure 2A-2B). Missense mutations were associated with intermediate survival, whereas non-missense mutations have worse survival compared with no mutations (BCSS: logrank p= 0.043; DFMS: p=4.1 E-05, Figure 2C-2D). Non-missense mutations in DNA-binding domains were associated with worse survival compared to missense mutations that affect DNA binding (Figure 2E-2F).
Figure 2A: Kaplan-Meier curves for DMFS in 213 breast cancer patients stratified according to wildtype and TP53 mutation. Logrank p-value = 0.03. Figure 2B: Kaplan-Meier curves for BCSS in 213 breast cancer patients stratified according to wildtype and TP53 mutation. Logrank p-value = 1.5E-04. Figure 2C: Kaplan-Meier curves for DMFS in 213 breast cancer patients stratified according to wildtype and TP53 mutation type (missense mutation versus non-missense mutations). Logrank p-value = 0.043. Figure 2D: Kaplan-Meier curves for BCSS in 203 breast cancer patients stratified according to wildtype and TP53 mutation type (missense mutation versus non-missense mutations). Logrank p-value = 4.1E-05. Figure 2E: Kaplan-Meier curves for BCSS in 213 breast cancer patients stratified according to wildtype and TP53 mutation classification (missense mutation affecting the DNA binding domain versus missense mutation outside the DNA binding domain versus non-missense mutation versus wildtype / silent mutation). Logrank p-value = 0.08. Figure 2F: Kaplan-Meier curves for DMFS in 213 breast cancer patients stratified according to wildtype and TP53 mutation classification (missense mutation affecting the DNA binding domain versus missense mutation outside the DNA binding domain versus non-missense mutation versus wildtype / silent mutation). Logrank p-value = 1.3 E-04.
**Predictive accuracy**

We explored the benefit of including clinicopathological variables in statistical models together with either PTEN [41] or P53 expression signatures [42] in terms of the predictive accuracy for both DMFS and BCSS in this dataset. Performance was also compared to the clinical staging systems Adjuvant! Online and the Nottingham Prognostic Index (NPI). In addition, we evaluated the predictive accuracy of nine available gene expression signatures in conjunction with either PIK3CA or TP53 mutation status. Full results are presented in Supplementary data file 2. The predictive inaccuracy for a model without no independent variables is 0.296 for this dataset, representing the maximum level of inaccuracy to predict either DMFS (BCSS: 0.322). Employing gene expression information or clinical staging could improve this performance significantly.

Using either the P53 (Miller et al. PNAS 2005) or PTEN loss signature [41, 42] in a Cox model to predict outcome resulted in a marginal improvement to the predictive inaccuracy (Table 3). When histological grade and matrix formation were added to the model, the predictive inaccuracy was more substantially improved in both cases, although the level of explained variation remained low at around 13%. Both of these models had lower predictive inaccuracies than the common clinical staging systems. Adding additional clinicopathological variables, which individually explained a reasonable amount of variation present in the dataset further reduced the predictive inaccuracies (Table 3). In particular, the “best” model found that incorporates the PTEN signature and several clinicopathological variables resulted in the signature no longer being significant in the multivariate model (p>0.05). Removing the PTEN signature from the model led to no appreciable drop in predictive inaccuracy or explained variation, regardless of outcome (DMFS and BCSS). This leads to the conclusion that the PTEN signature does not provide additional prognostic information to that contained in the standard clinicopathological variables, at least for this dataset. This observation is in line with the fact that the PTEN signature [41] was developed to identify tumors that have an activated PI3K pathway, e.g. as a biomarker to identify patients for PTEN/PI3K-pathway targeted therapy. It was not developed specifically for prognosis prediction, although Saal et al. [41] did observe some prognostic associations of the PTEN signature in breast cancer patients.

To investigate the impact of TP53 and PIK3CA mutation status on predictive accuracy, the data set was reduced to 202 patients in order to remove records with missing data. For all signatures, only TP53 mutation status, specifically the mutation classification, added information with respect to any gene expression signature, although the improvement was marginal at best in all cases (range of decrease in predictive inaccuracy: DMFS 0.002-0.012; BCSS 0.008-0.023). PIK3CA status does not appear to significantly improve predictive performance in this dataset (Supplementary file 2).
Discussion

We evaluated the association of PIK3CA and TP53 mutations with pathological features, clinical outcome and gene expression profiles in 295 breast cancers and we evaluated whether PIK3CA or TP53 mutation status, when combined with gene expression signatures, further improved prediction for patients with early stage breast cancer.

The frequency of PIK3CA (30%) and TP53 (33%) mutations in our series of breast carcinomas is similar to previous published results [32] [11, 13, 16, 18, 66]. Tumors with TP53 mutations are significantly associated with aggressive tumor behaviour. We were not able to verify previous observations by Alsner or Olivier et al. [16, 18] showing that missense mutations directly involved in DNA or zinc binding were associated with a poor outcome versus missense mutations outside these regions. As has also been reported previously by other groups, we found TP53 mutations to be associated with poor survival [16, 18, 66] as well as the “basal-like” and “HER2+” gene expression subgroups [66].

We have shown previously that oncogenic mutants of PIK3CA can act as a modulator of drug sensitivity, as activated PI3K signaling caused by PIK3CA mutation conferred resistance to trastuzumab in cell culture. In a cohort of 55 breast cancer patients, activation of the PI3K pathway, as judged by the presence of oncogenic PIK3CA mutations or low PTEN expression, was associated with poor prognosis after trastuzumab containing therapy, and the combined analysis of PTEN and PIK3CA identified twice as many patients at increased risk for progression compared to PTEN alone [33]. We were interested to know whether this previously observed association with outcome might be due to a prognostic effect rather than solely a predictive marker of resistance to trastuzumab in breast cancer patients. In this study of patients not treated with trastuzumab containing therapy, PIK3CA mutations (Exon 9 and Exon20) were not associated with prognosis of breast cancer patients. We also could not validate the previously found associations between PIK3CA mutation and nodal status [32, 67], HER2 [32] and grade III tumors [67] or Basal-like tumors [67].

We also investigated several aspects of the predictive accuracy of the TP53 and PIK3CA pathways. We found that neither TP53 nor PIK3CA mutation status significantly improved the predictive accuracy of any of the 9 well-known, validated breast cancer gene expression signatures investigated. In addition, the predictive accuracy performance of the P53 and PTEN gene expression signatures, in combination with several clinicopathological variables, was only marginally improved, although a promising improvement that may be implemented in common clinical staging systems.
List of abbreviations:

TP53: tumor protein p53
PI3K: phosphatidylinositol 3-kinase (PI3K)
PIK3CA: p110α catalytic subunit
PTEN: phosphatase and tensin homolog
MDM2: Mdm2 p53 binding protein homolog
PCR: Polymerase Chain Reaction
NKI: Netherlands Cancer Institute
TMA: Tissue Microarray
IHC: immunohistochemistry
CSR: Core Serum Response
GGI: Genomic Grade Index
HS: hypoxia signature
DMFS: Distant Metastasis Free Survival
BCSS: Breast Cancer Specific Survival
NPI: Nottingham Prognostic Index
BRCA1/2: breast cancer 1 and 2, early onset
RT-PCR: real time polymerase chain reaction
HER2: v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
ER: estrogen receptor
PR: progesterone receptor
ALTTTO: Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial
HR: Hazard ratio
CI: confidence interval
DNA: Deoxyribonucleic acid
RNA: Ribonucleic acid
CISH: chromogenic in situ hybridization

Competing interests: No potential conflict of interest relevant to this article was reported

Authors’ contributions: HMH, MJV, AL and NA designed the study. MJV, KM JBS and MR provided study materials and samples. HMH, AL, KB, AA, HH, KM, JBS, MR, HH and MJV gathered data. HMH, NA, AL interpreted data. HMH, NA, AL and MJV wrote the report. All authors gave their final approval to the report.

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All Supplementary tables are available online at: https://public.me.com/huugie
Reference List

PIK3CA and TP53 mutational analysis

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