Breast Cancer Research

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Breast Cancer Research 2012, **14**:R28 doi:10.1186/bcr3113

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ISSN 1465-5411

Article type Research article

Submission date 31 January 2011

Acceptance date 13 February 2012

Publication date 13 February 2012

Article URL http://breast-cancer-research.com/content/14/1/R28

PIK3CA mutation impact on survival in breast cancer patients and in ERα, PR and ERBB2-based subgroups

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ABSTRACT

Introduction: *PIK3CA* is the oncogene showing the highest frequency of gain-of-function mutations in breast cancer, but the prognostic value of *PIK3CA* mutation status is controversial.

Methods: We investigated the prognostic significance of *PIK3CA* mutation status in a series of 452 patients with unilateral invasive primary breast cancer and known long-term outcome (median follow-up 10 years).

Results: *PIK3CA* mutations were identified in 151 tumors (33.4%). The frequency of *PIK3CA* mutations differed markedly according to hormone receptor (estrogen receptor alpha [ERα] and progesterone receptor [PR]) and ERBB2 status, ranging from 12.5% in the triple-negative subgroup (ER-/PR-/ERBB2-) to 41.1% in the HR+/ERBB2- subgroup. *PIK3CA* mutation was associated with significantly longer metastasis-free survival in the overall population (P=0.0056), and especially in the PR-positive and ERBB2-positive subgroups. In Cox multivariate regression analysis, the prognostic significance of *PIK3CA* mutation status persisted only in the ERBB2-positive subgroup.

Conclusions: This study confirms the high prevalence of *PIK3CA* mutations in breast cancer. *PIK3CA* mutation is an emerging tumor marker which might become used in treatment-choosing process. The independent prognostic value of *PIK3CA* mutation status in ERBB2-positive breast cancer patients should be now confirmed in larger series of patients included in randomized prospective ERBB2-based clinical trials.

Key words: *PIK3CA*, Breast cancer, Estrogen receptor alpha, Progesterone receptor, *ERBB2* gene, Prognostic value.

Introduction

Dysregulation of tyrosine kinase receptor (TKR)-phosphatidylinositol 3-kinase (PI3K) signaling pathways is frequent in human cancers. Among the most important molecular events downstream of TKR activation is PI3K activation, which catalyzes the phosphorylation of inositol lipids to phosphatidylinositol-3,4,5-trisphosphate. Phosphatidylinositol-3,4,5-trisphosphate activates the serine/threonine kinase AKT, which in turn regulates several signaling pathways controlling cell survival, apoptosis, proliferation, motility, and adhesion [1]. PI3K is a heterodimeric enzyme composed of a p110α catalytic subunit encoded by the *PIK3CA* gene and a p85 regulatory subunit encoded by the *PIK3R1* gene [2].

Recently, gain-of-function mutations in *PIK3CA* have been found in several cancers, including breast cancer [1, 3, 4]. *PIK3CA* is frequently mutated at "hotspots" in exons 9 and 20, corresponding to the helical (E542K and E545K) and kinase (H1047R) domains, respectively. P110α carrying a hotspot mutation shows oncogenic activity: it can transform primary fibroblasts in culture, induce anchorage-independent growth, and cause tumors in animals [5, 6].

After the *TP53* suppressor gene, the *PIK3CA* oncogene is the most frequently mutated gene in human breast cancers, mutations being observed in 20% to 40% of cases [7, 8]. Mutation is an early event in breast cancer, and is more likely to play a role in tumor initiation than in invasive progression [9]. It is noteworthy that activating somatic mutations of other oncogenes (*EGFR, KRAS, HRAS, NRAF, BRAF, AKT1,* etc.) involved in molecular events downstream of TKR activation and frequently observed in other cancers are rare in breast cancer. Several studies of breast cancer suggest that *PIK3CA* mutations are more frequent in estrogen receptor alpha (ERα-positive) breast tumors (30-40%) than in ERα-negative breast tumors (10-20%) [3, 7, 10, 11].

The prognostic value of *PIK3CA* mutation status in breast cancer is controversial. Li et al.

suggested that mutations in any part of the gene may be related to poor clinical outcome [12]. On the contrary, Maruyama et al., Perez-Tenorio et al. and Kalinsky et al. suggested that *PIK3CA* mutations were significantly and independently associated with better recurrence-free survival [11, 13, 14]. In particular, Kalinsky et al. studied a series of 590 breast cancer patients with a median follow-up of 12.8 years and found 32.5% of *PIK3CA* mutations. *PIK3CA*-mutated status was associated with markers of good prognosis and with significant improvement in overall (P=0.03) and breast cancer-specific survival (P=0.004) [11]**.** A study focused specifically on recurrent and metastatic breast cancer found also a significant association of *PIK3CA* mutations and longer relapse-free survival [15]. Barbareschi et al. reported that only *PIK3CA* exon 9 mutations were independently associated with early recurrence and death, whereas exon 20 mutations were associated with favorable outcome [16]. Several teams have found no significant effect of *PIK3CA* mutations on patient outcome [7, 8, 17, 18]. It is however noteworthy, that Loi et al. identified an expression signature derived from exon 20 *PIK3CA*-mutated tumors. This signature predicted better outcome in estrogen receptor-positive breast cancer [18]**.** In particular, the clinical consequences of *PIK3CA* mutations might vary according to the status of well-known molecular markers in breast cancer, namely *ERα, PR* and *ERBB2*.

Here we examined the prognostic value of *PIK3CA* mutation status in a series of 452 patients with unilateral invasive primary breast cancer and known long-term outcome, taking *ERα, PR* and *ERBB2* status into account.

Materials and methods

Patients and Samples

We analyzed samples of 452 primary unilateral invasive primary breast tumors excised from women at the Institut Curie / Hôpital René Huguenin (Saint-Cloud, France) from 1978 to 2008. All patients who entered our institution before year 2007 were informed that their tumor samples might be used for scientific purposes and they had opportunity to refuse it. Since 2007, patients entering our institution give their approval also by signing informed consent. This study was approved by the Local Ethical Committee (Breast Group of René Huguenin Hospital). The samples were examined histologically and were considered suitable for this study if the proportion of tumor cells exceeded 70% with sufficient cellularity as was proven by evaluation of tumor samples stained by hematoxylin and eosin. Immediately following surgery the tumor samples were placed in liquid nitrogen until RNA extraction.

The patients (mean age 61.6 years, range 31-91) met the following criteria: primary unilateral non metastatic breast carcinoma, with full clinical, histological and biological data; no radiotherapy or chemotherapy before surgery; and full follow-up at Institut Curie / Hôpital René Huguenin.

One hundred and sixty patients (35.4%) had breast-conserving surgery plus locoregional radiotherapy, and 292 patients (64.6%) had modified radical mastectomy. Clinical examinations were performed every 3 or 6 months during the first 5 years according to the prognostic risk of the patients, then yearly. Mammograms were done annually. Three hundred and sixty-six patients received adjuvant therapy, consisting of chemotherapy alone in 94 cases, hormone therapy alone in 177 cases, and both treatments in 95 cases. None of the ERBB2-positive patients was treated with anti-ERBB2 therapy. The histological type and number of positive axillary nodes were established at the time of surgery. The malignancy of

infiltrating carcinomas was scored with Bloom and Richardson's histoprognostic system.
ER and PR status was determined at the protein level by using biochemical methods (dextran-coated charcoal method or enzymatic immunoassay) until 1999 and then using immunohistochemistry. Cutoff for estrogen and progesterone receptor positivity was set at 15 fm/mg (dextran-coated charcoal or enzyme immunoassay) and at 10% immunostained cells (immunohistochemistry). A tumor was considered ERBB2-positive by IHC if it scored 3+ with uniform intense membrane staining of > 30% of invasive tumor cells. Tumors scoring 2+ were considered to be equivocal for ERBB2 protein expression and were tested by FISH for ERBB2 gene amplification. In all cases the *ERα, PR* and *ERBB2* status was also confirmed by real-time quantitative RT-PCR with cutoff levels based on previous studies comparing results of the mentioned methods [19-22]. Based on HR (*ERα* and *PR*) and *ERBB2* status, we subdivided the 452 patients into 4 subgroups, as follows: HR+ (ER+ or/and PR+) / ERBB2+ (n=53), HR+ (ER+ or/and PR+) / ERBB2- (n=287), HR- (ER- and PR-) / ERBB2+ (n=48), and HR- (ER- and PR-) / ERBB2- (n=64).
Standard prognostic factors are reported in Additional File 1, Table S1.
Median follow-up was 10.0 years (range 13 months to 28.9 years). One hundred and seventy patients developed metastases.

RNA extraction

Total RNA was extracted from breast tumor samples by using the acid-phenol guanidium method. RNA quantity was assessed using NanoDrop Spectrophotometer ND-1000 with its corresponding software (Thermo Fisher Scientific Inc., Wilmington, DE). RNA quality was determined by electrophoresis through agarose gel and staining with ethidium bromide. The 18S and 28S RNA bands were visualized under ultraviolet light. DNA contamination was quantified by using a couple of primers locating in an intron of gene coding for albumin

(ALB, Gene ID: 213). Samples were further used only when cycle threshold (Ct) obtained using these ALB intron primers was greater than 40.

PIK3CA mutation screening

PIK3CA mutations were detected by screening cDNA fragments obtained by RT-PCR amplification of exons 9 and 20 and their flanking exons. Details of the primers and PCR conditions are available on request. The amplified products were sequenced with the BigDye Terminator kit on an ABI Prism 3130 automatic DNA sequencer (Applied Biosystems, Courtabæuf, France) with detection sensitivity of 5% mutated cells, and the sequences were compared with the corresponding cDNA reference sequence (NM_006218). All the detected *PIK3CA* mutations were confirmed in the second independent run of sample testing.

Statistical analysis

Relationships between *PIK3CA* mutation status and clinical, histological and biological parameters were estimated with the χ^2 test. Differences between the mutated and non mutated populations were judged significant at confidence levels greater than 95% ($p<0.05$). Metastasis-free survival (MFS) was determined as the interval between diagnosis and detection of the first metastasis. Survival distributions were estimated with the Kaplan-Meier method [23], and the significance of differences between survival rates was ascertained with the log-rank test [24]. Cox's proportional hazards regression model [25] was used to assess prognostic significance.

Results and discussion

PIK3CA mutations were identified in 151 (33.4%) of 452 primary breast tumors, in keeping with the results of the largest previous studies, showing mutation rates of 25% to 40% [7, 8, 11, 14, 16, 18, 26-30].

Sixty-four tumors bore *PIK3CA* mutations located in exon 9, 86 tumors bore mutations in exon 20, and one tumor bore mutations in both exon 9 and exon 20 (Table 1). Exon 20 was thus the most frequently mutated *PIK3CA* exon, in keeping with most other studies [7, 8, 11, 14, 26, 28-30]. Among the 151 tumors with *PIK3CA* mutations, 3 bore double mutations: 2 in exon 20 (D1029H and H1047R, H1047R and A1066V) and 1 in exon 9 and exon 20 (E542K and M1043V). Rare double *PIK3CA* mutations have also been reported elsewhere [7, 8, 30]. We also observed two c.3203dupA frameshift mutations that would change the last C-terminal amino acid (N1068K) of the PIK3CA protein and add another three amino acids. N1068K represents 50% of all *PIK3CA* mutations in hepatocellular carcinoma [28], but its possible role in tumor initiation or progression is unknown.

Table 2 shows links between *PIK3CA* mutation status and standard clinical, pathological and biological characteristics of breast cancer. *PIK3CA* mutations were significantly associated ($\chi 2$ test) with low histopathological grade, small macroscopic tumor size, and ERα-positive, PR-positive and ERBB2-negative tumors. For example, *PIK3CA* mutations were observed in 52.7% (29/55) of histopathological grade I tumors, 36.8% (84/228) of grade II tumors, and 23.3% (37/159) of grade III tumors. These relationships have also been found in most previous studies [3, 7, 10, 11]. For example, Kalinsky et al. [11] found, like us, that *PIK3CA* mutations were associated with low histopathological grade, and ERα-positive, PR-positive and ERBB2-negative tumors. It is noteworthy, however, that no significant association

between *PIK3CA* mutations and important clinical or pathological features was found in several studies [30]. A high frequency of *PIK3CA* mutations has also been found in lobular carcinoma [16, 31]. In agreement with other authors [27, 30], we observed a similar frequency of *PIK3CA* mutations in lobular carcinomas (34.5%; 10/29) and ductal carcinomas (33.2%; 129/388) of the breast (Table 2).

Functional genomic studies have recently shown that breast cancer is a highly heterogeneous disease. Several tumor subtypes, such as basal-like, *ERBB2*-positive, and hormone receptor-positive (luminal A and luminal B), can be distinguished on the basis of their gene expression profiles, pointing to the involvement of different oncogenetic pathways. In keeping with this possibility, we observed a marked difference in the *PIK3CA* mutation frequency across four major tumor subgroups: HR+ / ERBB2+ (28.3%, 15/53), HR+ / ERBB2- (41.1%, 118/287), HR- / ERBB2+ (20.8%, 10/48), and HR- / ERBB2- (12.5%, 8/64) (P=0.00009). *PIK3CA* mutations might thus be characteristic of the luminal subtype (HR+ / ERBB2-), being found in 41.1% of cases. We also observed a low frequency (12.5%) of *PIK3CA* mutations in triple-negative tumors (ER- / PR- / ERBB2-), a subgroup reported to overlap with the basal-like subtype of breast cancer. Stemke-Hale et al. also observed a marked difference in *PIK3CA* mutation frequency across breast tumor subtypes [8], *PIK3CA* mutations being more common in hormone receptor-positive tumors (39%) and ERBB2-positive tumors (25%) than in basal-like tumors (13%).

In the overall population of 452 patients, *PIK3CA* mutation was associated with more favorable MFS (P=0.0056) (Table 3, Figure 1A). The outcome of the 151 patients with *PIK3CA* mutations was thus significantly better than that of the 301 wild-type patients as was demonstrated by 5-year and 15-year survival in these two groups (5-year MFS 81.0% versus 69.6%; 15-year MFS 65.8% versus 53.4%). Differences in treatment are unlikely to account

for this difference, as *PIK3CA* mutations were as frequent in patients who received postoperative adjuvant chemotherapy and/or hormone therapy (126/366, 34.4%) as in those who received neither treatment (25/86, 29.1%).

These data confirm the results of smaller series of breast tumors, in which *PIK3CA* mutations were significantly associated with more favorable MFS [13, 14]. However, unlike Barbareschi et al., who found that mutations in the helical (exon 9) and kinase (exon 20) domains of the *PIK3CA* gene had different prognostic values [16], we found that MFS was similar in patients with mutations in one or other exon when we compared these two subgroups together and with the wild-type subgroup (Figure 1B).

More interestingly, *PIK3CA* mutation was associated with markedly better MFS in the patients with PR-positive tumors (P=0.0064) than in those with PR-negative tumors (P=0.71) (Table 3, Figure 2A), and also in patients with ERBB2-positive tumors (P=0.014) than in those with ERBB2-negative tumors (P=0.12) (Table 3, Figure 2B). In contrast, *PIK3CA* mutation was only associated with a trend towards better MFS in patients with ERα-positive (P=0.082) and ERα-negative tumors (P=0.098) (Table 3). Accordingly, Loi et al. did not find statistically significant difference in survival between *PIK3CA* wild-type and *PIK3CA*-mutated tumors in ER-positive population. However, it is noteworthy that these authors described a *PIK3CA* mutations-associated gene expression signature predicting favorable survival in ER-positive breast cancer [18].

Using a Cox proportional hazards model, we also assessed the MFS-predictive value of the parameters that were significant in univariate analysis, i.e. SBR histological grade, lymph node status, macroscopic tumor size, ERα, PR and ERBB2 status (Additional File 1, Table S1) and *PIK3CA* mutation status. The prognostic significance of *PIK3CA* mutation status

persisted in the ERBB2-positive tumor subgroup (P=0.023) (Table 4), but not in the total tumor population or in the PR-positive tumor subgroup. Since the patients were not treated with ERBB2-targeted treatment, these results address outcome of ERBB2-positive tumors affected by surgery and chemotherapy, but not targeted therapy like trastuzumab or lapatinib. The independent prognostic value of *PIK3CA* mutation status in ERBB2-positive breast cancer patients should now be tested in a larger series of patients included in randomized prospective ERBB2-based clinical trials.

PIK3CA mutation is also an emerging tumor marker which might become used in treatment-choosing process. Indeed, ERBB2 inhibitors (trastuzumab and lapatinib) are clinically active in women with ERBB2-positive breast cancer, but recent studies suggest that *PIK3CA*-mutated tumors could be resistant to these drugs [32, 33]. There is also evidence showing that tumors with PI3K/AKT pathway activation including PTEN loss and/or *PIK3CA* mutation are less sensitive to trastuzumab treatment [17]. Interestingly, this resistance appears to be reversed by mTOR or phosphatidylinositol 3-kinase (PI3K) inhibitors [33]. A final validation of *PIK3CA* mutation as an independent predictor of the response to trastuzumab treatment in ERBB2-positive breast cancer needs a prospective randomized study. Our results also support the emerging role of *PIK3CA* mutation status in the management of future gene-based therapies (ERBB2, mTOR or PI3K inhibitors used alone or in combination) for breast cancer, particularly in patients with tumors with activated PI3K/AKT pathway [34, 35]. *ERBB2* amplification and *PIK3CA* mutation were recently validated as biomarkers of sensitivity to single-agent PI3K inhibitor (GDC-0941) therapy in breast cancer models [35].

Conclusions

This study of 452 breast tumors confirms the high prevalence (33.4%) of *PIK3CA* mutations. The frequency of *PIK3CA* mutations differed markedly according to *ERα, PR* and *ERBB2* status, from 12.5% in triple-negative tumors to 41.1% in the HR+/ERBB2- subgroup. Subgroup analysis of patient survival identified *PIK3CA* mutation status as an independent prognostic value in ERBB2-positive breast cancer patients. These findings should be confirmed in larger series of patients included in a randomized prospective ERBB2-based clinical trial. Then *PIK3CA* mutation status could serve as a new independent prognostic tool when selecting targeted therapies for patients with ERBB2-positive breast cancer.

Abbreviations: *PIK3CA*, phosphatidylinositol 3-kinase, catalytic, alpha polypeptide gene; PI3K, phosphatidylinositol 3-kinase; MFS, metastasis-free survival; ERα, estrogen receptor alpha; PR, progesterone receptor; HR, hormone receptors; RT-PCR, reverse transcriptase-polymerase chain reaction; TKR, tyrosine kinase receptors.

Competing interests: The authors declare that they have no competing interests.

Author's contributions: AS, CA and SV conceived approach to mutational analysis and designed used primers, MC, AS and SV carried out the mutational analysis, CA performed the DNA extraction, GC-C and EF performed the statistical analysis, IB, RL, MC and KD drafted the manuscript, IB and RL conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Acknowledgements: This work was supported by the Conseil régional d'Ile-de-France, the Cancéropôle Ile-de-France, the Comité départemental des Hauts-de-Seine de la Ligue Nationale Contre le Cancer and the Association pour la Recherche en Cancérologie de Saint-Cloud (ARCS).

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Figure legends

Figure 1 Whole population survival curves. (A) MFS curves of patients with *PIK3CA* wild-type and mutated tumors. (B) MFS curves of patients with exon 9 *PIK3CA*-mutated tumors, exon 20 *PIK3CA*-mutated tumors and *PIK3CA* wild-type tumors. Comparison of these curves did not show any statistically significant difference.

Figure 2 Subgroup analysis survival curves. (A) MFS curves of PR-positive patients with *PIK3CA* wild-type and -mutated tumors. (B) MFS curves of ERBB2-positive patients with *PIK3CA* wild-type and -mutated tumors.

Table 1 *PIK3CA* mutation profiles.

Exon	Nucleotide	Codon	Number of mutations
9	**c.1634A>C**	**Glu545Ala**	**2**
9	**c.1636C>A**	**Gln546Lys**	**2**
9	**c.1624G>A**	**Glu542Lys**	**20**
9	**c.1634A>G**	**Glu545Gly**	**1**
9	**c.1633G>A**	**Glu545Lys**	**32**
9	**c.1633G>C**	**Glu545Gln**	**1**
9	**c.1490A>G**	**Asn497Ser**	**1**
9	**c.1636C>A**	**Gln546Lys**	**2**
9	**c.1637A>C**	**Gln546Pro**	**1**
9	**c.1637A>G**	**Gln546Arg**	**2**
20	**c.3203dupA**	**Asn1068Lys**	**2**
20	**c.3140A>T**	**His1047Leu**	**8**
20	**c.3140A>G**	**His1047Arg**	**70**
20	**c.3132T>A**	**Asn1044Lys**	**1**
20	**c.3145G>C**	**Gly1049Arg**	**2**
20	**c.3155C>A**	**Thr1052Lys**	**1**
20	**c.[3085>C(+)3140A>T]**	**p.[Asp1029His(+)His1047Leu]**	**1**
20	**c.[3140A>T(+)3197C>T]**	**p.[His1047Leu(+)Ala1066Val]**	**1**
9+20	**c.[1624G<A(+)3127A>G]**	**p.[Glu542Lys(+)Met1043Val]**	**1**
			Total =151

Table 2 Relationship between *PIK3CA* mutation status and standard clinical, pathological and biological features of breast cancer.

		Number of patients (%)		*P*-value[a]
	Total population (%)	*PIK3CA* wild-type	*PIK3CA* mutated	
Total	452 (100.0)	301 (66.6)	151 (33.4)	
Age				
≤50	96 (21.2)	66 (21.9)	30 (19.9)	NS
>50	356 (78.8)	235 (78.1)	121 (81.1)	
SBR histological grade[b,c]				
I	55 (12.4)	26 (8.9)	29 (19.3)	**0.00021**
II	228 (51.6)	144 (49.3)	84 (56.0)	
III	159 (36.0)	122 (41.8)	37 (24.7)	
Lymph node status[d]				
0	115 (25.5)	78 (26.0)	37 (24.5)	NS
1-3	237 (52.5)	157 (52.3)	80 (53.0)	
>3	99 (22.0)	65 (21.7)	34 (22.5)	
Macroscopic tumor size[e]				
≤25mm	217 (48.8)	135 (45.2)	82 (56.2)	**0.029**
>25mm	228 (51.2)	164 (54.8)	64 (43.8)	
ERα status				
Negative	117 (25.9)	97 (32.2)	20 (13.2)	**0.000014**
Positive	335 (74.1)	204 (67.8)	131 (86.8)	
PR status				
Negative	194 (42.9)	150 (49.8)	44 (29.1)	**0.000028**
Positive	258 (57.1)	151 (50.2)	107 (70.9)	
ERBB2 status				
Negative	351 (77.7)	225 (74.8)	126 (83.4)	**0.036**
Positive	101 (22.3)	76 (25.2)	25 (16.6)	
Histology				
Ductal	388 (85.8)	259 (86.0)	129 (85.5)	NS
Lobular	29 (6.4)	19 (6.3)	10 (6.6)	
Others	35 (7.8)	23 (7.7)	12 (7.9)	

[a]$\chi 2$Test. NS: not significant.

[b]Scarff Bloom Richardson classification.

[c]Information available for 442 patients.

[d]Information available for 451 patients.

[e]Information available for 445 patients.

Table 3 *PIK3CA* mutation status according to hormone receptor and ERBB2 status, and relation to metastasis-free survival.

	Number of patients	5-y MFS	HR (95% CI)[a]	*P*-value[b]
Total population	*452*			
Wild-type	301	69.6 %	1	**0.0056**
Mutated	151	81.0 %	0.62 (0.44-0.87)	
ERα-positive	*335*			
Wild-type	204	75.6 %	1	NS
Mutated	131	81.9 %	0.71 (0.49-1.04)	
ERα-negative	*117*			
Wild-type	97	56.9 %	1	NS
Mutated	20	75.0 %	0.46 (0.18-1.15)	
PR-positive	*258*			
Wild-type	151	77.8 %	1	**0.0064**
Mutated	107	86.6 %	0.52 (0.33-0.83)	
PR-negative	*194*			
Wild-type	150	61.3 %	1	NS
Mutated	44	67.6 %	0.91 (0.55-1.50)	
ERBB2-positive	*101*			
Wild-type	76	59.9 %	1	**0.014**
Mutated	25	88.0 %	0.31 (0.12-0.79)	
ERBB2-negative	*351*			
Wild-type	225	72.9 %	1	NS
Mutated	126	79.7 %	0.75 (0.51-1.08)	

[a]Hazard Ratio and 95% confidence interval.

[b]Univariate Cox analysis. NS: not significant.

Table 4 Multivariate Cox analysis of Metastasis-free survival in the total population and breast cance

Variables	Total population		ER-positive patients		ER-negative patients		PR-positive patients		
	HR (95% CI)[a]	*P*-value[b]	HR (95% CI)[a]	*P*-value[b]	HR (95% CI)[a]	*P*-value[b]	HR (95% CI)[a]	*P*-value[b]	H
SBR									
I	1	**0.038**	1	**0.019**	1	NS	1	**0.00077**	
II	1.34 (1.02-1.76)		1.45 (1.06-1.98)		1.02 (0.57-1.80)		1.88 (1.30-2.71)		0.
III	1.79 (1.03-3.11)		2.11 (1.13-3.92)		1.03 (0.33-3.26)		3.52 (1.69-7.32)		0.
pN									
0	1	**0.00014**	1	**0.00093**	1	NS	1	**0.0068**	
1-3	1.58 (1.25-1.99)		1.60 (1.21-2.11)		1.45 (0.94-2.25)		1.61 (1.14-2.27)		1.
>3	2.48 (1.56-3.96)		2.56 (1.47-4.47)		2.11 (0.88-5.05)		2.59 (1.30-5.18)		2.
pT									
≤25mm	1	**0.01**	1	**0.00041**	1	NS	1	**0.0023**	
>25mm	1.53 (1.11-2.13)		2.05 (1.38-3.05)		0.80 (0.44-1.47)		2.09 (1.30-3.36)		1.
ER									
Negative	1	NS	-	-	-	-	1	NS	
Positive	1.04 (0.68-1.60)		-		-		2.09 (0.28-15.63)		0.
PR									
Negative	1	NS	1	NS	1	NS	-	-	
Positive	0.77 (0.52-1.14)		0.81 (0.54-1.23)		0.31 (0.04-2.33)		-		
ERBB2									
Negative	1	NS	1	NS	1	NS	1	NS	
Positive	1.12 (0.77-1.62)		1.01 (0.60-1.70)		1.33 (0.74-2.38)		0.98 (0.50-1.93)		1.
PIK3CA									

[a]Hazard Ratio and 95% confidence interval.

[b]Multivariate Cox analysis. NS: not significant.

Additional files

Additional file 1 Table S1

Adode Acrobat type of file (.pdf)

Characteristics of the 452 primary breast tumors, and relation to metastasis-free survival.

A table showing metastasis free survival of the patients in relation to pathological data.

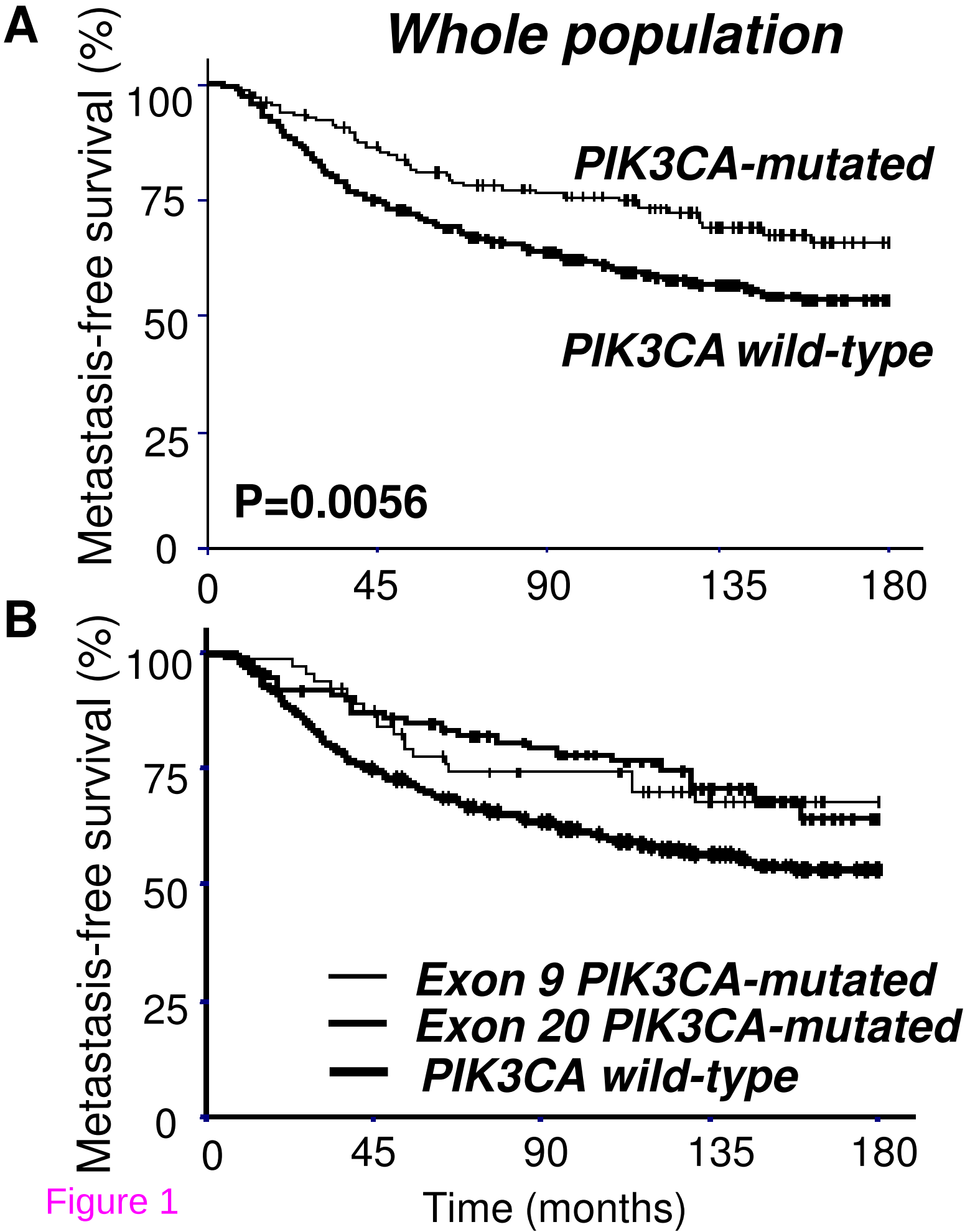
A
Whole population
Metastasis-free survival (%)
100
75
50
25
0
PIK3CA-mutated
PIK3CA wild-type
P=0.0056
0
45
90
135
180
B
Metastasis-free survival (%)
100
75
50
25
0
Exon 9 PIK3CA-mutated
Exon 20 PIK3CA-mutated
PIK3CA wild-type
0
45
90
135
180
Time (months)

Figure 1

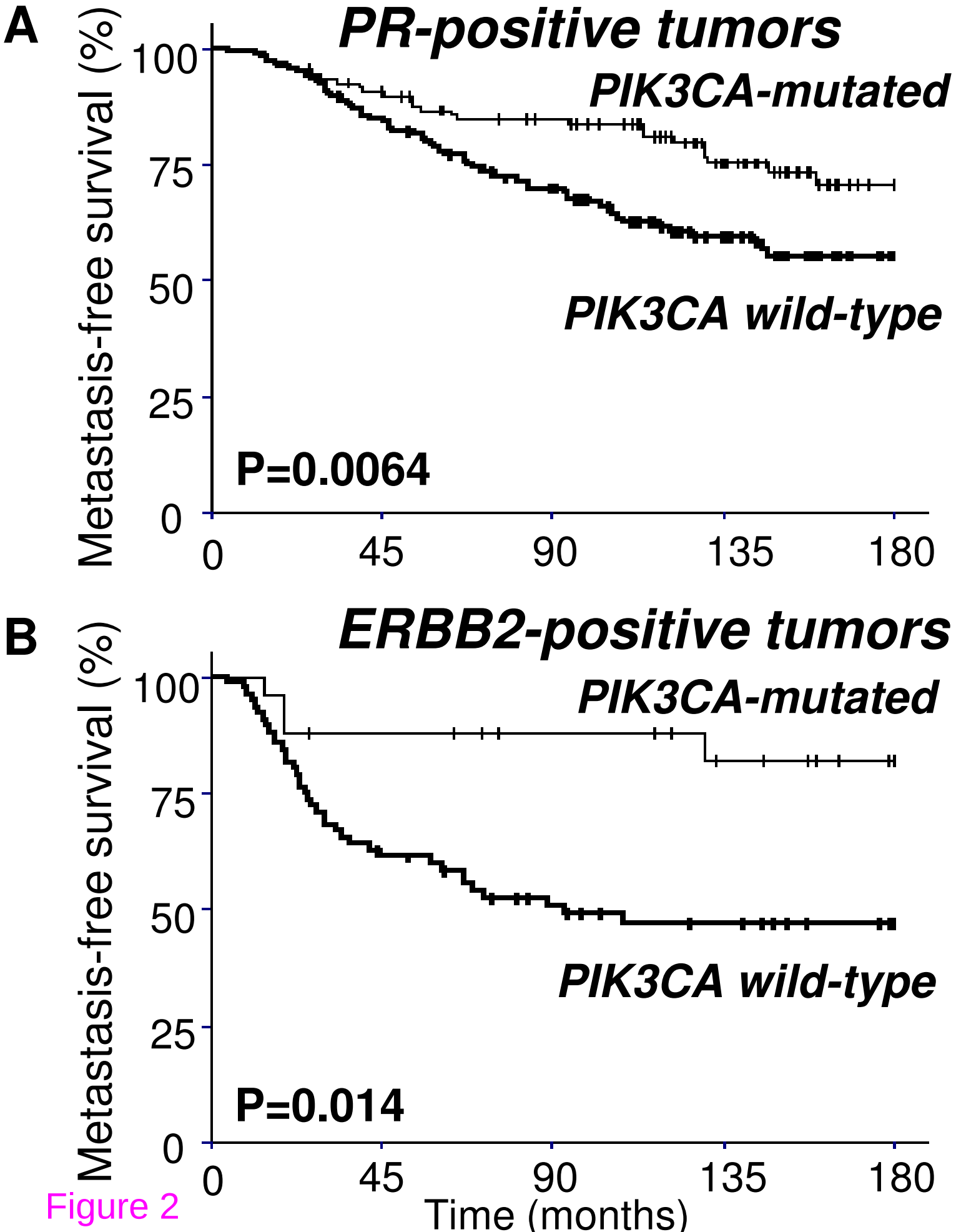
A
PR-positive tumors
PIK3CA-mutated
PIK3CA wild-type
P=0.0064
Metastasis-free survival (%)
100
75
50
25
0
0
45
90
135
180
B
ERBB2-positive tumors
PIK3CA-mutated
PIK3CA wild-type
P=0.014
Metastasis-free survival (%)
100
75
50
25
0
0
45
90
135
180
Time (months)

Figure 2

Additional files provided with this submission:

Additional file 1: sup2.pdf, 43K
http://breast-cancer-research.com/imedia/7587021266385656/supp1.pdf