Chinese Journal of Cancer

Original Article

# The favorable impact of *PIK3CA* mutations on survival: an analysis of 2587 patients with breast cancer

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**Abstract**

The phosphatidylinositol-3 kinase (PI3K) pathway regulates a number of cellular processes, including cell survival, cell growth, and cell cycle progression. Consequently, this pathway is commonly deregulated in cancer. In particular, mutations in the gene *PIK3CA* that encodes the p110α catalytic subunit of the PI3K enzymes result in cell proliferation and resistance to apoptosis *in vitro* and induce breast tumors in transgenic mice. These data underscore the role of this pathway during oncogenesis. Thus, an ongoing, large-scale effort is underway to develop clinically active drugs that target elements of the PI3K pathway. However, conflicting data suggest that gain-of-function *PIK3CA* mutations may be associated with either a favorable or a poor clinical outcome, compared with the wild-type *PIK3CA* gene. In the current study, we performed a systematic review of breast cancer clinical studies. Upon evaluation of 2587 breast cancer cases from 12 independent studies, we showed that patients with tumors harboring a *PIK3CA* mutation have a better clinical outcome than those with a wild-type *PIK3CA* gene. Importantly, this improved prognosis may pertain only to patients with mutations in the kinase domain of p110α and to postmenopausal women with estrogen receptor-positive breast cancer. We propose three potential explanations for this paradoxical observation. First, *PIK3CA* mutations may interfere with the metastasis process or may induce senescence, which results in a better outcome for patients with mutated tumors. Secondly, we speculate that *PIK3CA* mutations may increase early tumor diagnosis by modification of the actin cytoskeleton in tumor cells. Lastly, we propose that *PIK3CA* mutations may be a favorable predictive factor for response to hormonal therapy, giving a therapeutic advantage to these patients. Ultimately, an improved understanding of the clinical impact of *PIK3CA* mutations is critical for the development of optimally personalized therapeutics against breast cancer and other solid tumors. This effort will be important to prevent or explain therapeutic failures and select patients who are most likely to respond to new therapies that inhibit the PI3K pathway.

**Key words** PI3K, *PIK3CA* gene, mutation, breast cancer

Phosphatidylinositol-3 kinases (PI3Ks) are a well-characterized family of lipid kinases that were originally identified by their ability to phosphorylate the 3-hydroxy group of inositol phospholipids. In normal cells, this reaction is tightly regulated and leads to the activation of several cellular processes, including metabolism, proliferation, vesicle trafficking, and survival[1,2]. PI3Ks are divided into three different classes (I-III) based on structural homology and substrate[3,4]. The PI3K type that is dysregulated in cancer is the Class I heterodimer, which is composed of regulatory and catalytic subunits. This class is divided into Subclass IA and Subclass IB. Subclass IA members are activated by ligand binding of receptor tyrosine kinases (RTK), whereas Subclass IB members are activated by G protein-coupled receptors. A single activated receptor may then activate multiple downstream molecules, resulting in the signal amplification of a zymogen cascade. Specifically, activated PI3Ks catalyze the phosphorylation of

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**doi:** 10.5732/cjc.012.10032

phosphatidylinositol-4,5 bisphosphate ($PIP_2$) to produce the second messenger phosphatidylinositol-3,4,5 trisphosphate ($PIP_3$). The generation of $PIP_3$ activates downstream signaling effector proteins, including the serine/threonine kinase AKT. The activation of AKT molecules plays a key regulatory role by targeting multiple proteins, including Bad, FOXO, Cyclin D1, GSK3β , MDM2, P27, and the mammalian target of rapamycin (mTOR), resulting in cellular transformation, survival, and proliferation (Figure 1)[5,6]. The Subclass IA PI3K consists of a p85 regulatory subunit and a p110 catalytic subunit. Three genes, *PIK3R1*, *PIK3R2*, and *PIK3R3*, encode three isoforms of the p85 regulatory subunit. Additionally, the *PIK3R1* gene gives rise to two shorter isoforms through alternative splicing. The five p85 isoforms have a common core structure consisting of a p110-binding domain surrounded by two Src-homology-2 domains (SH2) (Figure 2). The three isoforms of the p110 catalytic subunit are encoded by three genes: *PIK3CA*, *PIK3CB*, and *PIK3CD*. All three isoforms possess an N-terminal p85-binding domain (also referred to an adaptor-binding domain), a Ras-binding domain, a C2 domain (membrane-binding domain), a helical domain (HD) of unknown function, and a C-terminal catalytic kinase domain (KD) (Figure 2).

In breast cancer, somatic mutations of *PIK3CA* on chromosome 3q26 are commonly found and are reported in the literature in 18% to 40% of cases[7-11]. The publically available COSMIC database includes 5838 breast tumor samples, wherein 1493 tumors harbor mutations in *PIK3CA*, giving a mutation rate of 26% [12]. Additionally, mutations are observed in more than half of breast cancer cell lines[13]. This frequency suggests an important role of this gene in tumorigenesis. Furthermore, *PIK3CA* mutations induce tumor formation in transgenic mice[14,15].

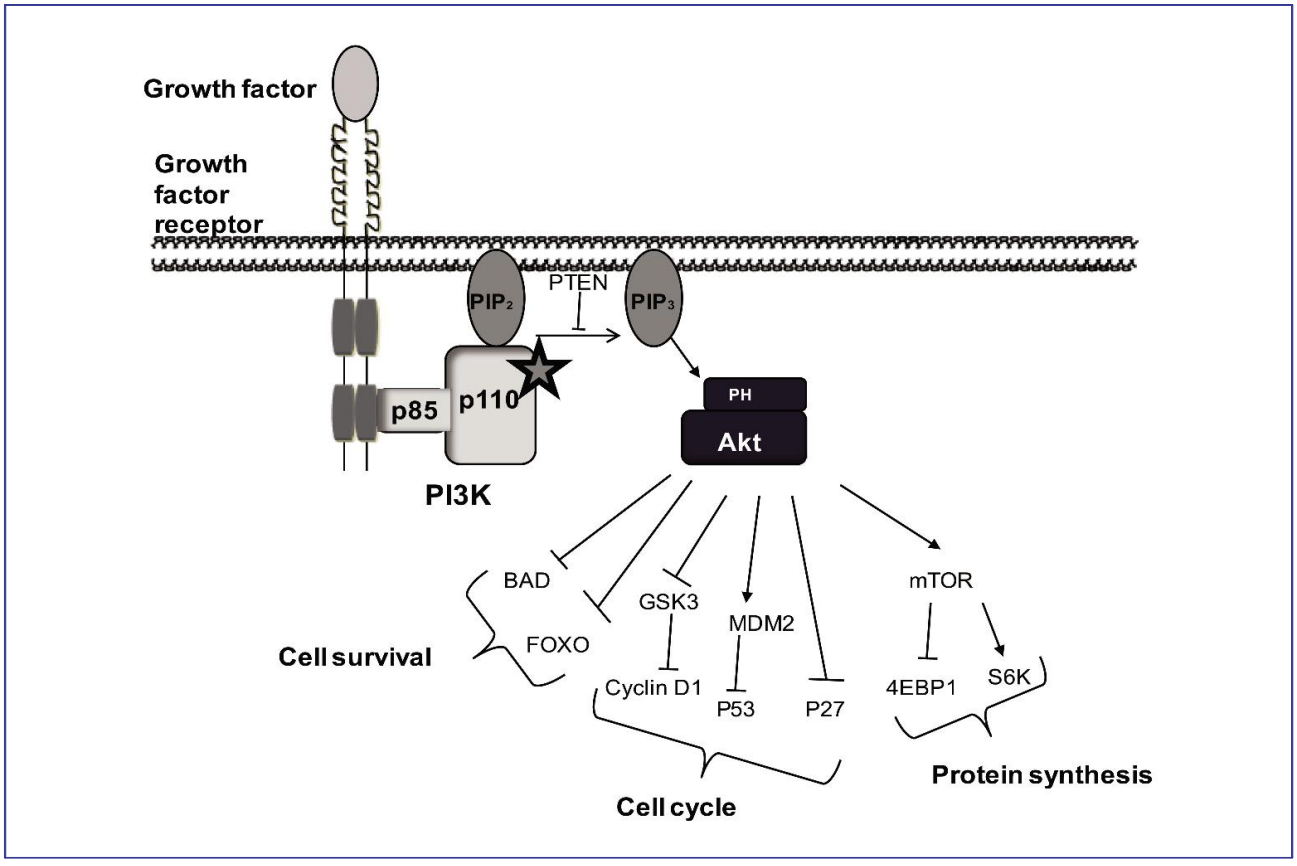


Figure 1. **Downstream signaling of phosphatidylinositol-3 kinase (PI3K) in cancer.** After activation and phosphorylation of growth factor receptors by a ligand, PI3K is recruited to the membrane through the p85 adaptor subunit, resulting in the activation of the p110 catalytic subunit and production of phosphatidylinositol-3,4,5 trisphosphate ($PIP_3$). This second messenger forms a docking site for cytoplasmic proteins that carry pleckstrin homology (PH) domains to the membrane, including the serine threonine kinase AKT. Once activated, AKT mediates the activity of several target proteins, which results in cell survival, proliferation, and protein synthesis. In this schematic representation, lines indicate either phosphorylation leading to activation (arrow) or inhibition (blunt end) of downstream targets. The star represents gain-of-function mutations in the p110 subunit.

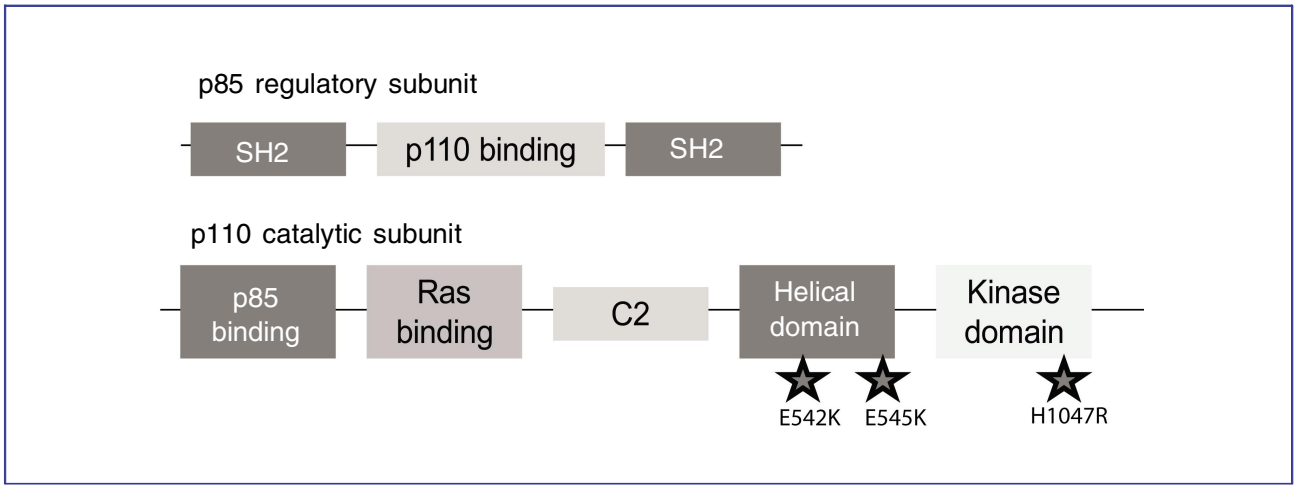


Figure 2. **PI3K proteins and functional domains.** The Subclass IA PI3Ks are heterodimers that consist of a p85 regulatory subunit and a p110 catalytic subunit. Key functional domains are identified. The three most common mutations in the *PIK3CA* gene are depicted with stars.

The majority of mutations occur at three hotspots: E542K, E545K, and H1047R. The first two hotspots are in the HD (exon 9), whereas the last hotspot is in the KD (exon 20) (Figure 2). These activating *PIK3CA* mutations enhance the lipid kinase activity to a level higher than that of wild-type *PIK3CA*, leading to increased phosphorylation and activity of downstream targets [11]. Different mechanisms lead to the gain-of-function mutations in the HD and the KD. Recent structural analyses have shown that mutations in the HD abrogate the inhibitory interaction of the SH2 domains of p85, whereas a mutation in the KD alters the tertiary structure of the catalytic subunit such that it increases the accessibility for substrates [2]. Mutations in the *PIK3CA* gene are not the only deregulations of the PI3K pathway described. Gene amplification of *PIK3CA*, loss of *PTEN*, and mutations of *AKT1* have also been reported.

Considering the key regulatory functions of the PI3K pathway and its common deregulation in breast cancer, we could predict that activating mutations of *PIK3CA* relates with a more aggressive tumor, resulting in poor patient prognosis and shorter survival. To test this hypothesis, we performed a systematic review of breast cancer clinical studies.

## *PIK3CA* Mutations and Breast Cancer Patient Survival: A Blurred Picture

To address the clinical impact of *PIK3CA* mutations on breast cancer, we performed a search on PubMed using the following keywords: "breast", "cancer", "pik3ca", and "mutation" (December 1st, 2011). We identified 12 studies[16-27] from the 119 abstracts evaluated. Clinical characteristics of the studies are summarized in Table 1. The 12 studies reported the outcomes of 2587 breast cancer patients. The discrepancies in observed clinical outcomes are likely due to variations in patient population and tumor characteristics. In fact, the effects of *PIK3CA* mutations are likely dependent on the subtype of breast cancer and the location where the mutation in *PIK3CA* occurs. Indeed, estrogen receptor (ER)-positive and HER2-positive breast tumors are two molecularly distinct diseases that differ in both biological and clinical behaviors[28]. Moreover, previous studies suggest that mutations in the HD and the KD have different biological and clinical consequences.

In the 12 studies analyzed, 4 studies reporting outcomes of 1211 women found that *PIK3CA* mutations was associated with either a longer disease-free survival (DFS) or overall survival (OS)[16-18,22] (Table 1). In these 4 studies, multivariate analyses confirmed that *PIK3CA* mutations were associated with a favorable prognosis regardless of other conventional prognostic factors. In the largest study, Kalinski *et al.* [16] analyzed samples from 590 patients with primary invasive ductal or lobular breast carcinomas, with a long-term patient follow-up of 12.8 years, using a Sequenom MassArray system. Patients with *PIK3CA* mutations had superior DFS, OS, and breast cancer-specific survival compared with those without *PIK3CA* mutations. Interestingly, analysis of *PIK3CA* mutation sites revealed that HD hotspot mutations were not associated with an improvement in OS (10-year OS rate was 62% for patients with wild-type *PIK3CA* vs. 59% for those with HD mutations, $P = 0.54$). In contrast, KD mutations was associated with superior OS when compared with wild-type *PIK3CA* (10-year OS rate was 62% for patients with wild-type *PIK3CA* vs. 76% for those with KD mutations, $P = 0.005$). A similar finding was also reported by Barbareschi *et al.* [18], in

**Table 1. Characteristics of the analyzed studies**

| Study | Total cases | Median follow-up (years) | Node positive (%) | HR positive (%) | *PIK3CA* mutation (%) | Association between *PIK3CA* mutation and prognosis (%) |
|---|---|---|---|---|---|---|
| Kalinsky *et al.* [16] | 590 | 12.8 | 42 | 69 | 33 | Better OS ($P$ = 0.03)[a] |
| Maruyama *et al.*[17] | 188 | 5.3 | 35 | 66 | 28 | Better RFS ($P$ = 0.02)[a] |
| Barbareshi *et al.*[18] | 163 | No data | 52 | 84 | 53 | Better OS ($P$ = 0.001)[a] |
| Loi *et al.*[19] | 173 | 9.6 | 50 | 100 | 26 | NS[b] |
| Michelucci *et al.*[20] | 176 | 6.8 | 51 | 76 | 38 | NS[b] |
| Boyault *et al.*[21] | 120 | 2 | 78 | 37 | 21 | NS[b] |
| Perez-Tenorio *et al.*[22] | 270 | 11 | 88 | 70 | 24 | Better local RFS ($P$ = 0.02)[a] |
| Saal *et al.*[23] | 292 | 4.4 | 56 | 55 | 26 | NS |
| Stemke-Hale *et al.*[24] | 157 | 7.5 | 35 | 100 | 35 | NS |
| Lai *et al.*[25] | 152 | 6.6 | 53 | 80 | 26 | NS |
| Li *et al.*[26] | 250 | 4.2 | 47 | 69 | 35 | Worse OS ($P$ = 0.004) |
| Lerma *et al.*[27] | 56 | 6.3 | 89 | 0 | 13 | Worse OS ($P$ = 0.015) |

HR, hormone receptor; OS, overall survival; RFS, recurrence-free survival; NS, not significant. The association between *PIK3CA* mutations and clinicopathologic parameters is shown in the 12 studies analyzed. Association of mutation status and PFS or OS was assessed by the Kaplan-Meier method and Cox proportional hazards models with 2-sided $P$ values. [a]Multivariate analysis confirmed that the prognostic factor is independent to other conventional prognostic factors. [b]Trend in favor of a longer OS.

which patients with KD mutations showed a significantly better OS. Data from this study also demonstrated that patients with HD mutations had a worse prognosis.

Six studies did not find a significant relationship between somatic *PIK3CA* mutations and either DFS or OS [19-21,23-25]. Nevertheless, 3 of these studies reported a favorable trend that related *PIK3CA* mutations with improved clinical outcomes. However, these differences were not significant, possibly due to the small sample size and the subsequent lack of power of these studies [19,20,24].

Against evidences presented by the above-mentioned studies, one study found that, although the OS was not significantly different in patients with all *PIK3CA* mutations, specific mutations in the KD were independently associated with a shorter OS [25]. Two additional studies that included 306 patients found that *PIK3CA* mutations were associated with a poor OS [26,27]. Nevertheless, these two studies have several limitations. In the first study, by Li *et al.*[26], the mutation status failed to reach significance as an independent prognostic factor of OS. The second study, by Lerma *et al.*[27], addressed only the role of *PIK3CA* mutations in HER-2- positive breast cancer while excluding patients with hormone receptor-positive tumors.

To analyze the association between *PIK3CA* mutations and ER status, relevant studies containing data on both of these factors were combined and a strong association between *PIK3CA* mutations and ER positivity was found ($P$ < 0.001) (Table 2). Furthermore, wild-type *PIK3CA* was associated with higher tumor stage ($P$ < 0.03) (Table 3). In both cases, we excluded studies that lacked necessary information.

We propose that the clinical benefit observed in the presence of *PIK3CA* mutations may pertain mainly to ER-positive breast tumors and mutations in the KD. The unexpected and counterintuitive observation that *PIK3CA* mutations appear to be associated with a better clinical outcome in some patients remains unsolved. Below, we propose three hypotheses to explain this paradoxical association of oncogene activation with better patient outcomes.

## *PIK3CA* Mutations May Suppress Metastasis and Induce Senescence

As metastatic disease is the leading cause of recurrence and death in breast cancer patients, we hypothesize that the PI3K pathway activation through *PIK3CA* gain-of-function mutations, in particular in the KD domain, may suppress metastasis, which could translate into observed clinical benefits. Indeed, our analysis found that *PIK3CA* mutations were associated with lower tumor stages (Table 3). Supporting this hypothesis, Pang *et al.* [29] have recently shown that expression of KD mutations in MDA-MB-231 cells rendered the cells less chemotactic and led to a decrease in blood intravasation and lung extravasation *in vivo*, compared with mutations in the HD. Additionally, Yoeli-Lerner *et al.*[30] have shown that overexpression of *AKT1*, the major downstream target of PI3K, in breast cancer cell lines resulted in a decrease in migration and invasion. In this hypothesis, *PIK3CA* mutations may

**Table 2.** ***PIK3CA*** **mutations and estrogen receptor (ER) status in breast cancer patients**

| Study | Estrogen receptor-positive status [% (*n*)] | |
|---|---|---|
| | Breast tumors with wild-type *PIK3CA* | Breast tumors with *PIK3CA* mutations |
| Kalinsky *et al.*[16] | 57% (225/398) | 73% (141/192) |
| Maruyama *et al.*[17] | 61% (82/134) | 78% (42/54) |
| Barbareschi *et al.*[18] | 83% (99/118) | 84% (38/45) |
| Michelucci *et al.*[20] | 70% (58/82) | 85% (40/47) |
| Perez-Tenorio *et al.*[22] | 67% (136/202) | 80% (52/65) |
| Saal *et al.*[23] | 52% (106/204) | 78% (56/72) |
| Li *et al.*[26] | 61% (99/162) | 78% (69/88) |
| **Total** | **62% (743/1208)** | **78% (415/535)** |

All data are expressed as "positive rate (positive cases / total cases)." Only studies containing data on both *PIK3CA* mutations and ER status were selected. The association between *PIK3CA* mutations and ER status was assessed by the $\chi^2$ test with 2-sided *P* values. *PIK3CA* mutations are associated with ER positivity ($P < 0.001$).

**Table 3.** ***PIK3CA*** **mutations and tumor stage in breast cancer patients**

| Study | Stage 3 or 4 tumors [% (*n*)] | |
|---|---|---|
| | Breast tumors with wild-type *PIK3CA* | Breast tumors with *PIK3CA* mutations |
| Kalinsky *et al.*[16] | 6% (24/398) | 4% (8/192) |
| Maruyama *et al.*[17] | 17% (19/112) | 6% (3/49) |
| Barbareschi *et al.*[18] | 14% (17/118) | 11% (5/45) |
| Saal *et al.*[23] | 8% (18/215) | 5% (4/77) |
| Stemke-Hale *et al.*[24] | 18% (18/101) | 13% (7/56) |
| **Total** | **10% (96/944)** | **6% (27/419)** |

All data are expressed as "positive rate (positive cases / total cases)." Only studies containing data on both *PIK3CA* mutations and ER status were selected. The association between *PIK3CA* mutations and tumor stages was assessed by the $\chi^2$ test with 2-sided *P* values. *PIK3CA* mutations are associated with few stage 3 or 4 tumors ($P = 0.03$)

interfere with metastatic progression of breast cancer cells, while simultaneously accelerating the process of primary tumor formation. Recently, Jensen *et al.* [31] assessed the *PIK3CA* mutation status in both primary breast tumors and corresponding metastases and showed a high discrepancy between primary tumors and metastases with either a gain or a loss of *PIK3CA* mutation in metastases. Furthermore, in this study, *PIK3CA* mutations were associated with a longer time to recurrence. Together, these data suggest that *PIK3CA* mutations did not confer significant advantages for metastasis and may, actually, decrease the metastasis formation. To definitively address this question, it would be useful to study spontaneous metastases in genetically engineered mice (GEM) carrying *PIK3CA* mutations in combination with other oncogenic events.

An alternative hypothesis is that *PIK3CA* mutations may induce senescence, resulting in a less aggressive phenotype compared with activation of other pathways. There is substantial evidence demonstrating that activated oncogenes can induce senescence *in vitro*, and this phenomenon is a potent antitumor barrier *in vivo* [32-34]. In this hypothesis, *PIK3CA* mutations may be important in the initiation of cell transformation but may result in senescence that has to be overcome in the tumor cell via additional genetic or epigenetic alterations.

## *PIK3CA* Mutations May Increase the Chance of Early Detection of Tumors

Early detection is believed to decrease patient mortality by allowing therapeutic intervention prior to tumor metastases. In breast cancer, a study indicated that mammography led to a 15% reduction in mortality, in particular in high risk patients [35]. In the current analysis, we found that *PIK3CA* mutations associated with lower tumor stages, which could be explained by an early diagnosis of these tumors. As a result, we

speculate that *PIK3CA* mutations may increase the probability of early detection by generating a tumor that is noticed earlier by the patient or her physician. This could be due to pain, location in the breast, or more firm nodularity. It is already well described that *PIK3CA* mutations can stimulate reorganization of the actin cytoskeleton in cells. For example, in the MCF-7 cell line, which carries a *PIK3CA* mutation, the major morphologic response to estrogen is the development of solid, large, multicellular nodules in postconfluent cultures[36,37]. In this case, the typical epithelial organization is lost to allow for multilayering and nodulation, which lead to changes in the architecture of the cytoskeleton system. This reorganization may change the density of the tumor, increasing the detectability of breast tumors by self-examination, physician examination, and x-ray mammography.

## *PIK3CA* Mutations May Be a Favorable Predictive Factor for Response to Hormonal Therapy

The last potential explanation for why *PIK3CA* mutations are associated with an increase in survival rate of some patients is that, although these mutations may enhance tumorigenicity, the same mutations may improve the response to treatments. In this hypothesis, *PIK3CA* mutations may increase hormonal therapy (tamoxifen) sensitivity and would not be a prognostic factor but rather a predictive factor for response. Our study found that *PIK3CA* mutations were significantly higher in ER-positive tumors than in ER-negative tumors (Table 2). We speculate that this positive association between *PIK3CA* mutations and ER-positive tumors is due to cross-talk between the PI3K and ER pathways. Indeed, it has been shown that AKT phosphorylates the Ser167 residue of ER and increases its transcriptional activity, resulting in the preferential growth of ER-positive cells in the presence of *PIK3CA* mutations. Additionally, it is possible that negative feedback secondary to PI3K activation yields insensitivity to growth factor receptor stimulation, delaying the development of hormonal therapy resistance. Given that almost all ER-positive breast cancer patients are treated with tamoxifen or aromatase inhibitors, the generally favorable prognosis observed in *PIK3CA*-mutated tumors may simply be the consequence of a specific sensitivity of these tumors to hormonal therapy.

## *PIK3CA* Mutations and the Clinical Development of PI3K Inhibitors

Several drugs targeting PI3K are currently being evaluated in clinical trials, particularly in breast cancer (Table 4)[38,39]. These molecules include pan-PI3K inhibitors, PI3Kα inhibitors, and dual PI3K/mTOR inhibitors. The association of *PIK3CA* mutations and good clinical outcomes does not mean that patients with *PIK3CA* mutations will not benefit from PI3K inhibitors. Some results from early clinical trials using PI3K inhibitors (BKM120, BEZ235, and XL147) are already available[40-43]. Although the data show a modest activity of these drugs as a single agent with a response rate of approximately 10%, patients with *PIK3CA* mutations seem to have a higher response rate than patients with wild-type

**Table 4. Agents targeting PI3K currently being tested in breast cancer clinical trials**

| Drug | Target | Clinical trial | Company |
|---|---|---|---|
| BEZ235 | PI3K/mTOR dual inhibitor | Phase I | Novartis |
| BGT226 | PI3K/mTOR dual inhibitor | Phase II | Novartis |
| BKM120 | pan-PI3K inhibitor | Phase II | Novartis |
| BYL719 | PI3Kα inhibitor | Phase I | Novartis |
| GDC0941 | PI3Kα inhibitor | Phase II | Genentech |
| GDC0032 | PI3Kα inhibitor | Phase I | Genentech |
| GSK1059615 | PI3K/mTOR dual inhibitor | Phase I | GSK |
| INK1117 | PI3Kα inhibitor | Phase I | Intellikine |
| PF04691502 | PI3K/mTOR dual inhibitor | Phase II | Pfizer |
| PKI587 | PI3K/mTOR dual inhibitor | Phase I | Pfizer |
| PX866 | pan-PI3K inhibitor | Phase I | Oncothyreon |
| XL147 | PI3Kα inhibitor | Phase II | Exelixis |
| XL765 | PI3K/mTOR dual inhibitor | Phase II | Exelixis |
| ZSTK474 | PI3K inhibitor | Phase I | Zenyaku Kogyo |

Information from www.ClinicalTrials.gov.

*PIK3CA*. Nevertheless, these phase I trials included few breast cancer patients and the results need to be confirmed in randomized clinical trials to draw definitive conclusions. Additionally, as is the case with other targeted therapies, a combined approach is more likely to produce a better clinical response than a single-agent strategy.

## Conclusions

The role of the PI3K pathway in oncogenesis has been extensively studied in breast cancer. Genetic aberrations of this pathway, such as *PIK3CA* mutations, loss of PTEN, and AKT activation, make this pathway a commonly activated one in breast cancer. As a result, this pathway has become an attractive target for drug development. To date, more than ten molecules targeting PI3K are being tested in clinical trials. The present analysis of several retrospective studies revealed a counterintuitive finding that gain-of-function mutations in *PIK3CA* are associated with superior clinical outcome in breast cancer patients, in particular for women with ER-positive tumors and mutations in the KD. As is frequently the case in translational research, these clinical data encourage us to look more closely at the biology of this pathway to develop a true understanding of the mechanisms underlying this paradoxical observation. This knowledge will have important implications for developing rational therapeutics for breast cancers harboring *PIK3CA* mutations.

## Acknowledgments

Part of this work has been supported by the University of Texas-Graduate School of Biomedical Sciences at Houston (A.G.D) and the French Association for Research against Cancer (S.N.D). We thank Drs Russell Broaddus, Laura Nelson, and Wei Zhang for helpful discussions.

Received: 2012-02-02; revised: 2012-03-07; accepted: 2012-03-14.

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