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New insights on PI3K/AKT pathway alterations and clinical outcomes in breast cancer

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Abstract

PI3K/AKT signaling pathway plays an important role in tumorigenesis and regulates critical cellular functions including survival, proliferation and metabolism. *PIK3CA* mutations and AKT activation by phosphorylation (pAKT) are often detected in many cancers and especially at high frequencies in breast cancer. Mounting data suggest that *PIK3CA* mutations or pAKT are mostly associated with better or insignificant outcomes in estrogen receptor-positive (ER+) early stage breast cancer and tend to be with worse prognosis in ER− disease. pAKT expression has been identified to predict paclitaxel chemotherapy benefit in node-positive breast cancer. Preclinical and neoadjuvant trial data suggest that *PIK3CA* alterations confer resistance to HER2-targeted therapy and are associated with lower pathological complete response (pCR) rate in HER2-positive breast cancer. However, recent results from randomized clinical trials of adjuvant and metastatic settings show that patients with mutant and wildtype *PIK3CA* tumors derived similar benefit from anti-HER2 therapy. This article, with our new insights, aims to decipher the mixed data and discusses the influence of the potential confounding factors in the assessments. We also share our views for validation of PI3K/AKT alterations in relation to clinical outcome in the context of specific breast cancer subtypes and treatment modalities towards further advance of the precision medicine for breast cancer treatment.

Keywords

Breast cancer; Chemotherapy; HER2; Paclitaxel; pAKT; *PIK3CA* mutations

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Conflict of interest

Dr. Sherry Yang is one of the inventors on the United States National Institutes of Health patent – US 8,546,091 B2. Drs. Polley and Lipkowitz declare no conflicts of interest.

Introduction

Breast cancer is the most commonly diagnosed cancer among women and the second leading cause of cancer death in women in the United States (http://www.cancer.org/Cancer/BreastCancer).

The phosphatidylinositol 3-kinase (PI3Ks) pathway comprises a family of intracellular signal transducer enzymes with three key regulatory nodes − PI3K, AKT, and mammalian target of rapamycin (mTOR) [1]. Somatic mutations have been identified in *PIK3CA* (36%), *PIK3R1* (3%), *PTEN* (3%) and *AKT1* (2%) genes in the pathway, with *PIK3CA* as the most frequently altered in breast cancer [2]. AKT activation by phosphorylation (pAKT) regulates critical cellular activities such as growth, proliferation, differentiation, metabolism and survival as well as tumorigenesis. Importantly, PI3K/AKT signaling is implicated in the pathogenesis of breast cancer and has been hypothesized to confer resistance to systemic treatments including chemotherapy and HER2-targeted therapy.

The relationship of *PIK3CA* mutations and AKT activation with prognosis and treatment benefit in breast cancer has been an area of intense investigation with mixed results. Given that chemotherapy and anti-HER2 treatment are standard management in breast cancer and a rapid advance of the targeted approach, it is imperative to diligently interpret the impacts of these alterations on the translational and/or clinical results. Here, we discuss *PIK3CA* mutations and pAKT for prognosis, and response to or benefit from standard therapy. We review the alterations with an emphasis on the translational research results of the randomized clinical trials in addition to the discussion of the relevant preclinical findings. We also share our views for validation of the pathway biomarkers pertaining to clinical outcomes in the context of specific cytotoxic agents or regimens in breast cancer subtypes.

Breast cancer subtypes and treatment modalities

Clinically, breast cancer is divided into the subtypes based on biologic or phenotypic markers. Estrogen receptor alpha-positive (ER+) and/or progesterone receptor (PR+) — hormone receptor-positive (HR+) – breast cancer (~70 –75%) is the most common clinical subtype. Patients with HR+ disease significantly benefit from endocrine therapy [3,4]. Human epidermal growth factor 2-positive (HER2+) breast cancer, accounting for about 20% of all cases of breast cancer, is a particularly aggressive form of breast cancer [5]. HER2+ disease is defined as tumors with either high expression of HER2 protein by immunohistochemistry (IHC) or amplification of *HER2* gene by fluorescence hybridization in situ (FISH) [6]. Standard systemic treatment for HER2+ disease includes chemotherapy in combination with HER2-targeted therapy [7]. Approximately half of HER2+ breast cancers are HR+, which are also managed with endocrine therapy. Triple-negative breast cancer (TNBC; ER−, PR− and HER2−) accounts for about 15% of all breast cancers. There are no approved targeted trerapy for TNBC and the standard treatment is cytotoxic chemotherapy. While the clinical HR+, HER2+ and TNBC subtypes are routinely used for management, breast cancer has been classified into the molecular subtypes by intrinsic gene expression signatures: luminal A (ER+ and HER2−, low proliferative, ~50%), luminal B (ER+, high

proliferative, HER2+ or HER2−), HER2-type (HER2+, ER+ or ER−; ~15%), and basal-like (accounting for the majority of triple-breast cancer; ~15%) [8–10].

PI3K/AKT pathway and alterations

Class IA PI3K is a heterodimeric lipid kinase consisted of a p110 catalytic subunit encoded by *PIK3CA* gene and a regulatory p85 subunit by *PIK3R1* gene. PI3K is activated in response to a variety of extracellular signals through a receptor tyrosine kinase (RTK) such as HER2, epidermal growth factor receptor (EGFR) or insulin-like growth factor 1 receptor (IGF1R) (Fig. 1A and B). The serine/threonine kinase AKT (protein kinase B) is a downstream multifunctional kinase, which serves as the central mediator of the pathway [11]. Upon activation, p110 PIK3CA phosphorylates phosphatidylinositol (3,4)-bisphosphate (PIP_2) to form phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). Binding of AKT to PIP_3 leads to AKT translocation from the cytoplasm to the plasma membrane, where the co-localization of 3-phosphoinositide dependent protein kinase-1 (PDK1) and AKT allows PDK1 to phosphorylate AKT at threonine 308. The full activation of AKT requires AKT to be phosphorylated at serine 473 (pAKT-S473 or pAKT) by mTOR/Rictor complex 2 (mTORC2) [12]. Based on this mechanism of action, antibodies to pAKT-S473 are commonly utilized to evaluate AKT activity [13]. Upon activation, AKT phosphorylates a large number of downstream substrates, which regulate cell growth and protein synthesis by regulating activity of the mTOR/Raptor complex 1 (mTORC1). This increases proliferation/cell-cycle progression. Through GSK-3ß and tau, pAKT regulates microtubule dynamics and organization [14]. pAKT promotes cellular survival via either direct inactivation by phosphorylation of multiple proapoptotic proteins or inhibition of the Forkhead box transcription factors that results in decreased expression of proapoptotic proteins [15]. The characteristic attenuation of apoptosis by pAKT has been hypothesized as a major mechanism of resistance to cancer treatment [16]. The PI3K/AKT pathway is negatively regulated by phosphatase and tensin homolog (PTEN), a lipid phosphatase that dephosphorylates PIP3 [17]. Loss or reduced expression (PTEN low) and function of PTEN occur frequently in breast cancer.

Noticeably, many studies have shown that AKT activity was modulated by chemotherapy agents and other cancer therapeutics [18–20]. Paclitaxel inhibits and doxorubicin augments AKT expression. Table 1 lists the agents that modulate AKT activity in vitro and patients. Of relevance is whether the treatment-induced changes in pAKT have an impact on resistance or sensitivity to specific treatments and long-term clinical outcomes.

Somatic mutations in the PI3K/AKT pathway genes have been identified in significant frequencies in breast cancer. About 90% of *PIK3CA* mutations, all missense, were located at hotspot clusters in the helical domain (HD) in exon 9 and kinase domain (KD) in exon 20. The activating mutations H1047R in the KD and E545K and E542K in the HD are the most prevalent alterations [21]. *PIK3CA* is mutated in ~30% of all breast cancers [22]. The mutation frequencies vary by breast cancer subtypes of 34.5–45%, 22.7–39% and 8.3–25% in HR+, HER2+ and TNBC, respectively [23,24,2,25]. The Cancer Genome Atlas (TCGA) breast cancer analysis found *PIK3CA* mutation rates of 45% in luminal A, 29% in luminal

B, 39% in HER2+, and 9% in the basal-like subtypes [2]. Association of *PIK3CA* mutations with AKT activation status was observed in some study cohorts [25–27].

PI3K/AKT alterations and prognosis

A factor for prognosis is defined as the one that is associated with clinical outcome in the absence of therapy or in the context of a standard treatment that all patients likely receive in a disease setting [28]. To date, the relationship between *PIK3CA* mutations/pAKT and prognosis are mixed in early breast cancer, some data demonstrating association with favorable outcome, others with poor prognosis, and a number of studies showing insignificant results (Table 2). That *PIK3CA* mutations or pAKT expression was associated with favorable outcomes has the following features. Over half of each study population received adjuvant endocrine therapy with or without chemotherapy or radiation therapy in studies including all subjects or in those with ER+ disease only (Table 2) [22,29–33]. Furthermore, *PIK3CA* mutation and a PIK3CA mutant-like expression signature derived from exon 20 *PIK3CA*-mutated tumors was associated with a favorable outcome in ER+ patients who received adjuvant tamoxifen by multivariate analysis [27]. Sabine et al. recently found that *PIK3CA* was mutated in ~40% of ER+ breast cancer samples in the Tamoxifen Exemestane Adjuvant Multinational (TEAM) phase III trial [33]. The mutations were associated with favorable clinicopathological factors (lower grade, less lymph node involvement, and PR expression), and a better 5-year distant relapse-free survival (DRFS). However, *PIK3CA* mutations were not an independent predictor of outcome in multivariate analysis. Consistent with these results, a recent meta-analysis confirmed that *PIK3CA* mutations were significantly associated with ER positivity, increasing age, lower grade and smaller tumor size. The genotype was correlated with better invasive disease-free survival (DFS) by univariate analysis, but not in multivariate analysis [34]. In a neoadjuvant study, *PIK3CA* KD mutations were prognostic of longer RFS in patients with HR+ tumors after receiving either letrozole or tamoxifen treatment [26]. Together, these results suggest that the clinical outcomes appear to be mostly driven by the intrinsic ER+ tumor characteristics, rather than by activating *PIK3CA* mutations [35]. Both PI3K and AKT kinases increase ER transcriptional activity in experimental models (Fig. 1A) [36]. MCF-7 cells transfected with an active AKT attenuated apoptosis induced by tamoxifen. However, clinical data may have suggested that the effect of cross-talk between PI3K/AKT signaling and ER could be largely inhibited by adjuvant endocrine therapy administered as a part of systemic therapy in the ER + breast cancer (Fig. 1A) [27,36]. A recent study by Bosch and colleagues demonstrated that the increase in ER activity following PI3K inhibition could be stimulated by estradiol and suppressed by tamoxifen and fulvestrant in vitro and patient-derived models as well as in tumors from patients [37].

In large studies with all subjects, evaluation of pAKT was not significantly associated with clinical outcomes, suggesting the possibility of confounding factors in the assessments (Table 2) [38–40]. By stratifying ER status, a study analysis recently demonstrated a significant difference for OS in patients with pAKT+ tumors with and without ER expression in doxorubicin–cyclophosphamide (AC) treatment arm ($P < 0.0001$) and AC followed by paclitaxel group ($P = 0.002$), respectively [41]. Particularly in the AC arm, OS at 10 years was similar in ER+ patients whether pAKT was present or not (77% in pAKT+

ER+, 75% in pAKT− ER+ tumors), but was much worse in ER− patients if pAKT was positive (66% in pAKT− ER− and 58% in pAKT+ ER− tumors).

Thus, it warrants further investigation for the divergent effects of pAKT on prognosis between ER+ and ER− breast cancers in the context of AC chemotherapy.

As for ER− breast cancer, what role may *PIK3CA* mutation or AKT activation play? pAKT or combined pAKT and total AKT expression appeared to be associated with unfavorable outcome in ER− disease treated with anthracycline-based chemotherapy (Fig. 1B) [41,42]. However, the results need to be validated in studies with sufficient numbers of ER− or TNBC breast cancer patients.

PI3K/AKT alterations and chemotherapy outcome

Anthracyclines (doxorubicin and epirubicin) are a class of chemotherapy agents that are commonly used for the treatment of breast cancer in the adjuvant and neoadjuvant settings [43]. The agents inhibit topoisomerase II and intercalate into DNA that cause DNA damage to produce cytotoxic effects. Doxorubicin has been shown to induce AKT activation, which mediates resistance to doxorubicin in both ER+ and ER− breast cancer cells [18,20]. Brown and colleagues recently reported that MERIT40 phosphorylation by AKT kinase facilitates assembly of the BRCA1 DNA repair complex, which contributes to DNA damage repair and cancer cell survival following doxorubicin treatment [20]. In the MCF7 breast cancer cell line, transfection of HER2 led to an increase in pAKT expression [44], which resulted in cellular resistance to multiple chemotherapy agents including microtubule-stabilizing paclitaxel, DNA-damaging etoposide, camptothecin and doxorubicin, and antimetabolite 5-fluorouracil. However, a subsequent study presented data that constitutive overexpression of HER2 was inadequate to augment pAKT upon exposure to doxorubicin in several HER2+ cell lines [45].

The treatment benefit can be evaluated through the interaction of a biomarker (predictive biomarker) status and treatment outcomes using any of the clinical endpoints such as response, overall survival (OS), disease-free survival (DFS) or progression-free survival (PFS) [28]. The relative clinical outcomes of the two treatments of comparing a new treatment to control in randomized clinical trials are assessed separately in the biomarker-positive and biomarker-negative patient groups [46].

A prospective-retrospective investigation examined pAKT expression in 823 primary breast tumors from patients who were randomized to receive no treatment or anthracycline-based adjuvant chemotherapy (Table 2). pAKT was neither significantly prognostic of DFS and OS nor predictive of efficacy of anthracycline-based chemotherapy [39]. The test for interaction was insignificant between pAKT status and efficacy with anthracycline-based chemotherapy. In the neoadjuvant setting, a research team evaluated 140 patients with stage II and III breast cancer and did not observe any association between *PIK3CA* mutation status and response to neoadjuvant anthracycline- and taxane-containing regimens [47]. Such results were ascribed to a limited number of patients categorized by ER status, mutation types and treatment regimens.

Taxanes including paclitaxel and docetaxel are another class of chemotherapy agents, which primarily stabilize microtubules to inhibit mitosis; they are commonly used for the treatment of breast cancer in all settings. Preclinical data did not demonstrate a clear correlative relationship between *PIK3CA* or PTEN mutation or AKT activation and paclitaxel (NSC125973) resistance in breast cancer cell lines. These include MCF-7 (*PIK3CA*-mutant, ER+ and HER2−; Log GI50, −7.9 or $10^{-7.9}$ M), MDA-MB-231 (PIK3CA/PTEN-wildtype, ER− and HER2−; −7.2), T47D (*PIK3CA*-mutant, ER + and HER2−; −7.0), MDA-MB-468 (PTEN-mutant, ER and HER2−; −7.7), Hs578T (*PIK3CA*/PTEN-wildtype, ER and HER2−; −8.1), and BT549 (PTEN-wildtype, ER− and HER2−; −7.6) (https://dtp.cancer.gov/dtpstandard/servlet/MeanGraph?searchtype=NSC&searchlist=125973&outputformat=HTML&outputmedium=page&chemnameboolean=AND&debugswitch=false&assaytype=&testshortname=NCI+Cancer+Screen+Current+Data&dataarraylength=60&endpt=GI50&button=Mean+Graph&highconc=−5.0). MDA-MB-468 cells that express high level of pAKT were more sensitive to paclitaxel than MDA-MB-231cells with low pAKT expression in vitro and *in vivo* [48].

Recently, pAKT, 1+ to 3+ expression detected by IHC (38%, 606/1581), was identified to predict benefit of adding paclitaxel to adjuvant AC chemotherapy. Women with node+ breast cancer were randomly assigned to 4 cycles of adjuvant AC or 4 cycles of AC followed by 4 cycles of paclitaxel chemotherapy in the National Surgical Adjuvant and Bowel Project (NSABP) B28 clinical trial [49,50]. In the pAKT+ breast tumors, addition of paclitaxel resulted in a 26% improvement in DFS and 20% in OS [41,50,51]. By contrast, adding paclitaxel to AC did not lead to any improvement in DFS and OS in patients with pAKT-negative tumors. An apparent interaction was detected between pAKT and treatment with sequential addition of paclitaxel to AC chemotherapy. Since pAKT regulates microtubule dynamics/organization and paclitaxel inhibits AKT phosphorylation, pAKT+ tumors may have incurred more damage from paclitaxel chemotherapy than pAKT− tumors (Table 1; Fig. 1B) [19,52–54].

Recently, Bartlett and colleagues reported that expression of pAKT, p70S6K and p90RSK were not significantly associated with either resistance or sensitivity to docetaxel-based chemotherapy [55]. The United Kingdom Taxotere as Adjuvant Chemotherapy Trial (TACT) tested 4 cycles of 5-fluorouracil, epirubicin and cyclophosphamide (FEC) followed by 4 cycles of docetaxel in comparison with 8 cycles of FEC or 4 cycles of epirubicin followed by 4 cycles of cyclophosphamide, methothexate and 5-flurouracial (CMF) regimens in the control arm (Table 3) [56]. The study did not identify any trend for interaction between the pathway biomarkers and docetaxel benefit. The analyses of the interactions between PI3K pathway proteins and addition of docetaxel were likely obscured by 4 additional cycles of FEC or CMF in the control arm. Thus, the interaction between the pathway biomarkers and docetaxel data presented can’t be directly inferred from the docetaxel only. For analysis of a biomarker-taxane interaction, it would be ideal to compare the addition of a taxane sequentially or concurrently to AC or FAC or FEC regimen in the experimental arm to equal cycle number of AC or FAC or FEC or CMF in the control group [49,57,58]. For more guidance, please refer to a recent publication on the “statistical and practical considerations for clinical evaluation of predictive biomarkers” [46]. Table 3 lists the randomized clinical

trials with and without a taxane, including those that are ideal for evaluation of the interaction between a biomarker and treatment outcomes.

PI3K/AKT alterations and clinical outcome in HER2+ breast cancer

HER2 overexpression and signaling result in the activation of two downstream signaling pathways – the RAS/RAF/ERK pathway and the PI3K/AKT pathway. Experimental observations with BT474 (luminal B phenotype) breast cancer cell model demonstrated that HER2+ breast cancer with an activating *PIK3CA* mutation and low PTEN expression were resistant to HER2-targeted therapy with trastuzumab [59]. Using a genetic approach to search novel modulators of lapatinib resistance in the BT474 breast cancer cells, Eichorn et al. found that loss of PTEN and *PIK3CA* activating mutations conferred resistance to lapatinib (a dual HER2/HER1 tyrosine kinase inhibitor) [60]. In a transgenic HER2+ mammary tumor mouse model, *PIK3CA* mutations cooperated with HER2 promoting tumor progression and inducing resistance to trastuzumab alone, and trastuzumab in combination with lapatinib or pertuzumab therapy [61].

In the phase II neoadjuvant Remagus 02 trial, patients with HER2+ breast tumors received epirubicin/cyclophosphamide (EC) preoperatively, and followed by docetaxel and trastuzumab either preoperatively or postoperatively [62]. With a median follow-up of 51 months, DFS was significantly worse in patients with *PIK3CA*-mutant tumors [63]. In a cohort of 240 patients who received adjuvant FEC followed by one year trastuzumab, worse OS and IDFS (invasive disease-free survival) were observed in early stage HER2+ breast cancer patients whose tumors harbor *PIK3CA* mutations [64]. Recently, results from the phase III CLEOPATRA trial (testing the addition of pertuzumab to docetaxel plus trastuzumab as first line therapy for metastatic breast cancer) showed that *PIK3CA* activating mutations were associated with a shorter PFS [65]. These data presented evidence of the association between *PIK3CA* alterations and poor prognosis in early and late stage HER2+ breast cancer. However, it is worthwhile to mention that in metastatic breast cancer with trastuzumab as first-line therapy, loss of PTEN but not *PIK3CA* mutations or pAKT was significantly associated with a shorter survival, and poor response to trastuzumab alone and to combination of trastuzumab with vinorelbine or taxane-based chemotherapy [66].

In the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial (NeoALTTO), patients received lapatinib plus paclitaxel or trastuzumab plus paclitaxel or trastuzumab in combination of lapatinib plus paclitaxel before surgery, followed by FEC chemotherapy plus anti-HER2 agent after surgery [67]. *PIK3CA* mutations were associated with worse outcome and a lower pCR rate [68]. However, PTEN status failed to distinguish a difference in total pCR to trastuzumab and lapatinib-based therapies [69]. Recently, *PIK3CA* mutations (21.4%) were evaluated in 504 patient tumors by combining three neoadjuvant trials – GeparQuattro [70], GeparQuinto [71] and GeparSixto [72–74]. HER2+ patients were treated with either trastuzumab or lapatinib plus anthracycline-docetaxel chemotherapy. The pCR rate was significantly lower in patients with *PIK3CA* mutations than those without by both univariate and multivariate analyses. However in this study, association of *PIK3CA* genotype with pCR did not translate into OS and DFS outcomes. Recently, another metaanalysis evaluated pCR rate in 967 patients from GEPAR,

NeoALTTO and CHER-LOB trials [75], and found a statistically significant lower pCR rate in HER2+/*PIK3CA*-mutant tumors than HER2+/*PIK3CA*-wildtype tumors [68,73,76]. Of note in the evaluation, the pCR rate in *PIK3CA*-mutant tumors was significantly lower than wildtype in HR+ subgroup; in contrast, the difference in pCR rates was not statistically significant in HR− subgroup [77].

Most recently, Slamon and colleagues conducted a combined analysis of the mTOR inhibitor everolimus from the BOLERO-1 and BOLERO-3 clinical trials in patients with locally advanced or metastatic HER2+ breast cancer [78]. The international BOLERO-1 clinical trial enrolled 719 patients with locally advanced or metastatic HER2+ breast cancer and randomized them to paclitaxel plus trastuzumab with or without everolimus as first-line therapy [79]. The BOLERO-3 trial recruited 572 women with locally advanced or metastatic HER2+ breast cancer previously treated with paclitaxel and trastuzumab, who were randomized to vinorelbine plus trastuzumab with or without everolimus [80]. The combined analysis demonstrated a significant PFS benefit in patients with *PIK3CA*-mutant or PTEN loss/low tumors treated with everolimus in combination with trastuzumab plus either paclitaxel or vinorelbine. By contrast, an everolimus benefit was not seen in patients with *PIK3CA* wildtype or PTEN normal status. These results are the first to demonstrate that *PIK3CA* mutations and/or PTEN loss predict everolimus efficacy in patients with advanced HER2+ breast cancer.

As for the predictive value of the PI3K/AKT pathway alterations to HER2-targeted therapy, several studies using samples from randomized clinical trials showed comparable results in patients with both *PIK3CA*-mutant and *PIK3CA*-wildtype tumors. Patients were randomly assigned to receive or not to receive nine weekly trastuzumab infusions after adjuvant chemotherapy with docetaxel or vinorelbine, followed by FEC in those with HER2+ disease in Fin-HER phase III trial [81]. There were no significant interactions between *PIK3CA* mutations and distant DFS or OS [82]. In NSABP B31 trial, all patients were randomly assigned to AC followed by paclitaxel chemotherapy with and without trastuzumab. Pogue-Geile and colleagues recently analyzed tumor *PIK3CA* mutation status and reported that patients with both *PIK3CA*- mutated and - wildtype HER2+ tumors similarly benefited from adjuvant trastuzumab [83]. In analysis of specimens from the BCIRG-005 and BCIRG-006 clinical trials [7,84], PTEN loss was associated with worse DFS and OS in HER2+ disease [85]. However, patients with PTEN− tumors, as those with PTEN+ tumors, also benefited from adjuvant trastuzumab. Extending this trend from the adjuvant setting, *PIK3CA* mutations were found not predictive of treatment resistance or benefit from addition of pertuzumab to trastuzumab/docetaxel regimen in HER2+ metastatic breast cancer [65]. The biomarker analysis in the EMILIA study also did not find an association between *PIK3CA* mutations and response to trastuzumab-emtansine conjugate (T-DM1) therapy in patients with HER2+ locally advanced or metastatic breast cancer [86].

Hence, mounting evidence from the randomized clinical studies has challenged the role of PI3K/AKT alterations to anti-HER2 therapy resistance or confounded by some cytotoxic treatment. *PIK3CA* mutations are mostly associated with poor prognosis and lower pCR rate in patients treated with neadjuvant chemotherapy in combination with trastuzumab and/or lapatinib in HER2+ breast cancer. The impact of administration sequence of paclitaxel and

anthracycline-based chemotherapy or paclitaxel versus docetaxel or vinorelbine with regard to PI3K/AKT alterations on the clinical outcome remains to be determined.

Conclusions

PI3K/AKT pathway is of critical importance in breast cancer pathogenesis unraveled after research spanning over several decades; nonetheless, data has been mixed for its implications in patient outcomes. As discussed, the factors complicating the translation primarily are (i) heterogeneous breast cancer population whose tumors may differ in the magnitude of dependence on the PI3K/AKT signaling or received different treatments; (ii) some and evolving treatment modalities that may have altered this pathway activity. As such, results were inconsistent with regard to *PIK3CA* mutations or AKT activation for prognosis in early stage breast cancer. These alterations were mostly associated with better or insignificant outcome in ER+ population and tend to be with worse outcome in ER− disease. Adjuvant endocrine therapy is effective, which may have largely overridden the effects of the PI3K/AKT pathway signaling in ER+ early breast cancer.

pAKT is identified to predict paclitaxel benefit in node+ breast cancer and the results on taxane benefit could be validated using samples from the randomized clinical trials listed in Table 3 or prospectively designed clinical trials. The question remains on whether anthracycline-based chemotherapy led to different outcomes with regard to AKT activation in ER+ and ER− breast cancers, respectively. It is important to determine if the alteration is a negative predictor to anthracycline-based chemotherapy in patients with ER− disease using sample sizes with adequate statistical power. Taxanes are the agents of choice in combination with trastuzumab or lapaninib. It is important to test their addictive effect with HER2-targeted agents in terms of inhibition of the PI3K/AKT signaling.

There have been challenges to retrospectively evaluate the interaction of a biomarker with the drug for treatment outcome in the randomized clinical trials. The elements that influence this type of analysis include (i) many of the conventionally designed treatment trials are not ideal or suitable to evaluate biomarker-drug interaction; (ii) modulation of the central mediator of PI3K pathway, AKT activity, by some chemotherapy or hormonal agents may have substantial impact on clinical outcome. In addition, (iii) much data have pointed out PI3K/AKT alterations in connection with the ER status that have impact on clinical outcome. The data may have implications on the choice of chemotherapy agents and other cancer therapeutics according to the ER status, shedding light on further advance of precision medicine in breast cancer treatment.

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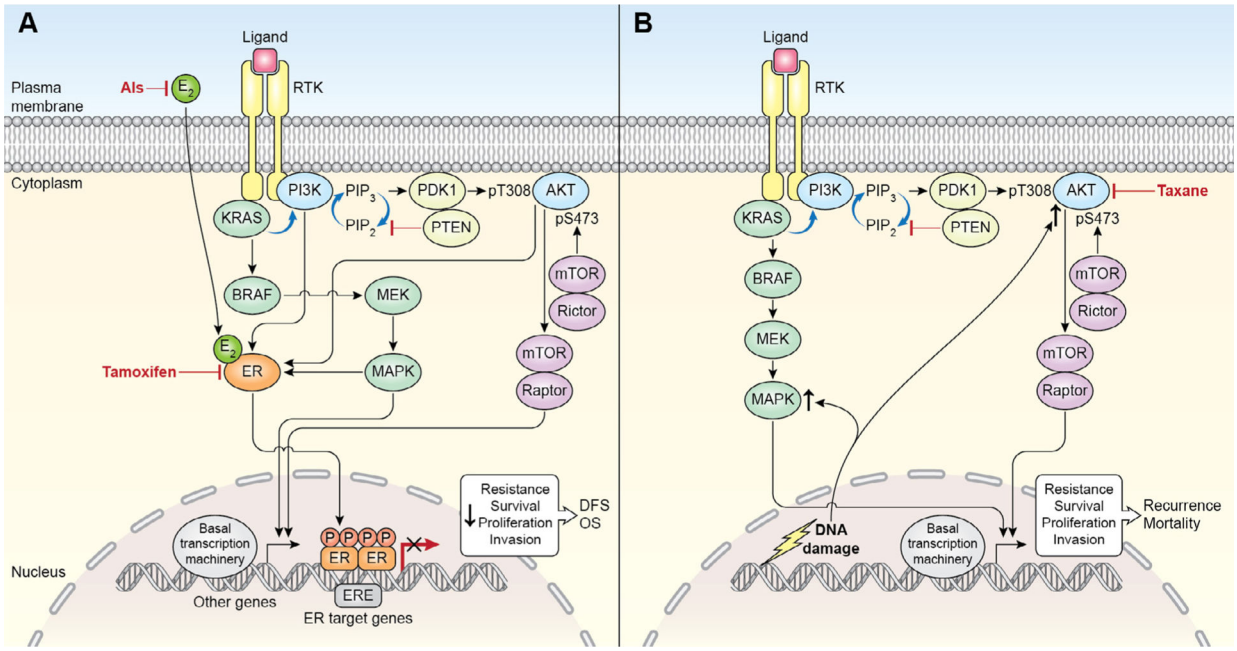

Fig. 1.

(A) ER and PI3K/AKT/mTOR signaling and endocrine therapy in ER+ early breast cancer. Schematic interaction of the two signaling pathways and dominant effects of adjuvant endocrine therapy on the cellular output and its overall impact within 10 years after surgery. Abbreviations: AI, aromatase inhibitors; DFS, disease-free survival; E2, estradiol; ERE, estrogen receptor element; OS, overall survival; PIP, phosphatidylinositol phosphate; RTK, receptor tyrosine kinase. (B) PI3K/AKT/mTOR signaling and chemotherapy in ER− early breast cancer. DNA damaging agents upregulate pAKT [20] that facilitates DNA repair, which may contribute to early recurrence [87]. Taxanes counteract AKT activity at certain extent [19,50,88]. Abbreviations: PIP, phosphatidylinositol phosphate; RTK, receptor tyrosine kinase.

Table 1

Anti-cancer agents that modulate AKT activity.

Drug	Modulation	Dose (μM)	In combination	References
Paclitaxel	Inhibition	0.25	MEK inhibitor	Mackeigan et al. [89]
Paclitaxel	Inhibition	0.05	Alone	Swanton et al. [19]
Paclitaxel	Inhibition	0.005, 0.1	Alone	Asakuma et al. [53]
Cisplatin	Inhibition	10	Alone	Asakuma et al. [53]
Topotecan	Inhibition	0.1, 1, 10	Alone	Nakashio et al. [90]
Docetaxel	Inhibition	0.05, 0.075	Alone or with MGd[†]	Ramos et al. [91]
Lapatinib	Inhibition	1	Alone	O'Brien et al. [92]
Letrozole	Inhibition	2.5 mg/daily[*]	Cyclophosphamide	Generali et al. [93]
Doxorubicin	Up-regulation	0.05, 0.5, 5	Alone	Clark et al. [18]

[*] Dose given to patients.

[†] Abbreviations: MGd, Motexafin gadolinium; NA, not available.

Table 2

PI3K/AKT pathway alterations and clinical outcomes in breast cancer.*

First author; year	No. of patients	Alteration (%)	Clinical outcome HR (95% CI), *P* value (mut vs. wt or positive vs. negative)†	Population	Therapy type (%)†	Other relevant information
Perez-Tenorio et al. [29]	270	PIK3CA (24.3)	Longer local RFS, *P* = 0.023	Postmenopausal	CMF, radiotherapy, tamoxifen	Univariate; associated with ER+ or HER2− status
Maruyama et al. [30]	188	PIK3CA (28.7)	RFS 2.34 (1.1–5.1), *P* = 0.03 (wt vs. mut)	All subjects	Chemotherapy (42.5). EC (17.5), tamoxifen (78)	Multivariate; associated with ER and pAKT expression
Barbareschi et al. [94]	163	−PIK3CA (27.6)	−OS *P* = 0.75; DFS *P* =0.61	−All subjects	CMF (30), anthracycline	−Univariate
		−Exon 20	−Better OS P = 0.01; DFS, *P* = 0.01	−All subjects	(17),	−Univariate
		−Exon 9	−OS 5.32 (2.1–13.2). *P* = 0.0003; DFS 4.6 (1.8–11.8). *P* = 0.001	−All subjects	Anthracycline + taxane (9), tamoxifen (68), AIs (3)	−Multivariate
Liedtke et al. [47]	140	PIK3CA(16.4)	pCR18% vs. 17%, *P* =1	All subjects	−Neoadjuvant FAC or FEC (45)	Univariate
					−TFAC or TFEC (55)	
Stemke-Hale et al. [25]	157	PIK3CA (34.5)	OS, *P* = 0.597; RFS, *P* = 0.919	ER+	Tamoxifen (100)	Univariate
Kalinsky et al. [31]	520	PIK3CA (32.5)	OS 71% vs. 62%, *P* = 0.03	All subjects	Unknown	Univariate; associated with ER expression
			BCSD 12% vs. 23%, *P* = 0.004			
Lopez-Knowles et al. [42]	292	PI3K pathway§	BCSD 4.2 (1.3–13.6), *P* = 0.02	All subjects	CMF or AC (38); Tamoxifen (49)	Univariate; associated with ER-negative status
Loi et al. [82]		PIK3CA-GS&				
	−717		−OS, 0.7 (0.4–1.1), *P*< 0.001	−ER+/HER2−	−No therapy	−Univariate
	−192		−OS, 1.3 (0.6–2.7), *P* = 0.4	−HER2+	−No therapy	−Univariate
	−280		−OS, 0.7(0.4–1.1), *P* = 0.3	−Triple−	−No therapy	−Univariate
	−302		−OS, 0.5 (0.3–0.8), *P* = 0.01	−ER+	−Tamoxifen (100)	−Multivariate
Ellis et al. [26]	−398	PIK3CA (32.0)				
	−125	−KD	−RFS.14 (1.9–105), *P* = 0.01 (wt vs. mut)	−ER+	−Neoadjuvant endocrine	−Multivariate
	−235	−KD&HD	−Negative interaction, *P* = 0.046	−ER+	−Neoadjuvant letrozole	−Less response to letrozole
Dupont Jensen et al. [95]	104	PIK3CA (45)	−Longer TTR, *P* =0.03	All subjects	Endocrine therapy (52), chemotherapy (33)	Univariate
Ramirez-Ardila et al. [96]	−1352	PIK3CA(31.3)				
	−342	−Exon 9	−MFS 1.04 (0.57–1.89), *P* = 0.9	−Node−	−No adjuvant therapy	−Univariate

First author; year	No. of patients	Alteration (%)	Clinical outcome HR (95% CI), *P* value (mut vs. wt or positive vs. negative)†	Population	Therapy type (%)†	Other relevant information
	−447	−Exon 20	−MFS 0.98 (0.63–1.54), *P* = 0.94	−Node−		
	−84	−Exon 9	−TTP 1.17 (0.87–1.57). *P* = 0.3	−ER+	−First line tamoxifen	−Univariate
		−Exon 20	−TTP1.01 (0.78–1.31), *P* = 0.93	metastatic		
		−Exon 9	−TTP 0.4 (0.17–0.95), *P* = 0.038	−ER+	−First line AIs	−Univariate and multivariate
		−Exon 20	−TTP 0.5 (0.27–0.91), *P* = 0.024	metastatic		
Beelen et al. [97]	563	PIK3CA		ER+	Tamoxifen vs. no treatment	Adjusted hazard ratios and interaction tests between PIK3CA Exon 9 or
		−Exon 9 (15.6)	−0.82(0.22–3.04), *P* = 0.51	postmenopausal		20 and tamoxifen, respectively
		−Exon20(18.1)	−0.77(0.25–2.36), *P* = 0.51			
Stal et al. [98]	280	pAKT, AKT1 and AKT2 combined (28)	Rate ratio 0.5 vs. 1, *P* = 0.04	Postmenopausal	CMF (57); radiotherapy (43); tamoxifen (49)	Multivariate
Kirkegaard et al. [99]	392	Cytoplasmic pAKT	Worse OS 1.65 (1.0–2.7). *P* = 0.04	ER+	Tamoxifen (100); Chemotherapy (24.8); Radiotherapy (27.6)	Univariate after excluding nuclear pAKT
Cicenas et al. [100]	156	pAKT (14)	2.09 (1.14–3.85), *P* = 0.02 (risk for relapse)	All subjects	Unknown	−Multivariate
Tokunaga et al. [101]	−252	pAKT (33)	−1.3 (0.7–2.3), *P* = 0.4 (pAKT− vs. pAKT+)	−All subjects	−Unknown	−Multivariate
	−107		−2.8 (0.9–5.0), *P* = 0.1 (pAKT− vs. pAKT+)	−107	−Tamoxifen and/or goserelin	−Multivariate
Andre et al. [39]	781	pAKT(15)	DFS 0.99 (0.72–1.36), *P* = 0.94	All subjects	−FAC or FEC (49)	Univariate for prognosis; Not predictive to anthracycline-based therapy
			OS 1.39 (0.97–2), *P* = 0.08		−No chemotherapy (51)	(tests for interactions P = 0.73 in OS and P = 0.95 in DFS)
Badve et al. [32]	−377	Nuclear pAKT	−Longer OS, *P* = 0.004	−ER+; 100	−Unknown	−Univariate
	−104		−Longer OS, *P* = 0.02	−ER+/PR+; 100	−Unknown	−Univariate
Yang et al. [50]	1581	pAICT (38)		Node+		Paclitaxel benefit by multivariate
			DFS: pAKT+: 0.74; *P* = 0.02		AC (48); AC → paclitaxel	
			pAKT−: 1.02, *P* = 0.81		(52)	
			OS: pAKT+: 0.8, *P* = 0.17		Tamoxifen (83.5)	

First author; year	No. of patients	Alteration (%)	Clinical outcome HR (95% CI), *P* value (mut vs. wt or positive vs. negative)†	Population	Therapy type (%)†	Other relevant information
			pAKT−: 0.97, *P* = 0.8			
			(AC-P arm v AC arm)			
Hartog et al. [40]	429	pAKT	DFS 0.78 (0.31–1.96), *P* = 0.6	All subjects	HT (35)	Univariate
			OS 0.73 (0.22–0.38), *P* = 0.5		Chemotherapy (28)	
Aleskandarany et al. [38]	1202	pAlCT (76)	BCSS*P* =0.1	All subjects	Unknown	Univariate
			MFS *P* = 0.13			
Cizkovo et al. [102]	452	P1K3CA (33.4)	Longer MFS 0.62 (0.44–0.87). *P* = 0.006)	All subjects	Radiation (35.4) Hormone (60) Chemotherapy (42)	Univariate; associates with ER/PR
Bostner et al. [103]	693	Nuclear pAKT (28)	pAKT−: 0.43 (0.28–0.65), *P*< 0.0001	Postmenopa usal node−	−Tamoxifen (46);	Multivariate
			pAKT+: 0.72 (0.39–1.33), *P* = 0.23 (RFS by Tam vs. No tam)		Radiation	
Bartlett et al. [55]	3321	pAKT (72.0)	No difference in DFS	High-risk node−	−FEC or E → CMF (50.5);	Univariate
				& node+	FEC → docetaxel (49.5)	
					Tamoxifen and/or AIs (69)	
Sabine et al. [33]	4272	PIK3CA (39.8)	−DRFS: 0.76 (0.63–0.91), *P* = 0.003	Postmenopausal	−Exemestane (50);	−Univariate
			−DRFS: 0.92 (0.75–1.12). *P* =0.401	ER+	Tamoxifen → exemestane (50)	−Multivariate
Panget al. [22]	5719	PIK3CA (range	RFS: 0.76 (0.59–0.98), *P* = 0.03	All subjects	NA	−Univariate, associated with ER/PR expression
		16.4–45)	OS: 1.14(0.72–1.82), *P* = 0.0.57			

*Full-text studies published in English are included if the patient numbers reach 100 or more, with a focus on those having treatment information; and if pAKT status was examined by immunohistochemistry.

†PIK3CA-mutant versus PIK3CA-wildtype or pAKTS473-positive versus pAKTS473-negative unless specifically narrated; adjuvant therapy unless specifically noted in the table.

‡Abbreviations: AC, doxorubicin-cyclophosphamide; AI, aromatase inhibitors; BCFS, breast cancer-free survival; BCSD, breast cancer specific death; BCSS, breast cancer specific survival; CMF, cyclophosphamide-methothexate-5-fluorouracil; CSS, cancer-specific survival; DFS, disease-free survival; DRFS, distant relapse-free survival; E, epirubicin; EC, epirubicin-cyclophosphamide; FAC, 5-fluorouracil-doxorubicin-cyclophosphamide; FEC, 5-fluorouracil-epirubicin-cyclophosphamide; HR, hazard ratio; HT, hormonal therapy; KD, kinase domain in exon 20; HD, helical domain in exon 9; MFS, metastasis-free survival; mut, mutant; pCR, pathological complete response; OS, overall survival; RFS, relapse-free survival; Tam, tamoxifen, TFAC or TFEC, paclitaxel followed by 5-fluorouracil-doxorubicin (or epirubicin)-cyclophosphamide; TTP, time to progression; TTR, time to recurrence; v, versus; wt, wildtype.

§PI3K pathway activation status includes *PIK3CA* mutations and gene copy numbers, pAKT, and PTEN status.

&PIK3CA-GS, *PIK3CA* mutation-associated gene signature.

Table 3

Randomized Phase III clinical trials that may test the interaction between pAKT or *PIK3CA* mutations and taxane therapy benefit in breast cancer.

PDQID	ClinicalTrials.gov ID	setting; phase	Regimens	Enrollment; population	References
CALGB 9344	N/A	Adjuvant, III	ACx4 vs. ACx4 → Px4	3121;Node+	Henderson et al. [57]
NSABP B-28	N/A	Adjuvant; III	ACx4 vs. ACx4 → Px4	3050; Node+	Mamounas et al. [49]
				1581; Node+	Yang (2010)
GEICAM 2003–02	NCT00129389	Adjuvant; III	FACx6* vs. FACx4 → wk Px8	1925; high-risk node− or node+	Martin (2013)
FRE-FNCLCC-PACS-01	N/A	Adjuvant; III	FECx6* vs. FECx3 → Dx3	1999; Node+	Roche (2006)
NSABP B-27	NCT00002707	Neoadjuvant &	Pre-surgery ACx4 vs. pre-surgery	2411; operable	Bear (2003)
		adjuvant; III	ACx4 → Dx4 vs.		Bear [58]
			ACx4 → post-surgery Dx4		
INT-23/96	NCT00003013	Adjuvant; III	Ax4* → CMFx4 vs. APx4 → CMFx4	1355; Operable	Gianni (2009)
TAX_IT1_302	NCT00174707	Adjuvant; III	Epidoxorubicin/CMF vs. epidoxorubicin/docetaxel/CMF	998; node+	N/A
TACT	N/A	Adjuvant; III	FECx8* or Ex4 → CMFx4* vs. FECx4 → Dx4	4162; high-risk node-	Ellis et al. [56]
				or node+	Bartlett et al. [55]

Source: //Clinicaltrials.gov (accessed January 2016).

Abbreviations: A, doxorubicin; D, docetaxel; E, epirubicin; FAC, fluorouracil, doxorubicin, cyclophosphamide; FEC, fluorouracil, epirubicin, and cyclophosphamide; M, methotrexate; N/A, not available; P, paclitaxel; w, weekly; +, positive;−, negative.

*Note: Trials that had extra cycles of kcomparator regimen in the control arm or a higher cumulative dose of doxorubicin in control arm in INT-23/96 trial. Such trials may not be ideal to evaluate the interaction between pAKT or *PIK3CA* mutation and taxane benefit.