Breast Cancer RESEARCH

REVIEW

# *PIK3CA* mutations in breast cancer: reconciling findings from preclinical and clinical data

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## Abstract

*PIK3CA* mutations represent one of the most common genetic aberrations in breast cancer. They have been reported to be present in over one-third of cases, with enrichment in the luminal and in human epidermal growth factor receptor 2-positive subtypes. Substantial preclinical data on the oncogenic properties of these mutations have been reported. However, whilst the preclinical data have clearly shown an association with robust activation of the pathway and resistance to common therapies used in breast cancer, the clinical data reported up to now do not support that the *PIK3CA* mutated genotype is associated with high levels of pathway activation or with a poor prognosis. We speculate that this may be due to the minimal use of transgenic mice models thus far. In this review, we discuss both the preclinical and clinical data associated with *PIK3CA* mutations and their potential implications. Prospective clinical trials stratifying by *PIK3CA* genotype will be necessary to determine if the mutation also predicts for increased sensitivity to agents targeting the phosphoinositide 3-kinase pathway.

## Introduction

Phosphoinositide 3-kinases (PI3Ks) comprise a family of lipid kinases, discovered in the 1980s, that are responsible for mediating important biological functions such as cell survival, differentiation and proliferation [1]. In breast cancer, mutations of the *PIK3CA* gene, which encodes the p110α catalytic subunit of PI3K, are highly frequent (2,257/9,095 = 24.82% according to the Catalogue of somatic mutations in cancer [2]), have been shown to be oncogenic, and are likely to represent important events in the initiation and progression of breast cancer. However, several characteristics of *PIK3CA* mutations in breast cancer have been observed, including a strong association with expression of the estrogen receptor (ER), a lack of an association with robust activation of the classical PI3K pathway, as well as a relatively good prognosis for patients with mutations compared with their wild-type counterparts. These features make it difficult to understand the functional and clinical relevance of *PIK3CA* mutations in breast cancer at present. In this article we review and summarize the preclinical and clinical data in breast cancer in an attempt to reconcile these findings.

## Background

Based on distinct structural characteristics and substrate specificity, PI3Ks can be divided into three classes, I to III. Class I can be further subdivided into class IA and IB kinases, with class IA activated by receptor tyrosine kinases (RTKs), G protein coupled receptors and other oncogenes such as RAS, and class IB activated exclusively by G protein coupled receptors [3]. Class IA PI3Ks represent the most extensively studied subclass, with implications in human carcinogenesis [3]. They are heterodimers consisting of a catalytic (p110) and a regulatory (p85) subunit, with the latter stabilizing the former in quiescent cells and suppressing PI3K activity. There are three different isoforms of the p110 subunit in mammals, p110α, p110β and p110δ, transcribed from the genes *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively, and three isoforms of the p85 subunit, p85α, p55α and p50α, deriving from three genes *PIK3R1*, *PIK3R2* and *PIK3R3*, respectively [4]. The p110α subunit consists of five domains: an amino-terminal domain termed adaptor-binding domain, a Ras-binding domain, a C2 domain, a helical domain and a kinase catalytic domain [5]. The p85α regulatory subunit also contains five domains: an amino-terminal SH3 domain, a Rho-GAP domain and two Src homology 2 (SH2) domains (one towards the amino terminus, nSH2, and one carboxy-terminal, cSH2), separated by an inter-SH2 (iSH2) domain [5].

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Upon growth factor stimulation p85 binds through its SH2 domains to phospho-motifs of RTKs, relieving its inhibitory effect over p110 and mediating the recruitment of PI3K to the plasma membrane. The activated p110 subunit catalyses the conversion of phosphatidylinositol-4, 5-bisphosphate to phosphatidylinositol-4,5-trisphosphate, which subsequently provides a docking site for the pleckstrin homology domain-containing proteins PDK1 and AKT [6]. The next step is a dual phosphorylation of AKT (on T308 and S473 residues), resulting in its activation and a subsequent intracellular cascade of phosphorylation of other proteins, including mammalian target of rapamycin (mTOR) [7]. The final functional outcome of this cascade of intracellular events is the induction of the multiple biologic effects of the PI3K/AKT/mTOR signaling pathway.

Activation of the PI3K/AKT/mTOR pathway has been demonstrated in all human cancers, with different aberrations variably affecting its different molecular components. In the setting of breast cancer, this represents the most commonly deregulated signaling pathway, with alterations that can be summarized as follows: i) overexpression of PI3K-activating RTKs; ii) inactivating events of negative PI3K pathway regulators (that is, phosphatase and tensin homologue (PTEN) and inositol polyphosphate 4-phosphatase type II); and iii) activating events of PI3K pathway components and/or positive regulators. Mutations of the *PIK3CA* gene, belonging to the third category, represent the most frequently reported molecular alterations of the PI3K signaling pathway in breast cancer.

## Preclinical data

### Oncogenicity of *PIK3CA* mutations

*PIK3CA* has been reported to be mutated frequently in human cancer, particularly in common cancer types such as breast, colorectal, endometrial and prostate [8-16]. This makes it an attractive target for therapeutic intervention. In the setting of breast cancer, *PIK3CA* mutations are extremely common, second only to *TP53* mutations [17-20]. The mutations display a non-random distribution, clustering within the helical domain (exon 9, commonly E542 and E545) and the kinase domain (exon 20, commonly H1047). When first reported, the presence of these 'hotspot' positions strongly implied that the mutant protein would be associated with increased kinase activity and oncogenic properties [21]. Such clustering of mutations in specific domains has been noted in other activating oncogenes, such as *BRAF*, *RAS* and *EGFR*. Interestingly, the non-class I PI3Ks have not been reported to be associated with oncogenic mutations.

The function of mutant PIK3CA protein compared with the wild type has been characterized in both human cancer cell lines and human mammary epithelial cells, mainly using gene targeting approaches [22-24]. Several investigators have reported that the mutation was strongly associated with AKT activation, growth factor-independent cell proliferation, resistance to apoptosis, as well as increased invasion and cell migration. Biochemical inhibition of the PI3K pathway was found to be effective in reversing these properties, particularly in *PIK3CA* mutant cell lines [22,23,25,26]. In human mammary epithelial cell lines, the two most common mutant alleles (H1047R and E545K) were found to activate PI3K signaling and could easily form tumors in nude mice [24,26]. Resistance to paclitaxel was also demonstrated [23]. Interestingly, significant increases in tumor angiogenesis have also been reported to be associated with oncogenic *PIK3CA* activity [26].

Differences between the helical and kinase domain mutants have also been extensively investigated. The data suggest that there are at least two different mechanisms by which mutant p110α can activate PI3K signaling. These differences are also supported by structural studies. The helical domain mutants require RAS binding for transformation and are independent of p85, whereas the H1047R mutant depends on p85 binding [27,28]. In another study, helical domain mutants produced a more aggressive phenotype than kinase domain mutants with regard to cellular motility and enhanced extravasation [29]. This study, however, used the MDA-MB-231 breast cancer cell line, which is known to be RAS mutant and ER-negative, so it is conceivable that the helical domain mutant could have synergized with these features. It is unclear how to extrapolate these data when, in breast cancer, *PIK3CA* mutations are strongly associated with an ER-positive phenotype and RAS mutations are extremely rare [29]. As a possible explanation for the phenotypic differences between the various *PIK3CA* mutations, a recent study has reported that helical domain but not kinase domain mutants acquire the capability to interact with IRS1, thus enhancing its ability to associate with the cellular membrane and subsequently activate the pathway [30]. This study highlighted that loss of p85 was not enough to result in growth factor-independent activity of p110α [30] and proposes a mechanistic reason for the differences seen between the helical and kinase domain mutations.

Crystal structure and biochemical analyses have also helped elucidate how different oncogenic *PIK3CA* mutations can change the PI3K architecture and promote oncogenicity dependent on the location of the mutated domain [31,32]. Mutations of the catalytic p110α subunit cluster around the activation loop involved in substrate recognition. In contrast, the helical domain mutants disrupt the interface between p110α and p85α, which likely increases the activity of the enzyme [31,32]. Besides these commonly occurring 'hotspot' *PIK3CA* mutations, rarer *PIK3CA* mutations on the C2 and RBD domains

have also been found in human cancers. These have mostly been found to also be oncogenic, although due to different mechanisms. For example, mutations in the C2 domain are thought to facilitate p110α localizing to plasma membrane by increasing the positive surface charge of this domain [33].

Interestingly, in breast cancer, the clinical difference between helical and kinase domain mutants is subtle [34,35]. Double mutants, or cases with two different *PIK3CA* mutations, have also been observed in breast cancer, albeit infrequently. There seems to be a higher incidence of *PIK3CA* mutations, particularly the helical domain mutants, in lobular cancer versus ductal invasive breast cancers (lobular 30.8% versus ductal 24.4%; $P = 0.14$) [34]. Also of note is that the common breast cancer cell lines used in preclinical experiments (MCF7 and T47D) contain a *PIK3CA* mutation (helical and kinase domains, respectively). These cell lines strongly express ER, are of the 'luminal A' phenotype and are sensitive to treatment with the hormonal agent tamoxifen [36].

### *PIK3CA* mutations and therapy resistance *in vitro*

*PIK3CA* mutations have been reported to be associated with resistance to human epidermal growth factor receptor 2 (HER2) and endocrine therapies in a number of preclinical cell line and xenograft models. In the setting of HER2-positive breast cancer, several preclinical studies have reported that *PIK3CA* mutations are associated with resistance to HER2 blockade with trastuzumab [37,38]. Another study also confirmed that these mutations could mediate resistance to trastuzumab, although the E545K- and H1047R-HER2 overexpressing breast cancer cell lines were sensitive to GDC-0941, a pan-PI3K inhibitor [39]. PI3K signaling pathway activation has also emerged as a molecular mediator of endocrine resistance in the setting of luminal breast cancer, with multiple lines of evidence supporting this notion [40-43]. Several studies have demonstrated a clear synergy between endocrine treatment and various PI3K blocking agents [41-44].

### Mouse models of *PIK3CA* mutations

Generation of transgenic mouse models can help us better understand the function of *PIK3CA* mutation *in vivo*, its contribution to mammary tumorigenesis, as well as its contribution to resistance of commonly used therapies.

Several different types of *Pik3ca*-driven mouse models of breast cancer have been reported (Table 1) [45]. Interestingly, in one study using the MMTV-Cre *Pik3ca*$^{H1047R}$ model high lethality (75%) was observed in mice younger than 4 months due to non-mammary tumor-related causes [46]. Leakiness of the mouse mammary tumor virus (MMTV) promoter resulting in harmful *Pik3ca*$^{H1047R}$ expression in tissues other than mammary gland was thought to be the cause. Similarly, another study with MMTV-Cre *Pik3ca*$^{H1047R}$ mice also showed a high lethality rate for reasons other than mammary tumors (43%), questioning the utility of a broad transgenic method [47]. The other approach has been to create endogenous levels of *Pik3ca*$^{H1047R}$ using a knock-in system under the control of a native promoter (combined with MMTV-Cre) [48,49]. These models are created to induce physiological expression of the mutant protein in the mammary gland only.

All the *Pik3ca*-driven models have produced mammary tumors of varying histologies in contrast to single histology mouse models such as Neu, Myc and the polyoma middle-T antigen. These included fibroadenomas, adenocarcinomas, adenosquamous carcinomas, sarcomas and spindle cell tumors. These tumors expressed ERα, as well as basal and luminal cytokeratin markers. Transgenic models resulted in far shorter latency periods, probably due to the overexpression of the mutant and wild-type protein induced by the exogenous promoters. In contrast, the knock-in models, which produce endogenous levels of the mutant protein, had extremely long latencies before the development of tumors, which was shorter in parous versus nulliparous mice, suggesting that pregnancy significantly accelerated *Pik3ca* mutation-mediated mammary oncogenesis. Notably, in one knock-in model, a significant increase in cell number in the ducts (hyperplasia), as well as the number of surrounding stromal cells, was observed [48]. These cells represented expansion of the luminal progenitor population, which demonstrated enhanced colony size and formation, though without signs of classical PI3K pathway activation [48]. The lack of activation of the pathway (pAKT and pS6) seems to more closely replicate the human observations. Overall, metastases have been rarely reported, perhaps suggesting that additional genetic alterations are needed. Two studies reported reduced latencies as a result of synergism between *PIK3CA* H1047R and p53 mutations [47,49]. Another study reported that *PIK3CA* mutant tumors could recur using both PI3K-dependent and -independent mechanisms or c-MET and MYC overexpression, respectively, the latter leading to resistance to a PI3K inhibitor [50].

These data highlight the importance of *Pik3ca* mouse models in contributing to a better understanding of *PIK3CA* mutant pathogenesis and breast cancer development, as well as investigating resistance mechanisms to commonly used therapeutics. They will provide a better understanding of mutation-related cell-extrinsic mechanisms as the tumors grow in the setting of intact immune systems and surrounding stroma. *In vivo* mouse models may perhaps also clarify some of the counterintuitive results that have been observed in the clinical setting, which we will discuss below. Phenotypic differences between knock-in and transgenic models are also evident, and clinical observations will eventually validate

**Table 1 Genetically engineered mouse models of *PIK3CA* mutations**

| Study | Mouse model | Transgenic versus knock-in | Inducible versus non-inducible | Penetrance | Tumor latency | Histology |
|---|---|---|---|---|---|---|
| Tikoo *et al.* [48] | MMTV-Cre Pik3ca$^{H1047R}$ | Site-specific | Non-inducible | 100% | Nulliparous mice: 484 days | Fibroadenoma (45%) |
| | | | | | | Adenosquamous carcinoma (10%) |
| | | | | | Biparous mice: 393 days | Osteosarcoma (2.5%) |
| Yuan *et al.* [49] | MMTV-Cre Pik3ca$^{H1047R}$ | Site-specific | Non-inducible | NR | Nulliparous mice: 492 days | Fibroadenoma (76.9%) |
| | | | | | | Adenocarcinoma (15.4%) |
| | | | | | Multiparous mice: 465 days | Spindle cell neoplasia (7.7%) |
| Liu *et al.* [50] | MMTV-rtTA TetO-Pik3ca$^{H1047R}$ | Transgenic | Inducible (doxycycline) | 95% | 7 months | Solid (33%) |
| | | | | | | Acinar (8%) |
| | | | | | | Glandular (5%) |
| | | | | | | Papillary (12%) |
| | | | | | | Squamous metaplasia (15%) |
| | | | | | | Mixed (28%) |
| Adams *et al.* [47] | MMTV-Cre$^{NLST}$ Pik3ca$^{H1047R}$ | Transgenic | Non-inducible | NR | 5 months | Adenosquamous carcinoma (51%) |
| | | | | | | Adenomyoepithelioma (45%) |
| | | | | | | Spindle cell neoplasia (1%) |
| | | | | | | Poorly differentiated adenocarcinoma (3%) |
| | MMTV-Cre$^{NLST}$ Pik3ca$^{H1047R}$; p53$^{f/+}$ | Transgenic | Non-inducible | NR | <5 months | Adenosquamous carcinoma (51%) |
| | | | | | | Spindle cell/EMT tumor (33%) |
| | | | | | | Radial scar lesion (10%) |
| | | | | | | Poorly differentiated adenocarcinoma (5%) |
| Meyer *et al.* [46] | WAPi-Cre Pik3ca$^{H1047R}$ | Transgenic | Non-inducible | NR | Nulliparous mice: 219 days | Adenosquamous carcinoma (54.6%) |
| | | | | | | Adenomyoepithelioma (22.7%) |
| | | | | | Parous mice: 140.3 days | Adenocarcinoma with squamous metaplasia (13.6%) |
| | | | | | | Adenocarcinoma (9.1%) |
| | MMTV-Cre Pik3ca$^{H1047R}$ | Transgenic | Non-inducible | 25% | Nulliparous mice: 214 days | Adenomyoepithelioma (100%) |

*EMT* epithelial-mesenchymal transition, *MMTV* mouse mammary tumor virus, *NR* not reported.

which model more closely represents human *PIK3CA* mutated breast cancer.

## Clinical data

### *PIK3CA* mutations, prognosis and treatment efficacy in breast cancer

The clinical relevance of *PIK3CA* mutations in newly diagnosed breast cancer disease has been extensively investigated. Surprisingly, *PIK3CA* mutations have been associated with good prognostic clinico-pathological features in breast cancer. These include positive expression of ER, smaller tumor size and low histological grade [51-54].

Whilst smaller studies initially reported inconsistent prognostic results, the larger studies now emerging seem to be trending in the same direction [40]. The largest published study evaluated *PIK3CA* genotype from 687 tumor samples from patients enrolled in the FinHER prospective, phase III clinical trial [34,55]. *PIK3CA* mutant compared with wild-type patients were noted to have a better prognosis in the first 3 years, which disappeared with longer follow-up [56]. Consistent with these results, a single center retrospective cohort analysis of 590 patients also reported that *PIK3CA* mutations were associated with significantly better clinical outcomes [51]. A retrospective pooled analysis of four neoadjuvant endocrine therapy breast cancer trials involving 278 women did not find that *PIK3CA* mutations were associated with endocrine therapy resistance [57]. Recently, published in abstract form, *PIK3CA* genotyping of the TEAM adjuvant endocrine study found a mutation

frequency of 39.8% (1,702/4,272) in post-menopausal patients with ER-positive tumors [35]. Again, significantly better survival was observed for the *PIK3CA* mutant breast cancers: hazard ratio 0.76 (95% confidence interval 0.63 to 0.91), $P = 0.003$.

*PIK3CA* mutations have been reported in ductal carcinoma *in situ* [58], suggesting that they are an early event, consistent with the knock-in mouse models. However, it seems that in breast cancer the mutation is not associated with high levels of PI3K pathway activation such as increased phosphorylated AKT (S473) and pS6 [18,59]. A genomic study reported that a gene signature developed from *PIK3CA* mutant human breast cancers was associated with low mTORC1 output and high *ESR1* signaling [44]. In contrast, *PIK3CA* mutant cell lines were associated with high levels of activation *in vitro*. This observation further supports the use of transgenic knock-in mouse models rather than breast cancer cell lines to investigate the functional effects of *PIK3CA* mutations. These data suggest several possibilities. Perhaps, similar to PTEN deficiency, high levels of PI3K pathway activation could be detrimental to the cell (that is, cause senescence); therefore, strong negative feedback is active in containing pathway activation until a 'second hit' disables this [60]. Alternatively, *PIK3CA* mutations may be weak activators of the PI3K pathway, due to the requirement for plasma localization and/or other activating factors, and require another hit(s) for full activation. We also speculate that the mutation may itself activate estrogen signaling given the strong cross-talk that exists between the two pathways. This would result in patients with *PIK3CA* mutations responding well to current endocrine therapies, which may explain the clinical observations.

With regards to HER2-positive disease, a number of single arm, cohort, single institutional series have suggested an association between the PI3K signaling pathway and trastuzumab and/or lapatinib resistance [61-65]. The majority of these have included PTEN loss or *PIK3CA* mutations to define activated PI3K pathway. The only data evaluating differences in treatment benefit from a randomized study did not observe that *PIK3CA* mutations were significantly associated with resistance to trastuzumab [56]. In fact, the opposite was observed. In contrast, in metastatic HER2+ disease, *PIK3CA* mutations have been associated with poor prognosis. Results from a retrospective biomarker analysis in the CLEOPATRA study, a phase III study assessing the trastuzumab, pertuzumab and docetaxel triplet versus trastuzumab, docetaxel and placebo in first-line treatment of HER2-positive metastatic breast cancer [66], were recently presented. *PIK3CA* genotype from the primary (not metastatic) tumor was found to be prognostic, with patients bearing a *PIK3CA* mutation having a worse clinical outcome ($P = 0.0001$) [67]. Interestingly, *PIK3CA* mutations did not predict for resistance to any type of HER2 blockade in this study, with significant clinical benefit of the triple combination of trastuzumab, pertuzumab and docetaxel persisting irrespective of its mutational status [67]. Further data will be required to confirm these findings. A more complete understanding of the genetic composition of these tumors, both primary and metastatic, will also be beneficial. It could be that, in the advanced setting, dual *HER2* amplification and *PIK3CA* mutation results in complete and robust activation of the PI3K pathway.

Hence, it is becoming clearer that *PIK3CA* mutations are associated with better outcomes in primary ER-positive disease. Generating firm associations with prognosis and clinical relevance could perhaps be achieved by a pooled analysis of all available data. This could result in *PIK3CA* genotype being integrated into clinical decision-making. However, its relevance in advanced disease is unclear and may be different from primary disease. However, the most interesting question remains: will a *PIK3CA* mutation predict for increased sensitivity to a PI3K inhibitor?

## Therapeutic targeting of *PIK3CA* mutated breast cancer

Currently, an abundance of targeted compounds are under clinical development targeting several components of the PI3K signaling pathway (Figure 1, Table 2) [68]. Preclinical evidence demonstrates sensitivity of *PIK3CA* mutated breast cancer cells to PI3K blocking agents [69,70] and, with p110α isoform-selective inhibitors being under clinical development, there is the promise for more potent target inhibition coupled with a milder toxicity profile [71]. Whilst the clinical development of those agents is still too preliminary for any definitive conclusions to be drawn, early data from phase I clinical trials do not support a strong association of antitumor activity by pan-class I PI3K blocking agents with *PIK3CA* genotype [72-74]. However, recent early results using the p110α isoform-selective inhibitors look promising in heavily pretreated *PIK3CA* mutant breast cancers [72]. BOLERO-2 was a phase III trial that randomized 724 patients with ER-positive metastatic breast cancer resistant to nonsteroidal aromatase inhibitors to receive exemestane and everolimus (an mTORC1 inhibitor) or placebo. The outstanding results have led to the registration of everolimus in this setting [75]. A biomarker analysis using available primary tumor from 227 (31%) patients from this study and a Foundation Medicine 182 cancer-mutation panel found *PIK3CA* was the most frequently mutated gene among the cases analyzed (48%). However, it was not found to be predictive, with similar treatment benefit derived from the everolimus plus exemestane therapy among *PIK3CA* mutated and wild-type breast cancer patients [76]. Hence, the

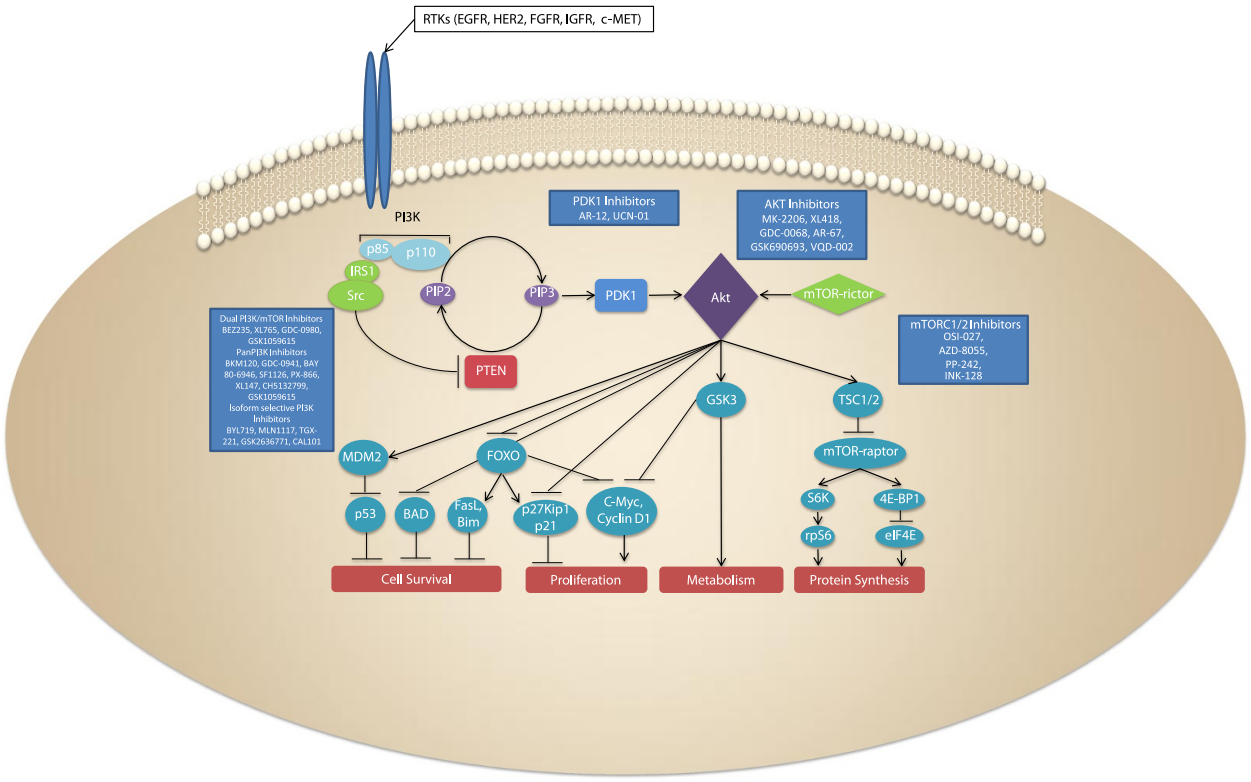


**Figure 1 Schema of phosphoinositide 3-kinase blocking agents currently under clinical development.** Dual phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitors (for example, BEZ235, XL765, GDC-0980, GSK1059615), pan-PI3K inhibitors (BKM120, GDC-0941, BAY 80–6946, SF1126, PX-866, XL147, CH5132799, GSK1059615), isoform-selective PI3K inhibitors (p110α selective: BYL719, MLN1117; p110β selective: TGX-221, GSK2636771; p110γ selective: AS-252424; p110δ selective: CAL-101), AKT inhibitors (MK-2206, XL418, GDC-0068, AR-67, GSK690693, VQD-002), mTORC1/2 inhibitors (OSI-027, AZD-8055, PP-242, INK-128), and PDK1 inhibitors (AR-12, UCN-01). $PIP_2$, phosphatidylinositol-4,5-bisphosphate; $PIP_3$, phosphatidylinositol-4,5-trisphosphate; RTK, receptor tyrosine kinase.

**Table 2 Ongoing clinical trials recruiting breast cancer patients with *PIK3CA* mutations**

| Agent | Class | Trial | Description | Patients (n) |
|---|---|---|---|---|
| BYL719 | α-Selective PI3K inhibitor | Phase I (NCT01219699) | Dose escalation in combination with fulvestrant | Postmenopausal women with MBC (160) |
| | | Phase Ib/II (NCT01708161) | Dose escalation in combination with AMG479 | Advanced solid tumors (70) |
| BKM120 | Pan-PI3K inhibitor | Phase I/II (NCT01589861) | Dose escalation in combination with lapatinib | HER2-positive, trastuzumab-resistant MBC (106) |
| MK2206 | AKT inhibitor | Phase II (NCT01277757) | Safety and efficacy of MK2206 monotherapy | Advanced breast cancer (40) |
| | | Phase II (NCT01776008) | Safety and efficacy of MK2206 and anastrozole with or without goserelin in the neoadjuvant setting | ER-positive breast cancer, stage II to IIIC (87) |
| AZD5363 | AKT inhibitor | Phase I (NCT01226316) | Dose escalation | Advanced solid tumors and MBC (107) |
| | | Phase I (NCT01625286) | Dose escalation in combination with paclitaxel | ER-positive MBC (110) |

*ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *MBC* metastatic breast cancer, *PI3K* phosphoinositide 3-kinase.

optimal PI3K pathway inhibition strategy in the setting of *PIK3CA* mutations also remains to be determined.

The only way to definitively determine the prognostic and predictive relevance of *PIK3CA* genotype in breast cancer will be through prospectively defined, upfront stratification in clinical trials. The 'NeoPHOEBE' trial (ClinicalTrials.gov study NCT01816594 [77]) is one such trial. This study will evaluate if the addition of BKM120, an oral pan-class I PI3K inhibitor, to trastuzumab improves response rates in HER2-overexpressing breast cancer. Eligible patients will undergo upfront *PIK3CA* genotyping as the trial will essentially have two identical cohorts in order to ensure that the *PIK3CA* mutant population is adequately powered. This trial will attempt to provide answers to the following important questions: i) is *PIK3CA* mutated, HER2-positive disease associated with trastuzumab resistance compared with wild type (prognostic implications), and ii) is *PIK3CA* mutation compared with wild-type associated with an increased response rate in the experimental arm with the PI3K inhibitor (predictive potential) (Figure 2). Only trials such as this one will be able to enlighten us on both the prognostic and predictive implications of this common aberration.

## Conclusion

*PIK3CA* mutations represent one of the most common molecular aberrations in breast cancer. Despite the counterintuitive findings concerning their prognostic significance, active investigation of PI3K pathway blockade is currently ongoing and still could prove to be a curative strategy for *PIK3CA* mutant breast cancers. Prospective clinical trials selecting patients on the basis of *PIK3CA* mutations are currently recruiting (Table 2), but upfront stratification will be required in order to ensure enough power is seen in the *PIK3CA* mutant subgroup. However, there is still much to be learnt about how the mutation contributes to breast cancer growth and, most of all, why high levels of classical PI3K signaling are not observed in human breast cancers. This may be critical to understanding who will respond to therapeutic PI3K inhibition. Recently developed mouse models will help to increase our understanding of cooperating pathways and mammary tumor pathogenesis, as well as immune and stromal influences. Detailed translational research correlative efforts will need to be systematically coupled with clinical trials evaluating efficacy of PI3K inhibitors in breast cancers, as this will enhance our understanding of responders and non-responders by providing the complete genomic

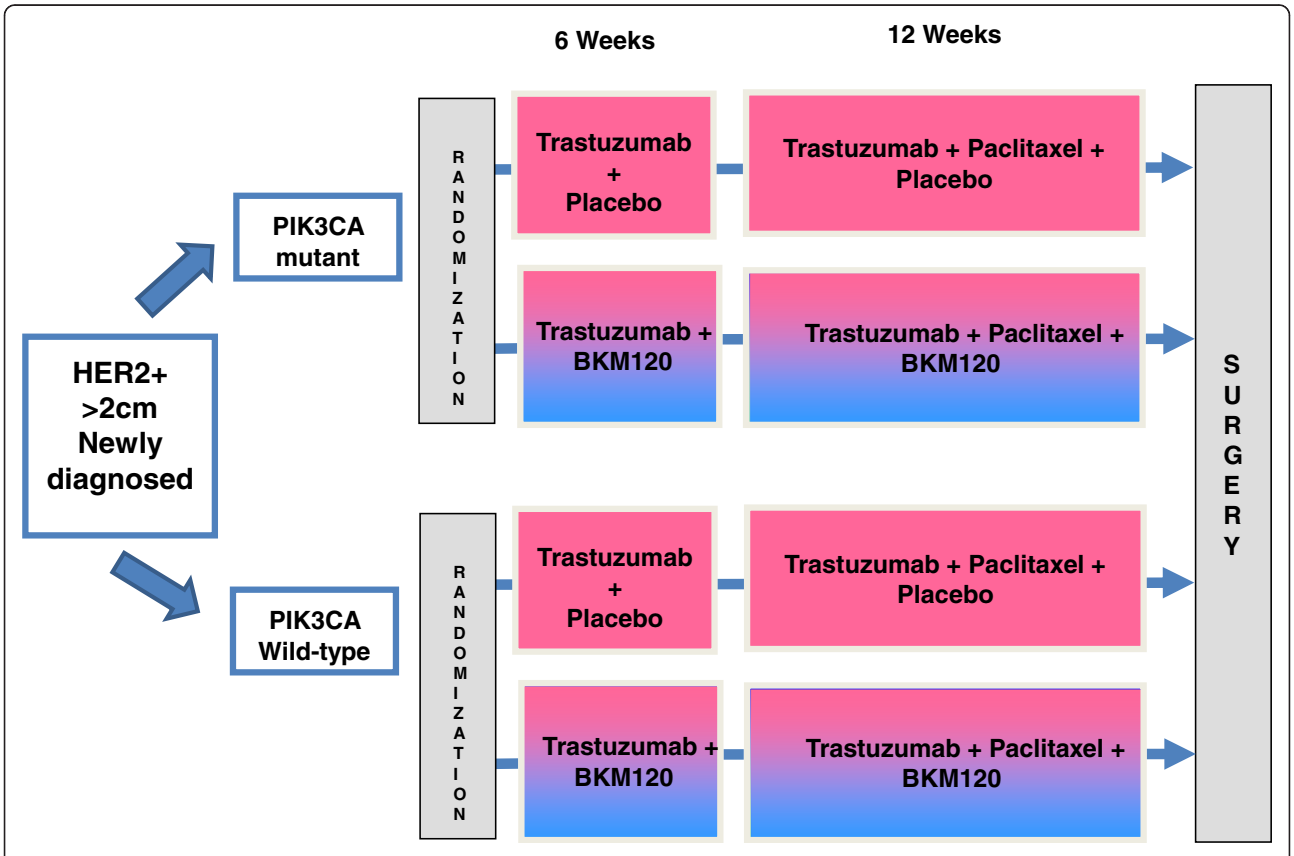


**Figure 2 Trial design of the 'NeoPHOEBE' trial with two identical cohorts and upfront *PIK3CA* genotyping to ensure adequate power for both mutant and wild-type cohorts.** HER2, human epidermal growth factor receptor 2.

landscape associated with *PIK3CA* mutations and treatment response.

#### Abbreviations

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; MMTV: Mouse mammary tumor virus; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositide 3-kinase; PTEN: Phosphatase and tensin homologue; RTK: Receptor tyrosine kinase.

#### Competing interests

DZ and WAP have no competing interests to declare. A provisional worldwide patent was filed by Université Libre de Bruxelles for *PIK3CA* mutation gene signature: prognosis and therapeutic responsiveness of HER2 overexpressing and estrogen receptor-positive breast cancer. SL is a named inventor, but receives no financial income. SL has acted as consultant for Novartis but has received no honoraria.

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