VOLUME 29 · NUMBER 33 · NOVEMBER 20 2011

JOURNAL OF CLINICAL ONCOLOGY

BIOLOGY OF NEOPLASIA

Phosphatidylinositol 3-Kinase and Antiestrogen Resistance in Breast Cancer

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Submitted April 22, 2011; accepted August 4, 2011; published online ahead of print at www.jco.org on October 17, 2011.

Supported by National Institutes of Health Grant No. K99CA142899 (T.W.M.); Breast Cancer Specialized Program of Research Excellence Grant No. P50CA98131; Vanderbilt-Ingram Cancer Center Support Grant No. P30CA68485; a grant from the Breast Cancer Research Foundation (C.L.A.); American Cancer Society Clinical Research Professorship Grant No. CRP-07-234 (C.L.A.); the Lee Jeans Translational Breast Cancer Research Program (C.L.A.); and Stand Up to Cancer/American Association for Cancer Research Dream Team Translational Cancer Research Grant No. SU2C-AACR- DT0209 (C.L.A.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/11/2933-4452/$20.00

DOI: 10.1200/JCO.2010.34.4879

ABSTRACT

Although antiestrogen therapies targeting estrogen receptor (ER) α signaling prevent disease recurrence in the majority of patients with hormone-dependent breast cancer, a significant fraction of patients exhibit de novo or acquired resistance. Currently, the only accepted mechanism linked with endocrine resistance is amplification or overexpression of the *ERBB2* (human epidermal growth factor receptor 2 [*HER2*]) proto-oncogene. Experimental and clinical evidence suggests that hyperactivation of the phosphatidylinositol 3-kinase (PI3K) pathway, the most frequently mutated pathway in breast cancer, promotes antiestrogen resistance. PI3K is a major signaling hub downstream of HER2 and other receptor tyrosine kinases. PI3K activates several molecules involved in cell-cycle progression and survival, and in ER-positive breast cancer cells, it promotes estrogen-dependent and -independent ER transcriptional activity. Preclinical tumor models of antiestrogen-resistant breast cancer often remain sensitive to estrogens and PI3K inhibition, suggesting that simultaneous targeting of the PI3K and ER pathways may be most effective. Herein, we review alterations in the PI3K pathway associated with resistance to endocrine therapy, the state of clinical development of PI3K inhibitors, and strategies for the clinical investigation of such drugs in hormone receptor–positive breast cancer.

J Clin Oncol 29:4452-4461. © 2011 by American Society of Clinical Oncology

INTRODUCTION

Approximately 75% of breast cancers express estrogen receptor (ER) α and/or progesterone receptor (PR). Hormone receptor expression typically indicates a degree of estrogen dependence for cancer cell growth. Treatment for these patients inhibits ER function either by antagonizing ligand binding to ER (tamoxifen and other selective ER modulators [SERMs]), downregulating ER (fulvestrant), or blocking estrogen biosynthesis (aromatase inhibitors [AIs]). Although endocrine therapies have changed the natural history of hormone-dependent breast cancer, many tumors exhibit de novo or acquired resistance. For example, more than 30% of patients with early ER-positive breast cancer relapse within 15 years after adjuvant therapy with tamoxifen, and 17% of patients treated with an AI relapse within 9 years.[1,2] An accepted mechanism of resistance to endocrine therapy involves overexpression of the *ERBB2* (*HER2*) proto-oncogene.[3-5] However, less than 10% of ER-positive breast cancers overexpress *HER2*, suggesting that for most ER-positive breast cancers, mechanisms of escape from endocrine therapy remain to be elucidated.

Tamoxifen has been a standard treatment for ER-positive breast cancer for more than 30 years. This SERM has dual agonistic/antagonistic effects on ER transcription and breast cancer cell growth.[6,7] In contrast, AIs induce estrogen deprivation without agonistic effects on ER. AIs are clinically equivalent if not modestly superior to tamoxifen.[2,8] Data on the clinical activity of fulvestrant as first-line therapy in the metastatic setting are limited but suggest it may be superior to AIs despite its inability to completely downregulate ER in patients' tumors.[9-11] It is unclear whether mechanisms of antiestrogen resistance are common to SERMs, AIs, and ER downregulators. Studies in human cell lines and xenografts have shown that growth factor receptor signaling pathways, particularly those that converge on phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK/ERK), can mediate resistance to all forms of endocrine therapy. PI3K is the most frequently altered pathway in breast cancer, with mutation and/or amplification of the genes encoding the PI3K catalytic subunits p110α (*PIK3CA*) and p110β (*PIK3CB*); the PI3K regulatory subunit p85α (*PIK3R1*); receptor tyrosine kinases (RTKs) such as HER2 (ERBB2) and fibroblast growth factor receptor 1 (FGFR1); K-Ras; PI3K effectors AKT1, AKT2, and PDK1; and loss of the lipid phosphatases phosphatase and tensin homolog (PTEN) and INPP4B (Table 1).

Table 1. PI3K Pathway Alterations Associated With Response to Endocrine Therapy

Gene	Protein	Aberration	Effect on Signaling	Frequency	Patient Prognosis	Reference
ERBB2	HER2	Gene amplification or overexpression	Hyperactivation of ErbB2 signaling (PI3K, MEK)	Approximately 10% of ER-positive tumors	Correlates with worse outcome	Ellis et al,[3] Arpino et al,[4] De Laurentiis et al[5]
PTEN		Loss-of-function mutation or reduced expression	Hyperactivation of PI3K signaling	37% to 44% of ER-positive tumors	No consistent correlation	Pérez-Tenorio et al,[12] Saal et al,[13] Shoman et al[14]
PIK3CA	p110α, PI3K	Activating mutation	Hyperactivation of PI3K signaling	28% to 47% of ER-positive tumors	No correlation or correlation with better outcome	Pérez-Tenorio et al,[12] Baselga et al,[15] Stemke-Hale et al,[16] Ellis et al,[17] Campbell et al[18]
PIK3CB	p110β, PI3K	Amplification	Unknown	5% of all cases	Unknown	Crowder et al[19]
IGF1R, INSR	IGF-1R, InsR	Receptor activation	Activates IGF-1R/InsR signaling (PI3K, MEK)	48% of ER-positive tumors	Unknown	Law et al[20]
FGFR1		Amplification	Hyperactivation of FGFR signaling (PI3K, MEK)	11.6% of ER-positive tumors	Correlates with shorter RFS	Turner et al[21]
RPS6K1	p70S6K	Amplification	Activates mTORC1, protein translation	8.8% to 12.5% of all tumors	Unknown	Monni et al[22]
INPP4B		Reduced expression or genomic loss	Hyperactivation of PI3K signaling	8.4% to 37.7% of ER-positive tumors	Correlates with worse outcome	Gewinner et al,[23] Fedele et al[24]
PIK3R1	p85α, PI3K	Inactivating mutation	Derepression of catalytic activity of p110α	2% of all tumors	Unknown	Jaiswal et al[25]
AKT1		Activating mutation	Hyperactivation of AKT signaling	2.6% to 3.8% of ER-positive tumors	Unknown	Stemke-Hale et al,[16] Loi et al,[26] Carpten et al[27]
AKT2		Amplification	Hyperactivation of AKT signaling	2.8% of all tumors	Unknown	Bellacosa et al[28]
EGFR		Amplification	Hyperactivation of EGFR signaling (PI3K, MEK)	0.5% of ER-positive tumors	Unknown	Al-Kuraya et al[29]
PDK1		Amplification or overexpression	Hyperactivation of PDK1 signaling (AKT, mTORC1)	21% of all tumors	Unknown	Maurer et al[30]
KRAS		Activating mutation	Hyperactivation of Ras signaling	4% to 6% of all tumors	Unknown	Rochlitz et al,[31] Di Nicolantonio et al,[32]

Abbreviations: EGFR, epidermal growth factor receptor; ER, estrogen receptor; FGFR, fibroblast growth factor receptor 1; HER2, human epidermal growth factor receptor 2; IGF-1R, insulin-like growth factor-1 receptor; InsR, insulin receptor; mTORC1, mammalian target of rapamycin complex 1; PI3K, phosphatidylinositol 3-kinase; RFS, relapse-free survival.

CROSSTALK BETWEEN ER AND PI3K PATHWAYS

PI3K is activated by growth factor RTKs and G-protein–coupled receptors (GPCRs). PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP_3).[33] In turn, PIP_3 recruits several pleckstrin homology (PH) domain–containing proteins to the plasma membrane such as PDK1, serine/threonine-protein kinase, and AKT, which on activation drive cell-cycle progression and survival.[34,35] Negative regulation of this pathway is conferred by PTEN and INPP4B, which dephosphorylate PIP_3 and PIP_2, respectively.[23,36] AKT activates the mammalian target of rapamycin (mTOR) –containing complex 1 (TORC1),[37] which regulates protein synthesis. mTOR is also part of another complex (ie, TORC2), which lies upstream of AKT.[38]

In addition to its pro-survival and growth-promoting roles, the PI3K pathway interacts with ER directly and indirectly (Fig 1). ER phosphorylation at Ser_{167} by AKT or p70S6K increases estrogen-induced, tamoxifen-induced, and ligand-independent ER transcriptional activity.[39,40] Additionally, PI3K and Ras promote c-Jun phosphorylation.[41-45] c-Jun complexes with c-Fos to form the AP-1 complex, which cooperates with ER transcription.[46-48] Other oncogenic kinase pathways (eg, MAPK, protein kinase C) also contribute to the modulation of ER and transcription cofactors.[49]

The activation of ER by growth factor RTK signaling is reciprocated in a feed-forward fashion, whereby ER promotes the transcription of genes encoding ligands, RTKs, and signaling adaptors (Fig 1). Clinical evidence also suggests that ER function may sustain PI3K pathway activation in breast cancer cells. Neoadjuvant treatment of patients with ER-positive breast cancer with the AI letrozole reduces $P\text{-}AKT_{S473}$ and $P\text{-}mTOR_{S2448}$ tumor levels (markers of PI3K activity), which correlates with clinical response and improved disease outcome.[50] Another study detected a reduction in P-S6 levels (marker of

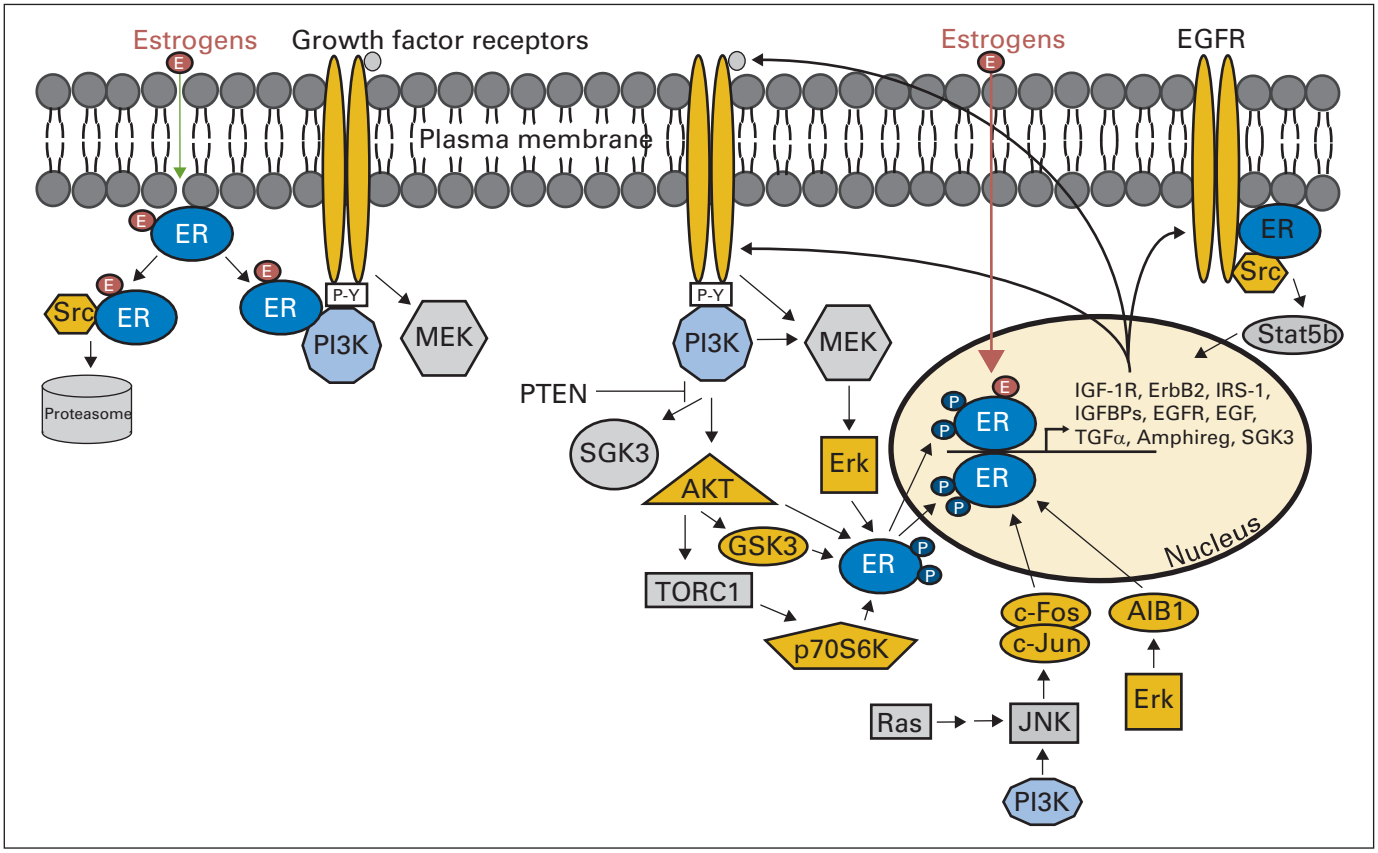

Fig 1. Reciprocal crosstalk between estrogen receptor (ER) α and growth factor receptor signaling pathways. Receptor tyrosine kinases (RTKs) and G-protein–coupled receptors activate phosphatidylinositol 3-kinase (PI3K; blue) and MEK signaling pathways. These signal transducers can then phosphorylate ER (green) and/or coactivators and corepressors to modulate ER transcriptional activity not necessarily dependent on ER ligands. In turn, ER transcribes genes encoding components of growth factor signaling pathways, thus completing signaling cycle of RTKs to ER to RTKs. ER also complexes with RTKs and Src to rapidly induce nongenomic signaling. ER-interacting proteins shown in color. EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor; IGFBP, insulin-like growth factor binding protein; IRS-1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; PTEN, phosphatase and tensin homolog; SGK3, serum/glucocorticoid-regulated kinase 3; TGFα, transforming growth factor alpha; TORC1, target of rapamycin complex 1.

TORC1 activity) after neoadjuvant letrozole.[15] Hence, estrogen deprivation may suppress ER-positive breast cancer cell growth in part by decreasing PI3K/AKT/mTOR signaling.

Experimentally, PI3K pathway activation has been causally associated with de novo and acquired resistance to endocrine therapy. RNAi-mediated knockdown of *PTEN* and overexpression of oncogenes that activate PI3K/AKT signaling (eg, *HER2*, type 1 insulin-like growth factor receptor (*IGF1R*), activated mutant *AKT1*) confer resistance to tamoxifen, fulvestrant, and estrogen deprivation in ER-positive breast cancer cells. In most such models, inhibition of PI3K has reversed antiestrogen resistance.[7,39,51] In another example, tamoxifen-resistant ER-positive MCF-7 breast cancer xenografts exhibited increased expression of IGF-1R, HER2, and epidermal growth factor receptor (EGFR).[52] One model of resistance to estrogen deprivation employs overexpression of aromatase in MCF-7 cells followed by selection with an AI. AI-resistant MCF-7/aromatase cells and xenografts have exhibited higher levels of HER2 and the EGFR ligand amphiregulin along with lower levels of ER.[53,54] We reported that four of four long-term estrogen-deprived (LTED) ER-positive breast cancer cell lines exhibited amplification of *PI3K/AKT/mTOR* signaling, and three of four lines showed hyperactivation of IGF-1R and/or InsR.[55] Chronic exposure of MCF-7 cells and xenografts to fulvestrant has been shown to elicit similar changes.[56-58]

Emerging evidence also implicates nongenomic, estrogen-induced signaling in the activation of PI3K. Estrogen stimulation rapidly initiates intracellular kinase signaling, including activation of IGF-1R/InsR, EGFR, Src, PI3K, and MEK (Fig 1).[51,59-61] This implies that a non-nuclear ER binds estrogen and transmits signals to membrane and cytosolic kinases. Several membrane ERs have been identified, including ERα, splice variants of ERα (ER-36, ER-46), ERβ, and GPCR 30.[62-64] Although ERα localizes to the plasma membrane in cultured cells, this has not been demonstrated in human tumors.[65] Membrane ER might activate oncogenic kinases to promote endocrine resistance; however, these mechanisms remain to be shown clinically.

PI3K pathway activation is required for growth of breast cancer cells resistant to endocrine therapy. Growth of four of four LTED cell lines in the absence of estrogen is inhibited by treatment with the PI3K/mTOR inhibitor BEZ235 or the TORC1 inhibitor everolimus.[55] Treatment of mice bearing estrogen-independent MCF-7 xenografts with the pan-PI3K inhibitor BKM120, or bearing letrozole-resistant MCF-7/aromatase xenografts with wortmannin, has been shown to slow tumor growth (unpublished data).[66,67] Also, treatment of MCF-7 and MCF-7/LTED cells with the Ras inhibitor farnesylthiosalicylic acid decreases mTOR signaling and hormone-independent growth.[68] Interestingly, a recent study showed that exogenous estrogen prevents

the apoptotic effects of BEZ235 and RNAi-mediated silencing of PI3K in ER-positive cells.[19] Most breast cancers that adapt to antiestrogen therapy retain ER and, presumably, estrogen sensitivity. These data imply that treatment of patients harboring ER-positive breast cancers with a PI3K-targeted therapy alone (without endocrine agent) might be insufficient to maximally inhibit tumor growth.

ASSOCIATION OF PI3K HYPERACTIVATION WITH RESISTANCE TO ENDOCRINE THERAPY

Gain-of-function oncogenes and/or loss of tumor suppressors in breast cancer cells may confer antiestrogen resistance via activation of PI3K. For example, HER2 overexpression predicts weaker response to neoadjuvant AIs or tamoxifen and worse outcome after adjuvant endocrine therapy compared with ER-positive/HER2-negative breast cancers.[3-5] Patients with FGFR1-overexpressing ER-positive tumors exhibit a shorter relapse-free survival (RFS) after adjuvant tamoxifen.[21] Patients with ER-positive/INPP4B-deficient tumors show worse survival compared with patients with ER-positive/INPP4B-positive tumors.[23] Although loss-of-function mutations in *PTEN* are rare in ER-positive breast cancer, immunohistochemical (IHC) studies have reported a wide range of PTEN loss but found no association of PTEN level with outcome after tamoxifen therapy. Whether other mutations in the PI3K pathway correlate with antiestrogen resistance remains to be determined.

Point mutations in *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, are the most common genetic alterations of this pathway in breast cancer. Up to 80% of *PIK3CA* mutations occur in hotspots within the helical (E542K and E545K) and kinase (H1047R) domains of p110α. They increase PI3K activity, induce cellular transformation in vitro and tumorigenicity in vivo when overexpressed in human mammary epithelial cells, and induce mammary tumor formation in transgenic mice.[69-72] Although such mutations occur in 28% to 47% of ER-positive breast cancers, their clinical significance remains unclear. In retrospective studies, *PIK3CA* mutations in primary ER-positive tumors have correlated with good long-term outcome.[12,15-18,26] In one report, *PIK3CA* mutations were linked with lower activation of PI3K (assessed by P-AKT) compared with PTEN deficiency in breast tumors.[16] In another study, a *PIK3CA*-mutant gene expression signature was associated with low P-AKT, low TORC1 signaling, and good outcome after adjuvant tamoxifen.[26] This association of *PIK3CA*-mutant status and good prognosis in ER-positive disease does not negate the possibility that combinations of antiestrogens and PI3K pathway inhibitors would be clinically more effective than antiestrogens alone in such tumors. Indeed, a recent analysis of a cohort of 217 patients with multiple solid tumor types enrolled onto phase I trials with PI3K/AKT/mTOR inhibitors showed a higher response rate among patients with *PIK3CA*-mutant versus *PIK3CA*–wild-type cancers.[73]

Understanding the relationship between *PIK3CA* mutations and endocrine resistance may be confounded by evidence suggesting that these genetic alterations may arise late in tumor development. For example, *PIK3CA* mutation status is discordant between invasive carcinoma and ductal carcinoma in situ in 33% of patient cases, between primary breast tumors and synchronous lymph node metastases in 13% of patient cases, between primary tumors and asynchronous metastases in 18% to 33% of patient cases, and even within microdissected regions of the same tumor.[74-76]

In addition to mutational analyses, tumor protein and gene expression profiling of PI3K pathway activation may provide a biomarker to identify patients with antiestrogen-resistant tumors. For example, a gene expression signature of PTEN loss, derived from a comparison of PTEN-positive and -negative tumors by IHC, was predictive of poor RFS after tamoxifen, whereas PTEN IHC status alone was not.[13] A gene expression signature of PI3K activation, based on levels of phosphoprotein markers (eg, P-AKT, P-p70S6K) in ER-positive tumors, was enriched in luminal B breast cancers.[77] This suggests that luminal B tumors have higher PI3K activity, which may contribute to their lower response to antiestrogens compared with luminal A tumors.[78] Similarly, we identified a tumor protein signature of PI3K pathway activation that predicts poor outcome after adjuvant endocrine therapy.[55] Therefore, signatures of PI3K activation may complement mutational analyses for the identification of PI3K-driven tumors.

RATIONALE FOR COMBINED USE OF ER AND PI3K INHIBITORS

One of the first PI3K pathway–targeted drugs to be tested in combination with endocrine therapy was the TORC1 inhibitor everolimus. Patients with ER-positive tumors were randomly assigned to neoadjuvant letrozole with or without everolimus for 4 months before surgery. The combination therapy induced a higher clinical response and greater suppression of tumor cell proliferation compared with letrozole alone.[15] In another study, patients with advanced disease who had progressed while receiving an AI were randomly assigned to tamoxifen with or without everolimus. Patients receiving tamoxifen plus everolimus showed a significantly improved rate of clinical benefit, time to progression, and disease-free survival compared with women receiving tamoxifen alone.[79]

Although combinations containing TORC1 inhibitors have shown clinical activity in breast cancer, inhibition of TORC1 relieves negative feedback on activators of PI3K (eg, IGF-1R, IRS-1, HER3), which in turn promote cell survival.[80-82] These results suggest that direct inhibitors of PI3K may be more effective. However, inhibition of PI3K or AKT also results in feedback upregulation/activation of several RTKs, which, by providing an upstream input to PI3K, may counteract drug action.[83-86] These data suggest that PI3K/AKT/TORC1 inhibitors should be combined with RTK inhibitors to induce an optimal antitumor effect. Consistent with this notion, studies with human xenografts have shown that combinations of inhibitors targeting HER2 and PI3K, HER2 and AKT, HER2 and TORC1, or EGFR and AKT are each superior to single-agent treatments.[80,83-85]

Additional rationales for combined inhibition of PI3K and ER come from studies in HER2-positive breast cancer. HER2 overexpression, a potent activator of PI3K, confers endocrine resistance.[87-90] Preclinical and clinical data suggest that ER-positive breast cancer cells initially inhibited by tamoxifen or estrogen deprivation can upregulate HER2 to bypass ER blockade.[88,91] The interdependence of these pathways is highlighted by examples in which HER2 inhibition with trastuzumab or the tyrosine kinase inhibitor lapatinib restores or upregulates ER levels or transcriptional activity in breast cancer cells and patient tumors.[92,93] Furthermore, treatment with AIs or downregulation of ER with fulvestrant or RNAi has inhibited the growth of HER2-positive tumors or cells that had progressed with trastuzumab or lapatinib.[92,93] These data suggest that combined inhibition of ER

and HER2 signaling may provide more effective control of ER-positive/HER2-positive tumors. This inference is supported by results from two clinical trials. In the TAnDEM (Trastuzumab in Dual Human Epidermal Growth Factor Receptor Type 2 [HER2] ER + Metastatic Breast Cancer) study, 207 patients with ER-positive/HER2-positive metastatic breast cancer were randomly assigned to the AI anastrozole with or without trastuzumab. A second study randomly assigned 219 patients with ER-positive/HER2-positive metastatic breast cancer to letrozole with or without lapatinib. In both trials, progression-free survival and clinical benefit were superior in the combination arm compared with the AI-alone arm, suggesting that both HER2 and ER should be simultaneously targeted for maximal therapeutic efficacy.[94,95] Whether combined inhibition of PI3K and ER is equally effective against ER-positive/HER2-positive tumors remains to be determined.

PI3K PATHWAY INHIBITORS IN CLINICAL DEVELOPMENT

Several drugs targeting multiple levels of the PI3K network (ie, PI3K, AKT, mTOR) are in clinical development. Class IA PI3K isoforms are heterodimeric lipid kinases that contain a p110 catalytic subunit and p85 regulatory subunit. Three genes (ie, *PIK3CA*, *PIK3CB*, and *PIK3CD*) encode the homologous p110α, p110β, and p110δ isozymes, respectively.[96,97] p110δ expression is largely restricted to immune and hematopoietic cells, whereas p110α and p110β are ubiquitously expressed.[98] p110α is essential for signaling and growth of tumors driven by *PIK3CA* mutations, RTKs, and/or mutant Ras, whereas p110β lies downstream of GPCRs and has been shown to mediate tumorigenesis in PTEN-deficient cells.[99] A number of ATP-mimetics that bind competitively and reversibly to the ATP-binding pocket of p110 are in early clinical development.[100,101] These include the pan-PI3K inhibitors BKM120, XL-147, PX-866, PKI-587, and GDC-0941; p110α-specific inhibitors BYL719, GDC-0032, and INK-1117; p110δ-specific inhibitor CAL-101; and dual PI3K/mTOR inhibitors BEZ235, BGT226, PF-4691502, GDC-0980, and XL-765.

The pan-PI3K and p110α-specific inhibitors are equally potent against oncogenic p110α mutants.[102-104] The rationale for the development of isozyme-specific antagonists is to allow higher doses of anti-p110α and anti-p110β drugs to be delivered without incurring adverse effects caused by pan-PI3K inhibitors. Interim results from a phase I trial with the p110δ-specific inhibitor CAL-101 in patients with hematologic malignancies showed that treatment reduced P-AKT levels by more than 90% in peripheral blood lymphocytes and induced objective clinical responses.[105] Recently completed phase I trials with BKM120, BEZ235, and XL-147 showed that treatment partially inhibited PI3K as measured by levels of P-S6 and P-AKT in patients' skin or tumors and [^{18}F]fluorodeoxyglucose uptake measured by PET. Main toxicities were rash, hyperglycemia, diarrhea, fatigue, and mood alterations. Few clinical responses were observed in patients with and without detectable PI3K pathway mutations, although screening for genetic lesions was not comprehensive.[106-108]

Both allosteric and ATP-competitive pan-inhibitors of the three isoforms of AKT are being developed. AZD5363, GDC-0068, GSK2141795, and GSK690693 are ATP-competitive compounds that have shown antitumor activity in preclinical models and recently entered phase I trials.[109,110] Allosteric inhibitors such as MK-2206 bind to the AKT PH domain and/or hinge region to promote a conformation incapable of membrane localization.[111] MK-2206 inhibits AKT signaling in vivo and suppresses growth of breast cancer xenografts harboring *PIK3CA* mutations or *ERBB2* amplification.[112] Phase I data have shown that treatment with MK-2206 decreases levels of P-AKT, P–proline-rich AKT substrate 40, and P-GSK3β in tumor cells, peripheral blood mononuclear cells, and hair follicles.[113,114]

The mTOR kinase is a component of PI3K-driven oncogenesis that functions within two signaling complexes: TORC1 and TORC2.[37,38] The macrolide rapamycin and its analogs complex with FK506-binding protein (FKBP12), which then binds to mTOR and inhibits the kinase activity of TORC1 but not TORC2.[38] The formulation problems of rapamycin prompted the development of analogs such as CCI-779 (temsirolimus), RAD001 (everolimus), AP-23573 (deferolimus), and MK-8669 (ridaferolimus). These rapalogs have shown cytostatic activity in preclinical models and clinical trials, particularly in patients with renal cell cancer and in those with mutations in the tuberous sclerosis complex (upstream of TORC1) who harbor renal angiolipomas. Compounds that target the ATP-binding cleft of mTOR (ie, OSI-027, AZD8055, INK-128), and are thus active against both TORC1 and TORC2, are also in phase I trials.[115]

CLINICAL INVESTIGATION OF PI3K PATHWAY INHIBITORS IN ER-POSITIVE BREAST CANCER

The somatic alterations described (ie, *PIK3CA* and *AKT1* mutations, PTEN and INPP4B loss, *PIK3CB* and *AKT2* amplification, and so on) identify cancers with aberrant activation of and potential dependence on the PI3K pathway. This is an important consideration for the selection of patients into trials with PI3K inhibitors. Like mutant PI3K, other somatic mutations in tumors have revealed molecules critical for cancer survival and progression. Pharmacologic targeting of these mutants has resulted in remarkable clinical responses in patients bearing tumors with such mutant oncogenes. Examples include imatinib and dasatinib in chronic myelogenous leukemia harboring the *BCR-ABL* oncogene, EGFR TKIs gefitinib and erlotinib in lung cancers with *EGFR*-activating mutations, HER2 antagonists trastuzumab and lapatinib in breast cancers with *HER2* gene amplification, and Raf inhibitors against metastatic melanomas containing B-Raf–activating mutations.[116]

As with other targeted therapies, only a fraction of patients with tumors containing PI3K pathway mutations will likely benefit from single-agent PI3K inhibitors. There is increasing agreement that initial phase II efficacy studies with PI3K inhibitors in patients with advanced disease should be enriched with, if not limited to, patients harboring mutations in this pathway. However, testing these drugs in single-arm phase II trials in patients with metastatic cancer with PI3K pathway alterations is intrinsically problematic because: first, the difficulty of obtaining biopsies from metastatic sites, and second, the limitations of assessing tumor response as a meaningful clinical end point in the absence of a placebo control arm. We will discuss alternative approaches that may address these issues.

PRESURGICAL AND NEOADJUVANT CLINICAL TRIALS

There are examples of short-term, pharmacodynamic trials providing information that can be later used for patient selection into trials with

novel targeted therapies such as PI3K antagonists. In ER-positive breast cancer, data from presurgical studies suggest that such trials can be used to predict longer-term outcome after adjuvant endocrine therapy. Dowsett et al[117] reported that patients with ER-positive tumors with high Ki67 scores after 2 weeks of neoadjuvant antiestrogen therapy had shorter RFS compared with those with low Ki67 scores. Ellis et al[118] found that Ki67 scores and ER status after 3 to 4 months of neoadjuvant endocrine therapy were predictive of RFS after adjuvant tamoxifen. Furthermore, ER-positive/HER2-negative tumors showed a reduction in Ki67 in response to neoadjuvant letrozole, whereas ER-positive/HER2-positive tumors did not.[3] Therefore, presurgical evaluation of molecular markers after short-term treatment may be valuable to predict long-term patient benefit. However, no single biomarker has been shown to accurately predict disease outcome in individual patients. Ki67 score was found to vary between biopsies from the same breast tumor such that a change greater than 36% to 50% in the post-treatment compared with pretreatment biopsy would be required to detect a potentially informative difference.[119,120] Furthermore, identifying patients who would benefit from neoadjuvant therapy using Ki67 score is challenging in tumors with rather low baseline cell proliferation.[120]

Although some PI3K pathway mutations (eg, *HER2*, *FGFR1* amplification) have been linked with antiestrogen resistance, the role of *PIK3CA* mutations is less clear.[3-5,21] Neoadjuvant trials provide a setting to investigate this role. For example, in a neoadjuvant study of letrozole with or without everolimus, $PIK3CA^{exon9}$ mutations were associated with a statistically lower reduction in cell proliferation in response to letrozole, as measured by a change in the Ki67 IHC score on day 15, compared with *PIK3CA*–wild-type tumors.[15] We have observed similar preliminary data in a presurgical trial of short-term letrozole (10 to 21 days) in patients with ER-positive/HER2-negative breast cancer (NCT00651976). In this study, analysis of the first 21 patients showed that *PIK3CA*-mutant tumors exhibited a statistically lower reduction in Ki67 score compared with tumors with wild-type *PIK3CA* (data not shown). However, another study in which tumor cell proliferation was assessed at baseline and after 4 months of antiestrogen therapy found no association between *PIK3CA* status and drug-induced change in Ki67 score.[17] This discrepancy may be the result of the different timing (2 to 3 weeks *v* 4 months) of the biopsies from which cell proliferation was assessed.

RANDOMIZED TRIALS OF ANTIESTROGENS WITH OR WITHOUT PI3K PATHWAY INHIBITORS

Although PI3K pathway activation may confer antiestrogen resistance, breast tumors that acquire hormone independence may still be stimulated by (host) estradiol. Thus, we speculate that PI3K inhibitors may have limited efficacy against ER-positive breast cancers as single agents. Because antiestrogen therapies are effective against a large fraction of ER-positive tumors as single agents, there is a need for randomized clinical trials of standard ER-targeted therapies versus combinations targeting both the ER and PI3K pathways.

In Table 2, we summarize the randomized trials in which inhibitors of TORC1, HER2, EGFR, IGF-1R, protein kinase C-β/PDK1/p70S6K, and farnesyltransferase have been combined with endocrine therapy. The molecular targets of these drugs rely on PI3K and have been linked to endocrine resistance. Neoadjuvant treatment with letrozole and the TORC1 inhibitor everolimus more effectively suppressed tumor cell proliferation and increased clinical response compared with letrozole alone in patients with early-stage ER-positive

Table 2. Prospective Randomized Trials Testing Combinations of PI3K Pathway Inhibitors and Endocrine Therapies

Kinase Target	Trial Design	Phase	Reference
mTOR	Letrozole ± everolimus in patients with early-stage ER-positive breast cancer	II	Baselga et al[15]
	Exemestane ± everolimus in ER-positive metastatic breast cancer after progression while receiving another AI	III	NCT00863655 (BOLERO-2; ongoing)
	Tamoxifen ± everolimus in ER-positive metastatic breast cancer after progression while receiving AI	II	TAMRAD; Bachelot et al[79]
HER2	Anastrozole ± trastuzumab in patients with ER-positive/HER2-positive metastatic breast cancer	III	Kaufman et al[95]
HER2/EGFR	Letrozole ± lapatinib in ER-positive metastatic breast cancer	III	Johnston et al[94]
	AI ± lapatinib or AI + fulvestrant ± lapatinib in ER-positive metastatic breast cancer after progression while receiving AI	III	NCT00688194 (ongoing)
	AI ± trastuzumab or lapatinib or both in patients with ER-positive/HER2-positive metastatic breast cancer	III	NCT01160211 (not yet open)
EGFR	Anastrozole ± gefitinib in early-stage ER-positive breast cancer	II	Smith et al[121]
	Anastrozole ± gefitinib in metastatic ER-positive patients	II	Cristofanilli et al[122]
PKCβ, PDK1, p70S6K	Fulvestrant ± enzastaurin in ER-positive metastatic breast cancer after progression while receiving AI	II	NCT00451555 (ongoing)
Farnesyl transferase	Letrozole ± tipifarnib in ER-positive metastatic breast cancer after progression on tamoxifen	II	Johnston et al[123]
IGF-1R/InsR	BMS-754807 ± letrozole in ER-positive metastatic breast cancer after progression while receiving AI	II	NCT01225172 (ongoing)
IGF-1R	IMCA12 ± same antiestrogen (AI, fulvestrant, or tamoxifen) after progression in patients with ER-positive metastatic breast cancer	II	NCT00728949 (ongoing)
	AI or fulvestrant ± AMG479 in ER-positive advanced or metastatic breast cancer after progression while receiving endocrine Tx	II	Kaufman et al[124]

Abbreviations: AI, aromatase inhibitor; BOLERO, Breast cancer trials of OraL EveROlimus; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IGF-1R, insulin-like growth factor-1 receptor; InsR, insulin receptor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; TAMRAD, Tamoxifen and RAD001.

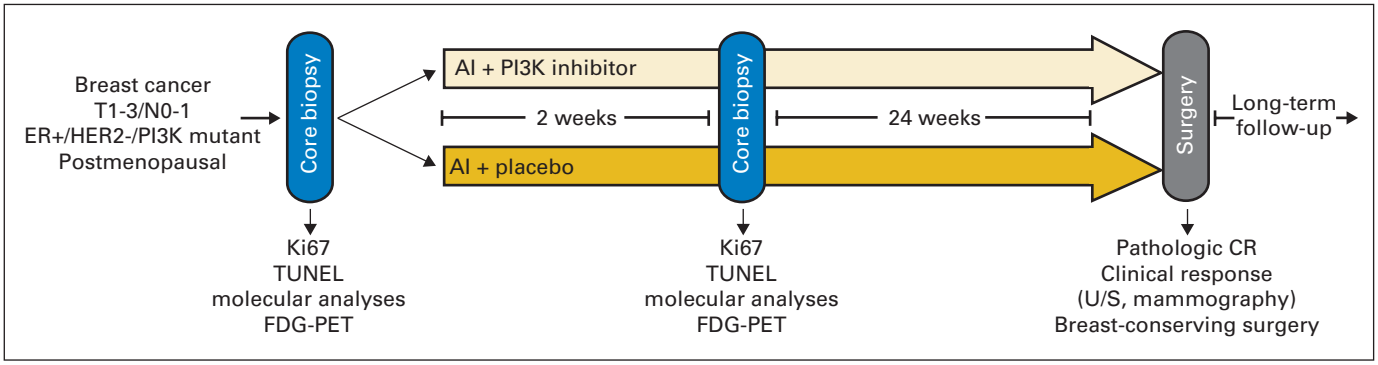

Fig 2. Diagram of neoadjuvant clinical trial with phosphatidylinositol 3-kinase (PI3K) pathway inhibitor. Patients with estrogen receptor (ER) –positive breast cancer eligible for neoadjuvant therapy randomly assigned to standard treatment (eg, aromatase inhibitor [AI]) with or without PI3K pathway inhibitor. Core biopsies obtained before and after 2 weeks of therapy to document effects on tumor cell proliferation, apoptosis, and pathway inactivation (ie, downregulation of P-AKT, P-S6, P–proline-rich AKT substrate 40 by immunohistochemistry). Incorporation of noninvasive [^{18}F]fluorodeoxyglucose (FDG) –positron emission tomography (PET) at 2 weeks could identify early metabolic changes; however, PI3K pathway inhibitors may induce hyperglycemia,[106-108] which could limit utility of FDG-PET. Primary end point of clinical response could be evaluated after approximately 4 to 6 months of therapy by measuring tumor with calipers, ultrasound (U/S), and/or mammography. Absence of tumor in surgical specimen would be scored as pathologic complete response (CR). Rate of breast-conserving surgery could be compared between both arms. Molecular mining of baseline, 2-week, and surgical biopsies could identify biomarkers that could be used for selection of patients enrolled onto subsequent studies of combination. HER2, human epidermal growth factor receptor 2; TUNEL, terminal deoxynucleotidyl nick-end labeling.

breast cancer.[15] In the TAMRAD (Tamoxifen and RAD001) trial, 111 patients bearing AI-resistant metastatic breast cancer (resistance defined as primary [relapse during adjuvant AI or $<$ 6 months after starting adjuvant AI in metastatic setting] or secondary [relapse $\geq$ 6 months on adjuvant AI or prior response to AI and subsequent metastatic progression]) were randomly assigned to tamoxifen with or without everolimus. Patients in the combination arm showed an improved clinical benefit rate (61% *v* 42%), time to progression (8.6 *v* 4.5 months), and overall survival compared with patients receiving tamoxifen alone. Patients with secondary but not primary AI resistance who received both drugs showed an increased time to progression compared with patients receiving tamoxifen alone.[79] In contrast, results from other studies have not favored the combination arm. For example, addition of the IGF-1R antibody AMG479 to fulvestrant or exemestane did not alter progression-free survival in patients with ER-positive breast cancer compared with endocrine therapy alone.[124] The addition of the farnesyltransferase inhibitor tipifarnib to letrozole did not improve response in patients with advanced ER-positive disease compared with letrozole alone.[123]

The neoadjuvant trial design depicted in Figure 2 illustrates an approach that can be used to determine whether to pursue combinations of PI3K inhibitors and antiestrogens in patients with ER-positive breast cancer. Such trials would have to be performed after safety of the combinations has been documented in phase I studies. Patients would be randomly assigned to standard endocrine therapy with or without a PI3K pathway inhibitor. A research biopsy could be obtained after 2 weeks to document effects on tumor cell proliferation, apoptosis, and ER/PI3K signaling and for wider exploratory mutational analysis. Incorporation of noninvasive imaging with [^{18}F]fluorodeoxyglucose–positron emission tomography at this time point could identify metabolic changes indicative of a pharmacodynamic effect. Study end points would be clinical and/or pathologic complete response (CR) after 4 to 6 months of therapy. Historically, pathologic CR has had limitations as an end point of neoadjuvant trials in ER-positive breast cancer. However, we cannot rule out that as we optimize targeted approaches such as antiestrogens in combination with PI3K inhibitors in ER-positive/PI3K-mutant tumors, a pathologic CR rate could be used as a primary end point for a trial of this design. This approach potentially addresses the following questions: First, is there an early (at 2 weeks) difference in cellular and molecular response between treatment arms? Second, is clinical and/or pathologic response superior in the arm containing the PI3K pathway inhibitor? A difference in favor of the combination would support further development of PI3K inhibitors and endocrine therapy in patients with advanced disease. Third, is there a tissue and/or imaging pharmacodynamic biomarker in the baseline, 2-week, and/or surgical specimen that correlates with response/resistance to the combination? If so, such a biomarker could be used to select patients with advanced disease who would likely benefit from the combination in phase II trials.

DISCUSSION

Alterations in the PI3K pathway are the most common somatic mutations in ER-positive breast cancer. Experimental and clinical evidence implies that such mutations are associated with antiestrogen resistance. Many PI3K pathway inhibitors are in clinical development. Early clinical data suggest that this strategy is feasible and that as single agents, these drugs are well tolerated. Although there is crosstalk between the ER and PI3K/AKT pathways, these networks also signal independently. Because most ER-positive breast cancers that acquire resistance to antiestrogens retain ER and responsiveness to estrogens, we speculate PI3K inhibitors should be used in combination with antiestrogens in patients who progress while receiving the latter. To determine if combined inhibition of PI3K and ER is more active than antiestrogen therapy alone against ER-positive tumors with mutations in the PI3K pathway, randomized clinical trials are required.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject

matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Carlos L. Arteaga, Roche (C), Monogram Biosciences (C), AstraZeneca (C), GlaxoSmithKline (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** Carlos L. Arteaga, U3-Amgen, AstraZeneca, Exelixis **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Manuscript writing: All authors
Final approval of manuscript: All authors

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