PIK3CA and PIK3CB Inhibition Produce Synthetic Lethality when Combined with Estrogen Deprivation in Estrogen Receptor–Positive Breast Cancer

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Abstract

Several phosphoinositide 3-kinase (PI3K) catalytic subunit inhibitors are currently in clinical trial. We therefore sought to examine relationships between pharmacologic inhibition and somatic mutations in PI3K catalytic subunits in estrogen receptor (ER)–positive breast cancer, in which these mutations are particularly common. RNA interference (RNAi) was used to determine the effect of selective inhibition of PI3K catalytic subunits, p110α and p110β, in ER+ breast cancer cells harboring either mutation (PIK3CA) or gene amplification (PIK3CB). p110α RNAi inhibited growth and promoted apoptosis in all tested ER+ breast cancer cells under estrogen deprived-conditions, whereas p110β RNAi only affected cells harboring PIK3CB amplification. Moreover, dual p110α/p110β inhibition potentiated these effects. In addition, treatment with the clinical-grade PI3K catalytic subunit inhibitor BEZ235 also promoted apoptosis in ER+ breast cancer cells. Importantly, estradiol suppressed apoptosis induced by both gene knockdowns and BEZ235 treatment. Our results suggest that PI3K inhibitors should target both p110α and p110β catalytic subunits, whether wild-type or mutant, and be combined with endocrine therapy for maximal efficacy when treating ER+ breast cancer. [Cancer Res 2009;69(9):OF1–8]

Introduction

Despite the use of adjuvant endocrine treatment, prognosis remains poor for a significant population of patients with estrogen receptor (ER)–positive breast cancer (1). The cellular basis for the efficacy of endocrine therapy treatment is principally through inhibitory effects on the tumor cell cycle (2) because, unlike cytotoxic chemotherapy, it has never been clearly shown that endocrine therapy promotes cell death through apoptosis (3). A logical approach to improving ER+ breast cancer treatment is, therefore, to inhibit gene activities that promote survival in the presence of ER-targeting agents. To address this hypothesis, we focused on combining endocrine agents with inhibitors of phosphoinositide 3-kinase (PI3K) because this pathway promotes cell survival in several tumor types (4).

Aberrant activation of the PI3K pathway through mutation and epigenetic silencing of genes within the PI3K signaling cascade frequently occurs in breast cancer. Gain-of-function mutations in the PI3Kα catalytic subunit (PIK3CA) occur in ~30% of ER+ breast cancer and, much less commonly, activating AKT1 mutations. Loss-of-function mutations affect the PI3K negative regulator PTEN and gene amplification in S6 protein kinase 1 (RPS6KB1) and AKT2 have also been reported (5–8). Whereas the precise consequences of these aberrations on the clinical outcome of ER+ disease remain to be fully defined, RPS6KB1 amplification and PTEN loss are both associated with poor prognosis and PTEF loss may correlate with endocrine therapy resistance in ER+ tumors (9–15). In contrast, PIK3CA presents a more complex picture and mutations may differentially affect prognosis depending on the affected PIK3CA functional domain (15). Finally, a role for PIK3CB, the gene encoding the PI3Kβ catalytic subunit, has also recently been postulated in breast cancer, although mutations in this gene have not been detected (16, 17).

Several PI3K catalytic subunit inhibitors are advancing toward phase II clinical testing (18). The targets for these agents are the products of the class 1A PI3K catalytic subunit genes (PIK3CA, PIK3CB, and PIK3CD). PIK3CA and PIK3CB are believed to be broadly expressed in breast cancer, whereas PIK3CD gene expression is more limited (19). We sought to address several issues related to the clinical development of these compounds. First, it is not clear if PIK3CA mutation status restricts the efficacy of PI3K inhibitors. Second, catalytic subunit targeting strategies for achieving maximum therapeutic effect have not been developed. Finally, a rationale for the combination of a PI3K inhibitor and endocrine therapy in ER+ breast cancer has not been established.

Materials and Methods

Human tumor samples. Fresh-frozen and formalin-fixed, paraffin-embedded human breast tumor biopsies for paired array comparative genomic hybridization (aCGH) and PIK3CB fluorescence in situ hybridization (FISH) were obtained from ER+ breast cancer patients undergoing preoperative letrozole treatment (20). RNA for transcriptional profiling and cDNA synthesis (samples >50% tumor) and DNA for aCGH (samples >70% tumor cellularity) were prepared from sectioned fresh-frozen samples using RNeasy Mini and QIAamp DNA Micro kits (Qiagen) for RNA and DNA extractions. Tumor enrichment was done using macrodissection or an
Arcturus Veritas laser capture microdissection instrument (Arcturus Bioscience). A human breast tissue microarray obtained at the Siteman Cancer Center Tissue Core Facility and used for PIK3CB FISH was described previously (21).

**Transcriptional profiling.** Details are provided in Supplementary Materials and Methods.

**Gene resequencing.** Details for PIK3CA and PIK3CB resequencing are provided in Supplementary Materials and Methods.

**Cell culture.** The HCC712 cell line (22) was provided by Dr. Adi Gazdar. Other cell lines were obtained from the American Type Culture Collection. Cell lines were propagated in RPMI 1640 containing 10% fetal bovine serum with antibiotics and supplements (50 μg/mL gentamicin, pyruvate, 10 mmol/L HEPES, and glucose to 4.5 g/L) in a humidified 37°C incubator containing 5% CO₂. To test the effects of estradiol (Sigma-Aldrich) treatment and withdrawal, cells were maintained in phenol red-free RPMI 1640 containing 5% charcoal-stripped serum (CSS; Invitrogen; CSS medium) for at least 7 days before small interfering RNA (siRNA) transfection or drug treatments.

**Protein extracts.** Details are provided in Supplementary Materials and Methods.

**RNA interference transfection.** Nuclease-resistant Stealth duplex siRNAs (Invitrogen) were used for RNA interference (RNAi) experiments. The following siRNAs were used: Universal Low GC Negative Control, PIK3CA siRNAs (target sequence 5’-GCGUGUCCGGAUUUCACACUUAU-3’ for primary siRNA duplex and 5’-CCCAGAAUACCUUCAGUAAGUUAUU-3’ for alternative siRNA duplex), and PIK3CB siRNAs (target sequence 5’-CGCUCAACUAGGGAUAAACCUU-3’ for primary siRNA duplex and 5’-GCCGUUGAAUUGAUUAACUGGAAA-3’ for alternative siRNA duplex). PIK3CA and PIK3CB siRNA knockdown efficiencies were determined by reverse transfection of siRNAs into cells and immunoblotting cell lysates prepared 3 days after transfection. Maximal knockdown efficiency (>70%) was achieved with 10 mmol/L PIK3CA or PIK3CB siRNA. Transfection efficiency assessed by the BLOCK-IT fluorescent oligo (Invitrogen) was >90% in all cell lines.

**Immunoblotting.** Details are provided in Supplementary Materials and Methods.

**Cell growth assay.** Details are provided in Supplementary Materials and Methods.

**Cell death assay.** Details are provided in Supplementary Materials and Methods.

**PIK3CB FISH.** Details are provided in Supplementary Materials and Methods.

**Statistical analysis.** Unless indicated otherwise, quantitative data are presented as mean ± SE. The effect of siRNA knockdowns and pharmacologic treatments on cell growth and apoptosis was analyzed using ANOVA. If within-group comparisons reached statistical significance (P < 0.05), comparisons between specific treatments were made with Student’s t test. Interactions between PAM50 subtypes or PIK3CA mutation status and PIK3CA, PIK3CB, and PIK3CD expression were analyzed by t tests using the SAS version 9.1 Statistical Package (SAS Institute).

**Results.**

**PIK3CB amplification in primary breast cancer.** aCGH analysis on ER⁺ primary breast tumors revealed PIK3CB copy gain in at least one tumor examined (1 of 35), which was confirmed by FISH (amplification ratio, 2.3; Fig. 1C). In another series of primary breast cancer samples from a breast tissue microarray, low-level PIK3CB amplification or copy number gain was observed by FISH in different breast cancer subtypes (1 ER⁺, 1 ER⁻, and 2 ER⁺/HER₂⁻), suggesting that PIK3CB copy number gain occurs with an incidence of ~5% (Supplementary Table S1). To determine if PIK3CB was mutant, the PIK3CB helical and kinase domains in 22 primary ER⁺ breast tumors were sequenced, including the PIK3CB amplified breast tumor illustrated in Fig. 1C and three breast cancer cell lines (HCC712, MCF-7, and T47D). No sequence anomalies were detected.

**PIK3CA and PIK3CB are expressed at higher levels in Luminal B breast cancer in comparison with Luminal A breast cancer.** The variation in PIK3CA and PIK3CB expression observed in breast cancer cell lines led to an expression analysis in a series of ER⁺ primary breast cancers. First, microarray studies were examined using the PAM50 model (23) to subtype cases into Luminal A (good prognosis - 31 cases) and Luminal B (poor prognosis - 44 cases; Table 1). There was strong evidence for higher expression of both PIK3CA and PIK3CB in poor prognosis Luminal B tumors when compared with Luminal A tumors. Higher expression of PIK3CD was also observed in Luminal B tumors, but the result was less striking. The presence of a PIK3CA mutation was associated with higher levels of PIK3CA mRNA but not PIK3CB or PIK3CD.

**p110h is the predominant mediator of PI3K signaling in breast cancer cells, but p110β contributes in a cell line–restricted manner.** To determine the individual effects of PIK3CA and PIK3CB on PI3K signaling, siRNAs were used to selectively knock down p110α and p110β expression (Fig. 2A). An analysis of signal transduction showed that PIK3CB RNAi had no effect on serum-stimulated Akt phosphorylation in MCF-7, T47D, and MDA-MB-231 cells but partially inhibited Akt phosphorylation in HCC712 cells (Fig. 2A). Knockdown of p110β had no clear effect on serum-stimulated phosphorylation of S6 protein in any of the cell lines tested. In contrast, PIK3CA RNAi suppressed serum-stimulated Akt phosphorylation in all cell lines tested. S6 phosphorylation was also significantly inhibited in MCF-7 and T47D cells but not in the HCC712 and MDA-MB-231 cell lines. Consistent with previous studies (24), we observed reductions in Akt and S6 protein levels in some experiments, particularly for MCF-7 cells. To test whether the lack of inhibition of serum-stimulated S6 phosphorylation by p110α knockdown in MDA-MB-231 and HCC712 cells was due to compensatory signaling through p110β, dual p110α/p110β knockdowns were done (Fig. 2B). Combined p110α/p110β knockdown had no clear effect on S6 phosphorylation in MDA-MB-231 cells. However, the combination partially inhibited S6 phosphorylation in HCC712 cells, indicating that both PIK3CA and PIK3CB must be inhibited to affect S6 kinase activation in this cell line. Overall, this analysis indicated that p110α is the major catalytic subunit that transduces PI3K pathway signals in ER⁺ breast cancer cells, but p110β significantly contributes to pathway activation, particularly in cells containing higher levels of p110β expression.

**PIK3CA and PIK3CB RNAi inhibit ER⁺ breast cancer cell growth and survival.** To more precisely determine the cellular
response of ER+ cells, the effects of RNAi-mediated p110α and p110β inhibition were examined under estrogen-dependent growth conditions (Fig. 3 A). PIK3CA RNAi inhibited growth in all cell lines, ranging from a modest reduction in growth in the MDA-MB-231 cell line to a >90% reduction in MCF-7 cells. In contrast, PIK3CB RNAi inhibited growth only in HCC712 cells. To determine if PIK3CA and PIK3CB RNAi promoted cell death, apoptosis was quantified in the presence and absence of estradiol (Fig. 3 B). Estrogen deprivation alone resulted in no significant increase in cell death in HCC712 and T47D cells but a modest (significant) increase in cell death in MCF-7 cells. However, PIK3CA RNAi resulted in significant activation of apoptosis in estrogen-deprived MCF-7, T47D, and HCC712 cells. In particular, p110α knockdown dramatically induced cell death in estrogen-deprived MCF-7 cells, with ~50% of cells dying via apoptosis 7 days after transfection (Fig. 3B). Consistent with data on cell growth, PIK3CB RNAi promoted apoptosis in HCC712 cells but not in the other cell lines examined. In contrast to the effects on ER+ cells, neither PIK3CA nor PIK3CB RNAi affected the survival of ER- MDA-MB-231 cells. Importantly, estradiol treatment suppressed the induction of apoptosis by PIK3CB RNAi in HCC712 cells and PIK3CA RNAi in all three ER+ cell lines, indicating that the combination of estrogen deprivation with specific PI3K inhibition caused synthetic lethality.

Combined PIK3CA/PIK3CB RNAi enhances apoptosis in estrogen-deprived ER+ breast cancer cells compared with either single-gene knockdown. Next, we examined the effects of simultaneous inhibition of PIK3CA and PIK3CB on cell growth and survival using RNAi (Fig. 4A and B). Dual knockdown of p110α and p110β reduced cell growth by ~90% in estrogen-deprived MCF-7 cells, similar to the inhibition of cell growth caused by p110α knockdown alone (Fig. 4 A). In contrast, dual p110α/p110β knockdowns produced a greater reduction in cell growth in estrogen-deprived T47D cells (90% inhibition of cell growth) in comparison with the single-subunit knockdowns (65% growth inhibition for PIK3CA RNAi and no significant growth inhibition for PIK3CB RNAi). Combined RNAi was also effective in inhibiting HCC712 cell growth; however, the growth of MDA-MB-231 cells was unaffected. Whereas dual p110α/p110β knockdown did not enhance cell death in estrogen-deprived MCF-7 cells compared with the marked effect already achieved by p110α knockdown alone, combined 110α/p110β knockdown resulted in ~4-fold higher levels of apoptosis in T47D and HCC712, similar to that achieved in MCF-7 cells with single PIK3CA knockdown. In contrast, the survival of MDA-MB-231 cells was unaffected by dual p110α/p110β knockdown (Fig. 4B). Importantly, estradiol treatment significantly rescued all three ER+ cell lines from cell death.

Figure 1. PIK3CB is amplified in breast cancer. A, p110α and p110β expression in breast cancer cell lines. Equal amounts (25 μg) of protein from each cell line were immunoblotted for the indicated proteins. Longer exposures revealed that both p110α and p110β proteins were expressed in all cell lines. B, PIK3CB aCGH analysis in breast cancer cell lines. Section of probes on chromosome 3 corresponding to the PIK3CB locus. Individual array probes indicate probable copy number gain (red), loss (green), or no change (gray) relative to female diploid DNA. C, aCGH and PIK3CB FISH in ER+ breast tumors. Top, aCGH probes corresponding to the PIK3CB locus. Arrows, tumor samples subjected to PIK3CB FISH. One of the FISH-tested tumor samples (asterisk) contained PIK3CB amplification. Bottom, FISH results from the PIK3CB-amplified breast tumor above. Red, CEP3 probe; green, PIK3CB-specific probe.
caused by dual p110α/p110β knockdown. It remained possible that the induction of apoptosis observed with RNAi knockdown was caused by off-target siRNA effects and that rescue of apoptosis with estradiol is not ER-dependent. However, dual p110α/p110β knockdowns in T47D cells with different siRNA than those used in Figs. 3A and B and 4A and B also induced apoptosis in estrogen-deprived cells. In addition, treatment with the ER-specific inhibitor fulvestrant abrogated rescue by estradiol, indicating that estradiol rescue was mediated by the ER (Fig. 4C).

**BEZ235 induces apoptosis in estrogen-deprived ER+ breast cancer cells.** The PIK3CA and PIK3CB RNAi experiments in the cell line panel provide a defined system for examining the potential of pharmacologic PI3K inhibitors in breast cancer cell lines. The effects of BEZ235 (a dual PI3K class 1 catalytic subunit/mTOR inhibitor) was therefore investigated. BEZ235 has been shown to potently inhibit wild-type and mutant p110α at low nanomolar concentrations (IC₅₀ ~5 nmol/L) and p110β at significantly higher concentrations (IC₅₀ ~75 nmol/L; ref. 25). Signaling effects in the cell line panel are consistent with a selective p110α inhibitor (summarized in Fig. 5A). Low concentrations (5 nmol/L) of BEZ235 significantly inhibited the growth of all three ER+ breast cancer cell lines in the presence and absence of estrogen (Fig. 5B). In contrast, only high concentrations (50 nmol/L) of BEZ235 inhibited MDA-MB-231 cell growth. When the effect of BEZ235 on cell survival in the presence and absence of estrogen was examined, marked differences between the three ER+ cell lines emerged (Fig. 5C). Treatment of estrogen-deprived MCF-7 and T47D cells with concentrations of BEZ235 as low as 5 nmol/L promoted cell death. However, effect of BEZ235 on survival was maximal using 5 nmol/L BEZ235 in T47D cells. In contrast, the level of apoptosis in MCF-7 cells increased with BEZ235 concentration and approached the levels observed with PIK3CA RNAi at 50 nmol/L. In estradiol rescue experiments, induction of cell death by 5 nmol/L BEZ235 in MCF-7 cells was completely blocked by estradiol, and in the presence of estradiol, 4-fold higher doses ≥20 nmol/L were required to induce cell death. Remarkably, estradiol abrogated the BEZ235-induced cell death in T47D cells at all doses tested. HCC712 cells were the least sensitive to BEZ235 treatment and required higher concentrations (≥20 nmol/L) to promote cell death under estrogen-deprived conditions. However, estrogen did suppress BEZ235-induced apoptosis in HCC712 cells such that cell death only occurred in estradiol treated cells at the highest concentration tested (50 nmol/L). Consistent with the PIK3CA and PIK3CB single and combination knockdown results, BEZ235 treatment did not induce apoptosis in MDA-MB-231 cells.

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NOTE: Gene expression in primary breast tumors was measured by whole-genome expression arrays and ER+ tumors were subtyped by PAM50 subclassification to Luminal A and Luminal B, and PIK3CA mutation status was determined. Mean (SD) was calculated for subtypes and mutation status. The 95% confidence intervals were calculated for the mean difference of Luminal B to Luminal A and PIK3CA mutant to wild-type. Two-sample t tests were used to determine differences in the expression of PIK3CA, PIK3CB, and PIK3CD based on Luminal B versus Luminal A subtypes and PIK3CA mutant versus PIK3CA wild-type tumors.

Figure 2. p110α is the predominant mediator of PI3K signaling in breast cancer cells. A, effect of p110α and p110β knockdown on PI3K signaling. Cells were transfected with control siRNAs (Control) or siRNAs against PIK3CB (PIK3CB) or PIK3CA (PIK3CA). Three days after transfection, serum-deprived cells were stimulated with 20% fetal bovine serum (final concentration) and lysates were analyzed for effects on PI3K pathway signaling through phospho-Akt (p-Akt) and phospho-S6 (p-S6) immunoblotting. Representative immunoblots obtained from at least three experiments per cell line. B, effect of p110α/p110β dual knockdown on PI3K signaling. Cells were transfected with control siRNAs or a mixture of PIK3CA and PIK3CB siRNAs (CA-CB), treated as above, and subjected to immunoblot analysis. Representative results obtained in at least two experiments per cell line.
Discussion

The role of estrogen in the proliferation of ER+ breast tumors is well established. However, the role of estrogen as a survival factor is less clear. Preclinical studies with the MCF-7 cell line showed that treatment with antiestrogens or estrogen deprivation increases apoptosis, as we can confirm (26–28). However, MCF-7 cells are unusual in this regard because we did not observe estrogen deprivation-induced apoptosis in the HCC712 or T47D cell lines, and in the neoadjuvant endocrine therapy setting, treatment did not increase apoptosis (3, 29). Our data indicate that signaling through the PI3K pathway may explain these observations because estradiol promotes survival only when PI3K is inhibited. The presence of two apparently independent cell survival mechanisms, one PI3K-dependent and one estradiol-dependent, creates an opportunity for synthetic lethality. At the current time, it is not known whether estrogen promotes survival through ER-dependent transcription or by rapid, nongenomic activation of signal transduction pathways (30, 31). Nevertheless, our data strongly suggest that the effectiveness of PI3K catalytic subunit inhibitors in treating ER+ breast cancer will be greatest when combined with endocrine therapy.

In vitro studies have shown that activated forms of p110α or p110β transform mammary epithelium (32, 33). Knock-in and knockout transgenic mouse studies confirmed these findings and p110β appears to be particularly important for ERBB2-driven...
A, dual PIK3CA/PIK3CB RNAi inhibits growth of ER+ cells. Cells in CSS medium were transfected with 20 nmol/L control (si Control) or 10 nmol/L each (20 nmol/L final) of PIK3CA and PIK3CB siRNAs (si CA/CB). Cells were left untreated or treated with 10 nmol/L estradiol and growth was assessed after 10 days. Growth is expressed relative to untreated control siRNA-transfected cells. Results from four to six experiments per cell line. * P < 0.05, significant differences between control siRNA and PIK3CA/PIK3CB siRNA-transfected cells in the presence or absence of estradiol. B, dual p110a/p110h knockdown enhances apoptosis in ER+ cells. Cells were transfected with 20 nmol/L control or 10 nmol/L each of PIK3CA and PIK3CB siRNAs. Cells were left untreated or treated with 10 nmol/L estradiol for 7 days and apoptosis was assessed by counting Hoechst-stained nuclei. Results from four experiments per cell line. * P < 0.05, significant differences between control siRNA and dual PIK3CA/PIK3CB siRNA-transfected cells in either the presence or the absence of estradiol. Estradiol significantly suppressed PIK3CA/PIK3CB RNA-induced apoptosis in MCF-7, T47D, and HCC712 cells. C, dual p110a/p110h knockdown was done in T47D cells with alternative PIK3CA and PIK3CB siRNAs. Cells were left untreated, treated with 10 nmol/L estradiol, or treated with estradiol + 300 nmol/L fulvestrant (Fulv) for 7 days. Apoptosis was assessed by counting Hoechst-stained nuclei. Results from four experiments. * P < 0.05, significant differences between control and PIK3CA/PIK3CA siRNA-transfected cells in either the presence or the absence of estradiol.

breast cancer (16, 34) and in the promotion of proliferation, survival, and invasiveness in a variety of cancer types (35–37). The data presented in this study are the first to show PIK3CB amplification in primary breast cancer and suggest that this amplification event may promote oncogenesis. Our initial screen indicates that PIK3CB copy number gain occurs at a low frequency (~5%) in tumors of breast cancer patients; however, this may be clinically significant, because breast cancer is common (38). We also find that PIK3CB is preferentially expressed in Luminal B breast cancer regardless of gene copy number, indicating that this isoform is a potentially important therapeautic target, perhaps as a conduit for the effects of other somatic mutations that activate the PI3K pathway such as PTEN loss (17). Interestingly, our in vitro data indicate that PIK3CB supports cell survival in HCC712 cells under estrogen-deprived conditions, implying that targeted p110β inhibition could be effective in treating PIK3CB-amplified breast tumors. However, our data also suggest that both PIK3CA and PIK3CB may have to be inhibited under these circumstances because high-level apoptosis only occurred when both catalytic subunits were targeted. Because PIK3CA is wild-type in HCC712 cells, we also conclude that PIK3CA gain-of-function mutations are not a prerequisite for the synthetic lethal effect when combining estrogen deprivation and PI3K inhibition. Additionally, inhibition of p110α also appears relevant in the presence of a PIK3CA mutation. T47D cells express modest levels of wild-type p110α as well as a mutant PIK3CA, raising the question of whether PIK3CB provides an escape from mutant p110α inhibition. A comparison between single and combined knockdowns suggests that this is the case, because both isoforms must be inhibited for maximal synthetic lethality. MCF-7 cells appear unusual in their extreme sensitivity to p110α inhibition alone; however, this may possibly reflect the low levels of PIK3CB expression in this cell line.

BEZ235 is an example of a new generation of PI3K inhibitors to enter clinical investigation in breast cancer (25). A comparison between the effects of BEZ235 and RNAi against PIK3CA and PIK3CB supports the conclusion that BEZ235 functions as a selective p110α inhibitor at low nanomolar concentrations (25). However, the apoptotic effect remained very modest in T47D cells, even at higher doses, consistent with lack of PIK3CB inhibition, which, based on the RNAi experiments, is necessary for the full synthetic lethal effect. Estradiol suppresses BEZ235-induced apoptosis in the three ER+ cell lines, but estrogen rescue was not as dramatic as that observed in the PIK3CA and PIK3CB RNAi experiments. The reduced sensitivity to estradiol rescue likely reflects inhibition of other kinases by BEZ235.

We conclude that there is a strong rationale for the combination of endocrine therapy and PI3K inhibitors. In terms of the population of patients suitable for a clinical trial of a PI3K inhibitor combined with endocrine treatment, the data suggest that eligibility should not be restricted by PIK3CA copy number gain, because high-level apoptosis only occurred when both catalytic subunits were targeted. Because PIK3CA is wild-type in HCC712 cells, we also conclude that PIK3CA gain-of-function mutations are not a prerequisite for the synthetic lethal effect when combining estrogen deprivation and PI3K inhibition. Additionally, inhibition of p110α also appears relevant in the presence of a PIK3CA mutation. T47D cells express modest levels of wild-type p110α as well as a mutant PIK3CA, raising the question of whether PIK3CB provides an escape from mutant p110α inhibition. A comparison between single and combined knockdowns suggests that this is the case, because both isoforms must be inhibited for maximal synthetic lethality. MCF-7 cells appear unusual in their extreme sensitivity to p110α inhibition alone; however, this may possibly reflect the low levels of PIK3CB expression in this cell line.
treatment strategy may be problematic because systemic inhibition of both catalytic subunits will cause derangements in insulin signaling and glucose homeostasis (16, 17, 39, 40), and metabolic toxicity could be further enhanced by estrogen deprivation. Nonetheless, it may be possible to pursue this strategy clinically because endocrine therapy in combination with a PI3K inhibitor is cytotoxic. Therefore, short-course high-toxicity combinations of PI3K inhibitors with endocrine therapy.
analogous to conventional chemotherapy, rather than prolonged exposure, may be sufficient to increase the cure rate for ER+ breast cancer.

Disclosure of Potential Conflicts of Interest

M.J. Ellis commercial research grants, Novartis, Astra Zenica, and Taiho; honoraria, Novartis and Astra Zenica; consultant, Novartis, Astra Zenica, and Pfizer. The other authors disclosed no potential conflicts of interest.

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