Review

# Targeting *PIK3CA* Alterations in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor-2–Negative Advanced Breast Cancer: New Therapeutic Approaches and Practical Considerations

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## Abstract

The phosphatidylinositol-3-kinase (PI3K) pathway is frequently dysregulated in human breast cancer. Approximately 30% of all patients with breast cancer will carry mutations of the *PIK3CA* gene, which encodes the PI3K catalytic subunit isoform p110α. Mutations in *PIK3CA* have been associated with resistance to endocrine therapy, HER2-directed therapy, and cytotoxic therapy. Early trials of pan-PI3K inhibitors showed little treatment benefit as monotherapy owing to disease resistance arising through enhanced estrogen receptor pathway signaling. Combining PI3K inhibition with endocrine therapy can help overcome resistance. Clinical trials of pan-PI3K inhibitors combined with endocrine therapy demonstrated modest clinical benefits but challenging toxicity profiles, facilitating the development of more selective PI3K-targeting agents. More recent trials of isoform-specific PI3K inhibitors in patients with *PIK3CA* mutations have shown promising clinical efficacy with a predictable, manageable safety profile. In the present review, we discuss the clinical relevance of mutations of *PIK3CA* and their potential use as a biomarker to guide treatment choices in patients with $HR^+$ $HER2^-$ advanced breast cancer.

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## Introduction

Breast cancer remains the most frequently diagnosed cancer in women and the leading cause of cancer-related deaths in women worldwide.[1] Approximately 70% of patients with breast cancer will have hormone receptor-positive ($HR^+$) and human epidermal growth factor receptor-2–negative ($HER2^-$) disease.[2] Although endocrine therapies (eg, tamoxifen, aromatase inhibitors [AIs]) and, more recently, cyclin-dependent kinase (CDK) 4/6 inhibitors, have improved the outcomes of patients with advanced breast cancer (ABC), which comprises both inoperable locally advanced and metastatic or stage IV breast cancer, resistance to these therapies will develop in virtually all patients.[3-5]

The phosphatidylinositol-3-kinase (PI3K) pathway (Figure 1) is frequently dysregulated in human cancer.[6,7] PI3Ks catalyze the phosphorylation of inositol lipids [phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol (3,4,5)-triphosphate], which then activate a variety of downstream effectors. In physiologic states, these include Ras, RAC1/CDC42, protein kinase C, protein kinase B (AKT)/mammalian target of rapamycin (mTOR), and transcription factors that control normal cellular processes, including cytoskeletal changes, apoptosis, DNA repair, cellular growth, and protein translation.[8,9] Additionally, PI3K signaling is key for activating insulin-dependent glucose uptake and stimulation of glycogen synthesis in peripheral tissues.[9] Intracellular phosphatase and tensin homolog (PTEN) buffers PI3K signaling by dephosphorylating phosphatidylinositol (3,4,5)-triphosphate and negatively regulating downstream signaling.[10] In pathologic scenarios, PI3K signaling is frequently hyperactivated, leading to

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oncogenic cell growth, proliferation, motility or invasion, and metabolic effects.[7,8]

PI3Ks have been classified into 3 classes determined by the structural characteristics and substrate specificity.[6] Of the 3 classes, class I PI3Ks are those most often altered in human cancer and have been most extensively studied. Class I PI3Ks function as heterodimers consisting of 1 of 4 catalytic p110 subunits (p110α, p110β, p110δ, or p110γ) and a regulatory subunit (p85 for class IA PI3Ks and p101 for class IB).[6,7,11] In the resting state, p85 suppresses the activity of p110. In response to growth factor stimulation, p85 binds to activated receptor tyrosine kinases, such as platelet-derived growth factor receptor and epidermal growth factor receptor, and relieves inhibition of p110.[12-15] When activated, p110 generates phosphatidylinositol-3,4,5-triphosphate, which, in turn, activates multiple downstream signaling cascades.[6]

PI3K signaling pathway disruption can occur through different mechanisms, including alteration of p85, p110, and PTEN at the genetic level. Other genomic alterations can cause oncogene activation or silencing of tumor suppressor genes, supporting PI3K pathway activation.[6,7,16]

In the present review, we consider the clinical relevance of *PIK3CA* mutations in human breast cancer and their potential use as biomarkers to guide the treatment choices for patients with HR$^+$, HER2$^-$ ABC.

## Role of *PIK3CA* Mutation in Breast Cancer

The association of PI3K with human cancer was made in the late 1990s, and only recent genomic analyses have revealed that multiple components of the PI3K pathway are frequently altered in human cancer.[6] Approximately 30% of all patients with breast cancer will carry mutations of the *PIK3CA* gene, which encodes the PIK3 catalytic subunit isoform p110α.[17-19]

The p110α subunit consists of 5 domains: an adaptor binding domain, a Ras-binding domain, a C2 domain, a helical domain, and a kinase catalytic domain.[17,20] The p110α catalytic subunit of PI3K, specifically, plays important roles in cell growth, proliferation, and survival and in insulin signaling.[10,21,22] Structural analysis of p110α and p85α has revealed that many of the mutations occur at residues lying at the interfaces between these subunits.[23] Other analyses have identified gain-of-function mutations with transforming capacity in exons 7, 9, and 20 of the gene encoding p110α.[10,24,25] Mutations in the C2 domain of exon 7 (commonly C420R) occur in a loop predicted to be part of the binding region of p110α.[25,26] Mutations in the helical domain of exon 9 (commonly located in E542K, E545K/A/D/G, and Q546E/R) occur at the interface between the helical domain of p110α and the N-terminal SH2 domain of p85α and are thought to abrogate the inhibitory effect of N-terminal SH2 in the p85 regulatory subunit.[10,23,26] Mutations in the kinase domain of exon 20 (commonly located in H1047L/R/Y) are thought to cause increased lipid kinase activity via membrane-bound phosphatidylinositol 4,5-bisphosphate and downstream tumorigenic signaling cascades.[10,26,27]

Other mutations, such as those located in the inter-SH2 domain of p85α (the region that interacts with the adapter-binding and C2 domains of p110α), have been shown to destabilize the inhibitory interaction with p110 (reducing p110 inhibition) but retain the ability to bind and stabilize p110.[28] Gain-of-function mutations in p110 and loss-of-function mutations in *PTEN*, which lead to reduced PTEN expression and loss of PI3K/AKT inhibition, contribute to the dysregulation of the balance between PI3K and PTEN often present in many cancers, including breast cancer.[29,30]

### *Prognostic Value of* PIK3CA *Mutation*

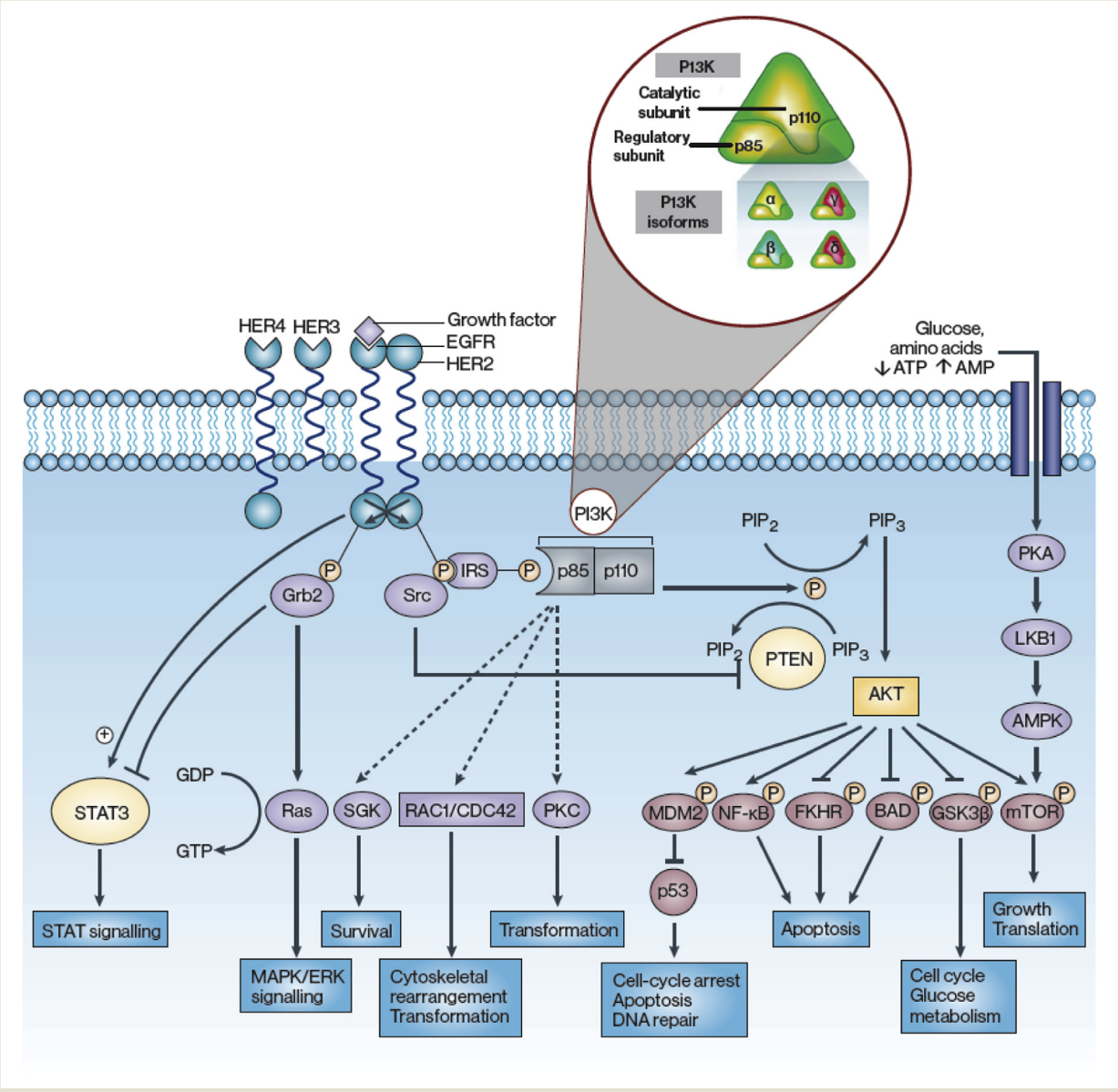
Alterations in the PI3K signaling pathway play important roles in tumor initiation and survival, angiogenesis, and the development of resistance to cancer therapies.[6,31] Mutations in *PIK3CA* have been associated with resistance to endocrine therapy, HER2-directed therapy, and cytotoxic therapy.[11] When 2 of the most common mutant alleles (H1047R and E545K) are expressed in human mammary epithelial cell lines, PI3K signaling will be activated, which leads to tumor formation in nude mice.[32,33] These mutations have been associated with AKT activation, growth factor-independent cell proliferation, resistance to apoptosis, and increased invasiveness and cell migration.[17,32,34,35] Such downstream activities can be blocked via inhibition of the PI3K pathway in *PIK3CA*-mutant cell lines.[17]

The clinical incidence of *PIK3CA* mutations differs according to the breast cancer subtype.[36] The incidence has ranged from 28% to 47% in HR$^+$ breast cancer, 23% to 33% in HER2$^+$ breast cancer, and 8% to 25% in basal-like (triple-negative breast cancer [TNBC]).[37] Although *PIK3CA* alterations appear to be associated with the presence of estrogen receptor (ER) and progesterone receptor expression,[38] the associations with prognostic outcomes have not been consistent[39] and could also differ according to breast cancer subtype.[40]

In a retrospective study of 590 patients with primary invasive breast cancer (all subtypes; nearly all with stage I-III disease) who had undergone surgery for primary breast cancer, the presence of *PIK3CA* mutations conferred a significant improvement in overall survival (OS) and breast cancer-specific survival.[39] The association between a *PIK3CA* mutation and a positive prognosis was further supported by a large pooled analysis of 19 cohorts with 10,319 patients with early-stage breast cancer. However, the investigators noted a weaker association on multivariate analysis compared with that found on univariate analysis, perhaps owing to the association of *PIK3CA* mutations with favorable clinical characteristics.[41] In contrast, in a meta-analysis of 1929 patients with breast cancer (all subtypes and various disease stages) who had received adjuvant chemotherapy, anti-HER2 therapy, endocrine therapy, or a combination of these, the presence of *PIK3CA* mutations was associated with reduced progression-free survival (PFS), disease-free survival, and OS.[42] The patient population in the meta-analysis was highly diverse, with different disease stages and multiple treatment lines and treatment types, in contrast to other studies that had included primary tissue from patients with an earlier disease stage.[39,41,42]

Recent analyses have shown that the presence of *PIK3CA* mutations might have a more favorable effect in HR$^+$ early-stage breast cancer.[43-45] For example, in postmenopausal patients with HR$^+$ early-stage breast cancer treated with adjuvant tamoxifen but no chemotherapy, a *PIK3CA* mutation was associated with a luminal A phenotype and a more favorable prognosis.[45] In contrast, findings from studies of ABC patients with *PIK3CA* mutations have ranged from a worse prognosis to no detectable effects from the *PIK3CA*

**Figure 1** Schematic of the Phosphatidylinositol-3-Kinase (PI3K) Pathway in Cancer. Reproduced with permission from Hennessy, et al. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* 2005; 4:988-1004. ©2019 Springer Nature Publishing AG




mutation. In an exploratory analysis of MONARCH-2 [a study of abemaciclib (LY2835219) combined with fulvestrant in women with hormone receptor positive HER2 negative breast cancer], patients with *PIK3CA*-mutated tumors had shorter PFS compared with those with wild-type tumors in both the abemaciclib-plus-fulvestrant (mutated, 15 months; wild type, 20 months) and the placebo-plus-fulvestrant (mutated, 5.7 months; wild type, 12.7 months) arms.[46] Similar results were observed in MONALEESA-3 (phase III randomized study of ribociclib and fulvestrant in hormone receptor−positive, human epidermal growth factor receptor 2−negative advanced breast cancer). In that study numerically shorter PFS was observed in patients with *PIK3CA*-altered tumors compared with patients with wild-type tumors in the same treatment arms in both the ribociclib-plus-fulvestrant (mutated, 16.4 months; wild type, 22.3 months) and placebo-plus-fulvestrant (mutated, 11.1 months; wild type, 16.5 months) groups.[47] A retrospective analysis of patients from the BOLERO-2 study (everolimus in combination with exemestane in the treatment of postmenopausal women with estrogen receptor positive locally advanced or metastatic breast cancer who are refractory to letrozole or anastrozole) study found that PFS was similar overall among the treatment groups, regardless of the *PIK3CA* mutation status (~7 months for everolimus plus exemestane and ~3 months for placebo plus exemestane for each group). However, differences were

observed when the patients were grouped by where the mutations had occurred in the *PIK3CA* gene.[48] In particular, patients with E545K/E542K helical mutations had numerically shorter PFS in both the everolimus-plus-exemestane (5.6 months) and the placebo-plus-exemestane (2.2 months) arms. Collectively, these data suggest that the prognostic value of *PIK3CA* mutations in breast cancer might be associated with time point in the course of the disease, as well as the type of *PIK3CA* alteration. The inclusion of preplanned analyses studying this association is paramount for building the body of evidence in support of the *PIK3CA* status as a determinant factor to consider during treatment planning.

## Clinical Studies With PI3K Inhibitors in HR$^+$ HER2$^-$ ABC and *PIK3CA* Mutation

### *Trials With Pan-PI3K and AKT Inhibitors*

Early clinical studies of pan-PI3K inhibitors (PI3Kis) evaluated their efficacy as single agents in advanced solid tumors but showed little treatment benefit as monotherapies and demonstrated challenging toxicity profiles and potential off-target effects.[49-51] The resistance mechanisms are thought to broadly involve incomplete inhibition of PI3K, reactivation of the PI3K pathway, and activation of alternative pathways.[52] The PI3K pathway involves numerous feedback loops, crosstalk with other pathways, and compensatory pathways that could enable resistance to PI3K inhibition. Also, although the mechanisms behind the resistance to PI3K inhibition have not been fully elucidated, activation of parallel pathways is a possibility.[53]

In preclinical models, suppression of PI3K signaling resulted in the induction of ER-dependent transcriptional activity, which was shown through changes in the expression of genes containing ER-binding sites and increased occupancy of promoter regions of upregulated genes by the ER.[54] Consistent increases in ER transcription and an induction of a luminal-type signature (typical of hormone-responsive breast cancer) were observed in cell lines, murine models, and patient samples on suppression of the PI3K pathway. Therefore, it has been proposed that increased ER activity might be a reactive, adaptive mechanism limiting the activity of PI3Kis and that combined PI3K and ER inhibition would be a rational approach to target such tumors. The causative role of ER activation on PI3Ki resistance was supported by the observation that the combination of the pan-PI3Ki buparlisib and fulvestrant (a specific ER inhibitor) induced near-complete tumor regression and was significantly more efficacious than either agent alone in mice.[54,55] This dual blockade of PI3K and ER pathways could potentially restore treatment sensitivity.

Protein kinase B (or AKT) is a key downstream enzyme in the PI3K pathway with increased signaling demonstrated when PI3K is mutated and constitutively activated. AKT has emerged as a potential therapeutic target in breast cancer when either *PIK3CA* or *AKT* has mutated.[8,17] The AKT inhibitor capivasertib has shown promising results both as a single agent and combined with fulvestrant.[56,57] In a phase Ib/II open-label study evaluating single-agent treatment with capivasertib for patients with ER$^+$ or HER2$^+$ ABC, some patients with *PIK3CA* mutations demonstrated tumor shrinkage in response to treatment.[56] A phase II study of ER$^+$ HER2$^-$ ABC involving patients treated with capivasertib plus fulvestrant recently reported significant clinical benefit (improved PFS and initial OS analysis), supporting further clinical evaluation in that breast cancer subtype in a phase III study.[57] However, PI3K pathway activation (defined as exon 9/20 hotspot mutations in *PIK3CA* or PTEN null status) did not appear to affect sensitivity to capivasertib in these patients. A phase III study of capivasertib combined with paclitaxel for TNBC is ongoing.[58] The AKT inhibitor ipatasertib showed antitumor activity when combined with docetaxel or paclitaxel in patients with HR$^+$ HER2$^-$ ABC or TNBC, including a subgroup of patients who had progressed during treatment with PI3Kis or had had *PIK3CA* mutations in early-phase studies.[59] Two phase III trials are currently ongoing involving ipatasertib in HR$^+$ HER2$^-$ ABC. The IPATunity150 study is evaluating ipatasertib plus the combination of palbociclib and fulvestrant.[60] The IPATunity130 study is evaluating the ipatasertib plus paclitaxel combination in both HR$^+$ HER2$^-$ ABC and advanced TNBC.[61]

*Pan-PI3Ki Buparlisib.* Phase I studies in which buparlisib, which inhibits all isoforms of class I PI3Ks, was used in combination with an endocrine therapy partner and demonstrated clinical activity with a clinical benefit rate of 31% and 58.6% with letrozole and fulvestrant, respectively, and tolerable safety profiles.[62-64] These encouraging results led to the phase III BELLE-2 study. The BELLE-2 study evaluated the use of buparlisib (or placebo) plus fulvestrant in 1147 postmenopausal women with HR$^+$ HER2$^-$ ABC whose disease had progressed during or after AI therapy and who had received $\leq$ 1 previous line of therapy for advanced disease.[65] Although all patients were eligible, randomization was stratified by PI3K pathway activation status (activated vs. nonactivated vs. unknown; assessed in tumor tissue). In the overall study population, the median PFS was significantly improved in the buparlisib versus placebo arms (6.9 vs. 5.0 months; hazard ratio, 0.78; $P = .00021$). Similarly, improved PFS was observed in patients with known *PIK3CA* mutation status (activated or not; n = 851; PFS, 6.8 vs. 4.5 months, respectively; hazard ratio, 0.80; $P = .0033$; Table 1) and for patients with an activated PI3K pathway (n = 372; PFS, 6.8 vs. 4.0 months, respectively; hazard ratio, 0.76; $P = .014$). However, substantial toxicity was observed with this pan-PI3Ki. The most common high-grade buparlisib-associated adverse events (AEs) included hyperglycemia (grade 3, 15%; grade 4, < 1%), increased alanine aminotransferase (ALT; grade 3, 19%; grade 4, 7%), increased aspartate aminotransferase (AST; grade 3, 15%; grade 4, 3%), and rash (grade 3, 8%; grade 4, < 1%). High-grade mood disorders such as depression (grade 3, 4%; grade 4, 1%) and anxiety (grade 3, 5%; grade 4, < 1%) were also observed. Serious AEs occurred more commonly in the buparlisib (23%) than in the placebo (16%) arms.

BELLE-3 was a phase III study that evaluated buparlisib (or placebo) plus fulvestrant in 432 postmenopausal patients with HR$^+$ HER2$^-$ ABC who had received previous AI and mTOR inhibitor therapy.[66] As a secondary biomarker analysis, *PIK3CA* mutation status detected by circulating tumor DNA (ctDNA) was assessed in hotspots from exons 9 and 20 on plasma samples from blood collected during screening or at day 1 of cycle 1. Similar to BELLE-2, the median PFS in the patients with BELLE-3 was improved in the buparlisib plus fulvestrant versus fulvestrant arms (3.9 vs. 1.8 months; hazard ratio, 0.67; $P = .00030$). Greater PFS benefit was

**Table 1** Median PFS in Subgroups of *PIK3CA*-Mutated or *PIK3CA*-Nonmutated Patients Treated With Targeted Therapy in Phase III Clinical Trials of PI3K Inhibitors

| | Buparlisib | | | | Taselisib | | Alpelisib | |
|---|---|---|---|---|---|---|---|---|
| | BELLE-2[65] (n = 587)[a] | | BELLE-3[66] (n = 432) | | (SANDPIPER[67]; n = 631) | | (SOLAR-1[68,69]; n = 572) | |
| Variable | BUP | PBO | BUP | PBO | TAS | PBO | ALP | PBO |
| *PIK3CA*-mutated[b] | | | | | | | | |
| Patients, n | 87 | 113 | 100 | 35 | 340 | 176 | 169 | 172 |
| PFS events, n | 48 | 90 | 61 | 30 | 194 | 119 | 99 | 120 |
| Median PFS, mo | 7.0 | 3.2 | 4.2 | 1.6 | 7.4 | 5.4 | 11.0 | 5.7 |
| Hazard ratio (95% CI) | 0.58 (0.41-0.82) | | 0.46 (0.29-0.73) | | 0.70 (0.56-0.89) | | 0.65 (0.50-0.85) | |
| *P* value | .001 | | .0005 | | .0037 | | <.001 | |
| *PIK3CA*-nonmutated[b] | | | | | | | | |
| Patients, n | 199 | 188 | 132 | 81 | 77 | 38 | 115 | 116 |
| PFS events, n | 124 | 126 | 97 | 65 | NA | NA | 47 | 57 |
| Median PFS, mo | 6.8 | 6.8 | 3.9 | 2.7 | 5.6 | 4.0 | 7.4 | 5.6 |
| Hazard ratio (95% CI) | 1.02 (0.79-1.30) | | 0.73 (0.53-1.00) | | 0.69 (0.44-1.08) | | 0.85 (0.58-1.25) | |
| *P* value | .557 | | .642 | | .1062 | | NA | |

Abbreviations: ALP = alpelisib; BUP = buparlisib; CI = confidence interval; PBO = placebo; PFS = progression-free survival; PIK3 = phosphatidylinositol-3-kinase; TAS = taselisib.
[a]BELLE-2 population reflects only the subset of patients with *PIK3CA* status determined by circulating tumor DNA.
[b]*PIK3CA* mutation status was determined by circulating tumor DNA in BELLE-2 and BELLE-3, tissue biopsy in SOLAR-1, and method not disclosed in the SANDPIPER trial.

found with buparlisib in patients with a *PIK3CA* mutation (4.2 vs. 1.6 months; hazard ratio, 0.46; $P = .00031$; Table 1) detected using ctDNA compared with patients with nonmutated *PIK3CA* (3.9 vs. 2.7 months; hazard ratio, 0.73; $P = .026$; Table 1). A limitation of the analysis, however, was that *PIK3CA* status, as assessed by ctDNA, was not initially planned as an exploratory endpoint. Determination of *PIK3CA* status was added as a secondary endpoint to the protocol of BELLE-3 in August 2015 because of the results for patients with a *PIK3CA* mutation in the BELLE-2 study. The most common grade 3 and 4 AEs in the buparlisib arm versus placebo arm were elevated ALT (22% vs. 3%), elevated AST (18% vs. 3%), hyperglycemia (12% vs. 0%), hypertension (6% vs. 4%), and fatigue (3% vs. 1%). However, because of the modest clinical benefit and substantial toxicity, development of this combination was discontinued.

Although buparlisib was not pursued further in the clinic, the findings from the BELLE-2 and BELLE-3 studies demonstrated that patients with *PIK3CA* mutation might derive greater benefit from PI3Kis as combination therapies compared with patients without mutated *PIK3CA*.[65,66] The results of BELLE-2 and BELLE-3 generated further interest in alternative PI3Kis with greater selectivity and improved clinical benefit/safety risk ratios.

*β-Sparing PI3Ki Taselisib.* Taselisib is a potent and selective β-sparing PI3Ki targeting the α, δ, and γ isoforms of PI3K.[70] Preliminary phase Ia clinical data demonstrated a favorable safety profile and signs of activity in 34 patients with locally advanced or metastatic solid tumors.[71] In that dose-escalation study, the participants started at the 3-mg dose level, with escalation ≤ 16 mg before testing of the final cohort at 12 mg. One of 10 patients treated at 12 mg and 2 of 11 patients treated at 16 mg experienced AEs that qualified as dose-limiting toxicities. For patients with *PIK3CA*-mutant disease, the confirmed response rate using Response Evaluation Criteria in Solid Tumors was 36% compared with 0% for patients without a known *PIK3CA* mutation. With these findings, additional development of taselisib was undertaken.

The phase III SANDPIPER trial evaluated taselisib combined with fulvestrant (vs. placebo plus fulvestrant) in 516 patients with $ER^+$, $HER2^-$ ABC with mutated *PIK3CA* (patients with nonmutated *PIK3CA* were randomized separately) and disease recurrence after AI therapy. The results were recently presented.[67] Improved median PFS per investigator review was observed in the taselisib plus fulvestrant versus placebo arms (7.4 vs. 5.4 months; hazard ratio, 0.70; $P = .0037$; Table 1), which was confirmed by a blinded independent central review (hazard ratio, 0.66). In the subset of patients with measurable disease at baseline (n = 264, taselisib plus fulvestrant arm; n = 134, placebo), overall response rate (ORR; 28% vs. 11.9%; $P = .0002$) and clinical benefit rate (51.5% vs. 37.3%) were greater in the taselisib than in the placebo arms, respectively. As an exploratory endpoint, efficacy was also assessed in 115 patients without *PIK3CA*-mutant tumors (median PFS, 5.6 vs. 4.0 months; hazard ratio, 0.69; $P = .1062$; Table 1). Diarrhea, hyperglycemia, colitis, and stomatitis were the most common AEs in the taselisib arm. The rates of grade 3/4 AEs were greater in the taselisib (50%) than in the placebo (16%) arms. These AEs most commonly

included diarrhea, hyperglycemia, rash, stomatitis, increased ALT/AST, and colitis. A much greater proportion of patients in the taselisib than in the placebo arms discontinued treatment (17% vs. 2%) or required dose reductions (37% vs. 2%) because of AEs. Because of the modest improvements in PFS and the challenging toxicity profile with taselisib plus fulvestrant compared with fulvestrant alone, the clinical development of taselisib was discontinued.

*α-Specific Inhibitor Alpelisib.* Alpelisib inhibits the α-subunit of PI3K (50% inhibitory concentration [$IC_{50}$], 4.6 nmol/L) more potently than the β- ($IC_{50}$, 1156 nmol/L), γ- ($IC_{50}$, 250 nmol/L), and δ- ($IC_{50}$, 290 nmol/L) isoforms and inhibits proliferation in *PIK3CA*-mutant cells in vitro.[72-74] In a single-arm phase Ib trial, postmenopausal women with *PIK3CA*-altered or *PIK3CA* wild-type ER$^+$ HER2$^-$ ABC, whose cancer had progressed during or after antiestrogen therapy, were treated with alpelisib plus fulvestrant.[75] The ORR for patients with *PIK3CA*-altered tumors (60% of evaluable patients; n = 49) receiving 300 to 400 mg of alpelisib plus fulvestrant was 29% (95% confidence interval, 17%-43%).

The phase III, randomized, double-blind SOLAR-1 study (study assessing the efficacy and safety of alpelisib plus fulvestrant in men and postmenopausal women with advanced breast cancer which progressed on or after aromatase inhibitor treatment) evaluated alpelisib (or placebo) at a dose of 300 mg plus fulvestrant in 572 patients with ER$^+$ HER2$^-$ ABC who had received previous treatment with an AI in the neoadjuvant or adjuvant setting and no previous chemotherapy for advanced disease.[68] Approximately 6% of patients in all treatment arms had had previous exposure to a CDK4/6 inhibitor. The *PIK3CA* mutation status of patients was determined from tumor tissue samples before enrollment and was also measured in plasma ctDNA at baseline.[76] Both *PIK3CA*-mutated and *PIK3CA* wild-type tumors were eligible for the trial. A total of 341 patients were found to have mutations in the 11 residues examined [C420R, E542K, E545A, E545D (1635G>T only), E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y].[26,68]

Locally assessed PFS in the *PIK3CA*-mutant cohort (the primary endpoint) was significantly improved in the alpelisib versus placebo arms (median PFS, 11.0 vs. 5.7 months, respectively; hazard ratio, 0.65; $P = .00065$; Table 1), meeting the primary study endpoint successfully.[69] The ORR was also improved with alpelisib (36% vs. 16%; $P = .0002$) for 262 patients in the *PIK3CA*-mutant cohort with measurable disease. However, a clinically relevant benefit was not observed with alpelisib in the *PIK3CA*-nonmutant cohort. The prespecified proof-of-concept criteria for the secondary endpoint of locally assessed PFS in the nonmutant cohort were not met (7.4 vs. 5.6 months; hazard ratio, 0.85; Table 1). Subgroup analyses demonstrated that the clinical benefit of alpelisib was consistent across the prespecified subgroups, including across the line of therapy and primary versus secondary endocrine resistance and regardless of treatment with any previous CDK4/6 inhibitor.[68,76]

Overall, the AEs observed with alpelisib treatment were manageable. The most common all-grade AEs included hyperglycemia, diarrhea, nausea, decreased appetite, and rash. The grade 3/4 AEs most commonly occurring in the alpelisib arm included hyperglycemia (37% vs. < 1% in the placebo arm) and rash (10% vs. < 1% in the placebo arm). Treatment discontinuations because of AEs were required for 25% versus 4% of patients in the alpelisib and placebo arms, respectively.[68] Hyperglycemia is an anticipated on-target effect related to the role of *PI3KCA* in insulin signaling and glucose homeostasis.[68,77] However, with conventional treatment (dose interruptions and concomitant antidiabetic medications), hyperglycemia will be manageable for most patients. Only 4% of the patients in the SOLAR-1 study had had diabetes at baseline (according to hemoglobin A1c [HbA1c] and fasting plasma glucose [FPG] values).[68] In addition, patients with type 1 or uncontrolled type 2 diabetes (presenting with a FPG > 140 mg/dL or HbA1c > 6.4%) were excluded from the trial. Therefore, the safety of alpelisib in these specific patient populations has not been established.

The SOLAR-1 study is the first to show a significant and clinically meaningful improvement in PFS with an α-specific PI3Ki with a manageable tolerability profile in patients with a *PIK3CA* mutation and HR$^+$ HER2$^-$ ABC.[68] The US Food and Drug Administration (FDA) approved alpelisib combined with fulvestrant for postmenopausal women, and men, with HR$^+$, HER2$^-$, *PIK3CA*-mutated (as detected by an FDA-approved test) ABC after progression during or after an endocrine-based regimen.[78]

### *AE Management: Practical Considerations*

PI3Ki-associated toxicities will generally be reversible if managed adequately.[79] Some of the commonly observed toxicities associated with PI3Ki treatment have included hyperglycemia, rash, and diarrhea.[65-68] Successful implementation of management strategies and monitoring schedules is vital for maximizing the clinical benefit to patients.

The regulatory guidelines for alpelisib have recommended a glucose baseline assessment using HbA1c and FPG.[78] Continued monitoring during treatment is recommended at a frequency of once each week for 2 weeks and at least once every 4 weeks for the remainder of the treatment, plus as clinically indicated for blood glucose/FPG and once every 3 months plus as clinically indicated for HbA1c. PI3Ki dose adjustments and pharmacologic intervention with metformin (preferred option) and other insulin sensitizers can be used to mitigate the severity of hyperglycemia.[78,80] Detailed guidance is available in the prescribing information for alpelisib. Lifestyle changes, such as adopting a low-carbohydrate diet, fasting, and exercise, have also been recommended because they may attenuate the development of hyperglycemia.[80,81] For patients with pre- or type 2 diabetes at baseline, close monitoring is advised to promptly detect and manage hyperglycemia.[68] The safety of PI3Kis in patients with type 1 or uncontrolled type 2 diabetes is unknown, because these patients were excluded from the SOLAR-1 study.

To reduce the frequency and severity of rash, prophylactic antihistamine treatment has been recommended, starting on the first day of PI3Ki treatment.[79] Guidance for rash management includes the use of topical steroids for lower grade events. Low-dose systemic corticosteroid treatment can be considered for managing grade 2 rash. The use of systemic steroids and PI3Ki treatment interruption and the use of systemic steroids have been recommended for grade ≥ 3 events.[78,82] Patients with any-grade rash might benefit from consultation with a dermatologist specialist.[78]

Overall, dose modifications and dose interruptions have been recommended for managing other PI3K-associated toxicities, as

medically indicated. Antidiarrheal medication, such as loperamide and/or diphenoxylate and atropine, has been advised to lessen the severity and duration of low-grade diarrhea.[78,82]

### *Patient Population Stratification and Practical Factors in PIK3CA Testing*

Although the high rates of toxicity in the BELLE trials presented a clinical challenge to the development of buparlisib, an important observation from these trials was the increased benefit derived from the treatment of patients with a *PIK3CA* mutation.[65,66] The predictive value of alterations in PI3K might depend on the timing of the tissue collection and the technology used to determine molecular alteration status.[83] It has been suggested that analyses of biomarkers should focus on both primary tumors and metastatic lesions to account for inherent disease heterogeneity, which only increases with disease progression.[84] Analysis of mutational profiles of primary breast tissue and paired metastases in 1 study (n = 23) found high interpatient mutational concordance in all but 1 patient profiled.[85] Another study assessed *PIK3CA* mutation status (exon 9 and/or exon 20) in tumor tissue samples from primary and paired asynchronous metastatic lesions from 104 patients.[86] The frequency of *PIK3CA* mutations in the primary tumors was 45% compared with 53% in the paired metastatic tumors. Importantly, one third of these patients had different mutational status in their primary and asynchronous metastatic lesions, with a change to mutant from wild type predominating. These findings have demonstrated that *PIK3CA* mutations can be acquired during disease progression. That analysis was subsequently supported by the findings from another study, which reported that the overall percentage of *PIK3CA* mutations (determined from tissue samples; n = 19) between the primary and metastatic lesions was similar (40.4% vs. 42.0%, respectively).[87] However, both gain (8%) and loss (10%) of *PIK3CA* mutations were detected in individual patients. Although these conclusions are limited by the small sample sizes and potential differences in testing methods (including mutational analysis and tissue availability), discordance between primary and metastatic tumor testing could have important clinical implications to consider. Failure to reassess *PIK3CA* status and to reevaluate treatment suitability in the metastatic setting could result in the exclusion of certain therapeutic options (ie, a patient with newly acquired *PIK3CA* mutations might benefit from PI3K-targeted therapy), which could substantially alter the clinical outcomes. Further studies and standardized testing practices are required to better characterize alterations in *PIK3CA* during the course of breast cancer progression.

ctDNA has recently emerged as a sensitive, minimally invasive tool to evaluate a tumor's *PIK3CA* mutation status.[65,88] Compared with archival tumor tissue, ctDNA mutational analysis can provide a more dynamic assessment of tumor heterogeneity, because it putatively integrates DNA shed from multiple tumor sites.[65,88,89] Various technologies have been used to detect ctDNA in plasma. Droplet digital polymerase chain reaction (PCR) and beads, emulsion, amplification, magnetics (BEAM)ing digital PCR methods have high sensitivity for point mutations but require knowledge of the mutation of interest, which can lead to the potential failure to identify rare mutations.[90,91] In BEAMing, DNA is mixed with magnetic beads coated with primers for specific genes. This technology allows for the detection of single-nucleotide variants, duplications, insertions, and deletions. The lower limit of detection with BEAMing is 0.01%, which is highly sensitive. Next generation sequencing (NGS) allows for the detection of new or rare somatic variants with reasonable sensitivity. However, the potential exists for false-negative results owing to low copies of mutated ctDNA (in patients with small tumors) or the lack of sensitivity.[91,92] Additionally, not all DNA alterations will affect expression, possibly confounding the interpretation of the results. Amplicon-capture NGS allows for the detection of single-base substitutions, duplications, insertions, and deletions and consists of multiplexed PCR amplification of regions of interest, followed by NGS and a bioinformatic analysis.[90,93] In hybridization-capture NGS, genomic DNA fragments are hybridized to specific probes and amplified. This technology allows for the detection of all the genomic alterations that amplicon-capture NGS detects and also for exon duplications, deletions, and gene copy number changes. Overall, NGS is a sensitive technique that can detect low-frequency mutations (allowing for mutation detection at an allelic frequency as low as 0.1%). However, it is expensive and requires sophisticated bioinformatic analyses of the data outputs.[90,94]

Studies have supported the potential use of ctDNA mutation assessment as a minimally invasive method to identify the current molecular status of tumor burden and to detect molecular changes that might be occurring over time.[88,95,96] One study found that matched primary tumor tissue and plasma (targeted gene screen [50 cancer genes] using a commercially available amplicon-capture high-coverage NGS) were concordant in 13 of 17 cases (76%). That study concluded that, for cases in which metastatic tumor biopsy is not feasible, a liquid biopsy method could be used as an alternative.[93] In the BELLE studies, ctDNA was detected using the Inostics BEAMing assay (Sysmex, Hamburg, Germany), with a panel of predefined *PIK3CA* mutations (BELLE-2, 15 mutations located on exons 1, 7, 9, and 20; BELLE-3, 8 mutations located on exons 9 and 20).[65,66] Concordance of the *PIK3CA* mutation status using this method in ctDNA and tumor tissue analysis (Sanger sequencing in exons 1, 7, 9, and 20 [BELLE-2]; real-time PCR covering exons 7, 9, and 20; [BELLE-3]) was 77% and 83% in the BELLE-2[65] and BELLE-3[66] trials, respectively. In both of these studies, patients with *PIK3CA*-mutant status determined by ctDNA analysis experienced longer PFS than that of patients with *PIK3CA*-nonmutant status.

The SOLAR-1 study included prospective analyses of *PIK3CA* mutations in ctDNA using PCR techniques to identify patients who might benefit from therapy.[68,76] The FDA approved a companion diagnostic, the *therascreen* PIK3CA RGQ PCR Kit, to detect *PIK3CA* mutations (real-time PCR; 11 mutations across exons 7, 9, and 20 were specifically detected in SOLAR-1) in breast tumor tissue or plasma-derived ctDNA.[26,97] The median PFS in patients with a ctDNA-detected *PIK3CA* mutation was 10.9 months for the alpelisib-plus-fulvestrant group and 3.7 months for the fulvestrant-only group (hazard ratio, 0.55).[76] Preplanned retrospective testing with hybridization-capture NGS (324 gene panel) was also performed in samples from patients randomized to treatment in SOLAR-1.[26,90] NGS allows for full coverage of encoding exons; therefore, additional *PIK3CA* mutations and amplifications were detected compared with PCR-based testing (including 60 different

mutations and 5 copy number variations across exons 1, 4, 5, 7, 8, 9, 13, 18, and 20). In SOLAR-1, 28 of 175 patients identified as not having *PIK3CA* mutations using PCR had a *PIK3CA* mutation found using NGS.[26] Despite this discrepancy, both tests demonstrated benefit for patients whose tumors had *PIK3CA* alterations overall.[68,98]

Sanger sequencing is usually performed on PCR products and can detect the same type of mutant variants as BEAMing, including unknown mutations. Sanger sequencing is, however, less sensitive, because it requires mutant DNA to be present at an abundance of 20% to 25%.[90] Real-time PCR has the limitation that only known target mutations can be detected. The limit of detection for *PIK3CA* mutations with this technology has been reported to be as low as 5% but varies broadly between detection kit brands and target exon.[97,99]

## Expert Perspectives

Although data from BELLE-2 and BELLE-3 have not supported the continued investigation of the buparlisib plus fulvestrant combination, both trials were critical in demonstrating the proof of concept to support further investigation with more selective (eg, α-specific) inhibitors for patients with HR$^+$ HER2$^-$ ABC and mutated *PIK3CA*.[65,66] These studies also demonstrated the need to use *PIK3CA* mutation testing to select patients for treatment (Table 1) and illustrated the feasibility of assessing tumor DNA using ctDNA versus archival tissue, which might not be reflective of molecular changes after endocrine and/or targeted therapy. For example, BELLE-3 demonstrated differences in the incidence of mutated *PIK3CA* in the buparlisib versus placebo arms as detected by ctDNA (43% vs. 30%, respectively) compared with the incidence detected using PCR on archival tissue (26% vs. 24%).[66] Therefore, the use of ctDNA versus archival tissue could have contributed to differences in the efficacy assessments in BELLE-3, highlighting the importance of stratification according to the *PIK3CA* mutation status as measured by ctDNA, specifically at randomization. Because of the potential risk of false-negative determinations, negative ctDNA results should be confirmed with tissue testing.[92]

A current standard for many patients with HR$^+$ ABC is to receive endocrine therapy with a CDK4/6 inhibitor in the first-line setting. This class of agents has dramatically improved PFS in HR$^+$ ABC.[100] Therefore, a common usage of the recently FDA-approved treatment of alpelisib plus fulvestrant will be in the second-line setting. Although only a few patients in the SOLAR-1 trial were previously exposed to this combination, we believe that this sequencing of drugs makes intrinsic sense until further clinical trials provide more information on whether any advantage or detriment exists with previous treatment with a CDK4/6 inhibitor before the use of a PI3K inhibitor. The BYLieve trial ClinicalTrials.gov identifier, NCT03056755), currently enrolling patients to examine the safety and efficacy of alpelisib plus endocrine therapy for patients who have previously received a CDK4/6 inhibitor-based regimen.[101]

PI3K activation has been associated with resistance to treatment in various breast cancer subtypes, prompting the evaluation of new combinations, including PI3K-targeted therapies. Resistance to treatment, which has often been observed in patients with HER2$^+$ tumors, has been associated with increased PI3K activation.[102] Lower response rates to anti-HER2 treatment have been reported in HER2$^+$ tumors with *PIK3CA* mutations compared with wild-type tumors.[103] The combination of alpelisib and ado-trastuzumab emtansine for patients with HER2$^+$ inoperable locally advanced or metastatic breast cancer with progression during or after previous trastuzumab or taxane therapy demonstrated encouraging efficacy in a phase I study.[104] In the TNBC setting, promising results from an early-phase study involving a combination of alpelisib with nab-paclitaxel in patients with *PIK3CA* mutations have supported further clinical evaluation of this combination.[105] Combinations with other targeted therapies are also under consideration owing to the potential for synergistic effects. Poly (ADP-ribose) polymerase (PARP) inhibitors have shown activity in the treatment of TNBC because they impede the formation of PARP-DNA complexes that cause abnormal homologous recombination repair of DNA in TNBC.[106] The combination of a PI3Ki with the PARP inhibitor olaparib is also currently under exploration for patients with recurrent TNBC in a phase I study.[107] In addition to these inhibitors, various early-phase trials are underway for other PI3Kis (eg, GDC-0077) investigating single-agent activity and PI3Ki-based combination regimens that include endocrine therapy or targeted agents.[108,109]

Many unanswered questions and important considerations remain. A need exists to better understand how *PIK3CA* mutations contribute to breast cancer growth and oncogenesis. Furthermore, evidence is available to suggest that tumor evolution might be influenced by the selective pressures of therapy.[110] For example, the sequencing of metastatic tumor sites with disease progression after *PIK3CA* treatment in a patient bearing an activating *PIK3CA* mutation showed acquired genetic alterations in *PTEN*, resulting in the loss of PTEN expression. Inducible PTEN knockdown in sensitive cells resulted in resistance to *PIK3CA* therapy. Having access to sequential ctDNA testing will allow us to decipher the clonal evolution of the tumor cells and be able to accurately identify the molecular mechanisms associated with the emergence of resistance. Despite the limitations, the increasing use and availability of ctDNA testing will also aid in the identification of patients who are candidates for treatment with alpelisib.

Models of resistance to PI3Ki therapy have also been demonstrated, and investigations into potential therapeutic strategies to overcome resistance to PI3Kis have identified CDK4/6 inhibitors as the strongest sensitizers of resistant cell lines to *PIK3CA* inhibition, highlighting the potential potency of using these agents in combination with PI3Kis and optimizing scheduling.[111,112] Biomarkers of PI3K activation, such as increased AKT phosphorylation, have not been reliably associated with *PIK3CA* mutation.[11] However, suppression of phosphorylated retinoblastoma protein could serve as a biomarker for the response to PI3Kis.[111] However, mutations in *PIK3CA* will not always predict for sensitivity to PI3K inhibition,[10] perhaps owing to the presence of simultaneous mutations mediating resistance.[113] Ongoing research into the mechanisms of primary and secondary resistance to PI3Kis should lead to novel clinical trial strategies and patient stratification.

## Conclusions

The PI3K pathway is the most frequently mutated pathway in breast cancer, although the prognostic and predictive value of these

mutations remains unclear at present.[17,114] Although pan-PI3Kis (eg, buparlisib) combined with endocrine therapy have shown efficacy in improving PFS, the challenging toxicity profile has been a concern with these agents.[65,66] Isoform-specific PI3Kis such as the α-specific inhibitor alpelisib offer improvements in efficacy and a more manageable safety profile compared with pan-PI3Kis.[68,76] The first α-specific inhibitor to be approved, alpelisib (combined with fulvestrant), is anticipated to quickly become an important part of the treatment options for $HR^+$ ABC. However, alpelisib and other α-specific agents will still require effort to manage class-specific AEs. Current trials of PI3K-targeted agents are evaluating new combinations, other methods of identifying targetable mutations, and the use of ctDNA to obviate the need for tissue in determining the eligibility to receive PI3Ki-based treatments.

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