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Breast Cancer

Exploring Biomarkers of Phosphoinositide 3-Kinase Pathway Activation in the Treatment of Hormone Receptor Positive, Human Epidermal Growth Receptor 2 Negative Advanced Breast Cancer

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Key Words. Breast neoplasms • Molecular targeted therapy • *PIK3CA* protein, human • Alpelisib • Buparlisib • Taselisib

Abstract

Resistance to endocrine therapy (ET) is common in patients with hormone receptor positive (HR+) advanced breast cancer (ABC). Consequently, new targeted treatment options are needed in the post-ET setting, with validated biomarkers to inform treatment decisions. Hyperactivation of the phosphoinositide 3-kinase (PI3K) signaling pathway is common in ABC and is implicated in resistance to ET. The most frequent mechanism of PI3K pathway activation is activating mutations or amplification of *PIK3CA*, which encodes the α-isoform of the catalytic subunit of PI3K. Combining buparlisib, a pan-PI3K-targeted agent, with ET demonstrated modest clinical benefits in patients with aromatase inhibitor-resistant, HR+, human epidermal growth receptor 2 negative (HER2−) ABC in two phase III trials. Importantly, greater efficacy gains were observed in individuals with *PIK3CA*-mutated disease versus *PIK3CA*-wild-type tumors. Although the challenging safety profile did not support widespread use of this treatment combination, isoform-selective PI3K inhibitors may improve tolerability. In early clinical trials, promising disease control benefits were demonstrated with the PI3K isoform-selective inhibitors alpelisib and taselisib in patients with *PIK3CA*-mutated HR+, HER2− ABC. Ongoing biomarker-guided phase II/III studies may provide further opportunities to identify patients most likely to benefit from treatment with PI3K inhibitors and provide insight into optimizing the therapeutic index of PI3K inhibitors. Challenges facing the implementation of routine *PIK3CA* mutation testing must be addressed promptly so robust and reproducible genotyping can be obtained with liquid and tumor biopsies in a timely and cost-effective manner. ***The Oncologist*** 2019;24:305–312

Implications for Practice: The development of phosphoinositide 3-kinase (PI3K) inhibitors, especially those that selectively target isoforms, may be an effective strategy for overcoming endocrine therapy resistance in hormone receptor positive, human epidermal growth receptor 2 negative advanced breast cancer. Early-phase studies have confirmed that patients with *PIK3CA* mutations respond best to PI3Kα-isoform inhibition. Ongoing phase III trials will provide further data regarding the efficacy and safety of PI3K inhibitors in patients with different biomarker profiles.

Introduction

Approximately 75% of breast cancers express the estrogen receptor (ER) and/or the progesterone receptor, indicating a degree of estrogen dependence for cancer cell growth and tumorigenesis [1, 2]. Endocrine-based single-agent or combination therapy is the established standard of care for postmenopausal women with hormone receptor positive (HR+), human epidermal growth receptor 2 negative (HER2−) advanced breast cancer (ABC) [3–5]. An estimated 20%–40% of patients with HR+ ABC respond to single-agent endocrine therapy (ET), with a median duration of response (DOR) of approximately 8–14 months [6]. However, many patients with HR+ ABC encounter de novo resistance (nonresponsiveness to

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first-line ET) or acquired resistance (relapse despite initial response), which poses a major clinical challenge [1, 6].

The molecular mechanisms of endocrine resistance may include disruption of the ER pathway itself or alterations in the cell cycle and cell survival signaling pathways [7, 8]. Dysregulation of the cyclin D-cyclin-dependent kinase (CDK)-retinoblastoma pathway is an important contributor to ET resistance, and several CDK4/6 inhibitors are now approved in combination with ET for the treatment of advanced/recurrent HR+, HER2− breast cancer [7, 9–11]. Another key mechanism of endocrine resistance is hyperactivation of the phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway [12]. PI3Ks regulate many cellular processes, including cell proliferation and differentiation, as well as cancer cell growth, survival, and metastasis [1, 13, 14]. Aberrant PI3K pathway signaling is associated with poor prognosis in several cancer types [15] and is the most commonly activated pathway in breast cancer. The main alterations are mutations in *PIK3CA* and, less frequently, mutations in *PIK3R1* encoding the PI3K regulatory subunit p85α, the PI3K effector AKT1 and 2, and loss of the lipid phosphatases phosphatase and tensin homolog (PTEN) and inositol polyphosphate-4-phosphatase type II B [14, 16]. These aberrations promote tumor growth, disease progression, and resistance to anticancer therapies [1, 15]. In vitro data indicate that endocrine-resistant cells rely on PI3K/mTOR signaling for growth and are extremely sensitive to inhibition of this pathway [12, 17]. Furthermore, PI3K and/or mTOR inhibition can restore sensitivity of anti-estrogen-resistant breast cancer cells to ET, providing strong rationale for PI3K/mTOR inhibition combined with ET in the treatment of HR+ breast cancer [12, 18, 19]. The effectiveness of this dual inhibition strategy was shown in a phase III study of the mTOR inhibitor everolimus, resulting in its approval in combination with the aromatase inhibitor (AI) exemestane for the treatment of postmenopausal women with HR+, HER2− ABC, recurring or progressing after prior nonsteroidal AI [20–22]. Inhibition of the PI3K/mTOR pathway may also help overcome acquired resistance to CDK4/6-targeted therapy. The PI3K/mTOR pathway has been shown to be active in breast cancer cells resistant to a CDK4/6 inhibitor; these drug-resistant cells remained sensitive to treatment with inhibitors of PI3K or mTOR combined with ET and/or CDK4/6 inhibitors [23, 24]. Consequently, use of PI3K inhibitors after progression on a CDK4/6 inhibitor is now being investigated in clinical settings [23, 24].

To further improve the treatment of HR+, HER2− ABC, additional therapies beyond the currently approved targeted agents (CDK4/6 and mTOR inhibitors) are needed to provide postprogression treatment options and delay chemotherapy for as long as possible. Moreover, biomarkers of response to different targeted therapies are needed to inform treatment decisions and provide the ideal sequence of targeted therapies. In this review, we summarize preclinical and clinical studies regarding potential biomarkers of PI3K pathway activation in HR+, HER2− ABC, and discuss emerging opportunities for PI3K-targeted therapy based on biomarker status in this patient population.

Materials and Methods

A comprehensive literature review of the PubMed database concerning PI3K in breast cancer was performed. The search terms "biomarker," "PI3K," and "breast cancer" were used. The search was limited to articles concerning human subjects published in English. Reference lists of key papers were also reviewed. In addition, unpublished abstracts were identified by searching resources such as the American Society of Clinical Oncology, the American Association for Cancer Research, the European Society for Medical Oncology, and the San Antonio Breast Cancer Symposium scientific programs.

The PI3K Pathway in ABC

Overactivation of the PI3K pathway can occur through loss of function of the tumor suppressor PTEN, a key negative regulator of PI3K pathway activity [15]. In addition to PTEN loss, constitutive mTOR complex 1 signaling can also result from loss of tuberous sclerosis complex (TSC1 and TSC2) function [1, 25]. However, the most common mechanism of PI3K pathway activation is activating mutations or amplification of *PIK3CA*, the gene that encodes the α isoform of the catalytic subunit (p110) of PI3K [15, 26]. *PIK3CA* is one of the most frequently mutated genes in breast cancer, with somatic mutations occurring in up to 40% of ER+ breast tumors, and these mutations also have prognostic value [16, 27–30].

At present, activated PI3K pathway biomarkers in HR+, HER2− ABC do not guide treatment decisions as no CDK4/6 or mTOR inhibitors currently approved for HR+, HER2− ABC have demonstrated superior efficacy in patients with versus without *PIK3CA*-mutated tumors [31–33]. However, phase III trials of investigational PI3K inhibitors suggest patients may derive differential benefit depending on their tumors' *PIK3CA* mutation status [34–36].

At present, activated PI3K pathway biomarkers in HR+, HER2− ABC do not guide treatment decisions as no CDK4/6 or mTOR inhibitors currently approved for HR+, HER2− ABC have demonstrated superior efficacy in patients with versus without *PIK3CA*-mutated tumors. However, phase III trials of investigational PI3K inhibitors suggest patients may derive differential benefit depending on their tumors' *PIK3CA* mutation status.

Clinical Studies Evaluating Pan-PI3K Inhibitors in HR+, HER2− ABC

Combining PI3K-targeted therapy and ET has clinical benefits in the AI-resistant HR+, HER2− ABC setting, demonstrated by the BELLE-2 and BELLE-3 phase III trials [34, 35]. These studies recruited postmenopausal women with AI-resistant, HR+, HER2− metastatic breast cancer (all patients had received prior mTOR therapy in BELLE-3), randomized to receive fulvestrant plus the pan-PI3K inhibitor buparlisib or placebo [34, 35]. Randomization was stratified by PI3K activation status (BELLE-2) and visceral disease (BELLE-2 and BELLE-3) [34, 35]. In the overall populations of both studies, median progression-free survival (PFS) in the buparlisib arm

Table 1. Progression-free survival in phase II/III studies of pan-PI3K inhibitors in hormone receptor positive, human epidermal growth receptor 2 negative advanced breast cancer

Study	PFS: overall, months	PFS: *PIK3CA* status: ctDNA, months		PFS: *PIK3CA*/PI3K pathway status: tumor, months	
BELLE-2 [34] Buparlisib + fulvestrant (*n* = 576) vs. placebo + fulvestrant (*n* = 571)	All (*n* = 1,147): 6.9 vs. 5.0 HR 0.78 (95% CI 0.67–0.89) *p* = .00021	*PIK3CA* mutant (*n* = 200): 7.0 vs. 3.2 HR 0.56 (95% CI 0.39–0.80) *p* = .0005	*PIK3CA* wild-type (*n* = 387): 6.8 vs. 6.8 HR 1.05 (95% CI 0.82–1.34) *p* = .642	PI3K pathway activated (*n* = 372):[a] 6.8 vs. 4.0 HR 0.76 (95% CI 0.60–0.97) *p* = .014	PI3K pathway nonactivated (*n* = 479):[a] Not reported
BELLE-3 [35] Buparlisib + fulvestrant (*n* = 289) vs. placebo + fulvestrant (*n* = 143)	All (*n* = 432): 3.9 vs. 1.8 HR 0.67 (95% CI 0.53–0.84) *p* = .00030	*PIK3CA* mutant (*n* = 135): 4.2 vs. 1.6 HR 0.46 (95% CI 0.29–0.73) *p* = .00031	*PIK3CA* wild-type (*n* = 213): 3.9 vs. 2.7 HR 0.73 (95% CI 0.53–1.00) *p* = .026	*PIK3CA* mutant (*n* = 109):[b] 4.7 vs. 1.4 HR 0.39 (95% CI 0.23–0.65) *p* < .0001	*PIK3CA* wild-type (*n* = 204):[b] 2.8 vs. 1.7; HR 0.81 (95% CI 0.59–1.12) *p* = .099
FERGI [37][c] Pictilisib + fulvestrant (part 1 *n* = 89; part 2 *n* = 41) vs. placebo + fulvestrant (part 1 *n* = 79; part 2 *n* = 20)	All part 1: (*n* = 168): 6.6 vs. 5.1 HR 0.74 (95% CI 0.52–1.06) *p* = .096	Not reported	Not reported	Part 1: *PIK3CA* mutant (*n* = 70):[d] 6.5 vs. 5.1 HR 0.73 (95% CI 0.42–1.28) *p* = .268 Part 2: *PIK3CA* mutant (*n* = 61): 5.4 vs. 10.0 HR 1.07 (95% CI 0.53–2.18) *p* = .84	Part 1: *PIK3CA* wild-type (*n* = 84):[d] 5.8 vs. 3.6 HR 0.72 (95% CI 0.42–1.23) *p* = .23

[a]In BELLE-2, PI3K pathway-activated tumor status was defined as any mutation detected by Sanger sequencing in *PIK3CA* exons 1, 7, 9, or 20; or loss of PTEN expression (<10% of cells with expression level 1+ by immunohistochemistry, and none with level >1+). PI3K pathway nonactivated tumor status was defined as no *PIK3CA* mutations observed and detectable PTEN expression.
[b]In BELLE-3, *PIK3CA* mutation status in tumor tissue was assessed by the Roche cobas *PIK3CA* PCR assay, covering exons 7, 9, and 20.
[c]Part 1 of FERGI recruited patients with *PIK3CA*-mutant and -wild-type tumors and part 2 enrolled patients with *PIK3CA*-mutant tumors only.
[d]In FERGI, *PIK3CA* mutation status in tumor tissue was assessed by quantitative real-time PCR.
Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; HR, hazard ratio; PCR, polymerase chain reaction; PFS, progression-free survival; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog.

versus the placebo arm was improved by approximately 2 months (BELLE-2: 6.9 vs. 5.0 months; BELLE-3: 3.9 vs. 1.8 months; Table 1) [34, 35].

Importantly, exploratory analyses from both studies suggest that assessment of *PIK3CA* mutations using circulating tumor DNA (ctDNA) may help identify patients who are more likely to benefit from adding a PI3K inhibitor to ET [34, 35]. A consistent treatment benefit from the addition of buparlisib to fulvestrant was observed in patients with *PIK3CA* mutations detected in ctDNA at study entry. In BELLE-2, 32% of patients harbored *PIK3CA* mutations, and median PFS was improved by approximately 4 months with buparlisib plus fulvestrant versus placebo plus fulvestrant in patients with *PIK3CA* mutations (7.0 vs. 3.2 months). Patients with wild-type *PIK3CA* in ctDNA experienced no PFS benefit from adding buparlisib versus placebo to fulvestrant (6.8 vs. 6.8 months; Table 1) [34]. This effect was also reflected in the tumor response rates [34]. In BELLE-3, patients with *PIK3CA*-mutated ctDNA (61%) who received buparlisib plus fulvestrant also had substantially longer median PFS than individuals treated with placebo plus fulvestrant (4.2 vs. 1.6 months). A smaller treatment benefit of buparlisib versus placebo was observed in patients with *PIK3CA*-wild-type ctDNA (3.9 vs. 2.7 months; Table 1) [35]. Concordance of *PIK3CA* status between archival tumor tissue and ctDNA was 77% and 83% in BELLE-2 and BELLE-3, respectively [34, 35]. In BELLE-3, when *PIK3CA* mutations were assessed by polymerase chain reaction (PCR) in DNA from archival tumor tissue, the benefit of adding buparlisib versus placebo to fulvestrant in patients with *PIK3CA* mutations (4.7 vs. 1.4 months, assessed by PCR) was similar to findings in ctDNA (4.2 vs. 1.6 months) [35]. However, in BELLE-2, the treatment benefit based on genotyping of archival tumor tissue (Sanger sequencing and/or PTEN loss by immunohistochemistry) was less pronounced than that observed in ctDNA (6.8 vs. 4.0 months and 7.0 vs. 3.2 months) [34]. The less pronounced benefit in patients with PI3K pathway-activated tumors as assessed in tumor tissue in BELLE-2 compared with BELLE-3 could have been due to differences in the classification of PI3K pathway-activated tumors (*PIK3CA* mutations and PTEN loss in BELLE-2, *PIK3CA* mutations only in BELLE-3), the sensitivity of the test used to assess *PIK3CA* in tumor tissue (Sanger sequencing in BELLE-2, PCR in BELLE-3), and the different treatment histories of the patient populations (mTOR inhibitor naïve in BELLE-2, mTOR inhibitor pretreated in BELLE-3) [34, 35].

In contrast to the BELLE trials, in the phase II FERGI study evaluating the pan-PI3K inhibitor pictilisib plus fulvestrant in ER+, HER2− advanced/metastatic breast cancer, *PIK3CA* mutation status was not associated with improved PFS (Table 1) [37]. In FERGI, all but one patient in part 2 of the study had a *PIK3CA* mutation, compared with approximately 41% of patients in part 1; the smaller patient numbers evaluated in this subanalysis, as well as the differing methods of detecting *PIK3CA* mutations, may make it difficult to interpret these findings compared with those in the BELLE studies [37].

Although the BELLE studies provided compelling evidence that drugs targeting PI3K may have preferential activity in patients with *PIK3CA*-mutated tumors, buparlisib had tolerability issues in some patients, likely as a result of the less selective nature of pan-PI3K inhibitors [38]. In both BELLE studies, adverse events (AEs) occurring more commonly with buparlisib versus placebo included hyperglycemia, elevated alanine aminotransferase and aspartate aminotransferase, diarrhea, nausea, and mood disorders [34, 35]. Overall, the risk-benefit profile did not support the widespread clinical use of buparlisib [34, 35, 38]. However, the promising clinical activity observed with buparlisib (despite only a 1.9-month median duration of exposure to buparlisib in BELLE-2) [34] suggests there could be a place for PI3K inhibitors in the treatment of ABC if their associated toxicity could be reduced. Currently, isoform-selective PI3K inhibitors are under clinical development and may offer efficacy benefits while avoiding some off-target toxicities seen with pan-PI3K inhibitors [1, 39].

Selectively Targeting PI3K Isoforms in the Clinic

PI3K isoforms have differing roles in physiologic and pathologic cellular processes. Consequently, isoform-selective and pan-PI3K inhibitors are anticipated to have differing side-effect profiles, and selectively targeting a particular PI3K subunit could reduce or prevent some AEs associated with pan-PI3K inhibition. For example, the PI3K catalytic subunits p110α and p110β play distinct roles in insulin responses and energy metabolism. Whereas the p110β isoform appears to drive tumorigenesis of *PTEN*-deficient tumors (e.g., brain, breast, prostate, and endometrium), p110α is responsible for glucose homeostasis and also participates in Ras-mediated signaling and cell proliferation [1, 40, 41]. Furthermore, p110α has a role in tumorigenesis of breast cancer; mutations in *PIK3CA* tend to occur early in breast tumor progression, and the high frequency of these mutations in HR+ tumors suggests a role in luminal tumor differentiation [1, 40, 41].

Consequently, selective inhibition of the PI3Kα isoform may be effective in *PIK3CA*-mutated cancers while reducing the AEs observed with pan-PI3K inhibitors [1, 40, 41]. The PI3K isoform-selective inhibitors alpelisib and taselisib have demonstrated promising antitumor activity in preclinical models [42–45] and clinical studies [46–50], summarized below. Both alpelisib and taselisib more potently inhibit p110α than p110β (approximately 250-fold and 30-fold greater specificity, respectively) [44, 51, 52]. Taselisib also inhibits PI3K-δ and PI3K-γ isoforms [53].

Alpelisib

Alpelisib was the first oral inhibitor selectively targeting the PI3Kα isoform to undergo clinical development, having demonstrated antitumor activity in cancer cell lines and ER+ breast cancer xenograft models, including those harboring *PIK3CA* mutations [42–44, 54]. Comprehensive in vitro pharmacologic profiling across a panel of cancer cell lines indicated that presence of *PIK3CA* mutation or amplification was associated with sensitivity to alpelisib [43]. Dose- and time-dependent antiproliferative effects were also observed in *PIK3CA*-altered breast cancer cells [42]. In another study, alpelisib potently inhibited the two most common *PIK3CA* somatic mutations, H1047R and E545K, and resulted in a dose-dependent and statistically significant antitumor effect in a PI3Kα-dependent mouse model [44].

Single-agent alpelisib displayed promising activity in a phase Ia study of 134 patients with advanced solid tumors (including those with ER+, HER2− ABC), 93% of whom had *PIK3CA*-mutated or -amplified disease (using archival or fresh tumor biopsies). Patients were heavily pretreated (median of 4 prior cancer therapies; range 1–19) [50]. A complete response was observed in 1 patient (1%), partial response (PR) in 7 (5%), and stable disease in 70 (52%) patients [50]. Drug-related AEs included hyperglycemia (52%; grade 3/4 in 24%), nausea (50%, grade 3/4 in 2%), decreased appetite (42%, grade 3/4 in 2%), and diarrhea (40%, grade 3/4 in 3%) [50].

The combination of alpelisib and fulvestrant demonstrated synergistic antitumor activity compared with either agent alone in *PIK3CA*-mutated ER+ breast cancer xenograft models [54, 55]. Of note, PI3K-mediated upregulation of ER messenger RNA and protein expression was mitigated by addition of fulvestrant, thus sensitizing ER+ tumors to PI3K inhibition [55]. Based on this preclinical rationale for combined inhibition of PI3K plus fulvestrant, alpelisib plus fulvestrant was investigated in a phase Ia, open-label study of heavily pretreated patients (median of 5 prior therapies; range 1–16) with ER+, HER2− breast cancer. Among 49 patients with *PIK3CA*-altered tumors, overall response rate (ORR) was 27% and the disease control rate was 80%. In the 32 patients with *PIK3CA*-wild-type, ER+, HER2− breast cancer, no objective tumor responses were observed. Median PFS was also improved with alpelisib plus fulvestrant in patients with *PIK3CA*-altered versus *PIK3CA*-wild-type, ER+, HER2− ABC (9 vs. 5 months, respectively) [49]. Treatment-related AEs included diarrhea (56%), hyperglycemia (48%), rash (48%), nausea (43%), and decreased appetite (38%) [49].

Alpelisib in combination with letrozole was investigated in a phase Ib study of 26 patients previously treated (median of 2 prior therapies; range 1–4) for metastatic ER+, HER2− breast cancer [48]. PR (25% vs. 10%) and clinical benefit rate (CBR; 44% vs. 20%) were notably higher in patients with *PIK3CA*-mutated tumors compared with individuals with *PIK3CA*-wild-type disease [48]. It is noteworthy that 6 of the 8 patients remaining on alpelisib plus letrozole beyond 12 months had *PIK3CA*-mutated tumors [48]. AEs included gastrointestinal events (73%), hyperglycemia (62%), fatigue (54%), and rash (42%), all of which were dose-dependent [48].

Taselisib

Taselisib is another PI3K inhibitor with improved selectivity against mutated versus wild-type p110α [45, 46, 51, 52]. In *PIK3CA*-mutated breast cancer models, treatment with taselisib plus ET resulted in enhanced antitumor activity compared with single-agent antiestrogens, including fulvestrant [52]. In a phase II, open-label, single-arm study of 60 postmenopausal patients with HR+, HER2− metastatic breast cancer with nonresponse or progression on ≥1 prior ET, taselisib plus fulvestrant demonstrated initial antitumor activity. Confirmed responses were observed among patients with mutated, wild-type, and unknown *PIK3CA* status, which was retrospectively and centrally evaluated on archival tumor tissue [47]. Rates of best confirmed response and clinical

benefit were numerically higher in patients with tumors harboring *PIK3CA* mutations (42% each) compared with *PIK3CA*-wild-type tumors (14% and 24%, respectively) [47]. Grade 3/4 AEs included colitis (13%), diarrhea (12%), hyperglycemia (7%), and pneumonia (5%); 18% of patients discontinued taselisib because of an AE [47].

Improved clinical benefit of taselisib plus fulvestrant in patients with *PIK3CA*-mutant tumors was further demonstrated in the phase III, double-blind, placebo-controlled SANDPIPER trial [36]. Postmenopausal women with HR+, HER2− locally advanced or metastatic breast cancer with recurrence or progression during or after AI therapy were randomized 2:1 to receive taselisib plus fulvestrant (n = 340) or placebo plus fulvestrant (n = 176) [36]. This was the first trial of a mutant-selective PI3K inhibitor with ET in a biomarker-defined population to report safety and efficacy results. The median investigator-assessed PFS of patients with *PIK3CA*-mutant tumors (assessed by central laboratory) treated with taselisib plus fulvestrant was significantly higher than that of patients treated with placebo plus fulvestrant (7.4 vs. 5.4 months; hazard ratio 0.70; p = .0037) [36]. Taselisib plus fulvestrant also demonstrated improved ORR (28%) versus placebo plus fulvestrant (12%; p = .0002) [36]. Both the CBR (52% vs. 37%) and DOR (8.7 vs. 7.2 months) also favored taselisib plus fulvestrant versus placebo plus fulvestrant in patients with *PIK3CA*-mutant tumors [36]. The safety profile of taselisib plus fulvestrant was consistent with previous studies. The most frequent grade ≥ 3 AEs with taselisib plus fulvestrant were diarrhea (12%), hyperglycemia (10%), colitis (3%), and stomatitis (2%). Gastrointestinal side effects were likely secondary to inhibition of p110δ as this has been seen in trials with p110δ inhibitors, such as idelalisib [56]. Frequent treatment discontinuations due to AEs seen with taselisib plus fulvestrant (17% vs. 2% with placebo plus fulvestrant) in this study suggest that the combination may have limited clinical benefit in this setting because of tolerability challenges [36].

The combination of taselisib plus letrozole was also evaluated as a neoadjuvant therapy in postmenopausal women with ER+, HER2− untreated, operable early breast cancer in the phase II randomized LORELEI trial [57]. Patients were treated with taselisib plus letrozole or placebo plus letrozole for 16 weeks prior to surgery. The ORR as assessed by central laboratory magnetic resonance imaging with taselisib plus letrozole was 50% versus 39.3% with placebo plus letrozole (odds ratio 1.55 [95% confidence interval (CI) 1.00–2.38]; p = .049) [57]. Among patients with *PIK3CA*-mutant tumors, taselisib plus letrozole demonstrated a more significant increase in ORR versus placebo plus letrozole (56.2% vs. 38%; odds ratio 2.03 [95% CI 1.06–3.88]; p = .033) [57]. There was no significant difference between treatment arms in the pathological complete response rate [57]. The most common grade 3/4 AEs with taselisib plus letrozole included gastrointestinal disorders (7.8%), infections (4.8%), and skin/subcutaneous tissue disorders (4.8%); grade 3/4 hyperglycemia occurred in 1.2% of patients [57].

Emerging Insights on *PIK3CA* Status Testing and Ongoing Biomarker-Based Trials

There are emerging opportunities to explore targeted patient selection and biomarker-guided treatment based on *PIK3CA* mutation status in HR+, HER2− ABC. Currently, tumor biopsies are the gold standard for molecular screening but are associated with practical, technical, and safety issues [58]. Obtaining biopsies is invasive, and tumor tissue is not always accessible or available. In addition, the quality and quantity of tumor tissue is often variable, sometimes with insufficient material available for genotyping. Furthermore, logistical issues are often encountered with archival tissue when retrieving patients' biopsies from different centers [59, 60].

The evolving and heterogeneous nature of tumors highlights the need for effective, real-time molecular profiling to permit adjustments in targeted therapy regimens to selectively address evolving cancer genotypes [58]. Such monitoring of disease status over time can determine changes in molecular alterations after the initial diagnosis of cancer, such as emergence of new mutations [61, 62]. Studies suggest that *PIK3CA* mutation status of the primary tumor from the initial surgical/archival specimen can differ from that obtained from a biopsy of a metastatic site, highlighting the importance of assessing *PIK3CA* status in the metastatic lesion for selection of PI3K inhibitor therapy [61, 63, 64]. Even then, however, *PIK3CA* mutations are frequently subclonal and not uniformly present across multiple metastatic sites [65]. Thus, obtaining ctDNA from peripheral blood is emerging as a sensitive, reliable, and less invasive way to measure current *PIK3CA* mutation status, tumor evolution, and potentially response to therapy [60, 61, 66–68]. Clinical studies demonstrated 73%–83% concordance for *PIK3CA* status in ctDNA and archival tumor tissue, measured using PCR [35, 61]. Good concordance (approximately 90%) has been demonstrated among next-generation sequencing, Sanger sequencing, and quantitative PCR for assessing wild-type *PIK3CA* or mutations in exon 9 or 20 of *PIK3CA* [48, 69]. However, it should be noted that PCR-based/hybridization methods or gene panels that focus on hotspot mutations may miss up to 20% of *PIK3CA* mutations [70]. The differing sensitivity and specificity of sequencing and PCR-based mutation detection techniques underscores the need to optimize technologies and standardize results [62]. Indeed, a key challenge to the routine assessment of *PIK3CA* mutation status in clinical practice is the development, implementation, and standardization of cost- and time-efficient technologies that detect biomarkers with sufficient sensitivity [58, 62]. Although the BELLE studies support measurement of biomarkers at study entry and the use of ctDNA in liquid biopsies to assess *PIK3CA* mutation status, key learnings include the need to standardize biomarker assessment, the timing of tissue collection, and the technology used [38, 62]. Future studies of *PIK3CA*-selected populations should incorporate the testing of technologies into their design for the clinical standardization of biomarker assessment [62].

There are several ongoing biomarker-guided phase II/III trials of alpelisib in patients with HR+, HER2− ABC (Table 2). The phase III, placebo-controlled SOLAR-1 trial is evaluating alpelisib plus fulvestrant in men and postmenopausal women who progressed on or after an AI. Patients are divided into *PIK3CA*-mutated versus -nonmutated cohorts (as measured by ctDNA) and also stratified by prior CDK4/6 inhibitor therapy and by presence of liver and/or lung metastases [71].

Table 2. Ongoing phase II/III clinical trials evaluating isoform-selective PI3K inhibitors in hormone receptor positive, human epidermal growth receptor negative advanced breast cancer [72]

Trial overview	Key eligibility criteria	Primary endpoint
Phase III		
SOLAR-1 Alpelisib (vs. placebo) + fulvestrant *n* = 572 NCT02437318	• Progressed on/after AI • Known *PIK3CA* status	PFS in the *PIK3CA*-mutated cohort
Phase II[a]		
BYLieve Alpelisib + fulvestrant or letrozole *n* ≈ 160 NCT03056755	• Progressed on or after a CDK4/6 inhibitor • *PIK3CA*-mutated tumors	Patients alive without disease progression
SAFIR PI3K Alpelisib + fulvestrant vs. chemotherapy (maintenance) *n* ≈ 90 NCT03386162	• ET-resistant disease • No progression after chemotherapy (6–8 cycles) • *PIK3CA*-mutated tumors	PFS
PIKNIC Alpelisib (single arm) *n* ≈ 34 NCT02506556	• PI3K-abnormal disease • Progression after ≥1 ET in the metastatic setting[b]	ORR

[a]Phase I/II trials not included.
[b]Patients with TNBC are also being recruited with progression after ≥1 prior systemic therapy.
Abbreviations: AI, aromatase inhibitor; CDK, cyclin-dependent kinase; ET, endocrine therapy; ORR, objective response rate; PFS, progression-free survival; PI3K, phosphoinositide 3-kinase; TNBC, triple-negative breast cancer.

The primary study objective is PFS in the *PIK3CA*-mutated cohort; a key secondary objective is overall survival in the *PIK3CA*-mutated cohort. PFS by baseline *PIK3CA* status, as measured in ctDNA, will also be evaluated [71]. Ongoing phase II studies in patients with HR+, HER2− ABC include BYLieve, which is investigating alpelisib plus fulvestrant or letrozole in men and women with *PIK3CA*-mutated tumors whose disease progressed on or after a CDK4/6 inhibitor [72]. SAFIR PI3K is comparing alpelisib plus fulvestrant versus chemotherapy in the maintenance setting in patients with *PIK3CA*-mutated disease, evaluated using tumor tissue or ctDNA collected at the time of disease progression [72]. PIKNIC is a single-arm study investigating tumor response to single-agent alpelisib in patients with advanced triple-negative breast cancer or PI3K pathway-altered, HR+ disease that has progressed on at least one line of ET for metastatic breast cancer [57].

Conclusion

Hyperactivation of the PI3K pathway in HR+, HER2− ABC is a key target of interest in ongoing clinical studies, particularly using p110α-selective inhibitors [1, 12]. Indeed, encouraging preclinical and clinical data support the rationale for pursuing isoform-selective PI3K inhibitors in combination with ET in patients with *PIK3CA*-mutated, endocrine-resistant ABC [36, 47–50]. Although the addition of PI3K inhibitors to ET has demonstrated modest clinical responses, the magnitude of benefit observed thus far has been below that initially expected. This may be due to several factors; for example, the presence of alternative aberrantly activated and compensatory signaling pathways (e.g., feedback activation of receptor tyrosine kinases, ER signaling, or Bcl-2) could be present clinically [40, 41, 73]. Further studies may be required to elucidate compensatory mechanisms and to further develop effective treatment combinations. Additionally, inhibitors targeting the PI3Kα isoform affect the wild-type protein as well as the mutant form, resulting in hyperglycemia and rash, thus limiting the potential for complete and sustained inhibition of the mutant enzyme. Furthermore, a dose-dependent increase in the plasma levels of fasting C-peptide and insulin, frequently associated with hyperglycemia, is an obligatory on-target pharmacodynamic surrogate of PI3K inhibition in trials with PI3K inhibitors [74, 75]. This obligatory surge in insulin secretion may activate insulin receptors and PI3K, particularly in tumors rich in insulin receptors, and limit the clinical activity of PI3K inhibitors [76]. Therefore, future development of inhibitors with greater selectivity for p110α mutant isoforms and reduced inhibitory activity against wild-type p110α protein is sorely needed to further advance the field.

Although the addition of PI3K inhibitors to ET has demonstrated modest clinical responses, the magnitude of benefit observed thus far has been below that initially expected. This may be due to several factors; for example, the presence of alternative aberrantly activated and compensatory signaling pathways could be present clinically.

In the meantime, data are eagerly awaited from phase III trials of isoform-selective PI3K inhibitors. As results are reported, it is important to consider the impact of p110δ and p110γ inhibition on the toxicity profiles observed. For instance, some of the potentially treatment-limiting AEs observed with isoform-specific PI3K inhibitors were gastrointestinal toxicities [36, 48], which are common with p110δ

inhibition [77]. Therefore, differences in specificity for other PI3K isoforms (β, δ, γ) may result in distinct toxicity profiles of isoform-selective PI3K inhibitors and may necessitate further evaluation of optimal dosing. Furthermore, ongoing biomarker-guided studies may provide opportunities to identify patients based on *PIK3CA* mutation status who are more likely to benefit from treatment with PI3K inhibitors and may aid in improving the therapeutic index of this class of drugs.

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Disclosures

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References

1. Ciruelos Gil EM. Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treat Rev 2014;40:862–871.

2. Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. J Clin Oncol 2011;29:4452–4461.

3. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Breast Cancer. Version 4.2017. Available at https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed April 27, 2018.

4. Rugo H, Rumble R, Macrae E et al. Endocrine therapy for hormone receptor-positive metastatic breast cancer: American Society of Clinical Oncology Guideline. J Clin Oncol 2016;34:3069–3103.

5. Cardoso F, Costa A, Senkus E et al. 3rd ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 3). Breast 2017;31:244–259.

6. Johnston SR. New strategies in estrogen receptor-positive breast cancer. Clin Cancer Res 2010;16:1979–1987.

7. Spring L, Bardia A, Modi S. Targeting the cyclin D-cyclin-dependent kinase (CDK) 4/6-retinoblastoma pathway with selective CDK 4/6 inhibitors in hormone receptor-positive breast cancer: rationale, current status, and future directions. Discov Med 2016;21:65–74.

8. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 2011;62:233–247.

9. Ibrance (palbociclib) [package insert]. New York, NY: Pfizer, Inc; 2017.

10. Kisqali (ribociclib) [package insert]. East Hanover, NJ: Novartis Pharmaceuticals Corp; 2017.

11. Verzenio (abemaciclib) [package insert]. Indianapolis, IN: Eli Lilly and Co; 2017.

12. Miller TW, Hennessy BT, González-Angulo AM et al. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. J Clin Invest 2010;120:2406–2413.

13. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: Divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 2015;15:7–24.

14. Hosford SR, Miller TW. Clinical potential of novel therapeutic targets in breast cancer: CDK4/6, Src, JAK/STAT, PARP, HDAC, and PI3K/AKT/mTOR pathways. Pharmgenomics Pers Med 2014;7:203–215.

15. Saal LH, Johansson P, Holm K et al. Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. Proc Natl Acad Sci USA 2007;104:7564–7569.

16. Mukohara T. PI3K mutations in breast cancer: Prognostic and therapeutic implications. Breast Cancer (Dove Med Press) 2015;7:111–123.

17. Yue W, Fan P, Wang J et al. Mechanisms of acquired resistance to endocrine therapy in hormone-dependent breast cancer cells. J Steroid Biochem Mol Biol 2007;106:102–110.

18. Boulay A, Rudloff J, Ye J et al. Dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer. Clin Cancer Res 2005;11:5319–5328.

19. Beeram M, Tan Q, Tekmal R et al. Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. Ann Oncol 2007;18:1323–1328.

20. Afinitor (everolimus) [package insert]. East Hanover, NJ: Novartis Pharmaceuticals Corp; 2017.

21. Baselga J, Campone M, Piccart M et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012;366:520–529.

22. Yardley D, Pritchard K, Burris H et al. Everolimus plus exemestane in postmenopausal patients with HR^+ breast cancer: BOLERO-2 final progression-free survival analysis. Adv Ther 2013;30:870–884.

23. Jansen VM, Bhola NE, Bauer JA et al. Kinome-wide RNA interference screen reveals a role for PDK1 in acquired resistance to CDK4/6 inhibition in ER-positive breast cancer. Cancer Res 2017;77:2488–2499.

24. O'Brien NA, Conklin D, Luo T et al. Antitumor activity of the PI3K/mTOR pathway inhibitors alpelisib (BYL719) and everolimus (RAD001) in xenograft models of acquired resistance to CDK-4/6 targeted therapy. Cancer Res 2017;77(suppl 13):4150a.

25. Fruman DA, Chiu H, Hopkins BD et al. The PI3K pathway in human disease. Cell 2017;170:605–635.

26. Huang WC, Hung MC. Induction of Akt activity by chemotherapy confers acquired resistance. J Formos Med Assoc 2009;108:180–194.

27. Saal LH, Holm K, Maurer M et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005;65:2554–2559.

28. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 2008;68:6084–6091.

29. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.

30. Isakoff SJ, Engelman JA, Irie HY et al. Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. Cancer Res 2005;65:10992–11000.

31. Cristofanilli M, Turner NC, Bondarenko I et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): Final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016;17:425–439.

32. Hortobagyi GN, Chen D, Piccart M et al. Correlative analysis of genetic alterations and everolimus benefit in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: Results from BOLERO-2. J Clin Oncol 2016;34:419–426.

33. Andre F, Stemmer S, Campone M et al. Ribociclib + letrozole for first-line treatment of hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (ABC): Efficacy by baseline tumor markers.

Abstract presented at: 108th Annual Meeting of the American Association for Cancer Research; April 1–5, 2017; Washington, DC.

34. Baselga J, Im SA, Iwata H et al. Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18:904–916.

35. Di Leo A, Johnston S, Lee KS et al. Buparlisib plus fulvestrant in postmenopausal women with hormone-receptor-positive, HER2-negative, advanced breast cancer progressing on or after mTOR inhibition (BELLE-3): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2018;19:87–100.

36. Baselga J, Dent S, Cortés J et al. Phase III study of taselisib (GDC-0032) + fulvestrant (FULV) in patients (pts) with estrogen receptor (ER)-positive, *PIK3CA*-mutant (MUT), locally advanced or metastatic breast cancer (MBC): Primary analysis from SANDPIPER. J Clin Oncol 2018;36(suppl 18):LBA1006a.

37. Krop IE, Mayer IA, Ganju V et al. Pictilisib for oestrogen receptor-positive, aromatase inhibitor-resistant, advanced or metastatic breast cancer (FERGI): A randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2016;17:811–821.

38. Chopra N, Turner NC. Targeting PIK3CA-mutant advanced breast cancer in the clinical setting. Lancet Oncol 2017;18:842–843.

39. Janku F. Phosphoinositide 3-kinase (PI3K) pathway inhibitors in solid tumors: From laboratory to patients. Cancer Treat Rev 2017;59:93–101.

40. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol 2010;28:1075–1083.

41. Jia S, Roberts TM, Zhao JJ. Should individual PI3 kinase isoforms be targeted in cancer? Curr Opin Cell Biol 2009;21:199–208.

42. Fritsch C, Schnell C, Chatenay-Rivauday C et al. NVP-BYL719, a novel PI3Kalpha selective inhibitor with all the characteristics required for clinical development as an anti-cancer agent. Cancer Res 2012;72(suppl 8):3748a.

43. Huang A, Fritsch C, Wilson C et al. Single agent activity of PIK3CA inhibitor BYL719 in a broad cancer cell line panel. Abstract presented at: 103rd Annual Meeting of the American Association for Cancer Research; March 31–April 4, 2012; Chicago, IL.

44. Fritsch C, Huang A, Chatenay-Rivauday C et al. Characterization of the novel and specific PI3Kalpha inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. Mol Cancer Ther 2014;13:1117–1129.

45. Song K, Edgar K, Kirkpatrick D et al. The PI3K inhibitor, taselisib, has a unique mechanism of action that leads to enhanced potency in PIK3CA mutant models. Abstract presented at: 108th Annual Meeting of the American Association for Cancer Research; April 1–5, 2017; Washington, DC.

46. Juric D, Krop I, Ramanathan RK et al. Phase I dose-escalation study of taselisib, an oral PI3K inhibitor, in patients with advanced solid tumor. Cancer Discov 2017;7:704–715.

47. Dickler MN, Saura C, Richards DA et al. A phase II study of the PI3K inhibitor taselisib (GDC-0032) combined with fulvestrant (F) in patients (pts) with HER2-negative (HER2-), hormone receptor-positive (HR+) advanced breast cancer (BC). J Clin Oncol 2016;34(suppl 15):520a.

48. Mayer IA, Abramson VG, Formisano L et al. A phase Ib study of alpelisib (BYL719), a PI3Kα-specific inhibitor, with letrozole in ER+/HER2− metastatic breast cancer. Clin Cancer Res 2017;23:26–34.

49. Juric D, André F, Rugo H et al. Combined alpelisib (BYL719) and fulvestrant in PIK3CA mutant or wild-type estrogen receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer. Abstract presented at: 33rd Annual Miami Breast Cancer Conference; March 10–13, 2016; Miami, FL.

50. Juric D, Rodon J, Tabernero J et al. Phosphatidylinositol 3-kinase α—selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: Results from the first-in-human study. J Clin Oncol 2018;36:1291–1299.

51. Juric D, Krop I, Ramanathan R et al. GDC-0032, a beta isoform-sparing PI3K inhibitor: Results of a first-in-human phase Ia dose escalation study. Abstract presented at: 104th Annual Meeting of the American Association for Cancer Research; April 6–10, 2013; Washington, DC.

52. Sampath D, Nannini MA, Jane G et al. The PI3K inhibitor GDC-0032 enhances the efficacy of standard of care therapeutics in PI3K alpha mutant breast cancer models. Abstract presented at: 36th Annual San Antonio Breast Cancer Symposium; December 10–14, 2013; San Antonio, TX.

53. Ndubaku CO, Heffron TP, Staben ST et al. Discovery of 2-{3-[2-(1-isopropyl-3-methyl-1H-1,2-4-triazol-5-yl)-5,6-dihydrobenzo[f]imidazo[1,2-d][1,4]oxazepin-9-yl]-1H-pyrazol-1-yl}-2-methylpropanamide (GDC-0032): A β-sparing phosphoinositide 3-kinase inhibitor with high unbound exposure and robust in vivo antitumor activity. J Med Chem 2013;56:4597–4610.

54. O'Brien N, Tong L, Ayala R et al. Targeting PI3K overcomes in vivo resistance to everolimus in estrogen receptor (ER+) breast cancer. Abstract presented at: 36th Annual San Antonio Breast Cancer Symposium; December 10–14, 2013; San Antonio, TX.

55. Bosch A, Li Z, Bergamaschi A et al. PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer. Sci Transl Med 2015;7:283ra51.

56. Cheah CY, Fowler NH. Idelalisib in the management of lymphoma. Blood 2016;128:331–336.

57. Saura C, Azambuja E, Hlauschek D et al. Primary results of LORELEI: A phase II randomized, double-blind study of neoadjuvant letrozole (LET) plus taselisib versus LET plus placebo (PLA) in postmenopausal patients (pts) with ER+/HER2-negative early breast cancer (EBC). Abstract presented at: ESMO 2017 Congress; September 8, 2017; Madrid, Spain.

58. Janku F. Tumor heterogeneity in the clinic: Is it a real problem? Ther Adv Med Oncol 2014;6:43–51.

59. Aung KL, Board RE, Ellison G et al. Current status and future potential of somatic mutation testing from circulating free DNA in patients with solid tumours. Hugo J 2010;4:11–21.

60. Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.

61. Higgins MJ, Jelovac D, Barnathan E et al. Detection of tumor *PIK3CA* status in metastatic breast cancer using peripheral blood. Clin Cancer Res 2012;18:3462–3469.

62. Sellami D, Dharan B, Wilke C et al. Circulating tumor DNA as a novel tool to shape clinical trial designs with the potential to impact outcomes: A focus on PI3K inhibitors. Ann Oncol 2017;28:2882–2887.

63. Dupont Jensen J, Laenkholm AV, Knoop A et al. *PIK3CA* mutations may be discordant between primary and corresponding metastatic disease in breast cancer. Clin Cancer Res 2011;17:667–677.

64. Gonzalez-Angulo AM, Ferrer-Lozano J, Stemke-Hale K et al. PI3K pathway mutations and PTEN levels in primary and metastatic breast cancer. Mol Cancer Ther 2011;10:1093–1101.

65. McGranahan N, Favero F, de Bruin EC et al. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. Sci Transl Med 2015;7:283ra54.

66. Garcia-Murillas I, Schiavon G, Weigelt B et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7:302ra133.

67. Rothé F, Laes JF, Lambrechts D et al. Plasma circulating tumor DNA as an alternative to metastatic biopsies for mutational analysis in breast cancer. Ann Oncol 2014;25:1959–1965.

68. Moynahan ME, Sung P, Asham M et al. Monitoring *PIK3CA* mutant allele fraction (AF) in cell-free DNA (cfDNA) in metastatic breast cancer (MBC) patients treated with PI3Kα–inhibitor plus letrozole (L) or exemestane (E). J Clin Oncol 2014;32(suppl 15):517a.

69. Papaxoinis G, Kotoula V, Alexopoulou Z et al. Significance of PIK3CA mutations in patients with early breast cancer treated with adjuvant chemotherapy: A Hellenic Cooperative Oncology Group (HeCOG) Study. PLoS One 2015;10:e0140293.

70. Richardson AL, Iglehart JD. BEAMing up personalized medicine: Mutation detection in blood. Clin Cancer Res 2012;18:3209–3211.

71. Rugo H, Andre F, Rubovszky G et al. A phase 3 study of alpelisib (ALP) plus fulvestrant (FUL) in men and postmenopausal women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) ABC progressing on or after aromatase inhibitor (AI) therapy: SOLAR-1. J Clin Oncol 2017;35(suppl 15):TPS1111a.

72. ClinicalTrials.gov. Accessed April 27, 2018.

73. Williams MM, Cook RS. Bcl-2 family proteins in breast development and cancer: Could Mcl-1 targeting overcome therapeutic resistance? Oncotarget 2015;6:3519–3530.

74. Bendell JC, Rodon J, Burris HA et al. Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 2012;30:282–290.

75. Juric D, Rodon J, Tabernero J et al. Phosphatidylinositol 3-kinase alpha-selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: Results from the first-in-human study. J Clin Oncol 2018;36:1291–1299.

76. Hopkins BD, Pauli C, Xing D et al. Suppression of insulin feedback enhances the efficacy of PI3K inhibitors. Nature 2018;560:499–503.

77. Greenwell IB, Ip A, Cohen JB. PI3K inhibitors: Understanding toxicity mechanisms and management. Oncology (Williston Park) 2017;31:821–828.