The phosphatidylinositol 3-kinase (PI3K) pathway is an intracellular pathway activated in response to progrowth signaling, such as human epidermal growth factor receptor 2 (HER2) and other kinases. Abnormal activation of PI3K has long been recognized as one of the main oncogenic drivers in breast cancer, including HER2-positive (HER2+) subtype. Somatic activating mutations in the gene encoding PI3K alpha catalytic subunit (PIK3CA) are present in approximately 30% of early-stage HER2+ tumors and drive therapeutic resistance to multiple HER2-targeted agents. Here, we review currently available agents targeting PI3K, discuss their potential role in HER2+ breast cancer, and provide an overview of ongoing trials of PI3K inhibitors in HER2+ disease. Additionally, we review the landscape of PIK3CA mutational testing and highlight the gaps in knowledge that could present potential barriers in the effective application of PI3K inhibitors for treatment of HER2+ breast cancer.
**CONTEXT**

**Key Objective**
Phosphatidylinositol 3-kinase (PI3K) pathway activation plays a key role in resistance to human epidermal growth factor receptor 2 (HER2)-targeted therapy. However, PIK3CA mutations are not routinely tested in HER2+ tumors, and PI3K inhibitors are not yet US Food and Drug Administration–approved for this breast cancer subtype. Currently available companion diagnostic test may miss up to 20% of activating PIK3CA mutations, whereas next-generation sequencing assays may yield variable results: not all mutations would have confirmed deleterious effects on protein function or a verified ability to predict response to PI3K inhibitors.

**Knowledge Generated**
Here, we summarize ongoing trials of PI3K inhibitors in HER2+ disease, outline the landscape of PIK3CA mutational testing, and examine preclinical and clinical evidence behind PIK3CA mutations detected by companion diagnostic and next-generation sequencing tests.

**Relevance**
This review provides a reference for translational and clinical investigators to accelerate research on PIK3CA inhibitors and their predictive biomarkers in HER2+ breast cancer to improve treatment of HER2+ disease.

Transgenic overexpression of HER2 and activating mutations in PIK3CA develop mammary tumors faster than those bearing only an HER2 transgene.17 HER2-positive (HER2+) breast cancer is an aggressive subtype characterized by rapid growth and visceral and brain metastases. Nearly 54,000 US women are diagnosed with HER2+ breast cancer every year. Despite advances in treatment, approximately 25% of patients experience recurrence within 5 years.18 Most patients with HER2+ metastatic breast cancer (MBC) develop resistance to HER2-targeted inhibitors (HER2i) leading to progressive disease and death.19,20 PIK3CA mutations are present in approximately 30% of HER2+ breast tumors (Table 1), contributing to aggressive tumor behavior and poor treatment outcomes.21 A large-scale shRNA screen identified PI3K pathway as a major modulator of sensitivity to HER2 monoclonal antibody trastuzumab.21 In vitro, PIK3CA-mutant HER2+ human breast tumor cell lines are resistant to trastuzumab and maintain AKT phosphorylation despite treatment.15,21 In vivo, PIK3CA-mutant HER2+ breast tumors grow despite treatment with trastuzumab, another HER2-mAb pertuzumab, or HER2 tyrosine kinase inhibitor (TKI) lapatinib; this drug resistance is reversed by addition of PI3K inhibitors (PI3Ki).17 In contrast to other HER2i, the antibody-drug conjugate ado-trastuzumab emtansine (T-DM1) is active in PIK3CA-mutant breast tumor cell lines and xenograft models,25 perhaps because of its unique mechanism of action via HER2-receptor mediated delivery of chemotherapeutic payload, which is independent of signaling downstream of HER2.23 PIK3CA mutations are associated with poor outcomes in patients with HER2+ early breast cancer.21-26 In NeoALTTO,27 GeparSixto,28 and NeoSphere29 studies, PIK3CA mutations were linked to low rates of pathologic complete response after neoadjuvant chemotherapy and HER2i, with 13%-20% absolute decrease in complete response rates in patients with PIK3CA-mutant versus wild-type tumors. In the adjuvant setting, PI3K/PTEN/AKT alterations were associated with poorer prognosis (hazard ratio, 1.35; 95% CI, 1.01 to 1.79) in the APHINITY study of adjuvant trastuzumab and pertuzumab.29 In contrast to APHINITY, the ExteNET trial of adjuvant neratinib (an irreversible TKI of HER1, HER2, and HER4) did not show the difference in outcomes between patients with PIK3CA-mutant and wild-type tumors.30 Similarly, KATHERINE trial showed that benefits of adjuvant T-DM1 on invasive disease-free survival are independent of PIK3CA mutational status.31 Among patients with HER2+ MBC enrolled in the docetaxel, trastuzumab, and pertuzumab arm of the CLEOPATRA study, those whose tumors had PIK3CA mutations experienced shorter progression-free survival (PFS) when compared with patients with PIK3CA wild-type tumors (13 v 22 months, respectively; hazard ratio, 0.67; 95% CI, 0.50 to 0.89).24,26 In the EMILIA trial, patients with PIK3CA-mutant tumors had shorter PFS and overall survival on capecitabine plus lapatinib treatment, but not on T-DM1 treatment,22,24 underscoring potential activity of T-DM1 against PIK3CA-mutant disease. Activity of T-DM1 against PIK3-mutant HER2+ MBC was confirmed in the TH3RESA trial, where T-DM1 was compared with treatment of the physician’s choice.32 Although published clinical trials suggest that T-DM1 and neratinib may be active against PIK3CA-mutant HER2+ breast cancer, the majority of the HER2i have diminished efficacy in this setting.21,23,27,28,33 Considering the presence of activating PIK3CA mutations in approximately 30% of HER2+ tumors and the strong association of these mutations with resistance to HER2i, there is an unmet need to develop combination regimens blocking HER2 and PI3K.
FIG 1. The PI3K pathway: normal and cancerous conditions and targeted agents. (A) Under normal conditions, the PI3K pathway contributes to the regulation of cell growth, proliferation, and survival via receptor tyrosine kinases including HER2 leading to downstream activation of AKT and mTOR complexes. (B) Inappropriate/excessive activation of the pathway caused by HER2 amplification, PIK3CA alteration, and/or PTEN loss-of-function can contribute to the formation of breast cancer via dysregulated cell growth, proliferation, and survival. (C) Pharmacologic agents targeting the PI3K pathway include PI3K, mTOR, and dual PI3K/mTOR inhibitors. HER2, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol diphosphate; PIP3, phosphatidylinositol 3-phosphate; PTEN, phosphatase and tensin homolog.
TABLE 1. Frequency of PIK3CA Mutations in HER2+ Breast Cancer

| Data Set | Frequency of PIK3CA Mutations |
|----------|-------------------------------|
| NeoSphere study | 32% (133 of 417) |
| MSKCC data set (Cancer Cell 2018) | 30% (54 of 185) |
| TCGA (Pan Cancer Atlas) | 29% (35 of 122) |
| METABRICK (Nature 2012, Nat Communication 2016) | 30% (103 of 342) |

Abbreviation: HER2, human epidermal growth factor receptor 2.

In this review, we summarize the targeted agents that block the PI3K pathway and ongoing clinical trials of these agents in HER2+ disease. Additionally, we highlight the landscape and challenges in PIK3CA mutational testing. Our goal is to provide a reference for translational and clinical investigators to accelerate research on PIK3CA inhibitors in HER2+ breast cancer.

Throughout this paper, we used the American College of Clinical Pathology’s definition for HER2+ and hormone receptor–positive (HR+) breast cancer. To ascertain the current landscape of clinical trials testing of PI3Ki in HER2+ disease, we performed a PubMed search of the published, English-language scientific literature through January 2021 using the keywords “breast cancer,” “HER2,” “human epidermal growth factor receptor 2,” “PI3K,” “PIK3CA,” and the generic names of specific inhibitors. The same keywords were used to search the NIH Clinical Trial Database ClinicalTrials.gov.

CHALLENGES IN DEVELOPING EFFECTIVE AND TOLERABLE PI3K INHIBITORS

Despite the key role of PI3K in numerous tumor types, development of effective and safe PI3Ki has been a major challenge. Reasons include off-target effects of nonselective PI3Ki leading to intolerable toxicities, drug resistance because of activation of alternative oncogenic pathways, mutations in PI3K downstream effectors, or PTEN inactivation. Pan-PI3Ki suppress activity of all PI3K class I isoforms and include buparlisib, pictilisib, pilparlisib, copanlisib, PX866, CH5132799, and SF1126. With the exception of copanlisib, no pan-PI3Ki have been approved for clinical use because of a lack of clinical activity and/or significant safety concerns. One of the most extensively tested pan-PI3Ki is buparlisib, which has results available from three randomized trials in HR+/HER2– MBC. Although it showed antitumor efficacy, including activity in brain metastases, its clinical development was abandoned because of toxicity. More than 20% of patients on buparlisib had grade ≥ 3 elevation of liver enzymes, and there were cases of severe depression leading to suicide attempts. Another pan-PI3Ki pictilisib failed to show improvement in PFS in HR+ breast cancer, perhaps because 24% of patients in the pictilisib arm discontinued treatment and an additional 24% required dose reduction because of adverse events (AEs). Development of pilparlisib, PX-866, and CH5132799 has been stopped because of lack of activity, whereas SF1126 is still in clinical trials.

Unlike other pan-PI3Ki, copanlisib is administered intravenously and has a better therapeutic index, with hyperglycemia and nausea being the most frequent yet manageable AEs. The drug demonstrated low incidence of high-grade GI and liver toxicity. Copanlisib showed activity in patients with hematologic malignancies, breast cancer, and endometrial cancer. Copanlisib is US Food and Drug Administration (FDA)–approved in patients with relapsed refractory follicular lymphoma irrespective of PI3K mutational status on the basis of the results of the CHRONOS-1 trial that showed durable responses in 59% of patients. Copanlisib is now being studied in solid tumors and hematologic malignancies in combination with chemotherapy, antihormonal therapy, targeted agents, or immunotherapy.

Further efforts to suppress the PI3K pathway progressed primarily in two directions. To improve efficacy and overcome drug resistance, dual PI3K/mTOR inhibitors were formulated, such as apitolisib, dactolisib, and gedatolisib. Alternatively, to improve the toxicity profile, isoform selectivity was prioritized and selective inhibitors were developed, such as taselisib (inhibiting p110α, p110γ, and p110δ, but sparing the p110β isoform) and alpelisib (selective p110α inhibitor).

Dual PI3K/mTOR inhibitors, dactolisib and apitolisib, have not achieved the hoped-for clinical efficacy, mainly because of frequent dose-limiting toxicities, such as diarrhea, hyperglycemia, mucositis, and liver toxicity. Broadening the spectrum of inhibition seemed to increase the toxicity of these inhibitors disproportionately to antitumor activity, likely because of the fundamental role of PI3K/mTOR pathway in normal tissues. Gedatolisib differs from other PI3K/mTOR inhibitors because of its intravenous formulation, such as apitolisib, dactolisib, and gedatolisib. Additionally, to improve the toxicity profile, isoform selectivity was prioritized and selective inhibitors were developed, such as taselisib (inhibiting p110α, p110γ, and p110δ, but sparing the p110β isoform) and alpelisib (selective p110α inhibitor).

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showed only modest activity and a challenging safety profile (diarrhea, nausea, vomiting, abdominal pain, stomatitis, fatigue, hyperglycemia, and rash), leading to cessation of its clinical development.

On the basis of the strong association with RTK signaling and frequent mutations in human cancers, PI3K p110α has become the clear target for inhibition. The selective PI3K p110α inhibitor alpelisib demonstrated a manageable safety profile and prolonged PFS among patients with the PIK3CA-mutant, HR+ MBC in the phase III clinical trial SOLAR-1. Main side effects were hyperglycemia, diarrhea, and rash. At a median follow-up of 20 months, PFS was 11 months (95% CI, 7.5 to 14.5) in the alpelisib-fulvestrant group compared with 5.7 months (95% CI, 3.7 to 7.4) in the placebo-fulvestrant group. Alpelisib received FDA approval for the HR+ MBC in May 2019, becoming a milestone of success on the difficult path toward PI3K inhibition in cancer. Although patients with brain metastases were not included in SOLAR-1, case reports indicate potential activity of alpelisib in brain metastatic disease.

Given the challenges of developing effective and tolerable PI3KI, researchers have explored inhibition of PI3K downstream effectors AKT and mTOR. A first-generation mTOR inhibitor everolimus is FDA-approved in HR+ MBC on the basis of the BOLERO-2 study, whereas the second-generation mTOR inhibitor, sapanisertib, and AKT inhibitors, capivasertib and ipatasertib, are in early clinical trials. Compared with everolimus, which predominantly inhibits mTORC1, sapanisertib may have an advantage because of its potential activity of alpelisib in brain metastatic disease.

**CURRENT CLINICAL TRIALS OF INHIBITORS OF THE PI3K PATHWAY IN HER2+ DISEASE**

Agents suppressing PI3K pathway were studied in several clinical trials in patients with HER2+ MBC (Table 2). The first-generation mTOR inhibitor, everolimus, has been tested in HER2+ disease in phase III randomized placebo controlled clinical trials BOLERO-1 and BOLERO-3. BOLERO-1 evaluated everolimus versus placebo in combination with trastuzumab and paclitaxel as a first-line therapy for HER2+ MBC. PFS did not differ between the everolimus and placebo groups. Although in the subgroup of patients with HR-/HER2+ disease PFS was 7.2 months longer on everolimus compared with placebo, it did not meet the prespecified criteria for significance. In BOLERO-3, women with HER2+ trastuzumab-resistant MBC previously treated with taxanes were randomly assigned to everolimus or placebo in combination with vinorelbine and trastuzumab. Median PFS was 7.0 months in the everolimus and 5.8 months in the placebo group (P = .0067). However, this small improvement in PFS came at the cost of increased toxicity, such as cytopenias, stomatitis, and fatigue, in the everolimus group. Serious AEs were reported in 42% of patients on everolimus and 20% of patients on placebo. Both BOLERO-1 and BOLERO-3 trials were conducted in a biomarker-unselected population of patients. These trials were not practice-changing because of two possible reasons: (1) the relatively weak activity of everolimus in suppressing the PI3K pathway, with mTORC2 mediating sustained AKT activation, and (2) the absence of a biomarker selection strategy. Subsequent exploratory analysis of the combined BOLERO-1 and BOLERO-3 trials suggested that patients with HER2+ MBC with aberrant PI3K pathway activation could derive significant PFS benefits from everolimus, whereas patients whose tumors lacked such activation do not benefit from mTOR inhibition.

Two clinical trials of buparlisib in HER2+ disease have been completed. In the phase II trial NEOFLAURA, patients with trastuzumab-resistant, PIK3CA mutation–unselected, HER2+ MBC were treated with a combination of buparlisib and lapatinib. The duration of treatment was 4-60 weeks, with a median duration of 40 weeks at maximum tolerated dose. The observed disease control rate was 79%, the clinical benefit rate (CBR) was 29%, and one patient obtained a complete response. AEs included diarrhea, nausea, rash, depression, anxiety, an increase in transaminases, and asthenia. The phase II trial PHOEBE randomly assigned HER2+ early breast cancer patients regardless of PIK3CA mutation status to receive either buparlisib or placebo plus trastuzumab in the first 6 weeks and then buparlisib or placebo with trastuzumab and paclitaxel. Although no significant differences were noted in the pCR rate between the buparlisib and placebo arms (32% vs 40%), a near-significant trend was observed in the overall response rate (68.6% vs 33%; P = .053) and a significant decrease was noted in Ki67 levels (75% vs 26.7%; P = .021) favoring buparlisib in the subgroup of patients with HR+/HER2+ tumors. Only eight of 50 enrolled subjects had PIK3CA mutations. The study planned to recruit 256 patients but suspended recruitment early because of hepatotoxicity. Both PIKHER2 and NEOFLAURA did not include PIK3CA mutations as a biomarker for selection, potentially affecting efficacy outcomes.

The results are available from a phase I study of alpelisib and T-DM1 in patients with HER2+ MBC who had progressive disease on trastuzumab and taxanes. In evaluable patients, overall response rate was 43% and CBR was 71%, with a median time on study of 7.6 months. Notably, even in patients with prior T-DM1 exposure, CBR of this combination reached 60%. This result is intriguing because T-DM1 is more active in patients with PIK3CA-mutant HER2+ breast tumors compared with other HER2-targeted agents. However, activation of the PI3K pathway may be partially responsible for acquired resistance to T-DM1, and inhibition of this pathway could induce resensitization to this agent. This early trial did not select patients on the basis of PIK3CA mutation status, although...
TABLE 2. Clinical Trials of Inhibitors of PI3K in HER2+ Breast Cancer

| Identifier (phase) | Name | Status | No. | Company/Organization |
|--------------------|------|--------|-----|----------------------|
| NCT0208178 (phase III) | Study of Alpelisib (BYL719) in Combination with Trastuzumab and Pertuzumab as Maintenance Therapy in Patients with HER2+ Advanced Breast Cancer with a PIK3CA Mutation | Recruiting | 588 | Novartis |
| NCT04108858 (phase IB/II) | Testing the Addition of an Anti-cancer Drug, Copanlisib, to the Usual Maintenance Treatment (Trastuzumab and Pertuzumab) After Initial Chemotherapy in a Phase Ib/Ii Trial for Advanced HER2+ Breast Cancer | Recruiting | 102 | National Cancer Institute |
| NCT02705859 (phase IB/II) | Phase I/II Trial of Copanlisib in Combination with Trastuzumab in HER2+ Breast Cancer (PANTHER Study) | Active, not recruiting | 26 | Cancer Trials Ireland |
| NCT03767335 (phase I) | MEN1611 with Trastuzumab (+/- Fulvestrant) in Metastatic Breast Cancer (B-PRECISE-01) | Recruiting | 48 | Menarini Group |
| NCT01589861 (phase IB/II) | Safety and Efficacy of BKM120 (Buparlisib) and Lapatinib in HER2+/PI3K-activated, Trastuzumab-resistant Advanced Breast Cancer (PIKHER2) | Completed | 106 | Institut Paoli-Calmettes |
| NCT01816594 (phase I) | NeoPHOEBE: Neoantivadant Trastuzumab + BKM120 (Buparlisib) in Combination with Weekly Paclitaxel in HER2+ Primary Breast Cancer (NeoPHOEBE) | Completed | 50 | Novartis |
| NCT02038010 (phase I) | Alpelisib (BYL719) + T-DM1 in HER2+ Metastatic Breast Cancer Patients Who Progressed on Prior Trastuzumab & Taxane Treatment | Completed | 17 | Northwestern University, National Cancer Institute |
| NCT00876395 (phase III) | Everolimus in Combination with Trastuzumab and Paclitaxel in the Treatment of HER2 Positive Locally Advanced or Metastatic Breast Cancer (BOLERO-1) | Completed | 719 | Novartis |
| NCT01007942 (phase III) | Daily Everolimus in Combination with Trastuzumab and Vinorelbine in HER2/ Neu Positive Women with Locally Advanced or Metastatic Breast Cancer (BOLERO-3) | Completed | 569 | Novartis |

NOTE. ClinicalTrials.gov accessed on November 08, 2021. No., number of patients (planned enrollment for ongoing studies and actual enrollment for completed studies).

Abbreviations: HER2, human epidermal growth factor receptor 2; PI3K, phosphatidylinositol 3-kinase.

approximately 50% of patients had tumors with PI3K pathway aberrations. Grade ≥ 3 AEs were observed in 59% of patients, and the most common events were rash, hyperglycemia, anorexia, and hypertension, but all were noted as manageable. Investigators concluded that the combination of alpelisib and T-DM1 is tolerable and has activity in patients with trastuzumab-resistant HER2+ MBC.

The results from these early trials indicate that PI3Ki will likely be essential in the treatment of HER2+ disease, and underscore the importance of a biomarker to identify patients who are most likely to benefit from addition of PI3Ki.

**CLINICAL TESTING FOR PIK3CA MUTATIONS: THE COMPANION DIAGNOSTIC VERSUS NONCOMPANION DIAGNOSTIC APPROACH**

One challenge in clinical studies of targeted agents is the development of a biomarker to identify potential responders. This biomarker should be fine-tuned to be specific, but also sensitive enough not to miss those who may derive clinical benefits. Challenges in the biomarker identification and development of a companion diagnostic (CDx) test can influence clinical fate of targeted agents.

Shortly after the 2019 FDA approval of alpelisib, the Therascreen PIK3CA RQ PCR Kit (QIAGEN Manchester Ltd, Manchester, UK) CDx test was approved for the identification of PIK3CA mutations in tumor tissue and/or plasma circulating tumor DNA. This assay (hereafter referred to as the CDx) is a real-time quantitative polymerase chain reaction (qPCR) assay that detects 11 mutations within exons 7, 9, and 20 (Table 3). These mutations can also be detected by using a variety of other platforms, including next-generation sequencing (NGS). A wide spectrum of NGS-based assays is used in clinical care, and most, if not all, cover the hotspots included in the CDx. Although the range of coverage of PIK3CA coding regions varies, nearly all NGS-based assays will detect alterations beyond those identified by the CDx. The ability of NGS-based tests to detect additional alterations raises important questions about the clinical implications of such findings.

Different groups have performed analyses to quantify the PIK3CA mutations in breast tumors that are missed by CDx testing alone. In an assessment of 5,813 breast tumors from publicly available data sets, the overall mutation rate of PIK3CA in the 763 HER2+ breast cancer specimens evaluated was 31%. This is similar to the prevalence of PIK3CA mutations previously observed in HER2+ tumors.
The mutation rate in HR+/HER2- (4,055 specimens) and HER2+ (4,055 specimens) tumors was 42% and 16%, respectively. The distribution of specific mutations across the three subtypes was similar, with five missense mutations (H1047R, H1047L, E542K, E545K, and N345K) constituting approximately 70% of all PIK3CA mutations. Specimens were obtained from patients across all clinical stages of disease and included some metastatic lesions.

A comparison of the mutations identified within these data sets with the mutations tested by CDx showed that 20% of tumors harboring PIK3CA mutations would be missed by the CDx, including approximately 25% of mutations identified in the HER2+ subtype. Notably, mutations with at least in vitro evidence of oncogenic activity were observed to occur at a higher rate than some of the variants included as part of the CDx. Corroborating these findings is an NGS-based assessment of 5,549 tumors from patients with MBC from a commercial laboratory database. Authors found more than 70 activating mutations not covered by the CDx, with 26% (626/2,435) of all activating mutations undetectable by the CDx. Moreover, in a retrospective reassessment of PIK3CA alteration status (originally determined by CDx) of tumors from SOLAR-1 participants, NGS revealed that 16% (28/175) of tumors originally designated as nonmutant did in fact harbor PIK3CA alterations. Retrospective analysis of SOLAR-1 reported 60 additional PIK3CA mutations and five copy-number alterations.

The identification of tumors with non–CDx-detectable PIK3CA mutations is of clinical importance, as non-CDx alterations may be oncogenic and may respond to alpelisib or another PI3Ki. To ascertain the spectrum of non-CDx PIK3CA mutations interrogated with alpelisib treatment to date, we performed a PubMed search of the published, English-language scientific literature through January 2021 using the terms “PIK3CA,” “alpelisib,” and “BYL719.” Preclinical and clinical studies were included regardless of tumor type. For in vitro studies using cell lines, PIK3CA mutation status was determined using the publicly available DepMap Portal data set. We initially identified 53 publications; however, studies evaluating only CDx mutations, or mutations of unknown significance were excluded. Twenty publications evaluating 37 alterations not detected by the CDx with a known or likely activating effect on the basis of OncoKB curated information annotated in the cBioPortal database were identified (Table 4, Fig 2). Alterations included additional point mutations within the C2, helical, and kinase domains, mutations within the p85 binding domain, amplifications, oncogenic deletions, and a nonstop frameshift mutation at the transcript terminus. In addition, two cell lines, two transduced cell lines, and one patient were reported as harboring a likely or known activating non-CDx mutation in combination with a known CDx-activating mutation (Table 4, Fig 2). Notably, several non-CDx mutations occur within the helical and kinase domains at or very near residues of CDx mutations (ie, E542*, E545*, Q546*, and G1049*). It is possible these mutations are detected by the CDx and misattributed. Such cross-reactivity can occur in PCR-based assays and was reported by the vendors as occurring between H1047R and H1047L in certain contexts.

With respect to current clinical testing, it is at the discretion of health care providers and patients whether a CDx or

| Exon | Region     | Mutation (c.)     | Percent of All PIK3CA Mutations in HER2+ Subtype | Frequency in HER2+ Subtype (%) | Overall Frequency in Breast Cancer (%) |
|------|------------|-------------------|------------------------------------------------|-------------------------------|---------------------------------------|
| Exon 7 | C2 domain  | C420R (1258T>C)   | < 2                                             | NA                            | 0.684                                 |
| Exon 9 | Helical domain | E542K (1624G>A)   | 10                                              | 3.1                           | 3.9                                   |
|       |            | E545K (1633G>A)   | 17                                              | 5.27                          | 6.1                                   |
|       |            | E545A (1634A>C)   | < 2                                             | NA                            | 0.18                                  |
|       |            | E545D (1635G>T)   | < 2                                             | NA                            | < 0.18                                |
|       |            | E545G (1634A>G)   | < 2                                             | NA                            | 0.18                                  |
|       |            | Q546R (1637A>G)   | < 2                                             | NA                            | 0.6                                   |
|       |            | Q546E (1636C>G)   | NA                                              | NA                            | NA                                    |
| Exon 20 | Kinase domain | H1047R (3140A>G) | 35                                              | 10.9                          | 12.5                                  |
|        |            | H1047L (3140A>T)  | 5                                               | 1.6                           | 1.4                                   |
|        |            | H1047Y (3139C>T)  | NA                                              | < 0.18                        | NA                                    |

NOTE. Data not reported by Martínez-Sáez et al, 2020. The Q546E variant was not identified in the cBioPortal data sets. It is, however, present in the Catalog of Somatic Mutations in Cancer (COSMIC) database, with three of eight documented mutations occurring in HER2+ disease.

Abbreviations: FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; NA, not available; qPCR, quantitative polymerase chain reaction.

*These mutations are part of the FDA-approved Companion Diagnostic Therascreen PIK3CA real-time qPCR panel, developed for assisting in the identification of patients with breast cancer eligible for treatment with the anti-PIK3CA, alpelisib.

Prevalence calculated from the distribution of PIK3CA mutation data provided by Martínez-Sáez et al, 2020.
| #   | Exon Domain | PIK3CA Alteration | Hotspot | Activating* | Preclinical Evidence | Clinical Evidence | Citations |
|-----|-------------|------------------|---------|-------------|---------------------|------------------|-----------|
| 1   | NA          | Amp              | NA      | Known       | ✓                   | ✓                | 74-76     |
| 2   | 1           | p85b             | R38C (c.112C>T) | Yes        | Likely             | ✓                | 79        |
| 3   | 1           | p85b             | R88Q (c.263G>A) | Yes        | Known              | ✓                | 79        |
| 4   | 1           | p85b             | R93W (c.277C>T) | Yes        | Likely             | ✓                | 79        |
| 5   | 1           | NA               | E110del (c.328_330del) | Known     |                      | ✓                | 77        |
| 6   | 1           | NA               | K111E (c.A331G) | Yes        | Known              | ✓                | 79        |
| 7   | 1           | NA               | K111N (c.G333T) | Yes        | Likely             | ✓                | 79,80-84 |
| 8   | 1           | NA               | K111R (c.332A>G) and H1047R | Yes        | Likely             | ✓                | 79        |
| 9   | 4           | C2               | V344G (c.1031T>G) | Yes        | Likely             | ✓                | 83        |
| 10  | 4           | C2               | N345K (c.1035T>G) | Yes        | Known              | ✓                | 87        |
| 11  | 5           | C2               | G364R (c.1090G>A) | Yes        | Likely             | ✓                | 88        |
| 12  | 5           | C2               | E365K (c.1093G>A) | Yes        | Likely             | ✓                | 79        |
| 13  | 5           | C2               | P366R (c.1097C>G) | Known     |                      | ✓                | 86        |
| 14  | 5           | C2               | C378F (c.1133G>T) | Yes        | Likely             | ✓                | 86        |
| 15  | 7           | C2               | P447_L455delPHGLEDL (c.1340_1366del27) | Yes        | Known              | ✓                | 89,00     |
| 16  | 7           | C2               | E453K (c.1357G>A) | Yes        | Known              | ✓                | 77,91     |
| 17  | 7           | C2               | E453Q (c.1357G>C) | Yes        | Likely             | ✓                | 86        |
| 18  | 9           | Helical          | P539R (c.1616C>G) | Yes        | Known              | ✓                | 79,80     |
| 19  | 9           | Helical          | E542A (c.1625A>C) | Yes        | Likely             | ✓                | 86        |
| 20  | 9           | Helical          | E545Q (c.1633G>C) | Yes        | Known              | ✓                | 77        |
| 21  | 9           | Helical          | Q546H (c.1638G>T) | Yes        | Likely             | ✓                | 86        |
| 22  | 9           | Helical          | Q546K (c.1636C>A) | Yes        | Known              | ✓                | 74,88     |
| 23  | 9           | Helical          | Q546P (c.1637A>C) | Yes        | Known              | ✓                | 77,91,89,02 |
| 24  | 19          | Kinase           | D939G (c.2816A>G) | Yes        | Likely             | ✓                | 89        |
| 25  | 19          | Kinase           | E970K (c.2908G>A) | Yes        | Likely             | ✓                | 77        |
| 26  | 20          | Kinase           | Y1021H (c.3061T>C) | Yes        | Likely             | ✓                | 86        |
| 27  | 20          | Kinase           | T1025A (c.3073A>G) | Yes        | Likely             | ✓                | 79        |
| 28  | 20          | Kinase           | T1025S (c.3073A>T) | Yes        | Known              | ✓                | 86        |
| 29  | 20          | Kinase           | M1043I (c.3129G>T) | Yes        | Known              | ✓                | 74,77,88 |
| 30  | 20          | Kinase           | M1043L (c.3127A>T) | Yes        | Likely             | ✓                | 77,91     |
| 31  | 20          | Kinase           | N1044K (c.3132T>G) | Yes        | Known              | ✓                | 88        |
| 32  | 20          | Kinase           | G1049R (c.3145G>C) | Yes        | Known              | ✓                | 79,80,88 |
| 33  | 20          | Kinase           | A1066V (c.3197C>T) | Likely    |                      | ✓                | 79        |
| 34  | 20          | NA               | N1068Kfs*5 (c.3203dup) | Likely    |                      | ✓                | 93        |
| 35  | 1           | NA               | K111R (c.332A>G) and H1047R | Yes        | Likely             | ✓                | 74,79     |
| 36  | 4           | C2               | D350N (c.1048G>A) and H1047R | Yes        | Likely             | ✓                | 80        |
| 37  | 7           | C2               | E453Q (c.1357G>C) and H1047R | Yes        | Likely             | ✓                | 91        |
| 38  | 9           | Helical          | P539R (c.1616C>G) and H1047R | Yes        | Known              | ✓                | 79,80,80,94 |
| 39  | 20          | Kinase           | M1043L (c.3127A>T) and E545K | Yes        | Likely             | ✓                | 91        |

**NOTE.** PIK3CA NCBI transcript NM_006218.4 used to determine position and codon. Exon numbering: to be most consistent with the published literature, exons are numbered by coding exons. The true exon 1 constitutes the 5'-UTR of the gene and has historically not been included in the exon count. As such, the exons listed above correspond to one exon higher when the noncoding exon is included (eg, coding exon 1 above corresponds to true exon 2; coding exon 20 corresponds to true exon 21).

Abbreviations: HER2, human epidermal growth factor receptor 2; NCBI, National Center for Biotechnology Information; UTR, untranslated region.

*Activating status established using the OncoKB curated information as annotated in the cBioPortal database (last accessed February 2021).

†Alteration reported to co-occur with other PIK3CA mutations of unknown significance or benign: (1) R38C and *1069W; (2) V344G and E978K; (3) P539R and I20M; (4) D939G, E78K, and E726K.

¶Transformed cells harbored engineered PIK3CA c-terminus frameshift mutations intended to recapitulate the N1068Kfs*5 mutation observed in human tumors.
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FIG 2. PIK3CA alterations tested in conjunction with alpelisib (BYL719). The 11 mutations identified by CDx testing (top portion of gene) constitute a subset of known or likely PIK3CA alterations. Alterations tested either preclinically or clinically with alpelisib (bottom portion of gene) include the amplified gene (not depicted) and span the full length of the gene. These mutations involve the p85-binding, C2, helical, and kinase domains as well as interdomain regions and the c-terminus. To date, 37 known or likely oncogenic non-CDx mutations have been tested in the context of alpelisib administration/exposure (for more information, see Table 3). Red: known activating alteration. Yellow: likely activating alteration. Orange: known and likely activating alteration reported for given amino acid position. *Mutations reported to co-occur with a known activating mutation: K111R, D350N, E453Q, and P539R co-occur with H1047R; M1043L co-occurs with E454K. P85_bd: p85 binding domain; PI3K_rbd: RAS binding domain; PI3K_C2: C2 domain; PIK_helical: helical domain; PI3_Pi4_kinase: kinase domain. Activating status determined using OncoKB curated information as annotated in the cBioPortal database (last accessed February 2021). Hotspots E545 and H1047 (marked by broken line in the lollipop) have a much higher frequency of mutations compared with other loci. Lollipop length does not correspond to relative mutation frequency. Gene structure adapted from cBioportal. CDx, companion diagnostic; PI3K, phosphatidylinositol 3-kinase.

non-CDx testing approach is optimal, as variables such as testing accessibility, insurance coverage, and pricing may influence the benefits and limitations of the two approaches. Ordering physicians should be mindful that the resulting formats of NGS-based assays are variable, and CDx and non-CDx mutations may be differentially emphasized depending on the specific mutations/disease combinations.

Data on whether specific PIK3CA mutations render differential responses to alpelisib are limited.93,98,99 Early evaluations have suggested that tumors with c-terminus H1047* mutations respond to alpelisib better compared with tumors with other PIK3CA mutations.93,99 In the SOLAR-1 study, patients whose tumors have PIK3CA mutations in the helical domain (E542*, E545*) or kinase domain (H1047*) had similar PFS benefits.98 In vitro analyses suggest that double PIK3CA-mutant tumors harbor increased sensitivity to PI3Ki including alpelisib.91 Such double PIK3CA mutations predominantly occur in the c/is-position and have been observed in 15% of HR+/HER2– and 5% of HER2+ and triple-negative tumors.91 Intriguingly, HER2+ breast tumor cell lines with and without PIK3CA mutation are more sensitive to inhibition by PI3Ki including alpelisib than cell lines without HER2 amplification.79,80 Notably, two of eight patients successfully treated for more than two years with alpelisib on SOLAR-1 trial had HER2+ disease.86

Extrapolation of the findings of any one study to infer overall clinical significance must be tempered, as response to alpelisib may vary depending on numerous clinical and genetic factors including breast cancer subtype, tumor burden, prior therapy, and the presence of concomitant mutations.74,89,100,101 Over time, a more nuanced approach is likely to emerge that incorporates other alterations known to influence the PI3K pathway such as PTEN, AKT1, and RAS aberrations.74,89,102,103 Indeed, the target- and/or pathway-specific focus can inadvertently neglect the influence of other pathways, rendering an oversimplified understanding of the clinical utility of targeted agents and their biomarkers. To this point, current preclinical data suggest combination therapy of alpelisib with other targeted inhibitors including neratinib, vandetanib, and OSI-027 (mTOR), erlotinib (epithelial growth factor receptor), erdafitinib (fibroblast growth factor receptor), AEW541 (insulin-like growth factor 1 receptor), and ribociclib (CDK4/6). Additionally, SGI-1776 and AZD-1208 (PIM kinase inhibitors) may overcome/delay resistance to alpelisib and produce additive/synergistic effects in tumor inhibition.104-113 As it is pertinent to breast cancer, alpelisib was synergistic with the CKD4/6 inhibitor ribociclib in triple-negative cell lines and PDX models,108 and showed synergy with the pan-HER TKI neratinib in inhibiting growth of HER2+ cell lines.113

In conclusion, development of a biomarker for PI3Ki is an evolving field. Currently, it is prudent to consider all non-CDx PIK3CA mutations on a case-by-case basis, recognizing that not all mutations identified by NGS assays have confirmed deleterious effects on protein function and a verified ability to respond to alpelisib. In the setting of certain PIK3CA alterations, it is biologically feasible that HER2+ disease may derive a unique benefit from alpelisib or other PI3Ki.

In conclusion, the PI3K pathway is fundamentally important for tumorigenesis of HER2+ tumors and escape from...
HER2\(\text{ii}\) that limits the survival of patients with HER2+ disease. Despite the challenges faced in the clinical testing for \(\text{PIK3CA}\) mutations and the high toxicities and abandonment of many PI3Ki, clinical development of PI3Ki and their predictive biomarkers in HER2+ breast cancer is ongoing. Effective PI3K blockade paired with a sensitive and specific biomarker of response may improve outcomes of thousands of patients with HER2+ breast cancer.

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