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review articles

PIK3CA Mutations Drive Therapeutic Resistance in Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer

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abstract

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.

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PHOSPHOINOSITIDE 3-KINASE PATHWAY AND ITS ROLE IN HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2–POSITIVE BREAST CANCER

The phosphoinositide 3-kinase (PI3K) pathway plays a key role in growth, proliferation, and survival of cancer cells.[1] PI3K transmits signals from oncogenic receptor tyrosine kinases (RTKs),[2] including human epidermal growth factor receptor 2 (HER2), platelet-derived growth factor receptor, insulin growth factor 1 receptor, and others.[3,4] Activated PI3K catalyzes phosphorylation of phosphatidylinositol diphosphate (PIP2) to phosphatidylinositol 3-phosphate (PIP3), which recruits serine-threonine kinase AKT to the membrane. Activation of AKT and mammalian target of rapamycin (mTOR) complex is a key consequence of RTK-based signaling.[5,6] AKT reduces apoptosis and promotes proliferation, epithelial-mesenchymal transition, invasion, metastases, and angiogenesis.[5] mTOR, a catalytic subunit of two protein complexes, mTORC1 and mTORC2, is the major regulator of cell growth.[7] Phosphatase and tensin homolog (PTEN) acts as a negative regulator of PI3K by dephosphorylating PIP3 to PIP2.[5,8] However, PTEN is frequently disabled in cancer cells by loss of heterozygosity, inactivating mutations, or epigenetic silencing,[5] which can augment the effects of PI3K activation (Fig 1).

Class I PI3K contains four isoforms of the catalytic subunit: p110α, p110β, p110δ, and p110γ.[9] These isoforms are subdivided according to their connections to upstream signaling. Class IA (p110α, p110β, and p110γ) can be activated by RTKs; and class IB (p110γ) by small G-proteins.[8] p110α and p110β are expressed ubiquitously, whereas p110δ and p110γ are mostly found in hematopoietic cells.[10] The p110γ isoform of PI3K regulates migration of CD4+ T lymphocytes[11] and may play a role in anticancer immunity.[12] Although p110α is the most studied in cancer, activity of any class IA PI3K isoform can sustain cell proliferation and survival.[9,13]

PI3K class IA consists of a p110 catalytic subunit and a p85 regulatory subunit. Genes *PIK3CA*, *PIK3B*, and *PIK3D* encode PI3K catalytic subunit isoforms p110α, p110β, and p110δ, respectively.[1] Notably, p110α or p110β inactivation differentially modulate RTK signaling: knockout of p110α allows all phosphosites to be occupied by p110β decreasing RTK signaling, whereas knockout of p110β leads to binding of p110α, which has a greater kinase activity and increases RTK signaling.[14,15] Out of all isoforms, p110α is particularly important in breast cancer because of its high RTK-dependent kinase activity and frequent activating mutations: mutations in p110α are seen in approximately 35% of breast tumors, compared with p110β (< 5%) and p110δ (not reported).[14]

PI3K p110α (encoded by *PIK3CA*) is critically important for the development of breast tumors overexpressing HER2. HER2 signaling is mediated almost exclusively through p110α.[15,16] *PIK3CA* knockout mice are completely resistant to HER2 transgene-mediated tumor formation,[15] whereas mice bearing

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,[22] 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 NeoSphere[23] 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 *PI3K*-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.

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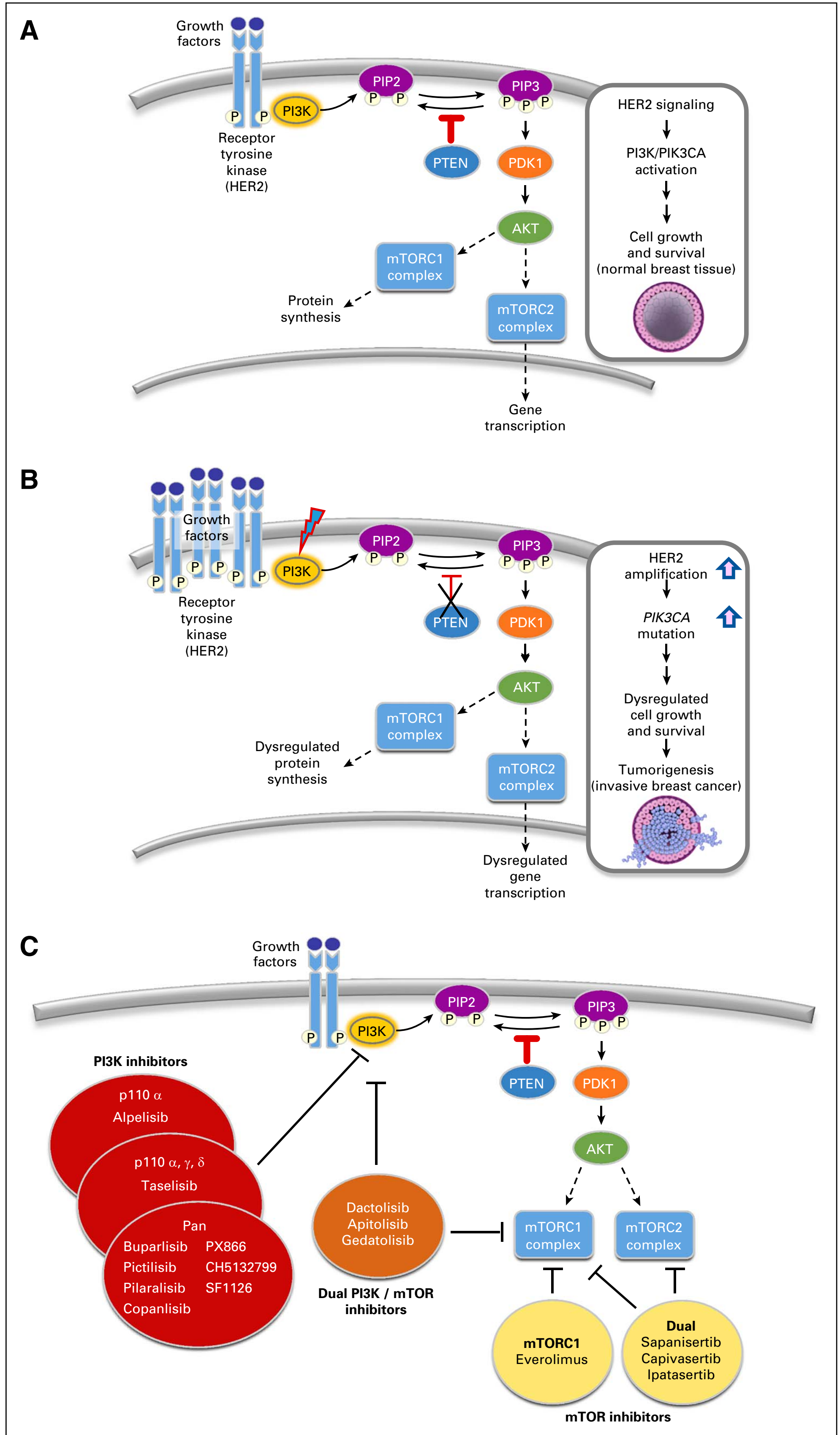

FIG 1. The PI3K pathway: normal and cancerous conditions and targeted agents. (A) Under normal conditions, the PI3K pathway contributes to the regulation of cell growth, proliferation, and survival via receptor tyrosine kinases including HER2 leading to downstream activation of AKT and mTOR complexes. (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 PI3/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[19]	32% (133 of 417)
MSKCC data set (Cancer Cell 2018)[a]	30% (54 of 185)
TCGA (Pan Cancer Atlas)[a]	29% (35 of 122)
METABRICK (Nature 2012, Nat Communication 2016)[a]	30% (103 of 342)

Abbreviation: HER2, human epidermal growth factor receptor 2.

[a]cBioPortal accessed on October 22, 2021. NeoSphere enrolled patients with early-stage HER2+ breast cancer. TCGA and METABRICK predominantly included patients with early-stage disease. MSKCC data set included patients with all disease stages.

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+[34] and hormone receptor–positive (HR+) breast cancer.[35] 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.[36]

Pan-PI3Ki suppress activity of all PI3K class I isoforms and include buparlisib,[37-39] pictilisib,[40,41] pilaralisib,[42] copanlisib,[43,44] PX866,[45,46] CH5132799,[47] and SF1126.[48] 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.[36] One of the most extensively tested pan-PI3Ki is buparlisib, which has results available from three randomized trials in HR+/HER2– MBC.[37-39] 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.[38] 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).[40] Development of pilaralisib, PX-866, and CH5132799 has been stopped because of lack of activity, whereas SF1126 is still in clinical trials.[36]

Unlike other pan-PI3Ki, copanlisib is administered intravenously and has a better therapeutic index,[36] with hyperglycemia and nausea being the most frequent yet manageable AEs.[43] The drug demonstrated low incidence of high-grade GI and liver toxicity.[49] Copanlisib showed activity in patients with hematologic malignancies, breast cancer, and endometrial cancer.[43] 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.[49] Copanlisib is now being studied in solid tumors and hematologic malignancies in combination with chemotherapy, antihormonal therapy, targeted agents, or immunotherapy.[36]

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.[50-52] 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 administration and is better tolerated. Although studies of gedatolisib in endometrial cancer (NCT01420081)[53] and hematologic malignancies (NCT02438761) were terminated because of low activity, studies evaluating combinations of gedatolisib with antihormonal drugs and targeted inhibitors of HER2, poly(ADP-ribose) polymerase, and CDK4/6 are ongoing in breast cancer (NCT03911973, NCT02626507, NCT03698383) as well as lung, pancreatic, and head and neck cancers (NCT03065062).

The first selective inhibitor of PI3K (taselisib) spared the p110β subunit, while inhibiting p110α, γ, and δ. From a signaling standpoint, this approach could alleviate the severity of some AEs without sacrificing antitumor efficacy. However, a phase III clinical trial of taselisib (SANDPIPER)

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showed only modest activity and a challenging safety profile (diarrhea, nausea, vomiting, abdominal pain, stomatitis, fatigue, hyperglycemia, and rash),[54] 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.[55] 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.[55] 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.[56]

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,[57] 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 combined inhibition of mTORC1 and mTORC2.[58] mTORC2 directly phosphorylates AKT, and this escape pathway is suppressed by sapanisertib.[58]

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[59] and BOLERO-3.[60] 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.[59] In BOLERO-3, women with HER2+ transtuzumab-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.[60] 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.[61]

Two clinical trials of buparlisib in HER2+ disease have been completed. In the phase IB PIKHER2 study, 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.[62] The phase II trial NeoPHOEBE 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.[63] Although no significant differences were noted in the pCR rate between the buparlisib and placebo arms (32% *v* 40%), a near-significant trend was observed in the overall response rate (68.6% *v* 33%; P = .053) and a significant decrease was noted in Ki67 levels (75% *v* 26.7%; P = .021) favoring buparlisib in the subgroup of patients with HR+/HER2+ tumors.[63] Only eight of 50 enrolled subjects had *PIK3CA* mutations.[63] The study planned to recruit 256 patients but suspended recruitment early because of hepatotoxicity. Both PIKHER2 and NeoPHOEBE 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.[64] Notably, even in patients with prior T-DM1 exposure, CBR of this combination reached 60%.[65] This result is intriguing because T-DM1 is more active in patients with *PIK3CA*-mutant HER2+ breast tumors compared with other HER2-targeted agents.[22,32] However, activation of the PI3K pathway may be partially responsible for acquired resistance to T-DM1,[66] 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
NCT04208178 (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 Ib/II Trial of Copanlisib in Combination with Trastuzumab in HER2+ Breast Cancer (PANTHER Study)	Active, not recruiting	26	Cancer Trials Ireland
NCT03767335 (phase IB)	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 II)	NeoPHOEBE: Neoadjuvant 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.[1,65] 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.[64,65] Investigators concluded that the combination of alpelisib and T-DM1 is tolerable and has activity in patients with trastuzumab-resistant HER2+ MBC.[64,65]

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* RGQ 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.[67,68] 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%.[69] This is similar to the prevalence of *PIK3CA* mutations previously observed in HER2+ tumors

TABLE 3. *PIK3CA* Mutations Currently Detected as Part of FDA-Approved Companion Diagnostic Testing in Breast Cancer[a]

Exon	Region	Mutation (c.)	Percent of All *PIK3CA* Mutations in HER2+ Subtype	Frequency in HER2+ Subtype (%)[b]	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.[69] 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.

[a]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.

[b]Prevalence calculated from the distribution of PIK3CA mutation data provided by Martínez-Sáez et al, 2020.[69]

assessed for hotspot mutations within exons 7, 9, and 20.[23] The mutation rate in HR+/HER2– (4,055 specimens) and triple-negative (995 specimens) tumors was 42% and 16%, respectively.[69] 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.[69] Specimens were obtained from patients across all clinical stages of disease and included some metastatic lesions.[69-71] 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.[69] 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.[69] Corroborating these findings is an NGS-based assessment of 5,549 tumors from patients with MBC from a commercial laboratory database.[67] 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.[67] 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.[68] Retrospective analysis of SOLAR-1 reported 60 additional *PIK3CA* mutations and five copy-number alterations.[68]

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.[72] 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.[73] 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).[94-96] 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 (Table 4, Fig 2). 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.[97]

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

TABLE 4. Noncompanion Diagnostic Alterations With Known or Likely Oncogenicity Tested With Alpelisib

#	Exon	Domain	*PIK3CA* Alteration	Hotspot	Activating[a]	Preclinical Evidence	Clinical Evidence	Citations
1	NA	NA	Amp	NA	Known	✓	✓	74-78
2	1	p85b	R38C (c.112C>T)[b]	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	✓		74,80-84
8	1	NA	P124L (c.371C>T)		Likely	✓		83
9	4	C2	V344G (c.1031T>G)[b]	Yes	Likely	✓		85
10	4	C2	N345K (c.1035T>G)	Yes	Known	✓	✓	77,80,86
11	4	C2	N345T (c.1034A>C)	Yes	Likely		✓	87
12	5	C2	G364R (c.1090G>A)	Yes	Likely		✓	88
13	5	C2	E365K (c.1093G>A)	Yes	Likely	✓		79
14	5	C2	P366R (c.1097C>G)		Known		✓	86
15	5	C2	C378F (c.1133G>T)	Yes	Likely		✓	86
16	7	C2	P447_L455delPHGLEDL (c.1340_1366del27)	Yes	Known		✓	89,90
17	7	C2	E453K (c.1357G>A)	Yes	Known		✓	77
18	7	C2	E453Q (c.1357G>C)	Yes	Likely	✓	✓	77,91
19	9	Helical	P539R (c.1616C>G)[b]	Yes	Known	✓		79,85
20	9	Helical	E542A (c.1625A>C)	Yes	Likely		✓	86
21	9	Helical	E545Q (c.1633G>C)	Yes	Known		✓	77
22	9	Helical	Q546H (c.1638G>T)	Yes	Likely		✓	88
23	9	Helical	Q546K (c.1636C>A)	Yes	Known	✓	✓	74,88
24	9	Helical	Q546P (c.1637A>C)	Yes	Known	✓	✓	77,86,89,92
25	19	Kinase	D939G (c.2816A>G)[b]	Yes	Likely		✓	89
26	19	Kinase	E970K (c.2908G>A)	Yes	Likely		✓	77
27	20	Kinase	Y1021H (c.3061T>C)	Yes	Likely		✓	86
28	20	Kinase	T1025A (c.3073A>G)	Yes	Likely	✓		79
29	20	Kinase	T1025S (c.3073A>T)	Yes	Known		✓	86
30	20	Kinase	M1043I (c.3129G>T)	Yes	Known	✓	✓	74,77,88
31	20	Kinase	M1043L (c.3127A>T)	Yes	Likely	✓	✓	77,91
32	20	Kinase	N1044K (c.3132T>G)	Yes	Known		✓	88
33	20	Kinase	G1049R (c.3145G>C)	Yes	Known	✓	✓	79,80,88
34	20	NA	A1066V (c.3197C>T)		Likely	✓		79
35	20	NA	N1068Kfs*5 (c.3203dup)[c]		Likely	✓		93
36	1	NA	K111R (c.332A>G) and H1047R	Yes	Likely	✓		74,79
37	4	C2	D350N (c.1048G>A) and H1047R	Yes	Likely	✓		80
38	7	C2	E453Q (c.1357G>C) and H1047R	Yes	Likely	✓		91
39	9	Helical	P539R (c.1616C>G) and H1047R	Yes	Known	✓		79,80,82,84
40	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.

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

[b]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.

[c]Transformed cells harbored engineered *PIK3CA* c-terminus frameshift mutations intended to recapitulate the N1068Kfs*5 mutation observed in human tumors.

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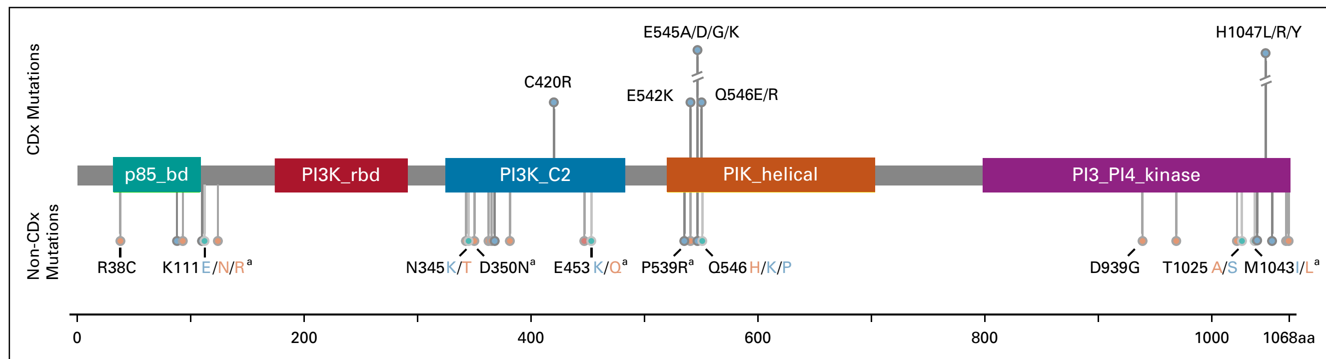

FIG 2. PIK3CA alterations tested in conjunction with alpelisib (BYL719). The 11 mutations identified by CDx testing (top portion of gene) constitute a subset of known or likely *PIK3CA* alterations. Alterations tested either preclinically or clinically with alpelisib (bottom portion of gene) include 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. [a]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.[94,95] 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.[89,98,99] Early evaluations have suggested that tumors with c-terminus H1047* mutations respond to alpelisib better compared with tumors with other *PIK3CA* mutations.[89,99] 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 *cis*-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, vistusertib, 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 summary, 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

HER2i that limits the survival of patients with HER2+ disease. Despite the challenges faced in the clinical testing for *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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REFERENCES

1. Verret B, Cortes J, Bachelot T, et al: Efficacy of PI3K inhibitors in advanced breast cancer. Ann Oncol 30:x12-x20, 2019
2. Paplomata E, O'Regan R: The PI3K/AKT/mTOR pathway in breast cancer: Targets, trials and biomarkers. Ther Adv Med Oncol 6:154-166, 2014
3. Hu P, Margolis B, Skolnik EY, et al: Interaction of phosphatidylinositol 3-kinase-associated p85 with epidermal growth factor and platelet-derived growth factor receptors. Mol Cell Biol 12:981-990, 1992
4. Klippel A, Escobedo JA, Fantl WJ, et al: The C-terminal SH2 domain of p85 accounts for the high affinity and specificity of the binding of phosphatidylinositol 3-kinase to phosphorylated platelet-derived growth factor beta receptor. Mol Cell Biol 12:1451-1459, 1992
5. Chalhoub N, Baker SJ: PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol 4:127-150, 2009
6. Mora A, Komander D, van Aalten DM, et al: PDK, the master regulator of AGC kinase signal transduction. Semin Cell Dev Biol 15:161-170, 2004
7. Sabatini DM: Twenty-five years of mTOR: Uncovering the link from nutrients to growth. Proc Natl Acad Sci USA 114:11818-11825, 2017
8. Engelman JA, Luo J, Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606-619, 2006
9. Denley A, Kang S, Karst U, et al: Oncogenic signaling of class I PI3K isoforms. Oncogene 27:2561-2574, 2008
10. Vanhaesebroeck B, Leevers SJ, Panayotou G, et al: Phosphoinositide 3-kinases: A conserved family of signal transducers. Trends Biochem Sci 22:267-272, 1997

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11. Thomas MS, Mitchell JS, DeNucci CC, et al: The p110gamma isoform of phosphatidylinositol 3-kinase regulates migration of effector CD4 T lymphocytes into peripheral inflammatory sites. J Leukoc Biol 84:814-823, 2008
12. Anderson K, Ryan N, Alkhimovitch A, et al: Inhibition of PI3K isoform p110γ increases both anti-tumor and immunosuppressive responses to aggressive murine head and neck squamous cell carcinoma with low immunogenicity. Cancers (Basel) 13:953, 2021
13. Foukas LC, Berenjeno IM, Gray A, et al: Activity of any class IA PI3K isoform can sustain cell proliferation and survival. Proc Natl Acad Sci USA 107:11381-11386, 2010
14. Thorpe LM, Yuzugullu H, Zhao JJ: PI3K in cancer: Divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 15:7-24, 2015
15. Utermark T, Rao T, Cheng H, et al: The p110alpha and p110beta isoforms of PI3K play divergent roles in mammary gland development and tumorigenesis. Genes Dev 26:1573-1586, 2012
16. Wang Q, Liu P, Spangle JM, et al: PI3K-p110α mediates resistance to HER2-targeted therapy in HER2+, PTEN-deficient breast cancers. Oncogene 35:3607-3612, 2016
17. Hanker AB, Pfefferle AD, Balko JM, et al: Mutant *PIK3CA* accelerates HER2-driven transgenic mammary tumors and induces resistance to combinations of anti-HER2 therapies. Proc Natl Acad Sci USA 110:14372-14377, 2013
18. Gianni L, Pienkowski T, Im YH, et al: 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): A multicentre, open-label, phase 2 randomised trial. Lancet Oncol 17:791-800, 2016
19. Gianni L, Pienkowski T, Im YH, et al: Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): A randomised multicentre, open-label, phase 2 trial. Lancet Oncol 13:25-32, 2012
20. Symmans WF, Wei C, Gould R, et al: Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol 35:1049-1060, 2017
21. Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395-402, 2007
22. Baselga J, Lewis Phillips GD, Verma S, et al: Relationship between tumor biomarkers and efficacy in EMILIA, a phase III study of trastuzumab emtansine in HER2-positive metastatic breast cancer. Clin Cancer Res 22:3755-3763, 2016
23. Bianchini G, Kiermaier A, Bianchi GV, et al: Biomarker analysis of the neosphere study: Pertuzumab, trastuzumab, and docetaxel versus trastuzumab plus docetaxel, pertuzumab plus trastuzumab, or pertuzumab plus docetaxel for the neoadjuvant treatment of HER2-positive breast cancer. Breast Cancer Res 19:16, 2017
24. Goel S, Krop IE: PIK3CA mutations in HER2-positive breast cancer: An ongoing conundrum. Ann Oncol 27:1368-1372, 2016
25. Seo Y, Park YH, Ahn JS, et al: *PIK3CA* mutations and neoadjuvant therapy outcome in patients with human epidermal growth factor receptor 2-positive breast cancer: A sequential analysis. J Breast Cancer 21:382-390, 2018
26. Baselga J, Cortes J, Im SA, et al: Biomarker analyses in CLEOPATRA: A phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 32:3753-3761, 2014
27. Majewski IJ, Nuciforo P, Mittempergher L, et al: *PIK3CA* mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies in breast cancer. J Clin Oncol 33:1334-1339, 2015
28. Loibl S, von Minckwitz G, Schneeweiss A, et al: *PIK3CA* mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (HER2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 32:3212-3220, 2014
29. Krop IE, Paulson J, Campbell C, et al: Genomic correlates of response to adjuvant trastuzumab (H) and pertuzumab (P) in HER2+ breast cancer (BC): Biomarker analysis of the APHINITY trial. J Clin Oncol 37, 2019 (suppl; abstr 1012)
30. Chia SKL, Martin M, Holmes FA, et al: *PIK3CA* alterations and benefit with neratinib: Analysis from the randomized, double-blind, placebo-controlled, phase III extenet trial. Breast Cancer Res 21:39, 2019
31. Denkert C, Lambertini C, Fasching PA, et al: Biomarker data from KATHERINE: A phase III study of adjuvant trastuzumab emtansine (T-DM1) versus trastuzumab (H) in patients with residual invasive disease after neoadjuvant therapy for HER2-positive breast cancer. J Clin Oncol 38, 2020 (suppl; abstr 502)
32. Kim SB, Wildiers H, Krop IE, et al: Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) vs. treatment of physician's choice in previously treated HER2-positive advanced breast cancer. Int J Cancer 139:2336-2342, 2016
33. Loibl S, Majewski I, Guarneri V, et al: *PIK3CA* mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: Pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. Ann Oncol 27:1519-1525, 2016
34. Wolff AC, Hammond MEH, Allison KH, et al: Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. J Clin Oncol 36:2105-2122, 2018
35. Allison KH, Hammond MEH, Dowsett M, et al: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 38:1346-1366, 2020
36. Janku F, Yap TA, Meric-Bernstam F: Targeting the PI3K pathway in cancer: Are we making headway? Nat Rev Clin Oncol 15:273-291, 2018
37. 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 18:904-916, 2017
38. 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 19:87-100, 2018
39. Martín M, Chan A, Dirix L, et al: A randomized adaptive phase II/III study of buparlisib, a pan-class i PI3K inhibitor, combined with paclitaxel for the treatment of HER2- advanced breast cancer (belle-4). Ann Oncol 28:313-320, 2017
40. 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 17:811-821, 2016
41. Vuylsteke P, Huizing M, Petrakova K, et al: Pictilisib PI3Kinase inhibitor (a phosphatidylinositol 3-kinase [PI3K] inhibitor) plus paclitaxel for the treatment of hormone receptor-positive, HER2-negative, locally recurrent, or metastatic breast cancer: Interim analysis of the multicentre, placebo-controlled, phase II randomised PEGGY study. Ann Oncol 27:2059-2066, 2016
42. Abramson VG, Supko JG, Ballinger T, et al: Phase Ib study of safety and pharmacokinetics of the PI3K inhibitor SAR245408 with the HER3-neutralizing human antibody SAR256212 in patients with solid tumors. Clin Cancer Res 23:3520-3528, 2017
43. Patnaik A, Appleman LJ, Tolcher AW, et al: First-in-human phase I study of copanlisib (BAY 80-6946), an intravenous pan-class I phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors and non-Hodgkin's lymphomas. Ann Oncol 27:1928-1940, 2016
44. Dreyling M, Santoro A, Mollica L, et al: Phosphatidylinositol 3-kinase inhibition by copanlisib in relapsed or refractory indolent lymphoma. J Clin Oncol 35:3898-3905, 2017

45. Bowles DW, Ma WW, Senzer N, et al: A multicenter phase 1 study of PX-866 in combination with docetaxel in patients with advanced solid tumours. Br J Cancer 109:1085-1092, 2013
46. Jimeno A, Bauman JE, Weissman C, et al: A randomized, phase 2 trial of docetaxel with or without PX-866, an irreversible oral phosphatidylinositol 3-kinase inhibitor, in patients with relapsed or metastatic head and neck squamous cell cancer. Oral Oncol 51:383-388, 2015
47. Blagden S, Omlin A, Josephs D, et al: First-in-human study of CH5132799, an oral class I PI3K inhibitor, studying toxicity, pharmacokinetics, and pharmacodynamics, in patients with metastatic cancer. Clin Cancer Res 20:5908-5917, 2014
48. Mahadevan D, Chiorean EG, Harris WB, et al: Phase I pharmacokinetic and pharmacodynamic study of the pan-PI3K/mTORC vascular targeted pro-drug SF1126 in patients with advanced solid tumours and B-cell malignancies. Eur J Cancer 48:3319-3327, 2012
49. Dreyling M, Santoro A, Mollica L, et al: Long-term safety and efficacy of the PI3K inhibitor copanlisib in patients with relapsed or refractory indolent lymphoma: 2-year follow-up of the CHRONOS-1 study. Am J Hematol 95:362-371, 2020
50. Tarantelli C, Lupia A, Stathis A, et al: Is there a role for dual PI3K/mTOR inhibitors for patients affected with lymphoma? Int J Mol Sci 21:1060, 2020
51. Fazio N, Buzzoni R, Baudin E, et al: A phase II study of BEZ235 in patients with everolimus-resistant, advanced pancreatic neuroendocrine tumours. Anticancer Res 36:713-719, 2016
52. Powles T, Lackner MR, Oudard S, et al: Randomized open-label phase II trial of apitolisib (GDC-0980), a novel inhibitor of the PI3K/mammalian target of rapamycin pathway, versus everolimus in patients with metastatic renal cell carcinoma. J Clin Oncol 34:1660-1668, 2016
53. Del Campo JM, Birrer M, Davis C, et al: A randomized phase II non-comparative study of PF-04691502 and gedatolisib (PF-05212384) in patients with recurrent endometrial cancer. Gynecol Oncol 142:62-69, 2016
54. Dent S, Cortés J, Im YH, et al: Phase III randomized study of taselisib or placebo with fulvestrant in estrogen receptor-positive, *PIK3CA*-mutant, HER2-negative, advanced breast cancer: The SANDPIPER trial. Ann Oncol 32:197-207, 2021
55. André F, Ciruelos E, Rubovszky G, et al: Alpelisib for *PIK3CA*-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 380:1929-1940, 2019
56. Batalini F, Moulder SL, Winer EP, et al: Response of brain metastases from *PIK3CA*-mutant breast cancer to alpelisib. JCO Precis Oncol 4:572-578, 2020
57. Baselga J, Campone M, Piccart M, et al: Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366:520-529, 2012
58. Wander SA, Hennessy BT, Slingerland JM: Next-generation mTOR inhibitors in clinical oncology: How pathway complexity informs therapeutic strategy. J Clin Invest 121:1231-1241, 2011
59. Hurvitz SA, Andre F, Jiang Z, et al: Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1): A phase 3, randomised, double-blind, multicentre trial. Lancet Oncol 16:816-829, 2015
60. André F, O'Regan R, Ozguroglu M, et al: Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): A randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 15:580-591, 2014
61. Errata. J Clin Oncol 37:357, 2019
62. Guerin M, Rezai K, Isambert N, et al: PIKHER2: A phase Ib study evaluating buparlisib in combination with lapatinib in trastuzumab-resistant HER2-positive advanced breast cancer. Eur J Cancer 86:28-36, 2017
63. Loibl S, de la Pena L, Nekljudova V, et al: Neoadjuvant buparlisib plus trastuzumab and paclitaxel for women with HER2+ primary breast cancer: A randomised, double-blind, placebo-controlled phase II trial (NeoPHOEBE). Eur J Cancer 85:133-145, 2017
64. Jain S, Shah AN, Santa-Maria CA, et al: Phase I study of alpelisib (BYL-719) and trastuzumab emtansine (T-DM1) in HER2-positive metastatic breast cancer (MBC) after trastuzumab and taxane therapy. Breast Cancer Res Treat 171:371-381, 2018
65. Jain S, Santa-Maria CA, Rademaker A, et al: Phase I study of alpelisib (BYL-719) and T-DM1 in HER2-positive metastatic breast cancer after trastuzumab and taxane therapy. J Clin Oncol 35, 2017 (suppl; abstr 1026)
66. Hunter FW, Barker HR, Lipert B, et al: Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. Br J Cancer 122:603-612, 2020
67. Kiedrowski LA, Juric D, Hardin AI, et al: *PIK3CA* mutational analysis using cell-free DNA next-generation sequencing detects activating mutations that may be missed with targeted hot-spot testing. Cancer Res 80, 2020 (abstr P4-10-15)
68. Rugo HS, Mayer I, Conte P, et al: Prevalence of *PIK3CA* mutations in patients with hormone receptor-positive, human epidermal growth factor-2-negative advanced breast cancer from the SOLAR-1 trial. Cancer Res 79, 2019 (abstr CT142)
69. Martínez-Sáez O, Chic N, Pascual T, et al: Frequency and spectrum of *PIK3CA* somatic mutations in breast cancer. Breast Cancer Res 22:45, 2020
70. Razavi P, Chang MT, Xu G, et al: The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 34:427-438.e426, 2018
71. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490:61-70, 2012
72. Ng PK, Li J, Jeong KJ, et al: Systematic functional annotation of somatic mutations in cancer. Cancer Cell 33:450-462.e410, 2018
73. Broad Institute: Explore the Cancer Dependency Map. https://depmap.org/portal/
74. Fritsch C, Huang A, Chatenay-Rivauday C, et al: Characterization of the novel and specific PI3Kα inhibitor NVP-BYl719 and development of the patient stratification strategy for clinical trials. Mol Cancer Ther 13:1117-1129, 2014
75. Elkabets M, Pazarentzos E, Juric D, et al: AXL mediates resistance to PI3Kα inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. Cancer Cell 27:533-546, 2015
76. Kim S, Kim TM, Kim DW, et al: Acquired resistance of met-amplified non-small cell lung cancer cells to the met inhibitor capmatinib. Cancer Res Treat 51:951-962, 2019
77. Razavi P, Dickler MN, Shah PD, et al: Alterations in *PTEN* and *ESR1* promote clinical resistance to alpelisib plus aromatase inhibitors. Nat Cancer 1:382-393, 2020
78. Toppmeyer DL, Press MF: Testing considerations for phosphatidylinositol-3-kinase catalytic subunit alpha as an emerging biomarker in advanced breast cancer. Cancer Med 9:6463-6472, 2020
79. Costa C, Ebi H, Martini M, et al: Measurement of PIP3 levels reveals an unexpected role for p110β in early adaptive responses to p110α-specific inhibitors in luminal breast cancer. Cancer Cell 27:97-108, 2015
80. Elkabets M, Vora S, Juric D, et al: mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in *PIK3CA*-mutant breast cancer. Sci Transl Med 5:196ra199, 2013
81. Garrett JT, Sutton CR, Kurupi R, et al: Combination of antibody that inhibits ligand-independent HER3 dimerization and a p110α inhibitor potently blocks PI3K signaling and growth of HER2+ breast cancers. Cancer Res 73:6013-6023, 2013
82. Le X, Antony R, Razavi P, et al: Systematic functional characterization of resistance to PI3K inhibition in breast cancer. Cancer Discov 6:1134-1147, 2016

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83. Vora SR, Juric D, Kim N, et al: CDK 4/6 inhibitors sensitize *PIK3CA* mutant breast cancer to PI3K inhibitors. Cancer Cell 26:136-149, 2014
84. Xu YC, Wang X, Chen Y, et al: Integration of receptor tyrosine kinases determines sensitivity to PI3Kα-selective inhibitors in breast cancer. Theranostics 7:974-986, 2017
85. Packer LM, Geng X, Bonazzi VF, et al: PI3K inhibitors synergize with FGFR inhibitors to enhance antitumor responses in FGFR2^{mutant} endometrial cancers. Mol Cancer Ther 16:637-648, 2017
86. Juric D, Janku F, Rodón J, et al: Alpelisib plus fulvestrant in *PIK3CA*-altered and *PIK3CA*-wild-type estrogen receptor-positive advanced breast cancer: A phase 1b clinical trial. JAMA Oncol 5:e184475, 2019
87. Konstantinopoulos PA, Barry WT, Birrer M, et al: Olaparib and α-specific PI3K inhibitor alpelisib for patients with epithelial ovarian cancer: A dose-escalation and dose-expansion phase 1b trial. Lancet Oncol 20:570-580, 2019
88. 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 36:1291-1299, 2018
89. 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 23:26-34, 2017
90. Croessmann S, Sheehan JH, Lee KM, et al: *PIK3CA* C2 domain deletions hyperactivate phosphoinositide 3-kinase (PI3K), generate oncogene dependence, and are exquisitely sensitive to PI3Kα inhibitors. Clin Cancer Res 24:1426-1435, 2018
91. Vasan N, Razavi P, Johnson JL, et al: Double *PIK3CA* mutations in cis increase oncogenicity and sensitivity to PI3Kα inhibitors. Science 366:714-723, 2019
92. Huijberts S, Boelens MC, Bernards R, et al: Mutational profiles associated with resistance in patients with BRAFV600E mutant colorectal cancer treated with cetuximab and encorafenib +/- binimetinib or alpelisib. Br J Cancer 124:176-182, 2021
93. Spangle JM, Von T, Pavlick DC, et al: PIK3CA C-terminal frameshift mutations are novel oncogenic events that sensitize tumors to PI3K-α inhibition. Proc Natl Acad Sci USA 117:24427-24433, 2020
94. Cerami E, Gao J, Dogrusoz U, et al: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401-404, 2012
95. Gao J, Aksoy BA, Dogrusoz U, et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6:pl1, 2013
96. Chakravarty D, Gao J, Phillips SM, et al: OncoKB: A precision oncology knowledge base. JCO Precis Oncol 1:1-16, 2017
97. US Food and Drug Administration: The *therascreen* PIK3CA RGQ PCR Kit—P190001 and P190004. Summary of Safety and Effectiveness Data (SSED), 2019
98. André F, Ciruelos EM, Juric D, et al: Alpelisib plus fulvestrant for *PIK3CA*-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: Final overall survival results from SOLAR-1. Ann Oncol 32:208-217, 2021
99. Janku F, Wheler JJ, Naing A, et al: *PIK3CA* mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res 73:276-284, 2013
100. Janku F, Wheler JJ, Westin SN, et al: PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring *PIK3CA* mutations. J Clin Oncol 30:777-782, 2012
101. Avivar-Valderas A, McEwen R, Taheri-Ghahfarokhi A, et al: Functional significance of co-occurring mutations in *PIK3CA* and *MAP3K1* in breast cancer. Oncotarget 9:21444-21458, 2018
102. Mosele F, Stefanovska B, Lusque A, et al: Outcome and molecular landscape of patients with *PIK3CA*-mutated metastatic breast cancer. Ann Oncol 31:377-386, 2020
103. Juric D, Andre F, Singer CF, et al: Clinical outcomes of alpelisib in hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer by next-generation sequencing-detected PIK3CA alteration status and phosphatase and tensin homolog loss: Biomarker analysis from the SOLAR-1 study. Cancer Res 80, 2020 (abstr P4-10-04)
104. Wong MH, Xue A, Julovi SM, et al: Cotargeting of epidermal growth factor receptor and PI3K overcomes PI3K-AKT oncogenic dependence in pancreatic ductal adenocarcinoma. Clin Cancer Res 20:4047-4058, 2014
105. Colamonici M, Blyth G, Saleiro D, et al: Dual targeting of acute myeloid leukemia progenitors by catalytic mTOR inhibition and blockade of the p110α subunit of PI3 kinase. Oncotarget 6:8062-8070, 2015
106. Leroy C, Ramos P, Cornille K, et al: Activation of IGF1R/p110β/AKT/mTOR confers resistance to α-specific PI3K inhibition. Breast Cancer Res 18:41, 2016
107. Iqbal A, Eckerdt F, Bell J, et al: Targeting of glioblastoma cell lines and glioma stem cells by combined PIM kinase and PI3K-p110α inhibition. Oncotarget 7:33192-33201, 2016
108. Yuan Y, Wen W, Yost SE, et al: Combination therapy with BYL719 and LEE011 is synergistic and causes a greater suppression of p-s6 in triple negative breast cancer. Sci Rep 9:7509, 2019
109. Eckerdt F, Clymer J, Bell JB, et al: Pharmacological mTOR targeting enhances the antineoplastic effects of selective PI3Kα inhibition in medulloblastoma. Sci Rep 9:12822, 2019
110. Eckerdt FD, Bell JB, Gonzalez C, et al: Combined PI3Kα-mTOR targeting of glioma stem cells. Sci Rep 10:21873, 2020
111. Chuang FC, Wang CC, Chen JH, et al: PI3K inhibitors (BKM120 and BYL719) as radiosensitizers for head and neck squamous cell carcinoma during radiotherapy. PLoS One 16:e0245715, 2021
112. Holzhauser S, Lukoseviciute M, Papachristofi C, et al: Effects of PI3K and FGFR inhibitors alone and in combination, and with/without cytostatics in childhood neuroblastoma cell lines. Int J Oncol 58:211-225, 2021
113. Zhao M, Scott S, Evans KW, et al: Combining neratinib with CDK4/6, mTOR, and MEK inhibitors in models of HER2-positive cancer. Clin Cancer Res 27:1681-1694, 2021