



Activation of PI3K/AKT/mTOR Pathway Causes Drug Resistance in Breast Cancer

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Although chemotherapy, targeted therapy and endocrine therapy decrease rate of disease recurrence in most breast cancer patients, many patients exhibit acquired resistance. Hyperactivation of the PI3K/AKT/mTOR pathway is associated with drug resistance and cancer progression. Currently, a number of drugs targeting PI3K/AKT/mTOR are being investigated in clinical trials by combining them with standard therapies to overcome acquired resistance in breast cancer. In this review, we summarize the critical role of the PI3K/AKT/mTOR pathway in drug resistance, the development of PI3K/AKT/mTOR inhibitors, and strategies to overcome acquired resistance to standard therapies in breast cancer.

Keywords: chemotherapy, targeted therapy, endocrine therapy, drug resistance, PI3K

INTRODUCTION

Breast cancer is the leading cause of cancer death in women around the world (Siegel and Miller, 2020). At the molecular level, breast cancer is a heterogeneous disease, divided into hormone/estrogen-receptor-positive (HR+/ER+), human epidermal growth factor receptor-2-positive (HER2+) and ER/PR/HER2 triple-negative breast cancer (TNBC) with corresponding treatment strategies according to molecular subtypes (Nagini, 2017). Common treatments include endocrine therapy (ET) for HR+ disease, HER2 targeted therapy for HER2+ disease, chemotherapy, and immunotherapy for TNBC patients as well as PARP inhibitors for BRCA-mutated TNBC patients. Acquired resistance leads to tumor relapse in breast cancer, which is associated with multiple but relatively independent mechanisms, including overexpression of breast cancer resistance protein (BCRP, also called ABCG2), modification of cell cycle checkpoints, inhibition of apoptosis, and activation of multiple signaling pathways (Kartal-Yandim et al., 2016).

The PI3K/AKT/mTOR pathway has emerged as a novel target for overcoming drug resistance in recent years (Keegan et al., 2018; Verret et al., 2019). Dysregulation of this pathway is closely related to tumor progression and resistance to standard therapies in breast cancer (Guerrero-Zotano et al., 2016). The PI3K/AKT/mTOR pathway is one of the most frequently activated pathways in several types of cancers (Alzahrani, 2019). This is also one of the most important reasons for intrinsic resistance. Several drugs against the PI3K/AKT/mTOR pathway are in clinical development. In this review, we summarize the current knowledge of the PI3K/AKT/mTOR pathway related to drug resistance in breast cancer and propose an effective drug development strategy.

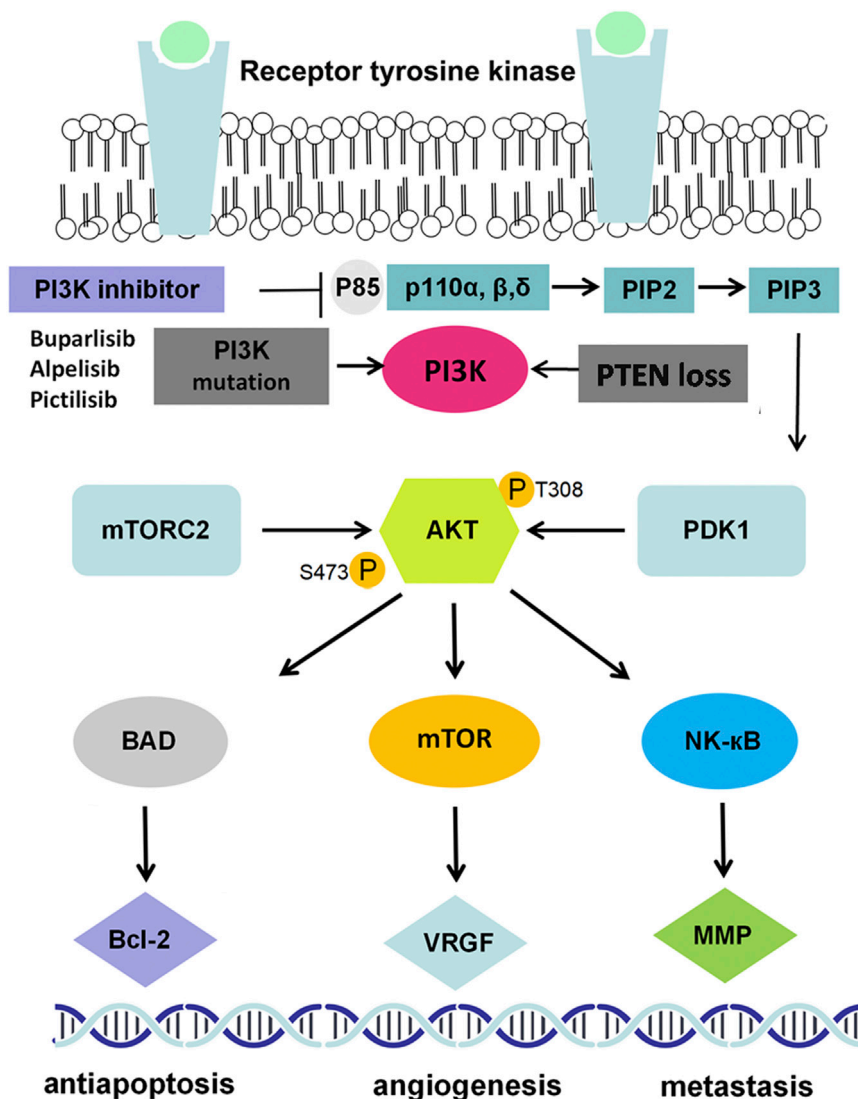


FIGURE 1 | PI3K/AKT signaling pathway in breast cancer. EGF(R): epidermal growth factor (receptor); PI3K: phosphatidylinositol 3-kinase; PDK1/2: 3-phosphoinositide dependent kinase-1/2; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor kappa-B; MMP9: matrix metallo protein 9; VEGF: vascular endothelial growth factor.

PI3K/AKT/MTOR PATHWAY AND THEIR INHIBITORS

PI3K/AKT/mTOR Pathway

The PIK3CA gene is one of the most frequently mutated genes in breast cancer (Mukohara, 2015), which leads to PI3K activation and serves as a key determinant of standard anticancer therapy resistance (Liu, et al., 2019; Luo, et al., 2018; Qiu, et al., 2019). The PI3K protein belongs to a large family of lipid kinases, classified into three classes on the basis of different structures and functions. Among them, class I PI3Ks, the most fully studied heterodimers, consist of a p110 catalytic subunit and a p85 regulatory subunit, which play a predominant role in many cancers (Dornan and Burke, 2018). The PI3K protein has the

dual activities of serine/threonine (Ser/Thr) kinase and phosphatidylinositol kinase and is activated in two ways: by interacting with various growth factor receptors, such as epithelial growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR), and by recruiting an adaptor protein to promote the binding of p110 and p85 to activate PI3K. Activated PI3K can convert phosphatidylinositol 3,4-bisphosphate (PIP₂) into 3,4,5-triphosphate (PIP₃), PIP₃ serves as the second messenger which can bind to phosphoinositide-dependent kinase-1 (PDK1) to phosphorylate AKT at Thr308. AKT can also be phosphorylated by mTORC2 at Ser473 (Figure 1). AKT is the key signal transduction protein that phosphorylates several substrates and downstream effectors,

TABLE 1 | Summary clinical RCT trials of PI3K/AKT/mTOR inhibitors in breast cancer.

Drug	Target	Study	Phase	Patients population	Therapy line	Regimen	Outcome
Buparlisib (BKM120), oral	Class I PI3K-	BELLE-2 (NCT01610284)	III	Postmenopausal, HR(+)/HER2(-), AI-resistant locally advanced or mBC	Second-line or later	Buparlisib + fulvestrant Placebo + fulvestrant	mPFS: 6.9 vs. 5.0 m (HR 0.78, $p = 0.00021$) mPFS: 6.8 vs. 4.0 m (HR 0.76, $p = 0.014$) in PIK3CA-mutant subset mOS: 33.2 vs. 30.4 m (HR 0.87, $p = 0.045$)
							mPFS: 3.9 vs. 1.8 m (HR 0.67, $p = 0.0003$)
	p110 α	BELLE-3 (NCT01633060)	III	Postmenopausal, HR(+)/HER2(-), mTOR inhibitor-resistant, locally advanced or mBC	Second-line or later	Buparlisib + fulvestrant Placebo + fulvestrant	mPFS: 3.9 vs. 1.8 m (HR 0.67, $p = 0.0003$)
	p110 δ						
Pictilisib (GDC-0941), oral	Class I PI3K-p110 α	NeoPHOEBE (NCT01816594)	II	HER2(+) primary BC	Neoadjuvant	Buparlisib + trastuzumab + paclitaxel Placebo + trastuzumab + paclitaxel	ORR: 69 vs. 33% ($p = 0.053$) pCR: 32 vs. 40% ($p = 0.811$)
		BELLE-4 (NCT01572727)	II/III	HER2(-) primary with locally advanced or mBC	First-line	Buparlisib + paclitaxel Placebo + paclitaxel	mPFS: 8.0 vs. 9.2 m (HR 1.18) mPFS: 9.1 vs. 9.2 m (HR 1.17) in PIK3CA-mutant subset
Pictilisib (GDC-0941), oral	Class I PI3K-p110 α	PEGGY (NCT01740336)	II	HR(+)/HER2(-) locally advanced or mBC	Second-line or later	Pictilisib + paclitaxel Placebo + paclitaxel	mPFS: 8.2 vs. 7.8 m (HR 0.95, $p = 0.83$) mPFS: 7.3 vs. 5.8 m (HR 1.06, $p = 0.88$) in PIK3CA-mutant subset
		FERGI (NCT01437566)	II	Postmenopausal, ER (+)/HER2(-), AI-resistant advanced or mBC	Second-line or later	Pictilisib + fulvestrant Placebo + fulvestrant	mPFS: 6.6 vs. 5.1 m (HR 0.74, $p = 0.096$) mPFS: 6.5 vs. 5.1 m (HR 0.74, $p = 0.268$) in PIK3CA-mutant subset
Alpelisib (BYL719), oral	Class I PI3K-p110 α	SOLAR-1 (NCT02437318)	III	PIK3CA-mutated, previously received endocrine therapy, HR (+)/HER2(-) advanced BC	First or second-line	Alpelisib + fulvestrant Placebo + fulvestrant	mPFS: 11.0 vs. 5.7 m (HR 0.65, $p = 0.00065$) in PIK3CA-mutant subset ORR: 26.6 vs. 12.8% in PIK3CA-mutant subset
		NEO-ORB (NCT01923168)	II	Postmenopausal, HR (+)/HER2(-) early stage BC	Neoadjuvant	Alpelisib + letrozole Placebo + letrozole	ORR: 63 vs. 61% in PI3K wide-type subset ORR: 43 vs. 45% in PI3K-mutant subset
Taselisib (GDC-0032), oral	Class I PI3K-p110 δ	LORELEI (NCT02273973)	II	HR (+)/HER2(-) operable early stage BC	Neoadjuvant	Taselisib + letrozole Placebo + letrozole	ORR: 50 vs. 39% (OR 1.55, $p = 0.049$) ORR: 56 vs. 38% (OR 2.03, $p = 0.033$) in PIK3CA-mutant subset
		SANDPIPER (NCT02340221)	III	HR(+)/HER2(-), AI resistant locally advanced or mBC	Second-line or later	Taselisib + fulvestrant Placebo + fulvestrant	mPFS: 7.4 vs. 5.4 m (HR 0.70, $p = 0.0037$)
Capivasertib (AZD-5363), oral	Akt1 Akt2 Akt3	FAKTION (NCT01992952)	II	HR(+)/HER2(-), AI-resistant advanced BC	Second-line or later	Capivasertib + fulvestrant Placebo + fulvestrant	mPFS: 10.3 vs. 4.8 m (HR 0.58, $p = 0.0035$) mOS: 26.0 vs. 20.0 m (HR 0.59, $p = 0.071$)
Ipatasertib (GDC-0068), oral	Akt1 Akt2 Akt3	LOTUS (NCT02162719)	II	Primary locally advanced or mTNBC	First-line	Ipatasertib + paclitaxel Placebo + paclitaxel	mPFS: 6.2 vs. 4.9 m (HR 0.60, $p = 0.037$) mPFS: 6.2 vs. 3.7 m (HR 0.59, $p = 0.18$) in PTEN-low cohort

(Continued on following page)

TABLE 1 | (Continued) Summary clinical RCT trials of PI3K/AKT/mTOR inhibitors in breast cancer.

Drug	Target	Study	Phase	Patients population	Therapy line	Regimen	Outcome
Everolimus, oral	mTOR1	BOLERO-2 (NCT00863655)	III	HR (+)/HER2(-), AI-resistant and postmenopausal advanced BC	Second-line or later	Everolimus + exemestane Placebo + exemestane	mPFS: 10.6 vs. 4.1 m (HR 0.36, $p < 0.001$) ORR: 7 vs. 0.4% ($p < 0.001$)
		MANTA (NCT02216786)	II	HR (+), postmenopausal and AI-resistant locally advanced or mBC	Second-line or later	Everolimus + fulvestrant Fulvestrant	mPFS: 12.3 vs. 5.4 m (HR 0.63, $p = 0.01$)
		PrE0102 (NCT01797120)	II	HR (+)/HER2(-), AI-resistant and postmenopausal mBC	Second-line or later	Everolimus + fulvestrant Placebo + fulvestrant	mPFS: 10.3 vs. 5.1 m (HR 0.61, $p = 0.02$) ORR: 18.2 vs. 12.3% ($p = 0.47$)
		BOLERO-1 (NCT00876395)	III	HER2(+), primary advanced BC	First-line	Everolimus + trastuzumab Placebo + trastuzumab	mPFS: 14.9 vs. 14.5 m (HR 0.89, $p = 0.12$) mPFS: 20.3 vs. 13.1 m (HR 0.66, $p = 0.0049$) in HR (-) tumors
		BOLERO-3 (NCT01007942)	III	HER2(+), taxane-pretreated and trastuzumab-resistant advanced BC	Second-line or later	Everolimus + trastuzumab + vinorelbine Placebo + trastuzumab+ vinorelbine	mPFS: 7.0 vs. 5.8 m (HR 0.78, $p = 0.0067$)
Temsirolimus (CCI-779), intravenous	mTOR	HORIZON (NCT00083993)	III	HR(+), postmenopausal, AI-naïve advanced BC	First-line	Temsirolimus + letrozole Placebo + letrozole	mPFS: 8.9 vs. 9.0 m (HR 0.90, $p = 0.25$) mPFS: 9.0 vs. 5.6 m (HR 0.70, $p = 0.009$) in age ≤ 65 years subgroup

AI, aromatase inhibitor; mBC, metastatic breast cancer.

including mTOR, matrix metalloproteinase (MMP), cyclin-dependent kinases (CDKs), and VEGF (Liu et al., 2009; Cidado and Park, 2012) (Figure 1).

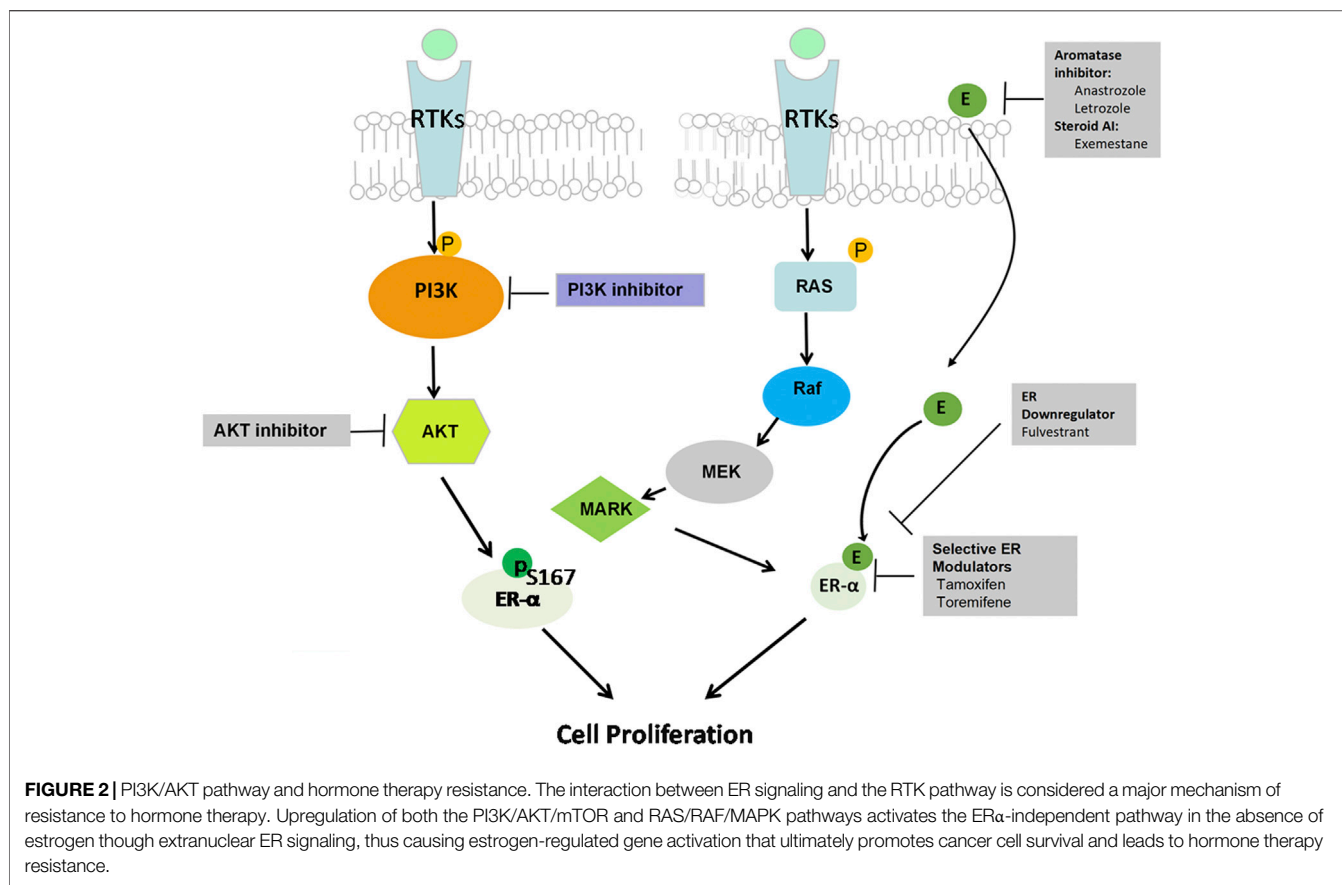
PI3K/AKT/mTOR Inhibitors

To date, several PI3K inhibitors (PI3Ki), including alpelisib, idelalisib and copanlisib, have been approved by the Food and Drug Administration (FDA) (Janku et al., 2018) in many types of cancer. According to the targeted isoforms of class IA PI3Ks, PI3Ki can be classified into pan-PI3Ki, such as buparlisib (BKM120) and pictilisib (GDC-0941), isoform-specific PI3Ki, such as taselisib (GDC-0032) and alpelisib (BYL719), and dual mTOR/pan PI3Ki, such as BEZ235. BYL719 is the first oral PI3Ki that targets the p110 α isoform selectively ($IC_{50} = 4.6$ nM) (Fritsch et al., 2014), and it was the first approved PI3K inhibitor combined with fulvestrant for patients with HR+/HER2- metastatic breast cancer (mBC) along with PIK3CA mutations that have progressed on or after endocrine-based regimens (Markham, 2019). Taselisib is another oral class I isoform-specific PI3K inhibitor, as it exhibits equal inhibition of p110 α , p110 γ and p110 δ (Ndubaku et al., 2013). Buparlisib, an oral pan-PI3K inhibitor, targets all four isoforms of class I PI3K (Hu et al., 2015). Pictilisib (GDC-0941) is another oral pan-PI3K inhibitor that shows equipotent efficiency in inhibiting the p110 α and p110 δ isoforms *in vitro* (Martín et al., 2017). Based on previous results of clinical trials, compared to pan-PI3K inhibitors, these isoform-specific

PI3K inhibitors show improved tolerability and increased anticancer efficacy (Krop et al., 2016; Baselga et al., 2017; André et al., 2019); thus, more selective PI3K inhibitors are expected in the future.

According to the structure and the binding site, AKT inhibitors (AKTi) can be divided into PH domain inhibitors: allosteric inhibitors and ATP competitive inhibitors. Several PH domain inhibitors and allosteric inhibitors were terminated in the preclinical stage due to poor pharmacokinetic properties and heavy toxicity effects. The current research hotspot is ATP competitive inhibitors such as ipatasertib (GDC-0068) and capivasertib (AZD-5363). As a downstream molecule of the PI3K/AKT pathway, mTOR inhibitors can inhibit breast cancer cell growth. Everolimus was the first approved mTOR inhibitor for HR+/HER2- breast cancer and showed an improved PFS in several clinical trials (Bachelot et al., 2012; Yardley et al., 2013), whereas another mTOR inhibitor, temsirolimus, showed only moderate activity in HR+ breast cancer patients with age ≤ 65 years, but this randomized phase III trial was terminated for futility as total PFS was not improved by temsirolimus plus letrozole as first-line therapy, so the best suitable population for temsirolimus needs to be further identified (Wolff et al., 2013). Detailed information on these PI3K/AKT/mTOR inhibitors is listed in Table 1.

In addition, activation of the PI3K/AKT pathway can upregulate multidrug resistance-associated protein-1 (MRP1), ABCG2 and P-glycoprotein (P-gp, also called ABCB1)



expression, which could be involved in multidrug resistance (Tazzari et al., 2007; Wang et al., 2016a). Dysregulation of the PI3K/AKT pathway has an impact on resistance to chemotherapy, endocrine therapy, HER2-targeted therapy, PARP inhibitors, and immunotherapy in breast cancer, and blockage of this pathway can enhance drug sensitivity and reverse resistance (Miller et al., 2011a; Hu et al., 2015; Pernas and Tolaney, 2019). Overall, accumulated evidence suggests that PI3K/AKT inhibitors could combine with other anticancer therapies to overcome drug resistance in breast cancer.

PI3K/AKT/MTOR PATHWAY INDUCES DRUG RESISTANCE IN BREAST CANCER

PI3K/AKT/mTOR Pathway in Endocrine Therapy-Resistant Breast Cancer

Several types of endocrine drugs, including selective ER modulators (tamoxifen), aromatase inhibitors (AIs, such as letrozole and exemestane), and selective ER degraders (fulvestrant), are the most important therapies for ER+ breast cancer. In addition to the well-known efficiency of these treatments, approximately 50% of patients develop disease progression due to endocrine resistance (Chen et al., 2020a). Therefore, it is important to improve the

survival and prognosis of breast cancer by exploring endocrine resistance mechanisms to develop new treatment strategies. At present, the known mechanisms of endocrine resistance are mainly related to alteration of ER structure and function, including downregulation or loss of ER expression, ER gene mutations, posttranslational modification of ER, abnormality of ER coactivators, and the interaction between ER and various intracellular signal molecules, such as HER2, EGFR, PI3K/AKT/mTOR and MAPK/ERK (Zhao and Ramaswamy, 2014).

Accumulating data indicate the role of PI3K activation in endocrine resistance, and the development of PI3K inhibitors is an attractive strategy to reverse endocrine resistance (Yap et al., 2015). Preclinical data support that PI3K and AKT can phosphorylate ER α at Ser167 to activate ER α independently in the absence of estrogen, so the interaction between ER and PI3K/AKT/mTOR pathway hyperactivation causes breast cancer cells to lose sensitivity to endocrine therapy (Campbell et al., 2001) (Figure 2). Upregulation of the PI3K/AKT pathway plays a critical role in endocrine treatment resistance through extranuclear ER signaling (Mukohara, 2015). During the progression of endocrine resistance, PI3K mainly activates mTOR substrate p70S6 kinase in MCF-7 and MDA-MB-361 cells after long-term estrogen deprivation (Miller, et al., 2010). In addition, PIK3CA mutations or PTEN loss can induce estrogen-

independent growth of breast cancer cells (Miller, et al., 2011b). PIK3CA mutations are detected in close to 50% of ER+ breast cancer patients, which contribute to endocrine resistance (Miller, et al., 2011c; Huang et al., 2019). Furthermore, the mechanism of resistance involves upregulation of receptor tyrosine kinases (RTKs), which leads to enhanced activation of the downstream PI3K/AKT signaling pathway (Mansouri et al., 2018) (**Figure 2**). PI3K/AKT inhibition combined with different types of endocrine drugs, such as tamoxifen, AIs, and fulvestrant, is a new strategy for ER+ breast cancer treatment (Sabine, et al., 2014; Hanamura and Hayashi, 2018). Miller et al. reported that endocrine-resistant cell proliferation depends on the PI3K pathway and becomes extremely sensitive when inhibiting this pathway (Miller et al., 2010). Then, in an *in vitro* study, Sanchez et al. demonstrated that PI3K inhibitors showed potent anti-proliferative activity when combined with endocrine therapy in ER+ MCF-7 breast cells (Sanchez et al., 2011). The ER degrader fulvestrant combined with a p110 α and p110 β isoform inhibitor induced apoptosis and suppressed PTEN-deficient xenograft tumor growth by inhibiting PI3K downstream substrates, indicating that it is an effective strategy to overcome endocrine resistance in the luminal B subtype of breast cancer due to reduced PTEN levels (Hosford et al., 2017). Therefore, these results implied that PI3K/AKT/mTOR inhibition can restore ER expression levels and activity and improve sensitivity to endocrine therapy.

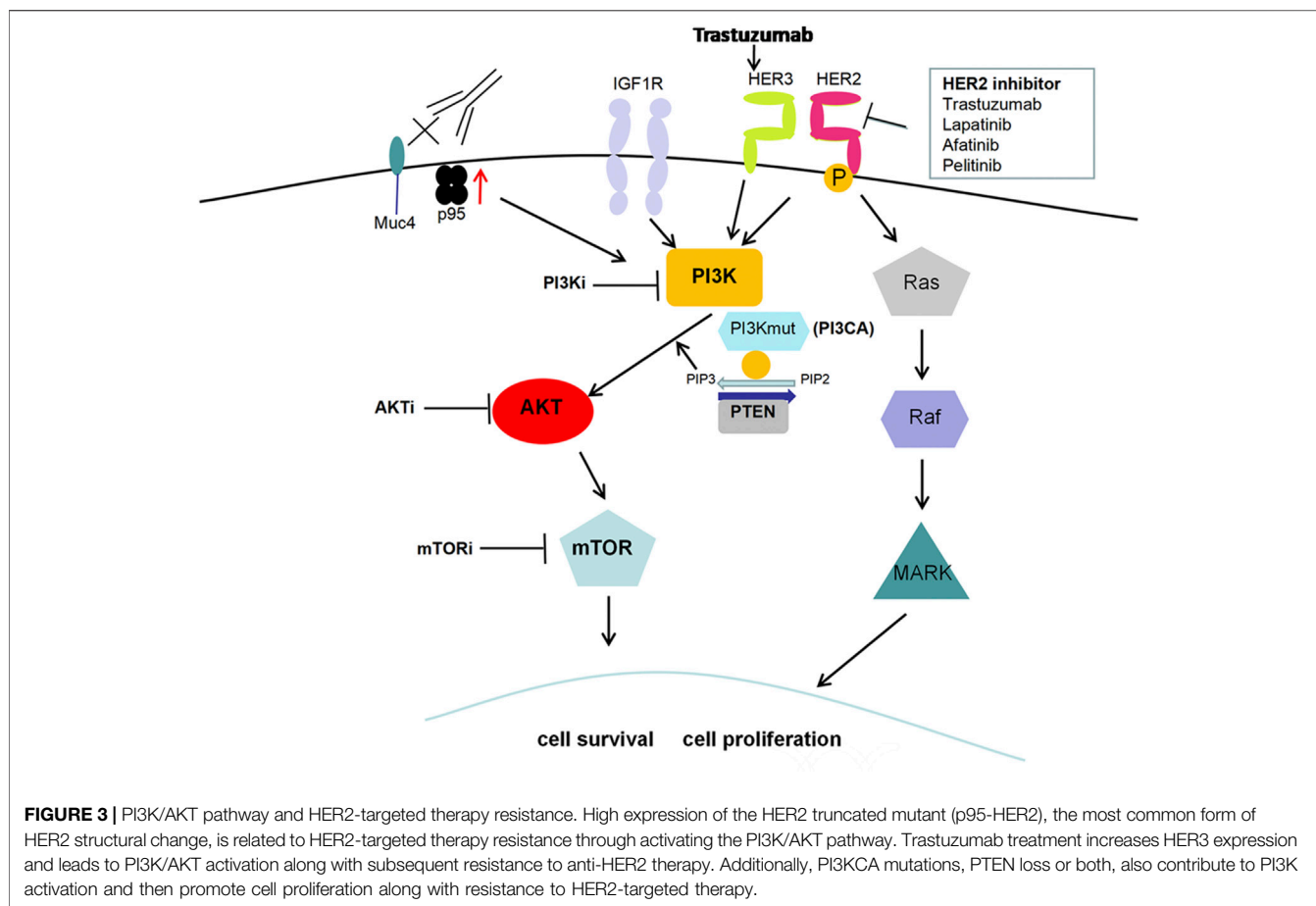
Until now, clinical trials using drugs targeting PI3K/AKT/mTOR plus hormone therapy have shown promising results in HR+/HER2- mBC. These results are summarized in **Table 1**. The combination of endocrine therapy with most PI3K inhibitors, such as buparlisib in the BELLE-2 study and the PI3K α inhibitors alpelisib and taselisib in the SOLAR-1 and SANDPIPER studies, respectively, has demonstrated potent anticancer effects in HR+/HER2- and AI-resistant breast cancer (Baselga et al., 2017; Baselga et al., 2018; André et al., 2019), although the pan-PI3K inhibitor pictilisib in the FERGI study showed limited efficacy in AI-resistant advanced or mBC. In addition, AKT inhibitor of capivasertib combined with fulvestrant (FAKTION study) also showed an improved PFS and OS in AI-resistant advanced BC (Jones et al., 2020). Furthermore, letrozole combined with the PI3K α inhibitor taselisib (LORELEI study) but not alpelisib (NEO-ORB study) showed a synergetic anticancer effect as neoadjuvant therapy in early stage breast cancer. Moreover, inhibition of mTOR, a downstream target of the PI3K pathway, has also been clinically shown to enhance the anticancer effect of endocrine therapy with AIs in HR+ advanced BC (Baselga et al., 2012). Consistently, patients with AI-resistant advanced BC have consistently been shown to obtain significant clinical benefits from everolimus in several phase II-III clinical trials that combined everolimus with endocrine therapy, including BOLERO-2 (NCT00863655), MANTA (NCT02216786) and PrE0102 (NCT01797120). Therefore, based on these promising preclinical and clinical data, several ongoing studies of PI3K inhibitors in combination with hormonal therapy as a strategy to overcome hormonal therapy-resistant

breast cancer are being conducted, including the BYLieve study (NCT03056755) and the SAFIR study (NCT02734615, NCT03386162, NCT01870505).

PI3K/AKT/mTOR Pathway in HER2-Targeted Therapy-Resistant Breast Cancer

In addition to HER2 monoclonal antibody drugs, including trastuzumab and pertuzumab, several small-molecule tyrosine kinase inhibitors, such as lapatinib, neratinib and pyrotinib, and an antibody-drug conjugate (T-DM1) have emerged for HER2+ breast cancer treatment in recent years. Anti-HER2 therapy has greatly improved the survival and prognosis of HER2+ breast cancer, but acquired resistance still occurs (Nagini, 2017). Further study of the mechanism of HER2-targeted therapy resistance is important. Attention has been given to the abnormal activation of the PI3K/AKT/mTOR signaling pathway, which is closely associated with anti-HER2 therapy resistance (Pernas and Tolaney, 2019). The common form of HER2 structural change is the HER2 truncated mutant (p95-HER2). High expression of p95-HER2 indicates poor prognosis and is related to the drug resistance of anti-HER2 therapy through activating the PI3K/AKT pathway (Arribas et al., 2011). HER3 is overexpressed in breast cancer and can potentially activate the downstream PI3K/AKT pathway, which promotes cancer cell survival and leads to drug resistance (Dey et al., 2015). It was reported that trastuzumab also increased HER3 expression, thus leading to trastuzumab resistance (Li et al., 2018). Additionally, PI3KCA mutations, PTEN loss or both, leading to PI3K activation, are related to tumor progression and reduced survival rate in breast cancer, which has an adverse effect on the therapeutic effect of trastuzumab (Razis et al., 2011) (**Figure 3**). PIK3CA somatic mutations mainly include two “hot spots,” H1047R or H1047L in exon 20 and E542K or E545K in exon 9. E545K and H1047R are prevalent in breast cancer, which leads to lapatinib resistance (Eichhorn et al., 2008). Mutation of the PIK3CA gene promotes anti-HER2 resistance via the catalytic subunit p110 α but not p110 β . The combination of alpelisib and lapatinib effectively reduced breast cancer cell growth in genetic models driven by HER2 activation and PTEN loss (Wang et al., 2016b). However, in recent years, some clinical studies have suggested that a single PI3KCA mutation or PTEN loss cannot accurately predict trastuzumab resistance, and more molecular markers need to be combined (Pogue-Geile et al., 2015; Stern et al., 2015).

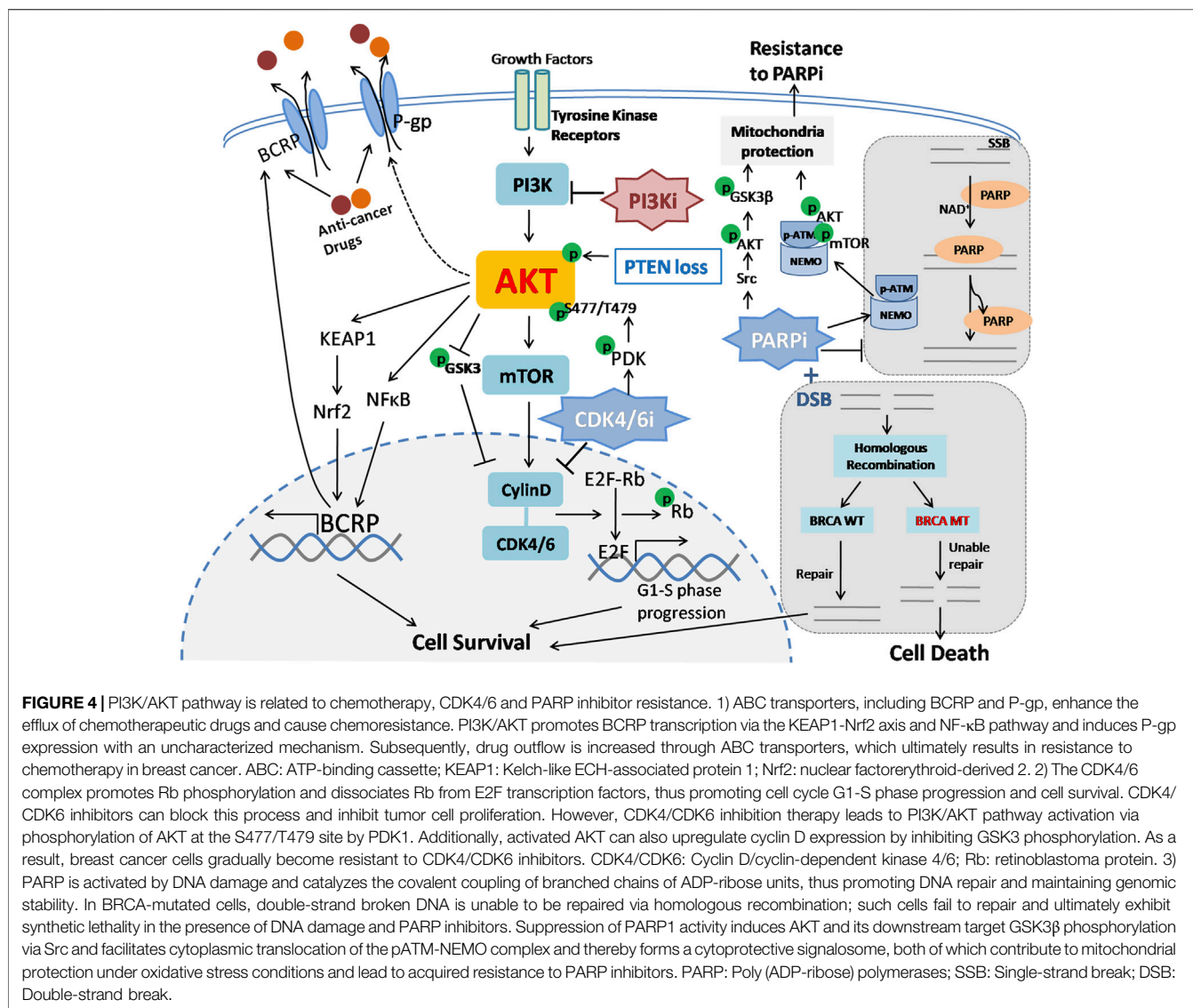
A number of preclinical studies suggest that PI3K/AKT/mTOR inhibition can overcome anti-HER2 therapy resistance. PI3K inhibition by BAY 80-6946 has been confirmed to resensitize trastuzumab and/or lapatinib-resistant cells to HER2-targeted therapies (Elster et al., 2015). Similar results suggest that alpelisib plus anti-HER2 Ab largely inhibit breast cancer tumor growth (Zhang et al., 2016). The pan-PI3K inhibitor NVP-BKM120 significantly suppresses PIK3CA-mutant tumor xenograft growth when combined with HER2-targeted therapy (Maira et al., 2012). In addition, PI3K inhibitors



and BEZ235 (dual PI3K/mTOR inhibitor) suppress trastuzumab-resistant breast cancer cell proliferation by inhibiting the PI3K/AKT/mTOR pathway (Kataoka et al., 2010; O'Brien et al., 2014). BEZ235 can also overcome resistance to lapatinib and EGFR/HER2 inhibitors in PI3KCA-activated or PTEN-deficient breast cancer cell lines (Brünner-Kubath et al., 2011). Furthermore, mTOR inhibition combined with lapatinib leads to higher synergistic tumor response rates in trastuzumab-resistant xenografts (Gayle et al., 2012). It was reported that lapatinib-resistant HER2+ breast cancer cells increased the expression levels of survivin and c-IAP2, two inhibitors of apoptosis (IAPs), and treatment with the mTOR kinase inhibitor AZD8055 reversed expression of IAPs and overcame lapatinib resistance in lapatinib-resistant cells (Brady et al., 2015). Moreover, targeted therapy against HER2 and the PI3K/mTOR pathway induce apoptosis and promote trastuzumab-resistant xenograft shrinkage (Vuylsteke et al., 2016; Kim et al., 2017).

Based on the results of these preclinical studies, many clinical trials were conducted as described in Table 1, NeoPHOEBE (NCT01816594) showed that the HER2-targeted therapy trastuzumab was enhanced when the pan-PI3K inhibitor buparlisib was combined in HER2+ primary breast cancer (Loibl et al., 2017). In addition, some other clinical trials

assessing the therapeutic effect of everolimus plus HER2-targeted therapy have also shown optimistic results, including BOLERO-3 (NCT01007942) and BOLERO-1 (NCT00876395) studies (André et al., 2014; Hurvitz et al., 2015). Currently, several phase I clinical trials of PI3K selective inhibitors in combination with HER2-targeted therapy designed to further evaluate these findings are ongoing. Firstly, a phase I trial (NCT02038010) combining PI3K α inhibitor alpelisib and trastuzumab-MCC-DM1 (T-DM1) in HER2+ mBC patients who have progressed from prior treatment with trastuzumab plus taxane-based chemotherapy is currently being conducted. Another new PI3K α selective inhibitor, MEN1611, is being tested in a phase Ib trial (NCT03767335) to evaluate the effect of MEN1611 plus trastuzumab combined with or without fulvestrant in PI3KCA-mutated patients who were diagnosed with HER2+, advanced or mBC and have progressed after HER2 targeted therapy. Furthermore, a Phase I, open-label study (NCT02167854) is ongoing to understand the best dose of alpelisib combined with LJM716 (anti-HER3 Ab) and trastuzumab in HER2+ mBC. In addition to PI3K α inhibitor, the PI3K δ isoform inhibitor copanlisib combined with trastuzumab, a phase Ib/II trial (NCT04108858), is being tested in HER2-targeted therapy-resistant breast cancer. Overall, PI3K/AKT/mTOR inhibition is emerging as a



potential therapeutic strategy to overcome HER2-targeted therapy resistance.

PI3K/AKT/mTOR Pathway in Chemotherapy-Resistant Breast Cancer

Chemotherapy also plays an important role during breast cancer treatment, especially in TNBC. However, chemotherapy failure often occurs because of the acquisition of anticancer resistance and acquired drug resistance (Olgen, 2018). Unfortunately, the underlying mechanisms of chemoresistance in breast cancer have not yet been clearly clarified. The biological causes of drug resistance are attributed to diverse molecular mechanisms, including reduced drug accumulation, metabolic detoxification, anti-apoptosis, autophagy, and other mechanisms (Ji et al., 2019). At present, studies have demonstrated that PI3K/AKT promotes chemotherapy resistance through multidrug resistance-related proteins and anti-apoptosis effects (Zhang et al., 2020). It was

reported that PI3K/AKT increases the outflow of chemotherapeutic drugs through the ATP-binding cassette (ABC) transporter superfamily, thus causing and maintaining the multidrug resistance of tumor cells (Kathawala et al., 2015) (Figure 4). In a number of MDR cancer cells, BCRP and P-gp proteins are overexpressed, which mediates the extracellular release of taxanes and anthracyclines (Kathawala et al., 2015). Additionally, AKT phosphorylation has been shown to be positively associated with cell viability, migration and apoptosis in breast cancer, which may induce chemoresistance (Gao et al., 2019). Therefore, blocking PI3K/AKT can restore the sensitivity of cancer cells to chemotherapy drugs and thus overcome drug resistance.

Several preclinical studies have indicated that PI3K/AKT/mTOR inhibitors have an important effect on reversing the chemoresistance of breast cancer. The PI3K inhibitor wortmannin nearly completely blocked cell proliferation and resistance to doxorubicin in MCF-7/ADR (ADR: Adriamycin resistance) breast cancer cells (Tsou et al., 2015). Similarly, our

previous study showed that the inhibition of PI3K by econazole significantly sensitized MDA-MB-231 and MCF-7 cells to adriamycin *in vitro* and *in vivo* (Dong et al., 2020). In addition, the pan-PI3K inhibitor BKM120 showed a potent antitumor ability in both sensitive and MDR breast cancer cells by inhibiting the PI3K/AKT/NF- κ B signaling pathway, and the combination of BKM120 with doxorubicin showed a synergistic effect (Hu et al., 2015). Moreover, doxorubicin combined with the pan-PI3K inhibitor buparlisib also revealed a strong synergistic anti-proliferative effect (Hu et al., 2015). Furthermore, it was reported that 3-methyladenine (3-MA) blocks the PI3K/AKT/mTOR pathway and reverses MDR by inhibiting agent-efflux transporters (Zou et al., 2014). Consistently, another study reported that BEZ235 combined with tunicamycin could promote autophagy and apoptosis and then enhance chemosensitivity in MCF-7 cells by suppressing the PI3K/AKT pathway (Zhong et al., 2017).

In the clinic, two randomized controlled trial (RCT) studies, PEGGY (NCT01740336) (Vuylsteke et al., 2016) and BELLE-4 (NCT01572727) (Martín et al., 2017), were conducted to evaluate the efficiency of adding PI3K inhibitors to paclitaxel in advanced BC. However, median PFS was only slightly prolonged but without significance in both studies, even in the PIK3CA-mutant subset. In addition, in the phase 2 LOTUS study (NCT02162719), inoperable, locally advanced or metastatic primary TNBC patients were recruited and randomly assigned to two groups treated with paclitaxel plus AKT inhibitor ipatasertib or placebo. The median PFS was improved in the ipatasertib arm (6.2 months vs 4.9 months, $p = 0.037$), and in the subset with low PTEN expression, the median PFS was also increased from 3.7 months to 6.2 months by ipatasertib treatment ($p = 0.18$), which is the first clinical result supporting AKT-targeted therapy for TNBC (Kim et al., 2017). These results are listed in **Table 1**. At present, several clinical trials, including the SAFIR study (NCT02379247), BEECH study (NCT01625286) and IPATunity130 (NCT03337724), are ongoing to assess the effect of the PI3K α inhibitor alpelisib or AKT inhibitor of AZD5363 or ipasertib plus paclitaxel in advanced BC or mBC. Overall, the potential role of PI3K/AKT inhibitors in overcoming chemoresistance is an attractive prospect in breast cancer therapy.

PI3K/AKT/mTOR Pathway in PARP Inhibitor-Resistant Breast Cancer

In recent years, the poly-ADP-ribose polymerase inhibitors (PARPis) olaparib and talazoparib were approved by the FDA for germline BRCA1 and BRCA2 mutation (gBRCA1/2+) mBC (Hoy, 2018; Tompa, 2018). Olaparib presents a significant clinical benefit, and a series of clinical trials showed that compared to standard chemotherapy, olaparib improved PFS in patients with gBRCA-mutated HER2-mBC, with a manageable toxicity profile (Griguolo et al., 2018). However, since acquired resistance is gradually emerging, there is an urgent need to develop strategies to overcome resistance to PARP inhibitors in breast cancer.

The mechanism of PARPi resistance is complex and includes drug efflux, homologous recombination repair restoration, dysfunction of the cell cycle and signal transduction (Bitler et al., 2017). Preclinical data have indicated that PI3K/AKT/mTOR

activation is related to PARPi resistance. PARP inhibitors can result in PI3K/AKT pathway activation via Src under oxidative conditions (Veres et al., 2003; Tapodi et al., 2005; Veres et al., 2004). Another study found that PARP suppression inhibits the PARylation of ATM and promotes an ATM-NEMO interaction, subsequently facilitating cytoplasmic translocation of this complex and forming a p-ATM-NEMO-Akt-mTOR cytoprotective signalosome (Tapodi et al., 2019), indicating that using combined inhibitors targeting the PI3K/AKT/mTOR pathway and PARPi may potentially obtain a better clinical benefit (**Figure 4**). In TNBC patient-derived primary tumor xenografts, dual inhibition of PI3K and PARP has been shown to significantly reduce the growth of tumors (Ibrahim et al., 2012). Similarly, the combination of BKM120 and olaparib dramatically extended the doubling time of tumors in an MMTV-Cre: Brca1(f/f): Trp53 (+/-) breast cancer mouse model (Juvekar et al., 2012). In addition, using rapamycin to inhibit S6 phosphorylation, an mTOR downstream effector, can restore the PARPi sensitivity of resistant BC cells, and the combination of rapamycin and PARPi can obviously suppress BRCA1-deficient tumor growth *in vivo* (Sun et al., 2014). Furthermore, it has been reported that compared to PARPi alone, the combination of PARP and PI3K inhibitors creates a synergetic antitumor ability to inhibit BRCA1-deficient breast tumor growth *in vitro* and *in vivo* (Zhang et al., 2019).

Currently, there are two clinical trials to determine the combined effect of PARPi and PI3K/AKT inhibitors in TNBC. One is a phase I study (NCT01623349) to assess olaparib combined with PI3K inhibitors BYL719 or BKM120 in patients diagnosed with mTNBC that have progressed from prior treatments including at least one cytotoxic agent. Another is a phase I/II study (NCT02208375), in which olaparib plus AZD 2014 (mTOR inhibitor) or AZD5363 (AKT inhibitor) regimens are being assessed in several types of cancers, including TNBC. Therefore, the combination of PARPi with PI3K/AKT/mTOR inhibitors may potentially increase clinical benefit and enhance the response to PARPi.

PI3K/AKT/mTOR Pathway in CDK4/6-Inhibited or CDK4/6-Resistant Breast Cancer

In addition to PARPi, CDK4/6 inhibitors, such as ribociclib, abemaciclib and palbociclib, have been approved for HR+/HER2- mBC treatment, and improved survival was observed by combining with endocrine therapy (Murphy, 2019). However, acquired resistance is inescapable, and the management of primary and acquired resistance to CDK4/6 inhibitors is urgent.

Until now, several cell cycle-related mechanisms have been determined as possible reasons for resistance, including RB1 or FAT1 loss mutations, CDK6 and CCNE1 overexpression or amplification, FGFR alterations and PI3K/mTOR-mediated CDK2 activation (Xi and Ma, 2020). PI3K/mTOR pathway activation is a potential mechanism for both primary and acquired resistance to CDK4/6 inhibitors, and the synergetic effect of PI3K inhibition post-CDK4/6 inhibitor treatment has been confirmed in several studies (Zhang et al., 2016; Jansen et al., 2017). Under ribociclib treatment, a kinome-wide siRNA

knockdown screen was conducted in MCF-7 cells and showed that PDK1 was identified as a key modifier of ribociclib sensitivity, which is a PI3K pathway component required for AKT activation (Jansen et al., 2017) (Figure 4). Combining PI3K inhibitors with CDK4/6i has been confirmed to have a synergistic effect to overcome intrinsic and adaptive resistance in multiple studies (Vora et al., 2014; Herrera-Abreu et al., 2016). CDK4/6 and PI3K inhibitors together have been shown to induce breast cancer cell apoptosis *in vitro* and suppress tumor growth in patient-derived tumor xenograft models (Herrera-Abreu et al., 2016). Additionally, mTORC1/2 inhibition has been shown to inhibit Rb phosphorylation, cyclin D1 expression and E2F-mediated transcription in CDK4/6i-resistant cells (Michaloglou et al., 2018). In this regard, the BYLieve (NCT03056755) study is being conducted to evaluate the effect of alpelisib in the subset of HR+/HER2- recurrent BC patients with PIK3CA mutations who have progressed on prior CDK4/6 inhibitor treatment. Another trial (NCT02088684) examined the anticancer effect of the triplet combination regimen of ribociclib, fulvestrant and buparlisib or alpelisib compared to the combination of ribociclib with fulvestrant. Several trials are assessing the efficacy of the triplet regimen in breast cancer, including NCT02871791, NCT02732119, NCT02088684, NCT02057133, NCT02389842, NCT02684032, and NCT01857193. In addition, several other ongoing studies (NCT02871791, NCT01857193, NCT02732119) are being conducted to test triplet regimens by combining CDK4/6i with exemestane and everolimus.

PI3K/AKT/mTOR Pathway in Immunotherapy-Resistant Breast Cancer

In the last decade, cancer immunotherapy has obtained marked clinical efficacy during cancer therapy. In breast cancer, several PD-1/PD-L1 inhibitors, such as pembrolizumab, avelumab and atezolizumab, are in clinical trials (Emens, 2018), although only atezolizumab as a first-line therapy has been approved by the FDA to treat patients with advanced metastatic TNBC expressing PD-L1 (Soare and Soare, 2019). In several preclinical studies, the PI3K/AKT/mTOR pathway has been preliminarily proven to be a critical pathway for sustaining the function of immunosuppressive Tregs. On the one hand, pan-AKT inhibitors such as wortmannin, IC87114 or MK-2206 enhanced CD8+ T cell infiltration in the tumor microenvironment (Abu-Eid et al., 2014). Similarly, the functions of CD8+ T cells were sustained under P110 δ inhibition (Patton et al., 2006). On the other hand, the differentiation process of memory T cells seems to be affected by the PI3K/AKT/mTOR network. Using rapamycin to inhibit mTOR significantly enhanced memory T cell differentiation (Araki et al., 2009). Another study demonstrated that AKT inhibition also potentially accelerated the differentiation of memory T cells (van der Waart et al., 2014). AKT can inhibit TCR/LEF/ β -catenin and FOXO transcription, which are required for the function of T cell memory (Lazarevic et al., 2013). Specifically, AKT inhibition can lead to a favorable immune profile in the breast tumor microenvironment, including an increased density of CD3+CD8+ cells and better expression of

interferon genes, providing a rationale for using AKT inhibition plus immunotherapy combination (Marks et al., 2020). Correspondingly, the PI3K β inhibitor AZD8186 enhanced anticancer efficacy by combining with anti-PD1 Ab in PTEN-deficient xenografts (Owusu-Brackett et al., 2020). Combining nanoscale PI3K-targeting supramolecular therapeutics with anti-PD-L1/PD-1 Ab exhibited a synergetic anticancer effect in breast cancer *in vivo* (Kulkarni et al., 2016). Therefore, these preclinical data suggest that the combination of PI3K/AKT/mTOR inhibitors may be effective in overcoming resistance to immune checkpoint blockade therapy in breast cancer. However, further clinical studies need to be conducted to understand the safety and efficacy of these combinations.

PI3K/AKT/mTOR Pathway in Radio-Resistant Breast Cancer

Aberrant upregulation and activation of PI3K/AKT/mTOR pathway is also correlated with resistance radiotherapy. A number of preclinical studies indicated that inhibition of PI3K/AKT/mTOR pathway can sensitize different types of cancer cells, including non-small cell lung cancer, breast cancer, nasopharyngeal carcinoma, oral squamous, and head and neck cancer, to radiotherapy (Yu et al., 2017; Chen et al., 2020b; Chen et al., 2020c; Glorieux et al., 2020; Schötz et al., 2020; Wanigasooriya et al., 2020). In breast cancer, IL-6 pre-treatment followed by SIRT1 activation by SRT1720 combined with dual PI3K/mTOR inhibitor NVP-BEZ235 obviously increased sensitivity of the cancer stem cells to radiation, so that late stage breast cancer cells therapeutic effect was effectively improved (Fatehi et al., 2018; Masoumi et al., 2020). Another dual PI3K/mTOR inhibitor PI103 could significantly induce prolongation of γ H2AX foci and also effectively sensitized SKBR3 cells to radiotherapy (No et al., 2009). Accordingly, further pre-clinical studies should be focused on dual PI3K/mTOR inhibitors. However, no relevant clinical trials have been conducted until now, thus early stage clinical studies are needed to understand the feasibility and efficacy of these dual PI3K/mTOR inhibitors in combination with radiotherapy in cancer patients.

POTENTIAL PREDICTIVE BIOMARKERS OF RESPONSE OR RESISTANCE TO PI3K INHIBITORS

PIK3CA Mutation Predicts the Response to PI3K Inhibitors

PI3K inhibitors show different activities in a range of different populations, indicating that there is a need for patient selection. PIK3CA mutation may serve as a potential predictive marker, which is of considerable interest in trials so far (Mollon et al., 2020). Notably, the combination of fulvestrant and alpelisib has shown synergistic antitumor activity against ER+/PIK3CA-mutated advanced BC (André et al., 2019). PIK3CA mutation-induced endocrine therapy resistance is of great interest since PIK3CA is frequently mutated in luminal tumors (Network,

2012). Therefore, PIK3CA mutations represent potential new therapeutic targets to overcome resistance and may be an appropriate therapeutic effect response predictor of PI3K inhibitors (Brandão et al., 2019).

PIK3CA somatic mutations mainly include two “hot spots,” which have presented a gain-of-function transforming capacity in PI3K signaling (Ching-Shian Leong et al., 2008). The synergistic effect of PI3K inhibitors has been shown to be much more dramatic in PIK3CA-mutated breast cancer cells than in PIK3CA WT cells, and E545K mutants in exon 9 have shown a relatively poor response to PI3K inhibitor (Liu et al., 2018). In the PALOMA-3 randomized phase III trial, decreased PIK3CA variants in patients were detected at the end of treatment compared to initial treatment (26.7 vs. 19.0%). Clonal evolution was frequently found during treatment by exome sequencing of plasma DNA, suggesting abundant subclonal complexity in breast cancer patients who had progressed on previous endocrine therapy (O’Leary et al., 2018). Therefore, it is rational to observe tumor clonal evolution prior to treatment and after disease progression to identify more predictive biomarkers.

However, there is a slight controversial correlation between the PIK3CA genotype and response to the combination PI3K inhibitor and endocrine therapy. In the BELLE-2 III trial as previously mentioned, those patients with PIK3CA mutations obtained an obviously improved PFS from the buparlisib plus fulvestrant therapy arm (Baselga et al., 2017). In contrast, a significant PFS benefit was not observed from the pan-PI3K inhibitor pictilisib plus fulvestrant regimen in the FERGI study (Krop et al., 2016). On the one hand, compared to PIK3CA WT tumors, PIK3CA mutant tumors tended to be resistant to these therapies. Previous trials have failed to confirm that PIK3CA mutations possess predictive value for RFS from tamoxifen treatment (Beelen et al., 2014). On the other hand, compared with patients with PIK3CA WT status, breast cancer patients with PIK3CA mutations treated with AIs showed an improvement in time to progression (Ramirez-Ardila et al., 2013). Studies have also shown that PI3K is hyperactivated in long-term estrogen-deprived cells and that fulvestrant plus BKM120 induced suppression of ER+ xenografts (Miller et al., 2011). Nevertheless, most of the current phase III PI3K inhibitor clinical trials are investigating endocrine therapy-resistant advanced BC by combining PI3Ki with endocrine therapy, which has a synergistic effect and may help reverse endocrine therapy resistance.

PTEN Loss Predicts the Response to PI3K Inhibitors

Activation of the PI3K/AKT/mTOR pathway is attributed to several factors, such as PIK3CA mutation, PTEN loss, RAS/MEK/ERK, ER or HER2 pathway activation, FGFR1/2 amplification, KRAS and TP53 mutations. PTEN, a key tumor suppressor gene, negatively regulates the PI3K/AKT pathway, and PTEN loss can lead to out-of-control PI3K signal transduction, which results in breast cancer cell growth (Li et al., 1997). It has been reported that nearly 40–50% of breast

tumors harbor heterozygous PTEN loss, whereas functional PTEN loss because of mutations has been detected in 5–10% of breast tumors, and the frameshift is the most common mechanism (Coughlin et al., 2010; Bazzichetto et al., 2019). Preclinical data indicate that breast cancer cells with PTEN expression loss are more sensitive to PI3K/AKT inhibitors (O’Brien et al., 2010). In patients who had progressed on prior alpelisib treatment, *de novo* PTEN expression loss has been reported in all metastatic lesions; accordingly, PTEN-null xenografts derived from these patients showed resistance to alpelisib (Juric et al., 2015). In addition, some PTEN-deficient tumors are dependent on PI3K β signaling because depletion of PIK3CA has no impact on cell growth in three PTEN-deficient cancer cell lines, whereas inhibition of the PIK3CB isoform, responsible for encoding p110 β , resulted in tumor growth suppression *in vitro* and *in vivo* (Wee et al., 2008). Furthermore, in the phase 2 LOTUS clinical trial (NCT02162719), inoperable, locally advanced or metastatic primary TNBC patients were recruited and treated with paclitaxel plus either ipatasertib (AKT inhibitor) or placebo, and in the low PTEN expression subset, median PFS was prolonged by ipatasertib as previously described (Kim et al., 2017).

To date, only the PIK3CA mutation has been proven to be a therapeutic efficiency predictive marker for PI3K α inhibitor treatment in advanced BC. For PTEN, more evidence from preclinical and clinical studies is needed to further investigate its predictive value for the efficiency of PI3Ki treatment in breast cancer patients.

Other Potential Biomarkers

In addition to two critical predictive biomarkers (PIK3CA mutation and PTEN loss) as described above, there are several potential biomarkers are under exploring. It was reported that patients with early metabolic response by ¹⁸F-FDG-PET/CT often showed an increased benefit from buparlisib and letrozole combination treatment in a phase Ib clinical trial (Mayer et al., 2014), that is to say, a decreased metabolism in tumors predict better response to PI3Ki. Additionally, results from OPPORTUNE trial demonstrated that patients with progesterone receptor-negative status present a better response from pictilisib (Schmid et al., 2016). What’s more, several possible mechanisms of PI3Ki resistance have been uncovered in recent years. Firstly, overexpression of ribosomal S6 kinases RPS6KA2 (RSK3) and RPS6KA6 (RSK4) promoted breast cancer growth upon PI3K inhibition treatment, which can be reversed by addition of MEK- or RSK-specific inhibitors (Serra et al., 2013). Secondly, it was reported that PI3Ki-resistant cell lines can be resensitized by AKTi MK-2206 (Vora et al., 2014). Moreover, PI3K inhibition can result in hyperglycaemia as PI3K mediates cellular responses to insulin, which leads to an increasing release of insulin in a feedback manner and re-activate PI3K pathway in tumor models in mice, thus ultimately causing PI3Ki resistance (Hopkins et al., 2018). High insulin levels may be a potential biomarker for predicting resistance to PI3Ki, which could be partially prevented by ketogenic diet and sodium-glucose cotransporter inhibitors (Hopkins et al., 2018). In a phase Ib trial, patients with TP53

mutations, KRAS, or FGFR1/2 amplification did not obtain clinical benefit from letrozole/alpelisib treatment (Mayer et al., 2017), thus these mutations or amplification may also serve as potential biomarkers to PI3Ki resistance.

SUMMARY

Breast cancer drug resistance is a complex phenomenon that results in treatment failure and disease progression. Inhibitors of the PI3K/AKT/mTOR pathway combined with existing treatment methods can act to overcome drug resistance in different subtypes of breast cancer. In this review, our perspective is that PI3K/AKT/mTOR pathway activation is related to conventional therapy resistance in breast cancer, including endocrine therapy, HER2-targeted therapy, CDK4/6 and PARP inhibitors, chemotherapy and immunotherapy. However, the poor selectivity of the inhibitors is an important factor leading to toxicity. The pan-PI3K inhibitors have shown mild results and significant toxicity. In contrast, the more selective PI3K inhibitors, such as alpelisib, have shown promising results, particularly in patients with PIK3CA mutations. Overall, PI3K/AKT/mTOR pathway inhibitors show the potential ability to restore sensitivity to standard treatments, which is an attractive strategy in overcoming resistance during breast cancer treatment.

A number of issues still should be focused on in the near future. Initially, more highly selective PI3K inhibitors should be developed to obtain better tolerability and anticancer efficiency. Second, further studies should be conducted to identify reliable biomarkers to predict response and resistance as well as avoid unnecessary side effects. Third, more optimized combination regimens need to be investigated for the best synergistic effect. Additionally, PI3K/AKT/mTOR inhibitor resistance should also be investigated. What's more, intrinsic resistance is inevitable besides acquired resistance since PI3K/AKT/mTOR pathway is

basally activated in multiple solid tumors, including breast cancer. Therefore, most importantly, more effective clinical trials should be conducted to adequately confirm or support the preclinical results so that the survival time and quality of life of resistant breast cancer patients may be largely further improved.

AUTHOR CONTRIBUTIONS

CC and JN conceived and designed this review. CD, JW, and YC performed the literatures investigation and reviewed literatures. CD, JW, and CC wrote and revised the manuscript. YC and JW checked the manuscript. All authors edited, read, and approved the manuscript. CD secured funding and administrative support for the project.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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