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REVIEW

Efficacy of PI3K inhibitors in advanced breast cancer

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The phosphoinositide 3 (PI3)-kinase/Akt signaling pathway has always been a focus of interest in breast cancer due to its role in cell growth, cell proliferation, cell migration and deregulated apoptosis. Its activation has been linked to endocrine resistance and worse prognosis in certain subgroups of breast cancer. In addition, deregulation of the PI3K/Akt pathway including *PIK3CA* activating mutation is frequently present in breast cancer. Multiple efforts have been carried out to target this pathway, initially with pan-PI3K inhibitors with some hint of activity but hampered by their limiting side-effects. A recent large randomized trial in patients with endocrine-resistant *PIK3CA*-mutant hormone receptor (HR)-positive tumors led to the approval of the first PI3K inhibitor, alpelisib, in combination with fulvestrant. The specificity of alpelisib against the p110α catalytic isoform provided additional efficacy and a better toxicity profile. In this review, we summarize the main research with PI3K inhibitors in breast cancer and we provide some insight of potential future combinations of this treatment in breast cancer patients.

Key words: PI3K inhibitors, breast cancer, targeted therapy

Introduction

The phosphatidylinositol 3-kinase (PI3K)—protein kinase B (PKB/AKT)—mammalian target of rapamycin (mTOR) axis regulates critical physiological functions and cellular processes, including cell proliferation, growth, survival, motility and metabolism [1]. This pathway has been extensively reviewed elsewhere [2].

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that are divided into three classes based on their structures and substrate specificities. In mammals, class I PI3Ks are further divided into subclasses IA and IB based on their modes of regulation. Class IA PI3Ks are heterodimers that contain a p110 catalytic subunit and a p85 regulatory subunit. The genes *PIK3CA, PIK3CB* and *PIK3CD* encode three highly homologous class IA catalytic isoforms: p110α, p110β and p110δ, respectively. These isoforms associate with any of the five regulatory isoforms collectively called p85-type regulatory subunits [3]. While p110α and p110β are ubiquitously expressed, p110δ expression is largely restricted to leukocytes [4]. Despite the fact that p110α and p110β isoforms are very similar in their catalytic and regulatory domains, they seem to play distinct roles in cellular signaling, growth, and tumorigenesis [3, 5, 6]. It has been shown, for example, that both p110α and p110β contribute to insulin action in the liver [7–10], whereas angiogenesis and vascular endothelial growth factor (VEGF) signaling require p110α but not p110β [11]. In addition, p110β has a role in platelet biology and thrombosis [12]. The p110δ isoform is mostly expressed in the hematopoietic system, including myeloid cells, B and T cells and play key roles in leukocyte signaling, proliferation, differentiation, activation, and chemotaxis [13]. A substantial number of clinical trials have led to the approval of different PI3K inhibitors in lymphoid and myeloid malignancies [14–16].

In breast cancer, the PI3K/AKT/mTOR pathway can be deregulated by a number of different mechanisms. First, *PIK3CA* activating mutations located either at the helical or the kinase domain are present in more than one-third of early breast cancer tumors (45% in luminal A, 29% in luminal B, 39% in HER2-enriched and 9% in basal-like tumors) [17–19]. A recent report has identified similar mutation rates in metastatic breast cancer (MBC) biopsies, confirming the clonal character of this mutation

[20]. Second, inactivating events might occur in tumor suppressor genes, mostly *PTEN*, but also *PIK3R1*, *INPP4B*, *TSC1*, *TSC2* and *LKB1*, leading to the activation of this pathway [18, 21, 22]. In addition, *PIK3CA* amplification and mutations in the *AKT* gene have been also described [20, 23–25].

The influence of these molecular aberrations on outcomes is still unclear. Whereas *PIK3CA* mutation in early hormone receptor positive (HR+)/HER2- breast cancer is associated with a better recurrence-free survival [26] and a better disease-free survival (DFS) [27], recent molecular profiling data from MBC patients seem to indicate that in advanced HR+/HER2- breast cancer, a *PIK3CA* mutation would lead to a certain resistance to chemotherapy and a poor outcome [28]. In the case of HER2-positive breast cancer, *PIK3CA* mutations seem to be associated with worse prognosis, either in the advanced and in the early setting [29, 30]. Moreover, the PI3K/Akt/mTOR pathway has been described as potentially intervening in secondary endocrine resistance in HR-positive breast cancer [31]. In preclinical models, long-term estrogen-deprived breast cancer cells and long-term exposure to tamoxifen are associated to an up-regulation of the PI3K pathway, leading to a ligand-independent activation of ER by its phosphorylation through the mTOR complex 1 (mTORC1)/S6K1 axis [31, 32]. Hence, there is a strong rationale to therapeutically target the PI3K/AKT/mTOR axis, especially in HR-positive breast cancer.

In this review, we will describe the clinical development and efficacy of different PI3K inhibitors in breast cancer and the potential future role for treatment.

Clinical data in HR+ HER2- advanced breast cancer

Pan-PI3K inhibitors

Early PI3K inhibitors (PI3Ki) targeted each of the four catalytic isoforms of class I PI3Ks, potentially for a broader activity in a number of tumor types with a range of molecular alterations. However, this broad inhibition may lead to potentially a higher risk of adverse events (AEs), which could limit the use of such agents at therapeutic doses.

Buparlisib. Buparlisib (BKM120; Novartis Pharmaceuticals, Basel, Switzerland) is an orally available pan-PI3Ki and the most clinically advanced agent in this class.

In vivo, buparlisib demonstrated antiproliferative and apoptotic activity in human tumor cell lines with PI3K pathway alterations and induced significant dose-dependent tumor growth delay or regression in *PIK3CA*-mutant tumor xenografts [33].

Due to the evidence supporting the hypothesis that activation of the PI3K pathway is a key mechanism of endocrine resistance in HR+/HER2- MBC(31), PI3Ki have been tested in combination with endocrine treatment in patients previously treated with hormone therapy.

In a phase I study (NCT01339442) of buparlisib in combination with fulvestrant in HR+ postmenopausal MBC patients previously treated with endocrine therapy ($n = 31$), the combination treatment achieved a clinical benefit rate (CBR) of 58.6% [34]. Treatment-related AEs were usually mild and resolved within 1–3 weeks after dose interruption or modification. The phase III BELLE-2 clinical trial (NCT01610284) randomized 1147 postmenopausal patients with HR+/HER2- locally advanced or MBC previously treated or who had progressed on treatment with an aromatase inhibitor to receive either buparlisib or placebo with fulvestrant [35]. The trial met its primary objective, demonstrating that the addition of buparlisib to fulvestrant prolonged progression-free survival (PFS) compared with fulvestrant alone [6.9 versus 5.0 months; hazard ratio (HR) 0.78; 95% confidence interval (CI) 0.67–0.89; $P < 0.001$). Frequent discontinuations because of AEs (transaminitis, hyperglycemia, rash, and mood disturbances) reduced the duration of treatment in the buparlisib arm, potentially limiting the efficacy of the combination therapy. Of note, this study included prespecified analyses of whether PI3K pathway activation in archival tissue (defined as *PIK3CA* mutation and/or loss of PTEN expression) was predictive of clinical benefit. The PFS improvement observed with buparlisib plus fulvestrant in the PI3K-pathway-activated population was not statistically significant when PI3K status was determined on tissue samples. However, in an exploratory analysis, when *PIK3CA* mutations were identified in ctDNA the buparlisib and fulvestrant combination was associated with a meaningful improvement in PFS compared with those treated with fulvestrant alone (7.0 months versus 3.2 months, HR 0.56; 95% CI 0.39–0.80, $P < 0.001$).

In the subsequent phase III BELLE-3 (NCT01633060) [36], patients with MBC pretreated with endocrine therapy and an mTOR inhibitor were randomized to receive a buparlisib-fulvestrant combination ($n = 289$) or placebo-fulvestrant ($n = 143$). The clinical trial design included end points such as efficacy by *PIK3CA* status (either determined by PCR in tumor tissue or by BEAMing using ctDNA from plasma samples). The study met its primary end point and the administration of buparlisib was associated with a higher PFS of 3.9 months versus 1.8 months for the fulvestrant-placebo group (HR 0.67; 95% CI 0.53–0.84; $P < 0.001$). Again, patients with *PIK3CA* mutations (both from tumor tissue and ctDNA) showed longer PFS than those with *PIK3CA*wt on the combination treatment arm.

Buparlisib has also been tested in combination with chemotherapy in breast cancer patients in the phase II/III clinical trial BELLE-4 (NCT 01572727) combining buparlisib or placebo with paclitaxel as the first-line treatment in 416 patients with HER2-negative MBC [37]. No significant differences were observed with the addition of buparlisib in median PFS (8.0 months in buparlisib group versus 9.2 months in placebo group). In this study, buparlisib treatment was associated with a higher frequency of serious AEs (30% in the buparlisib arm versus 21% in the placebo group) as well as a higher frequency of all grades AEs including diarrhea (55%), rash (43%), nausea and hyperglycemia (41% each), leading to a higher incidence of treatment discontinuation.

This increased toxicity had also been observed in the previous BELLE-2 and BELLE-3 clinical trials. The description of toxicities, such as transaminitis and psychiatric complications such as depression, anxiety and suicide attempts related to buparlisib, limited the potential for this agent to be adopted as standard of care.

Pictilisib. Pictilisib (GDC-0941; Genentech, San Francisco, CA) is another pan-PI3Ki that exhibits *in vitro* equipotent inhibition of

the p110α and –δ PI3K isoforms and less potent inhibition of p110β and –γ isoforms [38]. In a phase I dose-escalation clinical trial (NCT00876109) in unselected patients with advanced solid tumors ($n = 60$), pictilisib was overall safe with the most frequent grade 3/4 AEs were rash, hyperglycemia, and pneumonitis [39]. Some initial activity was observed with one partial response (out of 60 patients) and two patients reaching stable disease.

The phase II PEGGY clinical TRIAL (NCT01740336) randomized pre- and post-menopausal patients with HR-positive HER2-negative advanced breast cancer to receive paclitaxel with pictilisib or placebo [40]. One hundred and eighty-three patients were randomized to receive paclitaxel treatment combined with either pictilisib (n = 91) or placebo (n = 92). Approximately one-third of patients in each arm had detectable PIK3CA mutations (pictilisib 35.2%, placebo 32.6%). At the interim analyses, there was no significant PFS difference between arms in the ITT population or patients with PIK3CA-mutated tumors. The median PFS in the pictilisib arm was 8.2 months (n = 91) vs. 7.8 months in the placebo arm (n = 92). For the PIK3CA-mutated cohort, the median PFS was 7.3 months in the pictilisib arm (n = 32) and 5.8 months in the placebo arm (n = 30) (HR, 1.06; 95% CI 0.52–2.12; P = 0.88). Similarly, no significant differences were observed in terms of ORR between the two groups.

As with buparlisib, a randomized phase II clinical trial (FERGI, NCT01437566) was conducted to assess the benefit of adding pictilisib to fulvestrant in postmenopausal MBC patients previously treated with an aromatase inhibitor. The treatment combination did not significantly improve PFS as compared with fulvestrant-placebo, potentially due to higher toxicity in the combination arm including rash, diarrhea, transaminitis and fatigue, limiting the administrated dose of pictilisib [41]. Therefore, the development of pictilisib in this setting was discontinued.

In light of the results from these clinical trials with Pan-PI3Ki, there was clear need of treatments with greater PI3K selectivity to improve tolerability and increase efficacy in this patient population.

Isoform-specific PI3K inhibitors

Selective inhibition of specific PI3K isoforms may allow the administration of therapeutic doses of drugs without the off-target toxicity, although they require a narrower patient selection [42]. In breast cancer, the most common molecular alterations in the PI3K pathway are activating mutations in the *PIK3CA* gene, inducing hyperactivation of p110α. Hence, development in breast cancer patients has been focused on inhibitors with higher selection for this isoform.

Alpelisib

Alpelisib (BYL719; Novartis Pharmaceuticals, Basel, Switzerland) is the first oral PI3Ki to selectively target the class I p110α-isoform (IC_{50}=4.6 nM) [43]. A phase I trial (NCT01219699) included patients with *PIK3CA*-altered advanced solid tumors and showed sensitivity to alpelisib monotherapy [44]. The combination of alpelisib with fulvestrant demonstrated synergism when combined in xenografts models [31]. In a phase Ib dose expansion trial (NCT01219699), alpelisib plus fulvestrant led to a complete or partial response in 29% of heavily pretreated MBC patients with *PIK3CA*-mutated tumors [45] and a favorable safety profile in these patients with mainly on-target effects, notably hyperglycemia, nausea or diarrhea.

In light of these results, the phase III SOLAR-1 clinical trial (NCT02437318) was conducted to evaluate the efficacy and safety of alpelisib plus fulvestrant in HR+/HER2- MBC patients previously treated with endocrine therapy. The study was enriched with tumors harboring a *PIK3CA* mutation but included also a cohort of *PIK3CA* wild-type (wt) as a proof-of-concept of activity in this subgroup [46]. The primary end point was PFS in the *PIK3CA*-mutated cohort, whereas secondary end points included, among others, overall survival (OS) in the *PIK3CA*-mutated cohort and safety and efficacy in the *PIK3CA*wt group (determined by OS and PFS). *PIK3CA* status was centrally determined before entry using tumor tissue. In the *PIK3CA*-mutant cohort ($n = 341$), the median PFS was 11 months (95% CI, 7.5–14.5) in the alpelisib arm versus 5.7 months (95% CI, 3.7–7.4) in the fulvestrant-placebo group (HR, 0.65; 95% CI, 0.50–0.85; $P < 0001$). In contrast, in the *PIK3CA*wt cohort ($n = 231$), alpelisib administration was not associated with a significant effect in PFS (7.4 versus 5.6 months; HR 0.85; 95% CI, 0.58–1.25).

As observed with the initial phase I clinical trials, alpelisib toxicity was associated with specific p110α inhibition and included hyperglycemia (all-grade, 63.7% versus 9.8% for the alpelisib and placebo arms, respectively), diarrhea (57.7% 15.7%) and rash (35.6% versus 5.9%). Permanent discontinuation of alpelisib or placebo due to AEs occurred in 25% of patients in the alpelisib group versus 4.2% in the placebo arm; hyperglycemia and rash were the two main AEs leading to discontinuation of alpelisib. These toxicities were observed despite the exclusion of patients with diagnosed type 1 diabetes or uncontrolled type 2 diabetes. Moreover, the clinical trial was amended during its course to restrict the inclusion of patients with pre-diabetes and to provide guidelines for early management of hyperglycemia.

Results from the SOLAR-1 trial led to the approval by the Food and Drug Administration (FDA) of alpelisib in combination with fulvestrant for postmenopausal women, and men, with HR+/HER2-, *PIK3CA*-mutated, advanced or MBC as detected by an FDA-approved test following progression on or after an endocrine-based regimen. The currently approved companion diagnostic test is therascreen® *PIK3CA* RGQ PCR Kit (QIAGEN Manchester, Ltd., Germany), to select patients who have *PIK3CA* mutations in tumor tissue specimens and/or ctDNA isolated from plasma specimens. The recommendation by the FDA is to initially carry out the test in ctDNA and if the test is negative for *PIK3CA* mutations in plasma, patients should undergo testing for *PIK3CA* mutations in tumor tissue. Outside the United States, there is no mandatory companion diagnostic test to determine *PIK3CA* mutation status. A question remains as to whether to use tumor tissue or ctDNA for its determination. A subgroup analysis from the SOLAR-1 phase III trial evaluating PFS by *PIK3CA*-mutational status measured in ctDNA observed that assessing mutational status via liquid biopsy resulted in even larger clinical benefit compared with tissue biopsy, with improvement of median PFS from 3.7 months to 10.9 months. Indeed, while patients with *PIK3CA* mutations evaluated in tissue samples had a 35% reduction in risk for disease progression, the risk reduction was 45% for patients with *PIK3CA* mutations

identified in ctDNA [47]. Moreover, in the combined analyses from the BELLE-2 and BELLE-3 clinical trials, *PIK3CAmut* tumors derived more benefit from buparlisib treatment as compared with *PIK3CA*wt, although this benefit seemed to be numerically higher when the *PIK3CA* mutation was identified by BEAMing in ctDNA as compared with those identified by PCR in tumor tissue [48]. Based on these results, the easy accessibility of ctDNA and the good correlation of *PIK3CA* mutation status determined by ctDNA and tumor tissue [49] makes it plausible to initially use ctDNA and to carry out research for a *PIK3CA* mutation in the tumor tissue in the case of ctDNA negativity.

Taselisib

Taselisib (GDC-0032, Genentech, San Francisco, CA) is an oral class I PI3Ki, sometimes referred as β-sparing, as it exhibits equipotent inhibition of p110α, p110-γ and p110-δ, but inhibits p110β with 30-fold lower potency [50]. With this greater isoform selectivity, taselisib was expected to improve its efficacy in *PIK3CA*-mutant tumors as compared with pan-PI3Ki, with a potentially better toxicity profile. Preclinical studies with taselisib reported tumor suppression in *PIK3CA*-mutant xenografts models [51].

In a phase I dose-finding clinical trial, taselisib demonstrated clinical activity in patients with advanced solid tumors, particularly *PIK3CA*-mutant breast tumors, with an overall response rate (ORR) of 36% (versus 0% for *PIK3CA*wt) [52].

The phase III clinical trial SANDPIPER (NCT02340221) randomized patients with ER+/*PIK3CA*-mutant MBC previously treated with an aromatase inhibitor to receive fulvestrant with either taselisib or placebo [53]. The investigator-assessed median PFS was significant longer, albeit modest, in patients treated with taselisib (7.4 months versus 5.4 months; HR 0.70, $P < 0.01$). Taselisib administration was associated with higher severe AEs, notably diarrhea (grade 3/4 of 12% for taselisib arm versus <1% for placebo) and hyperglycemia (grade 3/4 11% versus <1%, respectively). Because of the modest PFS improvement at the cost of significant toxicity, taselisib will not be further developed in this population. The increased success rate of alpelisib compared with taselisib may be due to more potent and specific inhibition of p110α by alpelisib [43, 54], as evidenced by the higher rates of hyperglycemia seen in the SOLAR-1 trial as compared with the SANDPIPER trial.

Clinical trials in the early setting in HR+ HER2- breast cancer

The efficacy of PI3K inhibitors has already been tested in some phase II clinical trials. In the neoadjuvant Lorelei study (NCT02273973), 334 postmenopausal women with stage I–III HR-positive HER2-negative, PIK3CA-unselected and operable breast cancer, were randomized to letrozole plus taselisib vs. letrozole plus placebo for a total of 16 weeks [55]. The addition of taselisib to letrozole was associated with a higher proportion of patients achieving an objective response in all patients (39% in the placebo group vs. 50% in the taselisib group; OR 1.55, 95% CI 1.00–2.38; p = 0.049) and in the PIK3CA-mutant subset (38% vs. 56%; OR 2.03, 95% CI 1.06–3.88; p=0.033). Although no significant differences were observed in pathological complete response (pCR) between the two groups, either in the overall population (2% in the taselisib group vs. 1% in the placebo group; OR 3.07, 95% CI 0.32–29.85, p=0.37) or in the PIK3CA-mutant cohort (1% vs. 0%; OR not estimable, p=0.48). The most common adverse events in the taselisib group were gastrointestinal (diarrhea, nausea, and stomatitis), fatigue, hyperglycemia, rash, arthralgia, and hot flush; and grade 3 or worse adverse events occurred more frequently in the taselisib arm (26%) as compared to the placebo group (8%).

A similar study (NEO-ORB, NCT01923168) initially randomized T1c-T3, HR-positive HER2-negative postmenopausal operable breast cancer patients with known PIK3CA mutation status, to letrozole in combination with either alpelisib or buparlisib or placebo for 24 weeks in a 1:1:1 fashion [56]. Although the buparlisib was later closed due to the non-tolerable toxicity profile associated with this treatment. The addition to alpelisib was not associated to a higher ORR and pCR rate in either the PIK3CA-mutant or wild-type cohorts. ORR in the alpelisib versus placebo arm was 43% versus 45% and 63% versus 61% in the PIK3CA-mutant and wild-type cohorts, respectively. In the PIK3CA-mutant group, pCR was of 1.7% for alpelisib arm vs. 3.0% for placebo arm. In The wild-type cohort, observed pCR were 2.8% for the alpelisib arm vs. 1.7% for the placebo arm; all non-significant. At Cycle 1 Day 15, the combination of alpelisib plus letrozole demonstrated effective inhibition of PI3K signaling in the PIK3CA-mutant cohort as measured by a greater decrease in levels of phosphorylated AKT compared with that observed in the placebo plus letrozole arm, although this did not translate to higher anti-proliferative effect as determined by Ki-67 with alpelisib plus letrozole vs. placebo plus letrozole.

Several reasons might be responsible for this lack of significant efficacy with the addition of PI3K inhibitors in the early setting. One is in relation to the limitation of these studies in terms of treatment discontinuation in the PI3K inhibitors arms due to toxicity. 10% of the taselisib treated patients stopped investigational treatment in the Lorelei study vs. non in the placebo group. For the NEO-ORB trial discontinuation rates due to adverse events were of 25.7% and 31.7 both in the alpelisib arms for the PIK3CA-mutant and wild-type cohorts, respectively. This is of importance in the case of neoadjuvant treatment in early HR-positive HER2-negative early breast cancer were pCR following neoadjuvant endocrine therapy are usually low and in relation to longer treatment duration [57, 58].

Moreover, the significance of a PIK3CA mutation in early HR-positive HER2-negative breast cancer patients still remains unclear [59]. It has been suggested that early-stage PIK3CA-mutant breast cancers may be less dependent on PI3K signaling compared with recurrent or metastatic disease as dependence of the tumor on the PI3K pathway for growth and survival can differ between early- and late-stage disease [60] and they do not necessarily correlate with an active pathway [61]. In addition, the presence of a PIK3CA mutation in early breast cancer has not been identified as predictor of outcome or response to neoadjuvant endocrine treatment in previous clinical trials [62, 63], in agreement with results from both the Lorelei and the NEO-ORB clinical trials.

Role of PI3K inhibition in current clinical practice *(post CDK4/6 inhibitors era)*

Current standard first-line endocrine treatment for postmenopausal HR+/HER2- MBC patients consists of the administration of an aromatase inhibitor with a CDK4/6 inhibitor, palbociclib, ribociclib or abemaciclib, based on the results of phase III clinical trials demonstrating superior PFS as compared with aromatase inhibitor alone [64–66].

Recruitment for the phase III clinical trial SOLAR-1 started mid-2015, shortly after the FDA granted accelerate approval for palbociclib in association with an aromatase inhibitor as the first-line treatment. Due to these circumstances, only 5.9% of patients in the *PIK3CA*-mutated cohort had received a CDK4/6 inhibitor previous to enrolment in the SOLAR-1 trial. Therefore, it is still unclear if previous CDK4/6 treatment might have an effect on benefit to subsequent PIK3 inhibition.

There is evidence of a crosstalk between the CDK4/6 and the PI3K–mTOR pathways [67]. In fact, Goel et al. showed that inhibition of CDK4/6 not only suppressed Rb phosphorylation but also reduced the TSC2 phosphorylation, thereby partially hindering mTORC1 activity [68]. Additionally, Vora et al. demonstrated that CDK4/6 inhibitors were able to sensitize *PI3KCA* mutation-bearing breast cancer cell lines to PI3K inhibitors, as tumors cells insensitive to PI3Ki presented with persistent Rb phosphorylation [67]. These data were confirmed in *PIK3CA*-mutant mouse xenografts, in which co-treatment with CDK4/6 and PI3Ki elicited tumor regression, as well as in tumor samples from patients treated with alpelisib, where a tight correlation between patient response to alpelisib and suppression of Rb phosphorylation was observed.

On the other hand, it has been demonstrated that resistance to CDK4/6 inhibitors could be dependent on the activation of a compensatory PI3K non-canonical cyclin D1-CDK2 pathway, leading to the phosphorylation of Rb. Using ER-expressing breast cancer cell lines, two studies showed *in vitro* that a combination of treatment with PI3K and CDK4/6 inhibitors can overcome resistance to single-agent CDK4/6i because of the downregulation of cyclin D1 [67–69]. Subsequently, Herrera-Abreu et al. showed that combining CDK4/I and PI3Ki triggered cancer cell apoptosis both in breast cancer cell lines and in patient-derived xenografts [69]. Furthermore, the authors demonstrated that a combination of endocrine therapy, CDK4/6 inhibition and PI3K inhibition was even more effective at inducing tumor regression [69].

These preclinical observations led to a phase Ib/II study of ribociclib, alpelisib and letrozole (NCT01872260) in advanced postmenopausal ER+/HER2– breast cancer patients. In the dose-escalation part of the trial, the RP2Ds recommended were lower than the approved doses (ribociclib 300 mg/day, alpelisib 200 mg/day and letrozole 2.5 mg/day) as a consequence of the frequency of drug-related grade 3/4 AEs including elevated ALT (30%) and AST (26%), neutropenia (17%) and hyperglycemia (17%) [70]. Of the 43 patients who were evaluable for response, the ORR was 16% and the disease control rate was 70%, with a 24-week CBR of 26%. Nevertheless, toxicity associated with the triplet might be limiting for a long treatment period and it seems unlikely that the first-line treatment with this triple combination might be able to obtain greater benefit on overall PFS than the administration of each sequential line [71].

Despite that in the main clinical trials with CDK4/6 inhibitors the presence of a *PIK3CA* mutation did not seem to have an impact on benefit to CDK4/6 [72], further research from patients included in the PALOMA 3 trial identified a lower percentage of tumors with baseline *PIK3CA* mutation in the group of long-term responders to palbociclib (>18 months) versus those who benefited for less than 18 months, suggesting that this mutation could have an impact on long-term benefit from CD4/6 inhibition. Supporting this, O'Leary et al. observed in this same population that a relative increase in *PIK3CA* mutant copies/ml in ctDNA after 15-day treatment strongly predicts long-term benefit from palbociclib and fulvestrant treatment (HR 3.94, log-rank $P = 0.0013$) [73].

These results, if validated, could identify a subgroup of patients for whom treatment with a PI3Ki could be evaluated at an earlier stage than at clinical progression on CD4/6 inhibitors.

To further highlight the importance of determining tumor dynamics after CDK4/6 treatment, tissue analysis of paired samples from patients treated with CDK4/6 inhibitors revealed an enrichment in PTEN loss ($q < 0.005$), including in tumors harboring a clonal *PIK3CA* mutation [74]. PTEN loss has been previously described as a mechanism of secondary resistance to α-specific PI3Ki [75] as in the absence of PTEN, cells become more dependent on p110β to maintain PI3K pathway activation when p110α is blocked.

The currently ongoing clinical trial ByLieve (NCT03056755) is recruiting patients with advanced HR+/HER2- *PIK3CA*-mutant breast cancer who have had disease progression during or after a CDK4/6 inhibitor, or whose immediate prior treatment was chemotherapy or ET, to further assess the efficacy of alpelisib in this context.

The potential role of PI3K inhibitors in other breast cancer subtypes

Triple negative breast cancer

Activating *PIK3CA* mutations represent the second most frequent molecular aberration in triple negative breast cancer after *TP53* mutations. This is in addition to the rationale of combining PI3Ki with PARP inhibitors [76, 77], immune checkpoint inhibitors [78] and chemotherapy [79] might broaden further uses of PI3K inhibitors. For further details on the role and interest of PI3K pathway inhibition in TNBC, we refer the readers to the recent review by Pascual and Turner [80].

PI3K inhibition in HER2-positive breast cancer

There is a strong rationale to therapeutically target the PI3K pathway in HER2-positive breast cancer since it is highly deregulated in this disease and preclinical studies suggest that HER2 signaling is mediated almost entirely through p110α, rather than one of the other three catalytic subunits of PI3K [81].

In early HER2-positive breast cancer patients, the presence of a *PIK3CA* mutation was associated with a reduced pathological

complete response (pCR) rate in patients receiving a combination of neoadjuvant chemotherapy and anti-HER2 therapy [82]. In the advanced setting, several studies have described that *PIK3CA* mutation is associated with worse prognosis, although the mutation was not predictive of benefit to different anti-HER2 agents [30, 83].

There have been some clinical studies trying to determine the potential benefit of PI3K inhibition in HER2-positive breast cancer. The phase Ib trial PIKHER2 (NCT01589861), assessing the benefit of the combination of the panPI3K buparlisib and lapatinib was conducted on trastuzumab-resistant, *PIK3CA*-unselected, HER2-positive MBC patients [84]. The authors reported an RP2D dose for buparlisib of 80 mg (lower than for the HR+ clinical trials) and 1000 mg/daily for lapatinib. Observed CBR was of 29% with one patient (4%) experiencing a complete response.

Another phase Ib/II trial (NCT01132664) was conducted in patients with advanced HER2-positive breast cancer assessing the combination of buparlisib and trastuzumab in *PIK3CA*-unselected tumors resistant to trastuzumab [85]. The authors observed evidence of clinical activity with the combination (2% CR and 8% confirmed PR), although it did not meet the pre-established estimated primary endpoint of ORR $\geq$ 25%.

The NeoPHOEBE phase II trial (NCT01816594) randomized HER2-positive early breast cancer patients to either buparlisib or placebo in association with paclitaxel and trastuzumab. Only a small proportion of patients presented a *PIK3CA* mutation (16% for each group). The authors reported pCR rates of 32% for the buparlisib arm versus 40% for the placebo arm [86]. As expected, buparlisib administration was associated with higher incidence of serious AEs (36%) as compared with the placebo group (8%).

A phase I trial with the α-specific PI3Ki alpelisib in combination with trastuzumab emtansine (TDM-1) (NCT02038010) in trastuzumab-resistant patients determined the MTD of alpelisib as 250 mg [87]. In this study, the ORR was of 43%. In patients who had previously progressed on TDM-1 ($N = 10$), the ORR was 30% with 60% CBR. Despite the fact that patients were not selected based on *PIK3CA* status, 9 of the 17 patients included in the study (53%) presented a molecular aberration in the PI3K pathway (PTEN loss, AKT overexpression, or *PIK3CA* mutation). Five of these patients (56%) achieved CBR including three with previous progression on TDM-1 therapy [87]. Although reported side-effects were common (59% of patients experienced grade $\geq$3 toxicity), they were manageable, with evaluable patients receiving a median of 11 cycles of therapy. Results from this study suggest that, similarly to trastuzumab, activation of the downstream PI3K pathway might be a potential mechanism of resistance to TDM-1 [88].

Several other new α-specific PI3K inhibitors are currently been tested in the clinical setting targeting PI3K in breast cancer patients which provide more insight in the role of targeting this pathway in breast cancer patients.

GDC-0077 is a potent, orally available, and selective PI3Kα inhibitor (IC50=0.038 nM) that has demonstrated robust efficacy in *PIK3CA*-mutant breast cancer models [89]. Compared to the PI3K inhibitor, taselisib, the improved biochemical selectivity of GDC-0077 against PI3K delta is demonstrated in human CD69+ B-cells, which are primarily dependent on PI3K delta for proliferation and survival and were more sensitive to taselisib than GDC-0077. Mechanism of action studies indicate that GDC-0077 induces depletion of mutant PI3K alpha protein resulting in reduction of PI3K pathway biomarkers such as pAkt. It has also demonstrated greater inhibition of cell proliferation and increased apoptosis in human *PIK3CA* mutant breast cancer cell lines to a greater extent when compared to *PIK3CA* wild-type cells. In vivo efficacy in a PIK3CA-mutant human breast cancer xenograft model was also improved when GDC-0077 was combined with standard-of-care therapies for hormone-receptor positive (HR+) breast cancer such as anti-estrogens (fulvestrant) or CDK4/6 inhibitor (palbociclib) [89].

GDC-0077 is currently being tested in a Phase I dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of GDC-0077 as a single agent or in combination in combination with endocrine therapies including fulvestrant and letrozole and the CDK4/6 inhibitor palbociclib in advanced breast cancer patients harboring a PIK3CA-mutant tumor (NCT03006172). This trial is due to recruit more than 100 participants and includes one arm with GDC-0077+Palbociclib+Fulvestrant+Metformin to assess prevention of hyperglycemia.

MEN1611 is also a novel α-selective PI3K inhibitor in solid tumors with high inhibition potency the mutated forms of PI3Kα and PI3Kγ [90]. In an *in vivo* study, MEN1611 demonstrated activity either as monotherapy or in combination with targeted therapies in both xenograft and PDX models of breast cancer and in colorectal cancer and in NSCLC models bearing PI3Kα mutations. A reduced activity was instead observed with PIK3CA wild type had low levels of PTEN. In terms of pharmacodynamic activity both AKT and S6 phosphorylation were significantly inhibited mostly in those with PIK3CA mutation. In HER2-positive PIK3CA-mutated breast cancer cell lines and patient-derived xenograft models, MEN1611 seemed to act synergistically when combined with trastuzumab and induced a dose-dependent alpha-isoform depletion, modulated the macrophage polarization towards a pro-inflammatory phenotype, consistent with its capability to co-inhibit P110γ [91].

It is currently being tested in the phase Ib B-PRECISE-01 clinical trial, in combination with trastuzumab with or without fulvestrant in patients with PIK3CA-mutated, HER2-positive, advanced or metastatic breast cancer who have failed anti-HER2 based therapy (NCT03767335).

Conclusions

Enormous efforts and substantial research have been necessary to finally demonstrate evidence that PI3K is an important therapeutic target in breast cancer tumors with PI3K-dependance. Initial studies with pan-PI3K inhibitors led the way by identifying the target population of PI3K-aberrant tumors and the need for a combination with endocrine therapy, but their lack of specificity was associated to substantial toxicity that limited their use in clinical practice.

The development of isoform-specific PI3K inhibitors was able to overcome some of the previous issues and the p110α-specific alpelisib has recently incorporated into the arsenal available for the treatment of HR-positive MBC patients. Nevertheless, the non-negligible rates of dose reduction and discontinuation reported with alpelisib, despite being a selected population, emphasize the challenge associated with long-term systemic

inhibition of PI3Kα in current clinical practice. The impact of previous CDK4/6 inhibition on alpelisib efficacy is also still unknown. The ubiquitous interaction of the PI3K pathway merits studying further uses for PI3Ki including combinations with CDK4/6 inhibitors, anti-HER2, immune check point and PARP inhibitors, potentially providing a future role in the management of triple-negative and HER2-positive breast cancer patients.

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